Gladden, Nicola Louise (2021) Bovine parturition: welfare and production implications of assistance and ketoprofen analgesia. PhD thesis.

Bovine parturition: welfare and production implications of assistance and ketoprofen analgesia

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Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD)

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December 2021

Abstract

Parturition is a necessary event for productive dairy cows (and their calves) and assisted parturition is common. Although difficult parturition is believed by farmers and veterinary surgeons to be painful and stressful for cows and their calves, data to support this view are limited. Previous studies typically analysed the effects of assistance or analgesia as individual effects but inclusion of both in a factorial design is rare, so the association between pain and parturition assistance is not certain. Furthermore, there is a paucity of studies investigating calf birth-related experiences in general, and available work typically focuses on health and productivity rather than more sensitive measures of welfare (e.g. behaviour). Differences in study design further challenge the interpretation and practical application of available data; most studies refer to 'dystocia', but definitions of this term vary widely and important differences between veterinary and farmer provided assistance are not always acknowledged. Accordingly, it is currently difficult to develop evidence based recommendations for farmers and veterinary surgeons regarding the value of analgesic provision to cows and calves around parturition.

Farmers are recommended to closely monitor cows that may need assisted parturition to enable intervention to be optimised; however, this can be difficult to achieve particularly if staff availability is limited, and it is currently not possible to accurately predict when cows will give birth, or whether they are likely to need assistance. As such, some cows that experience difficult parturition may not receive timely assistance and conversely, some cows may be assisted unnecessarily — both are situations that may challenge welfare.

The studies presented in this thesis aimed to investigate the effects of farmer-assisted parturition and administration of the non-steroidal anti-inflammatory drug ketoprofen on the welfare, health, and productivity of commercially managed Holstein dairy cows and calves (*Bos taurus*) using a 2 x 2 factorial study design. Further work aimed to support the findings of initial studies using accelerometer generated data to analyse behavioural patterns of cows and calves for up to 48 h postpartum. A final aim was to assess the potential for data generated by animal-worn accelerometers to detect cows that are likely to need farmer-provided assistance at parturition.

Cows and calves subject to farmer-assisted and unassisted parturition were randomly assigned to receive either ketoprofen or saline within 3 h of parturition. Behaviour in the first 48 h postpartum was analysed using focal instantaneous sampling (visual observations) to investigate welfare outcomes. Detailed behavioural analysis was complemented with analysis of biomarkers indicative of health and welfare status (cortisol, creatine kinase [cows and calves]; L-lactate, plasma total protein [calves only]) in the first 7 d postpartum. Regardless of ketoprofen treatment, cows and calves subject to assisted birth showed behavioural differences consistent with a reduced welfare state (increased lateral recumbency [both] and reduced play [calves only]), compared to unassisted animals. Additionally, the plasma cortisol concentration of assisted cows was higher than unassisted cows immediately after parturition, suggesting assisted parturition is associated with heightened maternal stress. Irrespective of assistance status, cows and calves treated with ketoprofen engaged in behaviours consistent with pain and reduced welfare less than saline treated animals. Additionally, ketoprofen treated cows engaged in lying postures suggestive of improved comfort, and ketoprofen treated calves engaged in play behaviour more than saline treated cows and calves respectively (regardless of assistance status) — suggesting that all cows and calves experience pain after parturition that can be improved by ketoprofen. Results of further work using accelerometers to continuously monitor behaviour for 48 h after parturition corroborated these findings — ketoprofen treated cows and calves were more active than saline treated animals and ketoprofen treated calves engaged in increased play behaviour. Health and productivity data for cows and calves recruited in initial work were obtained from farm records: cow data were collected until the end of the subsequent lactation (approximately one year), calf data were collected until the end of the first lactation (approximately three years). Regardless of treatment status, parturition assistance was associated with increased postpartum disease and reduced maternal reproductive performance in the subsequent lactation. Birth assistance was associated with poorer growth of calves before first parturition and reduced reproductive performance in the first lactation (irrespective of treatment status). Ketoprofen treated cows had a 305 d mature equivalent milk yield 664 kg higher than saline treated cows, irrespective of assistance status. Ketoprofen treatment did not affect measures of calf productivity overall but ketoprofen treated assisted calves had a growth rate to weaning 0.1 kg/d higher than calves in the other assistance x treatment status interaction groups.

Accelerometer generated data (primarily step count) showed potential for detection of cows more likely to need assistance, although a threshold for detection could not be established with high accuracy. Additionally, the number of lying bouts exhibited by cows in the last 12 h of gestation showed promise for predicting the timing of parturition. These data suggest that leg-worn accelerometers may be a valuable tool to aid pre-partum management of dairy cows, and the results presented here offer a starting point for the development of pre-partum specific algorithms for use in future remote devices.

Collectively, the results presented in these studies indicate that parturition assistance is negatively associated with welfare and future productivity of cows and calves, and that ketoprofen administration immediately after parturition has beneficial effects on these outcomes. However, observed interaction effects were few, suggesting that a) farmer-assisted cows and calves experience challenges to welfare that extend beyond pain (i.e. challenges that cannot be manipulated using analgesia), and b) pain is experienced by all cows and calves after parturition, not just those that are assisted. These findings suggest that assistance at parturition should be provided judiciously and not be a routine management intervention. Furthermore, these results provide a robust basis on which inclusion of ketoprofen administration in parturition and newborn calf management protocols can be recommended to dairy farmers and veterinary surgeons seeking to optimise the welfare and productivity of Holstein cows and calves managed in a housed dairy system.

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Journal articles

Gladden, N., Ellis, K., Martin, J. and McKeegan, D. (2021) ‘Administration of ketoprofen affects post-partum lying behaviours of Holstein dairy cows regardless of whether parturition is assisted’, *The Veterinary Record*: e300. https://doi.org/10.1002/vetr.300.

Gladden, N., Cuthbert, E., Ellis, K. and McKeegan, D. (2020) ‘Use of a Tri-Axial Accelerometer Can Reliably Detect Play Behaviour in Newborn Calves’, *Animals*: 10(1137). https://doi.org/10.3390/ani10071137.

Gladden, N., Ellis, K., Martin, J., Viora, L. and McKeegan, D. (2019) ‘A single dose of ketoprofen in the immediate postpartum period has the potential to improve dairy calf welfare in the first 48h of life’, *Applied Animal Behaviour Science*: 212, pp. 19–29. https://doi.org/10.1016/J.APPLANIM.2019.01.007.

Gladden, N., McKeegan, D., Viora, L. and Ellis, K. (2018) ‘Postpartum ketoprofen treatment does not alter stress biomarkers in cows and calves experiencing assisted and unassisted parturition: A randomised controlled trial’, *The Veterinary Record*: 183, p. 414. https://doi.org/10.1136/vr.104913.

Conference presentations

Gladden, N., McKeegan, D., Martin, J., and Ellis, K. (2021) ‘Effects of calving assistance and pain-relief on the welfare and productivity of Holstein dairy cows’ [poster presentation], *Total Dairy Seminar*, Stratford upon Avon, UK, 10-11 November 2021.

Gladden, N., Ellis, K., Martin, J., and McKeegan, D. (2021) ‘Effects of calving assistance and pain-relief on the welfare and future productivity of Holstein dairy calves’ [poster presentation], *Total Dairy Seminar*, Stratford upon Avon, UK, 10-11 November 2021.

Gladden, N., McKeegan, D. and Ellis, K (2021) ‘Effects of farmer-assisted parturition and postpartum NSAID treatment on future productivity of dairy calves’ [oral presentation], *British Cattle Veterinary Association (BCVA) annual congress*, Newport, Wales, 14-16 October.

Gladden, N., McKeegan, D. and Ellis, K (2021) ‘Effects of farmer-assisted parturition and postpartum NSAID treatment on subsequent lactation performance’ [poster presentation], *British Cattle Veterinary Association (BCVA) annual congress*, Newport, Wales, 14-16 October.

Gladden, N., Ellis, K., Martin, J., Viora, L. and McKeegan, D. (2018) ‘Do newborn calves benefit from administration of analgesia in the immediate postpartum period?’ [oral presentation], *British Cattle Veterinary Association (BCVA) annual congress*, Hinckley, Leicestershire, UK, 18–19 October.

Gladden, N., Ellis, K., Martin, J., Viora, L. and McKeegan, D. (2018) ‘Effects of Postpartum Pain Relief on Behaviour of Calves Born to Both Assisted and Unassisted Parturition’ [oral presentation], *World Buiatrics Congress*, Sapporo, Japan, 28 August – 1 September.

Gladden, N., McKeegan, D., Viora, L. and Ellis, K (2017) ‘Behavioural effects of post-partum pain relief in calves born with and without assistance’ [oral presentation], *Association of Veterinary Teaching and Research Work (AVTRW) annual conference*, University of Surrey School of Veterinary Medicine, Guildford, UK, 12-13 September.

Gladden, N., Strazhnik, E., McGann, A., Viora, L., McKeegan, D., Ellis, K., (2017) ‘A Pilot Study to Investigate the Feasibility of Behavioural Monitoring of Periparturient Cows and Calves on a Commercial Dairy Farm in Scotland’ [poster presentation], *TotalDairy Seminar*, Keele, Staffordshire, UK, 14-15 June.

Gladden, N., Strazhnik, E., McGann, A., Viora, L., McKeegan, D., Ellis, K., (2016) ‘A Pilot Study to Investigate the Feasibility of Behavioural Monitoring of Periparturient Cows and Calves on a Commercial Dairy Farm in Scotland’ [poster presentation], *World Buiatrics Congress*, Dublin, Ireland, 3-8 July.

Acknowledgements

The journey to completion of this PhD thesis has been a long one, starting as an MVM(Res) project and encompassing completion of a Senior Clinical Scholarship (residency) before finally arriving at this point — almost 7 years after starting. This has not been a solo adventure and undoubtedly would not have been possible, or as enjoyable, without the support and friendship of the many people I have met along the way. I would like to take this opportunity to thank all of you.

First and foremost I must extend my eternal gratitude to my main supervisors Dr. Kathryn Ellis and Dr. Dorothy McKeegan for their unwavering support, encouragement, and friendship over the past few years without which the work presented in this thesis would not have been possible. Their doors have always been open for any reason at all and their encouragement has pushed me to expand my research and writing skills considerably. I am in no doubt that without your guidance I would not have achieved as much as I have during the past 7 years, and I will always be thankful to both of you.

I must also thank Lorenzo Viora (University of Glasgow) for his enthusiasm and support as co- supervisor during the initial stages of this project. I am also grateful to Dr. Jessica Martin (Royal [Dick] School of Veterinary Studies and the Roslin Institute) for providing statistical analysis for some of the studies in this thesis and for patiently answering all of my questions along the way, as well as kindly correcting my many misunderstandings. Dr. Martin's input provided not just analytic assistance but also invaluable guidance and has aided my statistical learning immeasurably.

Completion of this work would not have been possible without funding from several sources. I am grateful to the University of Glasgow James Herriot Scholarship Fund, the James Crawford Endowment Fund, Merial Animal Health (in particular Sioned Timothy) and Ceva Animal Health for providing financial support for these studies. Particular thanks must go to Kythé Mackenzie and Stéphane Floc'h (both of Ceva Animal Health UK) who saw the potential in this work and agreed to partially fund the additional work that facilitated the extension of this project from an MVM(Res) to a PhD. I must also thank IceRobotics for their assistance, in particular Catherine Malcolm and Dr. Vivi Thorup for their helpful advice and suggestions. Thanks also go to Dr. Dominic Mellor (University of Glasgow) at this point for the sourcing of some extra funding to purchase more IceTags as well as statistical assistance in the early stages of the project.

I would also like to thank all of the staff at the clinical pathology department at the University of Glasgow School of Veterinary Medicine for their assistance with the study reported in Chapter 6 — in particular James Harvie for running all of the samples and answering any and all technical questions I posed. My sincere thanks must also go to Erin Cuthbert, Allison McGann, Katie Ord, Emma Strazhnik, and the multiple BVMS Tier 2 students who have aided this work over the years by performing pilot projects as part fulfilment of their BVMS or MSc (Animal Welfare Science, Ethics and Law) degrees. All of your work has been immensely helpful and has made the journey that bit smoother. I would also like to thank Jean Wilson, Dr. Abigail Brown, Dr. Michael Wilkinson (all of the University of Glasgow), and Dr. Kim Willoughby at the Home Office, for their assistance and advice with all matters relating to A(SP)A. Additional thanks must go to everyone (in particular Marie Henderson) involved with the administration involved in changing my original MVM student status to a PhD, I know it wasn't always an easy job and I am grateful. Finally, I would like to express my gratitude to all of the clinicians, residents, interns, technicians (especially Malcolm McColl), and administrative staff I have worked with over the years at the Scottish Centre for Production Animal Health and Food Safety for the countless times they have provided invaluable assistance and support that are too numerous to mention.

An especial thanks goes to all of the farm staff for their assistance with this project, without which none of it would have been possible. In particular I sincerely extend my gratitude to Doug McMillan who kindly took the time and trouble to gain a Personal Licence in order to be able to provide assistance and uncomplainingly obtained blood samples, placed accelerometers on animals, and administered treatments at any time of the day or night as required whilst also looking after a herd of over 700 dairy cows. Thank you also for the many cups of tea and slices of cake supplied over the years! I must also thank David Hamilton for allowing these studies to be performed on his farm and allowing me full access to anything I needed, including (but not limited to) farm records, CCTV footage, and ladders. Further thanks also go to David Montgomery, Archie Hamilton, and Michael Wright who provided additional practical assistance on farm. Finally, I must also thank Archie Leitch (Almins Ltd.) and Will Tulley (Advanced Ruminant Nutrition) for kindly providing technical nutritional details (and answering any and all questions), as well as John Cook (World Wide Sires) for kindly providing DairyComp 305 technical assistance, even after moving onto a new company.

Last but not least I must thank my parents Andrea and Eddie Gladden for their unflinching support in my all my endeavours, as well as my sister Rosie Gladden, not just for her support and provision of perspective at times when I needed it, but also for offering to proof-read and for her artistic skills that appear in this thesis. Lastly, I'd like to thank Alfie for quietly keeping me company along the way.

Author’s Declaration

“I declare that except where explicit reference is made to the contribution of others, this thesis is solely the result of my own work and does not include work presented for another degree at the University of Glasgow or any other institution.”

Nicola Gladden
August 2021

Definitions/Abbreviations

° C	Degrees Celsius
"	Inch (es)
305ME	305 day mature equivalent
95% CI	95% confidence interval
a.m.	*ante merīdiem* ('before midday')
ACTH	Adrenocorticotropic hormone
AICc	Corrected Akaike's information criterion
APP	Acute phase protein
A(SP)A	Animal (Scientific Procedures) Act
AVI	Audio Video Interleave
BCS	Body condition score
BHB	Beta-hydroxybutyrate
BRD	Bovine respiratory disease
BVMS5	Bachelor of Veterinary Medicine & Surgery 5th (final) year
CA	California State
CART	Classification and Regression Tree Analysis
CCTV	Closed circuit television
CK	Creatine kinase
cm	Centimetre (s)
COX	Cyclo-oxygenase
CRH	Corticotropin-releasing hormone
CSV	Comma-separated values
d	Day (s)
DCAD	Dietary cation-anion difference
DIM	Days in milk
dL	Decilitre (s)
DMI	Dry matter intake
DOMS	Delayed onset muscle soreness
DVR	Digital video recorder
e.g.	*Exempli gratia* ('for example')
et al.	*et alia* ('and others')
FO	Fluoride oxalate
FPT	Failure of passive transfer
G	Gauge
g	Gram (s)
GLM	General Linear Model
GLMM	Generalised Linear Mixed Model
h	Hour (s)
HRV	Heart rate variability
Hz	Hertz
i.e.	*id est* ('that is to say')
ID	Identification number
IgG	Immunoglobulin G
IU/L	International units per litre
kg	Kilogram (s)

L	Litre (s)
LB	Lying bouts
LDA	Left displaced abomasum
LH	Lithium heparin
m	Metre (s)
mEq	Milliequivalent (s)
mg	Milligram (s)
MI	Motion Index
min	Minute (s)
mL	Millilitre (s)
mm	Millimetre (s)
mmol	Millimole (s)
n	Number (of samples or subjects)
NJ	New Jersey State
nmol	Nanomole (s)
NSAID	Non-steroidal anti-inflammatory drug
NY	New York State
OR	Odds ratio
p	*p*-value; statistical significance
p.m.	*post merīdiem* ('after midday')
PA	Pennsylvania State
PD	Pregnancy diagnosis
PGF2α	Prostaglandin F2α
pH	Potential of hydrogen; measure of acidity or basicity of a substance
PT	Passive transfer
r	Pearson's correlation coefficient
R^2	Square of the correlation coefficient
RFM	Retained foetal membranes
RI	Reference interval
s	Second (s)
SCK	Subclinical ketosis
SCPAHFS	Scottish Centre for Production Animal Health and Food Safety
SD	Standard deviation
SE	Standard error
TB	Terabyte
TMR	Total mixed ration
TP	Total protein
UK	United Kingdom
USA	United States of America
USG	Urine specific gravity
v	Version
W4MK	Week four milk yield
WA	Washington State
x g	Times gravity
χ^2	Chi-squared
VWP	Voluntary waiting period

Chapter 1 Introduction and Review of the Literature

1.1 Introduction

Dairy productivity is optimised by achieving a calving interval of approximately one year (Holmann *et al.*, 1984), thus parturition is an annual event for most dairy cattle. Although routine, parturition is considered by farmers and veterinary surgeons to be both painful and stressful for cows, especially if difficulty is incurred (Whay *et al.*, 2005a; Huxley *et al.*, 2007; Laven *et al.*, 2009; Moggy *et al.*, 2017; Remnant *et al.*, 2017) but data to support this view are limited. Although a common event affecting almost all cows, parturition is often overlooked in discussions of animal pain (e.g. Steagall *et al.*, 2021) and few studies have investigated the use of analgesia around parturition (Laven *et al.*, 2012). The welfare of farmed animals is of increasing importance to consumers (Ellis *et al.*, 2009; Ortega *et al.*, 2018) and the past three decades have seen an increase in the number of studies investigating the welfare of farmed cattle (von Keyserlingk *et al.*, 2017), but there is still a paucity of work investigating the welfare impacts of parturition. As pain is associated with negative welfare (Rutherford, 2002), the paucity of data regarding parturition-related pain (and other related welfare challenges) experienced by cows and calves represents an area where further study could have a wide positive impact on cattle welfare. This chapter reviews the existing literature around the welfare impacts of parturition on cows and calves with a specific focus on pain, and highlights areas where further research is merited. Behavioural and biochemical indicators of pain are reviewed, as are the effects of parturition on future productivity. Additionally, analgesia is discussed as an available method to reduce pain and thus potentially aid improvements in the welfare of postpartum cows and newborn calves.

1.2 Parturition

1.2.1 Normal parturition (eutocia)

Parturition is a complex process during which the foetus is expelled from the uterus, ending gestation. To be successful, foetal and maternal development need to synchronise to ensure that the foetus is able to adapt to extrauterine life and the dam is able to support neonatal survival (Challis *et al.*, 2005). However, moderate asynchrony resulting in pre-term parturition (defined as 266 to 277 d gestation) is common in cattle (Damaso *et al.*, 2018) and does not affect calf viability (Peters *et al.*, 1992). Whilst the complex hormonal interactions occurring at parturition in cattle are still not fully understood (Shenavai *et al.*, 2012), the important role of the foetus has been known for some time (Liggins, 1968). Prior to ruminant parturition, foetal adrenal gland sensitivity to adrenocorticotropic hormone [see Subsection 1.6.3] increases, resulting in a rapid increase in foetal plasma cortisol concentration. Parturition is initiated by this foetal cortisol peak (Liggins, 1968), and subsequently proceeds in three stages that gradually transition from one to the next:

> **Stage I**: Interaction of foetal cortisol and maternal oestrogen promotes placental prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) production leading to myometrial contraction [Appendix 1] (Challis *et al.*, 2005; Shenavai *et al.*, 2012). Uniquely in bovine parturition, $PGF_{2\alpha}$ production is further enhanced by oxytocin (Fuchs *et al.*, 1992). The luteolytic action of $PGF_{2\alpha}$ releases the maternal reproductive tract from progesterone control, facilitating relaxation and dilation of the cervix and contraction of the myometrium — positioning the foetus ready for expulsion (Mainau *et al.*, 2011). Stage I can last several hours in cattle (Mee, 2004) and during this time cows may be restless or seek isolation from the herd (Berglund *et al.*, 1987; Lidfors *et al.*, 1994; Proudfoot *et al.*, 2014; Rørvang *et al.*, 2018b). Visceral pain predominates (Mainau *et al.*, 2011).
>
> **Stage II**: This stage is characterised by the onset of abdominal contractions and rupture of the amniotic sac, followed by expulsion of the foetus (Mee, 2004; Mainau *et al.*, 2011). Increasing maternal plasma oxytocin concentration (in response to foetal movement into the pelvis) further enhances myometrial contractions. Stage II is typically completed within 2 h in cattle, although can be longer (Dufty, 1972; Miedema *et al.*, 2011a; Schuenemann *et al.*, 2011; Kovács *et al.*, 2016). Somatic pain predominates (Mainau *et al.*, 2011).

> **Stage III**: Myometrial contractions gradually reduce in frequency and intensity and placental expulsion occurs (Mainau *et al.*, 2011). In most cases stage III is completed within 6 h of expulsion of the foetus (Dufty, 1972) but can be longer and placental retention is not considered abnormal until 24 h postpartum (Sheldon *et al.*, 2008).

Stage II parturition in cattle is usually completed in a recumbent position (Houwing *et al.*, 1990) — although if human assistance is provided standing is more common (Edwards *et al.*, 1982; Houwing *et al.*, 1990; Jensen, 2012). Similar to other large ungulates (Hermes *et al.*, 2020), during normal parturition the bovine foetus is presented anteriorly with its head straight and both forelimbs stretched out forwards in a 'diving' position (Dufty, 1973; Homerosky *et al.*, 2017a) [Figure 1.1]. Variations from this foetal presentation are classed as malpresentations and can result in dystocia.

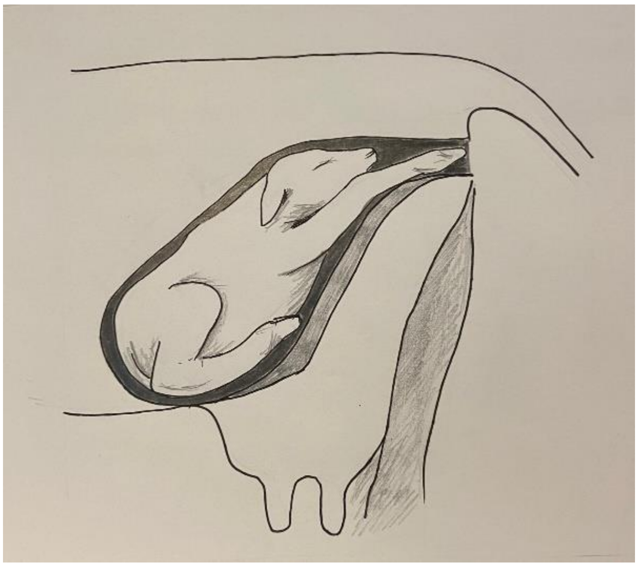

Figure 1.1: Anterior (normal) foetal presentation during bovine parturition
Image: Roseanne Gladden, reproduced with permission

1.2.2 Dystocia and assisted parturition

The term 'dystocia' originates from the Ancient Greek 'dys' meaning difficult and 'tókos' meaning childbirth. Several causes of dystocia are described [Table 1.1] but studies of domesticated cattle have found that foeto-maternal disproportion is the most common (Meijering, 1984; Citek *et al.*, 2011), with foetal malpresentation occurring less frequently in most studies (McDermott *et al.*, 1992; Holland *et al.*, 1993; Nix *et al.*, 1998).

Table 1.1: Described causes of dystocia
(Mee, 2008)

Aetiology	Time of occurrence	Origin[1]
Foetal sex	Conception or early gestation	F
Foetal abnormalities		F
Twins		F
Sire factors (e.g. breed, calving ease)		F
Dam factors (e.g. parity, calving history)		M
Gestation length	Late gestation	F
Foetal oversize		F
Dam undersize		M
Hypocalcaemia		M
Peri-parturient stress		M
Foeto-maternal disproportion	At parturition	M and F
Uterine torsion		M
Foetal malpresentation		F
Uterine inertia		M
Cervical or vulval stenosis		M

1. M = maternal. F = foetal.

Other less common causes of dystocia include congenital foetal abnormalities such as Schistosomus reflexus syndrome (Laughton *et al.*, 2005), uterine inertia, uterine torsion, and vulval, vaginal or cervical stenosis [Table 1.1]. De Amicis *et al.* (2018) found that after foetal malpresentations and foeto-maternal disproportion were accounted for, other types of dystocia were mostly of foetal origin (84%), with macrosomia (excessive foetal size) being most common (84%). The most frequent cause of maternal dystocia recorded by De Amicis *et al.* (2018) was uterine torsion (8.2%).

Dystocia is typically described in veterinary literature as difficulty either necessitating human assistance, or resulting in prolonged unassisted parturition (Blood *et al.*, 2002; Mee, 2004) but in practice these descriptions are highly subjective and accurate definition has proved difficult. Studies commonly use an ordinal scoring system in an attempt to describe parturition more objectively, but the wide variety and lack of standardisation of different systems used [Appendix 1] makes it difficult to compare results. Additionally, studies often report all cases of assisted parturition to be ‘dystocia’ (e.g. Barraclough *et al.*, 2020), use the terms ‘assisted parturition’ and ‘dystocia’ interchangeably (e.g. Whay *et al.*, 2003), or define dystocia independently of assistance score (Swartz *et al.*, 2018). Although sometimes treated as such, the terms ‘assisted parturition’ and ‘dystocia’ are rarely synonymous as most parturition assistance is provided by farmers (Egan *et al.*, 2001) and reasons other than dystocia (such as calf value and staff availability) are reported to influence farmer decision making (Villettaz Robichaud *et al.*, 2016). Accordingly, studies consistently find that although ‘true’ dystocia incidence is typically reported to be 5 to 10% [Table 1.2] the incidence of assisted parturition exceeds this (Mee *et al.*, 2011; Holmøy *et al.*, 2017), suggesting that the provision of parturition assistance in the absence of dystocia is routine on cattle farms.

Table 1.2: Reported incidence of bovine dystocia

Year of publication	Author	Country	Production type	Dystocia incidence
1992	McDermott *et al.*	Canada	Beef	5.8%
1998	Nix *et al.*	USA	Beef	6.0%
1998	Rajala and Gröhn	Finland	Dairy	2.1%
2001	Fourichon *et al.*	France	Dairy	6.6%
2011	Bonneville-Hébert *et al.*	Canada	Dairy	0.9%
2011	Mee *et al.*	Ireland	Dairy	6.8%
2012	Atashi *et al.*	Iran	Dairy	10.8%
2013	Uematsu *et al.*	Japan	Beef	8.6%
2014	Berge *et al.*[1]	France	Dairy	1.9%
		Germany		5.8%
		Italy		3.1%
		Netherlands		1.0%
		UK		3.0%
2014	Waldner	Canada	Beef	8.9%
2017	Daros *et al.*	Brazil	Dairy	11.0%
2017	Holmøy *et al.*	Norway	Beef	4.0%
2018	De Amicis *et al.*[2]	Italy	Dairy	6.2%
			Beef	4.4%
2021	Stefani *et al.*[3]	Brazil	Dairy	18.0%

1. Data for all countries reported together in a single study
2. Data for beef and dairy animals reported together in a single study
3. Only primiparous animals included

However, variability of farm records (Velasova *et al.*, 2015) and lack of standardised terminology make accurate determination of the frequency of assisted parturition difficult and as such a wide range in annual incidence of assisted parturition is reported (0 to 50%) (Egan *et al.*, 2001; Main *et al.*, 2003; Whay *et al.*, 2003; Hansen *et al.*, 2004; Kovács *et al.*, 2016). For the purposes of this literature review, references to 'dystocia' or 'assisted parturition' are aligned with the terminology used in the source material.

Dystocia has negative welfare and economic effects (Dematawewa *et al.*, 1997; Fourichon *et al.*, 2000; Tenhagen *et al.*, 2007; Barrier *et al.*, 2013b) but, although bulls can be selected to minimise dystocia risk (e.g. 'calving ease' index (AHDB, 2019)), production traits such as milk yield and fertility performance are typically prioritised by dairy farmers (Ahlman *et al.*, 2014; Martin-Collado *et al.*, 2015; Just *et al.*, 2018; Skjerve *et al.*, 2018). For instance, one study found that 'calving ease' was considered by dairy farmers to be the least important of ten selected genetic traits (Slagboom *et al.*, 2016). The reason for the low prioritisation of 'calving ease' by dairy farmers is unclear, although it has been suggested that other traits may be perceived to be more profitable (Skjerve *et al.*, 2018). Additionally, most negative economic effects of dystocia are related to reduced fertility and survival (McGuirk *et al.*, 2007) — delayed costs that may not be perceived by farmers to be associated with parturition difficulty. Nevertheless, Dematawewa *et al.* (1997) found that a single case of dystocia could cost up to $400[1], and Kerslake *et al.* (2018) found culling due to 'calving trouble' cost an average of NZ$2208[2] per cow, suggesting that farmer-perceived low profitability of 'calving ease' indices may be inaccurate.

Whilst prevention of dystocia is a worthwhile ultimate aim, it is not totally avoidable. As such, adequate training of veterinary surgeons and farmers for correction of dystocia is necessary to improve the likelihood of successful outcomes. Delivery of a live, healthy calf to a live, healthy dam depends on judicious, appropriately timed intervention and guidelines based on the expected duration of each parturition stage have traditionally been used to aid on-farm decision making; however, these are crude indicators and do not account for different causes of dystocia. Prolonged birth is known to have negative effects on neonatal respiratory function (Vannucchi *et al.*, 2018), but few data regarding optimisation of bovine parturition assistance are available — a single study found

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[1] Equivalent to £290 (calculated based on exchange rate on 2nd May 2021)

[2] Equivalent to £1140 (calculated based on exchange rate on 2nd May 2021)

that management of foetal malpresentation is optimised if parturition assistance is provided immediately (Schuenemann *et al.*, 2011). The same study found that if calves were presented normally, close monitoring for up to 65 min after appearance of foetal hooves (or 70 min after appearance of the amniotic sac) before providing assistance was optimal (Schuenemann *et al.*, 2011). This finding is supported by a more recent study that identified an increased risk of stillbirth and dam injury when assistance was provided too soon (Kovács *et al.*, 2016). Another study found that delaying parturition assistance for more than 1 h is also associated with an increased frequency of stillbirth (Villettaz Robichaud *et al.*, 2017a), suggesting that the 'Goldilocks principle' (Straker *et al.*, 2018) may be applied to the timing of parturition assistance in cattle, with intervening too quickly or too slowly both having detrimental effects. Whilst most farmers provide parturition assistance within 1 h of the appearance of foetal hooves, up to 18% routinely wait for 2 h or more (Egan *et al.*, 2001; Schuenemann *et al.*, 2013). Schuenemann *et al.* (2013) found that farm staff commonly reported being provided with unclear guidance for parturition management although since Schuenemann *et al.* (2013) published their study, more detailed guidelines have been developed for use on farm which may have led to improvements in understanding. For example, in the USA, guidance recommends to seek veterinary advice if hourly progress is not observed (Funnell *et al.*, 2016) and in Scotland a recommended standard operating procedure has recently been published (SRUC & SAC, 2021). It is too early to know the effects that these initiatives may have on the understanding of appropriate parturition management, but this would be of interest for future studies.

1.2.3 Prediction of parturition

The provision of timely and appropriate parturition assistance can be aided by accurate prediction of parturition, lessening the risk of injury (Kovács *et al.*, 2016) and frequency of stillbirth (Saint-Dizier *et al.*, 2015). Physical indicators of impending parturition (e.g. relaxation of pelvic ligaments, swelling of the teats and udder, vulval oedema and vaginal secretions) have traditionally been used to predict parturition in cattle. Whilst non-invasive and readily performed, these traditional methods require close inspection of cattle and rely on identification of changes that are sometimes subtle and can be missed by an inexperienced observer. Furthermore, traditional direct observations have been found to be imprecise predictors of parturition and attempts to accurately predict the timing of parturition using these measures have been disappointing (Lange *et al.*, 2017). Matsas *et al.* (1992) found maternal plasma progesterone concentration < 1.3 ng/mL was predictive of parturition occurring within 24 h, and more recent studies have shown that combining observation of relaxation of the pelvic ligaments with measurement of blood progesterone or oestradiol-17ß concentration accurately predicts the onset of parturition to within 12 to 24 h (Shah *et al.*, 2006; Streyl *et al.*, 2011). However, the routine application of these protocols on farms is limited by the requirement for cattle restraint, the need for staff to be trained in blood sampling techniques, and the expense and time taken to obtain laboratory results.

Koyama *et al.* (2018) employed an alternative approach using animal-worn sensors to measure ventral tail temperature. Whilst this method showed promise for detecting parturition occurring within 24 h, the accuracy of results was affected by environmental temperature which may limit use to the temperate climate studied. Furthermore, commercial availability of animal-worn temperature sensors is limited, whereas devices that measure activity are readily available. These types of devices have been increasingly used to monitor animal behaviour in the past two decades (Brown *et al.*, 2013) and have the potential to detect behavioural changes associated with imminent parturition. Pre-partum changes in maternal behaviour that are detectable by animal-worn remote devices include postural changes (Wehrend *et al.*, 2006; Miedema *et al.*, 2011b, 2011a; Jensen, 2012; Speroni *et al.*, 2018) and tail raising (Miedema *et al.*, 2011b, 2011a), and recent studies have focused on the potential for prediction of parturition using remote-monitoring technology. Titler *et al.* (2015) used data generated by commercially available accelerometers (IceQube, IceRobotics, South Queensferry, UK) to calculate an 'activity index' from step count, standing time and number of lying bouts. Activity index was found to peak 9.25 and 6.5 h prior to parturition of primiparous and multiparous animals respectively, and a 50% increase in activity index was observed approximately 6.25 h (range 2 to 14.25 h) prior to parturition in all animals (Titler *et al.*, 2015), suggesting accelerometer generated data might be able to predict parturition. This hypothesis is supported by Fadul *et al.* (2017) who found that lying behaviours detected by a leg-mounted accelerometer could accurately predict the onset of parturition to within 3 h. A recent pilot study evaluated the potential for a tail-mounted accelerometer to predict the timing of calf expulsion (Krieger *et al.*, 2018), finding the overall accuracy of prediction was better than previous studies (correct to within 52 min) but ranged from 6 to 121 min (Krieger *et al.*, 2018). Only five animals were studied and from a welfare perspective prediction of the onset of parturition is more valuable than prediction of calf expulsion as this allows earlier identification of the need for assistance. Nonetheless, Krieger *et al.* (2018) demonstrated that monitoring tail movement around parturition is feasible and has potential to predict parturition; a finding supported by a more recent study that also found the timing of parturition could be predicted using tail-mounted sensors (Miller *et al.*, 2020). These are novel and exciting findings that may be more practical for on-farm use than leg mounted accelerometers in some situations (e.g. if tail placement is safer than leg placement) and deserve further study.

1.2.3.1 Prediction of dystocia

Insufficient peri-partum monitoring has been found to be associated with poorer calf outcomes and as such, frequent monitoring of late-gestation cows has been recommended (Gundelach *et al.*, 2009). In recent decades there has been a global trend in the dairy industry for fewer, larger herds (Barkema *et al.*, 2015; Gargiulo *et al.*, 2018) and accurate identification of cows at risk of dystocia has the potential to aid the parturition management of these larger herds by allowing farmers to prioritise high risk cattle for monitoring. Although dystocia has been found to be associated with alterations in pre-partum behaviour such as increased postural changes (Proudfoot *et al.*, 2009), increased pawing at the ground and self-grooming (Wehrend *et al.*, 2006), and reduced appetite (Proudfoot *et al.*, 2009), a method to accurately predict dystocia has not yet been found. Use of internal sensors that monitor measures of reticulo-ruminal function such as temperature changes and rumination time have shown promise for predicting dystocia (Kovács *et al.*, 2017a) but few are commercially available, limiting their practical application. Leg- or neck-worn sensors are more widely available but just a single study has assessed the potential for this type of sensor to detect dystocia. Barraclough *et al.* (2020) studied accelerometer generated data for 4 d prior to parturition but did not identify any differences between cattle experiencing eutocia or dystocia. However, Barraclough *et al.* (2020) did not differentiate between dystocia and parturition assistance in their study, including score 2 assistance (defined as gentle manual traction by one person) in the dystocia group. The dystocia group comprised of 14 animals, but nine (64.3%) were classed as score 2 parturition assistance (Barraclough *et al.*, 2020), thus it is difficult to apply these findings in a wider context. It is possible that severe dystocia necessitating more advanced parturition assistance might be more readily predicted using accelerometer generated data.

Vincze *et al.* (2018) adopted a different approach to predicting dystocia, focusing on the calf rather than the cow. Using ultrasonography, this study measured metacarpal or metatarsal thickness and created an index relating this value to dam weight. A lower metacarpal/metatarsal index was associated with an increased dystocia risk (OR 2.074), but this was not found to be significant (95% CI 0.002 to 11.1; *p*-value not reported). Nonetheless, this novel approach — albeit limited to detection of dystocia caused by foeto-maternal disproportion — has potential for adoption on farms with the aid of veterinary surgeons or trained lay-ultrasonographers. Vincze *et al.* (2018) suggested that combining their approach with pelvimetry measurements might improve accuracy of dystocia prediction, an interesting hypothesis that deserves further study.

1.3 Pain and analgesia

1.3.1 What is pain?

Pain is defined by the International Association for the Study of Pain as "*an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage*" (Loeser *et al.*, 2008). This accepted definition highlights that pain is a subjective experience and draws a distinction with 'nociception' (detection of noxious stimuli that result in tissue damage). The basic pain pathway is illustrated in Appendix 1. In brief, specialised tissue receptors (nociceptors) attached to nerve fibres termed Aδ fibres (medium diameter, myelinated) and C fibres (narrow diameter, unmyelinated) detect noxious stimuli (internal and external) (Garland, 2012; Frandson *et al.*, 2015). An action potential is created at the nociceptor and is transmitted along neuronal pathways to the central and autonomic nervous systems, resulting in nociception (Garland, 2012; Yam *et al.*, 2018). Activation of the autonomic nervous system is responsible for sympathetic clinical signs observed in response to pain such as increased heart rate and pupillary dilation (Frandson *et al.*, 2015). In most cases, stimuli are simultaneously relayed to the brain (via the peripheral and central nervous systems), through the thalamus to regions of the cerebral cortex including the amygdala (where emotion is processed), resulting in the corresponding perception of pain (Garland, 2012) and related responses such as escape behaviours (Frandson *et al.*, 2015). A corresponding inhibitory response from the brain occurs that functions to reduce the intensity of pain perception. This process is termed ‘modulation’ and includes release of endogenous opioids, release of inhibitory neurotransmitters (e.g. glycine) and descending inhibitory signals (Das *et al.*, 2018). Pain perception may therefore be considered as a pathway comprising of four main events: transduction (stimulation of nociceptors), transmission (neuronal passage of the nociceptive stimulus), perception (stimulation of the sensory cortex in the brain resulting in the feeling of pain), and finally modulation (neural action to reduce pain perception) [Appendix 1].

Human patients describe different sensations of pain: a sharp, prickly sensation (due to activation of medium diameter myelinated Aδ fibres) and a dull, burning sensation (due to activation of small diameter unmyelinated C fibres) (Garland, 2012; Frandson *et al.*, 2015). It is unclear if cows experience similar pain sensations but the similarity in neuronal anatomy between mammals makes this likely. For clinical purposes pain is typically categorised as nociceptive, neuropathic, and inflammatory [Table 1.3]. Nociceptive and inflammatory pain are the primary types encountered in bovine medicine.

Table 1.3: Categorisation of pain

Type of pain	Description
Nociceptive	Nociceptor detection of noxious stimuli
	Functions to detect potentially harmful stimuli (e.g. thermal, chemical, or mechanical insult)
Neuropathic	Considered a form of neurological injury
	Non-functional ('pathologic') pain
	Associated with allodynia (pain response to non-painful stimuli)
Inflammatory	Interaction between inflammatory mediators and nociceptors
	May be acute or chronic

Pain is considered to be a negative affective state (Sneddon *et al.*, 2014) associated with reduced welfare (Rutherford, 2002), and as such was included in the ‘Five Freedoms’ paradigm developed more than 40 years ago with the aim to improve farm animal welfare (FAWC, 2009). In humans, a Likert scale (typically ranging from ‘no pain’ to ‘the most extreme pain imaginable’) or verbal descriptions of pain are commonly used for pain assessment (Younger *et al.*, 2009) but these tools are most suited to verbal patients and as such are not applicable to animals. Accordingly, assessment of animal pain typically involves measurement of functional, behavioural or physiological responses to perceived painful events such as surgery — an approach optimised by the inclusion of analgesia in a 2 x 2 factorial design (Weary *et al.*, 2006).

1.3.2 Analgesia in bovine veterinary practice

1.3.2.1 Perceptions of pain and use of analgesia in the cattle industry

A number of authors have surveyed farmer and veterinary surgeon attitudes to pain in cattle (Whay *et al.*, 2005a; Huxley *et al.*, 2007; Laven *et al.*, 2009; Thomsen *et al.*, 2012; Remnant *et al.*, 2017) finding that, whilst there is a high degree of pain recognition, perceptions vary widely. Furthermore, the proportion of respondents (both farmers and veterinary surgeons) routinely administering analgesia following a painful procedure (or to treat a painful condition) is frequently lower than the proportion of respondents that consider the same procedure or condition to be painful (Huxley *et al.*, 2007; Thomsen *et al.*, 2012; Remnant *et al.*, 2017) — suggesting that cattle may be routinely experiencing painful conditions without appropriate analgesic provision. This hypothesis is supported by studies of calf disbudding (a known painful procedure) that historically report disbudding to be frequently performed in the absence of pain relief (Misch *et al.*, 2007; Vasseur *et al.*, 2010); however, more recent studies have identified changing attitudes (Winder *et al.*, 2016; Hambleton *et al.*, 2017). Although the results of these studies are encouraging and will be contributing to improved welfare standards, there are still improvements to be made as no studies report 100% adoption of anaesthesia (or analgesia) at disbudding — even among veterinary surgeons. Furthermore, although current best practice recommendations for disbudding are a multimodal analgesic approach comprising local anaesthesia and non-steroidal anti-inflammatory drug (NSAID) analgesia (Winder *et al.*, 2018), this is rarely performed in practice with most farmers and veterinary surgeons choosing to just use local anaesthesia (Winder *et al.*, 2016;

Hambleton *et al.*, 2017). The lack of appropriate pain relief when performing a known painful procedure suggests that administration of analgesia to animals experiencing conditions less well studied may also be infrequent. This hypothesis is supported by Remnant *et al.* (2017) who found that as recently as four years ago, just 30% of UK cattle veterinarians reported using NSAID analgesia following castration. Barriers to analgesic use in farm animal practice include financial concerns, a lack of awareness of availability of analgesic drugs, and uncertainty regarding administrative techniques (Dwane *et al.*, 2013). Additionally, some countries restrict drug administration to veterinary surgeons, adding an extra barrier to analgesia usage (Hokkanen *et al.*, 2015). Interestingly, although veterinary surgeons perceive cost to be a significant barrier (Huxley *et al.*, 2006, 2007; Remnant *et al.*, 2017), farmers typically do not report this and awareness may be a greater barrier. For example, Whay *et al.* (2005a) found that 65% of veterinary surgeons believed cost of analgesia is a 'major issue' but when surveyed, few farmers reported concerns regarding cost of analgesia whereas over half of farmers stated that veterinary surgeons do not discuss options for pain control well enough (Huxley *et al.*, 2007). This suggests that a lack of communication between veterinary surgeons and farmers may be limiting the use of analgesia in cattle and may potentially limit progression of animal welfare standards. However, a more recent study by Andrighetto Canozzi *et al.* (2020) found that veterinary surgeons did not consider cost to be a 'major concern' for farmers, suggesting that veterinary attitudes may be changing. Indeed, Remnant *et al.* (2017) identified a decrease in the proportion of veterinary surgeons that thought analgesia cost was a 'major issue' (45% in 2017 compared to 65% in 2007) and an increase in the proportion of veterinary surgeons who thought farmers would be prepared to pay for analgesia (52% in 2017 compared to 37% in 2007). However, it needs to be considered that these studies were performed in different countries (Brazil and UK) and there are likely to be cultural differences affecting veterinary perception of farmer attitudes to pain relief. Furthermore, whilst both studies referred to 'major' concerns, this term was not defined in either study (Remnant *et al.*, 2017; Andrighetto Canozzi *et al.*, 2020) and subjective interpretation of the term 'major' by respondents may also have affected results. Accordingly, it is difficult to determine from these studies whether veterinary perceptions of barriers to analgesic use in cattle are changing or not. Nevertheless, Remnant *et al.* (2017) found that two-thirds of veterinary surgeons reported increased usage of analgesia in cattle during the previous decade with the majority (77%) citing changes in farmer attitude as a reason for this. Furthermore, the data also suggest that veterinary surgeons who qualified since 2010 are more aware of pain experienced by cattle than older veterinary surgeons (qualified prior to 1990) (Remnant *et al.*, 2017). Although it is not possible to determine from this study whether it is farmer attitudes *per se* that have changed or rather veterinary perception of farmer attitudes towards analgesia that has changed, this finding suggests that progress is being made in UK clinical farm practice and veterinary educational institutions regarding the recognition and treatment of pain in cattle. Farmer access to analgesic and local anaesthetic drugs typically requires veterinary involvement, either for prescribing (e.g. UK) or administering (e.g. Finland (Hokkanen *et al.*, 2015) and Brazil (Andrighetto Canozzi *et al.*, 2020)) thus it is important that veterinary surgeons maintain adequate communication with their clients to avoid developing potentially misplaced perceptions that may impede analgesic use in livestock. It is also important that veterinary education addresses these issues so that veterinary surgeons of the future are accurately informed regarding barriers to analgesic use in farmed species and can utilise this knowledge to encourage further adoption and enhancement of pain control in livestock, providing further improvements to welfare.

1.3.2.2 Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs are commonly used in farm animal veterinary practice for their antipyretic, anti-inflammatory and analgesic properties and a number of licensed formulations are available (Lees *et al.*, 2004; Whay *et al.*, 2005a; Hudson *et al.*, 2008). All NSAIDs have a common mechanism of action: inhibition of arachidonate cyclo-oxygenase (COX) enzymes, resulting in the inhibition of prostaglandin and thromboxane (Smith *et al.*, 1971; Vane, 1971). However, effects at a molecular level can vary between NSAIDs and some have central effects in addition to peripheral effects (Díaz-Reval *et al.*, 2004; Lees *et al.*, 2004; Stock *et al.*, 2015). The two principal COX enzymes are COX-1 and COX-2 and more recently a third COX enzyme has been identified (COX-3) (Chandrasekharan *et al.*, 2002), although the significance of COX-3 in cattle is yet to be elucidated. Cyclo-oxygenase-1 is expressed in most tissues and is considered a ‘house-keeping’ enzyme involved in cell to cell signalling and tissue homeostasis;

COX-1 is involved in blood clotting and maintenance of renal and gastric health (Rang *et al.*, 1999; Lees *et al.*, 2004; Radi, 2009). Cyclo-oxygenase-2 is expressed in inflammatory cells in response to injury or disease and is responsible for the production of inflammatory mediators including prostaglandins (Fu *et al.*, 1990; Radi, 2009). Cyclo-oxygenase selectivity of NSAIDs currently used in bovine veterinary practice is variable [Table 1.4].

Table 1.4: Non-steroidal anti-inflammatory drugs licensed for bovine use in the UK
(National Office of Animal Health, 2020)

NSAID	COX selectivity	Bovine plasma half-life
Carprofen	COX-2 preferential[2]	44.5 to 64.6 h[6]
Flunixin	COX-1 preferential[2]	20 min to 2.26 h[7]
Ketoprofen	Non-selective[3]	2 to 3 h[8]
Meloxicam	COX-2 preferential[2]	20 to 43.3 h[9]
Sodium Salicylate[1]	COX-1 preferential[4]	30 to 38 min[10,11]
Tolfenamic Acid	Non-selective[5]	2.5 to 13.5 h[12,13]

1. Licensed for use in calves only. 2. Beretta et al., 2005; 3. Singh et al., 2009; 4. Mitchell *et al.*, 1993: *5. Sidhu et al., 2005; 6. Ludwig et al., 1989; 7. Kissell et al., 2016; 8. Landoni et al., 1995; 9. Coetzee et al., 2009; 10. Coetzee et al., 2007; 11.* Whittem *et al.*, 1996*; 12. Landoni et al., 1996; 13. Lees et al., 1998.*

It is thought that NSAID use (especially if prolonged) may be associated with an increased risk of abomasal ulceration due to inhibited prostaglandin synthesis adversely affecting the abomasal mucosal barrier (Stock *et al.*, 2015; Hund *et al.*, 2018). However, a direct correlation between NSAID use in ruminants and abomasal ulceration has not been conclusively shown (Walsh *et al.*, 2016; Hund *et al.*, 2018), although this may be due to the shorter duration of administration typically used in farm animals (compared to companion animals and humans). In human and companion animal medicine more recent NSAID developments have resulted in the availability of COX-2 preferential and COX-2 selective NSAIDs which have fewer gastric and renal side effects than more traditional NSAIDs (Lees *et al.*, 2004), some of which are now available for use in cattle [Table 1.4] (Gorissen *et al.*, 2017; National Office of Animal Health, 2020) offering practitioners additional options for patients considered to be at higher risk of adverse side effects. This is an important development for bovine analgesia as concerns regarding adverse side effects may be a barrier to use (Whay *et al.*, 2005a).

1.3.2.3 Local and regional anaesthesia and alpha-2 adrenergic agonists

Alpha-2 adrenergic agonists activate α_2-adrenoreceptors and have analgesic and sedative properties, and are commonly used during surgical procedures (Hudson *et al.*, 2008; Stock *et al.*, 2015). Alpha-2 adrenergic agonists can also be used to prolong the duration of epidural analgesia (Grubb *et al.*, 2002; Ismail, 2016). Caudal epidural analgesia is commonly used in cattle for painful procedures concerning the reproductive tract (e.g. replacement of uterine prolapse, penile surgeries) and also to aid obstetrical manipulations during parturition (Edmondson, 2016). A number of other local and regional anaesthetic techniques are commonly used for surgical procedures in cattle including paravertebral nerve blocks for abdominal surgeries (Edwards, 2001; Edmondson, 2016), cornual nerve blocks for disbudding and dehorning (Edwards, 2001; Edmondson, 2016), and intravenous regional anaesthesia (IVRA) for digit amputation (Edmondson, 2016). These techniques provide localised anaesthesia and prevent acute pain; however, the duration of action can be short (e.g. the cornual nerve block provides anaesthesia for 2 to 4 h). To prolong the duration of pain relief and thus optimise the welfare benefits provided by local anaesthesia, inclusion of local nerve blocks in a multimodal analgesic approach including longer duration analgesia such as NSAIDs is recommended (Winder *et al.*, 2018).

1.3.3 Pain associated with parturition

In humans, parturition has been recognised to be painful for thousands of years; indeed, in the Old Testament God says to Eve "*in pain you shall bring forth children*" (Genesis 3:16, New Revised Standard Version). By contrast, parturition has historically been believed to be painless for animals (Melzack, 1984), but more recently this view has been challenged (Mainau *et al.*, 2011) and the notion of parturition pain affecting animals is now more widely accepted (e.g. McLennan, 2018). Nonetheless, the effects of unassisted parturition on cattle (e.g. pain experienced) have not been investigated and even in women, data regarding experiences of normal labour are limited (Olza *et al.*, 2018). Interestingly, neonatal pain following dystocia is typically not considered as severe as maternal pain (Huxley *et al.*, 2007; Laven *et al.*, 2009; Remnant *et al.*, 2017) which may be related to our incomplete understanding of neonatal pain processing and perception. Until quite recently, the limited capacity of babies to feel pain was a commonly held view (Barr, 1992), and although it is now known that newborn babies feel pain similarly to adults (Goksan *et al.*, 2015), there continues to be a lack of understanding of neonatal pain in human medicine (Vagnoli *et al.*, 2019) that is mirrored in the veterinary sphere. This assertion is supported by the findings of a recent study of animal professionals that identified analgesic use following painful procedures was higher in adult cattle than neonates (Andrighetto Canozzi *et al.*, 2020).

Substantial forces are applied to calves during assisted birth (Pearson *et al.*, 2020) which can result in injuries ranging in severity from subclinical tissue damage to fractures (Ferguson *et al.*, 1990; Pearson *et al.*, 2019c; Ishiyama *et al.*, 2020). Despite this, birth-related pain of calves and the potential benefits of analgesia are neglected areas of study (Laven *et al.*, 2012). By contrast, the negative effects of assisted birth on subsequent health and production of calves are better described (Lombard *et al.*, 2007; Tenhagen *et al.*, 2007; Barrier *et al.*, 2012a, 2013b, 2013a; Murray *et al.*, 2015b; Vannucchi *et al.*, 2015b; Homerosky *et al.*, 2017b; Norquay *et al.*, 2020). Four studies have investigated the effects of analgesia administration to calves at birth with a primary focus on its effects on measures of function (such as milk consumption) or productivity (such as growth rates) (Murray *et al.*, 2016; Pearson *et al.*, 2019a, 2019b; Clark *et al.*, 2020) rather than more sensitive measures of pain and welfare state, such as behaviour. Moreover, in the single study that included assisted and unassisted calves (Murray *et al.*, 2016) it is unclear if assistance x treatment status interaction effects were analysed. Thus, whilst it is probable that calves experience some pain after assisted birth, this is by no means certain based on currently available data. Furthermore, the compressive pelvic forces applied to calves during normal birth have not been quantified — although in humans these are considerable (Ami *et al.*, 2019) — and it is unknown if neonatal injury or pain occur during normal birth. As pain is a significant welfare concern (Rutherford, 2002), it is crucial that painful experiences are identified. Establishing whether pain is experienced by calves during birth, and establishing the magnitude of pain experienced, is important — especially for calves experiencing assisted birth. Although birth is unavoidable, birth assistance has the potential to be avoided in many cases and this is an area where there is the potential for welfare improvements to be made.

During normal parturition, maternal pain is initially visceral in origin and is associated with dilation of the cervix and distension of the uterus as the foetus is positioned for expulsion (Mainau *et al.*, 2011). As parturition progresses, somatic pain associated with distension of the pelvic muscles and perineum becomes more prominent (Mainau *et al.*, 2011). Humans report increasing intensity of pain as parturition progresses (Capogna *et al.*, 2010) and studies of rats indicate that the parturition experience of other mammals is likely to be similar (Catheline *et al.*, 2006; Tong *et al.*, 2008). It has been suggested that, even in the absence of dystocia, assisted parturition in cattle may be associated with more maternal pain than unassisted parturition due to iatrogenic tissue damage (Barrier *et al.*, 2012a; Schuenemann *et al.*, 2013). However, while assisted parturition is associated with an increased risk of vulvovaginal laceration (Kovács *et al.*, 2016; Vieira-Neto *et al.*, 2016), there are no data available to support the idea that more extensive iatrogenic injury occurs. Studies of maternal behaviour have identified differences in lying behaviours of assisted cows (compared to unassisted cows) that support the hypothesis that assisted parturition is painful (Proudfoot *et al.*, 2009; Barrier *et al.*, 2012b). Additionally, the adverse effects of dystocia and assisted parturition on maternal health, welfare and production are well described (Thompson *et al.*, 1983; Dematawewa *et al.*, 1997; Rajala *et al.*, 1998; Berry *et al.*, 2007; Atashi *et al.*, 2012; Hossein-Zadeh, 2014; Kovács *et al.*, 2016; De Amicis *et al.*, 2018). However, the potential extent of analgesic benefits are difficult to determine as most studies only include assisted cows (Newby *et al.*, 2013a; Barrier *et al.*, 2014) or report blanket treatment of all cows without analysis of assistance status [see Subsection 1.3.5] (Richards *et al.*, 2009; Stilwell *et al.*, 2014; Carpenter *et al.*, 2018). Just two studies have investigated the potential benefits of administering NSAID analgesia to cows experiencing dystocia and eutocia in a factorial design [see Subsection 1.3.4], but no dystocia x treatment interaction effects were observed (Swartz *et al.*, 2018; Barragan *et al.*, 2020a, 2020b). However, it is difficult to make direct comparisons between these studies as Swartz *et al.*, (2018) defined dystocia as parturition lasting more than 70 min (an unconventional definition that does not account for parturition assistance) whereas Barragan *et al.* (2020a, 2020b) defined 'dystocia' as ≥ 3 on a 5-point parturition assistance grading scale [Appendix 1], analysing the effects of assistance rather than duration of parturition.

1.3.3.1 Maternal postpartum pain

Postpartum uterine involution is known to be painful in humans (Deussen *et al.*, 2020) but pain experienced by cows following parturition has received little attention. It is unknown if cows experience postpartum pain related to uterine involution, or if postpartum pain is only experienced following parturition injury. A single study measured the degree of back arching as an indicator of uterine pain for up to 21 d (average 9 d) postpartum and found that cows with metritis were more painful than healthy cows at the same stage postpartum (Stojkov *et al.*, 2015). However, Stojkov *et al.* (2015) did not include cows later in lactation (i.e. when uterine pain due to either metritis or involution would not be expected) for comparison; therefore, it cannot be concluded that healthy cows experience no uterine pain post-partum — rather that metritis is associated with heightened pain. Furthermore, Stojkov *et al.* (2015) focused on visceral pain but it is possible that cows also experience somatic pain postpartum, especially if parturition related injuries (e.g. perineal tears) occur. Barrier *et al.* (2012a) found that whilst assisted cows engaged in self-grooming less than unassisted cows, all other postpartum pain-related behaviours studied (including lying behaviours, walking and posture changes) were unaffected. These results suggest that parturition assistance may not be associated with heightened postpartum pain, but Barrier *et al.* (2012a) only studied cows for 3 h postpartum during which the cow was left with the calf. Calf-directed maternal behaviours in the same study were unaffected by parturition experience (Barrier *et al.*, 2012a) and it is possible that behavioural indicators of pain are masked by the expression of maternal behaviours and attentional effects. This hypothesis is supported by previous work in chickens (Gentle, 2001) and cattle (Aitken *et al.*, 2013) that found pain-related behaviours were moderated when the animal was distracted, but attentional effects in the peri-partum period have not been studied. Additionally, ingestion of amniotic fluids whilst licking the neonate has been shown to have an analgesic effect for up to 1 h postpartum (Pinheiro Machado F *et al.*, 1997); therefore, it is also possible that pain-related behaviour was not observed by Barrier *et al.* (2012a) due to improved comfort of all cows, irrespective of assistance status. Separation of cows and calves soon after birth is common practice on dairy farms (Vasseur *et al.*, 2010) with one study reporting that calves are removed within 2 h of birth on more than 75% of farms (Klein-Jöbstl *et al.*, 2014). Although the effects of early cow-calf separation have been subject to much discussion, the effect that preventing cows licking their calves may have on postpartum pain has been overlooked. For example, Beaver *et al.* (2019) and Meagher *et al.* (2019) thoroughly reviewed cow-calf separation but failed to highlight maternal pain as an area that warrants further study.

In humans, postpartum pain is divided into three phases (acute, subacute and delayed) and can last for several months (Romano *et al.*, 2010; Eisenach *et al.*, 2013). Studies of pigs have identified behavioural effects of analgesia at 3 d postpartum but not sooner (Mainau *et al.*, 2012; Viitasaari *et al.*, 2014), suggesting that delayed postpartum pain may also be experienced by animals; but it is unclear if cows are similarly affected as studies investigating persistent or delayed parturition-related pain in cows are few and the results are conflicting [Table 1.5]. Collectively the results suggest that assisted cows maybe subject to additional welfare challenges for several days postpartum (compared to unassisted cows), but the effects of analgesia are inconsistent and as such, the role of pain is unclear.

1.3.3.2 Neonatal pain experienced after birth

Although calves are believed to experience pain after dystocia (Remnant *et al.*, 2017), data to support this view are almost absent. Assisted calves have been found to lie in lateral recumbency more (Barrier *et al.*, 2012a) and have elevated (plasma/serum) cortisol concentration compared to unassisted calves (Barrier *et al.*, 2013b; Kovács *et al.*, 2021), suggesting increased stress, but it is uncertain if this is due to pain or other factors. Whilst previous studies have identified limited beneficial effects of analgesia in newborn calves including improved milk consumption and improved growth in the first week of life [see Subsection 1.3.5.4] (Murray *et al.*, 2016; Pearson *et al.*, 2019a, 2019b; Clark *et al.*, 2020), these existing studies focus on production outcomes or measures of neonatal vigour and do not assess more specific indicators of pain. Furthermore, to date, a study including analgesia and birth assistance in a 2 x 2 factorial design has not been performed and as such, it is currently unknown whether calves experience pain during birth, or whether assisted calves experience heightened pain (compared to unassisted calves).

Table 1.5: Summary of studies investigating the effects of parturition assistance on pain experienced (by cows) in the postpartum period

Author	Duration studied (d)	Breed	Analgesic	Method of assessing pain	Results
Barragan *et al.*, (2020a, 2020b)	4	Holstein	Acetylsalicylic acid	Behaviour (accelerometer data)	Overall analgesia-treated cows were more active than placebo-treated cows irrespective of assistance status. Overall, dystocia[1] cows were less active and spent more time lying than eutocia cows, regardless of treatment status
				Biomarker (substance P)	Substance P elevated in dystocia cows at 7 d postpartum (no effect identified in eutocia cows)
Mainau *et al.*, (2014)	14	Friesian	Meloxicam	Behaviour (visual observations for the first 2 d only; pedometer data for complete 14 d)	Analgesia-treated primiparous cows were more active in first 2 d postpartum (detected using accelerometer generated data only). Activity of multiparous cows was not affected by treatment. No effect of assistance[2] status identified.
Swartz *et al.*, (2018)	7	Holstein and Jersey	Meloxicam	Behaviour (accelerometer data)	Analgesia-treated Jersey cows were found to be less active than saline treated cows on days six and seven postpartum. Jersey cows experiencing dystocia[3] were less active than Jersey cows experiencing eutocia regardless of treatment status. Holstein cows experiencing dystocia were found to be less active than eutocia cows on days four and five postpartum, regardless of treatment status.

1. Dystocia defined as parturition assistance ≥ 3 on a 5-point grading scale [Appendix 1].
2. Only unassisted and mildly assisted (defined as easy manual assistance provided by one person) cows included.
3. Dystocia defined as parturition lasting ≥ 70 min.

1.3.4 Analgesic usage around bovine parturition

Results of surveys of veterinary surgeons suggest that analgesia is more frequently administered to cows experiencing dystocia than calves (Huxley *et al.*, 2007; Laven *et al.*, 2009; Remnant *et al.*, 2017). Remnant *et al.* (2017) found that 80% of veterinary surgeons administer NSAIDs to cows experiencing dystocia at least 50% of the time but only 30% administer NSAIDs to calves. The same study found that respondents perceived dystocia to be a more painful experience for the cow than the calf, with maternal pain graded as 7/10 compared to 5/10 for neonatal pain (Remnant *et al.*, 2017). However, some authors have suggested that pain may be experienced more intensely by neonatal animals as they adapt to extra-uterine life (Mellor *et al.*, 2004) and human pain perception has recently been found to be similar in babies and adults (Goksan *et al.*, 2015). Thus, the view that dystocia is less painful for calves may be misplaced. Additionally, Huxley *et al.* (2007) and Laven *et al.* (2009) defined 'dystocia' as "*foeto-maternal disproportion requiring traction alone*" while Remnant *et al.* (2017) did not report a definition. This narrow definition of dystocia does not account for calf malpresentations or different grades of assisted parturition; therefore, it is likely that the findings of Huxley *et al.* (2007) and Laven *et al.* (2009) do not accurately represent the use of analgesia around parturition by veterinary surgeons in practice. Furthermore, studies of farmer attitudes to analgesia or parturition management practices do not evaluate the use of analgesia following farmer-assisted parturition (Huxley *et al.*, 2007; Villettaz Robichaud *et al.*, 2016). As pain is a welfare challenge, and parturition assistance is primarily provided by farmers (Egan *et al.*, 2001), obtaining accurate data regarding periparturient use of analgesia is crucial for evaluation of peri-parturient welfare in the cattle industry.

1.3.4.1 Analgesic effects on maternal postpartum behaviour

Whilst few studies have investigated the effects of peri-partum analgesia on maternal behaviours, the limited data available suggest that analgesia-treated cows are more active, and engage in feeding behaviours more frequently, than untreated or placebo treated cows (Newby *et al.*, 2013a; Mainau *et al.*, 2014; Stilwell *et al.*, 2014); however, studies typically only include assisted cows (Newby *et al.*, 2013a) or group assisted and unassisted cows together for analysis (Mainau *et al.*, 2014; Stilwell *et al.*, 2014), meaning the interaction between parturition and analgesia treatment cannot be determined. In the past decade, remote activity monitors have started to be used as an alternative to visual observations of behaviour [see Section 1.5] and two studies have investigated effects of analgesia on maternal activity using this approach (Swartz *et al.*, 2018; Barragan *et al.*, 2020a). Barragan *et al.* (2020a) did not identify a dystocia x analgesia interaction effect but found analgesia treated cows were more active overall and tended to engage in lying more on the day of parturition than saline treated cows. By contrast, Swartz *et al.* (2018) found that analgesia treated cows were less active than saline treated cows (both overall and in the dystocia group), but lying time was not affected by treatment status. These results suggest that, whilst analgesia has limited effects on postpartum lying behaviours, maternal activity is affected — although the direction of the effect is uncertain. Both studies used the same measure of activity (step count), thus the data collected are comparable. Differences in results might be explained by different NSAIDs used in each study: Barragan *et al.* (2020a) studied the effects of acetylsalicylic acid, whereas Swartz *et al.* (2018) studied the effects of meloxicam. Given the action of these NSAIDs differs [see Subsection 1.3.1], it is possible that the degree of postpartum pain relief provided also differs. An alternative explanation for the contrasting results reported by Barragan *et al.* (2020a) and Swartz *et al.* (2018) may be related to the different definitions of dystocia used in each study. Barragan *et al.* (2020a) used a one to five scoring system based on assistance severity, whereas Swartz *et al.* (2018) defined dystocia based on parturition duration. It is possible that different parturition experiences of cows may have interacted with analgesia efficacy, affecting the results. An additional challenge when interpreting the results of Barragan *et al.* (2020a) and Swartz *et al.* (2018) is the uncertainty around the interpretation of walking (the behaviour recorded by step count) as an indicator of pain. For example, whilst studies have found that walking behaviours of analgesia treated calves increased after disbudding (Pauly *et al.*, 2012) — suggesting walking is an indicator of comfort — other studies have found that mastitis (a painful condition) is also associated with increased walking behaviours (Siivonen *et al.*, 2011; Fogsgaard *et al.*, 2015). Therefore, it is possible that increased walking behaviours in cattle might be indicative of both comfort and discomfort depending on external factors such as age and pain aetiology.

1.3.4.2 Effects of analgesia on maternal postpartum health

A postpartum inflammatory response is a normal finding in cattle (Farney *et al.*, 2013a; Vailati Riboni *et al.*, 2015) and has been found to be greater in cows that subsequently develop postpartum disease (Humblet *et al.*, 2006; Qu *et al.*, 2014). Accordingly, there has been interest in the potential for postpartum NSAID administration to reduce the risk of developing postpartum disease, but results have been disappointing. Although postpartum NSAID administration has been associated with reduced serum haptoglobin and ß-hydroxybutyrate (BHB) concentration (Carpenter *et al.*, 2016; Pascottini *et al.*, 2019, 2020) — indicating a decrease in postpartum inflammation and improved metabolic adaptation — this does not appear to correlate with a consistent improvement in clinical postpartum disease incidence. For example, Barragan *et al.* (2021) found that NSAID treated cows had a lower risk of developing metritis but this finding was not supported by the results of Richards *et al.* (2009). Again, different NSAIDs were used in these studies (acetylsalicylic acid and ketoprofen respectively) which may explain the difference in results. Studies investigating the effects of NSAID administration on retained foetal membranes (RFM) have found that whilst meloxicam and salicylic acid do not affect RFM incidence (Swartz *et al.*, 2018; Barragan *et al.*, 2020a), ketoprofen shows a tendency towards a protective effect (Richards *et al.*, 2009), and flunixin is associated with an increased risk of developing RFM (Waelchli *et al.*, 1999; Newby *et al.*, 2017). Thus it is evident that, although all NSAIDs have anti-inflammatory properties, individual NSAIDs have differing effects on RFM incidence, and it is possible that other postpartum diseases are affected similarly.

1.3.4.3 Effects of analgesia on subsequent milk production and reproductive performance.

Studies investigating the effects of peri-partum analgesia on subsequent lactation performance have produced conflicting results [Table 1.6]; although, when appraised collectively, studies suggest that postpartum NSAID treatment may be associated with improvements in long-term milk production. Although some studies have found that postpartum NSAID administration is associated with improved milk production (Farney *et al.*, 2013b; Stilwell *et al.*, 2014; Carpenter *et al.*, 2016; Swartz *et al.*, 2018; Barragan *et al.*, 2020a) others have not found

Table 1.6: Summary of studies investigating the effects of peri-partum NSAID administration on subsequent milk production

Author	Study design	NSAID treatment	Control treatment	Milk production measure analysed	Effects of NSAID on studied measure of milk production
Barragan *et al.* (2020a)	2 x 2 factorial design assessing effects of dystocia and NSAID treatment	Sodium salicylate	Placebo	Daily milk yield for first 21 d of lactation. 305 d yield	Increased daily yield and a tendency towards increased DHIA test day yield. NSAID-treated dystocia[1] cows increased milk yield on some days but not overall.
Barragan *et al.* (2020c)	Assisted and unassisted included. Blocked by parity.	Sodium salicylate	Placebo	Daily milk yield and conductivity for first 60 d of lactation. 305ME milk, fat, and protein yield.	Daily milk yield of analgesia treated multiparous cows higher than untreated cows. Primiparous cows unaffected. No treatment effect on 305ME (any measure).
Bertoni *et al.* (2004)	Assistance status not reported	Acetyl-salicylate	Untreated	Milk yield on days 3, 7, 10, 14, 21, 28, 42, 56, 70, 90, and 120 postpartum	Increased
Carpenter *et al.* (2016)	Blocked by mastitis, breed, dystocia[1] and twin births	Meloxicam and sodium salicylate	Placebo	Whole lactation and 305 d milk yield	Both measures increased in both NSAID groups
Carpenter *et al.* (2018)	Assistance status not reported. Blocked by parity.	Sodium salicylate	Placebo	Daily milk yield (averaged by week) 305ME	No effect
Farney *et al.* (2013b)	Unassisted cows only. Blocked by parity	Sodium salicylate	Placebo	Daily milk yield for first 30 d of lactation. Test day yield for first 5 tests of lactation. 305 d yield	305 d yield increased in 3rd parity (and older) cows.
Mainau *et al.* (2014)	Unassisted and mildly assisted ('easy manual pull') cows only	Meloxicam	Placebo	Daily yield Yield per (twice daily) milking for first 30 d of lactation	No effect
Meier *et al.* (2014)	Assistance status not reported	Carprofen	Untreated	Weekly milk yield (representative[2]) for first 6 weeks of lactation	No effect
Newby *et al.* (2013)	Assisted cows only	Meloxicam	Placebo	Daily milk yield for first 14 d of lactation.	No effect
Newby *et al.* (2013a)	Blanket treatment. Assistance[3] included as fixed effect.	Flunixin	Placebo	Daily milk yield for first 14 d of lactation. Weekly milk yield thereafter.	Reduced milk production
Richards *et al.* (2009)	Blanket treatment.	Ketoprofen	Untreated	Milk yield at first test of lactation (mean 22.9 d postpartum)	No effect
Shwartz *et al.* (2009)	Assistance status not reported	Flunixin	Placebo	Daily milk yield for first 35 d of lactation.	No effect
Stilwell *et al.* (2014)	Blanket treatment	Carprofen	Untreated	Milk yield at 220 and 305 DIM	Increased milk yield of primiparous animals at 305 DIM. No effect on multiparous animals.
Swartz *et al.* (2018)	3 x 2 factorial design assessing effects of dystocia and pre- and postpartum NSAID treatment.	Meloxicam	Placebo	Daily milk yield for first 15 weeks of lactation.	Increased milk yield of eutocia[4] cows (pre-partum treatment had greater effect than postpartum treatment).

1. Defined as parturition grade ≥ 3 on a 1 to 5 scale
2. Milk production measured on one day per week as a representative sample of the daily yield for that week
3. Defined as ‘hard pull’ and surgical delivery
4. Defined as parturition duration < 70 min

similar positive effects (Bertoni *et al.*, 2004; Richards *et al.*, 2009; Shwartz *et al.*, 2009; Newby *et al.*, 2013a; Mainau *et al.*, 2014; Meier *et al.*, 2014; Carpenter *et al.*, 2018) [Table 1.6]. Differences in measures of milk production used may explain the contrasting results reported: studies that observed positive effects of NSAIDs (on milk production) typically analyse long-term parameters such as whole lactation or 305 d milk yield (Farney *et al.*, 2013b; Stilwell *et al.*, 2014; Carpenter *et al.*, 2016), whereas other studies measure daily or cumulative early lactation milk production (shorter-term measures) (Bertoni *et al.*, 2004; Richards *et al.*, 2009; Shwartz *et al.*, 2009; Newby *et al.*, 2013a, 2017; Mainau *et al.*, 2014; Meier *et al.*, 2014; Swartz *et al.*, 2018; Barragan *et al.*, 2020a). Therefore, it is possible that, had these studies continued to monitor the milk production for the full duration of the lactation, analgesia effects may have been identified. This hypothesis is supported by the study reported by Farney *et al.* (2013b) who found that whilst there was no effect of analgesia on milk production in the first three weeks of lactation, analgesia was associated with increased whole lactation production of older cows (lactation ≥ 3). No effect on whole lactation production of younger cows was identified, a finding the authors suggested could be due to increasing inflammatory pressure in the udder as cows age — an interesting hypothesis that deserves further study. It should be noted that one study has found that analgesia treatment was associated with reduced milk production (Newby *et al.*, 2017), suggesting that judicious use might be justified; however, Newby *et al.* (2017) evaluated the NSAID flunixin finding that (in addition to reduced milk production) flunixin was associated with increased risk of RFM and stillbirth. Accordingly, Newby *et al.* (2017) concluded that the reduced milk production seen in flunixin treated cows (compared to placebo treated cows) was due to the adverse effects of ill health associated with flunixin administration — a plausible explanation given RFM is known to have adverse production effects [see Subsection 1.7.1.1]. More direct effects of flunixin on milk yield could also be considered as a possible explanation for the findings of Newby *et al.* (2017) but this is not supported by the findings of other studies (Shwartz *et al.*, 2009).

It is difficult to assess whether postpartum analgesia administration to assisted cows offers enhanced production benefits as most studies report effects of blanket treatment of all cows (Bertoni *et al.*, 2004; Richards *et al.*, 2009; Mainau *et al.*, 2014; Carpenter *et al.*, 2016, 2018; Newby *et al.*, 2017), do not recruit both assisted and unassisted cows (Farney *et al.*, 2013b; Newby *et al.*, 2013a; Stilwell *et al.*, 2014), or do not report parturition assistance status (Shwartz *et al.*, 2009; Meier *et al.*, 2014). Just two studies have evaluated the effects of targeted analgesia treatment on milk production in a factorial design, with contrasting results. Barragan *et al.* (2020a) found that postpartum administration of acetylsalicylic acid was associated with increased milk production overall in cows experiencing dystocia, although when daily production was analysed this was effect was only observed on some days. By contrast, Swartz *et al.* (2018) found that only milk yield of cows classified as 'eutocia' was improved, with pre-partum analgesic administration having the greatest beneficial effect. Differences in study duration may have contributed to these contrasting results: Swartz *et al.* (2018) measured milk production for 15 weeks whereas Barragan *et al.* (2020a) only measured production for 30 d postpartum. However, it also needs to be considered that dystocia and eutocia were defined differently in each study [see Subsection 1.3.3] thus the parturition experience of cows in each study may not be comparable.

Fewer studies have investigated the effects of postpartum analgesia on milk components, but the available data are more in agreement, with studies finding that daily milk protein production is not affected by postpartum analgesia (Shwartz *et al.*, 2009; Barragan *et al.*, 2020a). Interestingly, Swartz *et al.* (2018) found that although daily milk protein yield was not affected by postpartum analgesia, milk protein production of cows treated with analgesia before parturition was increased. This novel finding suggests that the timing of analgesia administration in relation to parturition can alter production effects seen after parturition but, as no other studies evaluate pre-partum analgesia, cautious interpretation is warranted. Nonetheless, this finding merits further study as identification of optimal analgesia protocols for use around parturition will aid welfare improvements of postpartum cattle. Most studies have found that daily milk fat production is not affected by postpartum analgesia (Shwartz *et al.*, 2009; Meier *et al.*, 2014; Carpenter *et al.*, 2016; Barragan *et al.*, 2020a); although, by contrast, Swartz *et al.* (2018) found that analgesia administration was associated with increased milk fat production, with pre-partum administration having a greater effect than postpartum administration. It is unclear why the results of Swartz *et al.* (2018) are different to other studies but, as this is the only one of these studies that evaluated meloxicam, these findings may reflect differences in drug action or efficacy. No studies have investigated the effects of different NSAIDs on daily milk fat production of cows subject to the same management and study design; therefore it is difficult to interpret contrasting findings of existing studies and determine whether they are due to variations in NSAID choice rather than differences in animal and management factors (e.g. diet, genetics).

Effects of analgesia on longer term measures of fat and protein production are less consistent. Carpenter *et al.* (2016) found that 305 d milk fat production was not affected by administration of either meloxicam or sodium salicylic acid. By contrast, Farney *et al.* (2013b) found that sodium salicylic acid-treated cows in lactation three and older had higher 305 d milk fat production than placebo-treated cows but fat production of younger cows was not affected. Interestingly, analysis of 305 d protein production produced findings that were the reverse of the results of 305 d milk fat analysis. Carpenter *et al.* (2016) found 305 d protein yield of analgesia-treated cows was higher than placebo-treated cows, whereas Farney *et al.* (2013b) found analgesia treatment had a tendency to be associated with increased milk protein yield in older cows (lactation ≥ 3) only. However, Carpenter *et al.* (2016) only included cows entering their second lactation or higher and cows in lactation three and over made up a small majority (57%) of the study population. Therefore it is likely that the different results reported by Carpenter *et al.* (2016) and Farney *et al.* (2013b) reflect the different study populations in each study and it is possible that if Farney *et al.* (2013b) had only included older cows, a more significant effect of analgesia treatment consistent with the findings of Carpenter *et al.* (2016) may have been identified.

Cheong *et al.* (2017) found that non-ovulatory cows had elevated plasma haptoglobin concentration in the first 3 d postpartum, suggesting that postpartum inflammation has a negative impact on resumption of ovarian function after parturition. These results suggest that NSAIDs may have the potential to ameliorate the negative effects of postpartum inflammation on reproductive performance, but this is difficult to determine as few studies have investigated the effects of postpartum NSAID administration on subsequent reproductive performance; however, the limited available data are promising. Barragan *et al.* (2020a) found acetylsalicylic acid treated cows needed fewer insemination attempts to conceive and Barragan *et al.* (2021) found acetylsalicylic acid treated cows tended to conceive 18 d earlier than saline treated cows. These findings offer the exciting possibility that reproductive performance may be improved by administration of postpartum NSAIDs, but further work is needed to expand the available data and evaluate the potential benefits of NSAIDs other than acetylsalicylic acid.

1.3.4.4 Analgesia administration to newborn calves

Few data are available to evaluate the effects of post-birth analgesia treatment of newborn calves [Table 1.7]. Two studies have investigated the administration of post-birth analgesia to dairy calves, finding that meloxicam treated calves had a greater improvement in vigour and consumed more milk (Murray *et al.*, 2016) and more starter mix (Clark *et al.*, 2020) than placebo treated or untreated calves respectively. Although data are few, they suggest that NSAID treatment of newborn calves may be a promising tool to aid improvements in calf health. This view is supported by a study of beef calves that found weight-gain of analgesia treated calves was improved in the first 7 d of life (compared to placebo treated calves) (Pearson *et al.*, 2019b), although this finding was not replicated in a second study by the same group (Pearson *et al.*, 2019a). Whilst the results of some of these studies are promising, interaction effects between birth assistance and treatment status have not been investigated; therefore, it cannot be established whether assisted calves experience heightened benefits of analgesia (compared to unassisted calves). Furthermore, existing studies focus on health and production outcomes and as such, any specific welfare benefits of post-birth analgesia have not been determined. It would be of interest for future studies to focus on outcomes that are more sensitive indicators of welfare (e.g. behaviour) and investigate targeted administration of analgesia to assisted calves in order to improve the understanding of bovine neonatal pain and allow further assessment of newborn calf welfare.

Table 1.7: Summary of studies investigating the effects of administering NSAID analgesia to newborn calves

Author and year of study	Measured outcomes	Assistance status	NSAID treatment	Control treatment	Calf type[1]	Observed effects of NSAID (compared to control group)
Clark *et al.* (2020)	Performance biomarkers[2] Measures of passive transfer[3] Measures of growth[4] Time to consume milk replacer (recorded weekly)	Assisted and unassisted calves recruited but analysed by treatment group only	Meloxicam	Untreated	Holstein	Lower IgG at 12 h old. A tendency towards increased consumption of starter mix. A tendency towards increased blood ketone concentration.
Murray *et al.* (2016)	Calf VIGOR score [Appendix 1] Blood gases and oxygen saturation (pulse oximeter) Serum TP concentration Height and weight	Assisted and unassisted calves recruited. Unclear if assistance x treatment interaction effects were analysed.	Meloxicam	Placebo containing the base formulation of the NSAID product used[5]	Holstein	Improved vigour. Improved suckle reflex. Greater volume of milk consumed. Improved health outcomes before weaning (8 weeks)
Pearson *et al.* (2019a)	Biomarkers of pain and inflammation[6] Serum IgG concentration Calf vigour[7] Bodyweight	Assisted calves only	Meloxicam	Saline/vitamin solution[8]	Beef breed[9]	No effects observed
Pearson *et al.* (2019b)	Calf vigour[7] Serum IgG concentration Bodyweight Disease incidence and mortality before weaning[10]	Assisted calves only	Meloxicam	Saline/oxytetracycline[11] solution	Beef breed[9]	Greater average daily growth rate in the first 7 to 10 d of life

1. All studies recruited male and female calves
2. Plasma glucose, plasma urea nitrogen and blood ketone concentration (all measured at 0, 6, 12, 18 and 24 h of age then weekly for 6 weeks)
3. Serum total protein and IgG concentration (measured at 0, 6, 12, 18 and 24 h of age). Apparent efficiency of absorption was calculated at 24 h.
4. Body weight, hip height, withers height, length, and heart girth (all measured weekly).
5. Metacam, Boehringer Ingelheim, Germany
6. Cortisol, Substance P, Corticosterone (measured at 0 h, 4 h, and 24 h), haptoglobin and L-lactate (measured at 0 h and 24 h)
7. Vigour assessed using methodology described by Homerosky *et al.* (2017a, 2017b) [Appendix 1]
8. Vitamin solution mixed into saline at a rate of 1% to match the colour of meloxicam
9. Individual breeds not reported
10. Data obtained from farm records if available
11. Oxytetracycline mixed into saline at a rate of 2% to match the colour of meloxicam

1.4 Bovine behaviour around parturition

1.4.1 Pre-partum behaviour

Few studies provide detailed descriptions of bovine pre-partum behaviour. Wehrend *et al.* (2006) observed cattle performing nest building behaviour, similar to other mammals (Naaktgeboren, 1979); however, Wehrend *et al.* (2006) observed this behaviour immediately prior to parturition after cattle were moved into a dedicated parturition area — a much later stage than is typically observed (Naaktgeboren, 1979). Whilst it is possible that the timing of nest-building behaviour of domestic cattle varies from other mammals, the cattle studied by Wehrend *et al.* (2006) had recently been moved and the behaviour described may have been a type of exploratory behaviour rather than nest-building. Alternatively, as the bedding used was straw (easily gathered using a forelimb), this behaviour may also have been related to the ground pawing/scraping behaviour commonly observed in pre-partum wild and domesticated cattle (Rørvang *et al.*, 2018a). Wehrend *et al.* (2006) did not define 'nest-building', although did note that other authors describe this behaviour as "*looking for a protected area for delivery*". However, it should be considered that nest-building is uncommon among precocial mammals (Poindron, 2005) and is not described in other studies of cattle (Rørvang *et al.*, 2018a).

Some studies have observed cattle separating themselves from the herd prior to parturition, but this is an inconsistent finding that may be influenced by external factors. Consistent with some previous studies (Aitken *et al.*, 1982; Edwards, 1983; Lidfors *et al.*, 1994), Proudfoot *et al.* (2014) found that housed cattle sought isolation and a secluded area for parturition. By contrast, Jensen and Rørvang (2018) found that only 10% of cattle moved into a secluded area for parturition but 79% of cattle calved within 1.25 m of birth fluid (either their own or another cow's), leading to the conclusion that the presence of birth fluids may be more important to cattle than seclusion when choosing a parturition site. This hypothesis is supported by Rørvang *et al.* (2018b) who did not identify a strong motivation of dairy cattle to isolate for parturition and Edwards and Broom (1982) who reported that pre-partum isolation from the herd was an inconsistent finding. Interestingly, Rørvang *et al.* (2017) found that isolation from herd-mates mostly occurred when parturition was prolonged, suggesting that pre-partum isolation may be associated with difficult parturition. Wild ungulates such as fallow deer (*Dama dama*) and impala (*Aepyceros melampus*) seek concealed areas for parturition, a behavioural adaptation suggested to be due to the need to avoid predation (Jarman, 1976; Ciuti *et al.*, 2006). Thus, it is possible that the variations in pre-partum behaviour observed in domestic cattle may also be due to perceived threats in different cattle populations.

In contrast to other behaviours, changes in pre-partum activity and lying behaviours are consistent findings of studies of both beef and dairy cattle, with increased postural transitions and heightened activity reported (Huzzey *et al.*, 2005; Wehrend *et al.*, 2006; Miedema *et al.*, 2011a, 2011b; Jensen, 2012; Barraclough *et al.*, 2020). These behavioural observations are typically interpreted by authors as 'restlessness' but this terminology is poorly defined and carries little ethological meaning. Reasons for increased activity of pre-partum cattle are not understood, although some authors have suggested that increased walking behaviour may be due to an inability of housed cattle to isolate from the herd (Rørvang *et al.*, 2018a). Whilst this is a possible explanation, the limited data available suggest that the motivation of pre-partum cattle to separate from the herd is low (Jensen *et al.*, 2018; Rørvang *et al.*, 2018b) which does not support the hypothesis of Rørvang *et al.* (2018a). Alternatively, increased activity may be related to discomfort experienced in the pre-partum period, a hypothesis supported by the results of a study by Swartz *et al.* (2018), who found that Jersey cattle provided with pre-partum analgesia took fewer steps 24 h before parturition than placebo treated cows. Interestingly Swartz *et al.* (2018) only found that pre-partum activity of Jersey cattle, and not Holstein cattle, was affected by analgesia which may be due to the smaller size of Jersey cattle resulting in more abdominal discomfort.

1.4.2 Postpartum maternal behaviour

1.4.2.1 Activation of maternal behaviour

'Maternal behaviour' is a general term typically used to describe offspring-directed behaviours that have a care-giving function and contribute to neonatal survival (e.g. nest-building, licking the neonate and protective behaviours). Hormonal changes around parturition have been shown to play an important role in the activation of maternal behaviours of rats (*Rattus norvegicus domestica*) (Bridges, 1984; Sheehan *et al.*, 2002; Novakov *et al.*, 2005) and horses (*Equus ferus caballus*) (Berlin *et al.*, 2018). Other studies of rats (Yeo *et al.*, 1986) and small ruminants (Keverne *et al.*, 1983; Krehbiel *et al.*, 1987; Kendrick *et al.*, 1991; Romeyer *et al.*, 1994) have found that vagino-cervical stimulation during parturition is also important, but there are very little data available regarding activation of bovine maternal behaviour. A single study of nulliparous cattle found that factors previously identified as important in sheep (such as hormonal changes and genital stimulation) did not induce maternal behaviour in cattle (Williams *et al.*, 2001), suggesting that mechanisms of maternal behaviour may not be comparable across ruminant species. However, although the presence of different mechanisms of activation of maternal behaviour in cattle (compared to small ruminants) cannot be excluded, data are limited and — as nulliparous animals are less likely to show maternal behaviour (Yeo *et al.*, 1986; Krehbiel *et al.*, 1987; Geburt *et al.*, 2015) — studies of multiparous cows are needed before accurate conclusions can be drawn.

1.4.2.2 Postpartum behaviours of cows following normal parturition

Domestic cattle exhibit similar postpartum behaviours to comparable wild ungulates (Edwards *et al.*, 1982; Jensen, 2012; Rørvang *et al.*, 2018a) with calf-directed bonding behaviours predominating immediately after parturition in both cattle (*Bos taurus*) and buffalo (*Bubalus bubalis*) (Barrier *et al.*, 2012a; Jensen, 2012; Dubey *et al.*, 2018). Eventually the dam moves away from the calf and engages in other necessary behaviours (e.g. eating and drinking). The time (relative to parturition) that cows start to engage in non-calf-directed behaviours is variable but is related to parturition experience with more experienced cows engaging in calf-directed behaviours for a shorter duration of time (Edwards *et al.*, 1982; Barrier *et al.*, 2012a). Maternal-offspring bonding in cattle can occur with as little as 5 min of cow-calf contact (Hudson *et al.*, 1977) and once a bond has formed, other (non-recognised) offspring are rejected (Hudson *et al.*, 1977; Lidfors *et al.*, 1994). Maternal-offspring bonding ensures calf-directed care is correctly directed but, as immunoglobins do not pass across the ruminant placenta, it also has an important role in ensuring adequate passive transfer of colostral immunoglobulins (Romeyer *et al.*, 1994), failure of which can lead to poor neonatal health (Dewell *et al.*, 2006; Cuttance *et al.*, 2018; Lora *et al.*, 2018).

Parity effects on maternal postpartum behaviour have been reported, with some studies finding primiparous animals show fewer protective behaviours (Geburt *et al.*, 2015), take longer to stand, and longer to initiate bonding behaviours than multiparous animals (Edwards *et al.*, 1982; Houwing *et al.*, 1990). However, once initiated, calf-directed bonding behaviours are performed for longer by primiparous cattle (Edwards *et al.*, 1982). Jensen (2012) studied multiparous cows only and identified a similar parity effect with less experienced animals taking longer to both stand and to initiate calf-directed maternal behaviours; however, this was a short-term effect that was not observed after 24 h. By contrast, Houwing *et al.* (1990) did not observe differences between primiparous or multiparous cows in the timing of initiation of calf-directed behaviours, although a high prevalence of parturition assistance (68%) was reported which may have affected results. Reasons for contrasting results are unclear but, given similarly managed (individually housed) Holstein cattle were the subject of all three studies (Edwards *et al.*, 1982; Houwing *et al.*, 1990; Jensen, 2012), it is unlikely that differences in study methodology are involved.

1.4.2.3 Effects of parturition assistance

Maternal behaviour following difficult parturition has not been well studied in cattle although the few available data suggest that calf-directed maternal behaviours are not affected by parturition assistance (Barrier *et al.*, 2012a; Geburt *et al.*, 2015), suggesting that the motivation for expressing maternal behaviours may over-ride the suppressive effects of other factors (e.g. pain). Furthermore, it is difficult to determine the effects of assistance on calf-directed behaviours as studies typically do not report the prevalence of parturition assistance (Edwards *et al.*, 1982; Jensen, 2012), or management procedures that might affect calf-directed behaviours are performed (e.g. moving the calf in front of the cow following parturition assistance) (Houwing *et al.*, 1990; Barrier *et al.*, 2012a). By contrast, assisted parturition in sheep has been found to be associated with disrupted maternal behaviours (Winfield *et al.*, 1972). Winfield *et al.* (1972) analysed the effects of assistance on individual maternal behaviours (lamb desertion, allowance to suckle, and lamb grooming) and, similar to observations of calf-licking reported by Barrier *et al.* (2012a), found that lamb grooming was not affected by parturition assistance — although interestingly this was only observed in ewes with a low to moderate body condition score (BCS) (2.1/5). Well-conditioned assisted ewes (average BCS 4/5) engaged in lamb grooming less than unassisted ewes, suggesting that external factors such as nutritional status may also affect maternal grooming of the neonate. By contrast, assisted parturition was associated with an increased frequency of lamb desertion, irrespective of body condition. These findings suggest that offspring grooming might be an unreliable indicator of maternal behaviour and it is possible that neonatal desertion is a more appropriate choice for analysis if studying the effects of assistance. However, neonatal desertion is difficult to study in cattle because management factors often ensure the cow remains in the vicinity of the calf. Moreover, species differences also need to be considered and it is possible that desertion of offspring is a less common behaviour in cattle than sheep — a hypothesis supported by Geburt *et al.* (2015) who found that defensive behaviours exhibited towards handlers were common in cattle, suggesting that cattle are more motivated to protect their offspring from perceived threats.

1.4.3 Neonatal calf behaviour

1.4.3.1 Neonatal behaviour following normal birth

The predominant behaviour of newborn calves is lying (Houwing *et al.*, 1990), accounting for more than 80% of the time budget. Time engaged in non-lying behaviours (e.g. standing, teat-seeking, suckling) gradually increases until calves are 2 to 3 h old (Edwards *et al.*, 1982; Jensen, 2012); thereafter, short bouts of activity are interspersed between longer bouts of inactivity (lying) (Houwing *et al.*, 1990), although the proportion of the time budget engaged in lying behaviours remains high (Chua *et al.*, 2002; Hill *et al.*, 2013; Bonk *et al.*, 2016; Dennis *et al.*, 2018). Dam parity has been found to affect newborn calf behaviour, with calves born to dams in higher parities engaging in fewer lying bouts (Jensen, 2012) and more standing attempts (Houwing *et al.*, 1990) than calves born to younger dams. Although differences in study design mean it is difficult to make direct comparisons (Jensen (2012) only studied multiparous cows whereas Houwing *et al.* (1990) studied multiparous and primiparous cows), both studies found that calves born to older dams spend more time teat-seeking than calves born to younger dams (Houwing *et al.*, 1990; Jensen, 2012); a finding possibly related to anatomical differences increasing the difficulty of teat identification.

Studies of calf behaviour typically analyse behaviours related to calf vigour (e.g. time to achieve sternal recumbency or standing, and suckling behaviours) in the first 24 h of life (Edwards *et al.*, 1982; Houwing *et al.*, 1990; Barrier *et al.*, 2012a) or behaviours of calves that are more than 14 d old (Chua *et al.*, 2002; Bonk *et al.*, 2016), thus calf activity in the first few days of life is understudied. For example, Jensen (2011) found that in the first 11 d of life the total duration of lying behaviour gradually decreased, but did not start observing calf behaviour until 3 d after birth. Similarly, Hill *et al.* (2013) found that the time calves spent standing increased by approximately 0.5 min/d as calves age but again did not start recording activity until calves were 2 d old. More recently, Dennis *et al.* (2018) analysed activity, feeding and rumination of calves from the day of birth using ear-mounted tri-axial accelerometers, finding that inactive time decreased as calves aged, but detailed behavioural analysis was not performed and 'not active' was not defined.

Play behaviour of calves has been found to initially increase with time (Jensen, 2011) before decreasing between two to six weeks of age (Jensen *et al.*, 1998; Sutherland *et al.*, 2014). Play behaviour is considered to be a good indicator of welfare (Jensen *et al.*, 1998; Held *et al.*, 2011; Mintline *et al.*, 2013) that has been used to assess the welfare effects of cow-calf separation (Rushen *et al.*, 2016), different calf rearing practices (Sutherland *et al.*, 2014; Jensen *et al.*, 2015; Valníčková *et al.*, 2015), and painful procedures (Mintline *et al.*, 2013). However, normal play behaviour in very young calves is poorly described and effects of assisted birth have not yet been studied. A single study has evaluated remote-monitoring technology as a potential tool for detecting locomotor play in calves up to 48 h old (Gladden *et al.*, 2020) but, although a detailed ethogram for play behaviour was defined, detailed description of visual observations was not reported. Accordingly, the patterns of play behaviour exhibited by calves studied by Gladden *et al.* (2020) are unknown. Aside from the predominance of lying behaviours, very little data are available describing normal behavioural time-budgets of very young calves. As deviations from normal behaviour can be sensitive health and welfare indicators, it is important that normal behaviours are well described at all life stages. Therefore, further study is needed to improve the understanding of newborn calf behaviours and enable thorough assessment of newborn calf welfare.

1.4.3.2 Effects of assisted birth

A single study has investigated the effects of assisted birth on newborn calf behaviour, finding that assisted calves spent more time engaged in lying behaviours overall, and more time engaged in lateral recumbency, than unassisted calves (Barrier *et al.*, 2012a). Lateral recumbency is infrequently observed in cattle (Endres *et al.*, 2007; van Erp-van der Kooij *et al.*, 2019) and requires minimal effort to maintain. As such, lateral recumbency is considered an atypical posture adopted when animals are unwell or in pain (Molony *et al.*, 1995, 1997). It is possible that assisted calves in the study reported by Barrier *et al.* (2012a) spent more time in lateral recumbency due to pain or inflammation resulting from traumatic injuries sustained during birth, or alternatively may be due to assisted calves experiencing neonatal acidosis, although this was not assessed. Whilst Barrier *et al.* (2012a) did not report any clinically obvious birth related injuries, subclinical tissue trauma can occur during assisted birth (Pearson *et al.*, 2019c) and cannot be excluded. Barrier *et al.* (2012a) did not analyse calf behaviours beyond 3 h thus effects of assisted birth on lying behaviours of calves over 3 h old are unknown, but adverse effects of a difficult birth on calf feeding behaviour have been found to extend beyond the immediate postpartum period. De Passillé *et al.* (2016) found that whilst dystocia affected milk consumption of 2 d old calves, by 4 d old milk consumption of most affected calves had increased to average or above average volumes, suggesting that any adverse effects of dystocia may be short-lived and calves make a rapid recovery. Moreover, at 2 d old, calves in the bottom 10th percentile consumed 2.19 L of milk — a volume sufficient to meet maintenance requirements (Drackley, 2008). Thus, given the short duration of effects and adequate milk consumption of the lowest percentile reported by de Passillé *et al.* (2016), it is unlikely that the adverse effects of dystocia on milk consumption are likely to result in reduced calf welfare.

1.5 Use of remote-monitoring technology to monitor behaviour

Detailed continuous visual observations provide the most complete record of behaviour but are time consuming and laborious to perform (Altmann, 1984; Lehner, 1992; Martin *et al.*, 2009a) and are impractical for studies of long duration. As such, the use of continuous visual behavioural observations is limited to studies of short duration, or analysis of low frequency behaviours (Altmann, 1974). Furthermore, studied animals need to be within sight of the observer (either directly or using video footage), limiting the use of visual observations to monitoring the behaviour of cattle kept indoors or in small fields. Accordingly, there is interest in alternative methods for monitoring cattle behaviour, with the use of accelerometer technology gaining traction in the past two decades (Brown *et al.*, 2013). Tri-axial accelerometers (hereafter referred to as 'accelerometers') are animal-worn devices that continuously record acceleration in three planes to generate a data output that accurately represents three-dimensional movement (Shepard *et al.*, 2008; Brown *et al.*, 2013) and can be compared to visual observations to identify behaviours that are accurately detected. These types of devices are familiar to cattle farmers as pedometers (devices that count steps taken) have been used as oestrous detection aids for several decades (Kiddy, 1977; Stevenson *et al.*, 2017). Accelerometers are capable of generating more detailed motion data than pedometers (Bonomi *et al.*, 2009) and since their introduction to the livestock industry in the mid-2000s farmer-adoption has been rapid (Stevenson *et al.*, 2017), making them suitable for use in research performed in commercial farm settings. Although still primarily marketed as tools to aid oestrous detection (reflecting the importance of fertility performance), accelerometers now have a wider role in aiding livestock husbandry and management [Table 1.8] and some are incorporated into devices that contain other sensors (such as pH monitors) to enable continuous monitoring of multiple parameters (e.g. the smaXtec pH Bolus (smaXtec, 2021)). Accordingly, there is a wealth of data generated by accelerometers that are already in common usage on cattle farms that has potential to be a useful resource for future researchers.

Table 1.8: Examples of accelerometers commercially available in the UK for bovine use

Device	Company	Placement	Monitored variables	Primary marketed purpose
Breeder Tag System	Genus ABS, UK.	Leg mounted	Activity, lying duration (plus number of visits to, and duration at, the feed face if combined with Genus Feed Face system).	Oestrous detection and health monitoring.
IceQube and IceTag	IceRobotics, Scotland	Leg mounted	Step count, activity (number of bouts and duration), lying (number of bouts and duration)	Oestrous detection, health monitoring and lameness detection
Moo monitor+	Dairymaster, Ireland.	Neck collar	Activity, rumination, feeding, head position	Oestrous detection and health monitoring
Moocall Calving sensor	Moocall Ltd., Ireland	Tail mounted	Tail position	Prediction of time of parturition
Moocall HEAT	Moocall Ltd., Ireland	Leg (bull) and ear mounted (cow)	Activity and location of the bull in relation to each cow's location (tracked by the ear tag)	Oestrous detection
Silent Herdsman	Afimilk, Israel	Neck collar	Activity, rumination, feeding	Oestrous detection and health monitoring
Smartbow	Zoetis, UK	Ear mounted	Rumination, activity, animal location	Oestrous detection and health monitoring
Smarttag Leg	Nedap N.V., Netherlands	Leg mounted	Standing, lying, and walking behaviours. Step count and posture change count.	Oestrous detection and health monitoring
Smarttag Neck	Nedap N.V., Netherlands	Neck collar	Feeding, rumination, general activity	Oestrous detection and health monitoring
smaXtec Classic Bolus & smaXtec pH bolus	smaXtec animal care, Austria.	Internally (in the reticulo-rumen)	Rumination, core temperature, feeding and drinking, activity, rumen pH.	Health monitoring, oestrous detection, prediction of calving

Animal behaviour has long been identified as a sensitive indicator of health (Brownlee, 1939); however, visual indicators of ill health are subjective and can be unreliable. As such, most research comparing accelerometer generated data to direct visual observations of cattle behaviour has focused on behaviours considered to be consistent with good health, such as feeding and ruminating behaviours (Borchers *et al.*, 2016; Pereira *et al.*, 2018; Rombach *et al.*, 2018; Grinter *et al.*, 2019; Merenda *et al.*, 2019, 2020; Zambelis *et al.*, 2019). In calves, accelerometers have recently been used to detect early behavioural indicators of respiratory disease (Swartz *et al.*, 2017) and diarrhoea (Sutherland *et al.*, 2018); however, although ill health contributes to a poor welfare state, its absence does not necessarily equate to good welfare (Broom, 1988). The more recently developed concept of positive animal welfare includes consideration of positive experiences and emotions (Boissy *et al.*, 2007; Mellor, 2012; Vigors *et al.*, 2019) and has been gaining traction in the last decade as a response to the perception that animal welfare assessment is too focused on avoiding negative welfare states rather than promoting positive welfare (Lawrence *et al.*, 2019). Behaviour is a sensitive indicator of welfare and as such, accelerometers have the potential to be a useful tool in the assessment of positive welfare in cattle, but to be able to achieve this goal, accelerometers need to be able to generate data that allow the user to monitor behaviours that are not just indicative of health status but also of positive welfare. In young cattle, play behaviour is considered to have potential as an indicator of positive animal welfare (Ahloy-Dallaire *et al.*, 2018; Vigors *et al.*, 2019) and has a locomotor component detectable by accelerometers (Gladden *et al.*, 2020), but locomotor play is rarely observed in adult cattle (Brownlee, 1954). Ear posture has been described as an alternative indicator of positive welfare for adult cattle (Proctor *et al.*, 2014), but is not so easily detected by animal-worn accelerometers. Therefore, although accelerometers are valuable tools for remotely detecting and measuring behavioural patterns consistent with ill health, their value in assessment of positive welfare states is less clear. Nevertheless, as ill health can adversely affect animal welfare there is still a place for data generated by accelerometers to be used in bovine welfare assessment. Further study is needed, and it needs to be considered that, as remote monitoring technology is always evolving and improving, new devices may be developed in the future that are able to assess positive welfare of adult cattle more readily.

1.6 Stress, inflammation, and physiological status around parturition: clinical and biochemical indicators

1.6.1 Neonatal vigour

Neonatal vigour refers to the strength and appearance of a neonate immediately after birth; assessment aims to identify newborns that may have additional care needs in the immediate post-birth period. Neonatal vigour of babies is assessed within 1 min of birth using a scoring system developed by Dr. Virginia Apgar (commonly called the Apgar Score) that evaluates five measures: heart rate, respiratory effort, mucous membrane colour, muscle tone and reflex irritability (Apgar, 1953). Modified Apgar scoring systems have been developed for several species of veterinary importance and typically score selected parameters on an ordinal scale ranging from 0 (*abnormal*) to 2 (*normal*) [Appendix 1]. Individual scores are summed to calculate a single composite score — low values typically indicate poor vigour and high values indicate good vigour. Animals that are subject to difficult birth are at increased risk of having reduced neonatal vigour (Adams *et al.*, 1995; Dwyer, 2003; Riley *et al.*, 2004; Sorge *et al.*, 2009; Veronesi *et al.*, 2009; Barrier *et al.*, 2013b; Murray *et al.*, 2015a) with some studies finding a negative correlation between birth assistance score and Apgar score (Sorge *et al.*, 2009). Whilst Apgar scoring provides valuable information, identification of compromised calves has been inconsistent (Boyd, 1989; Sorge *et al.*, 2009; Feitosa *et al.*, 2012); additionally, Apgar assessment is complex and can be time consuming, resulting in poor adoption in practice (Homerosky *et al.*, 2017b). Calves with reduced vigour are weaker, take longer to attempt standing and longer to suckle than vigorous calves (Riley *et al.*, 2004; Barrier *et al.*, 2013b; Murray *et al.*, 2015b). Additionally, poor neonatal vigour of calves has been associated with impaired colostrum consumption (Vasseur *et al.*, 2009). Therefore, accurate identification of affected calves has important implications for calf health and welfare and as such, attempts to encourage the routine use of vigour assessment on farm have been made. For example, a smartphone app has been created (Murray-Kerr *et al.*, 2018) to aid on-farm use of a ten-point scoring system developed for assessment of dairy calf vigour (Murray *et al.*, 2015a), and an eight-point scale has been trialled in New Zealand (Gillingham *et al.*, 2018) [both systems are described in Appendix 1]. However, similar to other vigour scoring systems, these are time consuming to implement and development of more simplified methods for assessment of newborn calf vigour has also been attempted. For example, Schuijt and Taverne (1994) found latency to achieve sternal recumbency was a good indicator of vigour, and recently a protocol combining assessment of neonatal tongue withdrawal with dam parity and birth assistance score has been developed (Homerosky *et al.*, 2017a). The method proposed by Homerosky *et al.* (2017a) is straightforward and practical to implement and has been shown to perform well for vigour assessment of beef calves but to date has not been tested in dairy calves.

1.6.2 Heart rate variability and thermoregulation

Heart rate variability (HRV) is a measure of variation in the inter-beat interval (von Borell *et al.*, 2007) that reflects the antagonistic influences of the sympathetic (increases heart rate) and parasympathetic (decreases heart rate) nervous systems. Heart rate variability is therefore a non-invasive measure of the autonomic nervous system response to stress (Mohr *et al.*, 2002; von Borell *et al.*, 2007; Schmidt *et al.*, 2010; Stewart *et al.*, 2010; Kovács *et al.*, 2015b). Accordingly, HRV is a useful tool in welfare assessment of domestic species (von Borell *et al.*, 2007; Bergamasco *et al.*, 2010; Schmidt *et al.*, 2010; Kjaer *et al.*, 2011; Kovács *et al.*, 2014; Clapp *et al.*, 2015). A single study has measured HRV during parturition finding that (normal) parturition progress was associated with maternal HRV steadily increasing to a peak just before expulsion of the foetus, followed by a decrease immediately afterwards, suggesting that stage II is the most stressful stage of parturition for the cow (Kovács *et al.*, 2015a). Bovine foetal HRV increases during late gestation, reflecting maturation of the foetal autonomic nervous and cardiovascular systems (Trenk *et al.*, 2015; Nagel *et al.*, 2016a; Quevedo *et al.*, 2019), and peaks at birth (Nagel *et al.*, 2016a; Quevedo *et al.*, 2019). It is possible that birth is a stressful experience for calves, but further work is needed to explore this further.

Bovine neonatal temperature declines in the first hour of life (Vermorel *et al.*, 1989; Kovács *et al.*, 2017b; Vannucchi *et al.*, 2019) — likely due to the difference in ambient temperature of intrauterine and extrauterine environments. The effect of birth difficulty on neonatal temperature is currently unclear with previous studies reporting conflicting results (Vermorel *et al.*, 1989; Adams *et al.*, 1995; Bellows *et al.*, 2000; Barrier *et al.*, 2013b; Vannucchi *et al.*, 2015b, 2019) but some data suggest that the ability to thermoregulate might be adversely affected by severe birth assistance (Vermorel *et al.*, 1989; Adams *et al.*, 1995; Bellows *et al.*, 2000; Kovács *et al.*, 2017b). As such, severely-assisted calves may experience heightened benefits of husbandry measures to retain warmth (e.g. calf jackets, deep bedding). However, low environmental temperatures are associated with calf mortality irrespective of birth assistance (Hyde *et al.*, 2020); therefore in colder climates it is likely that all calves will benefit from additional husbandry measures intended to preserve warmth, regardless of assistance status.

1.6.3 Cortisol

Cortisol is an important glucocorticoid hormone essential for the maintenance of homeostasis that is secreted by the adrenal gland in response to adrenocorticotropic hormone (ACTH). Corticotropin-releasing hormone (CRH) regulates ACTH release as part of the hypothalamic-adrenal-pituitary (HPA) axis [Figure 1.2]. Regulation of CRH is complex but is affected by many factors, including stress. Accordingly, ACTH production is stimulated during times of physiological or emotional stress and cortisol secretion is subsequently increased, acting as a biochemical indicator of stress (Katsu *et al.*, 2015; Hulbert *et al.*, 2016).

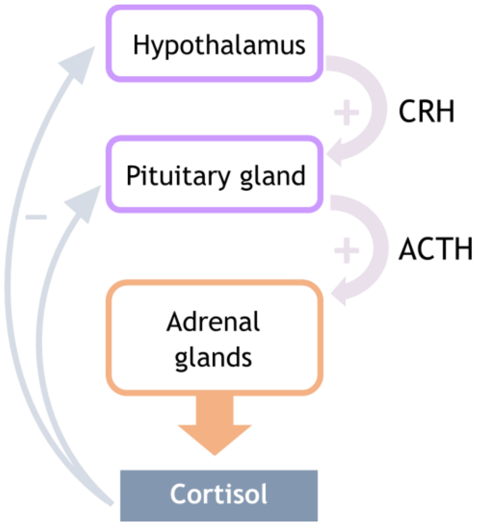

Figure 1.2: Schematic diagram of the hypothalamic-pituitary-adrenal axis
ACTH = adrenocorticotropic hormone. CRH = corticotropin-releasing hormone. **+** *indicates stimulatory aspect (right side of diagram).* **−** *indicates inhibitory aspect (i.e. negative feedback) (left side of diagram). (Engelking, 2012; Chang et al., 2013)*

Cortisol is readily measured in a variety of body fluids, tissues and products including blood (Becker *et al.*, 1985), saliva (Geburt *et al.*, 2015), faeces (Östl *et al.*, 1999) and hair (Gow *et al.*, 2010; Burnett *et al.*, 2015). Due to being a sensitive measure of stress and its ease of measurement, cortisol is a commonly measured biomarker in studies investigating the effect of events that are expected to be stressful (Becker *et al.*, 1985; Hemsworth *et al.*, 1989; Wagner *et al.*, 2013; Lockwood *et al.*, 2017). Studies assessing methods of ameliorating stressful experiences (for example studies investigating the benefits of analgesia during painful husbandry procedures (McMeekan *et al.*, 1998; Stilwell *et al.*, 2012)) also frequently measure cortisol concentration.

1.6.3.1 Effects of normal parturition on maternal cortisol

Elevated foetal blood cortisol concentration at birth is normal and is required to initiate parturition (Comline *et al.*, 1974; Liggins, 1994), but there is disagreement as to whether maternal blood cortisol concentrations also increase parallel to the foetus, or change only minimally. Some studies have identified a parturient increase in maternal cortisol concentration that mirrors the pattern identified in calves (Hoffman *et al.*, 1973; Patel *et al.*, 1996; Jacob *et al.*, 2001; Geburt *et al.*, 2015; Nagel *et al.*, 2016b), whereas other studies have not found any such changes (Comline *et al.*, 1974; Hunter *et al.*, 1977). Breed differences in maternal postpartum blood cortisol concentration have been identified in one study (Geburt *et al.*, 2015) which may contribute to the inconsistent results reported: Comline *et al.* (1974) and Hunter *et al.* (1977) studied Jersey cows, whereas most studies that identified a periparturient increase in maternal cortisol concentration involved other breeds of cattle [Table 1.9]. Breed has been found to affect the milk cortisol concentration of lactating cows (Sgorlon *et al.*, 2015) but, although periparturient maternal cortisol concentration of beef and dairy breeds has been found to differ (Geburt *et al.*, 2015), the effects of dairy breed are unknown. It is likely that breed differences affect the degree of parturient stress experienced, rather than there being more fundamental physiological differences between breeds. Most studies include a single breed [Table 1.9] thus controlling for potential breed effects within each study, but the wide variety of external factors contributing to stress (e.g. housing, study design) make comparisons between studies (and hence breeds) difficult. For instance, sampling methods used in some

Table 1.9: Studies of bovine maternal cortisol concentration changes around normal parturition

Author	Breed	Parity	Sampling method	Sampling frequency	Periparturient cortisol concentration increase reported?
Comline *et al.* (1974)	Jersey	Multiparous	Jugular catheterisation	Daily blood samples obtained between 09:00 and 10:00 each day	No
Geburt *et al.* (2015)	Simmental German Black Pied	Multiparous and primiparous	Mouth swab (saliva)	Single sample obtained at each of three sampling points: two weeks pre-partum, 1 d and 2 d postpartum	Yes
Hoffman *et al.* (1973)	Brown Swiss and Simmental	Multiparous	Jugular venepuncture	Daily at 12 h or 6 h intervals	Yes
Hudson *et al.* (1976)	Jersey and Jersey x Friesian	Multiparous	Jugular catheterisation	Three times daily (07:00, 15:00 and 23:00) plus additional samples during parturition (at the appearance of foetal feet, at calf expulsion and 1 h postpartum)	Yes
Hunter *et al.* (1977)	Jersey	Multiparous	Jugular catheterisation	Twice daily, more frequent sampling when parturition imminent.	No
Jacob *et al.* (2001)	Crossbred[1]	Not reported	Venepuncture (site not reported)	Single sample obtained at 3 d prior to parturition, on the day of parturition and 7 d postpartum	Yes
Nagel *et al.* (2016b)	Austrian Simmental (Fleckvieh)	Multiparous	Mouth swab (saliva)	Every 6 to 8 h between 36 h and 3 h pre-partum then every 15 to 30 min during parturition and for the first 2 h postpartum	Yes
Patel *et al.* (1996)	Holstein	Multiparous	Jugular venepuncture	Every third day from the day of embryo transfer until the last 10 d of gestation then daily from 10 d pre-partum to 1 d postpartum.	Yes
Smith *et al.* (1973)	Holstein	Primiparous	Jugular venepuncture	Twice weekly between 26 d and 6 d pre-partum then twice daily from 6 d pre-partum to 5 d postpartum. Further samples obtained twice on day nine postpartum.	Yes
Vannucchi *et al.* (2015a)	Holstein	Multiparous and primiparous	Coccygeal venepuncture	48 h pre-partum, during parturition, 20 min postpartum and 1 h postpartum	Yes

1. Individual breeds not reported

studies are more stressful than in other studies (e.g. repeated venepuncture versus mouth swabs) and may contribute to observed increases in blood cortisol concentrations. Nonetheless, similar results in the period approximately 14 d prior to parturition have been reported irrespective of sampling method, and only differ at parturition (Hoffman *et al.*, 1973; Comline *et al.*, 1974; Hunter *et al.*, 1977; Patel *et al.*, 1996; Geburt *et al.*, 2015). Furthermore, Nagel *et al.* (2016b) and Geburt *et al.* (2015) both measured salivary cortisol concentration — considered less stressful than obtaining blood samples — and found maternal cortisol concentration increased at parturition, suggesting that the effect of sampling method on maternal periparturient cortisol concentration is minimal.

1.6.3.2 Effect of dystocia on maternal cortisol concentration

Fewer studies investigate the effect of dystocia or assisted parturition on maternal blood cortisol concentration, but the findings are generally in agreement. A pilot study of three animals (Kindahl *et al.*, 2002) found that more difficult parturition was associated with higher plasma cortisol concentration. Whilst the small sample size casts doubt on the reliability of these results, other authors have reported similar findings (Hudson *et al.*, 1976; Nakao *et al.*, 1990; Civelek *et al.*, 2008). By contrast, Vannucchi *et al.* (2015a) found that dystocia was initially associated with a smaller increase in serum cortisol concentration compared to unassisted cows, and Barragan *et al.* (2020b) did not identify an effect of dystocia at all. However, these apparently conflicting results are likely to be due to differences in sampling times. Previous studies have measured maternal plasma cortisol concentration within 6 h of parturition (Nakao *et al.*, 1990; Civelek *et al.*, 2008), whereas Barragan *et al.* (2020b) first measured plasma cortisol concentration at 12 h postpartum. The half-life of cortisol is approximately 30 min in postpartum cattle (Dunlap *et al.*, 1981) and 12 h may have been too long after parturition to identify an effect of dystocia. Vannucchi *et al.* (2015a) sampled cows before parturition was complete (between allantochorion rupture and calf expulsion) and found that whilst cows experiencing dystocia had lower serum cortisol concentration at this point, maternal cortisol concentration continued to increase afterwards, exceeding serum cortisol concentration of unassisted cows at 20 min after calf expulsion — a similar sampling time to previous studies (Nakao *et al.*, 1990; Civelek *et al.*, 2008). The findings of Vannucchi *et al.* (2015a) suggest that dystocia may become more stressful as parturition progresses; however, a recent study of buffalo found that, compared to normal parturition, dystocia was associated with elevated serum cortisol concentration at the end of stage I parturition (Refaat *et al.*, 2013), suggesting that dystocia causes elevated stress for the duration of parturition. As both studies sampled animals at the same stage of parturition and defined dystocia similarly it is likely that species differences are contributing to the differences in these findings. Therefore, although it is likely that maternal cortisol increases at parturition, the effects of dystocia or assisted parturition are not yet clear.

1.6.3.3 Foetal cortisol concentration around parturition and the effects of dystocia

More than 40 years ago pivotal work demonstrated that in late gestation bovine foetal blood cortisol concentration increases gradually until the commencement of stage I parturition when a more rapid increase is observed until expulsion of the calf, shortly after which cortisol concentration starts to decline (Comline *et al.*, 1974; Hunter *et al.*, 1977). This pattern in foetal plasma cortisol concentration is normal and initiates ruminant parturition (Liggins, 1994). Although cortisol concentration is high in all calves at birth, dystocia is consistently associated with a heightened cortisol response (Stott *et al.*, 1978; Massip, 1980b; Hoyer *et al.*, 1990; Bellows *et al.*, 2000; Civelek *et al.*, 2008; Barrier *et al.*, 2013b; Vannucchi *et al.*, 2015a; Kovács *et al.*, 2021). Most studies measure neonatal cortisol concentration within 1 h of birth (Stott *et al.*, 1978; Massip, 1980b; Hoyer *et al.*, 1990; Bellows *et al.*, 2000; Civelek *et al.*, 2008; Vannucchi *et al.*, 2015a; Kovács *et al.*, 2021) but Barrier *et al.* (2013b) adopted a different approach, obtaining a single sample between 8 a.m. and 11 a.m. (within 24 h of birth) to measure salivary cortisol concentration. As neonatal plasma cortisol concentration declines rapidly in the first 24 h after birth (Comline *et al.*, 1974; Stott *et al.*, 1978; Hoyer *et al.*, 1990) it might be expected that the variation in time (relative to birth) that calves were sampled by Barrier *et al.* (2013b) may have affected results but, consistent with the results of other studies, Barrier *et al.* (2013b) found that salivary cortisol concentration was higher in calves born to dystocia, suggesting calves born to dystocia may experience heightened stress for up to 24 h. It is possible that calves born to dystocia experience heightened stress (compared to unassisted calves) beyond 24 h but this has not yet been studied.

Neonatal plasma or serum cortisol concentration are most commonly measured in existing studies (Massip, 1980b; Hoyer *et al.*, 1990; Bellows *et al.*, 2000; Civelek *et al.*, 2008; Vannucchi *et al.*, 2015a) but blood samples are invasive, can be technically challenging to obtain from newborn calves, and may create additional stress that affects results (Cook, 2012; Nemeth *et al.*, 2016). As such, measurement of salivary cortisol is of interest as a non-invasive measure (Kovács *et al.*, 2021). The response of adult bovine salivary cortisol to painful stimuli has been found to be delayed by approximately 10 min when compared to plasma cortisol concentration (Hernandez *et al.*, 2014) but it is unknown if neonatal cortisol responses are similar. Nonetheless, if repeated samples are taken as described by Kovács *et al.* (2021), or calves are sampled at a later time point as described by Barrier *et al.* (2013b), effects of dystocia on cortisol concentration are unlikely to be missed by salivary sampling. Kovács *et al.* (2021) recently examined neonatal salivary cortisol concentration as a measure of dystocia related stress and were able to sample calves every 15 min without the need for restraint, finding that salivary cortisol concentration was higher in calves born to dystocia (than eutocia). Although the findings of Kovács *et al.* (2021) were consistent with other studies, the concentration of cortisol measured was much lower than studies measuring neonatal plasma or serum cortisol concentration (Massip, 1980b; Civelek *et al.*, 2008; Vannucchi *et al.*, 2015a). Similar discrepancies between plasma and saliva cortisol concentration have previously been demonstrated following ACTH stimulation in adult cattle (Negrão *et al.*, 2004), pigs (Parrott *et al.*, 1989; Bushong *et al.*, 2000) and dogs (Vincent *et al.*, 1992), although all studies report good correlation between samples. These findings indicate that, whilst salivary cortisol concentration is a valuable, non-invasive measure of stress appropriate for use in studies of newborn calves, care must be taken when making comparisons between studies as cortisol concentration is affected by sampling method.

1.6.4 Creatine kinase

Creatine kinase (CK) is a muscle specific enzyme and as such is a sensitive indicator of muscle damage (Anderson *et al.*, 1976; Clark *et al.*, 1987; Braun *et al.*, 1995; Russell *et al.*, 2007; Baird *et al.*, 2012). As the half-life of CK in cattle is short (4 to 8 h (Lefebvre *et al.*, 1994)), serum concentration rapidly returns to normal in the absence of further insult (Russell *et al.*, 2007); therefore, prolonged elevation in serum or plasma CK concentration can be suggestive of continued or repeated muscle injury. Accordingly, increased serum CK concentration is a good indicator of the presence of recent muscle damage (and can be diagnostically useful when muscle injury is suspected but cannot be identified through clinical examination alone) but is not reflective of more historic injury and the prognostic value of CK concentration alone is limited (Shpigel *et al.*, 2003; Russell *et al.*, 2007).

Four major CK isoenzymes have been described [Table 1.10] and are found in a wide variety of tissues, although the main sites of bovine CK activity are skeletal muscle and the myocardium (Galitzer *et al.*, 1985). As such, elevated CK concentration is typically interpreted as indicative of myopathy in clinical veterinary practice (Russell *et al.*, 2007; Otter, 2013), as measurement of individual isoenzymes is not readily available.

Table 1.10: Creatine kinase isoenzymes and the primary site of activity
(Washington et al., 2012; Smith et al., 2013)

Isoenzyme[1]	Primary site of activity
CK-BB (CK-1)	Brain
CK-MB (CK-2)	Myocardium
CK-MM (CK-3)	Skeletal muscle
CK-Mt	Mitochondria

1. Alternative terminology in parentheses

1.6.4.1 Maternal periparturient creatine kinase concentration and the effect of dystocia

Creatine kinase activity is high in the gravid uterus (Payne *et al.*, 1993; Clark *et al.*, 1994) thus plasma CK concentration might be expected to be elevated in cows that experience dystocia (due to the tissue damage that can occur), and maybe also in all cows at parturition. It has been shown that serum CK concentration increases significantly in women at the time of normal childbirth (Abramov *et al.*, 1996) and, whilst there are few studies investigating changes in bovine CK concentration around the time of parturition, the current findings appear to be similar to humans. Hussein *et al.* (2008) found that serum CK concentration increased around the time of parturition in all cows studied irrespective of parturition experience, and West (1989) reported increased plasma CK concentration in periparturient cows compared to non-pregnant, non-lactating cows. Additionally, Hussein *et al.* (2008) found dystocia was associated with a greater increase in plasma CK concentration than normal parturition. However, it is difficult to determine whether this difference would be as apparent in 'on farm' situations as all of the cows experiencing dystocia were assisted by a veterinary surgeon and either a foetotomy or a caesarean section was performed (Hussein *et al.*, 2008). Not only are these procedures less common on commercial farms than farmer-assisted parturition (Egan *et al.*, 2001), but they also have the potential for a greater degree of tissue damage than the judicious application of traction. Nevertheless, another study including a range of parturition assistance severities identified similar findings of elevated plasma creatinine (a breakdown product of creatine) of cows experiencing dystocia (Civelek *et al.*, 2008). Although Civelek *et al.* (2008) only studied primiparous animals, the animal recruitment was more reflective of parturition management on commercial dairy farms and suggests that tissue damage occurs during assisted parturition of mild to moderate severity, not only when advanced or surgical assistance is required. It is possible that, due to their larger size and greater parturition experience, multiparous cows might experience less tissue injury at parturition than primiparous animals. Healthy primiparous cows have been found to have higher plasma CK concentration than multiparous cows in early and mid-lactation (Cozzi *et al.*, 2011), but a similar parity effect was not identified during late gestation (Brscic *et al.*, 2015). It is currently unknown if there is a parity effect on plasma CK concentration at parturition, or in the postpartum period.

1.6.4.2 Neonatal postpartum creatine kinase concentration and the effect of dystocia

Few studies have measured neonatal plasma or serum CK concentration after birth or investigated the effect of birth assistance. One study found that neonatal plasma CK was outside of the normal range for two out of 27 (7.4%) calves sampled 1 to 2 h after normal birth, suggesting that normal birth is sometimes (but not always) associated with neonatal tissue damage (Anderson *et al.*, 1976). However, a normal plasma CK reference interval (RI) has not been defined for newborn calves and Anderson *et al.* (1976) did not report the parameters of the range used in their study, thus it is difficult to be certain that only a small proportion of calves were affected. The hypothesis that normal birth might be associated with some tissue damage is supported by other studies that have also found neonatal plasma CK to be elevated at birth regardless of assistance status (Egli *et al.*, 1998; Knowles *et al.*, 2000). As traumatic injuries are reported to occur more often in calves experiencing assisted birth (Schuijt, 1990; Agerholm *et al.*, 1993; Pearson *et al.*, 2019c), it can be hypothesised that birth assistance might be associated with increased plasma CK concentration in newborn calves (compared to unassisted birth). Murray *et al.* (2015b) measured neonatal serum CK concentration at the time calves first achieved sternal recumbency and found it to be higher in calves experiencing severe assistance (score 3) than calves experiencing mild (score 2) or no birth assistance, suggesting that whilst intense birth assistance is associated with tissue damage, more gentle assistance is not more injurious than unassisted birth. This hypothesis is supported by a second study that found serum CK concentration of calves born to 'difficult assistance' (equivalent to score 3 in the study reported by Murray *et al.* (2015b)) was higher than serum CK concentration of calves experiencing 'easy assistance' (equivalent to score 2) or unassisted birth (Pearson *et al.*, 2019c). Pearson *et al.* (2019c) obtained samples at 24 h after birth therefore, as the half-life of CK in calves may be as short as 2 to 3 h (Anderson *et al.*, 1976), it is possible that tissue damage following (severely) assisted birth continues into the postpartum period. It is unknown for how long after birth neonatal plasma CK of calves born to severe assistance remains elevated, or whether any pain is associated with birth-related muscle injury. If so, this represents a welfare challenge for affected calves that has the potential to be improved; for example, with analgesic drugs or enhanced management such as provision of a softer or deeper bedding surface.

1.6.5 Lactate

Lactate is an end product of glucose metabolism formed during anaerobic glycolysis when pyruvate is converted by lactate dehydrogenase into lactic acid (Lagutchik *et al.*, 1996). Lactate occurs as two isomers: L-lactate and D-lactate, L-lactate being the primary isomer found in healthy animals (Kondoh *et al.*, 1992). Anaerobic glycolysis increases when tissues are hypo-perfused, therefore elevated blood L-lactate concentration can be indicative of hypoxia and subsequent tissue hypoperfusion. As such, plasma L-lactate concentration has been studied as a prognostic indicator following traumatic injury, surgery or severe illness

(Lagutchik *et al.*, 1996; Boulay *et al.*, 2014; Buczinski *et al.*, 2015; Yildiz *et al.*, 2017).

In contrast to L-lactate, D-lactate is produced by microbes and is primarily gastrointestinal in origin (Ewaschuk *et al.*, 2004), forming as a result of bacterial fermentation of carbohydrates. In pre-ruminant calves D-lactate acidosis occurs as a complication of neonatal diarrhoea (Omole *et al.*, 2001; Lorenz *et al.*, 2014), whereas in ruminating animals D-lactate acidosis is associated with excessive ingestion of highly fermentable carbohydrates (Snyder *et al.*, 2017).

1.6.5.1 Maternal lactate concentration around parturition

No studies have investigated changes in maternal plasma lactate concentration around normal or assisted parturition; although measurement of lactate has been assessed as a prognostic indicator for uterine torsion with studies finding high blood lactate concentration is associated with increased correction difficulty (of the torsion) and a poorer prognosis (Murakami *et al.*, 2017; Mahmoud *et al.*, 2020). Based on their results, Murakami *et al.* (2017) suggested blood lactate concentration cut-points that could be used as prognostic indicators for uterine torsion, but these are untested and further work is needed to assess their accuracy. Blood lactate concentration has been better studied in relation to gastrointestinal disorders and interestingly the findings of Murakami *et al.* (2017) are similar to the results of studies investigating the prognostic value of blood lactate concentration for conditions including abomasal displacement (cattle) (Boulay *et al.*, 2014; Buczinski *et al.*, 2015), gastric dilatation volvulus (dogs) (de Papp *et al.*, 1999; Zacher *et al.*, 2010; Green *et al.*, 2011), colic (horses) (Moore *et al.*, 1976; Johnston *et al.*, 2007) and upper gastrointestinal bleeding (humans) (Lee *et al.*, 2017). The similarity of results of studies that vary widely in both species and condition studied suggest that the threshold for blood lactate concentration to become abnormally elevated is consistent across mammalian species and is not related to individual disease states. It is unknown whether dystocia is associated with an elevation in maternal blood lactate concentration, or if (similar to uterine torsion) dystocia severity is positively associated with blood lactate concentration.

1.6.5.2 Neonatal lactate concentration at birth

In contrast to maternal parturient lactate concentration, relationships between neonatal blood lactate concentration and birth experience have been more extensively studied. A mixed metabolic-respiratory acidosis syndrome is often observed in newborn calves, resulting from the hypoxia and hypercapnia that all foetuses experience at birth (Varga *et al.*, 1998). Increased blood L-lactate concentration (metabolic component) contributes more to the pathogenesis of bovine neonatal acidosis than hypercapnia (respiratory component) (Bleul *et al.*, 2013), possibly due to compensatory improved lung function of acidotic calves (Varga *et al.*, 2001). Accordingly, neonatal plasma lactate concentration is highly (negatively) correlated with blood pH ($r > -0.8$) and as such is a good indicator of the severity of acidosis (Bleul *et al.*, 2013; Homerosky *et al.*, 2017a). Whilst mild neonatal acidosis (pH 7.0 to 7.2) at birth is normal and aids adaptation to extrauterine life (Varga *et al.*, 1998), more severe (or prolonged) acidosis is associated with negative outcomes such as an increased risk of failed passive transfer of immunoglobulins (Besser *et al.*, 1990; Murray *et al.*, 2015b; Homerosky *et al.*, 2017b) and increased morbidity and mortality (Szenci *et al.*, 1988; Schuijt *et al.*, 1994). Acidosis has an adverse effect on normal mentation and neurological function (Kasari *et al.*, 1986; Homerosky *et al.*, 2017a), therefore it might be expected to be correlated with Apgar score, but this is an inconsistent finding. Homerosky *et al.* (2017a) used traditional Apgar score parameters of vigour and found that neonatal blood lactate concentration was not related to most parameters measured. By contrast, Sorge *et al.* (2009) assessed calf vigour using a modified Apgar score on an ordinal one to eight scale and found that overall a high blood lactate concentration was associated with a low score (indicative of poor vigour), although the correlation between the score awarded and blood lactate concentration was low ($r = -0.251$), albeit statistically significant. Only 6% of calves studied by Sorge *et al.* (2009) had an Apgar score less than six (indicative of poor vigour) and blood lactate concentrations up to 19.8 mmol/L (normal RI: < 5 mmol/L) were observed in calves scored seven or eight (good vigour). It is possible that the imbalance in group sizes (vigorous/poor vigour) and wide variety of blood lactate concentration in vigorous calves has resulted in the low correlation between Apgar score and neonatal blood lactate concentration observed. Analysing these data in binary format comparing blood lactate concentration of vigorous calves (scores seven and eight) to calves with poor vigour (score ≤ 6) is an alternative approach that could have been employed to mitigate some of the effects of such an unbalanced dataset. Moreover, as vigour is associated with colostrum intake and calf health (Homerosky *et al.*, 2017b), and thus is the outcome of clinical importance to farmers and veterinary surgeons, this approach to analysis has more practical relevance.

Elevated blood lactate concentration in calves experiencing birth difficulty is a consistent finding (Vermorel *et al.*, 1983, 1989; Adams *et al.*, 1995; Diesch *et al.*, 2004; Feitosa *et al.*, 2012; Bleul *et al.*, 2013; Homerosky *et al.*, 2017a; Kovács *et al.*, 2017b) although neonatal blood lactate concentration shows a high degree of variability between individuals, irrespective of birth experience (Sorge *et al.*, 2009; Homerosky *et al.*, 2017a). Whilst most studies report blood lactate concentration of assisted calves in relation to unassisted calves (Vermorel *et al.*, 1983, 1989; Diesch *et al.*, 2004; Sorge *et al.*, 2009; Homerosky *et al.*, 2017a), prolonged unassisted parturition is also associated with elevated neonatal blood lactate concentration (Kovács *et al.*, 2017b); therefore, it cannot be assumed that calves born to unassisted birth will not experience neonatal acidosis. This is an important finding because it suggests that unassisted calves cannot always be relied upon to adopt important neonatal behaviours (such as suckling) and illustrates the importance of inspecting all calves to identify those that may need additional assistance — a view supported by Homerosky *et al.* (2017b) who found that high blood lactate concentration at birth was indirectly associated with poor health before weaning.

1.6.6 Glucose

Few studies have measured blood glucose concentration in periparturient cows. Vannucchi *et al.* (2015a) found that during parturition, blood glucose increased in cows experiencing both dystocia and normal parturition and at 1 h postpartum all cows were hyperglycaemic. Dystocia did not significantly affect maternal blood glucose concentration although it was associated with a numerical increase (Vannucchi *et al.*, 2015a). Similarly, Qu *et al.* (2014), Pascottini *et al.* (2020) and Jacob *et al.* (2001) all found that maternal blood glucose concentration was increased at parturition, but the effects of dystocia were not evaluated. It is likely that hyperglycaemia is a response to stress experienced during parturition and Vannucchi *et al.* (2015a) suggested that hyperglycaemia might have potential as a marker of parturition stress. This is a plausible hypothesis as stress hyperglycaemia is a well-recognised transient phenomenon in other domestic species, most notably cats (*Felis catus*) (Rand *et al.*, 2002). Although ruminant glucose metabolism is different to monogastric species (McDowell, 1983; De Koster *et al.*, 2013), hormonal regulation of blood glucose is similar (De Koster *et al.*, 2013) and it is reasonable to expect that stress hyperglycaemia may also occur in cows — indeed Janßen *et al.* (2016) found that the blood glucose of lame cows was higher than non-lame cows when claw trimming was performed. Brscic *et al.* (2015) and Quiroz-Rocha *et al.* (2009) did not identify a difference between pre- and post-partum maternal blood glucose concentration; however, both studies sampled cows a week or more before and after parturition — a timescale is unlikely to detect effects of parturition on maternal blood glucose concentration (due to the regulatory effects of homeostatic control mechanisms). Blood glucose is readily measured on farm using hand-held meters thus it is an ideal candidate for the study of parturition-related stress. However, it is possible that the potential for blood glucose concentration to be a useful marker of parturition-related stress (as suggested by Vannucchi *et al.* (2015a)) is limited by the necessity for samples to be obtained immediately after, or during, parturition. Fructosamine is a longer-term biomarker of hyperglycaemia widely used in companion animal practice but does not correlate well to blood glucose concentration in cattle (Sorondo *et al.*, 2009; Megahed *et al.*, 2018; Hasanabadi *et al.*, 2019), and as such is not a suitable alternative to blood glucose measurement. Therefore, further work focused on blood glucose is needed to elucidate the duration of parturition-related maternal hyperglycaemia in cattle and assess the value of hyperglycaemia as a marker of parturition-related stress. Establishment of the wider applicability to a commercial farm setting is needed before measurement of blood glucose for this purpose becomes more widely adopted.

Bovine neonatal blood glucose concentration increases in the first hour after birth before rapidly declining (Daniels *et al.*, 1974; Massip, 1980a; Kurz *et al.*, 1991; Chan *et al.*, 1993); thereafter, neonatal blood glucose concentration increases in response to colostrum feeding (Daniels *et al.*, 1974; Kurz *et al.*, 1991). Neonatal plasma cortisol and glucose concentration are positively correlated at birth

(Massip, 1980a) — an expected finding given cortisol stimulates an increase in hepatic gluconeogenesis, resulting in reduced glucose uptake from the blood (and thus increased blood glucose concentration) (Barnes *et al.*, 1978; Liggins, 1994). Therefore, it is plausible that dystocia may be associated with neonatal hyperglycaemia due to a heightened cortisol response associated with birth stress [see Subsection 1.6.3.3]. This hypothesis is supported by studies that found that dystocia is associated with elevated blood glucose concentration of newborn calves (Vermorel *et al.*, 1989; Chan *et al.*, 1993; Bellows *et al.*, 2000; Vannucchi *et al.*, 2015a); although this finding is not consistent across all studies — Homerosky *et al.* (2017a) did not detect an effect. Reasons for these inconsistent results are unclear but Homerosky *et al.* (2017a) suggested colostrum feeding could be a factor; however, this hypothesis is unlikely as, although Homerosky *et al.* (2017a) allowed calves to consume colostrum as soon as they were able, blood glucose concentration was measured at 10 min after birth when calves were not yet standing and as such were unlikely to have consumed colostrum. Dystocia-related challenges affecting newborn calves including hypoxia, acidosis, birth-stress, and cold temperatures are all thought to negatively affect gluconeogenesis (Warnes *et al.*, 1977; Massip, 1980a; Chan *et al.*, 1993; Bellows *et al.*, 2000). Although speculative (as comprehensive description of all of these factors are rarely included in studies), it is likely that varying challenges affecting calves in different studies has contributed to the inconsistent results reported.

Interestingly, some studies have found that calves subject to mild to moderate birth assistance have higher blood glucose concentration than calves experiencing more severe assistance (Vermorel *et al.*, 1989; Bellows *et al.*, 2000). This is an unexpected finding as severe birth assistance is associated with greater neonatal stress than more mild assistance (Hoyer *et al.*, 1990; Chan *et al.*, 1993), therefore might be expected to also be associated with a greater degree of hyperglycaemia. However, this is not a hypothesis supported by Chan *et al.* (1993) who, in contrast to other studies, found that increasing degrees of traction applied during assisted birth were associated with increasing blood glucose concentration. It is possible that, as neonatal acidosis is associated with a compensatory improved lung function (Varga *et al.*, 2001), calves subject to more severe birth assistance are less hypoxic (than more mildly assisted calves) and thus, gluconeogenesis is less affected. This hypothesis assumes calves experiencing severe assistance in the studies reported by Vermorel *et al.* (1989) and Bellows *et al.* (2000) were more acidotic than unassisted or more mildly assisted calves, and whilst this did occur in the study reported by Vermorel *et al.* (1989), blood pH was not reported by Bellows *et al.* (2000). Furthermore, this hypothesis is not supported by the results of Chan *et al.* (1993) who identified a negative relationship between blood pH and blood glucose concentration. The effects of dystocia and assisted birth on neonatal blood glucose concentration are not fully understood and in particular further study of the interactions of different dystocia-related challenges faced by neonatal calves would be helpful. It is possible that blood glucose concentration may be an indicator of birth-related stress in calves, but several different factors may have confounding effects that limit its use for this purpose.

1.6.7 Acute phase proteins

Acute phase proteins (APPs) are plasma proteins that are part of the innate immune system, increasing in response to inflammation, infection and trauma as part of a co-ordinated response that aims to restore homeostasis (Eckersall, 2000; Eckersall *et al.*, 2010; Tothova *et al.*, 2014). Many APPs have been identified and can be classified as 'negative' (plasma concentration decreases in response to insult) or 'positive' (plasma concentration increases in response to insult). Acute phase proteins can be further classified as 'minor', 'moderate' or 'major' depending on the degree of response observed, which is species specific [Table 1.11] (Murata *et al.*, 2004; Eckersall *et al.*, 2010).

Table 1.11: Acute phase proteins of veterinary importance
(Murata et al., 2004; Eckersall et al., 2010)

Acute phase protein	Classification		Species
Albumin	Negative	n/c	All
Alpha-1 acid glycoprotein	Positive	Moderate	Cat, cow, chicken, dog, pig
Caeruloplasmin	Positive	Moderate	Cow, chicken, horse
C-reactive protein	Positive	Major	Dog, pig
		Moderate	Horse, cow, sheep
Fibrinogen	Positive	Moderate	Horse, cow, sheep, pig, dog
		Minor	Chicken
Haptoglobin	Positive	Major	Cow, sheep
	Positive	Moderate	Cat, dog, horse, pig
Major acute phase protein	Positive	Major	Pig
Serum amyloid A	Positive	Major	All
Transferrin	Negative	Minor	Cow, pig

n/c = not classified

The major APP in cattle is haptoglobin, which has been found to increase more than 100-fold in response to infection (Eckersall *et al.*, 2010). Measurement of bovine plasma APPs (in particular serum amyloid A and haptoglobin) has shown promise as a sensitive and early biomarker of acute infections such as mastitis (Eckersall *et al.*, 2001, 2006; Nielsen *et al.*, 2004) and calf respiratory disease (Angen *et al.*, 2009; Orro *et al.*, 2009; Idoate *et al.*, 2015; Joshi *et al.*, 2018).

1.6.7.1 Acute phase proteins at parturition and birth

Several studies have found that bovine APPs increase in response to parturition (Alsemgeest *et al.*, 1993; Koets *et al.*, 1998; Humblet *et al.*, 2006; Chan *et al.*, 2010; Mainau *et al.*, 2014; Qu *et al.*, 2014; Aziz *et al.*, 2016; Pascottini *et al.*, 2020). Accordingly, there has been interest in the effects of dystocia and assisted parturition on maternal APPs (Mainau *et al.*, 2014; Pohl *et al.*, 2015; Aziz *et al.*, 2016; Barragan *et al.*, 2020b). Aziz *et al.* (2016) found serum haptoglobin concentration of all animals was increased similarly on the day of parturition, (irrespective of parturition experience); however, serum haptoglobin concentration at 3 d postpartum was higher in cows subject to dystocia than cows experiencing normal parturition (Aziz *et al.*, 2016). Barragan *et al.* (2020b) reported similar results with plasma haptoglobin concentration being moderately elevated at 12 h postpartum in all cows (irrespective of parturition experience) but at 24 h postpartum plasma haptoglobin concentration was higher in the dystocia group, a difference that persisted until 7 d postpartum. Pohl *et al.* (2015) employed a different approach, analysing the odds of plasma haptoglobin being above 1.4 g/dL (the upper limit of the RI), but the results were consistent with other studies: plasma haptoglobin concentration of assisted cows was twice as likely to exceed the RI than unassisted cows. By contrast, Mainau *et al.* (2014) did not identify an effect of calving difficulty on plasma APP concentration but only mild assistance (‘easy assists’) were studied. Additionally, very few calves were malpresented (7% compared to 54% in the study reported by Aziz *et al.* (2016)). It is likely that differences in inclusion criteria compared to studies that included more severe assistance explain these different results. As APPs are measures of inflammation, current evidence suggests that mild assistance does not cause heightened inflammation compared to unassisted parturition, a finding that further illustrates the importance of a careful, gentle approach to parturition assistance in cattle.

In cattle, increases in plasma haptoglobin concentration following (non-specific) infection occur over a period of 24 to 48 h (Eckersall *et al.*, 2010) and similarly, bovine plasma haptoglobin concentration has been observed to peak at 24 to 48 h postpartum following normal parturition (Alsemgeest *et al.*, 1993). This delay in haptoglobin response to inflammation likely explains why effects of dystocia on plasma haptoglobin concentration are not observed until at least 24 h postpartum (Aziz *et al.*, 2016; Barragan *et al.*, 2020b). Unusually for a major APP (Eckersall *et al.*, 2010; Tothova *et al.*, 2014), plasma haptoglobin concentration is reported to remain elevated for up to 10 d following parturition in cattle, irrespective of assistance status (Humblet *et al.*, 2006; Mainau *et al.*, 2014; Aziz *et al.*, 2016; Barragan *et al.*, 2020b, 2020c). This led Humblet *et al.* (2006) to suggest an alternative haptoglobin RI for use during the week after parturition. However, it should be noted that the haemoglobin:haptoglobin (Hb:Hp) complexes that form during intravascular haemolysis are cleared more quickly than free haptoglobin (Ceciliani *et al.*, 2012; Boretti *et al.*, 2014). Therefore these suggested RI are likely to be unreliable for use in cows diagnosed with post-parturient haemoglobinuria (Ellison *et al.*, 1986; Resum *et al.*, 2017) or other haemolytic conditions (e.g. Babesiosis, *Mycoplasma wenyonii* infection).

Evidence regarding the effects of birth on APPs of calves is scant, although a single study found that foetal serum amyloid A concentration did not change around birth (Alsemgeest *et al.*, 1993). Additionally, a more recent study found that neonatal serum haptoglobin concentration at 24 h old was similar to older calves (2, 5 and 7 weeks old) (Yu *et al.*, 2019). Calf assistance status was not reported in these studies (Alsemgeest *et al.*, 1993; Yu *et al.*, 2019), but Pearson *et al.* (2019c) did not identify any effects of birth assistance. This is an interesting finding as the same study found serum CK concentration (suggestive of subclinical muscle trauma) of assisted calves was elevated (Pearson *et al.*, 2019c) and as such, an associated increase in serum haptoglobin might be expected. Pearson *et al.* (2019c) measured all blood parameters at 24 h after birth, which may have been too early to identify effects of assistance on haptoglobin; although this hypothesis is not supported by another study that found serum haptoglobin up to

8 d after birth was not affected by assistance status (Murray *et al.*, 2014). However, it should be noted that less than 40% of calves studied by Murray *et al.* (2014) were subject to birth assistance of any kind and only 6.7% experienced moderate to severe birth assistance, thus these findings may not be reliable.

1.6.8 Substance P

Substance P is a neuropeptide released from the spinal cord dorsal horn in response to noxious stimuli as part of the pain response [Appendix 1] (DeVane, 2001). It acts as both a neurotransmitter and a neuromodulator and has an important role in mediating nociception as well as integrating the stress responses (DeVane, 2001; Onuoha *et al.*, 2001). Additionally, substance P is involved in some responses mediated through the amygdala (Shaikh *et al.*, 1993; Brodin *et al.*, 1994) and as such, may be an indicator of pain-related affective state.

Although detailed physiology of substance P in cattle (such as half-life) has not been reported, results of studies that have measured changes in plasma substance P concentration in response to painful and stressful experiences suggest that it shows promise as a biomarker of pain and stress (Coetzee *et al.*, 2008, 2012; Van Engen *et al.*, 2014). Only two studies have evaluated the measurement of substance P around parturition in adult cattle, but the results suggest that substance P may have potential as a biomarker of postpartum pain. Cows diagnosed with clinical metritis were found to have higher plasma substance P concentration than healthy cows (Barragan *et al.*, 2018), supporting the findings of Stojkov *et al.* (2015) who identified behavioural changes suggestive of pain in cows diagnosed with metritis. By contrast, dystocia was not found to affect plasma substance P concentration in the first 48 h postpartum, but at 7 d postpartum a moderate increase in cows experiencing dystocia (compared to eutocia) was observed (Barragan *et al.*, 2020b). This finding suggests that dystocia related pain may be delayed onset in cattle, an interesting finding that may relate to delayed onset muscle soreness (DOMS). Delayed onset muscle soreness follows strenuous exercise and is well described in humans (e.g. Cheung *et al.*, 2003) but has not been described in non-human animals. It is possible that a similar phenomenon is occurring in postpartum cattle; however as data are limited, and the half-life of substance P in cattle is unknown, this result should be interpreted with caution.

1.6.9 Summary of biochemical indicators

When appraised collectively, studies of biomarkers of stress and inflammation suggest that parturition and birth are stressful events associated with inflammation for both cows and calves. However, the impact of parturition assistance is less clear although the data suggest that assisted birth may result in additional stress for calves. Effects of parturition on maternal and neonatal biochemical indicators of stress and inflammation are briefly summarised in Tables 1.12 and 1.13.

Table 1.12: Summary of effects of normal (unassisted) and difficult parturition on some maternal biochemical analytes reported in existing studies

Biochemical analyte	Serum/plasma half-life[1]	Effects of normal parturition	Effects of dystocia/parturition assistance (compared to unassisted parturition)
Cortisol	30 min[2]	Mild to moderate increase reported in most studies. No effect reported in a minority of studies.	Increased
Creatine kinase	Up to 8 h[3]	Increased	Increased
L-Lactate	13.5 to 15.5 min[4]	Not studied	Not studied
Glucose	Up to 45 min[5,6,7]	Increased	Unclear due to paucity of data. Non-significant increase reported in one study.
Haptoglobin	Not reported[8]	Increased	No difference on the day of parturition. Elevated 24 h to 7 d postpartum.
Substance P	Not reported[9]	Uncertain	A single study found no difference for first 48 h postpartum but elevated at 7 d postpartum.

1. Adult cattle
2. In postpartum cattle. Dunlap *et al.*, 1981
3. Lefebvre *et al.*, 1994
4. Prior, 1983
5. González-Grajales *et al.*, 2019
6. Kouider *et al.*, 1978
7. González-Grajales et al., 2018
8. Reported to be 5 d in humans and rodent models (Körmöczi *et al.*, 2006; Shih *et al.*, 2014)
9. Reported to range from seconds to tens of minutes in laboratory animals (Mashaghi *et al.*, 2016)

Table 1.13: Summary of effects of normal (unassisted) and difficult birth on neonatal biochemical analytes reported in existing studies

Biochemical analyte	Serum/plasma half-life	Effects of normal birth	Effects of dystocia/birth assistance (compared to unassisted birth)
Cortisol	Not studied	Marked increase at the time of birth followed by rapid decline in first 24 h of life	Increased
Creatine kinase	2 to 3 h[1]	Unclear — a single study found CK increased in some calves but not others	Increased in calves experiencing severe assistance.
L-Lactate	Not reported	Variable — no effect in most calves but elevated in some calves	Increased
Glucose	~30 min[2]	Increases in first hour of life before declining until colostrum is consumed[3]	Unclear. Some studies report increased blood glucose, but others report no effect.
Haptoglobin and serum amyloid A	Not reported[4]	No effect reported	No effect on haptoglobin. Serum amyloid A not studied.
Substance P	Not reported[5]	Not studied	Not studied

1. Anderson *et al.*, 1976
2. Grutter *et al.*, 1991
3. Blood glucose increases in response to colostrum consumption
4. Reported to be 5 d in humans and rodent models (Körmöczi *et al.*, 2006; Shih *et al.*, 2014)
5. Reported to range from seconds to tens of minutes in laboratory animals (Mashaghi *et al.*, 2016)

1.7 Health and productivity: effects of parturition assistance

1.7.1 Maternal health and productivity

It has been known for some time that assisted parturition has an adverse effect on the subsequent health and productivity of cows. A recent study (McConnel *et al.*, 2017) attempted to quantify the long-term effects of several disease states seen in cattle by calculating a measure similar to the Disability-Adjusted Life Year — a summary measure of overall population health disease burden commonly used in human population health medicine (GBD 2015 DALYs and HALE Collaborators, 2016). McConnel *et al.* (2017) sought expert opinion of the relative impact of several different dairy cow diseases on cow health and milk production, asking respondents to rank each disease from one (*least impact*) to ten (*most impact*). Parturition trauma was assigned to disability weight class III (out of IV) and had a calculated individual disability weight of 0.57 (on a 0 to 1 scale), indicating that injuries sustained during parturition are considered to have a significant adverse long-term effects on dairy cattle. McConnel *et al.* (2017) did not define 'parturition trauma', resulting in a wide variety of responses (range 2 to 10) that likely reflected variations in personal experience. Accordingly, the long term effects of assisted parturition *per se* cannot be determined from this study as it is unknown whether respondents considered unassisted parturition to be traumatic. However, as it is often suggested that parturition injuries are more likely when parturition is assisted (Barrier *et al.*, 2012a; Schuenemann *et al.*, 2013), it is possible that some respondents to McConnel *et al.* (2017) considered parturition trauma to be analogous to assisted parturition. McConnel *et al.* (2018) compared the results of their previous study to data obtained from three US dairy farms, finding that — although uncommon — parturition trauma was one of the three highest ranked conditions for causing death, supporting the high score assigned by McConnel *et al.* (2017). However, McConnel *et al.* (2018) analysed farm recorded data, and parturition assistance data were unavailable therefore its impact cannot be determined. Additionally, parturition trauma was only recorded by farmers if affected animals were sold or died, but was not recorded if animals remained in the herd (McConnel *et al.*, 2018). Thus, it is likely that the true frequency of parturition trauma was underestimated, and the impact on mortality may have been overestimated. Nonetheless, the high (adverse) impact of parturition trauma identified by these studies is still likely to be a robust finding as non-lethal effects (e.g. reduced reproductive performance) that were not studied are also likely to increase the adverse impact.

The development of a metric to assess disease impact on dairy herds is an interesting concept and has the potential to improve welfare through improved transparency and monitoring of dairy cow health (Croney *et al.*, 2011). Additionally, as health and welfare is of high importance to consumers (Jackson *et al.*, 2020), these types of metrics may also become of increasing interest to consumers seeking to make considered and sustainable food choices in the future. ‘Parturition trauma’ is a general term that does not reflect the variety of bovine parturition experiences; therefore, it would be advantageous for the metric developed by McConnel *et al.* (2017) and McConnel *et al.* (2018) to be further refined to assess the effects of different parturition experiences. This would allow a more accurate assessment of the effects of different types of parturition on future production of dairy cows thus potentially offering more benefits for monitoring of cattle health and welfare in the postpartum period.

1.7.1.1 Postpartum disease

Postpartum diseases have important adverse effects on future productivity of dairy cows. For example, uterine disease is associated with poorer reproductive performance (Fourichon *et al.*, 2000; Elkjær *et al.*, 2013; Ribeiro *et al.*, 2013, 2016; Toni *et al.*, 2015; Piccardi *et al.*, 2016) and subclinical ketosis (SCK) is associated with reduced milk and protein production (Kaufman *et al.*, 2018). Cow welfare is also affected by postpartum disease as metritis is a painful condition (Stojkov *et al.*, 2015; Barragan *et al.*, 2018) associated with increased time spent standing (Lomb *et al.*, 2018), and metritis and SCK are associated with reduced feed consumption (Schirmann *et al.*, 2016). As such, it is important that risk factors for postpartum disease are accurately identified so that evidence-based measures can be implemented on farms to reduce their incidence as much as possible. Assisted parturition is a known risk factor for developing diseases related to parturition and the transition period; most importantly ketosis, uterine diseases, retained foetal membranes, hypocalcaemia and displaced abomasa (McArt *et al.*, 2013; Vergara *et al.*, 2014; Kovács *et al.*, 2016). Vergara *et al.* (2014) assessed risk factors for developing disease in the first 30 d postpartum using a metric termed TXR30 (defined as cows that received ≥ 1 treatment for ≥ 1 disease of interest, were removed from the herd, or both, within 30 d postpartum) and found that a cow experiencing abnormal parturition had a three-fold increased risk of meeting the criteria to be classified as TXR30. Although this metric does not provide detailed information regarding risk factors for individual diseases, and Vergara *et al.* (2014) defined abnormal parturition broadly (twins, stillbirth and assistance score ≥ 3 out of 5 were all included), this finding highlights that assisted parturition is not an isolated event with short term effects and illustrates the importance of appropriate parturition management.

1.7.1.2 Uterine disease

Postpartum uterine contamination by a wide variety of bacteria is a normal finding in healthy cows (Bicalho *et al.*, 2017); however, in up to 40% of cows, normal postpartum bacterial contamination progresses to uterine disease (Kelton *et al.*, 1998; Giuliodori *et al.*, 2013, 2017; Toni *et al.*, 2015; Bogado Pascottini *et al.*, 2017). Reasons for this are not yet fully understood but trauma to the reproductive tract (incurred during parturition) (Potter *et al.*, 2010), alterations in bacterial populations (Bicalho *et al.*, 2017), and altered immune status around parturition (Jacob *et al.*, 2001; Moyes *et al.*, 2010; Crookenden *et al.*, 2016) are all likely to be involved. Five main uterine diseases are described based on their clinical appearance and the time after parturition each disease is diagnosed: metritis (puerperal and clinical), endometritis (subclinical and clinical) and pyometra [Table 1.14]. More recently, it has been suggested that the term ‘purulent vaginal discharge’ (PVD) is more appropriate than ‘clinical endometritis’ because PVD is not specific for endometrial inflammation (Dubuc *et al.*, 2010a), but this terminology has not been universally adopted and some studies use PVD as a broader term encompassing both metritis and clinical endometritis (Giuliodori *et al.*, 2017).

Table 1.14: Postpartum uterine disease definitions
(Sheldon et al., 2006, 2008; Dubuc et al., 2010a)

Uterine disease	Definition
Puerperal metritis	Foetid, watery-brown uterine discharge with associated pyrexia (T > 39.5 °C) and systemic signs diagnosed < 21 d postpartum.
Clinical metritis	Purulent uterine discharge diagnosed < 21 d postpartum in the absence of systemic signs.
Subclinical (cytological) endometritis	Based on results of uterine cytology samples at defined times postpartum: ▪ 21 to 33 d postpartum: > 18% neutrophils present ▪ 34 to 47 d postpartum: ≥ 6 to 10% neutrophils present ▪ ≥ 56 d postpartum: ≥ 4% neutrophils present
Clinical endometritis / purulent vaginal discharge	Purulent (> 50% pus) uterine discharge ≥ 21 d postpartum or mucopurulent (approximately 50:50 pus:mucus) ≥ 26 d postpartum in the presence of an open cervix.
Pyometra	Purulent material within the uterine lumen in the presence of a corpus luteum and a closed cervix.

Numerous studies are in agreement that parturition difficulty is associated with an increased risk of uterine disease (Curtis *et al.*, 1985; Correa *et al.*, 1993; Emanuelson *et al.*, 1993; Dubuc *et al.*, 2010b; Potter *et al.*, 2010; Hossein-Zadeh *et al.*, 2011; Giuliodori *et al.*, 2013, 2017; Prunner *et al.*, 2014; Vieira-Neto *et al.*, 2016; Kelly *et al.*, 2020; Pascal *et al.*, 2021). However, the detailed effects of parturition assistance are difficult to determine because studies employ a single generalised definition for ‘dystocia’ or ‘parturition assistance’ (Curtis *et al.*, 1985; Correa *et al.*, 1993; Dubuc *et al.*, 2010b; Prunner *et al.*, 2014; Vieira-Neto *et al.*, 2016; Pascal *et al.*, 2021), include assisted parturition in a broad ‘abnormal parturition’ category (encompassing assisted parturition, dystocia, twin births and stillbirths (Emanuelson *et al.*, 1993; Giuliodori *et al.*, 2013, 2017)), or do not provide a definition at all (Potter *et al.*, 2010; Hossein-Zadeh *et al.*, 2011). A single study has assessed the association of different degrees of parturition assistance with postpartum disease, finding that whilst severe parturition assistance was associated with an increased risk of uterine disease, there was no effect of mild assistance (Kelly *et al.*, 2020). Again, assistance scores were broadly defined (‘some assistance’ and ‘considerable assistance’) and were obtained from farm records, therefore were likely to have varied in their interpretation. Nonetheless, these are important findings as they further support the hypothesis that optimisation of bovine parturition assistance can have long-term benefits.

1.7.1.3 Retained foetal membranes

Bovine RFM is typically defined as failure to expel the placenta within 24 h of parturition (Sheldon *et al.*, 2008), although some studies use an earlier threshold (Vannucchi *et al.*, 2017; Pascal *et al.*, 2021). Difficult parturition (including assistance and dystocia) is a known risk factor for RFM (Joosten *et al.*, 1991; Correa *et al.*, 1993; Emanuelson *et al.*, 1993; Han *et al.*, 2005; Hossein-Zadeh *et al.*, 2011; Kovács *et al.*, 2016; Daros *et al.*, 2017; Mahnani *et al.*, 2021), with increasing severity of assistance being associated with an increased risk (Mahnani *et al.*, 2021). Furthermore, Kovács *et al.* (2016) found that almost 80% of cows experiencing inappropriately timed (early) parturition assistance developed RFM, compared to 25% of appropriately assisted cows — suggesting that RFM risk in assisted cows can be minimised by judicious management. This finding is supported by Vannucchi *et al.* (2017) who found that for assisted cows, a longer duration of parturition was associated with reduced time to expel the placenta, suggesting that early assistance impedes placental expulsion more than delayed assistance. However, Vannucchi *et al.* (2017) defined RFM as failure to expel the placenta within 8 h of parturition and 14 h was the maximum time placental expulsion occurred. As such, although Vannucchi *et al.* (2017) reported an increased risk of RFM following parturition assistance, none of the cows met the more traditional criteria for a diagnosis of RFM. This makes it difficult to compare these results with other studies; nevertheless, despite differences in RFM definition, the incidence of RFM following early parturition assistance reported by Vannucchi *et al.* (2017) is comparable to Kovács *et al.* (2016) (75.0% vs. 78.9% respectively). Retained foetal membranes is itself a risk factor for other postpartum diseases (Han *et al.*, 2005), and also results in impaired reproductive performance (Fourichon *et al.*, 2000; Han *et al.*, 2005) — thus presenting an important welfare challenge with potential adverse economic effects. As such, RFM incidence should be minimised as much as possible, and optimisation of parturition assistance may be one approach to help achieve this aim.

1.7.1.4 Subclinical ketosis

Fewer studies investigating risk factors for SCK have evaluated dystocia or assisted parturition (Stengärde *et al.*, 2012; Vanholder *et al.*, 2015; Daros *et al.*, 2017; Tatone *et al.*, 2017; Xu *et al.*, 2020), with a meta-analysis not identifying a relationship between dystocia and SCK (Raboisson *et al.*, 2014). McArt *et al.* (2013) found that severe parturition assistance was associated with an increased risk of SCK in the first 5 d postpartum, although interestingly this effect was identified on only one of the four farms studied, suggesting that animal management can compensate for the effects of assisted parturition. As SCK is related to negative energy balance (NEB) which is managed by optimising nutrition in the pre-parturition period, this is perhaps to be expected. However, it needs to be considered that the parturition data analysed by McArt *et al.* (2013) were obtained from farm records, thus subjectivity around scoring parturition assistance may have affected the results and — given that the nutritional management around parturition is unknown — it is difficult to draw conclusions regarding the effects of assisted parturition on SCK from this study. Berge *et al.* (2014) collated data from 131 farms in five countries and accounted for farm effects in the statistical analyses, showing that dystocia was not a risk factor for ketosis (clinical or subclinical). Whilst this finding seems consistent with some of the findings of McArt *et al.* (2013), Berge *et al.* (2014) defined dystocia as "*assistance with parturition due to difficulties with calving*", a broad definition that does not differentiate degrees of assistance severity. Therefore, it is possible that Berge *et al.* (2014) may have missed effects of severe parturition assistance on ketosis risk. Additionally, Berge *et al.* (2014) measured milk concentration of BHB for up to 21 d postpartum with a cut-point correlating to serum BHB concentration range 1.0 to 1.4 mmol/L, whereas McArt *et al.* (2013) measured blood BHB concentration 3 to 16 d postpartum with a more precise cut-point of ≥ 1.2 mmol/L. As such, it is difficult to compare the results of these two studies.

As data are limited, a clear relationship between parturition assistance and SCK cannot be determined as yet. Subclinical ketosis is an economically important condition that is associated with an increased risk of developing other diseases including left displaced abomasum (LDA), metritis, mastitis and lameness (Raboisson *et al.*, 2014), thus it is important to identify all potential risk factors to aid management and prevention. Accordingly more research is warranted to further investigate the relationship between parturition assistance (in particular different severities of assistance) and SCK.

1.7.1.5 Reproductive performance and survival

Despite variations in design and measured outcomes, studies investigating the effects of assisted parturition on subsequent maternal survival and reproductive performance are in agreement that assisted parturition has a negative impact on maternal reproductive performance and survival in the subsequent lactation. Reasons for the negative relationship between assisted parturition and subsequent reduced reproductive performance are not fully understood but Richards *et al.* (2009) found that assisted parturition was associated with delayed resumption of ovarian cyclicity (as measured by the ultrasonographic presence of a corpus luteum on one or both ovaries at 21 to 31 d postpartum). Whilst assessing ovarian cyclicity based on a single examination is a crude measure, this finding is supported by other studies that have found assisted parturition to be associated with increased time to first breeding (Thompson *et al.*, 1983), increased time to conception (Tenhagen *et al.*, 2007), reduced likelihood of conception by 100 and 200 days in milk (DIM) (Hayes *et al.*, 2012), and increased number of unsuccessful insemination attempts (Bonneville-Hébert *et al.*, 2011) — all expected consequences of delayed resumption of ovarian cyclicity. Furthermore, farmer attitudes to affected animals may also have an effect (e.g. farmers may choose to apply different breeding protocols assisted cows) (Fourichon *et al.*, 2000). Tenhagen *et al.* (2007) categorised parturition as ‘mild dystocia’, ‘severe dystocia’ and ‘caesarean section’ and found that severe dystocia was associated with an increased time to conception. However, Tenhagen *et al.* (2007) did not identify an effect of mild assistance and concluded that mild parturition assistance does not affect reproductive performance. By contrast, Dematawewa and Berger (1997) defined parturition assistance using a one (*unassisted*) to five (*substantial difficulty*) grading system and found that, overall, the time taken to conceive and the number of inseminations before conception were positively associated with parturition score. However, although the effect of parturition assistance on the time taken to conceive was similar for all cows, a parity x parturition score interaction effect on the number of insemination attempts was observed, with a positive relationship between parturition score and insemination attempts being evident in primiparous animals (Dematawewa *et al.*, 1997). This finding is in contrast to studies that have found older cows require more insemination attempts to achieve conception (Bonneville-Hébert *et al.*, 2011). However, Bonneville-Hébert *et al.* (2011) did not study the interaction between parity and parturition assistance, thus it is possible that parturition assistance has less of an adverse effect on the reproductive performance of multiparous animals. Given nulliparous cows have a higher prevalence of parturition assistance than older animals (Bonneville-Hébert *et al.*, 2011; Mee *et al.*, 2011), and poor reproductive performance is commonly found to be a primary reason for removal of cows from a herd (Seegers *et al.*, 1998; Hadley *et al.*, 2006; Kerslake *et al.*, 2018), this merits further study.

Assisted parturition is consistently associated with an increased risk of leaving the herd early in the subsequent lactation (through either death or sale) (Thompson *et al.*, 1983; Dematawewa *et al.*, 1997; López de Maturana *et al.*, 2007; Tenhagen *et al.*, 2007; Bonneville-Hébert *et al.*, 2011; Hayes *et al.*, 2012), with one study finding that up to 12% of animals that have 'calving trouble' leave the herd early (although the term 'calving trouble' was not defined and as such it cannot be certain that this referred to assisted parturition) (Kerslake *et al.*, 2018). Dematawewa and Berger (1997) found a positive relationship between parturition grade and likelihood of cow death in the subsequent lactation, irrespective of cow age. This is supported by a previous study that found foetal malpresentation at birth was associated with an increased risk of the cow leaving the herd in the subsequent lactation (Mangurkar *et al.*, 1984). Interestingly, Mangurkar *et al.* (1984) found the risk of early culling of cows was affected by calf survival, with a higher culling risk for all grades of assistance (including unassisted) if the calf was stillborn or died within 24 h of birth. Reasons for this finding are unclear, although Mangurkar *et al.* (1984) suggested that calf death may be associated with increased dam stress, resulting in reduced production. This hypothesis is supported by best linear unbiased estimates (BLUEs) for milk production and reproductive performance calculated as part of the same study (Mangurkar *et al.*, 1984), and the findings of Bicalho *et al.* (2008) who found stillbirth was associated with reduced milk production. However, both studies performed retrospective analysis of data obtained from farm records and as such it cannot be determined from the available data that this effect was related to dam stress. Reasons for calf death were not reported by Mangurkar *et al.* (1984) or Bicalho *et al.* (2008) and it is possible that factors causing calf death may also have had more direct effects on subsequent production (e.g. infectious disease).

1.7.1.6 Subsequent lactation milk production

Studies investigating the effect of dystocia or assisted parturition on milk production in the subsequent lactation have produced contradictory findings. Some studies have found that cows experiencing assisted parturition have reduced milk production in the subsequent lactation compared to cows not receiving parturition assistance (Dematawewa *et al.*, 1997; Barrier *et al.*, 2011; Atashi *et al.*, 2012, 2021; Hossein-Zadeh, 2014), with more severe parturition assistance having a greater negative effect (Dematawewa *et al.*, 1997; Hossein-Zadeh, 2014). However, other studies have found that while milk yield in early lactation was negatively affected by parturition assistance, effects were not evident later in lactation and parturition assistance did not have whole lactation effects (Thompson *et al.*, 1983; Tenhagen *et al.*, 2007). The difference in findings between studies may be due to the different parameters evaluated: Tenhagen *et al.* (2007) analysed monthly milk records whereas Atashi *et al.* (2012, 2021) and Hossein-Zadeh (2014) analysed cumulative measures of milk production. It also needs to be considered that multiple factors affect milk production, and it is possible that variations in animal management between studies has also contributed to results. Most studies analyse combined milk recording data obtained from multiple farms (Djemali *et al.*, 1987; Eaglen *et al.*, 2011; Atashi *et al.*, 2012, 2021; Hossein-Zadeh, 2014) but do not analyse individual farm data. Therefore, it is not possible to identify possible confounding farm effects on milk production, but the hypothesis that animal management may contribute to conflicting results is supported by the findings of Barrier *et al.* (2011) who compared data from two farms and found that negative effects of parturition assistance on milk production was farm dependent.

Fewer studies report effects of parturition assistance on milk fat and protein production, but the results are more consistent, with most studies finding parturition assistance is associated with decreased production (Dematawewa *et al.*, 1997; Atashi *et al.*, 2012; Hossein-Zadeh, 2014). In contrast to other studies, Atashi *et al.* (2021) and Eaglen *et al.* (2011) found that milk fat and protein production were not affected by parturition assistance. However, these studies only included primiparous animals (Eaglen *et al.*, 2011; Atashi *et al.*, 2021), which is a departure from other studies and is a likely reason for the differences in results compared to other studies.

1.7.1.7 Feed consumption and rumination

Pre-partum dry matter intake (DMI) and water consumption have both been shown to be lower in cows that subsequently experience dystocia (Proudfoot *et al.*, 2009). Reduced pre-partum water consumption in cows that experienced dystocia was compensated for by a tendency towards increased postpartum water intake (compared to cows experiencing eutocia) but a similar pattern was not observed for DMI, although cows experiencing dystocia did approach the feed barrier sooner after parturition than cows not subject to dystocia (Proudfoot *et al.*, 2009). The importance of maintaining adequate DMI in the periparturient period has been recognised for over four decades (Marquardt *et al.*, 1977) and it is well established that periparturient DMI and postpartum metabolic health are closely linked (Drackley, 1999). Dystocia is reported to be risk factor for metabolic disorders in the postpartum period (McArt *et al.*, 2013; Vergara *et al.*, 2014) and it is possible that differences in pre-partum DMI in cows experiencing dystocia contribute to this. Rumination time is known to be reduced for up to 48 h prior to parturition (Büchel *et al.*, 2014; Fadul *et al.*, 2017; Kovács *et al.*, 2017a) and there has been recent interest in the potential for monitoring rumination time (using remote sensors) in order to predict parturition (and possibly also dystocia) (Büchel *et al.*, 2014; Fadul *et al.*, 2017; Kovács *et al.*, 2017a). Kovács *et al.* (2017a) found that although postpartum rumination times were lower in cows experiencing dystocia, no differences were observed in the pre-partum period. Whilst this is an important finding with regard to postpartum management of cows experiencing dystocia, the ability to predict dystocia using rumination times was not proven and DMI was not reported. Although DMI might be expected to be correlated with rumination time, Clément *et al.* (2014) demonstrated that this is not the case. This may explain why, although differences have been reported in pre-partum DMI between cows experiencing dystocia and those experiencing normal parturition, no differences in rumination time have been reported. This makes it less likely that rumination time will be of use in the future in predicting cows that are likely to go on to have dystocia; however, the study by Kovács *et al.* (2017a) was small (only 18 animals) and both Proudfoot *et al.* (2009) and Kovács *et al.* (2017a) studied housed animals fed a total mixed ration (TMR). Larger studies and studies including animals in different management systems (e.g. at pasture) are needed to draw definitive conclusions regarding the effect of dystocia on pre-partum rumination time.

1.7.2 Calf health and future productivity

1.7.2.1 Neonatal mortality

An association between difficult birth and an increased risk of calf mortality is well described (Sellers *et al.*, 1968; Laster *et al.*, 1973; Patterson *et al.*, 1987; Arthur *et al.*, 2000; Tenhagen *et al.*, 2007; Lombard *et al.*, 2007; Gundelach *et al.*, 2009; Hoedemaker *et al.*, 2010; Bleul, 2011; Barrier *et al.*, 2013b, 2013a; Hossein-Zadeh, 2014; Mee *et al.*, 2014; Norquay *et al.*, 2020). Additionally, several studies have found that increasing severity of birth assistance is associated with an increasing likelihood of perinatal mortality (Laster *et al.*, 1973; Patterson *et al.*, 1987; Arthur *et al.*, 2000; Meyer *et al.*, 2000; Tenhagen *et al.*, 2007; Lombard *et al.*, 2007; Bleul, 2011; Barrier *et al.*, 2013a; Hossein-Zadeh, 2014; Juozaitiene *et al.*, 2017; Hohnholz *et al.*, 2019; Norquay *et al.*, 2020), with one study finding that perinatal losses exceeded 20% for calves born to severe assistance (Norquay *et al.*, 2020). However, Berger *et al.* (1992) found that unassisted birth was associated with a higher risk of perinatal calf mortality — a departure from other studies. Berger *et al.* (1992) retrospectively analysed data collated from farm records (obtained through the American Angus breeding association) and as such reasons for calf death were not detailed, although Berger *et al.* (1992) did suggest that management practices may be involved, noting that parturition assistance is intended to "*increase the chances of obtaining a live calf*". It is possible that compromised unassisted calves were not attended to soon enough after birth, or that birth was very prolonged for some unassisted calves and earlier assistance may have improved outcomes (Villettaz Robichaud *et al.*, 2017a), but this cannot be determined from the data available. Furthermore, other studies of beef cattle have not identified similar effects (Laster *et al.*, 1973; Patterson *et al.*, 1987; Norquay *et al.*, 2020), suggesting that the findings of Berger *et al.* (1992) are not replicated in the wider beef industry.

Several mechanisms have been proposed to explain the increased incidence of perinatal mortality of severely-assisted calves [Table 1.15].

Table 1.15: Some proposed mechanisms affecting calf survival following assisted birth

Proposed mechanism affecting calf survival	Reference
Neonatal acidosis and hypoxia	Breazile *et al.* (1988) Szenci *et al.* (1988) Yildiz *et al.* (2017)
Impaired neonatal thermoregulation	Mellor *et al.* (2004)
Inadequate passive transfer of immunoglobulins	Tyler *et al.* (1998) Cuttance *et al.* (2018)
Traumatic injuries sustained during birth	Barrier *et al.* (2013a) Mellor *et al.* (2004) Schuh *et al.* (1988)

Traumatic injuries sustained at birth occur more frequently in assisted calves (Schuijt, 1990; Agerholm *et al.*, 1993; Barrier *et al.*, 2013a) and may result in cardiopulmonary and cerebral damage that can impair neonatal survival (Mellor *et al.*, 2004; Barrier *et al.*, 2013a). Furthermore, serious injuries (e.g. vertebral fractures) incurred as a result of application of substantial traction may result in sequalae (e.g. paralysis) that necessitate euthanasia for welfare reasons (Schuh *et al.*, 1988), also contributing to perinatal mortality.

Neonatal thermoregulation and passive transfer (PT) of immunoglobulins can be impaired in assisted calves [see Section 1.6] (Vermorel *et al.*, 1989; Bellows *et al.*, 2000; Barrier *et al.*, 2013b; Homerosky *et al.*, 2017b) and are reported to affect calf survival (Tyler *et al.*, 1998; Mellor *et al.*, 2004; Cuttance *et al.*, 2018). Additionally, neonatal thermoregulation and PT have been found to interact, with Beam *et al.* (2009) finding that on farms where heat was not provided during cold ambient temperatures, assisted calves were 1.6 times more likely to have failure of passive transfer (FPT) than unassisted calves. Furthermore, results of previous studies have found that the rate of immunoglobulin absorption is reduced in cold-stressed calves (Olson *et al.*, 1980) supporting the hypothesis that impaired thermoregulation contributes to FPT — a condition associated with increased perinatal mortality (Tyler *et al.*, 1998; Cuttance *et al.*, 2018).

1.7.2.2 Passive transfer of immunoglobulins

Calves are born almost agammaglobulinaemic and the importance of adequate colostrum ingestion within the first few hours of life is well described (Stott *et al.*, 1979b; Besser *et al.*, 1990; Weaver *et al.*, 2000; Hulbert *et al.*, 2016; Cuttance *et al.*, 2018; Fischer *et al.*, 2018; Godden *et al.*, 2019). The absorptive capacity of the bovine neonatal intestine for large molecules (such as immunoglobulins) rapidly decreases soon after birth (termed 'closure') and by 24 h old is almost absent in most calves (Stott *et al.*, 1979b). Intestinal closure is delayed for up to 48 h in lambs if colostrum is not fed soon after birth (Campbell *et al.*, 1977), but this has not been observed in calves (Fischer *et al.*, 2018; Inabu *et al.*, 2018); thus, FPT is more likely to occur if colostrum consumption is delayed, with consumption within 4 h of birth being associated with optimal immunoglobulin absorption (Chigerwe *et al.*, 2008; Beam *et al.*, 2009). Failure of passive transfer is well known to be associated with increased calf morbidity and mortality (McGuire *et al.*, 1976; Robison *et al.*, 1988; Wittum *et al.*, 1995; Donovan *et al.*, 1998; Cuttance *et al.*, 2018; Lora *et al.*, 2018), known welfare challenges (Vasseur *et al.*, 2010). Adverse effects of FPT extend beyond the neonatal period and include reduced growth rates, continued susceptibility to disease, reduced milk production in the first lactation and an increased likelihood of failing to reach the second lactation (Robison *et al.*, 1988; DeNise *et al.*, 1989; Wittum *et al.*, 1995; Cuttance *et al.*, 2018).

Several studies have found birth assistance is associated with impaired PT in calves (Vermorel *et al.*, 1983; Donovan *et al.*, 1986; Besser *et al.*, 1990; Tyler *et al.*, 1991; Waldner *et al.*, 2009; Barrier *et al.*, 2013b; Murray *et al.*, 2015b; Homerosky *et al.*, 2017b). For example Barrier *et al.* (2013b) found that assisted calves were less likely to have a zinc sulphate turbidity test result ≥ 20 units (indicative of adequate passive transfer (Hogan *et al.*, 2015, 2016)) and Beam *et al.* (2009) found calves born on farms where veterinary assistance was not sought to aid management of foetal malpresentation were 2.6 times more likely to have FPT. However, this relationship between birth assistance and FPT is not a consistent finding: Stott and Reinhard (1978) and Adams *et al.* (1995) found that immunoglobulin absorption was not affected by dystocia, findings supported by the results of Perino *et al.* (1995) who found that although plasma immunoglobulin G (IgG) concentration of assisted calves was lower than unassisted calves at 10 h after birth, this difference was not statistically significant once factors including mastitis and age of dam were controlled for. Similarly, Burton *et al.* (1989) found that although birth assistance was associated with numerical differences in serum IgG concentration, statistically significant differences were not present. Differences in colostrum management between older and more modern studies [Table 1.16] may explain inconsistent findings. Whilst the colostrum management described by Stott and Reinhard (1978) and Burton *et al.* (1989) [Table 1.16] was not unusual for the time (Godden *et al.*, 2009), it is not consistent with modern standards for colostrum management: current recommendations are to ensure a calf consumes at least 4 L (or approximately 10% of bodyweight) of good quality colostrum within 4 h of birth (Beam *et al.*, 2009; Vasseur *et al.*, 2010; Godden *et al.*, 2019). Additionally, older studies report the use of pooled colostrum [Table 1.16], a practice associated with an increased risk of FPT (Weaver *et al.*, 2000). Alternatively, variations in tests for FPT [Table 1.16] used in different studies could also explain inconsistent findings. For example, Barrier *et al.* (2013b) assessed FPT using the zinc sulphate turbidity test (an indirect measure of immunoglobulin transfer that provides an estimate of plasma IgG concentration (McEwan *et al.*, 1970)) whereas other studies measured FPT using radial immunodiffusion (a direct measure of serum IgG concentration considered to be the optimal test for assessing FPT) (Hogan *et al.*, 2015; Cuttance *et al.*, 2017a).

The underlying aetiopathogenesis of the negative effect of assisted birth on FPT risk is still the subject of debate. As assisted birth has been associated with reduced neonatal vigour and delayed time to stand and suckle (Barrier *et al.*, 2012a; Murray *et al.*, 2015b; Homerosky *et al.*, 2017b), it has been suggested that failure to consume adequate volumes of colostrum within 4 h of birth is the reason for the increased frequency of FPT observed in assisted calves. However, FPT of assisted calves has also been found in studies where calves are fed a measured volume of colostrum using a bottle or oesophageal feeder (Barrier *et al.*, 2013b; Murray *et al.*, 2015b); therefore, poor vigour cannot solely explain the negative effects observed. Assisted birth is associated with a greater degree of hypoxia and neonatal acidosis (Vermorel *et al.*, 1983) — conditions found to negatively affect the absorptive capacity of the neonatal intestine (for immunoglobulins)

Table 1.16: Summary table of studies investigating the effect of dystocia or assisted birth on passive transfer of immunoglobulins

Study	Production type	Volume of colostrum provided	Timing of colostrum feed relative to birth	Method of colostrum administration	Method of FPT assessment	Birth assistance effect identified?	Comments
Barrier *et al*. (2013b)	Dairy	2 L	Within 6 h of birth	Oesophageal feeder	Zinc sulphate turbidity test	Yes	Exact timing of colostrum feed not reported.
Burton *et al*. (1989)	Dairy	1st feed: 1.75 L 2nd feed: 1.2 L	1st feed: within 6 h of birth 2nd feed: within 12 h of 1st feed	Bottle	Radial immunodiffusion	No	Pooled colostrum at 2nd feed. Unclear whether all calves consumed all offered colostrum. Definition of easy, moderate, and difficult parturition not reported.
Homerosky *et al*. (2017b)	Beef	Not measured	Within 4 h of birth	Suckling from dam	Radial immunodiffusion	Yes	Overall mean colostrum quality = 159 g/L IgG. Calves that failed to suckle from the dam within 4 h of birth were assisted by farm personnel or administered colostrum using a bottle or oesophageal feeder (volume range 0.5 to 1 L).
Murray *et al*. (2015b)	Dairy	Colostrum replacer (180 g in 3 L)	2 h after birth	Oesophageal feeder	Radial immunodiffusion	Yes	Apparent efficiency of IgG absorption calculated as a measure of passive transfer
Perino *et al*. (1995)	Dairy x beef[1]	Not measured	Within 10 h of birth	Suckling from dam	Radial immunodiffusion	No	Calves with IgG concentration < 4.8 g/dL at 10 h old fed colostrum replacement product (30g IgG)
Stott and Reinhard (1978)	Dairy	1st feed: 1 L 2nd feed: 1 L	1st feed: 4 h postpartum 2nd feed: 12 h after 1st feed	Not reported	Radial immunodiffusion	No	Pooled colostrum
Waldner and Rosengren (2009)	Beef	Not recorded at calf level (82% of herds ensured colostrum volume equivalent to 10% of birthweight within 12 h of birth)		Not recorded	Radial immunodiffusion	Yes	

1. Individual breeds not reported. Managed in a beef system.

(Besser *et al.*, 1990; Tyler *et al.*, 1991) — and it is possible that impaired absorption of IgG is also a contributing factor.

1.7.2.3 Future production of calves

There is a paucity of research on the effects of assisted birth on the future health and production of calves after weaning; however, the few data available suggest that birth assistance is likely to have a negative effect on future production (Arthur *et al.*, 2000; Eaglen *et al.*, 2011; Heinrichs *et al.*, 2011; Barrier *et al.*, 2012c). Goonewardene *et al.* (2003) found that assisted cross-breed calves (a mixture of beef-cross and dairy x beef breeds) had a reduced growth rate to weaning. This results contrasts with Arthur *et al.* (2000) who measured individual productivity of beef calves using average daily liveweight gain and found that, whilst assisted birth did not have an effect on individual productivity, birth assistance was associated with a reduction in overall herd productivity. This result was due to assisted birth being associated with an increased likelihood of pre-weaning mortality, thus reducing the number of calves that reached slaughter age (Arthur *et al.*, 2000). This is an interesting finding and highlights the differences in production outcomes important to beef and dairy herds. Accordingly, it is likely that assisted birth has different impacts on future production of calves in beef and dairy systems as different measures of production may not all be affected in the same manner.

Heinrichs *et al.* (2011) studied Holstein calves for a decade and found that birth assistance was associated with reduced first lactation 305 day mature equivalent yield (305ME) as well as reduced whole lactation milk and protein production in the first lactation, a finding supported by the results of Eaglen *et al.* (2011). Additionally, Heinrichs and Heinrichs (2011) found that each one grade increase in birth assistance severity was associated with an average decrease in 305ME and whole lactation yield of 195 kg and 285 kg respectively. Furthermore, a recent study analysing over half a million farm records (from 1999-2013) found birth assistance was associated with reduced length of productive life (Dallago *et al.*, 2021). These results suggest that the negative effects of birth assistance on calf production are long-lasting and further supports the value of judicious birth assistance.

Only two studies have investigated the effects of birth assistance on reproductive performance of calves, analysing performance at different life stages. Barrier *et al.* (2012c) assessed reproductive performance before first calving, whereas Eaglen *et al.* (2011) assessed reproductive performance in the first lactation, but both studies failed to detect an effect of birth assistance.

1.8 Summary and study aims

Although dystocia is considered by veterinary surgeons and farmers to be painful, with some veterinary surgeons and farmers providing pain relief to affected cows and calves (Moggy *et al.*, 2017; Remnant *et al.*, 2017), data to support these views are scarce. Only two previous studies have employed a 2 x 2 factorial design (considered optimal for investigating animal pain) to investigate dystocia-related pain in cows (Swartz *et al.*, 2018; Barragan *et al.*, 2020a, 2020b) but these did not perform detailed behavioural analysis (a sensitive indicator of pain). The effects of postpartum analgesia on subsequent production has been more widely studied but the potential extent of analgesic benefits are difficult to determine as most studies only include assisted cows (Newby *et al.*, 2013a; Barrier *et al.*, 2014), or report blanket treatment of all cows without analysis of assistance status [Table 1.6] (Richards *et al.*, 2009; Stilwell *et al.*, 2014; Carpenter *et al.*, 2018). Similarly (to cows), few previous studies have investigated the effects of post-birth analgesia on calves and the primary focus of these is health and production outcomes such as passive transfer and growth rate [Table 1.7] (Murray *et al.*, 2016; Pearson *et al.*, 2019a, 2019b; Clark *et al.*, 2020). Furthermore, existing calf studies do not employ a factorial design, therefore it is unclear how birth assistance affects neonatal pain. Additionally, the long-term effects of post-birth analgesia on future calf production (into and including the first lactation) have not been studied and as such are unknown.

Most parturition assistance is provided by farmers (Egan *et al.*, 2001), with up to 50% of dairy cows (and their calves) affected (Mee, 2008). However, previous studies have not focused on farmer-provided assistance in general, but rather focus on the effects of dystocia — a lower prevalence condition (typically 5 to 10% [Table 1.2]). ‘Dystocia’ is inconsistently defined by previous studies and as such, it is difficult to determine whether the negative effects of dystocia reported are due to dystocia *per se* or related to parturition assistance in general. Additionally, some studies that dichotomise assistance status into ‘assisted’ and ‘unassisted’ groups for analysis include cows subject to mild assistance in the unassisted group (e.g. Newby *et al.*, 2017), leading to further difficulties in drawing accurate conclusions regarding the effects of parturition assistance on cows and their calves. Although parturition assistance has been shown to be a stressful event for cows and their calves in the short-term (Hudson *et al.*, 1976; Nakao *et al.*, 1990; Kindahl *et al.*, 2002; Civelek *et al.*, 2008), the longer term effects are uncertain as only a single study (Barragan *et al.*, 2020b) has investigated biomarkers of stress (such as cortisol) beyond the first few hours postpartum in cows, and there are no similar studies of calves. Prolonged stress can be associated with an increased risk of developing pathologies (Romero *et al.*, 2009) and has the potential to compromise welfare, thus further study is merited.

Improvements in the understanding of maternal and neonatal pain following parturition in cattle are needed. In particular, it is unclear if unassisted parturition is painful for cows and their calves and if assisted parturition results in heightened postpartum pain. As pain is an important welfare concern, this represents an area where incomplete knowledge has the potential to compromise animal welfare and where improved understanding has the potential to contribute to improved welfare of both cows and calves in the postpartum period.

The studies presented in this thesis represent a detailed investigation into parturition-related pain experienced by Holstein dairy cows and calves and aim to determine the effects of farmer-assisted parturition and immediate postpartum administration of ketoprofen on the postpartum welfare of cows and calves, with a particular focus on postpartum pain. A further aim of these studies is to establish the effects of farmer-assisted parturition and immediate postpartum administration of ketoprofen on maternal productivity on the subsequent lactation and neonatal productivity until the end of the first lactation. The integrative nature of this series of studies encompassing behavioural, biochemical and production analysis aims to provide a well-rounded view of the potentially wide-ranging effects of parturition and the potential benefits of analgesia in cattle.

Chapter 2 General Materials and Methods

2.1 Ethical approval

All studies presented in this thesis were performed under UK Home Office Project and Personal Licence authority in accordance with regulations laid out in the Animals (Scientific Procedures) Act 1986 (A(SP)A). When each study was completed, all recruited animals were discharged from the controls of A(SP)A and rehomed back to the herd following veterinary inspection. No recruited animals were used in subsequent research projects. All personnel implementing research procedures held a Home Office personal licence for performing animal research. Animals were recruited in two periods: 7th March to 15th December 2016 and 19th June to 12th November 2020.

2.2 Animal husbandry and farm management

All studies in this thesis were performed on a single dairy farm in central Scotland, UK. In 2016 the farm milked approximately 700 Holstein cows (*Bos taurus*) and the total number of animals in the herd (including calves and youngstock) was approximately 1300. By 2020, the herd size had increased to approximately 800 lactating cows and approximately 1500 animals in total. All animals on the farm are female purebred Holstein cattle, apart from a small number of (male and female) Holstein x beef breed calves that are sold at one to two weeks old to be reared for the beef industry. There are no adult bulls on the farm; all breeding is performed using artificial insemination and all replacement animals are homebred, farm breeding protocols are described in Appendix 2. All cows are examined 28 to 35 d post-insemination by a veterinary surgeon from the Scottish Centre for Production Animal Health and Food Safety (SCPAHFS) using trans-rectal ultrasound to identify pregnancy. All pregnant cows are re-examined one month later to confirm continuation of a viable pregnancy. If a cow is not pregnant at one of these examinations, the decision whether to continue to breed these animals or not is made by senior farm staff, guided by established farm breeding protocols.

The farm has been closed for more than 10 years and is bovine viral diarrhoea (BVD) accredited free in accordance with both the Premium Cattle Health Scheme

(PCHS) and the Scottish BVD Eradication Scheme. The herd is a member of the National Johne's Management Plan (NJMP) and no cases of Johne's disease (*Mycobacterium avium* subsp. *paratuberculosis*) had been diagnosed until July 2020. The adult herd is vaccinated against *Leptospira hardjo*, *Salmonella dublin*, bovine herpes virus-1 (BoHV-1), and clostridial diseases. Calves and youngstock are vaccinated against BVD, bovine parainfluenza type 3 virus (PI-3), bovine respiratory syncytial virus (BRSV), BoHV-1 and clostridial diseases.

2.2.1 Management of adult cows

Sixty to 90 cows calve each month in a year round system. Lactating cows are housed all year round in a cubicle system; cubicles are deep bedded (depth 10 cm) with a 2:1 mixture of oat husks and calcium carbonate (lime — added to control bacterial growth). Lactating primiparous animals are housed as a single group (i.e. not with multiparous lactating animals), whereas lactating multiparous animals are housed in two groups: a high yielding group (≥ 35 kg per day) and a low yielding group (< 35 kg per day). Due to the natural lactation curve of cattle, the low yielding group is made up of animals later in their lactation than the high yielding group. Cows are milked three times daily (at 8 h intervals) through a 50-point DeLaval rotary parlour; average annual yield for the herd is 12,000 kg (average daily yield 40 kg) and average percentage milk fat and protein is 3.5% and 3.1% respectively.

Cows cease to be milked and are moved to a 'far-off' dry cow group (housed in cubicles at a separate site approximately two miles from the main farm) eight weeks before the expected parturition date. Three weeks before expected parturition cows are moved into a 'close-up' dry cow group at the main farm (housed in a straw-bedded group pen [15.2 m x 22.5 m]). Animals remain here until parturition is complete (i.e. cows do not calve in individual pens). Heifers and cows are housed together in the 'close-up' group; an extra pen is available for use when needed (to avoid overstocking the primary pen) but is used primarily to house heifers. Immediately after parturition all animals are offered 40 L of a highly palatable rehydration solution formulated for postpartum cows (Farm-O-San Reviva, Farm-O-San, Amersfoort, Netherlands). Most cows voluntarily drink this solution however if this does not occur, it is administered orally by experienced personnel using an Aggers rumen drenching set. Cows in lactation three and older also receive an oral calcium supplement as a bolus (Bovikalc, Boehringer Ingelheim, Ingelheim am Rhein, Germany). At, or before, the next milking (after parturition) cows are moved to an adjacent straw-bedded group pen (15.2 m x 22.5 m). Due to the fixed (8 h) intervals between milking the maximum potential delay before moving a cow into the postpartum pen is 8 h. Both heifers and cows are housed together in the same postpartum pen; multiparous animals routinely remain in the postpartum group for one week before moving into cubicle accommodation whereas primiparous animals remain in the postpartum group for 3 d. If postpartum disease is diagnosed [see Appendix 2], affected animals are treated as advised by the attendant veterinary surgeon and remain in the postpartum pen until treatment is no longer needed. For recording purposes, ‘postpartum disease’ is defined as a veterinary diagnosis of metritis, RFM, SCK, LDA, or a combination of any of these diseases, within the first 8 d postpartum. All multiparous animals are routinely examined by a veterinary surgeon. Primiparous animals are not routinely examined unless requested by the farmer but for the purposes of these studies, all recruited primiparous animals were subject to a postpartum veterinary examination.

Nutritional advice and ration formulation is provided by an external company (Evidence Based Veterinary Consultancy, Carlisle, UK) who work closely with both the farmer and the farm veterinary surgeons (SCPAHFS, University of Glasgow School of Veterinary Medicine, Glasgow, UK) to monitor the nutritional health of the herd. All lactating cows are fed a grass silage based TMR (35% forage, 65% concentrates on dry matter [DM] basis) *ad libitum*; extra concentrate feed is not provided in the milking parlour. Non-lactating (dry) cows have *ad libitum* access to a straw/whole-crop based total mixed ration. In the last three weeks of gestation, ‘close-up’ dry cows are fed a straw/whole-crop based TMR (48% forage, 52% concentrates on a DM basis) with a calculated dietary cation-anion difference (DCAD) of −100 to −200 mEq/kg [Appendix 2]. *Ad libitum* fresh water is available to all cows at all times.

2.2.2 Calf management

All calves are fed 4.0 to 4.5 L colostrum within 4 h of birth. Colostrum is initially fed from a bottle, but if the calf does not drink the full 4.5 L an oesophageal feeder is used. Calves that drink the full 4.5 L enthusiastically and are subjectively assessed by farm staff to be hungry are offered another bottle (2.5 L) of colostrum and allowed to consume as much or as little of this extra bottle as needed to meet their appetite. Stored colostrum (single source) is usually fed to ensure colostrum is provided before the calf is 4 h old; most calves do not receive colostrum from their own dam. Colostrum is harvested (by machine) at the first milking after parturition (i.e. hand-milking is not employed).

Within 8 h of birth (typically within 4 h) calves are removed from their dam and are weighed and the umbilicus is dipped (7.5% iodine solution) before being moved into age-matched groups of four to six, housed in pens measuring 3.1 m x 2.9 m (1.5 to 2.2 m^2 per calf). Calves are fitted with an approved ear tag within 36 h of birth (Commission Regulation [EC] 911/2004). When housed in group calf pens, calves are fed 3.5 to 4.0 L of powdered milk replacer (Provimilk; 21.0% crude protein, 18.0% fat) twice daily with a group teat feeder. If calves fail to suckle well, or are pushed away from the feeder by other calves, they are fed individually with a bottle or an oesophageal feeder as deemed necessary by the farmer. Concentrate feed (Vantage Calf Starter Pellets; 18.0% crude protein, 4.0% crude oil and fats, 10.9% crude fibre) is provided *ad libitum* from one to two weeks and increases with age in accordance with Scottish legislation (The Welfare of Farmed Animals (Scotland) Regulations, 2010); *ad libitum* good quality straw is also provided as an additional source of fibre. Calves are provided *ad libitum* fresh water from birth.

Beef-cross calves remain in small groups until they are sold, whereas Holstein calves remain here until they are 10 to 14 d old — at this stage they are moved into larger pens in age-matched groups of ten until calves are weaned. Calves are weaned between six and nine weeks old using a step-down programme (reducing daily milk volume by 2 L each week for three weeks). After weaning, calves are moved into larger groups of 30 until approximately 9 months old when they move to a second farm in south west Scotland and are housed in larger groups in a cubicle system. Youngstock are bred at this farm (artificial insemination) and return to the main farm four to eight weeks prior to parturition[3].

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[3] Before the last 10% of gestation (Council Regulation [EC] 1/2005)

2.2.3 Study treatment protocols

At the time of recruitment, animals in all studies were randomly allocated to receive either the non-steroidal anti-inflammatory drug (NSAID) ketoprofen (Ketofen 10%, Merial Animal Health (2016) and Ketofen 10%, Ceva Animal Health Ltd., Amersham, UK (2020)) or isotonic saline (Vetivex No. 1, Dechra, Northwich, UK). Ketoprofen was administered by deep intramuscular injection at the manufacturer's recommended dose rate of 3 mg/kg (equivalent to 1 mL/33 kg bodyweight). Isotonic saline was administered in the same manner (i.e. 1 mL/33 kg administered by deep intramuscular injection). All calves were weighed prior to treatment administration to ensure accurate dosing; the calf weigh crate (Ritchie 345GE calf weighing crate, Ritchie Agricultural, Forfar, Scotland and Ezi-Weigh 5, Tru-test, Datamars UK, Selkirk, Scotland) was purchased for study use and accuracy of measurement was confirmed prior to data collection. Facilities were not available to weigh adult cattle therefore treatment dosage (volume) was calculated based on weight estimates made by the administrator. All calves were injected in the cranial aspect of the quadriceps femoris muscle using a 2.5 cm (1"), 20G needle attached to a 2.5 mL syringe. All cows were injected in the caudal thigh muscles (semimembranosus and semitendinosus) using a 3.8 cm (1.5"), 18G needle attached to a 30 mL syringe. All treatments were administered within 3 h of parturition by either a veterinary surgeon (NG) or an experienced stockman holding a personal licence in accordance with A(SP)A. Details of the product and volume administered to each animal were recorded by the administrator in a dedicated hand-written record and kept in the farm office for the duration of each study.

Both ketoprofen and saline are licensed in the UK for use in adult dairy cattle (National Office of Animal Health, 2020); when administered by the intramuscular route ketoprofen has zero milk withdrawal period. Administration of ketoprofen to young calves (under 6 weeks) is not licensed, but safety data are available for administration to very young calves (3 d old) (National Office of Animal Health, 2017). Calves would not contribute to the milking herd until at least 2 y after completion of the study meaning administration complied with UK prescribing legislation for 'off-licence' usage (7 d milk and 28 d meat withdrawal periods (Veterinary Medicines Directorate, 2015)).

Chapter 3 Newborn calf behaviour is affected by both birth assistance and ketoprofen treatment

3.1 Introduction

Dystocia is believed to be painful for calves (Huxley *et al.*, 2007; Laven *et al.*, 2009; Remnant *et al.*, 2017), but data to support this view are limited. It is reasonable to assume that significant injuries (e.g. fractures (Ferguson *et al.*, 1990; Schuijt, 1990)) sustained during severe or mismanaged birth assistance are painful, but it is less certain whether milder or more appropriately provided birth assistance also causes pain. Pearson *et al.* (2019c) found that severe assistance at birth (defined as use of a mechanical foetal extractor or traction applied by more than two people) was associated with increased neonatal serum concentrations of creatine kinase (CK) and aspartate aminotransferase compared to unassisted and more mildly assisted calves. As these compounds are released following muscle injury Pearson *et al.* (2019c) concluded that severe birth assistance is associated with subclinical soft tissue injury — a likely cause of pain. As pain has a negative effect on welfare (Broom, 1991) it is accepted that the use of analgesic drugs can contribute to improvements in animal welfare. The beneficial welfare effects of analgesia have been widely studied in the context of painful calf husbandry procedures such as disbudding (e.g. Stilwell *et al.*, 2012; Mintline *et al.*, 2013) and castration (Nalon *et al.*, 2021), but the potential for analgesia to improve the welfare of newborn calves has largely been ignored — a matter highlighted almost a decade ago by Laven *et al.* (2012). Since then, four studies have investigated the effects of administering analgesia to newborn calves [see Chapter 1], but none have performed detailed behavioural observations or employed a 2 x 2 factorial design considered optimal for the investigation of animal pain (Weary *et al.*, 2006). Pearson *et al.* (2019b) found that assisted beef calves treated with the non-steroidal anti-inflammatory drug (NSAID) meloxicam had a higher growth rate in the first week of life than calves treated with a placebo drug. This result suggests that birth related pain may affect the early growth of calves although these results were not replicated in a second (larger) study (Pearson *et al.*, 2019a). Two similar studies of dairy calves found that meloxicam treatment was associated with improved neonatal vigour and milk consumption (Murray *et al.*, 2016) and a tendency towards increased starter mix consumption (Clark *et al.*, 2020) (compared to placebo-treated or untreated calves respectively) but, although birth assistance was recorded in both studies, calves were grouped together for analysis and its effects were not investigated. Therefore, it is currently unknown whether assisted calves experience different (i.e. heightened) effects of analgesia than unassisted calves. Moreover, health and production outcomes such as those reported in existing studies (Murray *et al.*, 2016; Pearson *et al.*, 2019a, 2019b; Clark *et al.*, 2020) can be insensitive indicators of welfare and have been suggested to be better suited to herd level screening than individual assessment (de Vries *et al.*, 2014). As a more sensitive indicator of welfare (Dawkins, 2003), behavioural analysis is commonly included in animal welfare assessments (Yon *et al.*, 2019): interpretation is based on the principle that adverse welfare states are associated with differences in the expression of behaviours seen during optimal conditions (e.g. Siivonen *et al.*, 2011; Mintline *et al.*, 2013) or contribute to the development of abnormal behaviours such as stereotypies (e.g. Redbo, 1992; Redbo *et al.*, 1997). Behavioural analysis also shows promise for identification of positive animal welfare, the importance of which is increasingly being recognised (Boissy *et al.*, 2007).

By contrast, some studies have investigated the effects of birth assistance on calf behaviour but these typically focus on limited measures such as calf vigour (Homerosky *et al.*, 2017b), latency to sternal recumbency or standing (Schuijt *et al.*, 1994; Murray *et al.*, 2015b) or very early behaviours within the first 3 h of life (Barrier *et al.*, 2012a; Murray *et al.*, 2015b; Homerosky *et al.*, 2017b). Therefore, the effects of assisted birth on the behaviour of neonatal calves older than 3 h, are unclear. Furthermore, most existing studies include calves subject to veterinary-assisted birth or caesarean section (Schuijt *et al.*, 1994; Barrier *et al.*, 2012a; Homerosky *et al.*, 2017b) but, as studies have indicated that most birth assistance is provided by farmers (Egan *et al.*, 2001), this may not reflect the experience of most calves. Accordingly, the present study focused on farmer-assisted births rather than more severe (i.e. veterinary) assistance.

Work presented in this chapter has been published (Gladden *et al.*, 2019); parts of the current chapter draw on this publication with permission from Applied Animal Behaviour Science (Elsevier).

3.1.1 Study aims and hypotheses

This study aimed to determine the effects of farmer-assisted birth and ketoprofen analgesia on the behaviour of neonatal calves in the first 48 h of life using a 2 x 2 factorial design — a novel approach for investigating the effects of birth assistance and analgesia on calf behaviour. A further aim was to determine whether assisted calves experience heightened benefits of ketoprofen administration compared to unassisted calves. Three hypotheses were tested:

1. Farmer-assisted birth results in pain that is experienced by calves in the first 48 h after birth.

2. Neonatal pain will be ameliorated by immediate post-birth administration of the NSAID ketoprofen.

3. Assisted calves will experience heightened benefits of ketoprofen analgesia compared to unassisted calves (i.e. assisted birth is likely to be more painful than unassisted birth).

3.2 Materials and methods

3.2.1 Calf management and ethical approval

Calves were managed as described in Chapter 2, Section 2.2.2. Calves recruited to the study were subject to the same management as all other female Holstein calves on the farm. UK Home Office approval for this study was obtained as described in Chapter 2, Section 2.1.

3.2.2 Animal recruitment and study design

Calves (and their dams) that met the inclusion criteria described in Table 3.1 were recruited at the time of birth and randomly allocated to either a ketoprofen or placebo group in a 2 x 2 factorial design. Treatment administration and recording followed protocols described in Chapter 2, Section 2.3.

Table 3.1: Calf inclusion and exclusion criteria

Recruitment decision	Criteria
Included	Female Holstein (purebred) Unassisted birth Farmer-assisted birth Apparent good health
Excluded	Male Female twin to male calf Holstein x beef cross breed Veterinary assisted birth Caesarean section Congenital abnormalities

The decision to provide parturition assistance was made by an experienced stockperson who provided parturition assistance if deemed necessary. For study purposes parturition assistance was scored in accordance with a grading scheme modified from Barrier *et al.* (2013b) and Proudfoot *et al.* (2009) [Table 3.2].

Table 3.2: Grading of parturition assistance

(Gladden et al., 2018, 2019, 2021)

Grade	Description	Category
1	No assistance provided.	Unassisted
2	Mild assistance. No repositioning of the calf or mechanical calving aid required. Traction applied by one person only.	Assisted
3	Moderate to severe assistance. Calf is malpresented and requires repositioning and/or mechanical calving aid is required to deliver calf. Traction may be applied by more than one person.	Assisted
4	Assistance provided by a veterinary surgeon. Caesarean section.	Veterinary assistance (excluded)

To control for potential seasonal effects, recruitment of assisted and unassisted calves was time-matched: assisted calves (less frequent) were recruited first followed by recruiting the next eligible unassisted calf that was born — no unassisted calves were recruited without prior recruitment of a time-matched assisted calf. The high frequency of unassisted births on farm allowed most assisted calves to be time-matched with an eligible unassisted calf within two weeks. Animal identification number (ID) (the last four digits of the official ear tag number), the date and time of birth, the degree of assistance provided (1 to 3), the time the calf was moved into a calf pen, the pen number (1 to 6), calf birthweight (kg) and the amount of colostrum consumed (L) were all recorded contemporaneously by farm staff in a laminated chart positioned next to the calf pens. These data were photographed and manually transcribed into an Excel spreadsheet (Excel 2013, Microsoft, Redmond, WA, USA) at regular intervals for the duration of the project.

All calves were marked with coloured agricultural marker spray on the lumbosacral region to identify recruited calves as subject to the controls of A(SP)A and aid identification on video footage. This type of marking was not used for any other purpose on the farm. To further aid (video) identification, photographs were taken of all recruited calves. Sequential jugular blood samples were obtained as described in Chapter 6. All calves were recruited during a single period between the 7th of March and the 15th of December 2016.

3.2.3 Behavioural monitoring

Closed circuit television (CCTV) cameras (Sony CCD, Vari-focal, 700TVL, Sony, Minato, Tokyo, Japan) were positioned to continuously film each calf pen on wooden mounts created as part of pilot projects (Glasgow University School of Veterinary Medicine BVMS5 students, unpublished) [Figure 3.1]. Additional wooden mounts were built to the same design as required.


a *b*

Figure 3.1: Camera positioning to monitor calf behaviour *in situ* (a) and close-up view (b)

One camera was positioned on each calf pen (total 6 cameras) at a height of 2.17 m, achieving a maximum field of view (at adequate quality) whilst not interfering with animal behaviour or farm machinery [Figure 3.2]. For study purposes, calf pens were numbered one to six (clockwise starting with the top left pen) and correlated with the appropriate input port of the digital video recorder (DVR) (i.e. the camera on pen 1 was input through port 1, camera 2 was input through port 2 and so on).

a *b*

Figure 3.2: Examples of video footage obtained during daylight hours (a) and night hours (b)

3.2.3.1 Video filing and storage

Footage was continuously recorded and stored on DVRs (Guardian II+ DVR 8 Channel, Digital Direct Security, Huntingdon, Cambridgeshire, UK) on farm. The required 48 h of footage for each calf was backed up regularly in Audio Video Interleave (AVI) format onto a portable external hard drive (Seagate 1TB portable external hard drive, Seagate Technology LLC, Cupertino, CA, USA) for transportation back to the University of Glasgow. Video footage from the point that the calf entered the pen to the end of the 49th hour of life was filed and stored for all calves on larger external hard drives (Seagate 2TB expansion desktop drive, Seagate Technology LLC, Cupertino, USA, and Toshiba 6TB Canvio desktop external hard drive, Toshiba Europe GmBH, Hammfelddamm, Neuss, Germany). Calf video footage was also backed up to a 4TB storage drive provided by the University of Glasgow. Video footage was saved as individual files for most calves but if more than one recruited calf was born on the same day and housed together in the same calf pen footage was saved in a single file to avoid duplication and save space. All files were labelled with the calf ID and the date of birth. The time of entry into the calf pen was recorded in the file name of the appropriate individual video file. Surplus video footage was kept until data analysis was complete, then deleted.

3.2.4 Behavioural analysis

3.2.4.1 Behavioural definitions and recording methodology

An ethogram for calf behaviour established in pilot work (Emma Strazhnik, unpublished) was modified to develop the final ethogram used in the current study [Table 3.3]. Individual behaviours were categorised as ‘primary behaviours’ (mutually exclusive behaviours observable at all time points) or ‘secondary behaviours’ (behaviours nested within with primary behaviours) [Table 3.3]. The primary behaviour category comprised of five defined behavioural patterns: lying, standing, attempting to stand, walking or play. Visible calves were assigned to one of these five behaviours at every observation. Secondary behaviours were recorded whenever they occurred, but this did not have to be at all observations.

Calves were excluded from behavioural analysis if ≥ 24 h of footage was missing; if < 24 h footage was missing, calves were included and a record of missing data (classified as Missing Completely at Random) was made for the relevant observation. If footage was available but the subject calf could not be identified, a record of ‘not visible’ was made for that observation.

Focal instantaneous sampling (Altmann, 1974; Martin *et al.*, 2009a) was used to record behaviour and construct a time budget. This methodology was chosen due to the long study duration (48 h), for which continuous observations would have been impractical. Sample intervals of every five minutes every other hour were selected based on pilot work (Emma Strazhnik, unpublished) which showed that this interval resulted in an accurate representation of activity. Behavioural observations started at 2 h 0 min after birth, a start point selected based on pilot work (BVMS5 veterinary students, unpublished) that found most calves were moved into individual calf pens within 2 h of birth.

Table 3.3: Ethogram of calf behaviours

(Gladden et al., 2019)

Behaviour	Description	Code	Category
Sternal recumbency	Calf is lying on sternum. Each forelimb may be positioned on each side of the body, tucked under the sternum, or both forelimbs may be on the same side of the body in a flexed position. Further defined by head position: head up [NU] – no part of the head or face is in contact with the ground or body. Head down [NH] – any part of the head or face is in contact with the ground or body. Head position cannot be identified [NK].	NU/NH/NK	Primary behaviours (lying)[1]
Lateral recumbency	Calf is lying on its side (either left or right) with both forelimbs positioned to the same side of the body. The dependent shoulder is in contact with the ground. Further defined by head position: head up [RU] – no part of the head or face is in contact with the ground or body. Head down [RH] – any part of the head or face is in contact with the ground or body. Head position cannot be identified [RK].	RU/RH/RK	
Unknown lying position	Calf can be determined to be lying but the body position cannot be identified from the footage available (e.g. only part of the calf is visible). Further defined by head position if the head is visible: head up [KU] – no part of the head or face is in contact with the ground or body. Head down [KH] – any part of the head or face is in contact with the ground or body. If the head position also cannot be determined this is recorded as unknown [KK].	KU/KH/KK	
Standing	Calf is supported in an upright position by all four limbs. All four limbs are extended for a duration of more than three seconds, are in contact with the ground and the animal is not moving in any direction.	T	Primary behaviours (active)
Attempting to stand	Calf is transitioning from lying to standing and pauses in a partially standing position supported by one, two, or three limbs extended with the remaining limbs flexed (which differentiates this from standing). All four limbs are in contact with the ground. The head may be up or in contact with the ground. Completion of standing is not achieved within 3 s.	A	
Walking	The calf is in an upright position with all four limbs extended and takes more than two steps in any direction. Three out of four feet are in contact with the ground at any one time during movement (differentiating walking from solo play).	W	
Play behaviours	Calf is running, jumping, bucking, or skipping – two or more feet are simultaneously lifted away from the ground during these activities and speed is faster than a walking pace. Play can be solitary or social. The body may twist or change direction whilst the calf is elevated form the ground or may remain in the same plane of direction.	P	
Grooming self	Calf is licking or nibbling any part of own body or legs. This can be performed whilst the calf is standing or lying.	S	Secondary behaviours
Grooming others	Calf is licking or nibbling the legs, head, face, or any part of the body of another calf or calves. This can be performed whilst the subject calf is standing or lying.	O	
Investigatory behaviours	Calf is investigating surroundings by sniffing, licking, chewing, rubbing, nuzzling, moving with foot/nose any inanimate object (including water/feed containers, bars of pen and bedding).	I	
Other social behaviours	Calf is engaging in social behaviour with other calves that does not meet the definition of grooming or social locomotor play. Includes head rubbing, head resting, chin resting, cross-suckling, sniffing or aggressive interactions with another calf.	B	
Feeding/drinking directed behaviours	Calf is showing behaviours consistent with consuming either food (solid or liquid) or water. This primarily includes milk feeding but also includes drinking water and eating solid foodstuffs.	F	
Other secondary behaviour	Calf is engaging in rare miscellaneous secondary behaviours not described in detail in the ethogram. This includes interaction with humans at times other than feeding times.	X	
Not visible	Calf is not identifiable on camera at the time point of analysis and behaviour cannot be determined.	V	

1. Examples of lying behaviours are presented in Appendix 3

Observations were recorded every 5 min of every even hour (i.e. 2 h post-birth, 4 h post-birth and so on) in four 12 h time periods selected based on expected analgesic duration [Table 3.4]. Behavioural observations ended at 48 h 55 min after birth. The observer was blind to both assistance and treatment status.

Table 3.4: Number of neonatal behavioural observations in each 12 h time period

Time period	Number of observations
0 h to 12 h	60
12 h to 24 h	72
24 h to 36 h	72
36 h to 48 h	84
Total	*288*

3.2.4.2 Analysis of video footage

Calves were initially identified on video footage using the coloured lumbosacral spray mark applied at recruitment before further confirming calf identity (prior to starting observations) by comparing the individual coat patterns of each subject to photographs. Each calf was observed individually as a single focal subject. Video footage was viewed using Windows Media Player (Windows Media Player v.12, Microsoft, Redmond, WA, USA). Videos were played at normal speed for the purposes of behavioural observation and were manually skipped forward to each sampling time point. Behavioural observations were entered into an Excel spreadsheet (Excel 2013, Microsoft) in the form of letter codes corresponding to the ethogram [Table 3.3].

3.2.4.3 Construction of time budgets

For every calf, the number of sample points at which each behaviour was observed was counted and a time budget was produced for each behaviour. Both a total time budget (number of times a behaviour was observed divided by total number of available sample points) and a visible time budget (number of times a behaviour was observed divided by the number of sample points that the subject was visible) were calculated for each calf. Time budget data for every calf were combined into a single spreadsheet for analysis together with the calf ID, time period, median number of calves in the pen (in each time period), time of birth, date of birth, birthweight (kg), amount of colostrum consumed (L), dam lactation number and dam parity (primiparous or multiparous). Assistance and treatment status were included after all observations were complete.

3.2.5 Statistical analysis

All raw data were combined into a single Excel spreadsheet (Excel 2013, Microsoft) and exported into statistical software for further analysis. To balance the group sizes (and improve power) all assisted calves (grades 2 and 3) were analysed together as a single 'assisted' group. For all analyses, the visible time budget was used and is hereafter referred to as the 'time budget'. The threshold of statistical significance was set at $p < 0.05$ for all tests. A tendency towards significance was considered if $p \geq 0.05$ and $p \leq 0.08$.

3.2.5.1 Descriptive analysis and univariate analysis of birth metadata

Data were exported into Minitab (Minitab v.18, Minitab Inc., State College, PA, USA) for descriptive analysis and univariate analysis of birth-related data and to assess data distribution. For the purposes of analysis, the time and date of birth were formatted as categorical data relating to defined time periods of equal length as presented in Tables 3.5 and 3.6.

Table 3.5: Definition of each time of birth category

Time of birth (hh:mm)	Time category	Combined category for χ^2 analysis[1]
02:00 to 05:59	Early morning	Morning
06:00 to 09:59	Morning	
10:00 to 13:59	Lunchtime	Afternoon
14:00 to 17:59	Afternoon	
18:00 to 21:59	Evening	Night
22:00 to 01:59	Night	

1. Categories combined to meet assumptions of Chi-square analysis regarding sample size

Table 3.6: Definition of each date of birth category

Date of birth	Season category
1st March to 31st May	Spring
1st June to 31st August	Summer
1st September to 30th November	Autumn
1st December to 29th February	Winter

Data distributions were assessed using Anderson Darling statistics and visual assessment of histograms. Chi-squared tests of association and two sample t-tests were used to investigate the relationships between assistance and treatment status and categorical and continuous birth-related data respectively. To prevent violation of Chi-square analysis assumptions regarding sample size, time categories were further collapsed into three equal categories [Table 3.5] and dam lactation was categorised as lactation 1, 2 and ≥ 3. The amount of colostrum consumed was analysed as both the raw data (continuous) and also categorised into two groups defined as 'standard' and 'extra' (> 4.5 L) to align with farm colostrum feeding practices [see Section 2.2.2]. This approach allowed consideration of factors that might affect a calf's motivation to voluntarily consume more colostrum over and above the standardised minimum volume offered. A Pearson correlation coefficient was calculated to analyse the relationship between calf birthweight and volume of colostrum consumed.

3.2.5.2 Multivariate analysis

Multivariate statistical analysis of calf behaviour was performed by Dr. Jessica Martin, University of Edinburgh, UK. Data were exported into Genstat (Genstat v.14, VSN International, Hemel Hempstead, UK) for inferential statistical analyses. Data were analysed using Generalised Linear Mixed Models (GLMM) (Poisson distribution) with log-link function.

A priori, covariate data were selected for inclusion based on known or hypothesised effects on neonatal calf behaviour; hypothesised effects were informed by clinical knowledge. Covariate factors included the median number of calves in the pen, birthweight, and dam lactation number. Calf ID was included in each model as a random effect. Birth assistance status (assisted/unassisted), treatment status (ketoprofen/placebo) and time period (0 to 12 h, 12 to 24 h, 24 to 36 h and 36h to 48 h) as well as assistance x treatment status interactions, and treatment x time interactions were all included as fixed effects in each model.

Time budgets for each individual behaviour were analysed individually as well as combined into progressively larger behavioural categories [Appendix 3]. Body posture (sternal/lateral/unknown) and head position (up/down/unknown) were analysed separately as individual postures and together as combined lying behaviours [Appendix 3]. Active behaviours were analysed in two ways: as a proportion of the total time budget and as a proportion of the active (non-lying) time budget. Rare behaviours accounting for less than 0.5% of the time budget were not analysed as individual behaviours but were included in the analysis of the relevant combined behavioural categories.

3.3 Results

3.3.1 Neonatal study population

3.3.1.1 Descriptive results

Ninety-five calves were recruited to the study with 10 a.m. to 2 p.m. being the most common time for births [Figure 3.3]. Most calves (66%) were born between 6 a.m. and 2 p.m.

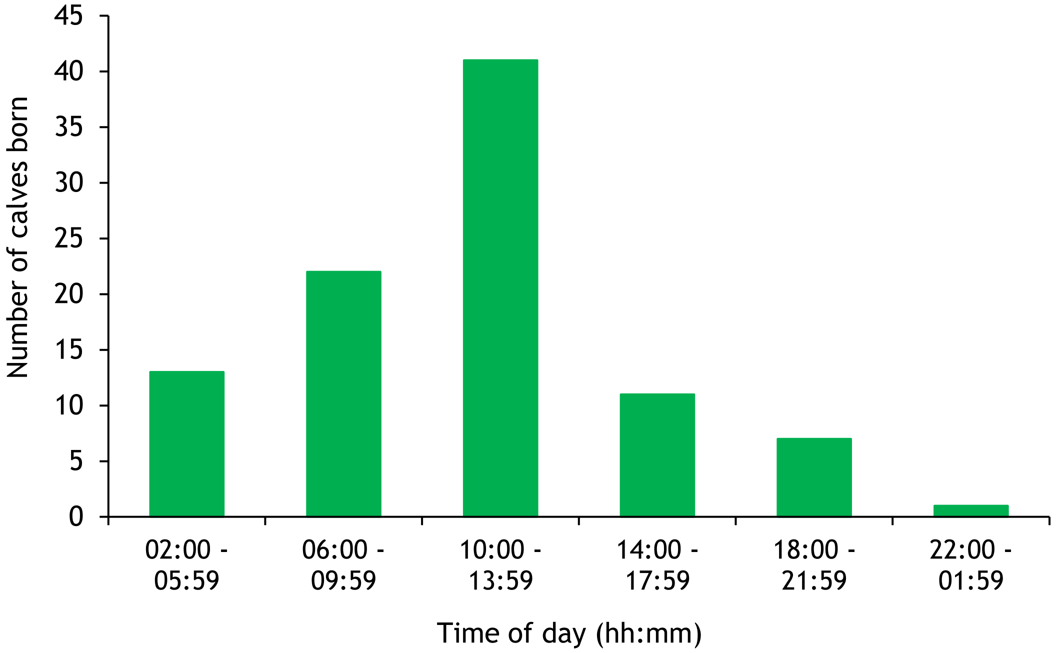


Figure 3.3: Frequency of calf births by time of day
Raw data presented

Fifty-two calves (54.7%) were born to primiparous dams and 43 (45.3%) were born to multiparous dams. The mean volume of colostrum consumed was 4.8 L (range 3.0 L to 7.0 L); 61 calves (64%) voluntarily consumed more colostrum than 4.5 L. Mean calf birthweight was 42.8 kg (range 27.6 to 62.8 kg) [Table 3.7].

Table 3.7: Calf birthweight (mean, standard deviation, maximum and minimum) overall, and for each dam parity group

Calf birthweight	*n*	Mean	SD	Min.	Max.
Primiparous dams	50	41.1	5.21	27.6	48.6
Multiparous dams	43	44.8	6.45	31.6	62.8
Overall	93	42.8	6.07	27.6	62.8

All values measured in kg. SD = Standard deviation. Min. = minimum. Max. = maximum. n = 93 as birthweight data for two calves not available due to weigh scale failure.

3.3.1.2 Univariate analysis of birth data

Assisted calves were 2.9 kg heavier at birth than unassisted calves (p = 0.022). Treatment status was not associated with any birth-related data. Birthweight was moderately (positively) correlated with the volume of colostrum consumed (r = 0.46; p < 0.001; R^2 = 0.21). Data are presented in Appendix 3.

3.3.2 Behavioural analysis

3.3.2.1 Descriptive results

Twenty calves were excluded from behavioural analysis — the most common reason for exclusion was incomplete footage (< 24 h available) due to technical failure [Table 3.8]. In total, 75 calves (38 ketoprofen; 37 placebo) were included in the final behavioural analysis [Table 3.9].

Table 3.8: Number of calves excluded at each stage prior to analysis and reasons for exclusion

Reason for exclusion	Stage of exclusion	*n*
Incomplete video footage (equipment failure)	Behavioural analysis	16
Illness	Data collection stage	2
Recording errors	Statistical analysis	2
Total		*20*

Table 3.9: Number of calves included in the final behavioural analysis for each assistance x treatment interaction group

Assistance status	*n*	Group	*n*
Assisted	39	Assisted saline (AP)	21
		Assisted ketoprofen (AT)	18
Unassisted	36	Unassisted saline (UP)	16
		Unassisted ketoprofen (UT)	20
Total	*75*		*75*

Lying was the most common behaviour observed (85% of the time budget) [Figure 3.4]. Calves in all assistance x treatment status groups engaged in lying behaviour for a similar proportion of time to the mean, although a (non-significant) numerical increase in saline treated assisted calves was observed [Table 3.10].

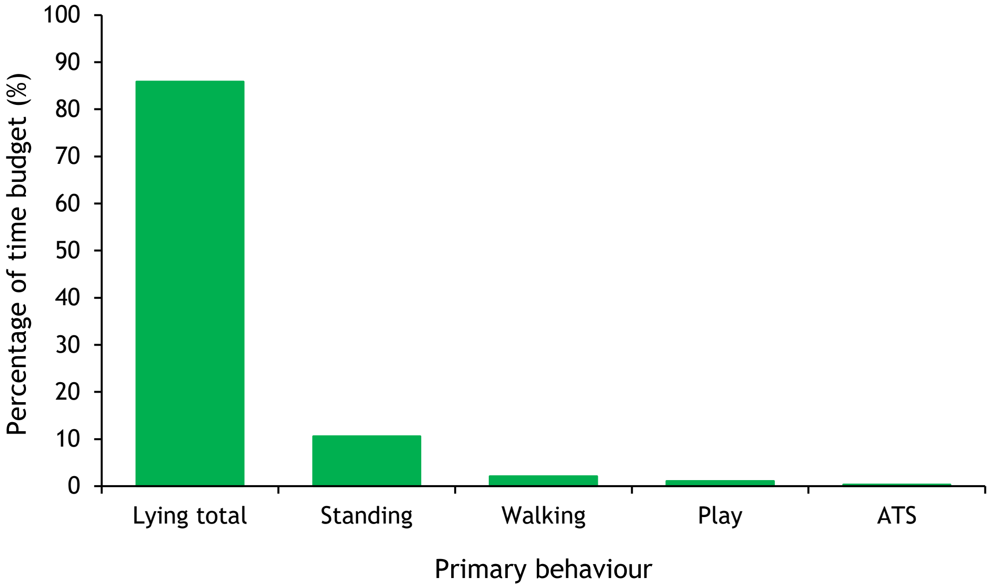


Figure 3.4: Percentage of visible time budget accounted for by each primary calf behaviour
All assistance x treatment groups combined (n = 75). Raw data presented. Lying total = all lying behaviours combined into a single category. ATS = attempting to stand.

Secondary behaviours were rarely observed and even when grouped together into a single category, they accounted for only 5.3% of the time budget. Investigatory behaviour was the most common individual secondary behaviour observed (1.8%); other secondary behaviours accounted for less than 1% of the time budget [Table 3.10].

For the combined 48 h observation period the mean proportion of the time budget that calves could not be observed was 4.6%. 'Not visible' was recorded most frequently during the 0 to 12 h time period (14.7% of observations).

Table 3.10: Mean (± SE) calf behavioural data for each assistance x treatment status group

Gladden et al., 2019; reproduced with permission

Behaviour	AP		AT		UP		UT	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Sternal recumbency, head up	28.5	1.4	31.4	1.3	30.9	1.4	32.9	1.3
Sternal recumbency, head down	49.1	1.5	47.3	1.6	46.5	1.6	47.4	1.3
Sternal recumbency, unknown head position	0.7	0.2	0.2	0.1	0.5	0.2	1.2	0.3
Lateral recumbency, head up	1.8	0.4	1.5	0.3	2.6	0.6	0.7	0.2
Lateral recumbency, head down	5.9	1.0	4.3	0.8	4.7	0.8	2.2	0.6
Attempting to stand (% of total visible time budget)	0.4	0.1	0.4	0.1	0.4	0.1	0.2	0.1
Standing (% of total visible time budget)	9.9	0.7	10.5	0.7	10.8	0.7	11.3	0.7
Walking (% of total visible time budget)	1.7	0.2	2.6	0.3	2.2	0.3	2.0	0.2
Play (% of total visible time budget)	0.6	0.1	1.2	0.2	1.1	0.2	1.4	0.2
Attempting to stand (% of active time budget)	3.2	0.8	3.8	1.0	2.8	1.0	1.6	0.6
Standing (% of active time budget)	72.5	2.8	69.1	2.6	76.7	2.1	76.4	1.7
Walking (% of active time budget)	13.9	1.9	16.1	1.5	14.0	1.7	12.9	1.4
Play (% of active time budget)	5.6	1.1	7.0	1.0	6.5	1.1	9.1	1.2
Feeding directed behaviour	0.8	0.1	0.9	0.2	1.1	0.2	1.3	0.2
Self-grooming	0.8	0.1	1.2	0.2	0.7	0.1	1.1	0.2
Investigatory behaviours	2.1	0.3	1.9	0.2	1.3	0.2	1.9	0.2
Other social behaviours	0.8	0.2	1.1	0.2	1.0	0.2	1.1	0.2
Total lying in sternal recumbency	78.3	1.4	78.8	1.3	77.9	1.3	81.4	1.1
Total lying in lateral recumbency	7.8	1.2	5.8	1.0	7.2	1.3	2.8	0.7
Total lying in unknown position	1.3	0.5	0.7	0.4	0.5	0.2	0.9	0.4
Total lying	87.4	0.9	85.3	0.9	85.6	0.9	85.1	0.8
Total active behaviours	12.6	0.9	14.7	0.9	14.4	0.9	14.9	0.8
Total secondary behaviours	5.0	0.5	5.6	0.5	4.3	0.4	6.0	0.5
Total head down	55.3	1.5	51.8	1.6	51.3	1.6	49.8	1.3
Total head up	30.4	1.3	33.1	1.3	33.5	1.3	33.7	1.3

Data are presented as a proportion of the visible time budget (%). Raw means are presented. AP = Assisted saline group. AT = Assisted ketoprofen group. UP = unassisted saline group. UT = Unassisted ketoprofen group. SE = standard error of the mean.

3.3.2.2 Effects of assisted birth

Irrespective of treatment status, assisted calves engaged in play behaviour for a smaller proportion of the time budget than unassisted calves (*p* = 0.019) [Figure 3.5 and Table 3.11]. When play behaviour was analysed as a proportion of active time, the same effect was observed but with reduced significance (*p* = 0.065) [Table 3.11].

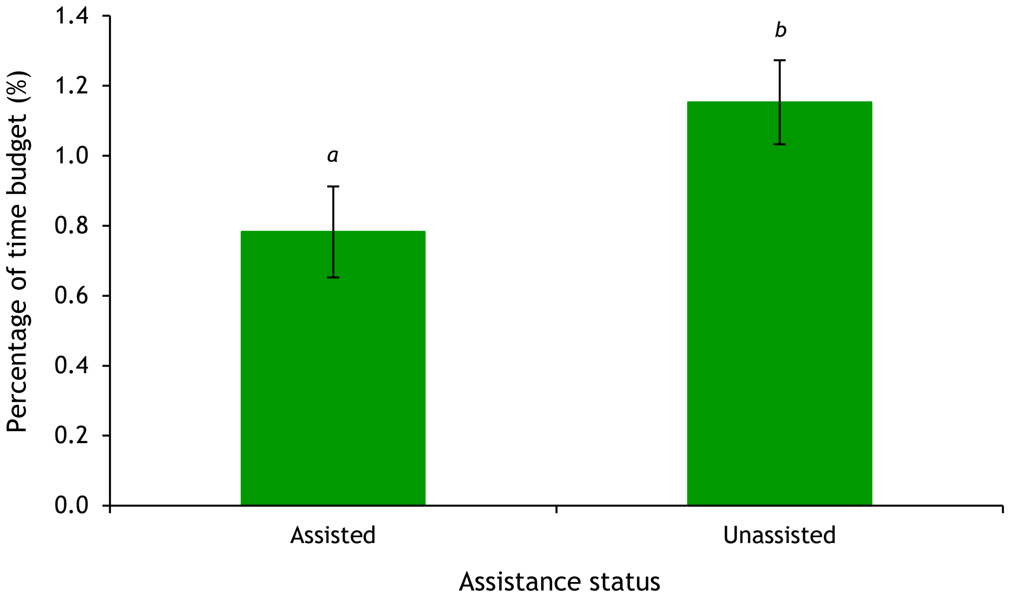


Figure 3.5: Mean (± SE) percentage of visible time budget assisted and unassisted calves engaged in play behaviour, irrespective of treatment status
Assisted calves n = 39. Unassisted calves n = 36. Different letters indicate significant differences (p = 0.019). Back transformed estimated marginal means (± SE) presented.

Irrespective of treatment status, assisted calves spent a greater proportion of the time budget with the head rested ('head down') (*p* = 0.008) and a smaller proportion of the time budget their head held up (*p* = 0.038) than unassisted calves [Table 3.11]. Additionally, irrespective of treatment status, assisted calves engaged in investigatory behaviours and showed a tendency to lie in lateral recumbency more than unassisted calves (*p* = 0.036 and *p* = 0.08 respectively) [Table 3.11].

Table 3.11: Mean (± SE), maximum and minimum percentage of time budget engaged in different behaviours for calves in each assistance group, irrespective of treatment status. *Bold font indicates significant difference ($p < 0.05$). Back transformed estimated marginal means (± SE) and raw maximum and minimum presented. Gladden et al. (2019), reproduced with permission*.

Behaviour	Assisted			Unassisted			F-statistic	*p*-value
	Mean (± SE)	Min (%)	Max (%)	Mean (± SE)	Min (%)	Max (%)		
Lying sternal recumbency	78.4 ± 0.01	35.3	100	79.8 ± 0.03	50.0	98.8	1.08	0.299
Lying lateral recumbency	7.60 ± 0.15	0.00	41.2	4.70 ± 0.51	0.00	40.4	3.09	0.080
Total lying behaviours	86.3 ± 0.01	58.3	100	85.4 ± 0.01	57.1	98.8	1.20	0.274
Attempting to stand (proportion of total time budget)	0.23 ± 0.16	0.00	4.17	0.21 ± 0.24	0.00	3.33	0.12	0.728
Walking (proportion of total time budget)	2.10 ± 0.06	0.00	8.33	2.20 ± 0.21	0.00	10.0	0.00	0.976
Play (proportion of total time budget)	**0.78 ± 0.13**	**0.00**	**6.94**	**1.15 ± 0.12**	**0.00**	**6.98**	**5.61**	**0.019**
Play (proportion of active time budget)	5.40 ± 0.12	0.00	50.0	7.14 ± 0.34	0.00	40.0	3.43	0.065
Total active behaviours	13.7 ± 1.04	0.00	41.7	14.7 ± 1.04	1.19	42.9	1.23	0.269
Feeding directed	0.90 ± 0.14	0.00	5.95	1.10 ± 0.17	0.00	8.33	1.45	0.230
Grooming Self	0.90 ± 0.11	0.00	5.95	0.79 ± 0.20	0.00	9.52	0.82	0.367
Investigatory behaviours	**2.10 ± 0.05**	**0.00**	**11.7**	**1.50 ± 0.05**	**0.00**	**8.33**	**4.45**	**0.036**
Total secondary behaviours	5.38 ± 0.01	0.00	16.7	4.96 ± 0.02	0.00	19.4	0.58	0.446
Total head up	**31.3 ± 0.02**	**5.00**	**71.9**	**34.3 ± 0.05**	**6.25**	**70.0**	**4.33**	**0.038**
Total head down	**54.0 ± 0.02**	**8.77**	**91.7**	**49.9 ± 0.05**	**16.7**	**85.7**	**7.05**	**0.008**

SE = standard error of the mean

3.3.2.3 Effects of ketoprofen treatment

Irrespective of assistance status, ketoprofen treated calves engaged in play behaviour and combined secondary behaviours for a greater proportion of the time budget than saline treated calves (p = 0.017 and p = 0.011 respectively) [Figures 3.6 and 3.7 and Table 3.12].

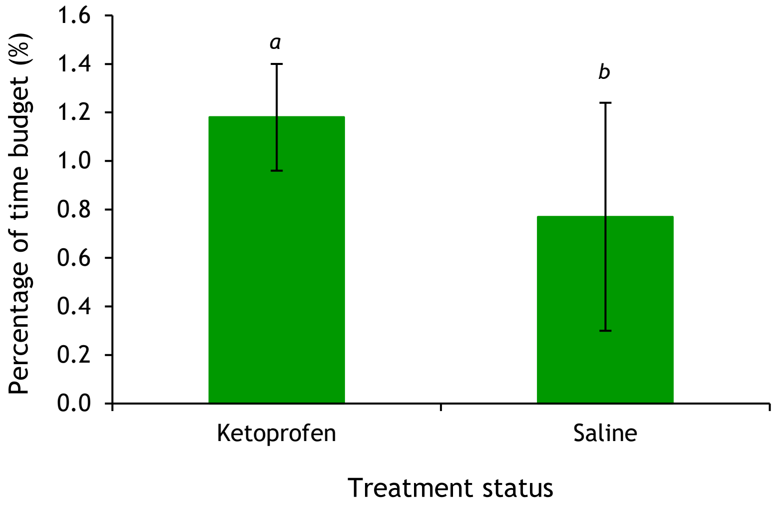


Figure 3.6: Mean (± SE) percentage of the time budget ketoprofen and saline treated calves engaged in play behaviour, irrespective of assistance status

Different letters indicate significant differences (p = 0.017). Ketoprofen treated calves n = 38. Saline treated calves n = 37. Back transformed estimated marginal means (± SE) presented.

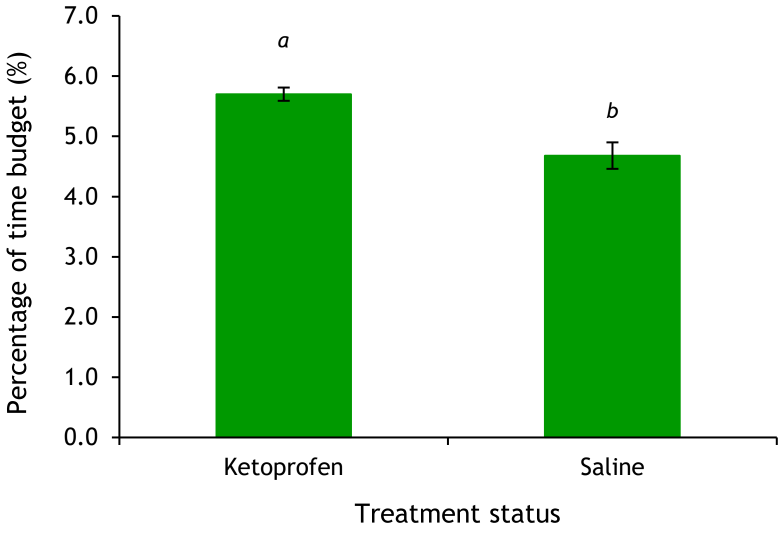


Figure 3.7: Mean (± SE) percentage of the time budget ketoprofen and saline treated calves engaged in combined secondary behaviours, irrespective of assistance status

Different letters indicate significant differences (p = 0.011). Ketoprofen treated calves n = 38. Saline treated calves n = 37. Back transformed estimated marginal means (± SE) presented.

Table 3.12: Mean (± SE), maximum and minimum percentage of time budget engaged in different behaviours for calves in each treatment group, irrespective of assistance status. *Bold indicates significant difference (p < 0.05). Back transformed least square means (± SE) and raw maximum and minimum presented. Gladden et al. (2019), reproduced with permission.*

Behaviour	Ketoprofen			Saline			F-statistic	*p*-value
	Mean (± SE)	Min (%)	Max (%)	Mean (± SE)	Min (%)	Max (%)		
Lying sternal recumbency	80.0 ± 0.01	40.0	100.0	78.3 ± 0.34	35.3	100.0	1.83	0.177
Lying lateral recumbency	4.46 ± 0.20	0.00	37.5	7.92 ± 0.27	0.00	41.2	3.79	0.052
Total lying behaviours	85.4 ± 0.01	57.1	100.0	86.3 ± 0.01	58.3	100.0	1.41	0.236
Attempting to stand (proportion of total time budget)	0.22 ± 0.16	0.00	3.39	0.21 ± 0.24	0.00	4.17	1.93	0.166
Walking (proportion of total time budget)	2.28 ± 0.12	0.00	10.0	2.03 ± 0.26	0.00	8.93	0.99	0.321
Play (proportion of total time budget)	**1.18 ± 0.22**	**0.00**	**6.94**	**0.77 ± 0.47**	**0.00**	**6.98**	**5.72**	**0.017**
Play (proportion of active time budget)	7.30 ± 0.23	0.00	40.0	5.20 ± 0.53	0.00	50.0	2.99	0.085
Total active behaviours	14.7 ± 0.05	0.00	42.9	13.6 ± 0.11	0.00	41.7	0.99	0.321
Feeding directed	1.09 ± 0.22	0.00	7.69	0.91 ± 0.25	0.00	8.33	2.38	0.124
Grooming Self	**1.04 ± 0.24**	**0.00**	**9.52**	**0.69 ± 0.42**	**0.00**	**5.56**	**12.5**	**< 0.001**
Investigatory behaviours	1.93 ± 0.14	0.00	11.1	1.66 ± 0.26	0.00	11.7	0.28	0.600
Total secondary behaviours	**5.70 ± 0.11**	**0.00**	**19.4**	**4.68 ± 0.22**	**0.00**	**16.7**	**6.56**	**0.011**
Total head up	33.3 ± 0.05	6.25	62.5	32.2 ± 0.12	5.00	71.9	1.00	0.318
Total head down	50.9 ± 0.03	23.3	83.3	53.0 ± 0.04	8.77	91.7	3.14	0.078

SE = standard error of the mean

When secondary behaviours were analysed individually, ketoprofen treated calves treated spent a greater proportion of the time budget self-grooming than saline treated calves ($p < 0.001$) [Table 3.12]. Ketoprofen treated calves showed a tendency to spend less time lying in lateral recumbency ($p = 0.052$) and more time with the head held in a rested position ($p = 0.078$) than saline treated calves (irrespective of assistance status) [Table 3.12].

3.3.2.4 Effects of assistance x treatment status interactions

Calves in the assisted ketoprofen (AT) group spent a greater proportion of the time budget engaged in walking behaviours than calves in any of the other three interaction groups ($p = 0.004$). Calves in the unassisted ketoprofen (UT) group engaged in lateral recumbency less frequently than calves in the other three groups ($p = 0.005$) [Figure 3.8]. Additionally, there was a tendency for calves in the UT group to lie in sternal recumbency more frequently than calves in the other three groups ($p = 0.056$).

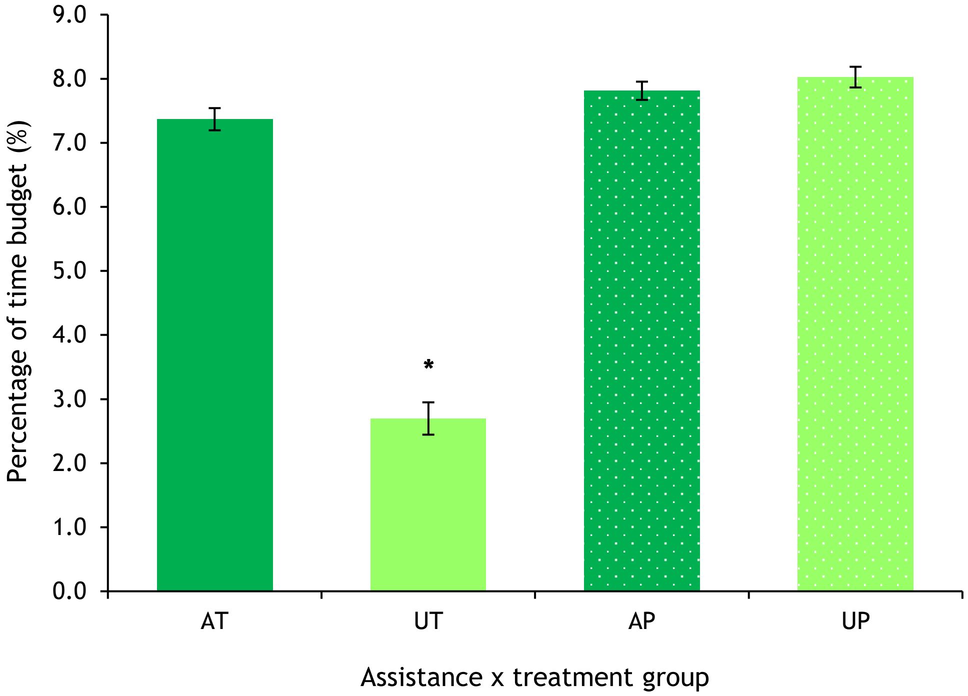


Figure 3.8: Mean (± SE) percentage of visible time budget engaged in lateral recumbency for calves in each assistance x treatment status group

***** *Indicates significant difference (p = 0.005). AT = assisted ketoprofen treatment group (n = 18). UT = unassisted ketoprofen treatment group (n = 20). AP = assisted saline placebo group (n = 21). UP = unassisted saline placebo group (n = 16). Assisted calves represented by dark green, unassisted calves represented by light green. Ketoprofen treatment represented by solid bars. Saline treatment represented by patterned bars. Back transformed estimated marginal means (± SE) presented.*

When analysed as a proportion of the active (non-lying) time budget, UT calves tended to engage in play behaviour more than calves in the other three interaction groups (*p* = 0.062) [Figure 3.9].

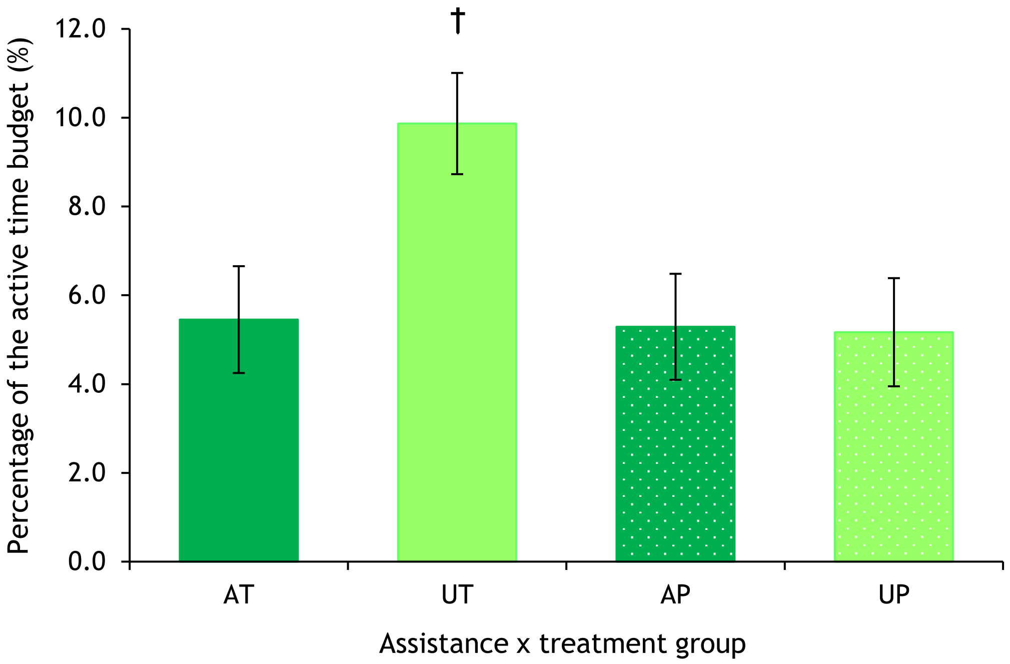


Figure 3.9: Mean (± SE) percentage of active time budget engaged in play for calves in each assistance x treatment status group

† Indicates tendency towards significant difference (p = 0.062). AT = assisted ketoprofen treatment group (n = 18). UT = unassisted ketoprofen treatment group (n = 20). AP = assisted saline placebo group (n = 21). UP = unassisted saline placebo group (n = 16). Assisted calves represented by dark green, unassisted calves represented by light green. Ketoprofen treatment represented by solid bars. Saline treatment represented by patterned bars. Back transformed estimated marginal means (± SE) presented.

3.3.2.5 Effects of time period

Irrespective of assistance or treatment status, play behaviour was observed most frequently in the 12 to 24 h time period (*p* = 0.001). The same effect was observed (with increased significance) when play behaviour was analysed as a proportion of the active time budget (*p* < 0.001). The youngest calves engaged in attempting to stand behaviour (ATS) most frequently (*p* < 0.001) [Figure 3.10]; ATS was observed 14.4 times more in the 0 to 12 h time period than in the 36 to 48 h time period.

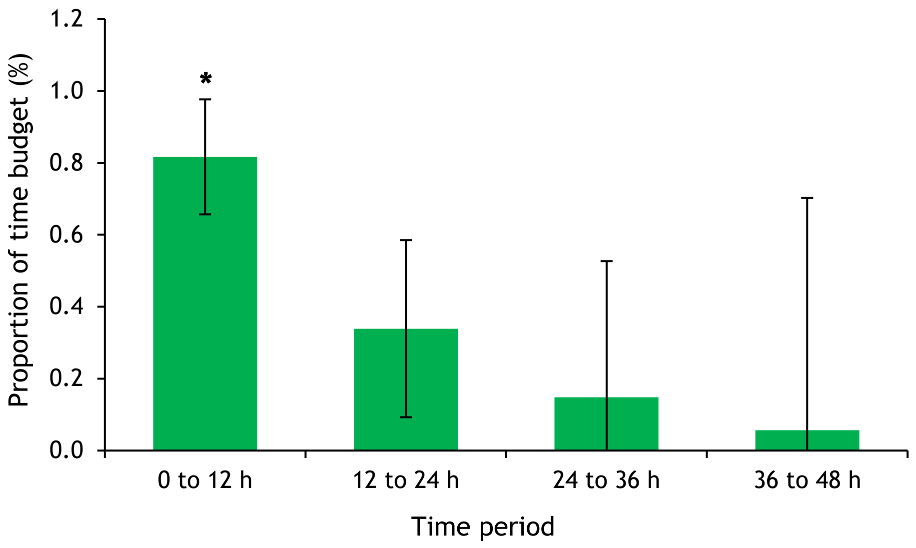


Figure 3.10: Mean (± SE) percentage of the time budget calves engaged in attempting to stand behaviour irrespective of assistance or treatment status
** Indicates significant result (p < 0.001). n = 75. Back transformed estimated marginal means (± SE) presented.*

Overall, the proportion of the time budget engaged in combined secondary behaviours increased from 4.23% in the 0 to 12 h time period to 6.84% in the 24 to 36 h time period ($p < 0.001$). When secondary behaviours were analysed individually, only self-grooming was affected by time: the proportion of the time budget calves engaged in self-grooming increased from 0.38% in the 0 to 12 h time period to 1.57% between 24 to 36 h ($p < 0.001$). There was a tendency for feeding behaviour to increase as time progressed (from 0.70% between 0 to 12 h to 1.34% in the 24 to 36 h time period) ($p = 0.060$). In the second 24 h time period, calves showed a tendency to spend less time with the head in a rested position as time progressed (48.9% in the 24 to 36 h time period compared to 54.4% in the 36 to 48 h time period) ($p = 0.069$).

3.3.2.6 Effects of dam lactation, number of pen-mates and birthweight

Irrespective of assistance or treatment status, calves born to dams in lactation four and over spent less time in sternal recumbency ($p = 0.002$), and more time in lateral recumbency ($p = 0.014$), than calves born to younger dams. Time engaged in (combined) active behaviours also increased with increasing dam lactation ($p = 0.047$), as did time spent engaging in investigatory behaviours and combined secondary behaviours (both $p = 0.007$).

The frequency of observations for which calves were recorded as not visible increased as the number of calves in the pen increased ($p = 0.011$). Additionally, as the number of calves in the pen increased, calves were observed to hold the head held down in a rested position less often ($p = 0.045$).

Birthweight was positively associated with time spent with the head held elevated ($p = 0.023$) and the proportion of the time budget engaged in walking ($p = 0.042$). By contrast, birthweight was negatively associated with time spent with the head rested ($p = 0.029$), engaged in self-grooming behaviours ($p = 0.022$), and combined secondary behaviours overall ($p = 0.01$).

3.3.3 Summary

Both birth assistance and ketoprofen treatment affected newborn calf behaviour but variable effects were observed. Key results are summarised in Table 3.13.

3.4 Discussion

3.4.1 Behavioural analysis

As hypothesised, behavioural differences (such as decreased play behaviour) were evident among assisted calves (irrespective of treatment status) and also in ketoprofen treated calves (irrespective of assistance status). Additionally, the interaction between assistance and treatment status affected some behaviours (such as lateral recumbency), suggesting that ketoprofen analgesia can ameliorate some of the adverse effects of assisted birth. The reported duration of clinical effect of ketoprofen is up to 24 h (Landoni *et al.*, 1995). Accordingly, it was hypothesised that there might be differences in observed calf behaviour consistent with a reduction in the efficacy of ketoprofen after 24 h. However, no treatment x time interaction effect was identified, suggesting that the duration of clinical effect of ketoprofen possibly exceeded 24 h. There is some evidence in adult cattle to support this hypothesis (Whay *et al.*, 2005b) and an extended duration of clinical effect of ketoprofen has also been reported in humans — thought to be due to delayed clearance from inflamed tissues (Kantor, 1986).

Table 3.13: Summarised results of main and interaction effects (assistance status, treatment status, assistance x treatment status and time period) on newborn calf behaviours

Behaviour	Summarised result	Significance
Attempting to stand	Most frequent in 0 to 12 h time period	***
Walking	Ketoprofen treated assisted calves engaged more frequently than calves in other interaction groups	**
Play (proportion of total time)	Assisted calves engaged in play less than unassisted calves	*
	Ketoprofen treated calves engaged in play more than saline treated calves	*
	Play observed most frequently in 12 to 24 h time period	**
Play (proportion of active time)	Assisted calves tended to engage in play for less than the active time budget than unassisted calves	†
	Ketoprofen treated unassisted calves tended to engage in play for more of the active time budget than other calves	†
	Play observed most frequently in 12 to 24 h time period	***
Feeding directed behaviour	Tended to increase as calf aged (i.e. with increasing time post-birth)	†
Self-grooming	Observed more frequently in ketoprofen treated calves than saline treated calves	***
	Increased as calf aged	***
Investigatory behaviours	Observed more frequently in assisted calves than unassisted calves	**
Total lying in sternal recumbency	Ketoprofen treated unassisted calves tended to lie in sternal recumbency more than calves in other groups	†
Total lying in lateral recumbency	Assisted calves tended to lie in lateral recumbency more than unassisted calves	†
	Saline treated calves tended to lie in lateral recumbency more than ketoprofen treated calves	†
	Ketoprofen treated unassisted calves engaged in lateral recumbency less than calves in other groups	**
Combined secondary behaviours	Ketoprofen treated calves engaged in combined secondary behaviours more than saline treated calves	*
	Secondary behaviours increased as calves aged	***
	Assisted calves held the head rested more than unassisted calves	**
	Ketoprofen treated calves tended to hold the head rested less than saline treated calves	†
	Calves tended to hold the head rested less as time progressed	†
Total head up (elevated)	Assisted calves held the head elevated less than unassisted calves	*

********* *$p < 0.001$.* ******** *$p < 0.01$.* ******* *$p < 0.05$.* † *= tendency ($p \geq 0.05$ and $p \leq 0.08$). Effects on behaviours not presented were not significant. Active behaviours presented as proportion of total time budget unless otherwise noted*

Lying was the most common behaviour observed, accounting for over 80% of the time budget — a normal finding in young calves (Chua *et al.*, 2002; Trénel *et al.*, 2009; Hill *et al.*, 2013). To account for the large proportion of the time budget spent lying, active behaviours were analysed both as a proportion of the total time budget (inclusive of lying) and a proportion of the active (non-lying) time budget. No meaningful differences between these approaches were identified, although some results were found to be more robust statistically when the proportion of active time was analysed (e.g. the effect of time period on play behaviour).

The most common lying posture observed was sternal recumbency with the head rested. This position is commonly adopted by older calves and adult cattle when sleeping (Ruckebusch, 1975; Hänninen *et al.*, 2003, 2008; Hunter *et al.*, 2021) and likely also represents sleep in the calves studied here. Newborn babies also spend a large proportion of the time budget sleeping in the first few days of life (Parmelee *et al.*, 1961) and it is likely that birth and subsequent adaptation to extra-uterine life is a tiring experience. The current study was not designed to investigate the sleep patterns of newborn calves, but this result highlights the importance of sleep for newborn calves and as such may have implications for newborn calf welfare. Although an important behavioural state, sleep has not been studied in young calves and is not currently considered as part of welfare assessment. For example, individual housing of calves is associated with diminished time engaged in sternal recumbency (Webster *et al.*, 1985) — possibly compromising the ability of newborn calves to sleep — but is currently allowed up to eight weeks old under EU legislation (Council Directive 2008/119/EC). Accordingly, sleep patterns of newborn calves and the welfare impacts of sleep alterations deserve further study.

Standing accounted for 11% of the total time budget and 74% of the active time budget making this the most common active primary behaviour observed. Locomotory behaviours (play and walking) accounted 21% of the active time budget and approximately 3% of the total time budget. By contrast, Chua *et al.* (2002) found that calves spent less than 5% of the time budget engaged in standing behaviour and approximately 1% engaged in (non-specific) moving behaviours. The differences in findings of these two studies may be due to variations in calf management, because the calves studied by Chua *et al.* (2002) were housed as individuals or pairs, whereas in the current study calves were housed in small groups — possibly facilitating more activity. This hypothesis is supported by the results of another study that found that calves housed together in small groups engaged in mobile behaviours (walking and play) approximately six times more than individually housed calves (Tapki, 2007).

3.4.1.1 Lying behaviours

Ketoprofen treated unassisted calves were observed lying in lateral recumbency less frequently than calves in the other three assistance x treatment status interaction groups. Lateral recumbency is an abnormal lying posture infrequently adopted by cattle (Webster *et al.*, 1985; Hänninen *et al.*, 2003), and is often exhibited when in pain or unwell (Molony *et al.*, 1995; Petherick *et al.*, 2014). As such, this finding suggests that the analgesic effect of ketoprofen may be heightened in unassisted calves (compared to assisted calves). Alternatively, it is possible that factors other than pain adversely affect assisted calves more than unassisted calves. This hypothesis is supported by results that indicated some behaviours were affected by birth assistance regardless of treatment status [Table 3.11]. Irrespective of treatment status, assisted calves held the head in a rested position more (and an elevated position less) than unassisted calves. An elevated (alert) head position has been identified as a reliable indicator of comfort in adult cattle (Gleerup *et al.*, 2015), but this has not been assessed in calves. It is possible that assisted calves held their head elevated less often than unassisted calves due to pain, although head position was not affected by ketoprofen, as might be expected following mild to moderate birth assistance. A rested head position is adopted when cattle are asleep (Ruckebusch, 1975; Hänninen *et al.*, 2003, 2008; Hunter *et al.*, 2021), and so it is possible that assisted birth is associated with increased neonatal fatigue. This is corroborated by previous studies that report assisted calves take longer to stand (Diesch *et al.*, 2004; Hickson *et al.*, 2008), have poorer vigour (Riley *et al.*, 2004), and spend a greater amount of time lying on their flank (Barrier *et al.*, 2012a) than unassisted calves.

Pain scoring systems such as the Glasgow Composite Measure Pain Scale (Reid *et al.*, 2007) are commonly used in veterinary medicine to objectively assess pain of companion animals and accordingly, establishment of a bovine pain evaluation system based on selected behaviours has been attempted (Gleerup *et al.*, 2015). However, only adult cattle were assessed and the findings of the current study suggest that this evaluation system may not be sensitive enough to detect pain experienced by newborn calves (possibly due to the confounding effect of factors other than pain), although only one of the behavioural indicators described by Gleerup *et al.* (2015) (head position) was assessed in the current study. It would be of value to establish the lower age limit for which the pain evaluation scheme described by Gleerup *et al.* (2015) is valid and define an alternative pain scoring system for calves younger than this. The data collected here has generated detailed time budgets for newborn calves and provides information on their behavioural repertoire in group housing. The 2 x 2 design used has enabled identification of some behaviours that are affected by pain (e.g. play, self-grooming) and may provide a starting point to aid future researchers in the selection of behaviours suitable for analysis when evaluating pain experienced by very young calves.

Interestingly, lying behaviours were affected by dam lactation with calves born to older dams (lactation four and over) engaging in sternal recumbency less (and lateral recumbency more) than calves born to younger dams. This was an unexpected finding as in the study reported in Chapter 6, dam lactation was negatively associated with neonatal plasma creatine kinase concentration, a biomarker of muscle injury (Anderson *et al.*, 1976) (a finding most likely due to the smaller pelvic size of younger dams resulting in greater compressive forces being applied to the calf during birth) and as such, calves born to older dams might have been expected to spend less time in lateral recumbency (compared to calves born to younger dams). As calves were separated from their dams shortly after birth, this finding is unlikely to be related to maternal behaviour. It is possible that factors such as exhaustion or fatigue affected calves born to older dams more than calves born to younger dams, but it is difficult to determine this from the data available and further study is warranted.

3.4.1.2 Play behaviour

Although play behaviour of young animals has been discussed for more than 80 years (Brownlee, 1939, 1954), its purpose is still not fully understood (Martin *et al.*, 1985; Held *et al.*, 2011; Pellis *et al.*, 2019). Play is not typically essential for survival and is usually observed when animals' fundamental needs (e.g. nutrition) are met (Boissy *et al.*, 2007). Absence of play behaviour has long been recognised as an indicator of poor health (Brownlee, 1939) and more recently play has been employed as an indicator of positive animal welfare (Boissy *et al.*, 2007; Held *et al.*, 2011; Mintline *et al.*, 2013). In the current study play behaviour was observed less frequently in assisted calves, suggesting that birth assistance adversely affects early-life welfare. A reduction in play behaviour has been observed when calves are exposed to painful experiences (Mintline *et al.*, 2013) and, as hypothesised, ketoprofen treated calves engaged in play more than saline treated calves; although no assistance x treatment interaction effect was identified. These results suggest that assisted birth has a greater adverse welfare effect than unassisted birth, but this is possibly due to challenges other than (or in addition to) pain. However, this result needs to be interpreted with caution as the power to detect interaction effects on infrequent behaviours in the present study was limited. Additionally, the optimised parturition management on the study farm may have contributed to this result. Parturition assistance on the study farm is provided by experienced personnel and the duration of traction provided during assisted birth was short (< 1 min). It is possible that, when parturition assistance is optimised, assisted and unassisted births are similar calf experiences and result in similar degrees of pain. If this were the case, a similar magnitude of analgesic effect would be expected in both assisted and unassisted calves. Nevertheless, these data suggest that — even on farms that practise optimised parturition protocols — pain is experienced by all newborn calves irrespective of birth experience. This important finding implies that all *per vaginum* births are potentially painful for calves, and the administration of ketoprofen immediately after birth contributes to improved early life welfare of all calves — not just those born to assisted parturition. Whilst pain associated with assisted birth had been hypothesised, identification of behavioural changes suggestive of neonatal pain following unassisted birth was unexpected and has not previously been reported. Researchers typically consider birth related neonatal pain to be a consequence of human intervention (e.g. Remnant *et al.*, 2017) due to external forces applied by traction (Pearson *et al.*, 2020) but significant compressive forces are experienced by babies during normal labour (Ami *et al.*, 2019) and it is possible that similar compressive forces are experienced by calves, causing pain that persists after birth. The neonatal experience of birth is poorly understood, and further work is merited to improve our understanding of birth experience and allow further optimisation of newborn calf management.

Play behaviour was also affected by time, with play being observed most frequently in the 12 to 24 h time period irrespective of treatment or assistance status. In 1954 Dr. Alexander Brownlee, an early pioneer of the study of neonatal play behaviour, reported that play behaviour of newborn calves becomes more accomplished with age; however, detailed analysis of temporal patterns in the first 48 h of life was not described (Brownlee, 1954). More recent studies of young calves (Tapkı *et al.*, 2006), dolphins (Mann *et al.*, 1999) and baboons (Owens, 1975) have also found that play increases with age, but none describe play behaviour in animals as young as those studied here. It is possible that the temporal pattern of calf play behaviour (relative to birth) observed here reflects normal bovine development in the first 48 h of life. However, the calves studied in all chapters of this thesis were recruited on the same farm and were subject to the same environmental and management conditions. As such, it is difficult to be certain that this finding is a manifestation of the normal behavioural development of *Bos taurus* calves in general, and not more reflective of the development of group-housed dairy calves reared in a specific farm environment. The effects of environmental and rearing practices on calf welfare is of great interest to industry stakeholders and consumers alike (Ventura *et al.*, 2013; Sumner *et al.*, 2018). As play behaviour can be used as an indicator of welfare, further research into the development of play behaviour in very young animals is warranted and will allow for a greater understanding of rearing practices that contribute to deviations from normal development of play behaviour.

3.4.1.3 Attempting to stand, walking and secondary behaviours

Ketoprofen treated assisted calves were observed walking more frequently than calves in the other three assistance x treatment status interaction groups. Calves in the current study were not encouraged to walk or managed in a way that necessitated walking, therefore engagement in walking behaviours was voluntary. Previous work found that after dehorning, analgesia treated calves walked more than saline treated calves (Pauly *et al.*, 2012). By contrast, other studies have reported that the number of steps taken by adult cattle diagnosed with clinical mastitis (a painful condition) was higher than unaffected cows (Siivonen *et al.*, 2011; Fogsgaard *et al.*, 2015). It is possible that the effect of pain on walking behaviours in cattle is affected by the source of pain, or other factors such as age. As such, walking is a behavioural state not well suited for use as a solo indicator of pain in cattle and careful interpretation alongside analysis of other behaviours is needed. In the present study, it may be assumed that — similar to locomotor play — voluntary walking is more likely to be exhibited by calves that are comfortable. Accordingly, the results suggest that assisted calves experienced heightened benefits of ketoprofen analgesia — seemingly in contrast to the assistance x treatment interaction effects on lateral recumbency. Reasons for this apparent disparity in results are unclear but it may be due to the different behavioural states analysed, rather than conflicting effects *per se*. It is likely that other challenges associated with birth assistance that are not expected to be affected by analgesia (e.g. exhaustion) are interacting with (or obscuring) pain to affect behaviours analysed in this study and may have contributed to these findings. For example, it is possible that fatigue experienced by assisted calves has a greater effect on lying in lateral recumbency than pain, obscuring the effects of analgesia that were observed in unassisted calves (likely to be less fatigued). Whilst the use of analgesia and placebo drugs to aid the identification of animal pain is well described (Weary *et al.*, 2006), identification of other welfare challenges associated with assisted birth (such as fatigue) is more difficult as accepted methodologies are not available. Assessment of fatigue has been attempted in mice (e.g. Wolff *et al.*, 2018) but these studies typically investigate changes in exercise tolerance as rodent models for human fatigue (often associated with disease). As such, results of these studies are not applicable to newborn calves in a farm environment and — as fatigue represents a welfare challenge — further work is needed to investigate potential methods for identification of fatigue (and exhaustion) in newborn calves, and other species of veterinary interest.

Ketoprofen treated calves engaged in self-grooming behaviours more than saline treated calves, regardless of assistance status. It has been suggested that animals in pain minimise unnecessary movement (Molony *et al.*, 1997) and it is possible that — as self-grooming requires stretching and turning — ketoprofen treated calves self-groomed more due to improved comfort. Increased engagement in self-grooming by ketoprofen treated calves might be due to these calves being in a more positive emotional state, a hypothesis supported by a previous study that found self-grooming behaviour of sick calves was reduced (Borderas *et al.*, 2008). However, poor welfare states have also been associated with increased self-grooming (Krohn, 1994) and as such (similar to walking behaviours) interpretation of self-grooming requires caution. Whilst grooming is important for the maintenance of a healthy coat and skin (Rich, 1973; Kohari *et al.*, 2009) it is also a pleasurable and relaxing activity associated with an endogenous opioid response (Keverne *et al.*, 1989), and may be a displacement activity. Self-grooming accounted for approximately 1% of the time budget in the current study, similar to that reported for young Jersey calves (Pempek *et al.*, 2016) and is likely to be normal for calves of this age. Given that the proportion of the time budget engaged in self-grooming was not excessive, and management of calves in both treatment groups was the same, it is considered likely that here the positive association between self-grooming and ketoprofen treatment represents improved comfort and a more positive affective state.

As calves aged, they spent a smaller proportion of the time budget attempting to stand. ‘Attempting to stand’ was defined as an abnormal behaviour where calves paused for a longer duration than expected (> 3 s) during the transition between lying and standing and was observed 14 times more often in the 0 to 12 h time period (i.e. the youngest calves) than between 36 to 48 h. Engagement in ATS behaviour was interpreted as difficulty achieving completion of the transition from lying to a fully standing posture. The strength of calves improves linearly with age (Nishimura *et al.*, 1996), therefore this finding likely reflects the reduced strength of the youngest calves (i.e. increased difficulty in elevating the body to standing).

3.4.2 Study design and methodology

Although a 2 x 2 factorial design is considered optimal for the study of animal pain and analgesia (Weary *et al.*, 2006), it has not been utilised by similar previous studies (Murray *et al.*, 2016; Pearson *et al.*, 2019a, 2019b; Clark *et al.*, 2020). As such, the approach of the current study is novel. Furthermore, whilst previous work has focused on the effects of analgesia on newborn calf vigour (Murray *et al.*, 2016) or physiological parameters such as growth rate and serum immunoglobulin concentration (Pearson *et al.*, 2019a, 2019b; Clark *et al.*, 2020), the effects of analgesic treatment on longer-term calf behaviours (up to 48 h) has not previously been studied. Additionally, whilst the effects of assisted birth on neonatal lying and standing behaviours immediately after birth have been reported (Diesch *et al.*, 2004; Hickson *et al.*, 2008; Barrier *et al.*, 2012a), the effects of parturition assistance on calf behaviour beyond 3 h has not previously been analysed and detailed behavioural time budgets of assisted and unassisted calves have not been described. Focal instantaneous sampling was used and is appropriate for the construction of time budgets (Altmann, 1974; Martin *et al.*, 2009a), allowing behaviour to be monitored when continuous observations would be impractical, for example over long durations of time or when multiple different behaviours need to be recorded simultaneously. Time budgets constructed from instantaneous sampling have been shown to correlate well with continuous observations of common or long duration behaviours in adult cattle (Mitlöhner *et al.*, 2001); however, infrequent or short duration behaviours can be missed using instantaneous sampling and consequently these behaviours can be underestimated (Chen *et al.*, 2016). Sample intervals of every 5 min every other hour were selected for use in the current study and are similar to sampling intervals suggested by Hämäläinen *et al.* (2016) for the analysis of feeding behaviours in adult cattle. Sample intervals of 5 min have also been found to be adequately correlated (R^2 = 0.79) with continuous observations of feeding duration in calves (Miller-Cushon *et al.*, 2011), although the same study found that correlation with meal frequency was low (R^2 = 0.39). With the exception of feeding (Miller-Cushon *et al.*, 2011), appropriate observation intervals for visual instantaneous sampling of calf behaviours have not been proposed, although short to medium length sample intervals are typically used. For example, Hill *et al.* (2013) described using 5 min sample intervals to analyse standing times in calves with animal-worn data logging devices and Chua *et al.* (2002) used 10 min sampling intervals for visual instantaneous sampling of calf behaviour, but neither has been validated against continuous observations to determine accuracy. Studies of adult cattle have found that intervals of 15 to 30 min are appropriate for analysis of longer duration behaviours such as standing and lying (Mitlöhner *et al.*, 2001; Chen *et al.*, 2016; Hämäläinen *et al.*, 2016); thus, time budgets of longer duration behaviours (e.g. lying) constructed in the current study are considered likely to be accurate. This contention is supported by Pullin *et al.* (2017) who found that 5 min sample intervals provided an accurate representation of lying, feeding and standing behaviours in lambs when compared to continuous observations (Pullin *et al.*, 2017). However, whilst 5 min sample intervals are likely to have accurately captured longer duration behaviours displayed by calves in this study, the proportion of the time budget calves engaged in short duration sporadic behaviours (e.g. play) may be underestimated. Although continuous observations have been suggested to be more appropriate for detecting short duration or sporadic behaviours (Lehner, 1992; Pullin *et al.*, 2017) their time consuming nature (Lehner, 1992), and the need to analyse multiple different types of behaviour, some of which occurred concurrently, made continuous observations impractical for use in this study. Behavioural analysis of all calves was performed by the same observer who used the same technique to analyse the behaviour of each calf and was blind to both assistance and treatment status. Therefore, any inaccuracies resulting from instantaneous sampling are likely to have affected all calves equally. Moreover, the identification of significant effects on infrequent behaviours in this study (e.g. play and self-grooming) suggests that the findings are robust despite the proportion of the time budget calves engaged in these behaviours possibly being underestimated. Whilst instantaneous sampling provides a reasonably accurate representation of length of time engaged in behaviours that have a long duration relative to the sample interval (e.g. lying behaviours), it does not allow for absolute duration of time engaged in more sporadic behaviours to be calculated (Martin *et al.*, 2009a). This is a limitation of the current study and further work using continuous analysis would provide valuable additional information to augment the data presented here. Advances in remote-monitoring technology has allowed animal behaviour to be monitored continuously over longer time periods more efficiently than continuous visual observations and has recently been validated for measuring play behaviour in calves (Gladden *et al.*, 2020). Similar work utilising remote monitoring devices to record the activity of newborn calves has been performed and is presented in Chapter 5.

In the current study calves could not be identified for 4.6% of observations. Whilst indirect observation using video footage prevents human presence affecting observed behaviours (unlike direct observations where this can be difficult to achieve), the possibility that subjects may be outwith the field of view of the camera at any particular sample point is a limitation of this method of observation that can be difficult to avoid (Martin *et al.*, 2009b). Inability of the observer to identify the subject animal in a group or technical failure causing loss of footage can also contribute to missing data (Payne *et al.*, 2017). To minimise the risk of the observer being unable to identify the subject calf, all calves were marked with agricultural marker spray and photographed from a minimum of three viewpoints to aid identification during visual observations. This method, together with the small group sizes, ensured that there were no instances where the subject calf could not be identified among a group. To minimise the likelihood of calves being out of the camera field of view, video footage of each pen was assessed on farm prior to starting data collection and the position of each camera was modified as needed to ensure visibility was maximised. Additionally, during data collection, cameras were regularly inspected to ensure the position had not moved (e.g. following impact from farm machinery). During camera positioning the small area of the pen directly underneath each camera was identified as an unavoidable blind spot where a calf could not be seen. The presence of these unavoidable, albeit small, blind spots underneath the cameras may explain the positive association between the proportion of observations that the calf was recorded as ‘not visible’ and the group size. It was noted during behavioural observations that calves chose to lie closer to the centre of the pen when the pen contained few calves but when the pen was more occupied, calves positioned themselves at the perimeter of the pen more often. Calves were most commonly out of view during the 0 to 12 h observation period because 42 calves (56%) were not moved into the calf pens within the first 2 h after birth. Most of these calves were moved into the calf pens by the end of the second observed hour (i.e. by 5 h after birth); however, this was still an unexpected finding as pilot work had indicated that most calves were moved into the calf rearing pens within the first 2 to 3 h. Consequently, some very early calf behaviours will have been missed however — as neither assistance status nor treatment status were associated with the proportion of observations that calves were not visible — this is unlikely to have affected the overall results.

3.5 Conclusion

Assisted birth is associated with reduced welfare of newborn calves for up to 48 h. Whilst these results suggest that this is in part due to pain, assisted calves also show behaviours consistent with excess fatigue — a further challenge to welfare. Additionally, the results presented here suggest that pain is experienced by all newborn calves (irrespective of birth assistance), a finding not previously reported. These results indicate that a single dose of ketoprofen immediately after birth has the potential to improve the welfare of newborn calves (regardless of birth experience), although there was some evidence that assisted calves may experience heightened benefits. Accordingly, administration of ketoprofen to calves (particularly after assisted birth) should be considered for inclusion in routine parturition protocols by farmers and veterinary surgeons seeking to optimise newborn calf welfare.

Chapter 4 Ketoprofen administration affects lying behaviours of cows experiencing both assisted and unassisted parturition

4.1 Introduction

Parturition is essential for dairy production but is widely considered likely to be both painful and stressful for cows, particularly if dystocia occurs (Huxley *et al.*, 2006, 2007; Kielland *et al.*, 2009; Laven *et al.*, 2009; Remnant *et al.*, 2017). Reported dystocia prevalence in cattle ranges from 2% to 7% (Mee, 2008) but the prevalence of assisted parturition consistently exceeds this, being reported as high as 50% by some authors (Kovács et al., 2016; Mee, 2008). Although parturition assistance is common, research into how assisted parturition affects welfare is limited and it is currently unclear how it is related to pain. It has been suggested that, due to an increased likelihood of iatrogenic soft tissue trauma, assisted parturition is likely to be more painful than unassisted parturition (Barrier *et al.*, 2012a; Schuenemann *et al.*, 2013); however, no data are available to support this belief. Furthermore, the study reported in Chapter 6 did not identify an assistance effect on maternal plasma creatine kinase concentration (a marker of muscle injury) — challenging the commonly accepted idea that assisted parturition inevitably causes some degree of tissue damage. Proudfoot *et al.* (2009) described increased pre- and postpartum 'restlessness' among cows experiencing dystocia (compared to normal parturition) and Barrier *et al.* (2012a) reported that assisted cows engaged in postpartum self-grooming behaviour less frequently than unassisted cows. The authors of both studies concluded that the behavioural differences identified were likely to be due to pain relating to parturition assistance, but neither study assessed the effects of analgesia and as such it is possible that other factors (e.g. exhaustion, stress) may explain the differences observed.

Non-steroidal anti-inflammatory drugs (NSAIDs) are known to provide effective and reliable pain relief to cattle following surgical procedures (Newby *et al.*, 2013b, 2014a; Barrier *et al.*, 2014) but few studies report the effect of NSAIDs on bovine postpartum pain, even though NSAIDs are commonly administered by veterinary surgeons following dystocia (Remnant *et al.*, 2017). Moreover, most available data have been obtained from studies investigating the effects of blanket treatment of all cows rather than the targeted treatment of assisted cows (Richards *et al.*, 2009; Stilwell *et al.*, 2014; Carpenter *et al.*, 2018), with the few studies that do report analgesic treatment of assisted cows typically not including an unassisted control group (Newby *et al.*, 2013a; Barrier *et al.*, 2014). Additionally, analgesic effects on subsequent production parameters (e.g. milk yield, reproductive performance) are the outcomes of interest most commonly assessed by existing studies (Richards *et al.*, 2009; Carpenter *et al.*, 2018) with very few studies assessing postpartum behaviour. Two previous studies have investigated the effects of analgesia and parturition assistance in a 2 x 2 design (Swartz *et al.*, 2018; Barragan *et al.*, 2020a); however, although cow activity was analysed (using remote devices), detailed behavioural analysis was not performed.

Although the effects of assisted parturition and postpartum analgesia have been studied separately in cattle, the paucity of studies that employ a factorial design means that it is still uncertain whether assisted parturition is a more painful experience for cattle than unassisted parturition, or whether parturition of any type is painful. It is well known that pain has a negative effect on welfare (Rutherford, 2002), so there is a need to establish whether the provision of immediate postpartum analgesia provides welfare benefits, and how this relates to assistance status.

Work presented in this chapter has been published (Gladden *et al.*, 2021); parts of the current chapter draw on this publication with permission from The Veterinary Record.

4.1.1 Study aims and hypotheses

Most parturition assistance is provided by farmers (Egan *et al.*, 2001), therefore the present study focused on farmer-provided assistance and aimed to establish how farmer-provided parturition assistance affects cow welfare in the first 48 h postpartum. A further aim was to determine whether immediate postpartum administration of the NSAID ketoprofen provides postpartum welfare benefits. Three hypotheses were tested:

1. Farmer assisted parturition is painful for cows and pain may be experienced for up to 48 h after parturition (as demonstrated by behavioural assessment).

2. Pain experienced by cows subject to assisted parturition will be ameliorated by the administration of ketoprofen within the first 3 h postpartum.

3. Farmer assisted parturition is more painful for cows than unassisted parturition, therefore cows subject to assisted parturition may experience greater beneficial effects of ketoprofen.

4.2 Materials and methods

4.2.1 Cow management and ethical approval

Adult purebred Holstein dairy cows (*Bos taurus*) were recruited; management was as described in Chapter 2, Section 2.2.1. Recruited cows were subject to the same management protocols and procedures as all other adult cows on the farm — cow management was not affected by either assistance or treatment status. UK Home Office approval for this study was obtained as described in Chapter 2, Section 2.1.

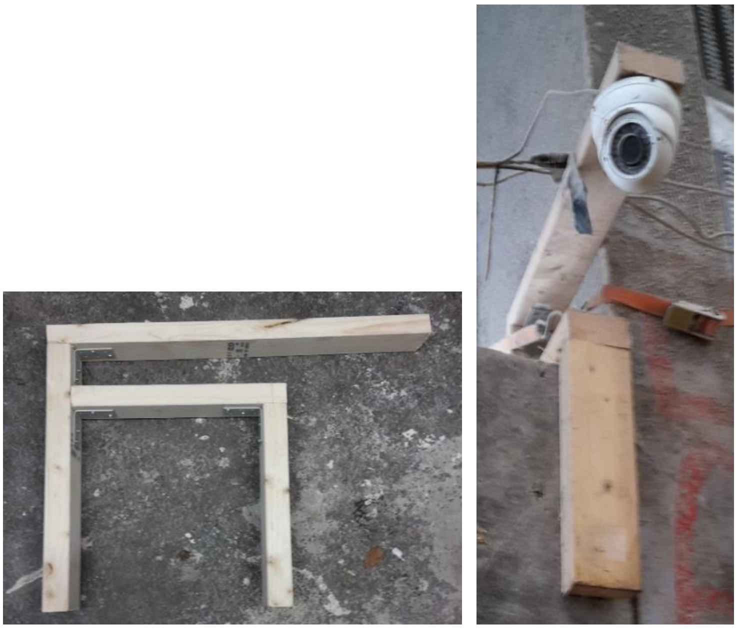
4.2.2 Animal recruitment and study design

Cows were recruited with their calves as paired units during a single period from 7th March to 15th December 2016. Cow-calf pairs were eligible for recruitment if the calf met the criteria described in Chapter 3 — recruitment of cows was therefore determined by the inclusion eligibility of their calf. Due to a communication error, one cow was recruited as an individual (i.e. its calf was not recruited to the study).

Farmer-assisted cows (as well as time matched unassisted cows) were recruited and randomly assigned to the same treatment group as their calves (see Chapter 2, Section 2.2.3). To aid identification, all recruited cows were marked on the lumbo-sacral area with agricultural marker spray and photographs (lateral, caudal, dorsal, and cranial views) of each animal were taken at the time of recruitment. Treatment selection, administration and recording protocols are described in Chapter 2, Section 2.3. Sequential coccygeal blood samples were obtained from all recruited cows on the day of parturition and also 24 h, 48 h and 7 d after parturition for the study reported in Chapter 6.

4.2.3 Data collection and behavioural monitoring

Closed circuit television (CCTV) cameras (Sony CCD, Vari-focal, 700TVL, Sony, Minato, Tokyo, Japan) were positioned to continuously film the parturition pen (four cameras) and the postpartum pen (three cameras) [Appendix 4]. Cameras were fixed onto wooden mounts designed and built during pilot work (Allison McGann, unpublished). Additional wooden mounts were built as required for this study using the same design [Figure 4.1]. Optimal camera positioning was determined by moving the cameras until the maximum area of both pens was visible.



a ***b***

Figure 4.1: Camera mount used to monitor cow behaviour in construction (a) and *in situ* (b)

Parturition related data were recorded contemporaneously by farm staff as described in Chapter 3, Section 3.2.2. Dam parity and postpartum disease data were obtained with permission from farm and veterinary records. Body condition score was assessed on a 1 to 5 scale by the author within 7 d of parturition and at 60 d postpartum (±3 d) using the Penn-State method (AHDB, 2020).

4.2.3.1 Video filing and storage

Footage was continuously filmed and stored on a DVR (Guardian II+ DVR 8 Channel, Digital Direct Security, Huntingdon, Cambridgeshire, UK) on farm. Twice weekly the on-farm DVR was replaced with another DVR and taken back to the University of Glasgow to enable the footage to be downloaded to an external hard drive (Toshiba 6TB Canvio desktop external hard drive, Toshiba Europe GmBH, Hammfelddamm, Neuss, Germany). This method was adopted (rather than downloading data on farm) due to it taking several hours to download footage. All recorded footage was downloaded in AVI format and the required 48 h of footage for each cow was selected and stored on one of three external hard drives (Toshiba 6 TB Canvio desktop external hard drive, Toshiba Europe GmBH, Hammfelddamm, Neuss, Germany); surplus footage was stored until completion of the data analysis and was then deleted. Video footage was saved as individual files for most cows; if more than one recruited cow calved on the same date these were grouped together into a single file to avoid duplication of video footage and save space. All files were labelled with the animal's identification number(s) (the last three or four digits of the official ear tag number) and the date of parturition. The time of parturition was recorded in the file name of the video file containing footage of the parturition event and the time that the cow was moved into the postpartum pen was recorded in the file name of the appropriate individual video file.

4.2.4 Behavioural analysis

4.2.4.1 Behaviour definition and recording methodology

A preliminary ethogram defined using published literature and pilot observations (Allison McGann, unpublished) was further refined to develop the ethogram used in the current study [Table 4.1]. Initial work established that focal instantaneous sampling (Altmann, 1974; Martin *et al.*, 2009a) at intervals of every twenty minutes, every other hour was appropriate. Cow behaviour was recorded every 20 min of every even hour postpartum (i.e. 0 h, 2 h, 4 h and so on); data recording began at the point that parturition was complete and ended at 48 h 40 min after completion of parturition. Completion of parturition was defined as the point at which the whole of the calf was expelled (anterior or posterior presentation), or the body up to and including the hips were fully expelled with the hindlimbs

Table 4.1: Ethogram of cow behaviours

(Gladden et al., 2021)

Behaviour	Description	Code	Category	Classification
Sternal recumbency	Cow is lying with the sternum in contact with the ground; the shoulder does not contact the ground. Hindlimbs may be positioned either one on each side of the body, or both on the same side of the body. Further defined by head position: head up [NU] — head is held in an elevated position not in contact with the ground or any part of the body. Head down [NH] — head is held in a rested position and any part of the head or face has contact with either the ground or any part of the body. If the body position can be identified but the head position cannot, head position is recorded as 'unknown' [NK].	NU/NH/NK	Lying behaviours[1]	Primary behaviours
Lateral recumbency	Cow is laying on one side with both forelimbs on one side of the body. The dependent shoulder is in contact with the ground. Further defined by head position: head up [RU] — head is held in an elevated position not in contact with the ground or any part of the body. Head down [RH] — head is held in a rested position and any part of the head or face has contact with either the ground or any part of the body. If the body position can be identified but the head position cannot, head position is recorded as 'unknown' [RK].	RU/RH/RK		
Unknown lying position	Cow can be determined to be lying but the body position cannot be identified from the footage available (e.g. only part of the cow is visible). Further defined by head position: head up [KU] - no part of the head or face is in contact with the ground or body. Head down [KH] - any part of the head or face is in contact with the ground or body. If the head position also cannot be determined this is recorded as unknown [KK].	KU/KH/KK		
Standing	Cow is in an upright position with all four limbs extended and in contact with the ground. The animal is not moving in any direction.	T	Active behaviours	
Walking	The cow is in an upright position with all four limbs extended and takes two or more consecutive steps in a forward direction.	W		
Feeding directed	The cow is standing at the feed face with the head positioned between the bars of the head yokes to enable access to feed.	F		Secondary behaviours
Drinking directed	The cow is positioned at the water trough with the face/nose directed to the water.	D		
Self-grooming	Cow is licking/nibbling/scratching self.	S		
Grooming others	Cow is licking/nibbling/scratching other adult cow(s).	O		
Social behaviours	Non-grooming behaviours directed towards other adult cows in the group — chin resting, head rubbing, suckling, mounting and aggressive interactions.	B		
Investigating calf[2]	Cow engages in licking/nuzzling/sniffing/head rubbing the calf.	C		
Allowing suckling[2]	Cow allows calf to teat seek and/or suckle. Calf is seen with nose intentionally at the ventral abdomen and/or udder or teats and this behaviour is allowed and/or encouraged by the cow.	A		
Other secondary behaviour	Cow engages in undefined secondary behaviours.	X		
Not visible	Cow is out of the field of view of the cameras or cannot be positively identified on footage for the sample point scored.	V		

1. Examples of lying behaviours are presented in Appendix 4.
2. Only recorded during the 0 to 12 h time period due to farm management practices.

partially remaining in the vagina (anterior presentation only) (Barraclough *et al.*, 2020). The timing of parturition was recorded to the nearest second from the video timestamp.

4.2.4.2 Behavioural observations

Observations of cow behaviour were performed using focal instantaneous sampling. Cow behavioural observations were performed in four time periods relative to the time of parturition: 0 to 12 h, 12 to 24 h, 24 to 36 h (18 observations each) and 36 h to 48 h postpartum (21 observations, as the 48th hour was included), resulting in a total of 75 behavioural observations being recorded for each cow. Maternal behaviour was classified as primary or secondary in the same way as neonatal behaviour (Chapter 3, Section 3.2.4.1). In contrast to neonatal primary behaviours, maternal primary behaviours comprised of one of only three mutually exclusive behavioural patterns: lying, standing, or walking [Table 4.1]. Similar to neonatal behaviours, maternal secondary behaviours were nested within primary behaviours and included grooming behaviours, feeding/drinking directed behaviours and social behaviours [Table 4.1].

Behavioural observations were entered into an Excel spreadsheet (Excel 2013, Microsoft, Redmond, Washington, USA) in the form of letter codes corresponding to the ethogram [Table 4.1]; an individual worksheet was completed for each cow. Where video footage was incomplete, cows were excluded if ≥ 24 h (i.e. ≥ 50%) of video footage were unavailable. If < 24 h were unavailable, cows were included, and data were recorded as ‘missing’ for relevant scans. Missing data were classified as Missing Completely at Random. If video footage were available but the subject cow could not be positively identified, a record of ‘not visible’ was made for that observation.

The number of observations of each behaviour were counted and a 48 h time budget was constructed for each cow. Both a total time budget (number of times a behaviour was observed divided by total number of available sample points) and a visible time budget (number of times a behaviour was observed divided by the number of sample points that the subject was visible) were calculated. The time and date of parturition, calf birthweight, parity (primiparous/multiparous) and postpartum disease status (Y/N) were also entered into each worksheet. Data were linked to each cow as identified by the last three or four digits of the cow's individual ear tag number. Due to the time that recording started and the direct visual nature of analysis, the observer could not be blinded to assistance status and was only blind to treatment status. Treatment status was entered into the Excel spreadsheet after all behavioural observations were complete.

4.2.5 Statistical analysis

4.2.5.1 Descriptive analysis

All raw data were combined into a single Excel spreadsheet (Microsoft Excel 2013, Microsoft, Redmond, Washington, USA) and exported into Minitab (Minitab v.18, Minitab Inc., State College, Pennsylvania, USA) for descriptive analysis. Graphs were produced in Minitab (boxplots) and Excel (bar charts).

4.2.5.2 Inferential analysis

Inferential statistical analysis of maternal behavioural data was performed by Dr. Jessica Martin, University of Edinburgh, UK. Data were exported into Genstat (Genstat v.14, VSN International, Hemel Hempstead, UK) and were analysed using Generalised Linear Mixed Models (GLMM) (negative binomial distribution with a log-link function). Rare behaviours accounting for less than 1% of the time budget were not analysed individually, although data obtained from observations of these behaviours did contribute to analysis of the relevant larger behavioural categories (e.g. the combined secondary behaviour category). For all analyses, the visible time budget was used and is hereafter referred to as the 'time budget'.

A priori, covariate data were selected for inclusion based on known or hypothesised effects on postpartum cow behaviour; hypothesised effects were informed by clinical knowledge. Assistance status (assisted/unassisted), treatment status (ketoprofen/placebo) and time period (0 to 12 h, 12 to 24 h, 24 to 36 h and 36 to 48 h) as well as treatment x assistance status interactions, treatment x time interactions and assistance status x time interactions were all included as fixed effects in each model. Covariate factors included calf birthweight, parity (primiparous or multiparous) and postpartum disease status. Cow identification number was included in each model as a random effect. Time period was included in the statistical model for all behaviours except for calf-directed behaviours (due to cow-calf separation occurring in the first time period). The other factors were included in all models.

Time budgets for each individual behaviour were analysed separately and were also combined into larger behavioural categories [Appendix 4]. Lying behaviour was further defined by head position, examples of individual lying behaviours are presented in Appendix 4. Additionally, different head positions were analysed separately as individual categories irrespective of body position [Appendix 4].

4.3 Results

4.3.1 Study population

In total 94 cows were recruited; 72 cows were included in the final analysis [Table 4.2].

Table 4.2: Number and percentage of cows excluded and included in the final behavioural analysis

Animals excluded from the final analysis			
Stage of exclusion	Reason for exclusion	*n*	Percentage[1]
Data collection	Illness	2	2.1
Video processing	Loss of ≥ 24 h video footage (technical failure)	18	19.1
Behavioural analysis	Recording errors	2	2.1
Total		*22*	*100*

Animals included in the final analysis		
Group	*n*	Percentage[2]
Assisted placebo (AP)	20	27.8
Assisted ketoprofen (AT)	17	23.6
Unassisted placebo (UP)	17	23.6
Unassisted ketoprofen (UT)	18	25.0
Total	*72*	*100*

1. Percentage of total number recruited (*n* = 94)
2. Percentage of total number included in behavioural analysis (*n* = 72)

Most parturition events occurred between 10 a.m. and 2 p.m.; data are further presented in Chapter 3, Section 3.3.1 and Appendix 3. Thirty-four (47.2%) multiparous cows (lactation range 2 to 8) and 38 (52.7%) primiparous cows were recruited, reflecting the wider farm population. Of the cows included in behavioural analysis, eight (11%) were subsequently diagnosed with postpartum disease (see Chapter 2, Section 2.2.1 and Appendix 2); six of these (75%) were assisted. Postpartum disease is further analysed and reported in Chapter 7.

4.3.2 Behavioural analysis

4.3.2.1 Descriptive results

Cows engaged in active and lying behaviours for 53.75% and 46.25% of the time budget respectively. The most common individual behaviour observed was standing (49%) [Figure 4.2].

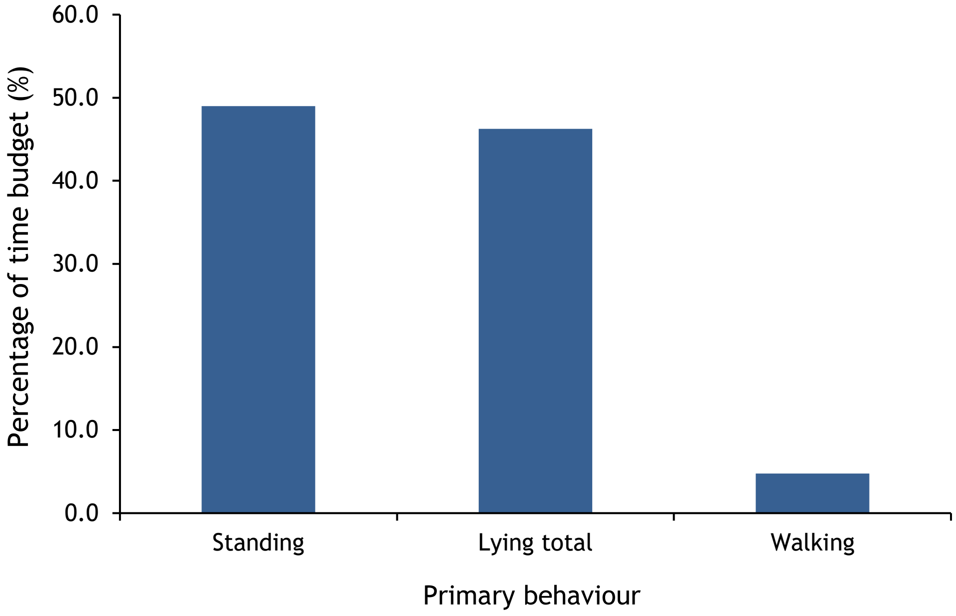


Figure 4.2: Proportion of visible time budget accounted for by each maternal primary behaviour
n = 72. Lying total = all lying behaviours combined into a single category.

Lying in sternal recumbency with the head elevated was the most commonly observed lying behaviour (26.2%); lying in sternal recumbency with head rested and lying in lateral recumbency (any head position) were less frequently observed (9.7% and 3.6% respectively). Combined secondary behaviours accounted for 23.4% of the time budget with feeding directed behaviour being the most common individual secondary behaviour (15.2%). The least common behaviours observed were lateral recumbency with unknown head position, lying in an unknown position, grooming others, and allowing the calf to suckle – these behaviours each represented less than 1% of the time budget and are not further presented.

Overall, the mean proportion of the time budget cows were recorded as not visible was 14.7% (range 0% to 90.5%); this was similar for each 12 h observation period. The most commonly recorded reason for cows being out of view of the cameras was due to cows leaving the postpartum pen to be milked — 59 cows (82%) were recorded as not visible for at least one observation for this reason. Due to differences in the timing of parturition relative to the fixed times of daily milking, the proportion of the time budget in each 12 h observation period spent milking was variable and ranged from 0% to 33.3% (mean 7.8%).

4.3.2.2 Effects of parturition assistance and ketoprofen treatment

Irrespective of treatment status, assisted cows spent more time in lateral recumbency both with their head rested (p = 0.049) and overall (p = 0.008) than unassisted cows [Table 4.3].

Ketoprofen treated cows spent less time in lateral recumbency with their head rested (p = 0.008) and less time in lateral recumbency overall (p = 0.031) than saline treated cows, regardless of assistance status [Table 4.4]. When lying in sternal recumbency, ketoprofen treated cows spent less time with the head elevated (p = 0.038) and more time with their head rested (p = 0.009) than saline treated cows [Table 4.4]. Ketoprofen treated assisted (AT) cows tended to spend more time engaged in feeding directed behaviours than cows in the other three interaction groups (p = 0.079).

Table 4.3: Mean (± SE) percentage of time budget engaged in different behaviours for cows experiencing assisted and unassisted parturition, irrespective of treatment status, and statistical differences (*p*-value and F-statistic)

Bold font indicates significant result. Back transformed results presented. Gladden et al. (2021) reproduced with permission.

Behaviour	Assisted parturition			Unassisted parturition			F-statistic	*p*-value
	Mean (± SE)	Min (%)	Max (%)	Mean (± SE)	Min (%)	Max (%)		
Lying in sternal recumbency, head elevated	26.9 ± 1.06	0.00	64.3	29.6 ± 1.07	0.00	83.3	0.77	0.381
Lying in sternal recumbency, head rested	11.0 ± 1.09	0.00	41.2	10.3 ± 1.12	0.00	38.9	1.73	0.190
Lying in lateral recumbency, head elevated	1.13 ± 1.37	0.00	14.3	0.08 ± 584	0.00	14.3	0.90	0.343
Lying in lateral recumbency, head rested	**1.28 ± 1.41**	**0.00**	**27.8**	**1.02 ± 1.45**	**0.00**	**15.4**	**3.92**	**0.049**
Walking	3.63 ± 1.20	0.00	31.3	3.06 ± 1.22	0.00	20.0	2.19	0.140
Standing	45.5 ± 1.23	5.90	100	46.4 ± 1.26	8.30	100	0.01	0.907
Feeding directed behaviour	15.7 ± 1.09	0.00	55.6	14.2 ± 1.06	0.00	50.0	0.54	0.465
Drinking directed behaviour	1.04 ± 1.35	0.00	16.7	1.72 ± 1.78	0.00	16.7	0.40	0.526
Grooming self	1.59 ± 1.26	0.00	12.5	1.95 ± 1.29	0.00	26.7	1.15	0.285
Total lying in sternal recumbency (any head position)	42.5 ± 1.04	0.00	92.9	43.4 ± 1.05	0.00	83.3	0.04	0.843
Total lying in lateral recumbency (any head position)	**2.78 ± 1.27**	**0.00**	**37.5**	**1.87 ± 1.31**	**0.00**	**28.6**	**7.12**	**0.008**
Total head elevated (all body positions)	28.4 ± 1.06	0.00	64.3	30.6 ± 1.09	0.00	83.3	0.29	0.588
Total lying	48.6 ± 1.04	0.00	92.9	47.6 ± 1.03	0.00	91.7	0.36	0.550
Total active behaviours	50.1 ± 1.04	7.10	100	50.3 ± 1.04	8.30	100	0.42	0.519
Total secondary behaviours	22.8 ± 1.06	0.00	66.7	22.0 ± 1.08	0.00	69.2	0.13	0.720

Assisted n = 35. Unassisted n = 37.

Table 4.4: Mean (± SE) percentage of time budget engaged in different behaviours for cows treated with ketoprofen or saline, irrespective of assistance status, and statistical differences (*p*-value and F-statistic).

Bold font indicates significant result. Back transformed results presented. Gladden et al. (2021) reproduced with permission.

Behaviour	Ketoprofen			Saline			F-statistic	*p*-value
	Mean (±SE)	Min (%)	Max (%)	Mean (±SE)	Min (%)	Max (%)		
Lying in sternal recumbency, head elevated	**25.8 ± 1.06**	**0.00**	**65.0**	**30.7 ± 1.07**	**0.00**	**83.30**	**4.35**	**0.038**
Lying in sternal recumbency, head rested	**12.1 ± 1.10**	**0.00**	**41.2**	**9.33 ± 1.15**	**0.00**	**33.30**	**7.02**	**0.009**
Lying in lateral recumbency, head elevated	0.34 ± 24.5	0.00	14.3	0.27 ± 24.5	0.00	14.30	0.35	0.554
Lying in lateral recumbency, head rested	**0.77 ± 1.45**	**0.00**	**18.2**	**1.70 ± 1.40**	**0.00**	**27.80**	**7.24**	**0.008**
Walking	3.79 ± 1.19	0.00	25.0	2.93 ± 1.23	0.00	31.30	2.19	0.685
Standing	46.9 ± 1.23	5.90	100	45.0 ± 1.25	7.10	100	0.01	0.915
Feeding directed behaviour	14.8 ± 1.09	0.00	55.0	15.1 ± 1.10	0.00	50.00	0.54	0.465
Drinking directed behaviour	1.22 ± 1.32	0.00	16.7	1.47 ± 1.31	0.00	16.70	0.40	0.526
Grooming self	1.73 ± 1.26	0.00	13.3	1.80 ± 1.28	0.00	26.70	0.10	0.756
Total lying in sternal recumbency (any head position)	42.6 ± 1.04	0.00	83.3	43.4 ± 1.05	0.00	92.90	0.02	0.894
Total lying in lateral recumbency (any head position)	**1.83 ± 1.29**	**0.00**	**18.2**	**2.85 ± 1.28**	**0.00**	**37.50**	**4.69**	**0.031**
Total head elevated (all body positions)	**27.1 ± 1.06**	**0.00**	**65.0**	**32.0 ± 1.07**	**0.00**	**83.30**	**4.14**	**0.043**
Total head rested (all body positions)	13.2 ± 1.09	0.00	47.1	12.3 ± 1.10	0.00	55.60	NA	0.264
Total lying	47.4 ± 1.04	0.00	83.3	48.9 ± 1.04	0.00	92.90	0.21	0.648
Total active behaviours	51.2 ± 1.04	16.7	100	49.2 ± 1.04	7.10	100	0.35	0.553
Total secondary behaviours	22.4 ± 1.07	0.00	66.7	22.3 ± 1.07	0.00	69.20	0.12	0.731

Ketoprofen n = 35. Saline n = 37

4.3.2.3 Effects of time period

Irrespective of assistance or treatment status, cows were most active (and engaged in lying behaviours least) during the first 12 h postpartum (both *p* < 0.001). When analysed as a combined behavioural category, secondary behaviours were also observed most frequently in the first 12 h postpartum (*p* < 0.001). Lying in sternal recumbency both overall and with the head elevated were most frequently observed in the 12 to 24 h and 36 to 48 h time periods (both *p* < 0.001). The same temporal pattern was observed for lying in sternal recumbency with the head rested (*p* < 0.001) [Figure 4.3]. Similarly, cows adopted an elevated head position (irrespective of body position) most frequently in the 12 to 24 h and 36 to 48 h observation periods (*p* < 0.001).

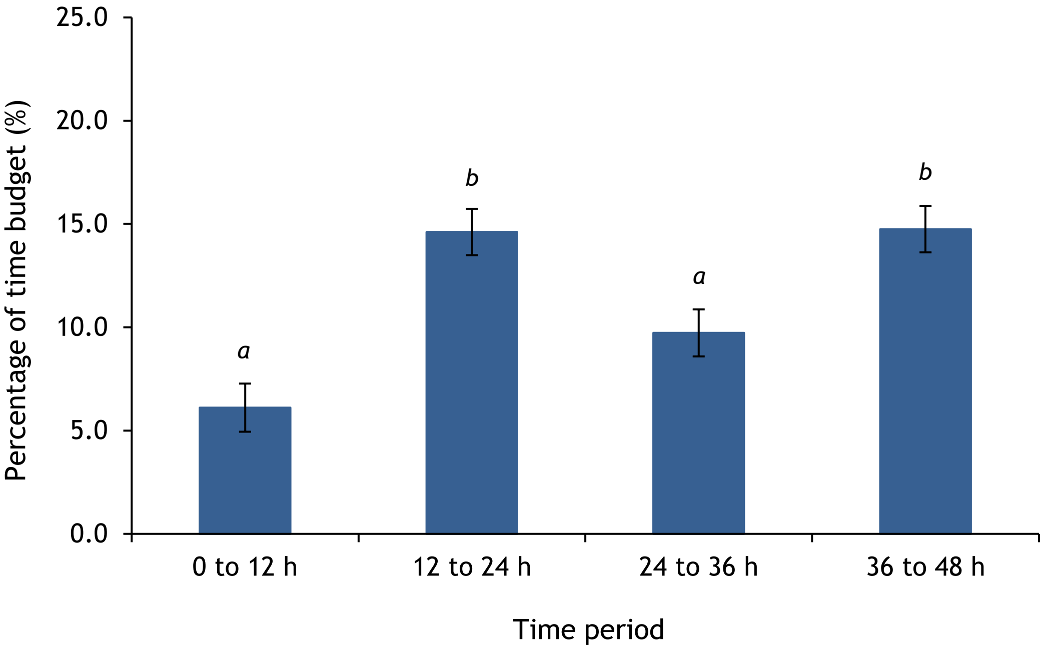


Figure 4.3: Mean (± SE) percentage of visible time budget spent lying in sternal recumbency with the head rested for each 12 h observation period irrespective of assistance or treatment status

Different letters indicate statistical differences (p < 0.001). Estimated marginal means (± SE) presented.

In the first 24 h postpartum, assisted cows showed a tendency to engage in drinking directed behaviours less frequently than unassisted cows (*p* = 0.063) (irrespective of treatment status). This effect was not observed beyond the first 24 h postpartum.

4.3.2.4 Effects of parity and postpartum disease

Cows that were subsequently diagnosed with postpartum disease spent more time lying in sternal recumbency both with the head elevated (p = 0.023) and overall (p = 0.013) than healthy cows.

Primiparous cows engaged in lateral recumbency with the head rested 2.5 times more frequently than multiparous cows (p = 0.002) [Figure 4.4]. Similarly, primiparous cows engaged in lateral recumbency (any head position) more than multiparous cows (p = 0.004).

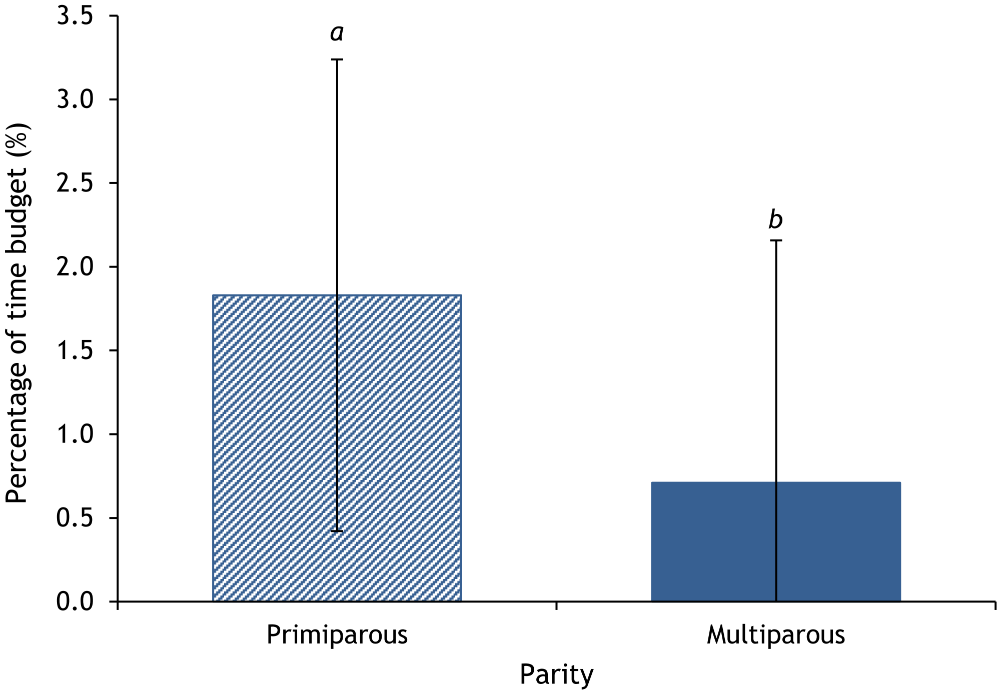


Figure 4.4: Mean (± SE) percentage of visible time budget spent in lateral recumbency with head rested for primiparous and multiparous cows, irrespective of assistance or treatment status.

Different letters indicate significant differences (p = 0.002). Primiparous cows represented by patterned bar (n = 34). Multiparous cows represented by solid bar (n = 38). Estimated marginal means (± SE) presented.

4.4 Discussion

4.4.1 Behavioural analysis

Standing was the most common behaviour observed, accounting for 49% of the time budget, matching previous studies which reported that group housed dairy cattle engage in standing behaviours for 50% of the time budget (both generally and in the postpartum period) (Endres *et al.*, 2007; Itle *et al.*, 2015). However, since cows were recorded as 'not visible' during milking (and cows stand to be milked), it is possible that the proportion of time spent standing was slightly underestimated in the present study. Cows engaged in lying behaviours for 46.3% of the time budget, a finding consistent with the widely accepted cumulative daily lying duration required by dairy cows (10 to 14 h, equivalent to 41% to 58% of the daily time budget) (Krawczel *et al.*, 2019). The most common lying posture was sternal recumbency with the head held elevated, followed by sternal recumbency with the head rested. These findings are similar to previous work that also found that lying cows most commonly adopt an elevated head position (Endres *et al.*, 2007), probably because cows adopt this posture to chew the cud — an important part of ruminant digestion.

4.4.1.1 Effects of parturition assistance and ketoprofen treatment

Regardless of treatment status, assisted cows spent more time in lateral recumbency (both overall and with the head rested) than unassisted cows. Lateral recumbency is infrequently adopted by cattle (Endres *et al.*, 2007; Petherick *et al.*, 2014; van Erp-van der Kooij *et al.*, 2019), and is a posture that is favoured when cows are in pain or diseased (Molony *et al.*, 1995; Petherick *et al.*, 2014). These results therefore suggest that postpartum discomfort is greater among assisted cows (compared to unassisted cows). By contrast, Barrier *et al.* (2012a) did not identify an assistance effect on maternal postpartum lying behaviours, but this was based on behaviour analysed for only 3 h postpartum. Lying behaviours were observed less frequently in the first 12 h postpartum (compared to later time points) in the current study, a finding consistent with the results of three recent studies that all found that postpartum lying behaviours increase over time (Campler *et al.*, 2019; Hendriks *et al.*, 2019; Perier *et al.*, 2019). Thus, it is possible that by focusing on the very early postpartum period Barrier *et al.* (2012a) may have missed assistance effects on maternal lying behaviours identified using a longer period of behavioural analysis.

Ketoprofen treated cows spent less time in lateral recumbency (overall and with the head rested) than saline treated cows, suggesting that ketoprofen analgesia improves postpartum comfort in all cows, irrespective of parturition experience. This hypothesis is further supported by the finding that, when lying in sternal recumbency, ketoprofen treated cows spent more time with the head rested than saline treated cows (irrespective of assistance status). A rested head posture is less commonly adopted by lying cattle than an elevated head posture (Endres *et al.*, 2007) but is typically adopted by cows in deep (rapid eye movement) sleep (Ruckebusch, 1975; Hunter *et al.*, 2021). Stress is known to adversely affect sleep in cattle (Ruckebusch, 1975) and it is possible that pain has a similar effect. Insomnia has been associated with postpartum pain in women (Sivertsen *et al.*, 2017), and the effect of ketoprofen treatment on head position suggests that parturition related pain may also adversely affect sleep in cattle. Nevertheless, it needs to be considered that sleep patterns of the cattle studied may also have been affected by stress resulting from recent cow-calf separation. All cows were separated from their calves at similar times relative to parturition and as such all cows will have been affected similarly, but individual temperament related differences in responses to cow-calf separation could not be controlled for; however, the randomised design of this study does mean that animals with a temperament predisposed to experiencing a greater stress response were equally likely to be assigned to each treatment group. Data describing sleep in cattle are limited and further work is needed to fully elucidate the effects of pain on sleep quality. Remote monitoring technology has shown promise as a possible tool for measurement of bovine sleep (Klefot *et al.*, 2016) and future technological advances may lead to more studies of bovine sleep and greater awareness of this important behavioural state.

Swartz *et al.* (2018) found that NSAID treated cows experiencing dystocia were less active (as measured by step count) than placebo treated dystocia cows and overall, dystocia cows showed fewer lying bouts of longer duration than cows experiencing eutocia. By contrast, Barragan *et al.* (2020a) did not identify a NSAID treatment x dystocia interaction but did find that overall dystocia was associated with increased lying and reduced activity in the first week postpartum. In contrast to Swartz *et al.* (2018) and Barragan *et al.* (2020a) the proportion of the time budget engaged in lying or active behaviours in the current study was not affected by either treatment or assistance status but lying postures were affected. Differences in data collection methods may explain the differences in findings: visual observations of behaviour were analysed in the current study, whereas Swartz *et al.* (2018) and Barragan *et al.* (2020a) monitored activity using animal-worn accelerometers that do not allow for analysis of behaviour in as much detail as visual observations and cannot easily record different lying or head postures.

It was hypothesised that assisted cows would experience greater benefits of ketoprofen analgesia (compared to unassisted cows), but no assistance x treatment status interaction effect was identified, although AT cows tended to engage in feeding directed behaviours more than cows in other groups. Accurate measurement of feeding behaviours of cattle can be difficult using visual observations and ‘feeding directed behaviour’ is typically defined as occurring when the cow has its head through the feed barrier (Chen *et al.*, 2016). Whilst this approach is straightforward, it does not account for cows that spend time at the feed face without eating and therefore results may not accurately reflect feed consumption or motivation to feed. This limitation is of particular importance if cows are restrained at the feed face for management purposes. Nevertheless, the importance of maintaining the appetite of postpartum cattle cannot be overstated and the tendency for ketoprofen treated assisted cows to spend increased amounts of time at the feed face (compared to unassisted or saline treated assisted cows) is an exciting finding that deserves further attention. In contrast to Swartz *et al.* (2018), no other assistance status x treatment status interaction effects were observed. Swartz *et al.* (2018) found that NSAID treated dystocia cows engaged in lying behaviours more than NSAID treated eutocia cows. However, Swartz *et al.* (2018) defined dystocia as parturition lasting ≥ 70 min and although parturition assistance (defined as human intervention to extract the calf) was recorded, the effects of assisted parturition were not reported. Although the effects of prolonged parturition on the calf have been studied (Vannucchi *et al.*, 2015a) there is a paucity of data on maternal effects. Prolonged parturition is reported by women to be associated with a high degree of pain (Nystedt *et al.*, 2006) and it is possible that prolonged parturition (even if unassisted) is more painful for cows than appropriately timed assisted parturition. Duration of parturition was not recorded in the current study as the start of 2nd stage parturition could not be accurately determined from the video footage. It is possible that cows experiencing prolonged parturition (irrespective of assistance status) may experience heightened benefits of analgesia. Further work is needed to investigate the effects of prolonged parturition on dairy cows and any possible welfare improvements that might be achieved through analgesic provision.

4.4.1.2 Effects of time period

Irrespective of assistance or treatment status, cows were most active (and engaged in combined secondary behaviours most frequently) during the first 12 h postpartum — a finding consistent with previous studies (Campler *et al.*, 2019; Hendriks *et al.*, 2019; Perier *et al.*, 2019). It is possible that, similar to other painful conditions (Siivonen *et al.*, 2011; Veissier *et al.*, 2017), this result is due to postpartum discomfort. However, ketoprofen treatment did not affect active behaviours, as might be expected if pain were the sole contributor to this finding. It is likely that postpartum activity is affected by multiple factors (e.g. stress or exhaustion), a view supported by the results presented in Chapter 6 that suggest that parturition-related stress is short term (lasting less than 24 h).

Cows were observed to be lying in sternal recumbency and adopt an elevated head position (irrespective of body position) more frequently in the 12 to 24 h and 36 to 48 h time periods than in the 0 to 12 h and 24 to 36 h time periods. Sternal recumbency is a posture commonly adopted by resting cows (Phillips, 2002) for the purposes of both sleep (Ruckebusch, 1975) and chewing the cud. Postpartum cattle rest more at night than during the day (Dechamps *et al.*, 1989) but as time in this study was expressed relative to the timing of parturition (not time of day) it is unlikely that this observed alternating pattern of resting behaviour is related to daylight. This finding may instead reflect effects of parturition on daily resting patterns of cattle that last for up to 48 h and might represent a normal recovery period following parturition where resting patterns are altered compared to later in lactation. This is the first study to analyse postpartum cow behaviour at a level of detail that enables the capture of different lying postures at 12 h observation intervals. It would be of interest for future studies to continue similar behavioural observations beyond 48 h postpartum to investigate the duration of this effect.

In the first 24 h postpartum assisted cows tended to spend less time engaged in drinking directed behaviours than unassisted cows. By contrast Proudfoot *et al.* (2009) found that cows experiencing dystocia (equivalent to grade 3 assistance in the present study) tended to drink more during the first 24 h postpartum than unassisted cows. Proudfoot *et al.* (2009) suggested that this finding may be related to heightened pain experienced by cows in the dystocia group, but this hypothesis was not further investigated and is not supported by the findings of the current study. Although painful events have been shown to affect suckling behaviours of calves (Turner *et al.*, 2020), the effect of pain on drinking behaviours of adult cattle is less certain and other factors affecting postpartum behaviour (e.g. exhaustion) may also contribute to this finding. This hypothesis is further supported by the absence of an identifiable ketoprofen effect on drinking behaviours, although it needs to be considered that the farm practice of providing an electrolyte solution immediately after parturition [see Chapter 2] may also have affected results. Further work is needed to investigate factors other than pain that might be affecting postpartum drinking behaviours of assisted cows. Identification of these factors might allow for farmers to identify cows that require additional rehydration support following parturition, thus further optimising postpartum health and welfare.

4.4.1.3 Effects of parity and postpartum health status

Irrespective of treatment or assistance status, primiparous animals spent a greater proportion of the total 48 h time budget lying in lateral recumbency with the head rested than multiparous animals. As lateral recumbency is an abnormal lying behaviour for cattle, this result suggests that primiparous cows may experience more pain following parturition (regardless of assistance status) than multiparous cows. Their smaller size and lack of parturition experience may result in primiparous cows experiencing more parturition-related muscle and uterine tissue trauma than multiparous cows, although plasma creatine kinase concentration (a biomarker of muscle injury) in the same animals as those reported here was not affected by age [see Chapter 6 and Appendix 6]. It is also possible that postpartum pain experienced by primiparous cows may be less responsive to NSAID analgesia than pain experienced by multiparous animals. The possibility of primiparous cows experiencing heightened postpartum pain is an interesting contrast to some human and pig studies that have reported increased postpartum pain in multiparous patients (Jangsten *et al.*, 2005; Ison *et al.*, 2018). Further study is warranted to enable greater insight into how postpartum pain in cows is affected by parity. Separate management of lactating primiparous and multiparous animals is already a familiar strategy on large dairy farms; a more comprehensive understanding of the relationship between parity and postpartum pain in cattle would allow farmers to extend this concept to management in the peri-partum period, thereby further improving peri-parturient bovine welfare.

Cows that were subsequently diagnosed with postpartum disease engaged in sternal recumbency with the head elevated more frequently than healthy cows — an unexpected finding. Sepúlveda-Varas *et al.* (2014) found that primiparous grazing cattle experiencing postpartum disease spent more time lying than healthy animals but lying postures were not reported and lying behaviours of multiparous animals were unaffected. Similarly, Piñeiro *et al.* (2019) found that postpartum disease was associated with increased lying times in the first six days after parturition; however, in contrast to Sepúlveda-Varas *et al.* (2014), only multiparous animals were affected (Piñeiro *et al.*, 2019). Whilst neither of these studies describe lying postures, the reported effects of postpartum disease on lying times are in contrast to the results of the current study which suggest that whilst postpartum disease is associated with differences in lying posture, the proportion of the time budget spent lying is unaffected. Differences in study design may explain the contradictory findings of the current study and earlier work; however, very few of the animals in the current study experienced postpartum disease, therefore findings should be interpreted with caution.

4.4.2 Study design and limitations

The 2 x 2 factorial design employed by the current study is considered optimal for establishing whether a defined behaviour is affected by pain or another factor (Weary *et al.*, 2006) but is rarely used in studies of bovine postpartum pain. Although optimal, the sensitivity of this type of design for detecting pain can be affected by different types of pain as well as variations in analgesic efficacy (Gleerup *et al.*, 2015). The current study aimed to optimise pain detection by using an analgesic drug with known efficacy in cattle (ketoprofen) for treating the type of pain expected in the immediate postpartum period (i.e. musculoskeletal and visceral pain related to inflammation) (National Office of Animal Health, 2020); but, as postpartum cows' experience of pain is not fully understood, it remains possible that not all pain experienced by cows in the current study was ameliorated by ketoprofen. Nevertheless, the results of the current study do suggest a beneficial effect of ketoprofen in the immediate postpartum period. The degree of analgesia provided is difficult to elucidate (and it is likely that there are variations between individuals) but, as pain has a negative effect on welfare (Rutherford, 2002), the identification of any improvement is important and will have positive welfare impacts.

An unavoidable limitation of studies where video footage is analysed is the possibility that subjects may be outside of the camera field of view for some observations (Martin *et al.*, 2009b). In the current study, cameras were positioned to maximise the field of view but there were still some observations for which the subject cow could not be identified, which may have meant some behaviours were underrepresented in the analysis. Nonetheless, there were no differences between assistance or treatment groups in the proportion of the time budget the cows were recorded as not visible; therefore, it is unlikely that the number of observations where the subject cow could not be identified affected the results. The primary reason for cows being out of the camera field of view was cows leaving the postpartum pen for milking. It was not possible to position cameras in the milking parlour and — as the timing of parturition could not be fixed in relation to the time of milking — it was also not possible to ensure all cows were affected for an equal proportion of the 48 h time budget. Being not visible due to milking accounted for 7.8% of the time budget which is similar to the proportion of the day accounted for by milking of postpartum cows on the study farm (approximately 120 min [8.3% of 24 h]). Moreover, the majority of studied cows were affected (although all cows were milked, the fixed times of milking relative to timing of parturition and the duration of milking (< 1 h) meant some cows were not milked during their observation periods), the primary behaviour of all cows is the same during milking (i.e. standing), and the provision for secondary behaviours to be expressed in the milking parlour is limited (cows are not fed during milking on the study farm) therefore it is unlikely that time spent milking affected the overall results. Outside of milking times, the most common reason for cows to be recorded as not visible was due to their position in the pen relative to the cameras. The lens design of the cameras allowed for a wide area to be captured but also meant that increasing distance from each camera was associated with a reduction of image quality. The design of the shed meant it was only possible to fix the cameras to the back and side walls of the pen [see Appendix 4], therefore the closer a subject was positioned towards the front or middle of the pen, the more difficult it was to positively identify. Additionally, the deep straw bedding obscured some ventral markings of lying cows and contributed to difficulties in identification. As a result, if cows were lying in a position far away from all three cameras, positive identification was not always possible. The postpartum pen on the study farm typically houses approximately 25 cows at any one time. Fewer cows in the pen might have made positive identification of subjects easier and reduced the proportion of time budget recorded as 'not visible' but, as the current study was performed on a commercial farm, altering the number of cows in the postpartum pen was not possible.

Instantaneous sampling is an appropriate sampling method for construction of time budgets (Altmann, 1974; Martin *et al.*, 2009a) and allowed behavioural analysis over a 48 h period for which continuous observation would have been impractical. The sampling interval used was established during the initial stages of video analysis and is similar to sampling intervals used in other studies of cattle behaviour (Mitlöhner *et al.*, 2001; Overton *et al.*, 2002; Neisen *et al.*, 2009; Mattachini *et al.*, 2013). Previous studies have found that instantaneous sampling intervals up to 30 min are well correlated with continuous observations for standing and lying behaviours in cattle but intervals of 15 min or shorter are needed to capture walking, drinking and feeding behaviours accurately (Mitlöhner *et al.*, 2001; Chen *et al.*, 2016; Hämäläinen *et al.*, 2016). The time budgets constructed for lying and standing behaviours in the current study are likely to be an accurate representation of the proportion of time engaged in these behaviours, but it is possible that the proportion of the time budget for more sporadic behaviours such as drinking, feeding, and walking are underestimated. Whilst this study was designed to allow for detailed behavioural analysis of cows for 48 h postpartum, a survey of the daily behavioural patterns of postpartum cattle was not the intended outcome. Rather, this study aimed to analyse the effects of parturition assistance status and analgesic treatment on cattle behaviour by comparing the time budget of animals in different groups. All individuals in this study were observed using the same methodology, therefore all would have been similarly affected by any inaccuracies, and so it is unlikely that the findings are affected.

The decision to intervene with parturition assistance was made by an experienced stockman and was not influenced by the researcher. Animals are monitored 24 h a day on the study farm and early parturition assistance is only provided in cases of calf malpresentation. Although this approach has been shown to be optimal for achieving a balance between the interests of the calf (i.e. achieving a timely delivery to reduce the risk of stillbirth) and the interests of the dam (i.e. allowing enough time to elapse for soft tissue relaxation and dilation to reduce the risk of iatrogenic parturition trauma) (Schuenemann *et al.*, 2011) a study of routine practices on dairy farms has indicated that it may not be reflective of the wider dairy industry (Egan *et al.*, 2001). Whilst performing this study on a single farm has ensured consistency of animal management, it does not allow for comparison of different farm approaches to parturition management which would be of interest for future studies. Few effects of assistance x treatment status interaction were identified in the current study, although behaviours were affected by both assistance status and treatment status individually. It is possible that — due to the optimised parturition assistance provided on the study farm — assisted cows did not experience as much soft tissue trauma (and associated pain) as cows subject to less optimal parturition management. Albeit difficult to determine, it is possible that on farms with less optimised parturition assistance protocols, a greater effect of assistance x treatment status interaction might be observed.

4.5 Summary

These results suggest that postpartum pain is experienced by all cows (irrespective of parturition experience) and that the NSAID ketoprofen has the potential to ameliorate postpartum pain (and thus improve welfare) for up to 48 h. Farmer-assisted parturition was associated with abnormal lying postures suggestive of an adverse effect on cow welfare. This finding was not affected by treatment status indicating that, although pain is experienced, there are likely to be other factors affecting postpartum cow welfare that are not affected by analgesia (e.g. exhaustion).

A single dose of ketoprofen in the immediate postpartum period has the potential to improve the welfare of all post-parturient cows for up to 48 h. As such, it is recommended that farmers and veterinary surgeons consider the routine provision of postpartum analgesia to optimise parturition protocols and improve post-parturient welfare on dairy farms.

Chapter 5 Remote monitoring of pre- and postpartum behaviour: prediction of assistance, and effects of postpartum ketoprofen treatment

5.1 Introduction

Animal behaviour is a valuable indicator of welfare and behavioural analysis is recommended to be included in on-farm welfare assessment protocols (Mellor, 2012). Whilst traditional visual observations based on an ethogram allow construction of very detailed behavioural time budgets, this type of analysis is labour intensive and time consuming (Barrell, 2019), and is a barrier to routine application of behavioural analysis as a tool for welfare assessment. Additionally, behaviour is most accurately recorded by continuous visual observations but this methodology is impractical when long observation periods are required (Martin *et al.*, 2009a). In recent years there has been increasing interest in the potential for automatic monitoring of behaviour using animal-worn devices. Pedometers (devices that measure the number of steps taken) were first explored as possible herd management tools (particularly as aids to oestrous detection) in the 1990s (Maatje *et al.*, 1997) and are widely used in the dairy industry today. More recently, advances in technology have enabled the development of animal-worn tri-axial accelerometers that can measure acceleration in three dimensions simultaneously, allowing for the generation of a data output that (when combined) provides a realistic representation of movement (Brown *et al.*, 2013). Step count and animal position (standing or lying) can also be measured by most commercially available accelerometers, providing more detailed information than traditional pedometers. Although accelerometer generated data are not as detailed as visual behavioural observations, this is compensated for by the advantages conferred by remote devices — notably the ability to generate continuous records of activity data over long periods of time. Accordingly, there is wide interest in the potential for utilising accelerometers to aid behavioural analysis, with domestic cattle being particularly well studied (Brown *et al.*, 2013). Accelerometers have been validated against visual observations for measuring lying behaviours in both cattle and calves (Martiskainen *et al.*, 2009; Borchers *et al.*, 2016; Finney *et al.*, 2018), as well as non-lying behaviours such as walking (Tullo *et al.*, 2016), locomotor play (Gladden *et al.*, 2020), and feeding (Tullo *et al.*, 2016; Zambelis *et al.*, 2019). Whilst accelerometer generated data have been used to detect adverse health events (Garcia *et al.*, 2014; Thorup *et al.*, 2015; Swartz *et al.*, 2017), this technology is rarely employed in the postpartum period with only three studies reporting the use of either maternal or neonatal accelerometer generated data immediately after parturition (Swartz *et al.*, 2018; Barragan *et al.*, 2020a; Gladden *et al.*, 2020). A method analogous to visual one-zero sampling for using IceTag generated data to accurately detect play behaviour was developed as part of previous work (Gladden *et al.*, 2020). This methodology was utilised in the current study to enhance the analysis of active behaviours and enabled the detection of play behaviour — a measure of positive welfare.

5.1.1 Study aims and hypotheses

The studies reported in Chapters 3 and 4 identified differences in the behaviour of cows and their calves treated with ketoprofen (compared to saline) suggestive of improved welfare, irrespective of assistance status. However, some of the behavioural changes were sporadic and of short-duration (e.g. play in calves) and based on intermittent scans of video recordings. The magnitude and patterning of change may be recorded more accurately using continuous observations. The aim of the current study was to investigate the effects of postpartum ketoprofen analgesia on cow and calf activity as measured by a tri-axial accelerometer. It was hypothesised that, although accelerometer generated data are less detailed than visual observations, such measures would capture beneficial ketoprofen effects on activity.

5.2 Materials and methods

5.2.1 Animal management and ethical approval

UK Home Office approval was obtained as described in Chapter 2, Section 2.1. In accordance with the regulations of A(SP)A, animals that were recruited to the studies reported in Chapters 3, 4, 6 and 7 were not recruited to the current study.

Animals were managed as described in Chapter 2, Section 2.2.2 and were subject to the same management protocols and procedures as all other animals (at the same life stage) on the farm.

5.2.2 Animal recruitment and study design

All healthy, non-lame, cows that were not recruited to the studies reported in Chapters 3, 4, 6 and 7 were eligible for inclusion; all recruited cows were purebred Holstein dairy cows (*Bos taurus*). Female Holstein calves and all Holstein x beef breed calves were initially eligible for recruitment but, due to logistic challenges related to the timing of their sale, recruitment of Holstein x beef calves was stopped early on in the study and calves that had been recruited thus far were excluded from further analysis. Unassisted and farmer-assisted cows and calves were eligible but, as the focus of this study was the effect of analgesia, the study design was not balanced for assistance status (unlike other studies in this thesis). Nevertheless, video footage obtained from cameras positioned on the main parturition pen (described in Chapter 4, Section 4.2.4, and Appendix 4) was used to confirm the recorded assistance status and the time of parturition (to the nearest second). For parturition events occurring in the second parturition pen, farm security camera footage was used for the same purpose. Parturition was considered to be complete when the whole body (up to and including the hips) of the calf was expelled. If video footage was not available, or the subject animal could not be identified, the timing of parturition was taken as recorded on the farm record.

Immediately following parturition or birth, cows and calves were randomly allocated to either a ketoprofen treatment group or a saline placebo group. Unlike other studies in this thesis, cows and calves were recruited individually (i.e. not as pairs) therefore randomisation was performed separately for cows and calves. Animals were randomised in blocks of 10 using a random number generator (*www.random.org*, 2020): odd numbers were attributed to the saline group and even numbers to the ketoprofen group. Treatments administered to both cows and calves (including the route of administration and the dose rate) were as described in Chapter 2, Section 2.3. The timing and recording of treatment administration were as described in Chapter 2, Section 2.3. The ketoprofen used in this study was manufactured by Ceva UK (Ketofen 10%, Ceva Animal Health Ltd., Amersham, Buckinghamshire, UK). Calves were weighed at birth by farm staff and the body condition score (BCS) of cows was assessed and recorded by the author at the first visit following parturition (0 to 5 d postpartum). A voided (free-catch) urine sample was obtained from a subset of recruited calves (*n* = 18 for each treatment group) at approximately one week of age to measure urine specific gravity (USG) as an indicator of renal function.

Details regarding recruited animal identification number (ID), animal category (cow/calf) the date and time of parturition (or birth), assistance grade (1, 2, 3), and the person providing assistance were recorded for all animals. The time of IceTag placement, birthweight, and the amount of colostrum consumed were recorded for calves only. All records were made contemporaneously by farm staff in a laminated chart provided and kept in the farm office. These data were photographed (iPhone SE [2nd gen.], Apple Inc. Cupertino, CA, USA) and manually transcribed into an Excel spreadsheet (Microsoft Excel v.1908, Microsoft, Redmond, WA, USA) twice weekly throughout the duration of the project.

At the time of recruitment, all cows and calves were marked on the lumbosacral region with coloured agricultural marker spray to identify recruited animals as subject to the controls of A(SP)A; this type of marking was not used for any other purpose on the farm. To enable further confirmation of identification of subjects on the video footage, photographs were taken of all recruited animals by the author (iPhone SE [2nd gen.], Apple Inc).

5.2.3 Accelerometer technology employed to monitor maternal and neonatal activity

The activity of both cows and calves was monitored remotely using commercially available tri-axial accelerometers (IceQube and IceTag, IceRobotics, South Queensferry, Scotland, UK) positioned on the lateral aspect of one hindlimb. Maternal activity was monitored using the IceQube tri-axial accelerometer (IceRobotics, Scotland). This is a small, approximately cube shaped device [Figure 5.1] that was held in place using a plastic strap that could be cleaned and reused on multiple cows [Figures 5.2 and 5.3].

Figure 5.1: IceQube tri-axial accelerometer

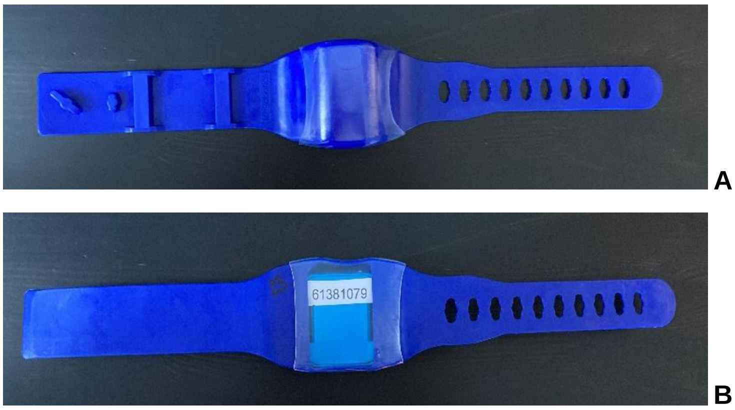


Figure 5.2: IceQube tri-axial accelerometer positioned within a plastic strap for animal placement *A: Front view (faces away from the animal). B: Back view (in contact with the skin)*

Figure 5.3: IceQube placement on the lateral aspect of one hindlimb

Due to its higher data collection frequency (more suited to capturing sporadic behaviours), neonatal activity was monitored using the IceTag tri-axial accelerometer (IceRobotics, Scotland). The IceTag is a small, cuboid device

[Figure 5.4] and was held in place using cohesive bandage (Wrapz cohesive bandage, Millpledge Veterinary, Clarborough, Notts., UK) [Figure 5.5]. Prior to placement, IceTags were placed inside a fabric child's sock to provide cushioning and ensure calf comfort using a method developed during pilot work (Katie Ord, unpublished).

Figure 5.4: IceTag tri-axial accelerometer
From L to R: Back view (in contact with the leg), front view (faces out from the animal), side view.

Figure 5.5: IceTag placement on the lateral hindlimb using cohesive bandage

Thirty IceQubes and six IceTags were used in rotation to monitor activity of cows and calves respectively. Data collection frequency, available output resolutions, and data storage ability differ between the two devices [Table 5.1].

Table 5.1: Comparison of IceQube and IceTag accelerometers
Adapted from https://www.icerobotics.com/researchers/#sensor-specifications (IceRobotics, 2021)

	IceQube	IceTag
Dimensions	55 mm x 55 mm x 27 mm	66 mm x 55 mm x 27 mm
Weight	74.5 g	117 g
Data collection frequency	4 Hz	16 Hz
Storage time	10 d and 4 d options available	60 d
Output resolution	-	1 s
	-	1 min
	15 min	15 min
	1 h	1 h
	2 h	2 h
	1 d	1 d
	2 weeks	1 week
	overall summary[1]	overall summary[1]

1. Cumulative data output for a complete time period as defined by the user (e.g. 13:00:00 to 01:00:00)

Both devices contain an accelerometer that measures acceleration in three dimensions and combines the generated data to produce an output that accurately represents the animal's activity. Standing activity is recorded when the device is vertically orientated, lying activity is recorded when the device is horizontally orientated, and a lying bout is recorded when the device is recorded to move from vertical to horizontal and back to vertical orientation. Accelerometer generated data are downloaded into the IceManager software (IceManager, IceRobotics, Scotland) (IceRobotics, 2019) and a report is generated that can be exported in comma-separated values (CSV) format for further analysis. The overall format of the data output is determined by the manufacturer; however, the generated report can be modified by the user to meet their own needs. Output resolution (i.e. sample interval) is selected by the user from a range of pre-determined options [Table 5.1]. Variables included in reports produced by the IceManager software are presented in Table 5.2; reports can also be modified to include the animal ID and other details as required. Standing time, lying time and motion index (MI) can be generated in numerical or graphical formats but step count and number of lying bouts (LB) are only available in numerical format. Generated reports are the same for both the IceQube and IceTag devices, with the exception of the differing availability of output resolutions for each device [Table 5.1]. As well as the summarised number of LB reported in the primary report, detailed LB data (start date/time, end date/time, and individual LB duration) can also be generated.

Table 5.2: Variables included in reports produced by IceManager software for data generated by IceQube and IceTag accelerometer devices

Variable	Definition
Device ID	Eight digit identification number of the device (determined by the manufacturer)
Date of sampling	The date that data were recorded
Time of sampling	The time that data were recorded
Lying/standing time	The duration of time spent lying and standing in the defined sampling period respectively. Both lying and standing time are available as either absolute duration (hh:mm:ss) or as a proportion of the total time budget (%)
Step count	The number of steps taken in the defined sampling period
Number of lying bouts	The number of lying bouts in a defined sampling period. A single lying bout is defined as the animal moving from standing to lying (recorded when the device orientation changes from vertical to horizontal) and back to standing again (recorded when the device orientation changes from horizontal to vertical)
Motion index (MI)	A proprietary metric that indicates the magnitude (i.e. vector sum) of three-dimensional acceleration in each second summed over the period of interest. The value of MI is equivalent to the gravitational acceleration applied (during the period of interest) x 10.

5.2.4 Accelerometer data generation and processing

IceQubes were positioned on the lateral aspect of one hindlimb of adult cows [Figure 5.3] approximately one week prior to the expected parturition date. IceTags were positioned on the lateral aspect of one hindlimb of calves after birth at the same time as treatment administration. Prior to placement, all devices were activated using an IceReader wireless download device (IceReader, IceRobotics, South Queensferry, Scotland) [Figure 5.6] together with the IceManager software (IceManager, IceRobotics, Scotland).

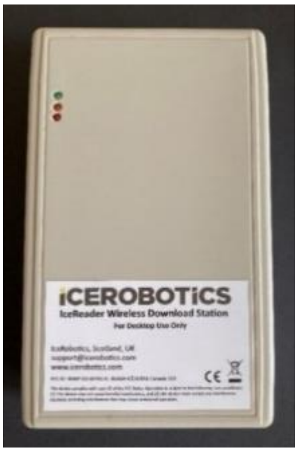


Figure 5.6: IceReader wireless download station

5.2.4.1 Accelerometer data extraction

Twice weekly, devices were removed from cows and calves (by the author with assistance from farm staff) after 48 h had elapsed postpartum. IceQubes were removed from standing cows in the milking parlour during midday milking (11:15 to 12:00 for postpartum cows). At the time of IceQube removal the ID number of the cow from which the IceQube had been removed was immediately written on each IceQube to ensure correct identification of the data (this number was removed after data were downloaded). Prior to analysis this was cross-referenced with the IceQube number recorded at placement to confirm that the data file was correctly labelled. IceTags were removed from standing or lying calves (on the same day as cows) at any available time (although this was usually prior to milking). At the time of IceTag removal the ID of each calf and the number of the IceTag removed from that calf, as well as the time of removal, were all recorded. Prior to analysis the IceTag and calf ID were cross-referenced with the placement records to confirm correct identification of data. IceQube and IceTag generated data were extracted from each device individually using the IceReader wireless download station [Figure 5.6] and the IceManager software. Raw data files were labelled automatically by the IceManager software and file names were modified at the time of download to include the animal ID for identification during data storage and analysis.

5.2.4.2 IceQube data processing (maternal data)

Overall summary data (MI, step count, number of LB, lying and standing time) were exported in CSV format to Microsoft Excel (Microsoft Excel v.1908, USA) in four defined postpartum time periods (0 to 12 h, 12 to 24 h, 24 to 36 h and 36 to 48 h) and the same time periods pre-partum (i.e. 0 to −12 h, −12h to −24 h and so on) and combined into a single spreadsheet for analysis of each dataset. Detailed LB data (date/time and duration of individual LB) were exported separately in the same format (due to separate generation of these data in IceManager).

5.2.4.3 IceTag data processing (neonatal data)

As IceTags were placed on calves at variable times after birth (mean 01:52:51; range 00:03:06 to 03:31:32), commencement of neonatal data analysis was standardised to start at 3 h after birth (i.e. the first 3 h of life were excluded from analysis thus a total of 45 h of data were analysed). This start time for data analysis was selected as the majority of IceTags (93%) were placed before 3 h old and calves are typically moved into calf pens in the first 3 h of life.

Overall summary data for each 12 h sample period as well as the raw LB data were exported and processed as described in Subsection 5.2.4.2. In addition, data for the same defined sample periods were exported at output resolutions of 1 min and 15 min for the analysis of play behaviour as described in Gladden *et al.* (2020) and combined in a single Excel spreadsheet.

5.2.5 Statistical analysis

All data were summarised in an Excel spreadsheet (Microsoft Excel v.1908, USA) and exported to SPSS (v.27, IBM, Armonk, NY, USA) for further analysis. Maternal pre-partum data were exported to Minitab (v.19, Minitab, State College, PA, USA) for Classification and Regression Tree (CART) analysis. Descriptive analyses were performed in SPSS (v. 27) and Microsoft Excel (v.1908). Graphs were produced in Microsoft Excel (v.1908).

Chi-squared tests for association and Mann-Whitney U tests (Minitab v.19) were used to check relationships between parturition metadata and treatment status. As USG results were positively skewed, the Mann-Whitney U test was performed (Minitab v.19) to compare results for calves in each treatment group.

5.2.5.1 Data processing

Log_{10} transformation was applied to maternal MI and step count data and neonatal MI data to meet residual data distribution assumptions. In accordance with the manufacturer's advice, neonatal step count data were not analysed as these data are not accurately recorded for calves. The proportion of the time budget engaged in lying and standing behaviours were used for analysis of lying and standing behaviours. Absolute standing and lying duration (hh:mm:ss) were analysed descriptively only.

Short LB of duration < 33 s (maternal data) and ≤ 8 s (neonatal data) were classified as false LB records and removed prior to analysis. These thresholds were chosen based on previous studies assessing the reliability of IceQube generated LB data for cows (Kok *et al.*, 2015) and calves (Finney *et al.*, 2018). Filtered LB data were summarised in an Excel spreadsheet (v. 1908) and exported to SPSS (v.27) for further analysis.

Motion index thresholds of ≥ 23 for 1 min interval analysis and ≥ 62 for 15 min interval analysis were selected to detect play behaviour in accordance with methodology validated by Gladden *et al.* (2020). The number of sample intervals in each 12 h time period exceeding the relevant MI threshold were calculated and all data were combined in an Excel spreadsheet that was exported to SPSS (v.27) for further analysis.

5.2.5.2 Multivariate analysis

Prior to analysis, data distributions were assessed visually using histogram plots for each variable. Residual and histogram plots were examined for all models to ensure model assumptions were met. Proportion of the time budget engaged in lying and standing behaviours, motion index and step count were all analysed using Linear Mixed Models. Neonatal LB and play data (1 min intervals) were analysed using Generalised Linear Mixed Models (GLMM) negative binomial distribution (log link). Neonatal play data (15 min intervals) and maternal LB data were analysed using GLMM Poisson distribution (log link). Animal ID was entered into all models as random effect to account for repeated measures. Effects included in each model are presented in Table 5.3.

Table 5.3: Fixed, covariate and interaction effects included in statistical models used for multivariate analyses
X indicates included effect

Type of effect	Variable	Analysis: Maternal pre-partum behaviour	Analysis: Maternal postpartum behaviour	Analysis: Neonatal behaviour
Fixed effects	Treatment (ketoprofen/saline)		X	X
	Assistance status (assisted/unassisted)	X	X	X
	Time period[1]	X	X	X
	Parity/dam parity (primiparous/multiparous)	X	X	X
	Season (summer/autumn)	X	X	X
	Time of parturition/birth (early morning, morning, lunchtime, afternoon, evening, and night)	X	X	X
	Parturition/birth site (1 or 2)	X	X	X
	Body condition score	X	X	
	Postpartum disease (Y/N)	X	X	
Covariates	Calf birthweight			X
	Volume of colostrum consumed (L)			X
Interaction effects[2]	Treatment x assistance status		X	
	Treatment x time period		X	X
	Assistance x time period	X		

1. 12 h time period relative to timing of parturition. 0 to −12 h, −12 to −24 h, −24 to −36 h and −36 to −48 h for pre-partum analyses and 0 to 12 h, 12 to 24 h, 24 to 36 h and 36 to 48 h for postpartum analyses.

2. Assistance interactions were not analysed for neonatal data due to the small number of assisted calves ($n = 6$)

Factors were sequentially removed using backward elimination if $p > 0.1$ and Corrected Akaike's information criterion (AICc) were compared to aid selection of the final model. Statistical significance was set at a threshold of $p < 0.05$ for all tests. A statistical tendency was considered if $p \geq 0.05$ and $p < 0.08$.

Associations between pre-partum IceQube generated data and assistance status were explored using a case-control design with each measure of activity analysed separately (as the outcome variable), in an approach similar to Barraclough *et al.* (2020). Predictive analysis was performed using Classification and Regression Trees (CART) (Breiman *et al.*, 1984).

5.2.5.3 Classification and Regression Tree analysis

Following regression analysis [Subsection 5.2.5.2], CART analysis was performed to investigate the ability of selected IceQube generated data to predict the timing of parturition and to identify cows more likely to need assistance. Classification and Regression Tree analysis is a recursive partitioning process that employs binary splitting of a dataset to construct a decision tree that can be used to make clinical predictions (Izenman, 2013; Zimmerman *et al.*, 2016). Results of regression analysis were used to inform the data included in CART analysis. Validation was performed using 5-fold cross-validation.

Discriminant analysis was performed to assess the performance of data thresholds selected using CART. Sensitivity (true positive rate) and specificity (true negative rate) were obtained from the statistical output of CART analysis. The confusion matrix (contingency table) produced by CART analysis was exported to Microsoft Excel for calculation of positive and negative predictive values where appropriate (Dohoo *et al.*, 2009).

5.3 Results

In total 88 cows and 90 calves were recruited. Eight cows and 10 calves were excluded prior to data analysis [Table 5.4].

Seventy-eight cows (97.5%) were included in both pre-partum and postpartum analyses; two cows (2.5%) were included in pre-partum analysis only (due to missing data [n = 1] and a treatment recording error [n = 1]) and two cows (2.5%) were included in post-partum analysis only (both due to incomplete pre-partum data).

Technical failure affected three IceQubes (one failure episode each) and four IceTags (three IceTags experienced a single failure, and one was affected twice).

Urine specific gravity was not affected by treatment status. Data are presented in Appendix 5.

Table 5.4: Cows and calves excluded prior to analysis of each dataset

Excluded animals	Reason	*n*
Cows excluded prior to pre-partum analysis	IceQube technical failure	3
	Incomplete data	2
	Illness/injury	2
	Recording errors	1
	Total	*8*
Cows excluded prior to postpartum analysis	IceQube technical failure	3
	Incomplete data	1
	Illness/injury	2
	Recording errors	2
	Total	*8*
Calves excluded prior to analysis	IceTag technical failure	5
	IceTag detached from animal	2
	Beef-cross breed	2
	Illness	1
	Total	*10*

5.3.1 Maternal pre-partum behavioural analysis

5.3.1.1 Study population and descriptive analysis

Eighty cows were included in the final analysis. Parity and frequency of assistance and postpartum disease status are presented in Table 5.5. Seventeen cows (21.3%) were subsequently diagnosed with postpartum disease: 15 (88%) were multiparous animals and three (17.6%) had experienced assisted parturition. Forty-five cows (56.3%) were recruited during the summer months (June – August), the remaining 35 cows (43.8%) were recruited in the autumn (September – November).

Lying and standing behaviours accounted for 54.4% (26 h 5 min) and 45.6% (21 h 54 min) of the pre-partum 48 h time budget, respectively. Mean pre-partum step count was 1357 (range 551 to 3537) and mean MI was 5486 (range 2566 to 12707). Cows engaged in 32.2 LB (range 12 to 54) of mean duration 48 min 35 s (range 00:00:33 to 06:28:46) in the 48 h pre-partum. Ninety-eight (3.7%) false LB of mean duration 10 s (range 2 to 32 s) were recorded; the cumulative duration of false LB recorded for all cows was 16 min 32 s, accounting for 0.007% of the cumulative pre-partum time budget for all cows (80 x 48 h).

Table 5.5: Number and proportion of cows in each assistance status, parity and postpartum disease status group and number diagnosed with each individual postpartum disease

Parameter	Variable	*n*	Proportion[1] (%)
Assistance status	Unassisted	57	71.3
	Assisted	23	28.8
Parity	Primiparous	22	27.5
	Multiparous[2]	58	72.5
Postpartum disease overall	No	62	77.5
	Yes	17	21.3
	Missing[3]	1	1.3
Individual postpartum diseases[4]	Subclinical ketosis	7	41.2
	Metritis	5	29.4
	Retained foetal membranes	7	41.2
	Left displaced abomasum	0	0
	Clinical hypocalcaemia	0	0

1. Assistance status, parity and postpartum disease status presented as a proportion of all animals (*n* = 80). Individual postpartum diseases presented as a proportion of animals diagnosed with postpartum disease (*n* = 17).
2. Lactation numbers 2 to 8
3. Results of postpartum examination not recorded for one cow (missing data).
4. Individual postpartum disease sum greater than 100% due to some cows being diagnosed with more than one disease concurrently.

5.3.1.2 Relationships between pre-partum activity and assistance status

Irrespective of time period, there was a tendency for number of LB to be higher in assisted cows than unassisted cows in the 48 h pre-partum (p = 0.064) [Figure 5.7]. Similarly, step count also tended to be higher in assisted cows (p = 0.054) [Figure 5.7], suggesting that assisted cows spend more of their non-lying time walking than unassisted cows.

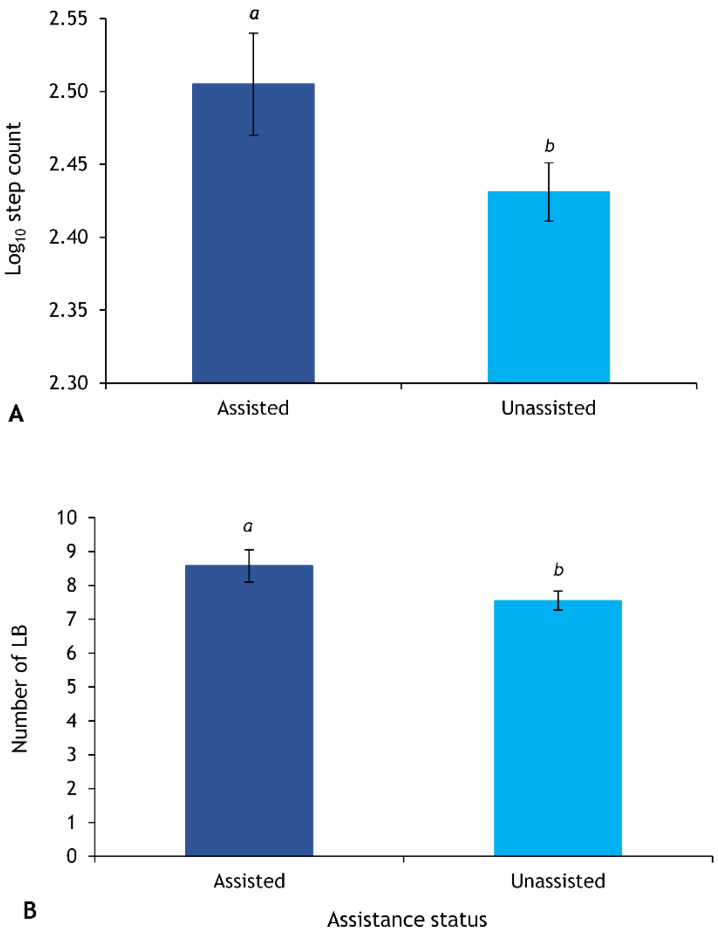


Figure 5.7: Mean (± SE) pre-partum $\log_{10}$ step count [A] and lying bouts [B] for assisted and unassisted cows.

Different letters indicate tendency toward significant results (p = 0.054 for step count and p = 0.064 for lying bouts). Assisted cows represented by dark blue bar (n = 23). Unassisted cows represented by light blue bar (n = 57). Estimated marginal means (± SE) presented.

5.3.1.3 Effects of covariates

Cows that calved in the morning (06:00 to 09:59) had higher step count (p = 0.035) and MI (p = 0.020) than cows that calved in the evening (18:00 to 21:59) [Figure 5.8]. Additionally, cows that calved in the early morning (02:00 to 05:59) had higher MI than cows that calved in the evening (p = 0.020) [Figure 5.8].

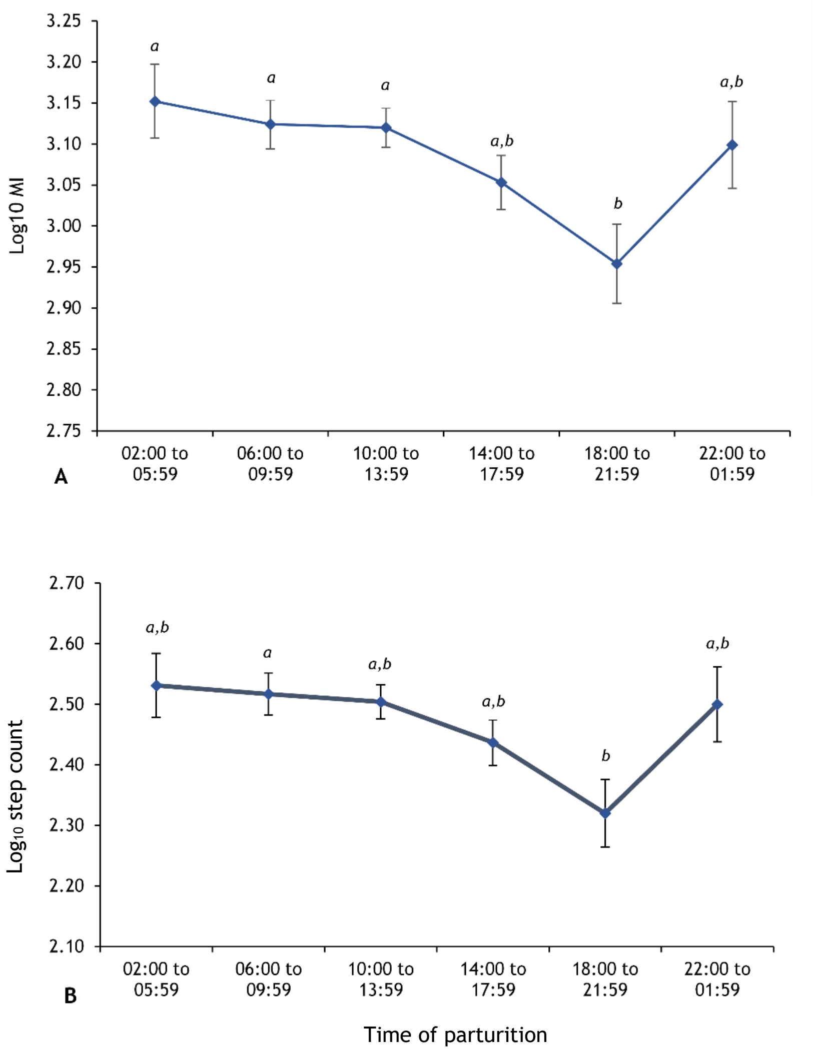


Figure 5.8: Association between pre-partum mean (± SE) $\log_{10}$ MI [A] and $\log_{10}$ step count [B] and time of parturition

Different letters indicate significant differences ($p = 0.020$ for $\log_{10}$ MI; $p = 0.035$ for $\log_{10}$ steps). 02:00 to 05:59 $n = 8$. 06:00 to 09:59 $n = 18$. 10:00 to 13:59 $n = 26$. 14:00 to 17:59 $n = 15$. 18:00 to 21:59 $n = 7$. 22:00 to 01:59 $n = 6$. Estimated marginal means (± SE) presented.

All analysed behaviours were affected by time period irrespective of subsequent assistance status (all $p < 0.001$) [Figure 5.9]. The number of LB recorded in the final 12 h pre-partum was double that recorded in the other time periods ($p < 0.001$) [Figure 5.9E].

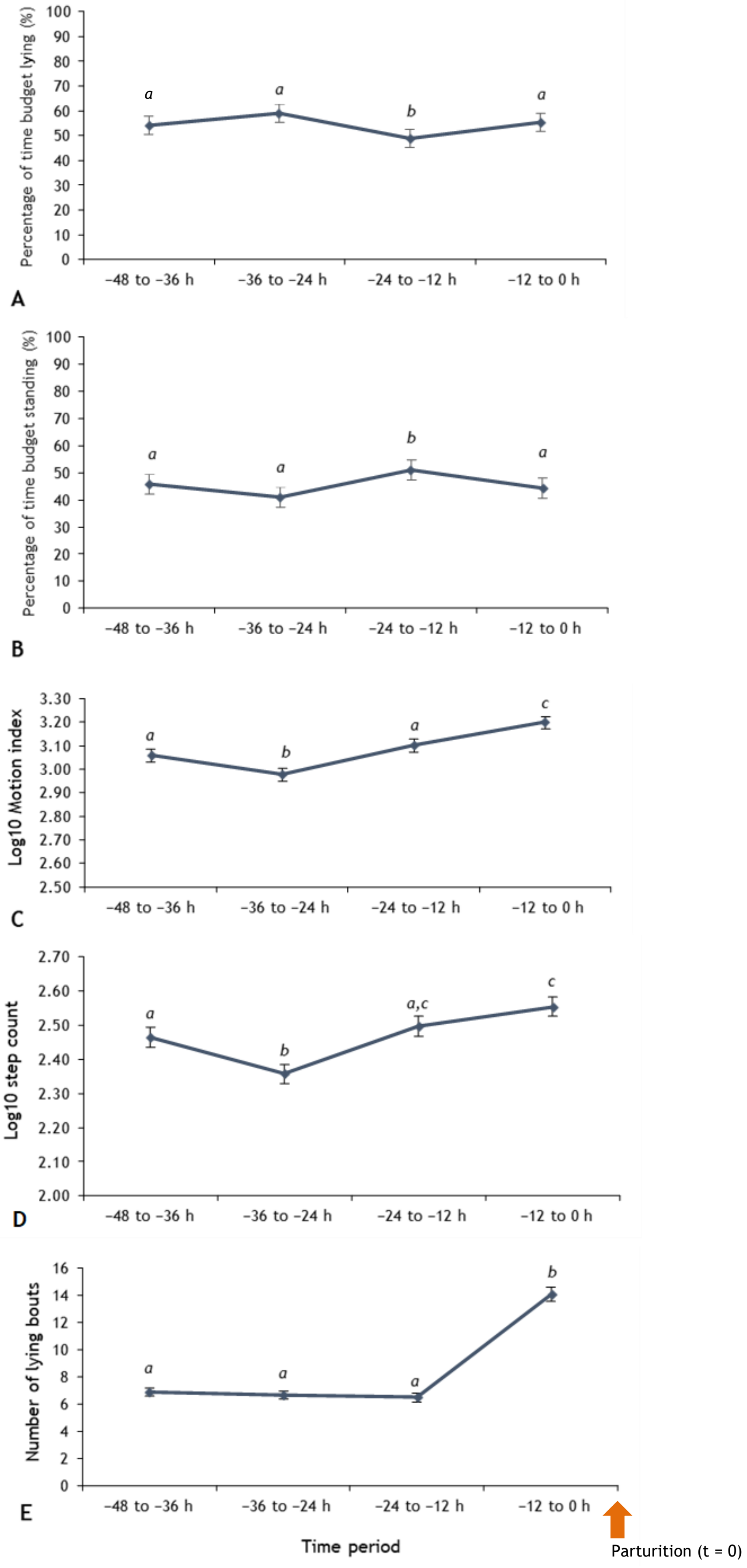


Figure 5.9: Mean (± SE) pre-partum behaviours (percentage of the time budget spent lying [A] and standing [B], $\log_{10}$ motion index [C], $\log_{10}$ step count [D] and lying bouts [E]) for each 12 h time period pre-partum

Different letters indicate significant differences within each behaviour ($p < 0.001$). Estimated marginal means (± SE) presented.

Overall, primiparous animals engaged in lying behaviours for less (and standing behaviours more) of the time budget than multiparous animals (both $p < 0.001$) [Figure 5.10].

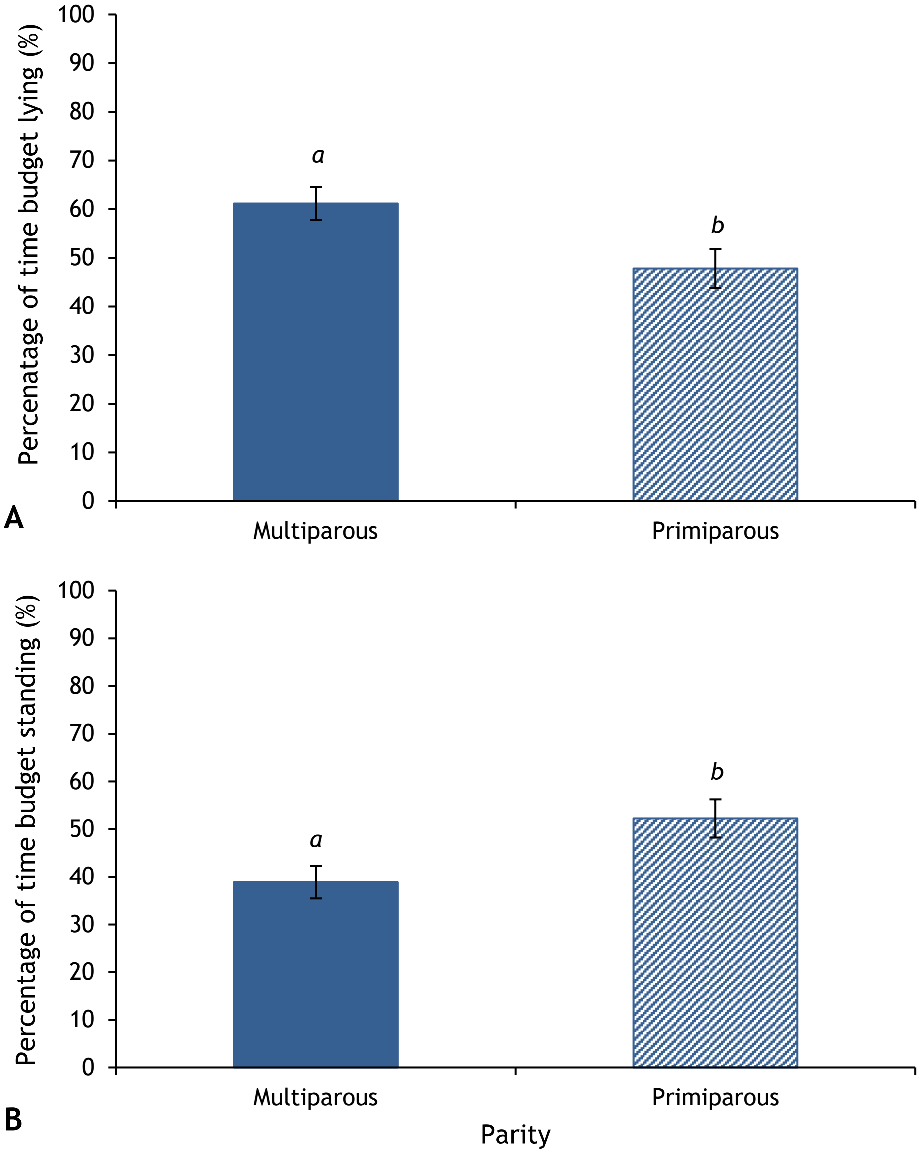


Figure 5.10: Mean (± SE) percentage of the time budget engaged in lying [A] and standing [B] behaviours by cows in each parity group (primiparous/multiparous)
Different letters indicate significant differences within each behaviour (p < 0.001). Multiparous cows represented by sold bar (n = 58). Primiparous cows represented by patterned bar (n = 22). Estimated marginal means (± SE) presented.

Additionally, cows that subsequently developed postpartum disease engaged in lying behaviours for a greater proportion of the pre-partum time budget (and standing behaviours for a smaller proportion of the time budget) than cows that did not develop postpartum disease (both $p = 0.040$) [Figure 5.11].

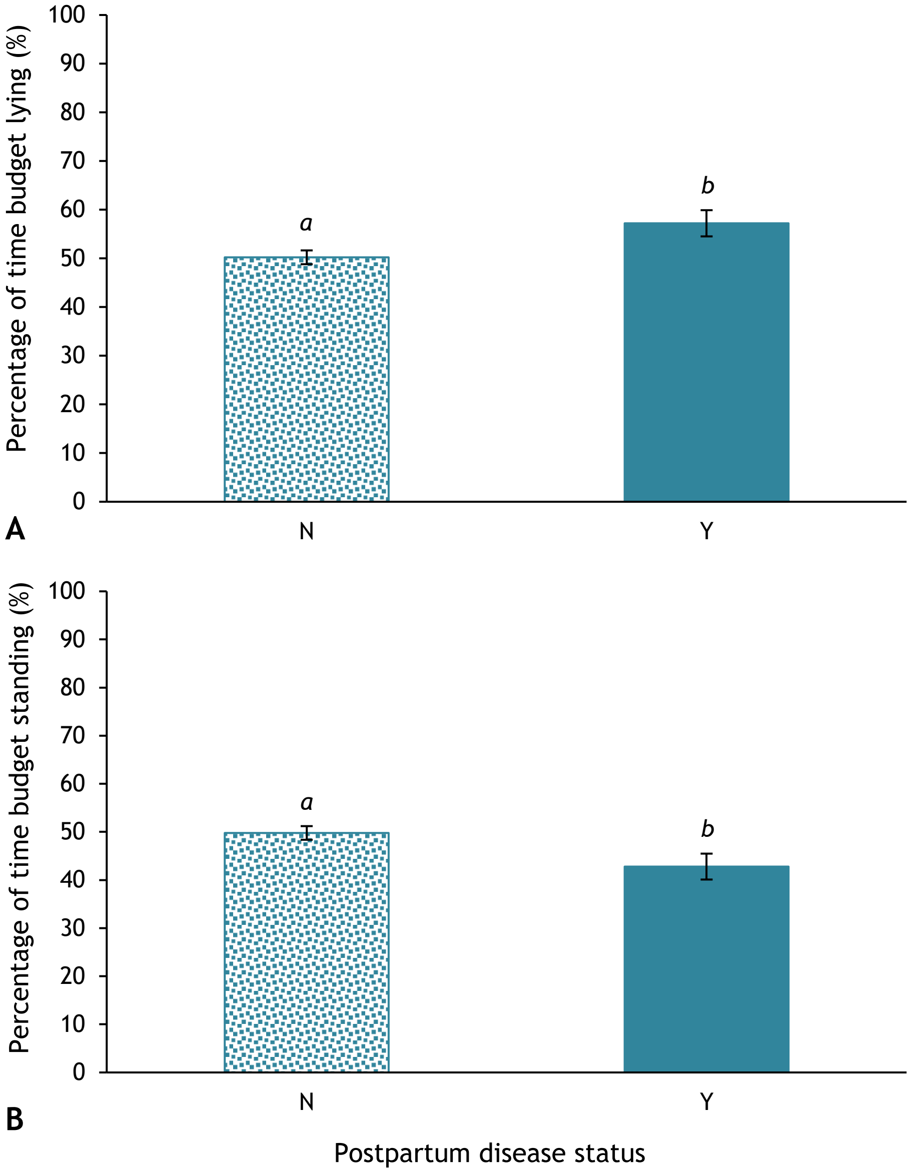


Figure 5.11: Mean (± SE) percentage of the time budget engaged in lying [A] and standing [B] behaviours by cows subsequently affected (Y) or not affected (N) by postpartum disease
Different letters indicate significant differences within each behaviour (p = 0.040). Estimated marginal means (± SE) presented. N = postpartum disease not subsequently diagnosed (represented by patterned bar; n = 62). Y = postpartum disease subsequently diagnosed (represented by solid bar; n = 17). Positive postpartum disease diagnosis recorded if one or a combination of metritis, RFM, SCK or LDA diagnosed 1 to 8 d postpartum.

5.3.1.4 Classification and Regression Tree analysis

Classification and Regression Tree analysis indicated that step count had the greatest association with subsequent assistance status. Based on this finding and the results of multivariate analysis, pre-partum step count was selected for CART analysis as a possible predictive variable for assisted parturition. The optimal tree (based on minimum classification cost) was a four-node tree [Appendix 5]. This was pruned to a two node tree for simplicity and to explore the use of a single threshold value for predicting assistance status. Using this technique, a step count > 883 was selected as the optimum threshold [Figure 5.12].

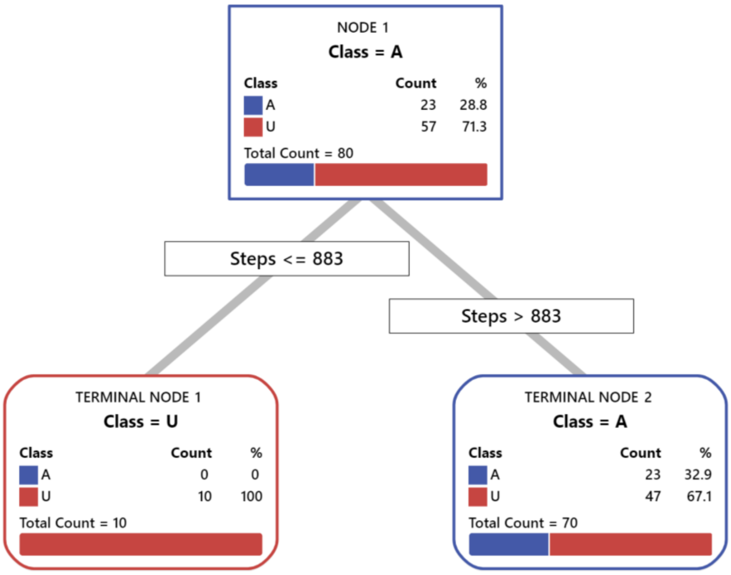


Figure 5.12: Two node Classification and Regression Tree indicating optimum threshold for pre-partum step count to predict assisted parturition
Blue = event (assisted [A]). Red = non-event (unassisted [U]).

Discriminant analysis indicated this threshold had an acceptable sensitivity (69.6%) but low specificity (31.6%) for detecting subsequent parturition assistance. The positive predictive value for this threshold (steps > 883) was 29.1% and the negative predictive value was 72.0%.

Classification and Regression Tree analysis of time period as a multinomial response variable (0 to −12 h, −12 to −24 h, −24 to −36 h and −36 to −48 h) identified that the last 12 h gestation could be predicted by a threshold of > 10.5 lying bouts with an accuracy of over 75%. Identification of the other three pre-partum time periods was poor (accuracy range from 27.5% to 43.1%), therefore CART analysis was repeated with time period characterised as a binary response: ‘last 12 h gestation (Y/N)’. This methodology allowed discriminant analysis to be performed and the ability of IceQube generated data to detect the final 12 h of gestation to be investigated.

Classification and Regression Tree analysis identified the number of LB to be the most important predictor for the last 12 h of gestation. Second most important was MI, with a relative importance (defined as percentage improvement with respect to the top predictor, i.e. LB) of 19.4% [Appendix 5]. A LB threshold > 10.5 was able to predict the last 12 h of gestation with a balanced accuracy of over 80% [Table 5.6].

Table 5.6: Results of discriminant analysis of IceQube generated data for differentiating the last 12 h of gestation from other time periods.

Variable	Threshold[2]	Training set[1]			Test set[1]		
		Sensitivity (%)	Specificity (%)	Balanced accuracy (%)	Sensitivity (%)	Specificity (%)	Balanced accuracy (%)
Lying bouts	10.5	76.3	92.5	84.4	78.8	87.1	82.7
Motion index	1268	75.0	62.9	69.0	75.0	50.4	62.7
Step count	257.5	81.3	47.1	64.2	76.3	37.5	56.9
Percentage of time budget engaged in lying (%)	72.8	95.0	15.4	55.2	62.5	36.7	49.6

1. Sensitivity and specificity refer to proportion of time periods correctly positively and negatively identified, respectively. Balanced accuracy refers to the proportion of correctly identified time periods and is the arithmetic mean of sensitivity and specificity.

2. Thresholds determined using CART analysis.

5.3.2 Maternal activity postpartum

5.3.2.1 Study population and descriptive analysis

Eighty cows (40 in each treatment group) were included in the final analysis. Distribution of assistance and postpartum disease status were as for pre-partum analyses [Table 5.5].

Twenty-one (26.3%) primiparous and 59 (73.8%) multiparous cows were included in analysis of postpartum data. Median BCS at parturition was 3.0 (range 2.25 to 3.75). Forty-six cows (57.5%) were recruited in the summer months (June – August), the remaining 34 cows (42.5%) were recruited in the autumn (September – November). Parturition most commonly occurred between 10 a.m. and 2 p.m. (32.5% of parturition events); the majority (55%) of parturition events occurred between 6 a.m. and 2 p.m. [Figure 5.13].

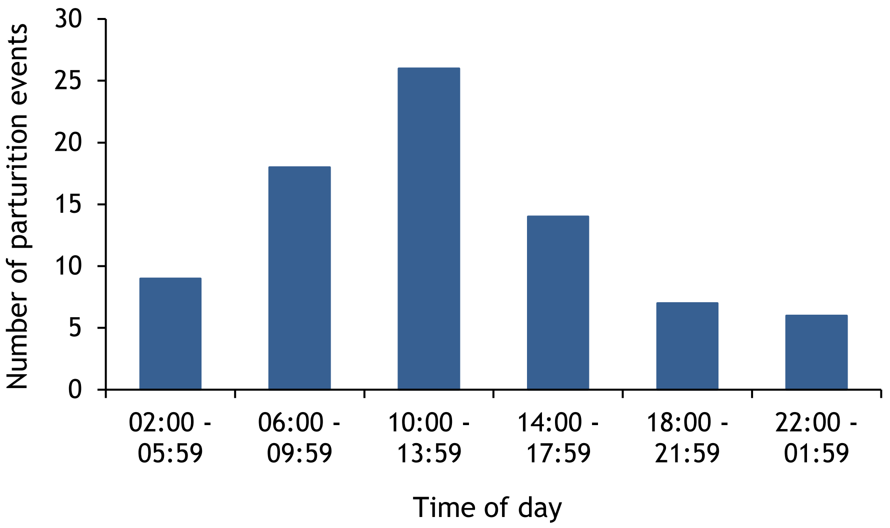


Figure 5.13: Number of parturition events at each time of day
Time presented in 24 h format (hh:mm). n = 80.

Overall, cows were recorded as lying for 42.4% and standing for 57.6% of the time budget in the first 48 h postpartum (20 h 21 min and 27 h 38 min respectively). Mean MI and step count were 10745 (range 4485 to 48497) and 2851 (range 1225 to 13561) respectively. Twenty-six LB (range 11 to 46) with mean duration of 46 min 48 s (range 00:00:34 to 05:32:18) were recorded during the first 48 h postpartum. Sixty-four (2.9%) false LB with a mean duration of 10 s (range 0 to 32 s) were recorded. The cumulative duration of false LB recorded for all cows was 10 min 27 s, accounting for 0.005% of the cumulative postpartum time budget for all cows (80 x 48 h).

5.3.2.2 Effects of ketoprofen treatment

Overall, ketoprofen treated cows had higher MI and step count than saline treated cows (p = 0.021 and p = 0.012 respectively) [Table 5.7].

In the 12 to 24 h time period ketoprofen treated cows spent a greater proportion of time lying [Figure 5.14] (and accordingly a smaller proportion of time standing) than saline treated cows (both p = 0.009). During the same time period (12 to 24 h), ketoprofen treated cows had more LB than saline treated cows (p = 0.027) [Figure 5.14].

Table 5.7: Mean (± SE), range and statistical differences (*p*-value and F-statistic) of each IceQube output for cows in each treatment group (ketoprofen/saline)

Bold font indicates statistically significant result ($p < 0.05$).

IceQube output	Ketoprofen			Saline			F-statistic	*p*-value
	Mean (± SE)	Min	Max	Mean (± SE)	Min	Max		
Motion index[1]	**3023 ± 1.14**	**696**	**21314**	**2489 ± 1.15**	**696**	**7262**	**5.606**	**0.021**
Step count[1]	**795 ± 1.15**	**166**	**6019**	**634 ± 1.16**	**183**	**2282**	**6.697**	**0.012**
Standing (%)	58.1 ± 2.92	24.2	97.9	59.0 ± 3.18	22.7	97.4	0.232	0.631
Lying (%)	41.9 ± 2.92	2.10	75.8	41.0 ± 3.18	2.60	77.3	0.233	0.631
Lying bouts	6.82 ± 0.29	1	15	6.38 ± 0.26	1	20	1.425	0.233

Min = minimum. Max = maximum. SE = standard error. Estimated marginal means presented. Ketoprofen n = 40. Saline n = 40.

1. Back-transformed data presented

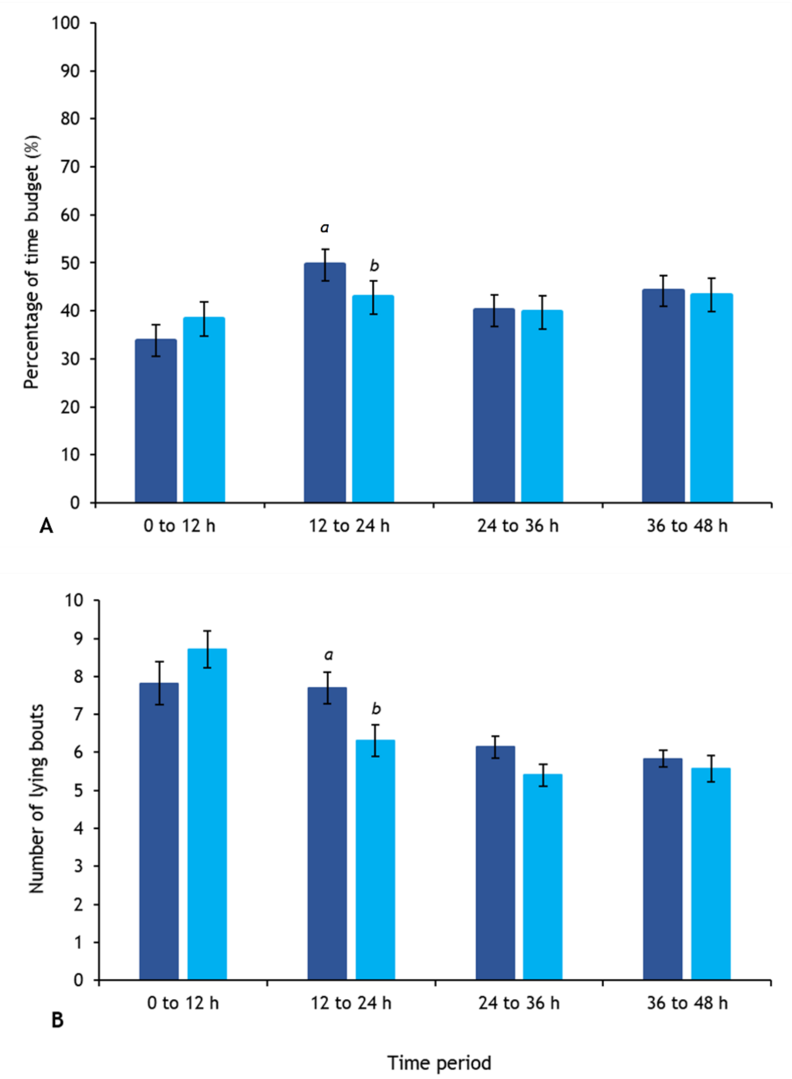


Figure 5.14: Mean (± SE) percentage of maternal time budget engaged in lying behaviour [A] and number of lying bouts [B] during each postpartum time period for cows treated with ketoprofen or saline

Different letters indicate significant results ($p = 0.009$ for lying proportion of time budget and $p = 0.027$ for lying bouts). Paired bars with no letters indicate no significant difference. Comparisons made within each time period. Ketoprofen treated cows represented by dark blue bars (n = 40). Saline treated cows represented by light blue bars (n = 40). Estimated marginal means (± SE) presented.

5.3.2.3 Effects of time period

Irrespective of treatment status, cows spent the greatest proportion of time standing and the least proportion of time lying in the first 12 h postpartum (both *p* < 0.001). Accordingly, step count, MI and number of LB were all highest in the first 12 h postpartum (all *p* < 0.001) [Figure 5.15].

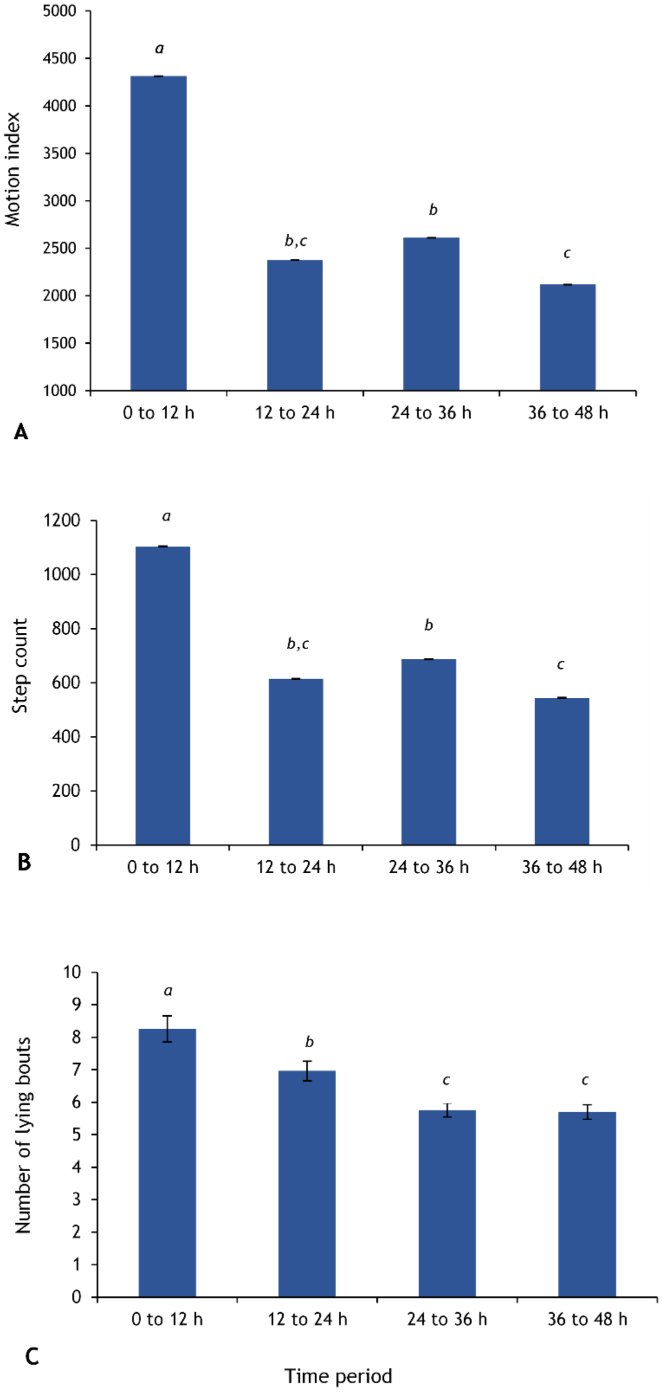


Figure 5.15: Mean (± SE) maternal motion index [A], step count [B] and number of lying bouts [C] in each postpartum time period

Different letters indicate significant differences (all behaviours p < 0.001). Estimated marginal means (± SE) presented. n = 80 for all behaviours.

5.3.2.4 Effects of parity, postpartum disease, and time of parturition

Overall (irrespective of treatment status), primiparous animals spent a greater proportion of time standing (and a smaller proportion of time lying) than multiparous animals (both $p < 0.001$). Primiparous animals had higher MI and step count than multiparous animals ($p = 0.030$ and $p = 0.020$ respectively) but LB was unaffected by parity.

Irrespective of treatment status, cows that were diagnosed with postpartum disease had a lower step count ($p = 0.045$) and spent a greater proportion of time engaged in lying behaviour (and a smaller proportion of time engaged in standing behaviours) (both $p = 0.003$) than healthy cows. There was also a tendency for MI to be lower in cows diagnosed with postpartum disease (compared to healthy cows) ($p = 0.050$).

Cows that experienced parturition at lunchtime (10:00 to 13:59) had a higher number of LB than cows that experienced parturition in the morning (06:00 to 09:59) (7.5 LB compared to 6.4 LB) or the afternoon (14:00 to 17:59) (7.5 LB compared to 5.8 LB) ($p = 0.027$).

5.3.3 Neonatal activity

5.3.3.1 Study population and descriptive results

Eighty calves (40 in each treatment group) were included in the final analysis. Six (7.5%) calves were assisted (equally divided between score 2 and score 3). Thirty-six calves (45%) were born to multiparous dams (lactation range 2 to 7), the remaining 55% of calves (*n* = 44) were born to primiparous dams. Calf birthweight for each dam parity group is presented in Table 5.8.

Table 5.8: Summary statistics (mean, standard error, standard deviation, and range) for calf birthweight overall and for each dam parity group (primiparous/multiparous)

Dam parity	*n*	Calf birthweight (kg)				
		Mean	SE	SD	Min.	Max.
Primiparous	44	35.9	0.64	4.27	27.6	44.5
Multiparous	36	41.1	0.80	4.79	28.3	49.6
All calves combined	80	38.3	0.58	5.18	27.6	49.6

SE = standard error (of the mean). SD = standard deviation. Min. = minimum. Max. = maximum

Birth most commonly occurred between 10 a.m. and 2 p.m. (26.3% of births) with most calves (67.5%) born between 2 a.m. and 2 p.m. Thirty-seven calves (46.3%) were born in the summer months (June – August); 43 calves (53.8%) were born in the autumn (September – November).

Overall, calves engaged in lying behaviours for 39 h 56 min and standing behaviours for 5 h 03 min, accounting for 88.8% and 11.2% of the time budget respectively. Calves engaged in 53.9 LB (range 32 to 192) of mean duration of 44 min 24 s (range 00:00:09 to 14:23:39). Mean MI was 8412 (range 2944 to 17005). The median number of 1 min intervals where play behaviour was detected was 108 (4%) (range 26 to 214). The median number of 15 min intervals where play behaviour was detected was 30 (17%) (range 13 to 51).

5.3.3.2 Effects of ketoprofen treatment

Overall, ketoprofen treated calves engaged in lying behaviours for a smaller proportion, and non-lying behaviours for a greater proportion, of the time budget than saline treated calves (both $p = 0.014$).

An interaction between time and treatment status was also identified with ketoprofen treated calves engaging in non-lying behaviours more frequently (and lying behaviours less frequently) than saline treated calves in the first 12 h of life (both $p = 0.002$) [Figure 5.16]. Additionally, there was a tendency for MI of ketoprofen treated calves to be higher than saline treated calves in the first 12 h after birth ($p = 0.058$). This was a short-term effect that was not observed at later time periods.

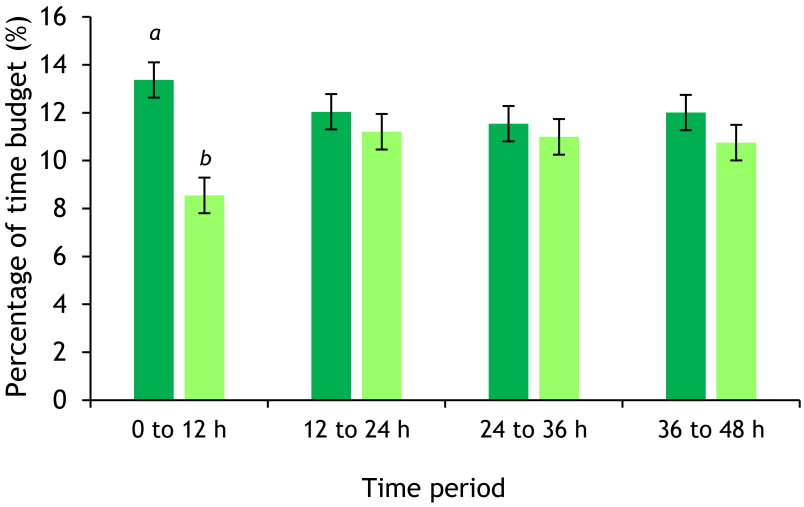


Figure 5.16: Mean (± SE) percentage of the time budget calves in each treatment group engaged in active behaviours at each time period

Different letters indicate significant differences (p = 0.002). Paired bars with no letters indicate no significant difference. Comparisons made within each time period. Ketoprofen treated calves represented by dark green bars (n = 40). Saline treated calves represented by light green bars (n = 40). Estimated marginal means (± SE) presented.

When analysed in 15 min intervals, ketoprofen treated calves engaged in play behaviour more frequently than saline treated calves in the first 12 h of life (p = 0.013) [Figure 5.17]. When analysed in 1 min intervals, the same effect was identified but with reduced significance (p = 0.056).

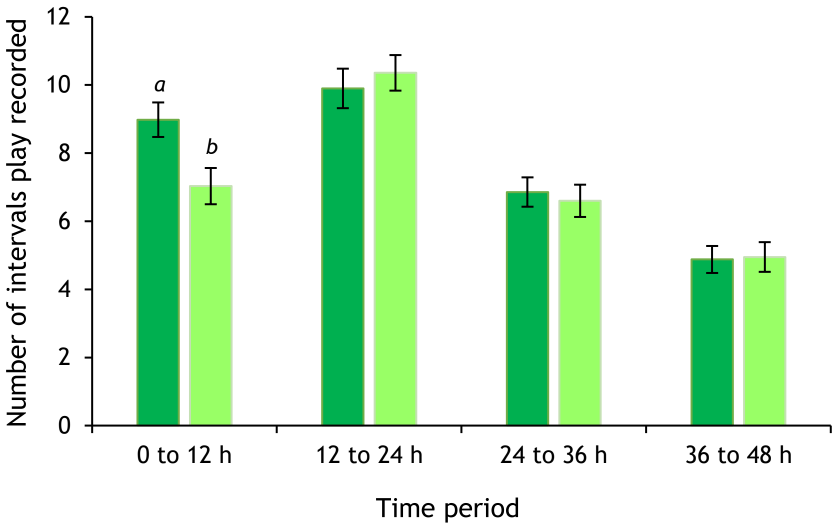


Figure 5.17: Frequency of play behaviour at each time period for calves in each treatment group when data analysed in 15 min intervals

Different letters indicate significant differences (p = 0.013). Paired bars with no letters indicate no significant difference. Comparisons made within each time period. Ketoprofen treated calves represented by dark green bars (n = 40). Saline treated calves represented by light green bars (n = 40). Estimated marginal means (± SE) presented.

5.3.3.3 Effects of time period

Overall (irrespective of treatment status), neonatal MI and LB were highest in the 12 to 24 h time period and lowest in the 36 to 48 h time period (both $p < 0.001$) [Figure 5.18].

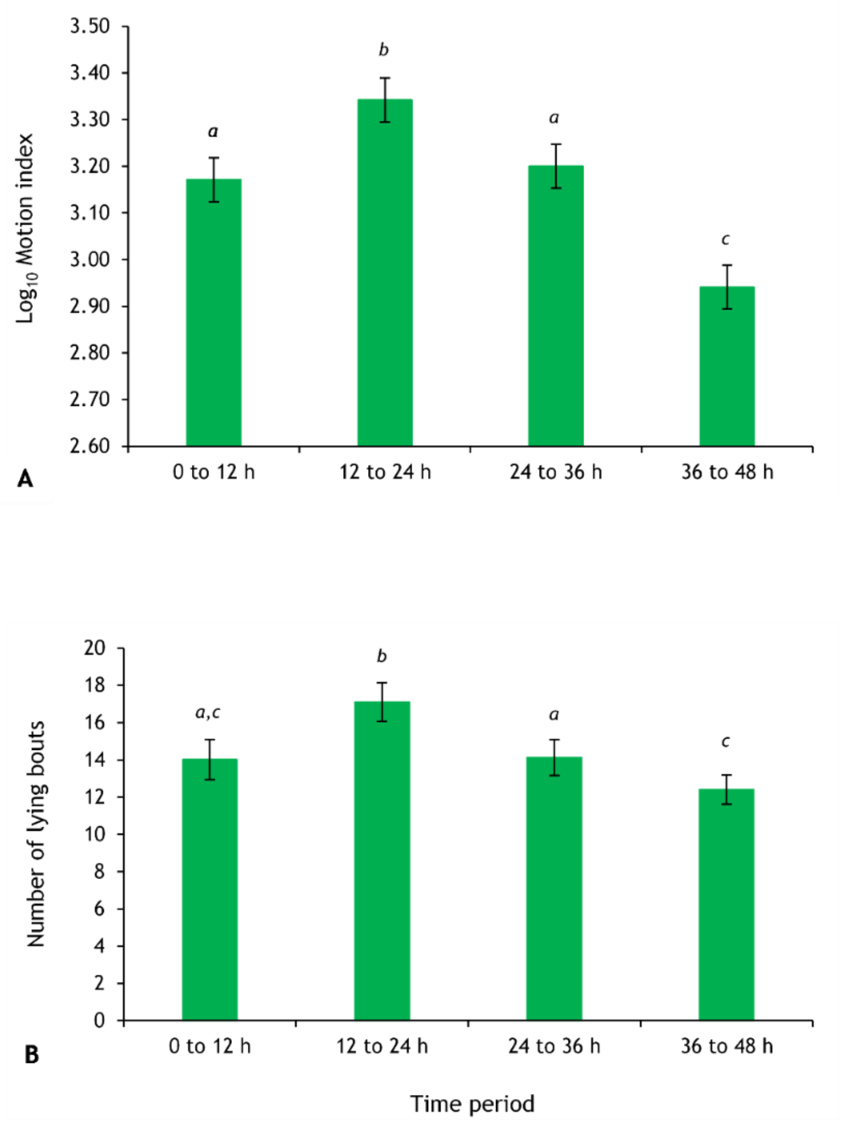


Figure 5.18: Mean (± SE) neonatal $\log_{10}$ Motion Index [A] and lying bouts [B] for each time period, irrespective of treatment status

Different letters indicate significant differences ($p < 0.001$). Estimated marginal means (± SE) presented. n = 80.

When analysed in 1 min sample intervals, calves engaged in play behaviour most frequently in the 12 to 24 h time period and least in the 36 to 48 h time period ($p < 0.001$) [Figure 5.19]. The same pattern was observed when analysed in 15 min intervals ($p < 0.001$).

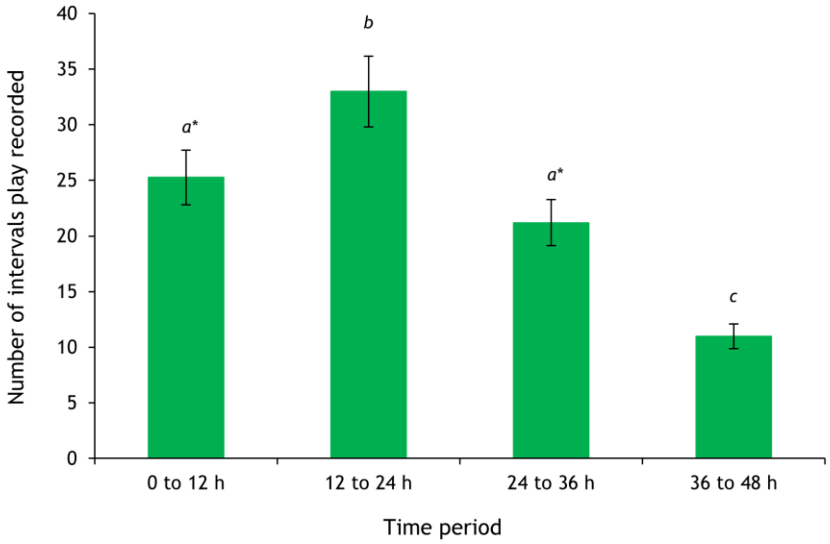


Figure 5.19: Frequency of neonatal play behaviour in each 12 h time period (irrespective of treatment status) when analysed in 1 min sample intervals
*Different letters indicate significant differences (p < 0.001). *Post hoc Bonferroni pairwise comparison indicated a tendency toward a difference in frequency of play behaviour between the 0 to 12 h and 24 to 36 h time periods (p = 0.052). Estimated marginal means (± SE) presented. n = 80.*

5.3.3.4 Effects of assistance status, dam parity, and season of birth

When data were analysed in 1 min intervals, calves born to assisted parturition engaged in play behaviour less frequently than calves born to unassisted parturition (p = 0.014). This finding was not apparent when data were analysed in 15 min intervals. Overall (irrespective of treatment status), there was a tendency for assisted calves to have lower MI than unassisted calves (p = 0.064).

Irrespective of treatment status, calves born to multiparous dams engaged in non-lying behaviours for a greater proportion of the time budget and had a higher LB, higher MI and engaged in play behaviours more frequently than calves born to primiparous dams (all p < 0.05) [Table 5.9]. Additionally, calves born to multiparous dams engaged in lying behaviours for a smaller proportion of the time budget than calves born to primiparous dams (p = 0.030) [Table 5.9].

When analysed in 15 min intervals, calves born during the autumn months more frequently engaged in play behaviour than calves born during the summer months (p = 0.031), this effect was not observed when data were analysed in 1 min intervals.

Table 5.9: Estimated marginal mean (± SE), range and statistical differences (*p*-value and F-statistic) of each IceTag output for calves born to primiparous and multiparous dams

IceTag output	Dam parity						F-statistic	*p*-value
	Primiparous			Multiparous				
	Mean (± SE)	Min	Max	Mean (± SE)	Min	Max		
Motion index[1]	1247 ± 1.12	37	5641	1702 ± 1.10	287	7645	10.31	0.002
Standing (%)	10.4 ± 0.53	0.00	31.3	12.2 ± 0.59	2.10	30.7	4.90	0.030
Lying (%)	89.6 ± 0.53	68.7	100.0	87.8 ± 0.59	69.3	97.9	4.91	0.030
Lying bouts	12 ± 0.4	1	36	15 ± 0.9	4	78	15.09	< 0.001
Play behaviour (1 min analysis)	18 ± 1.7	0	79	24 ± 1.9	1	94	11.26	0.002
Play behaviour (15 min analysis)	6.5 ± 0.3	0	16	7.9 ± 0.3	0	21	10.88	0.002

Primiparous n = 44, multiparous n = 36. Min = minimum. Max = maximum. SE = standard error.

1. Back transformed data presented

5.3.4 Overall summary of results

Effects of ketoprofen treatment, parturition assistance, and time period on measures of pre- and postpartum maternal and postpartum neonatal activity are summarised in Table 5.10.

5.4 Discussion

5.4.1 Pre-partum maternal behaviour

Cows that were subject to parturition assistance tended to be more active in the 48 h pre-partum than (subsequently) unassisted cows — although interestingly there was no interaction between pre-partum time period and assistance status, suggesting that behavioural changes related to difficult parturition are present for at least 48 h pre-partum. A single previous study attempted to identify behavioural changes indicative of impending dystocia solely using accelerometer generated data (Barraclough *et al.*, 2020) but — in contrast to the present study — did not identify a relationship between pre-partum activity and subsequent parturition experience. Barraclough *et al.* (2020) studied approximately half the number of cows recruited here (44 compared to 80) and did not report sample size or power calculations. Therefore it is possible that, as the effect identified in the current study was small, Barraclough *et al.* (2020) may have missed more subtle pre-partum behavioural differences associated with parturition experience. Additionally, Barraclough *et al.* (2020) analysed data for 4 d pre-partum in 24 h time periods, with the last 24 h analysed in 2 h time periods; whereas the current study analysed data for 48 h pre-partum (a duration selected based on the results of Proudfoot *et al.* (2009)) in 12 h time periods. It is possible that differences in analytic methodology have contributed to differences in the results and further work is needed to determine the optimum methodology for analysing IceQube generated pre-partum data.

Previous work found that cows experiencing dystocia engaged in more postural changes during the last 24 h of gestation (Proudfoot *et al.*, 2009). Although the current study identified a tendency for pre-partum LB to be associated with subsequent parturition assistance, CART analysis indicated that pre-partum step count had the greatest association with prediction of parturition assistance. This

Table 5.10: Summarised results of multivariate analysis of effects of ketoprofen treatment, parturition assistance, and time period on measures of maternal (pre- and postpartum) and neonatal activity generated by IceQube (maternal data) and IceTag (neonatal data) accelerometers

Dataset	Variable	Effect		
		Treatment status	Assistance status	Time period
Maternal pre-partum	Lying[1]	-	NS	−24 to 12 h < other time periods
	Standing[1]	-	NS	−24 to 12 h > other time periods
	Motion Index	-	NS	Increased between −36 h to 0 h
	Step count	-	A > U†	Increased between −36 h to 0 h
	Number of lying bouts	-	A > U†	−12 to 0 h > other time periods
Maternal postpartum	Lying[1]	NS	NS	Lowest in 0 to 12 h time period
	Standing[1]	NS	NS	All variables highest in 0 to 12 h time period
	Motion Index	T > P	NS	
	Step count	T > P	NS	
	Number of lying bouts	NS	NS	
Neonatal	Lying[1]	T < P (both overall and in 1st 12 h)	-	NS
	Standing[1]	T > P (both overall and in 1st 12 h)	-	NS
	Motion Index	T > P (in 1st 12 h†)	-	All variables highest in 12 to 24 h time period and lowest in 36 to 48 h time period
	Number of lying bouts	NS	-	
	Play (1min)	T > P (in 1st 12 h†)	-	
	Play (15 min)	T > P (in 1st 12 h)	-	

- Indicates variables or effects not analysed. NS = result not significant nor tending towards significance (i.e. $p \geq 0.08$). † = tendency ($p \geq 0.05$ and $p < 0.08$). Unless otherwise stated all summarised results $p < 0.05$. T = ketoprofen treatment group. P = saline treatment group. A = assisted U = unassisted.

1. Percentage (%) of the time budget

suggests that future studies investigating the potential for accelerometer generated data to predict assisted parturition should consider analysing measures of active behaviour, with a particular focus on step count. The results presented here indicated behaviours that may have potential for predicting the need for parturition assistance and attempted to establish a predictive threshold using CART analysis. This approach has been employed to predict human diseases such as influenza (Zimmerman *et al.*, 2016) and types of cancer (Barlin *et al.*, 2013) but it has not previously been applied as a method of predicting parturition assistance in cattle. Although an optimum threshold with high sensitivity and specificity for predicting parturition assistance could not be identified, CART analysis was able to utilise pre-partum IceQube generated data to attempt prediction of parturition assistance. The number of animals in the current study is a smaller sample size than is typically recruited for CART analysis and it would be of interest to analyse similar data generated by larger sample sizes using the same approach to further explore the possibility of predicting parturition assistance.

Pre-partum data were analysed retrospectively based on the known time of parturition. Whilst this was necessary to enable accurate analysis of timing of behaviours (relative to parturition), application of the results to a wider context is limited by the reliance on accurate prediction of the timing of parturition, a method for which has not yet been established. Consistent with previous findings (Huzzey *et al.*, 2005; Speroni *et al.*, 2018; Hendriks *et al.*, 2019), cow activity increased in the last 36 h of gestation with LB increasing markedly in the last 12 h — observations that may have potential for predicting the time of parturition. Barraclough *et al.* (2020) also found that the number of LB increased in the last 24 h of gestation (by up to 45%). The present study identified a greater increase in LB than Barraclough *et al.* (2020) but this is likely due to differences in analytic method used: Barraclough *et al.* (2020) analysed data in 24 h time periods, whereas the current study analysed data in 12 h time periods and found that LB did not increase in the −12 to −24 h time period. As such, the longer time period analysed by Barraclough *et al.* (2020) has likely included a period of time where LB did not change, thus affecting the mean LB recorded in the last 24 h of gestation. The present study was not designed to predict the timing of parturition and further work is needed to determine the time that LB increase relative to parturition more accurately. Nevertheless, the relationships between time pre-partum and cow behaviour identified in the current study offer the exciting possibility that future technology may be able to utilise different types of accelerometer generated data to predict the timing of parturition and the likelihood of parturition assistance. As such, accelerometers have the potential to become a valuable tool in parturition management and may also contribute to parturient welfare improvements by enabling farmers to identify cows that are more likely to have additional parturition-related challenges for targeted management.

5.4.2 Postpartum maternal behaviour

Comparable to the findings of previous studies (Endres *et al.*, 2007; Itle *et al.*, 2015), cows in the current study engaged in standing behaviours for 57.6% of the postpartum time budget. Lying behaviours accounted for 42.4% of the postpartum time budget with an average of 13 LB per day (range 5.5 to 23). Interestingly, this finding is the reverse of the pre-partum time budget where lying was the predominant behaviour, accounting for 54.4% of the time budget. These results are consistent with those of Campler *et al.* (2019) who found that cows engaged in lying behaviours for 14 h a day (58.3%) before parturition and 10.5 h a day (43.8%) in the first 2 d postpartum. In contrast to Campler *et al.* (2019), cows studied here had a lower number of LB per day both 24 h pre-partum (10 compared to 14) and postpartum (13 compared to 15.9). As the proportion of the time budget engaged in lying behaviours was similar in both studies, an explanation for this is that cows in the current study engaged in fewer, longer lying bouts than cows studied by Campler *et al.* (2019). These contrasting results may be explained by differences in farm management of peri-parturient cows: although the frequency of cow entry to the parturition pen reported by Campler *et al.* (2019) was similar to the farm protocols of the current study (once weekly), the parturition pens and space allowance described by Campler *et al.* (2019) were smaller than those used in the current study (7.0 m^2 per cow compared to approximately 9 m^2 per cow). As such, cow movement into and out of the pen may have resulted in an increased frequency of cow disturbance in the study reported by Campler *et al.* (2019) compared to the current study, reflected in increased postural changes.

5.4.2.1 Effects of ketoprofen treatment

Ketoprofen treated cows were more active during the non-lying time budget than saline treated cows. However, the number of lying bouts was not affected by treatment status, suggesting that increased activity was due to increased movement (i.e. walking or running) rather than an increased number of postural transitions. The proportion of the time budget engaged in lying behaviours was not affected by treatment status. It is possible that, whilst postpartum discomfort was not substantial enough to affect lying behaviours, it was enough to cause pain during behaviours that required a more musculoskeletal engagement (i.e. locomotory behaviours). Accordingly, ketoprofen treated cows possibly experienced heightened comfort whilst moving and were more active than saline treated cows. This hypothesis is supported by the results presented in Chapter 4 that suggested improved comfort of ketoprofen treated cows.

Maternal lying behaviours were not affected overall by treatment status, although ketoprofen treated cows spent a greater proportion of time engaged in lying behaviours in one time period (12 to 24 h). By contrast, Barrier *et al.* (2014) found that meloxicam treated cows engaged in lying behaviours overall for a longer duration (following caesarean section) than placebo treated cows, but the findings of Swartz *et al.* (2018) and the study reported in Chapter 4 are more consistent with the current findings and it is possible that following caesarean section, cows experience different types and intensities of pain than that following *per vaginum* delivery. The study reported in Chapter 4 employed detailed visual behavioural observations, unlike both the current study and Swartz *et al.* (2018), and therefore was able to identify a treatment effect on particular lying postures which cannot be assessed using leg-mounted accelerometers. If included here, visual observations may have identified subtle differences in lying behaviours (e.g. lying posture or head position) in this study population. Whilst accelerometer technology allows behaviours of a large number of animals to be analysed efficiently, the loss of detail incurred compared to visual observations is an unavoidable limitation which should be considered at the design stage, a point highlighted in a recent review (Hendriks *et al.*, 2020). Nevertheless, the different parturition experiences of studied cattle also need to be considered when comparing related studies. Swartz *et al.* (2018) and Barrier *et al.* (2014) studied cows experiencing dystocia whereas in the current study most cows (71.3%)

experienced normal parturition; thus, it is possible that the cows in the studies reported by Swartz *et al.* (2018) and Barrier *et al.* (2014) experienced greater parturition-related discomfort. It is possible that accelerometer generated data may not be sensitive enough to detect more subtle differences in behaviour associated with mild to moderate discomfort. This hypothesis is further supported by Swartz *et al.* (2018), who did not identify an analgesic treatment effect on the lying behaviours of cows experiencing eutocia (defined as parturition lasting less than 70 min). Alternatively, the type and anatomical location of postpartum pain may not be accurately reflected in lying behaviours. It is well known that lying behaviours are affected by lameness and mastitis — albeit in opposing directions (Siivonen *et al.*, 2011; Fogsgaard *et al.*, 2015; Solano *et al.*, 2016; Weigele *et al.*, 2018) — and abdominal surgeries (Barrier *et al.*, 2014; Newby *et al.*, 2014a), but few studies have investigated other painful conditions (Tucker *et al.*, 2021) and as such it is unclear how lying behaviours of cattle are affected by pain that does not originate from the udder or limbs, or is post-surgical. Since ketoprofen would be expected to alleviate pain, the results of the current study suggest that active behaviours are more sensitive to postpartum pain than lying behaviours. Lying behaviour is prioritised by cows (Munksgaard *et al.*, 2005; Cooper *et al.*, 2007) and as such is considered a key indicator of welfare during farm assessments (Vasseur *et al.*, 2012; de Vries *et al.*, 2014; O'Connor *et al.*, 2019), but it is possible that non-lying behaviours are a more sensitive indicator of mild to moderate pain. More work is needed to explore this possibility as the current focus on lying behaviours has the potential to miss some more subtle welfare challenges, thus limiting the optimisation of dairy cow welfare.

5.4.2.2 Effects of other factors

All measures of maternal activity were affected by time (irrespective of treatment status), with cows being most active (and lying least) in the first 12 h postpartum. This is consistent with the results presented in Chapter 4 as well as previous studies reported by Campler *et al.* (2019) and Hendriks *et al.* (2019) that found cows spent less time lying on the first day than the second day postpartum. Similarly, Perier *et al.* (2019) studied lying behaviours in the first 24 h postpartum and found that cows spent the least amount of time lying in the first 6 h. Proudfoot *et al.* (2009) found that drinking and eating behaviours decrease in the 24 h pre-partum and increase in the 24 h postpartum — a likely compensatory effect for the pre-partum reduction in feed and water consumption. It is likely that early postpartum activity observed in the current, and previous (Campler *et al.*, 2019; Hendriks *et al.*, 2019; Perier *et al.*, 2019), studies is related to cows engaging in behaviours that are not performed (by healthy cows) when lying — in particular eating and drinking.

Primiparous animals were more active overall and spent less time lying than multiparous animals (irrespective of treatment status). This is consistent with previous studies that have also found that primiparous animals are more active in the immediate postpartum period (than multiparous animals) (Barragan *et al.*, 2020a). It is possible that this heightened postpartum activity is due to younger animals experiencing more postpartum discomfort; however, it also needs to be considered that primiparous animals do not have prior parturition experience and animals were moved soon after parturition into postpartum accommodation shared with multiparous animals. Novelty has been associated with increased locomotor behaviours in young calves (Pempek *et al.*, 2017) and adolescent rats (Stansfield *et al.*, 2006), and it is possible that the increased postpartum activity of primiparous cows is related to the novel experience of parturition, as well as moving into new accommodation and a novel social group. This hypothesis is further supported by previous work that found that changing social groupings disrupts cow behaviour for up to 3 d (von Keyserlingk *et al.*, 2008).

Cows that were diagnosed with postpartum disease spent a greater proportion of the time budget engaged in lying behaviours (and less time engaged in non-lying behaviours) than healthy cows. This finding is similar those reported by Piñeiro *et al.* (2019) who found that each 1 h increase in postpartum lying time was associated with a 3.7% increased risk of developing ketosis within the first 14 days postpartum. Similarly, Sepúlveda-Varas *et al.* (2014) found that postpartum disease was associated with increased lying times but only in primiparous animals. The current study was not designed to investigate the effects of postpartum disease on lying behaviours and as such, the small number of animals affected by postpartum disease has precluded analysis of the effect of individual postpartum diseases or parity x health status interactions as described by Sepúlveda-Varas *et al.* (2014) and Piñeiro *et al.* (2019). It would be of interest for future studies to investigate this further. Nevertheless, in the current study cows diagnosed with postpartum disease had a lower (postpartum) step count (and tended to have lower MI) than healthy cows — a finding that may be due to the depressive effects of illness (Tizard, 2008). Few studies have investigated the effects of postpartum disease on activity and typically only study a single condition. For example, Barragan *et al.* (2018) found step count was not affected by metritis, Itle *et al.* (2015) found that standing time of ketotic cows was higher than healthy cows, and Jawor *et al.* (2012) found subclinical hypocalcaemia was associated with increased time standing, but other postpartum diseases were not included these studies. Edwards and Tozer (2004) included ketosis, RFM, milk fever and LDA in their study but only reported the effects of LDA and ketosis, finding that cows diagnosed with ketosis engaged in walking behaviours less than healthy cows in the first 5 d postpartum. The findings of the present study indicate that postpartum disease is associated with increased lying behaviours and decreased activity in the first 48 h postpartum, suggestive of reduced welfare of affected cows. These general findings are consistent with previous studies (Edwards *et al.*, 2004; Sepúlveda-Varas *et al.*, 2014; Piñeiro *et al.*, 2019), although it is not possible to make more detailed comparisons due to differences in study design.

Cows that experienced parturition between 10 a.m. and 2 p.m. had a higher number of LB than cows that experienced parturition during the 4 h before or after this time period. This time of day includes midday milking on the study farm, and it is possible that human activity around milking and the milking procedure disrupted postpartum cow behaviour and contributed to this finding. Lying behaviour of cows experiencing parturition during other times of day that include milking (02:00 to 05:59 and 18:00 to 21:59) was not affected by time of parturition but few cows experienced parturition during these times (n = 9 and n = 7 respectively) therefore this finding needs to be interpreted with caution. The effects of milking on postpartum behaviour have not previously been studied and more work is needed to investigate this finding since these results suggest that milking immediately after parturition may be a source of postpartum stress. Colostrum IgG concentration is negatively associated with time of harvest relative to parturition (Silva-del-Río *et al.*, 2017), and it is well established that poor colostrum quality is associated with poorer calf health and increased mortality (Stilwell *et al.*, 2011; Raboisson *et al.*, 2016; Cuttance *et al.*, 2018; Turini *et al.*, 2020). Therefore, delaying milking of the dam is likely to have detrimental effects on calf health and welfare, and cannot be recommended. Due to the necessity of harvesting colostrum soon after parturition, it is important to determine with more clarity the effects of milking on dam welfare to allow farmers to optimise the postpartum care of their cattle. For instance, cows that need to be milked in the first few hours after parturition might benefit from entering the milking parlour first, separately from other cows (avoiding the social mixing that occurs whilst cows wait to be milked in the collecting yard).

5.4.3 Neonatal behaviour

Lying was the predominant behaviour exhibited by calves in this study, accounting for 88.8% of the time budget. This is normal for newborn calves and is a similar proportion of the time budget to the results presented in Chapter 3 and in previous studies (Chua *et al.*, 2002; Hill *et al.*, 2013), all of which found lying accounted for more than 80% of the time budget. The small proportion of time engaged in non-lying behaviours is an unavoidable limitation of studies of newborn calves, but the continuous nature of remote activity monitoring enables accurate capture of active behaviours that may be missed by visual observations recorded using focal instantaneous or one-zero sampling. Although remote activity monitoring enables continuous detection of activity, individual behaviours are captured in less detail than visual observations and detection of active behaviours of calves has been found to be less accurate than detection of lying behaviours (Trénel *et al.*, 2009). When generated in selected sample intervals (e.g. 1 min, 15 min) MI data is analogous to data collected using visual one-zero sampling but is collected at frequencies much higher than would be possible using visual observations, allowing accurate recording of sporadic short-duration behaviours such as play. The methodology used here to detect locomotor play has previously been found to provide an accurate representation of play bouts when compared to visual one-zero sampling (Gladden *et al.*, 2020). Accordingly, the IceTag device has allowed robust analysis of play behaviour in a much less laborious fashion than visual observations as performed in Chapter 3. As play behaviour is a good indicator of positive welfare, IceTag accelerometers may have potential in the future for use as a tool in welfare assessment of newborn dairy calves.

5.4.3.1 Effects of ketoprofen treatment

Overall, ketoprofen treated calves engaged in active behaviours and play behaviour more than saline treated calves — suggesting that ketoprofen treatment is associated with improved welfare. Most (92.5%) calves in the current study were from unassisted births, thus these results suggest that calves experience pain after normal birth — as suggested in Chapter 3, where ketoprofen treatment was associated with changes in behaviours consistent with improved welfare of all calves (irrespective of assistance status). It is likely that compressive forces experienced during birth result in subclinical injury that causes discomfort in the first days of life. This is an important finding that has not previously been demonstrated outside of this body of work and further supports the importance of calf comfort (e.g. deep bedding) and the value of analgesia provision for optimising the welfare of newborn calves.

5.4.3.2 Effects of other factors

Assisted birth was associated with a decreased frequency of play behaviour, and a tendency towards reduced MI. Although only six calves (7.5%) were assisted, these results support the hypothesis that assisted birth represents a welfare challenge. It is likely that the adverse welfare impact of assisted birth is related to a combination of factors including pain and exhaustion. The design of the current study does not allow for detailed investigation into reasons for reduced welfare of assisted calves, but this is considered further in Chapters 3, 5 and 7.

Neonatal activity (as measured by MI) was highest in the 12 to 24 h time period. This result correlated with the highest frequency of play behaviour — a pattern very similar to the results presented in Chapter 3. Reasons for a reduction in calf play after 24 h are unclear; however, it may be related to delayed onset muscle pain resulting in reduced activity. Delayed onset muscle soreness following exertion is well described in humans but has not been reported in animals, although (given muscle structure varies little between mammals) it has been suggested that it would be reasonable to assume animals do experience DOMS (Steiss, 2002). This hypothesis is supported by results presented in Chapter 6, but this phenomenon deserves further work.

Calves born to primiparous dams engaged in lying behaviours more, and active behaviours (including play) less, than calves born to multiparous dams (irrespective of treatment status). This finding is similar to results presented in Chapter 3 that indicated calves born to older dams (lactation ≥ 4) spent more time engaged in active behaviours than calves born to younger dams. These results suggest that calves born to primiparous dams experience greater challenges to welfare than calves born to multiparous dams. As calves are separated from cows soon after birth on the study farm this finding is unlikely to be related to maternal care, but it is possible that calves born to primiparous dams experience more discomfort earlier in life due to greater compressive forces applied during birth (due to the smaller pelvic size of younger animals). This hypothesis is supported by the results presented in Chapter 6 that showed younger dam age was associated with higher neonatal plasma creatine kinase concentration (a biomarker of muscle injury). The relationship between dam age and newborn calf behaviour has not previously been studied thus data are limited, but the findings presented in this thesis suggest that calves born to younger dams may experience a greater challenge to welfare in the first 2 d of life and as such, may benefit from enhanced management to optimise welfare.

5.5 Conclusions

Results presented here show that IceQube generated data have the potential to be used to predict the timing of parturition and to identify cows more likely to need parturition assistance. These results could underpin future computational advances which may be able to produce algorithms that can identify data thresholds that may be used to alert farmers of higher risk cows, enabling farmers to direct resources towards ensuring the welfare of these animals.

Ketoprofen treatment was associated with behavioural differences associated with improved welfare of both cows and calves. These findings support the results presented in Chapters 3 and 4 and demonstrate that accelerometer technology is a suitable alternative to visual observations for behavioural assessment of postpartum cows and newborn calves. A single dose of ketoprofen was not associated with any adverse effects and as such, routine postpartum ketoprofen treatment of cows and calves should be considered by farmers and veterinary surgeons seeking to optimise welfare immediately after parturition or birth.

Chapter 6 Maternal and neonatal biomarkers of postpartum stress and inflammation: effects of parturition assistance and ketoprofen treatment

6.1 Introduction

Objective measurement of pain in animals is difficult. Whilst behavioural indicators of pain are sensitive, they are often time consuming to record and analyse (Barrell, 2019) and can be open to subjective interpretation by the observer. Additionally, behavioural indicators of animal pain are often not immediately obvious to human observers, or are altered in human presence, which further adds to the challenges encountered when attempting to detect and measure pain (Sorge *et al.*, 2014; McLennan *et al.*, 2019). The availability of technologies using animal-worn sensors is creating additional opportunities to study behaviour in a more objective and less labour intensive manner (Brown *et al.*, 2013), but to date the value of remote sensors for assessment of behaviours relating to postpartum pain is not well described compared to behaviours more directly related to production (e.g. oestrous behaviour, lameness). As pain is closely associated with stress (Molony *et al.*, 1997), biochemical measures of stress are often employed as proxy measures of pain in animal studies. For example, in studies where pain is expected (e.g. studies involving surgical interventions), cortisol concentration is widely measured as a biochemical indicator of stress and, by extension, the presence of pain across a range of animal species (e.g. Robertson *et al.*, 1994; McMeekan *et al.*, 1998; Stilwell *et al.*, 2010, 2012; Hempstead *et al.*, 2018). Although less sensitive than behavioural analysis as an indicator of welfare (Mormède *et al.*, 2007), measurement of biochemical analytes confers certain advantages over behavioural analysis: laboratory testing is easily accessible, samples are usually straightforward to obtain (from domestic species) and there is typically less subjectivity in the interpretation of results. Moreover, biochemical analysis can be used alongside behavioural analysis in studies of painful conditions or procedures, thus augmenting the results obtained from behavioural analysis and providing a more comprehensive investigation (Mormède *et al.*, 2007). A number of different biochemical analytes have merit for inclusion in the study of the welfare impacts of parturition on cows and their calves including cortisol, creatine kinase, L-lactate, and plasma total protein.

Cortisol is a glucocorticoid hormone synthesised in the adrenal glands [see Chapter 1] and is important for the maintenance of homeostasis as well as being produced in response to stressful experiences (Katsu *et al.*, 2015). An increase in cortisol concentration at parturition is considered normal (Comline *et al.*, 1974), but a heightened cortisol response is reported in both cows and calves experiencing dystocia (Hoyer *et al.*, 1990; Nakao *et al.*, 1990; Kindahl *et al.*, 2002; Civelek *et al.*, 2008; Barrier *et al.*, 2013b; Vannucchi *et al.*, 2015a). As cortisol concentration is known to increase in response to painful procedures (Robertson *et al.*, 1994; Stafford *et al.*, 2003; Stilwell *et al.*, 2012; Winder *et al.*, 2018) it is possible this is due to pain experienced during dystocia. However, although scarce, the available data do not support this hypothesis. A single study found NSAID analgesia did not affect maternal serum cortisol concentration for up to 7 d postpartum, regardless of assistance status (Barragan *et al.*, 2020b). Similarly, Pearson *et al.* (2019a) found that post-birth NSAID administration did not affect neonatal plasma cortisol concentration, although this study did not analyse assistance status. It is possible that factors associated with dystocia not affected by analgesia contribute to the heightened cortisol response reported in affected animals, but more study is needed to explore this further.

Creatine kinase (CK) is a muscle specific enzyme (Anderson *et al.*, 1976; Lefebvre *et al.*, 1994) that is detected in increased concentrations (in plasma or serum) when muscle injury has occurred [see Chapter 1]. Creatine kinase has a high specificity for detecting muscle injury (albeit indirectly), therefore plasma CK concentration can be used as a proxy indicator of pain caused by muscle injury; however its use for detecting pain more generally is limited by the inability to detect pain of other aetiologies (i.e. pain caused by factors other than muscle injury are not detected). Available data regarding bovine plasma CK concentration in the peripartum period are limited but it has been reported that a more difficult parturition is associated with increased maternal plasma CK concentration (Civelek *et al.*, 2008; Hussein *et al.*, 2008). Uterine tissues have high levels of CK activity (Payne *et al.*, 1993; Clark *et al.*, 1994; Sattler *et al.*, 2004) and (similar to skeletal muscle injury) uterine injury may be detected by measuring plasma CK concentration (Sattler *et al.*, 2004). However, routine laboratory tests for CK are non-specific therefore results need to be interpreted alongside other measures (e.g. clinical examination) to determine which tissues are affected.

Bovine neonatal CK concentration has rarely been measured, although serum CK measurement has been suggested to have value as an indicator of birth trauma in human neonates (Rudolph *et al.*, 1966). Serum neonatal CK concentration has been found to be elevated in severely assisted calves (compared to mildly assisted or unassisted calves) (Murray *et al.*, 2015b; Pearson *et al.*, 2019c) but it is unknown if tissue damage persists as neonatal serum CK concentration beyond 24 h has not been studied. Related work has measured plasma creatinine concentration as a proxy measure for CK and did not observe any effects of dystocia (Civelek *et al.*, 2008). However, Civelek *et al.* (2008) obtained neonatal blood samples 'immediately after delivery' but, as creatinine is a breakdown product of creatine (created during a process catalysed by CK), it would not be expected to be detected immediately following the insult. The half-life of CK in cattle is 4 to 8 h (Anderson *et al.*, 1976; Lefebvre *et al.*, 1994), therefore care is needed when interpreting the findings reported by Civelek *et al.* (2008) as it is likely that (at the time of sampling) the breakdown of neonatal plasma CK to creatinine was limited which may have affected results.

Neonatal blood lactate concentration is strongly (negatively) correlated with blood pH and as such is a good biomarker of neonatal acidosis [see Chapter 1]. Neonatal acidosis typically comprises a mixed metabolic (hyperlactatemia) and respiratory (hypercapnia) acidosis but, as the metabolic component is dominant, measurement of L-lactate concentration (which only assesses metabolic acidosis) accurately reflects the degree of acidosis experienced by newborn calves (Homerosky *et al.*, 2017a). Plasma L-lactate concentration is higher in assisted calves (than unassisted calves) in the first 24 h of life (Sorge *et al.*, 2009; Murray *et al.*, 2015b; Homerosky *et al.*, 2017a), but it is unknown whether these differences can be detected beyond 24 h. It is uncertain if analgesia can aid the resolution of neonatal acidosis, although a single study found that bovine neonatal plasma L-lactate concentration at 24 h after birth was not affected by meloxicam analgesia (Pearson *et al.*, 2019b); however, only assisted beef calves were studied. The effects of analgesia on neonatal L-lactate concentration of assisted and unassisted calves using a factorial study design have not yet been studied, therefore further work is needed before the effects of analgesia on neonatal plasma L-lactate concentration can be determined more conclusively.

Plasma total protein (TP) concentration accurately represents plasma immunoglobulin G (IgG) concentration of young calves and as such is a good (albeit indirect) measure of passive transfer (PT) of immunoglobulins (Calloway *et al.*, 2002; Hernandez *et al.*, 2016). Assisted birth is a known risk factor for FPT in both dairy and beef calves [see Chapter 1] (MacFarlane *et al.*, 2015; Bragg *et al.*, 2020; Renaud *et al.*, 2020), with one study finding that colostrum consumption in the first 4 h of life was impaired (and FPT was more likely) in assisted calves, even if calves had a strong suckle reflex (Homerosky *et al.*, 2017b). It is likely that blood-gas disturbances associated with birth assistance adversely affect central nervous system function and muscle tonicity, thus impairing affected calves' ability to stand and achieve successful suckling (necessary for adequate PT) within 4 h (Homerosky *et al.*, 2017b). Although this hypothesis is supported by studies that have found delayed consumption of colostrum is associated with impaired PT (Stott *et al.*, 1979a), negative effects of birth assistance on PT have also been observed in studies where adequate colostrum consumption is ensured (Barrier *et al.*, 2013b; Murray *et al.*, 2015b), thus impaired consumption cannot solely explain these findings. Some studies have found that (when colostrum volume is controlled for) increased neonatal plasma CK and L-lactate concentration (at birth) are associated with an increased risk of FPT (Boyd, 1989; Besser *et al.*, 1990). Accordingly, it has been hypothesised that assisted birth may be associated with a reduced capacity for the neonatal bovine gastrointestinal tract to absorb IgG (possibly due to neonatal acidosis) but the data are inconsistent and others have reported no adverse effect (Drewry *et al.*, 1999).

Whilst some effects of assisted parturition on the aforementioned biochemical analytes have previously been reported, data are typically available for just the first 24 h postpartum (Nakao *et al.*, 1990; Civelek *et al.*, 2008; Murray *et al.*, 2015b; Homerosky *et al.*, 2017b; Pearson *et al.*, 2019c) or only a single sample is analysed (Waldner *et al.*, 2009; Pearson *et al.*, 2019b). Just a single study has analysed parturition-related biomarkers of maternal stress beyond the first 24 h postpartum (Barragan *et al.*, 2020b) and no data for neonatal biomarkers after 24 h are available. Furthermore, analgesic effects on the biochemical analytes measured in the current study are rarely studied. Therefore, the longer-term effects (beyond 24 h) of assisted parturition and the effects of pain on these postpartum biomarkers are unclear.

Work presented in this chapter draws on published work (Gladden *et al.*, 2018) with permission from The Veterinary Record.

6.1.1 Study aims and hypotheses

This study aimed to address some of the gaps in the current knowledge by investigating the effects of farmer-assisted parturition and immediate postpartum ketoprofen administration on plasma cortisol and CK concentration (cows and calves) and plasma L-lactate and TP concentration (calves only) for up to 7 d postpartum using the same factorial design described in Chapters 3, 4 and 7. The four hypotheses addressed by this study were:

1. Farmer-assisted parturition is a) more stressful than unassisted parturition and b) results in a greater degree of iatrogenic muscle injury to both cow and calf (reflected in increased plasma cortisol and CK concentration compared to unassisted controls).

2. Pain experienced by Holstein cows and their calves following farmer-assisted parturition can be ameliorated by administration of ketoprofen (as indicated by decreased plasma cortisol and CK concentration compared to placebo treated animals).

3. Farmer-assisted birth is associated with a mixed metabolic and respiratory acidosis in newborn calves (reflected by an increased calf plasma L-lactate concentration compared to unassisted control calves).

4. Farmer-assisted birth is associated with impaired passive transfer of immunoglobulins as indicated by a lower plasma TP concentration at 48 h old compared to unassisted calves.

6.2 Materials and Methods

6.2.1 Animal management and ethical approval

UK Home Office approval was obtained as described in Chapter 2, Section 2.1. Farm management and animal husbandry were as described in Chapter 2, Section 2.2. Study design, animal recruitment, treatment protocols and collection of animal metadata were as described in Chapter 2, Section 2.3, Chapter 3, Section 3.2. and Chapter 4, Section 4.2. Video footage of all animals was obtained as part of the wider study [see Chapters 3 and 4].

6.2.2 Blood sample collection and processing

Four sequential lithium heparin (LH) anticoagulated coccygeal blood samples were obtained using 7 mL Vacutainer blood collection tubes (Vacutainer™, BD Medical, NJ, USA) and 2.5 cm (1”), 18 G Vacutainer needles (BD Medical, NJ, USA) from each cow at parturition (within 6 h), 24 h (± 6 h), 48 h (± 6 h), and 7 d after parturition (± 12 h). Four LH and fluoride oxalate (FO) anticoagulated jugular blood samples were obtained from calves in the same manner [Table 6.1].

Table 6.1: Details of blood samples (anti-coagulant, biochemical analyte, and volume) obtained from cows and calves

Animal	Anticoagulant	Volume per sample (mL)	Biochemical analyte analysed	Total volume^{1,2} obtained per animal (mL)
Cows	Lithium heparin	7	Cortisol Creatine kinase	28
Calves	Lithium heparin (LH)	7	Cortisol Creatine kinase Total protein	56
	Fluoride oxalate	7	L-lactate	

1. Cumulative total of blood obtained from sequential sampling
2. Total volume obtained was within recommended limits for all animals (Diehl *et al.*, 2001; National Centre for the Replacement Refinement & Reduction of Animals in Research, 2020).

All blood samples were marked at the time of sampling with the animal identification number (ID) and the date of sampling. Plasma from all blood samples was separated using a centrifuge located at the farm [Appendix 6]. All plasma samples were divided into two plastic 2 mL screw-top containers (Henry Schein Animal Health, Dumfries, UK) for short- and long-term storage at −20 ºC and −80ºC respectively. Additionally, a small aliquot of plasma (approximately 0.2 mL) from each LH anti-coagulated calf sample was separated into a third 2 mL plastic screw-top container for plasma TP analysis [Appendix 6]. All plasma samples were labelled with the animal ID, the date of sampling, the anti-coagulant used when the sample was obtained and sampling time (0 h, 24 h, 48 h or 7 d). All samples except the 0.2 mL aliquot of calf plasma were initially stored at −20 ˚C on farm and were periodically moved from the farm to the University of Glasgow School of Veterinary Medicine in appropriate containers to ensure samples remained frozen during transit. After transportation, samples were sorted into two groups, each containing one replicate of every sample. Half of the sample replicates were moved to long-term storage at −80 ˚C and the remaining half were moved to short-term storage at −20 ˚C prior to laboratory analysis [Appendix 6]. The 0.2 mL calf plasma samples were refrigerated on farm immediately after separation and transported back to the University of Glasgow School of Veterinary Medicine on the same day for measurement of plasma TP concentration (by the author).

Occasionally a blood sample was not obtained from an animal due to human error — these were all recorded as missing samples. The missing sample rate for cow and calf samples was 3.3% (12/364) and 3.0% (11/372) respectively.

6.2.3 Biochemical analysis

6.2.3.1 Plasma cortisol, creatine kinase and L-lactate concentration

Samples were referred to an external veterinary laboratory (University of Glasgow Veterinary Diagnostic Services, Garscube Campus, Glasgow, UK) for measurement of plasma cortisol, CK and L-lactate concentration. Cow and calf samples were analysed separately in batches of 20 to 30; each batch was identified with a letter code (A to X) and the type of sample (cow or calf and LH or FO), individual samples within each batch were identified by the corresponding animal ID. Plasma CK concentration and plasma L-lactate concentration were measured using a Siemens Dimension Xpand clinical chemistry analyser and the Siemens Immulite 2000 XPi chemiluminescence system (Siemens Healthcare Diagnostics, Erlangen, Germany).

Plasma cortisol concentration was measured using solid-phase competitive chemiluminescence on the Siemens Immulite XPi analyser (Siemens Healthcare Diagnostics, Erlangen, Germany). Biochemical analyses were performed in accordance with International Federation of Clinical Chemistry (IFCC) parameters and laboratory technicians performing biochemical analyses were blinded to both assistance and treatment status.

6.2.3.2 Plasma total protein concentration

Calf plasma TP concentration was measured by the author using a hand-held optical refractometer (DIGIT 012 clinical pocket refractometer, CETI, Medline Scientific, Oxfordshire, UK). Calibration of the refractometer using distilled water was performed prior to analysis of each batch of samples. When calibration was complete, a drop of plasma was placed on the platform of the refractometer and the scale representing TP concentration (g/dL) was read [Figure 6.1]; the platform of the refractometer was cleaned with distilled water between each sample.

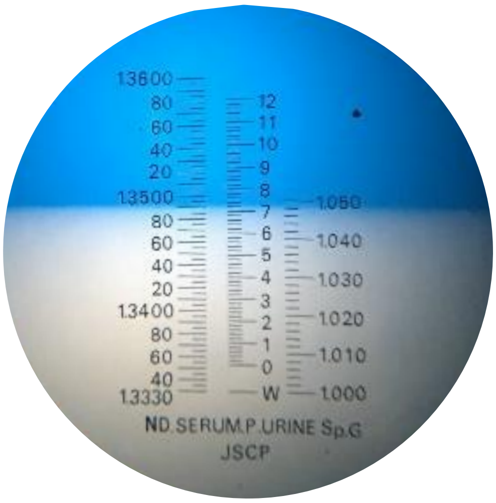


Figure 6.1: Optical refractometer measurement of plasma total protein
The middle scale is read where it is crossed by the division between the white and blue sections (in this case plasma TP concentration is 7.2 g/dL)

6.2.4 Statistical analysis

Raw data for all biochemical analytes were summarised in an Excel spreadsheet (Excel 2013, Microsoft, USA) and exported into Minitab (Minitab v.19, Minitab Inc., State College, PA, USA) and SPSS (SPSS v.27, IBM Corporation, Armonk, NY, USA) for statistical analysis. Grades 2 and 3 parturition assistance were combined into a single ‘assisted’ group for analysis of all maternal and neonatal datasets. Descriptive analysis and data exploration of all datasets was performed in Minitab (v.19). Relationships between parturition-related metadata and main effects (assistance and treatment status) were explored using Minitab (v.19).

Data were appraised for normality using Anderson Darling statistics and visual appraisal of histograms. As all data were positively skewed transformation techniques were applied. Cortisol, L-lactate, and TP concentration were all transformed using logarithmic (base 10) transformation. Creatine kinase (both maternal and neonatal) data could not be adequately transformed using standard transformations; capability analysis (Minitab v.19) indicated that neonatal CK data could be transformed using Johnson transformation with the formula:

$$y = -3.39114 + 0.981996 \times \mathrm{Asinh}\left((x - 34.6282) / 7.53297\right)$$

Where y is the transformed result and x is the measured neonatal plasma CK concentration.

Maternal CK data exhibited non-constant variance that could not be stabilised using transformation techniques. To manage this, maternal CK data were converted from continuous to binary format and categorised as being either above [1] or below [0] the upper limit of the laboratory RI for bovine plasma CK concentration (196 IU/L). The lower limit of the RI was not included as a third category due to the small number of samples that met the criteria ($n = 5$) and the absence of any known clinical significance of a low plasma CK concentration in cattle.

The threshold of statistical significance was set at $p < 0.05$ for all tests. A tendency towards significance was considered if $p \geq 0.05$ and $p \leq 0.08$.

6.2.4.1 Univariate analysis of parturition-related metadata

Univariate analysis of data collected at parturition was performed as described in Chapter 3, Subsection 3.2.5.1. Data are presented in Appendix 3.

6.2.4.2 Multivariate analysis

Biochemical analytes were each analysed separately using mixed effects models (normally distributed transformed data) and generalised linear mixed models (GLMM) (binomial distribution) [Table 6.2] with individual subject (i.e. animal ID) entered as a random effect in all models to account for repeated measurements.

Table 6.2: Details of statistical analysis (transformation type, distribution, and statistical model) for analysis of each biochemical analyte

Animal	Analyte	Transformation[1]	Distribution	Statistical model
Cow	Cortisol	Logarithmic	Normal[2]	Mixed effects model
	Creatine Kinase	NA	Binomial	GLMM (binomial distribution) with logit link
Calf	Cortisol	Logarithmic	Normal[2]	Mixed effects model
	Creatine Kinase	Johnson	Normal[2]	Mixed effects model
	L-lactate	Logarithmic	Normal[2]	Mixed effects model
	TP	Logarithmic	Normal[2]	Mixed effects model

1. Logarithmic transformations all base 10
2. Post-transformation distribution

Assistance status (assisted/unassisted), treatment status (ketoprofen/placebo) and sampling time (0 h, 24 h, 48 h and 7 d) were entered as fixed effects in all models. Interaction terms entered into all models as fixed effects were assistance status x treatment status; assistance status x time; treatment status x time; and assistance status x treatment status x time. *A priori,* covariate data were selected for inclusion based on known or hypothesised effects on the analytes of interest; hypothesised effects were informed by clinical knowledge. Cow lactation number was entered as a covariate factor in all statistical models used to analyse maternal datasets. Dam lactation and calf birthweight were entered as covariate factors in all models used to analyse neonatal datasets. Volume of colostrum consumed was included as a covariate factor in the model for analysis of neonatal plasma TP concentration only. Models were finalised for all biochemical analytes using backward stepwise elimination; terms were removed sequentially if $p > 0.1$ with the exception of assistance and treatment status (these factors were retained in all models). Factors retained in the final statistical model used to analyse each dataset are presented in Appendix 6.

Corrected Akaike's information criterion was inspected to assess how well each model fitted the data and to compare the fit of different models for the same outcome variable. Residual plots were visually appraised to verify that the assumptions of each model were met.

6.2.4.3 Correlation between neonatal plasma L-lactate concentration at birth and failure of passive transfer

For the purposes of analysis of neonatal plasma TP concentration a cut-point of < 5.6 g/dL at 48 h old was considered indicative of FPT (MacFarlane *et al.*, 2014). The correlation between birth neonatal plasma L-lactate concentration and 48 h neonatal plasma TP concentration was assessed using Pearson's correlation analysis (Minitab v.19) (calf 48 h TP data were normally distributed) and the square of the Pearson's correlation coefficient (R^2) was calculated manually. Data were further analysed by creating two groups using an approach similar to Boyd (1989). Calves with plasma L-lactate concentration at birth greater than the median value were categorised as 'high L-lactate' and calves with birth plasma L-lactate concentration below the median value were categorised as 'low L-lactate'. This analysis was repeated with samples categorised as being either above or below 7.5 mmol/L — the plasma L-lactate concentration indicative of acidosis in newborn calves (Homerosky *et al.*, 2017a). For both analyses, Mann-Whitney *U* tests were performed prior to analysis to confirm plasma L-lactate concentration differed between high and low categories. Similarly, two-sample t-tests were used to determine whether 48 h plasma TP concentration differed between categories.

6.3 Results

6.3.1 Study population and descriptive results

In total, 94 cows and 95 calves were recruited; study population data are presented in Chapter 3 [Subsection 3.3.1] and Chapter 4 [Subsection 4.3.1]. Three cows and two calves were excluded at the data collection stage due to animal illness (*n* = 4) and sample collection errors (*n* = 1). A further two cows and two calves were excluded at the data analysis stage due to data recording errors being identified. In total, 89 cows and 91 calves were included in the final analysis

[Table 6.3]. Summary statistics for each biochemical analyte are presented in Appendix 6.

Table 6.3: Number of animals in each assistance x treatment group included in the final biochemical analysis
(Gladden et al., 2018)

Animal	Assistance x treatment group	*n*
Cows	Assisted saline	24
	Assisted ketoprofen	23
	Unassisted saline	20
	Unassisted ketoprofen	22
	Total	*89*
Calves	Assisted saline	25
	Assisted ketoprofen	22
	Unassisted saline	20
	Unassisted ketoprofen	24
	Total	*91*

6.3.2 Maternal and neonatal plasma cortisol concentration

6.3.2.1 Effects of parturition assistance, ketoprofen treatment and sampling time

Assistance status did not affect either maternal or neonatal plasma cortisol concentration overall. However, immediately after parturition maternal plasma cortisol concentration was higher in assisted cows than unassisted cows ($p = 0.023$) [Figure 6.2].

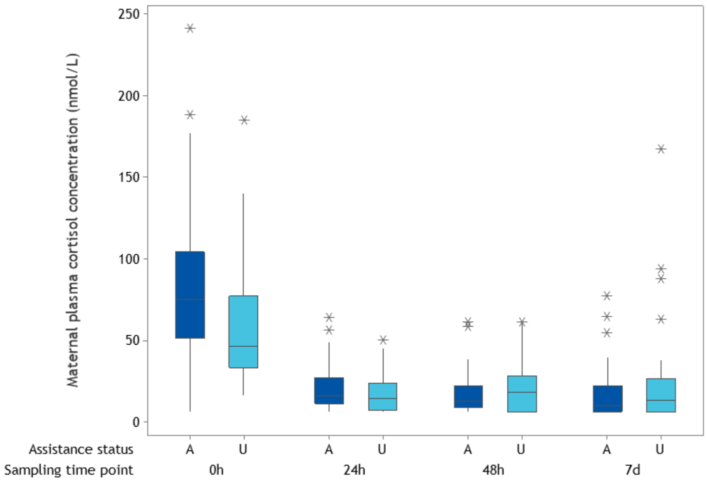


Figure 6.2: Maternal plasma cortisol concentration (nmol/L) at each sampling time point for cows experiencing assisted parturition and unassisted parturition, irrespective of treatment status
*A = assisted cows (n = 47). U = unassisted cows (n = 42). * Indicates outliers, central line indicates median result, box indicates interquartile range, whiskers indicate full range of results. Back-transformed results presented*

Neonatal plasma cortisol concentration was not affected overall by treatment status but at 48 h after birth neonatal plasma cortisol concentration was higher in saline treated calves than ketoprofen treated calves (*p* = 0.044) [Figure 6.3]. Treatment status did not affect maternal cortisol concentration.

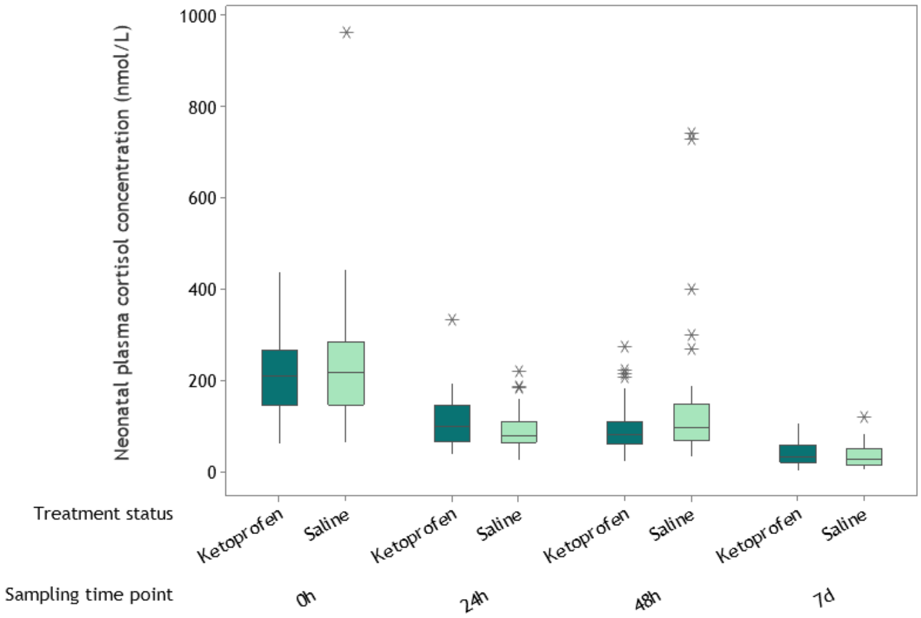


Figure 6.3: Neonatal plasma cortisol concentration (nmol/L) at each sampling time point for calves treated with ketoprofen and saline, irrespective of assistance status
*Ketoprofen treated calves n = 46. Saline treated calves n = 45. * Indicates outliers, central line indicates median result, box indicates interquartile range, whiskers indicate full range of results. Back-transformed results presented.*

Maternal and neonatal plasma cortisol concentrations were both higher at the time of parturition than at any other sampling point (*p* < 0.001), regardless of assistance or treatment status.

6.3.3 Maternal and neonatal plasma creatine kinase concentration

6.3.3.1 Effects of assistance and treatment status

Overall (compared to saline) ketoprofen was associated with an increased number of maternal samples having a plasma CK concentration exceeding the upper limit of the laboratory RI, regardless of assistance status (*p* < 0.001). Additionally, a treatment status x time interaction effect was identified with plasma CK concentration of more ketoprofen treated cows than placebo treated cows exceeding the upper limit of the laboratory RI at 24 and 48 h postpartum

(p < 0.001). Neonatal plasma CK concentration was not affected by assistance or treatment status.

6.3.3.2 Effects of time and lactation number

Irrespective of treatment or assistance status, the number of samples exceeding the laboratory RI for maternal plasma CK concentration was highest at 24 h postpartum ($p < 0.001$). *Post hoc* Chi-squared analysis indicated that (irrespective of assistance or treatment status) the frequency of samples exceeding a CK concentration of 196 IU/L was higher than expected at 24 h postpartum and lower than expected at 7 d postpartum (χ^2 [3, n = 342] = 48.5; $p < 0.001$).

The number of samples with high plasma CK concentration exceeded the number of samples within the RI for primiparous cows only ($p < 0.001$) [Table 6.4].

Table 6.4: Number of samples above and below the upper limit of the laboratory reference interval (196 IU/L) for cows in each lactation

Lactation number	Plasma CK concentration exceeds the upper limit of the reference interval (196 IU/L)		Total (*n*)
	Yes (*n*)	No (*n*)	
1	119	72	191
2	20	26	46
3	23	33	56
4	7	16	23
5	6	10	16
6	2	6	8
8	1	1	2

n = number of samples

Irrespective of assistance or treatment status, neonatal plasma CK concentration was highest in the first 24 h postpartum and declined over time ($p < 0.001$). Each increase in dam lactation number was associated with a decrease in neonatal plasma CK concentration of 0.094 IU/L ($p = 0.004$).

6.3.4 Neonatal plasma L-lactate concentration

Plasma L-lactate concentration was not affected by either assistance status or treatment status either overall or at any individual sample time point. Irrespective of assistance or treatment status, plasma L-lactate concentration was highest at birth and declined over time ($p < 0.001$) [Figure 6.4].

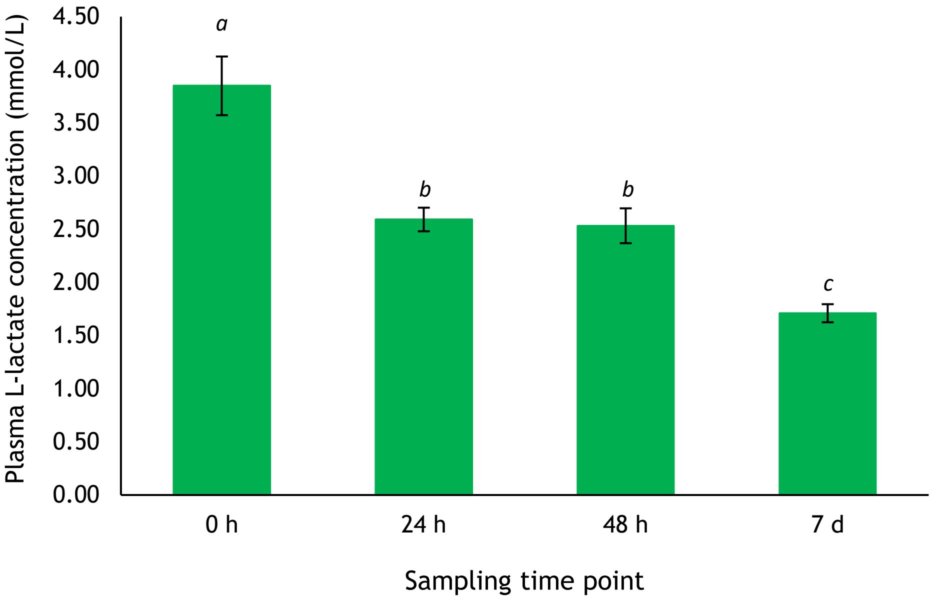


Figure 6.4: Back-transformed mean and standard error of neonatal plasma L-lactate concentration (mmol/L) at each sampling time point, irrespective of assistance or treatment status
Different letters indicate significant differences.

Neonatal plasma L-lactate concentration was negatively associated with both dam lactation and calf birthweight. Every 1 kg increase in calf birthweight was associated with a 0.005 mmol/L decrease in neonatal plasma L-lactate concentration (p = 0.022). Each increase in dam lactation was associated with a 0.018 mmol/L decrease in neonatal plasma L-lactate concentration (p = 0.039).

6.3.5 Neonatal plasma total protein concentration

6.3.5.1 Failure of passive transfer prevalence

Within study prevalence of FPT was 5.7% (5/87 samples). Seventy samples (80%) exceeded the plasma TP concentration cut-point associated with minimal calf morbidity and mortality (≥ 6.2 g/dL) (Lombard *et al.*, 2020).

6.3.5.2 Effects of assistance and treatment status, sampling time and colostrum consumption

Irrespective of assistance status ketoprofen treated calves had lower plasma TP concentration than saline treated calves (p = 0.001) at 48 h, but not at other time points. However, although a difference was identified, this was not associated with FPT — mean neonatal plasma TP concentration in both treatment groups exceeded 5.6 g/dL at 48 h.

Neonatal plasma TP concentration was lowest immediately after birth and increased with time until 48 h postpartum regardless of assistance or treatment status ($p < 0.001$) [Figure 6.5]. Neonatal plasma TP concentration was not affected by the volume of colostrum consumed.

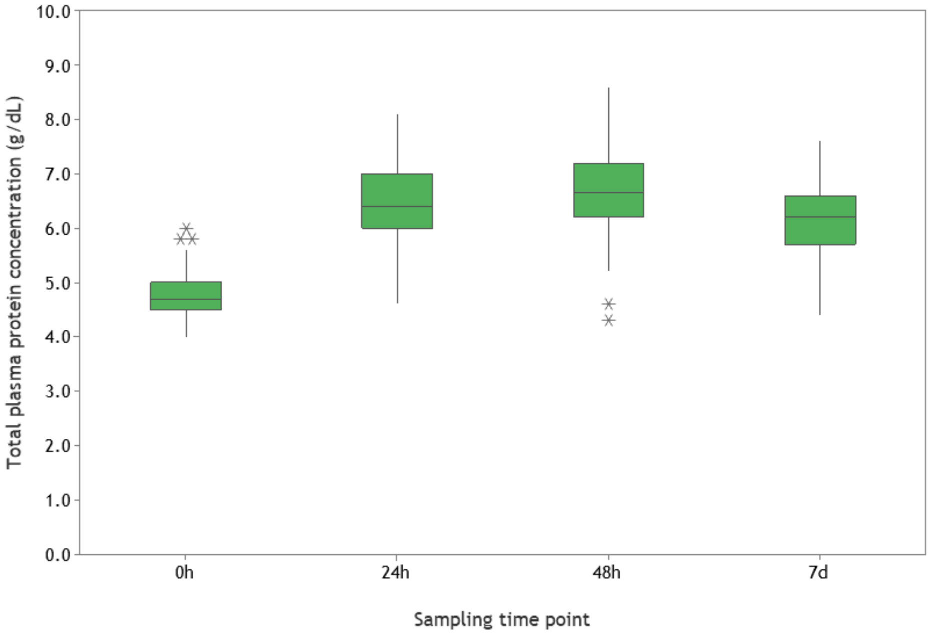


Figure 6.5: Calf total plasma protein concentration (g/dL) at each sampling time point, irrespective of assistance or treatment status
** Indicates outliers, central line indicates median value, boxes indicate interquartile range, whiskers indicate range. Back transformed results presented. n = 91.*

6.3.5.3 Association between birth plasma L-lactate concentration and passive transfer of immunoglobulins

There was a tendency for L-lactate concentration at birth to be mildly (positively) correlated with 48 h TP concentration ($r = 0.191$; $p = 0.077$) but the proportion of the variance in plasma TP concentration (at 48 h after birth) explained by birth plasma L-lactate concentration was negligible ($R^2 = 3.6\%$).

Median plasma L-lactate concentration at birth was 3.4 mmol/L; a threshold of plasma L-lactate concentration ≥ 3.4 mmol/L was therefore used to define samples as ‘high lactate’ (and < 3.4 mmol/L as ‘low lactate’). Plasma L-lactate concentration in these two groups was confirmed to differ ($p < 0.001$) [Table 6.5].

When this threshold was used, plasma L-lactate category did not affect passive transfer of immunoglobulins [Table 6.5]. Passive transfer of immunity was also not affected by birth L-lactate concentration when a higher threshold of 7.5 mmol/L was used to assign calves to 'high lactate' and 'low lactate' groups [Table 6.6].

Table 6.5: Comparison of neonatal plasma L-lactate and plasma total protein concentration in samples categorised as 'high' and 'low' lactate at birth using a threshold of 3.4 mmol/L

Biochemical analyte	Category		*p*-value
	High lactate	Low lactate	
Plasma L-lactate concentration at birth (mmol/L)[1]	4.5	2.5	< 0.001
Plasma total protein concentration at 48 h (g/dL)[2]	6.7	6.6	0.576

1. Median plasma L-lactate concentration presented
2. Mean plasma TP concentration presented

Table 6.6: Comparison of neonatal plasma L-lactate and plasma total protein concentration in samples categorised as 'high' and 'low' lactate at birth using a threshold of 7.5 mmol/L

Biochemical analyte	Category		*p*-value
	High lactate	Low lactate	
Plasma L-lactate concentration at birth (mmol/L)[1]	13.5	3.4	0.001
Plasma total protein concentration at 48 h (g/dL)[2]	6.9	6.7	0.620

1. Median plasma L-lactate concentration presented.
2. Mean plasma TP concentration presented

6.4 Discussion

6.4.1 Analysis of maternal biochemical analytes

6.4.1.1 Effects of parturition assistance

Maternal plasma cortisol concentration was the only biochemical analyte affected by assistance status: immediately postpartum (but not at other sample points), plasma cortisol concentration of assisted cows was higher than unassisted cows. This result suggests that farmer-assisted parturition is more stressful for cows than unassisted parturition and is consistent with the findings of previous studies (Nakao *et al.*, 1990; Kindahl *et al.*, 2002; Civelek *et al.*, 2008). It is possible that this finding is due to increased pain, but it should also be considered that human presence can also be associated with a stress response (Hemsworth *et al.*, 1987) and may have affected results. The effect of assisted parturition on maternal plasma cortisol concentration in the present study was a short-term effect and by

24 h postpartum plasma cortisol concentration did not differ between assisted and unassisted cows, a novel finding. Short-term increases in adrenocortical activity in response to acute stressors (such as parturition) form part of an adaptive response and are thus considered to be normal (Barnett *et al.*, 1990); as such, the relevance of such short-term stress responses to welfare can be difficult to interpret. Nevertheless, acute stressors should not be ignored and, although parturition cannot be avoided, animal management should aim to minimise additional stressors as far as possible. As parturition assistance is consistently reported to exceed dystocia incidence (Mee, 2008; Mee *et al.*, 2011; Holmøy *et al.*, 2017) it is probable that some cattle (and their calves) are experiencing unnecessary assistance at parturition. The recognition of animals that truly require assistance at parturition is a vital part of obstetrical training of farm staff and will ensure that the number of cattle experiencing assisted parturition is minimised. When assistance at parturition is unavoidable, appropriate timing of intervention — as well as appropriate techniques — are important factors that can be included in farm training programmes to minimise stress (Schuenemann *et al.*, 2011, 2013). Indeed, it has been argued that parturition assistance provided by personnel who have received good quality obstetrical training may even reduce parturition associated stress in cattle (Schuenemann *et al.*, 2011, 2013).

Parturition was associated with a general increase in maternal plasma CK concentration but in contrast to previous work (Civelek *et al.*, 2008; Hussein *et al.*, 2008), no assistance effect was observed. It should be noted that although Civelek *et al.* (2008) and Hussein *et al.* (2008) found that plasma CK concentration of assisted cows was higher than unassisted cows, both studies report the effects of more intensive parturition assistance than the current study (including foetotomy and caesarean section), and it is probable that these differences in study design explain differences in results. The results of the present study suggest that — whilst parturition in cattle always results in some muscular injury — judicious provision of mild to moderate parturition assistance does not inevitably cause more severe muscle injury than unassisted parturition; however, the effects of more severe assistance cannot be determined. Nevertheless, this is an important finding that further supports the hypothesis that careful and judicious parturition assistance provided by well-trained personnel need not be a traumatic event.

Similar to the results presented here, plasma CK concentration has been reported to be elevated at parturition in women (Abramov *et al.*, 1996) and guinea-pigs (Clark *et al.*, 1994). The laboratory RI for CK used in this study (≤ 196 IU/L) is very similar to the RI for bovine CK typically used for female cattle at all stages of production (< 200 IU/L) (Sattler *et al.*, 2004; Otter, 2013). Whilst it has been suggested that stage in lactation may affect plasma CK concentration (Cozzi *et al.*, 2011), to date different reference values for bovine plasma CK appropriate to different stages of production have not been established. Given that RI are defined as the range of values that comprise 95% of the healthy population (Friedrichs *et al.*, 2012), and that parturition itself is associated with an increase in plasma CK (Payne *et al.*, 1993; Clark *et al.*, 1994; Abramov *et al.*, 1996), it is possible that the standard RI for bovine plasma CK concentration is too low to be accurately applied immediately postpartum. Whilst it is likely that parturition always results in some muscle and uterine injury, it is important for veterinary surgeons to be able to determine when the degree of injury is excessive. Establishment of appropriate RI for bovine maternal plasma CK concentration in the first week postpartum would provide valuable information and aid accurate interpretation of biochemical profiles in clinical practice.

6.4.1.2 Effects of ketoprofen treatment

Consistent with Barragan *et al.* (2020b) ketoprofen treatment did not affect maternal plasma cortisol concentration (irrespective of assistance status). This result suggests that pain may not be the foremost source of parturition-related stress experienced by dairy cattle (even when assistance is provided). However, as the onset of analgesic action of ketoprofen is not immediate (and the first blood sample was obtained at the same time as ketoprofen was administered), it needs to be considered that any effects of ketoprofen on maternal plasma cortisol concentration occurring in the first 24 h postpartum may have been missed. Due to the short postpartum half-life of cortisol (approximately 30 min (Dunlap *et al.*, 1981)), very frequent blood sampling at short intervals is needed to accurately capture post-parturient bovine cortisol responses (Hudson *et al.*, 1976). The current study was designed to investigate longer term (up to 7 d) effects of parturition assistance and analgesia on biochemical analytes, therefore multiple blood samples were not obtained in the first 24 h postpartum. A single study has investigated the effects of analgesia on maternal plasma cortisol concentration in the first 24 h postpartum but multiple short-interval samples (< 12 h) were not obtained (Barragan *et al.*, 2020b). As such, the effects of analgesia on maternal cortisol concentration in the first hours postpartum remain unknown and merit further attention.

At 24 and 48 h postpartum plasma CK concentration was more likely to exceed the upper limit of the RI in ketoprofen treated cows than saline treated cows. Plasma CK concentration is known to be increased in cattle for up to 48 h after intramuscular injection of NSAIDs (Pyörälä *et al.*, 1999), whereas serum CK concentration is not affected by intramuscular administration of saline (as it is a less irritant product) (Lefebvre *et al.*, 1994; Pyörälä *et al.*, 1999). Therefore, it is probable that the effect of ketoprofen treatment on maternal plasma CK concentration identified resulted from localised muscle irritation at the injection site.

6.4.1.3 Effects of time and lactation number

Consistent with previous studies (Comline *et al.*, 1974; Hudson *et al.*, 1976; Patel *et al.*, 1996; Barragan *et al.*, 2020b) maternal plasma cortisol concentration was highest at parturition (irrespective of assistance or treatment status) and declined thereafter. Timing of blood sampling was relative to the time of parturition; therefore the time of day blood samples were obtained varied between individuals. Unlike some other species (Mesbah *et al.*, 1982; McMillen *et al.*, 1987; Torres-Farfan *et al.*, 2008; Cordero *et al.*, 2012), bovine cortisol profiles do not exhibit diurnal variation (Hudson *et al.*, 1975; Lefcourt *et al.*, 1993), thus the different times of day that blood samples were obtained is unlikely to have affected results.

Maternal plasma CK concentration was highest at 24 h postpartum (irrespective of treatment or assistance status) where it was more likely to exceed the upper limit of the laboratory RI than at any other time point. Hussein *et al.* (2008) also found that maternal plasma CK concentration was more elevated 24 h postpartum than immediately postpartum; however (in contrast to the results of the current study), this effect was only observed in cows that had experienced assistance at parturition (Hussein *et al.*, 2008). It is possible that cows experience persistent muscle injury following parturition, resulting in the observed rise in plasma CK concentration in the first 24 h postpartum. Although administration of ketoprofen might have been expected to ameliorate any muscle injury occurring after injection, this was not observed. It is possible that the underlying reason for the increase in maternal plasma CK concentration between parturition and 24 h postpartum was not affected by the anti-inflammatory effect of ketoprofen, or that localised muscle injury caused by ketoprofen administration confounded any anti-inflammatory effect. This has implications for whether intramuscular administration of NSAIDs is suitable for future studies where plasma CK concentration is measured. Intravenous administration may be a more appropriate route if plasma CK concentration is the outcome of interest, but this can be a more stressful method of administering a product to cattle and the benefits need to be carefully evaluated in any future study design. Alternatively, different NSAIDs that do not require intramuscular or intravenous administration could be considered, for example meloxicam is administered via the subcutaneous route (National Office of Animal Health, 2020).

Maternal plasma CK concentration in primiparous cows was most likely to exceed the upper limit of the laboratory RI (196 IU/L). This result may be due to the lack of parturition experience in primiparous cows as these animals are typically smaller with a reduced expansion of pelvic space at parturition (relative to multiparous cows). Accordingly, there is likely to be an increased risk of muscle injury being sustained by primiparous cows during parturition. This finding therefore suggests that primiparous cows may experience more pain — and benefit from enhanced care — in the postpartum period. This hypothesis is supported by the results of the study reported in Chapter 4 that found differences in lying behaviours of primiparous cows suggestive of heightened discomfort and reduced welfare compared to multiparous cows.

6.4.2 Analysis of neonatal biochemical analytes

6.4.2.1 Effects of birth assistance

In contrast to previous studies (Hoyer *et al.*, 1990; Civelek *et al.*, 2008; Vannucchi *et al.*, 2015a), neonatal plasma cortisol concentration was not affected by assistance status. Provision of parturition assistance is restricted to experienced personnel on the study farm and is focused on welfare. Therefore, it is possible that even the most severe (grade 3) birth assistance was not traumatic enough to cause increased stress in affected calves (relative to unassisted calves); which may explain the differences in findings. This hypothesis is supported by Hoyer *et al.* (1990) who found that plasma cortisol concentration of calves born to caesarean section or mildly assisted birth did not differ from unassisted calves, but plasma cortisol concentrations of severely assisted calves (defined as two or three personnel applying traction for more than 5 min — a severity and duration of assistance not observed in the present study) was numerically (albeit not significantly) elevated. Similarly, Vannucchi *et al.* (2015a) found that plasma cortisol concentration of calves born to dystocia was higher than unassisted calves and (normally presented) calves subject to prolonged birth. Therefore, although initially seeming to be contrasting results, the findings of the current study are more consistent with those reported by Vannucchi *et al.* (2015a) and Hoyer *et al.* (1990) when differences in study design are taken into account (i.e. mild to moderate assistance at birth is no more stressful for calves than unassisted birth). Unlike previous studies (Hoyer *et al.*, 1990; Civelek *et al.*, 2008), veterinary assistance and caesarean section were excluded from the current study and it is possible that assistance effects on neonatal plasma cortisol concentration may have been identified if calves born to more severe assistance were included. However, the current study was designed to reflect commercial practices and as such focused on farmer provided assistance (Egan *et al.*, 2001). It is difficult to determine whether neonatal cortisol concentration would be unaffected by assistance status on other farms where parturition assistance may not be as optimised as the study farm; however, these results suggest that if judiciously applied, birth assistance need not be stressful for calves. This important finding suggests that even as early as during birth there are opportunities to improve the welfare of calves through appropriate education and training of farmers, stock-personnel, and veterinary surgeons.

Only two previous studies have reported the effects of assisted birth on neonatal plasma CK concentration (Murray *et al.*, 2015b; Pearson *et al.*, 2019c). Both used similar birth assistance grading schemes [Appendix 1], finding that compared to unassisted birth, severe (but not mild) birth assistance was associated with increased neonatal plasma CK concentration (Murray *et al.*, 2015b; Pearson *et al.*, 2019c). By contrast, the current study did not identify an assistance effect on neonatal plasma CK concentration but it is possible that (although grade 3 assistance in this study was analogous to difficult birth assistance in the studies reported by Murray *et al.* (2015b) and Pearson *et al.* (2019b)) the optimised nature of birth assistance provided on the study farm resulted in comparatively less muscle injury to calves. Alternatively, differences in study design may also explain contrasting results. Pearson *et al.* (2019b) and Murray *et al.* (2015b) analysed the effects of individual grades of assistance whereas the current study grouped all calves into a single 'assisted' category. Whilst this approach ensured adequate statistical power, it has prevented detailed comparisons of the effects of different levels of birth assistance to be made. However, the majority (68%) of assisted births in the current study were grade 3 — equivalent to the category of 'difficult assist' that Pearson *et al.* (2019b) and Murray *et al.* (2015b) found was associated with increased neonatal plasma CK concentration. As such, whilst it would have been of interest for the current study to compare the effects of grade 2 with grade 3 birth assistance, it is unlikely to have changed the overall findings.

Previous work has consistently found assisted birth to be associated with elevated neonatal L-lactate concentration (Diesch *et al.*, 2004; Sorge *et al.*, 2009; Homerosky *et al.*, 2017a) but this was not observed in the current study. It is possible that this is related to the parturition assistance provided on the study farm. Prolonged traction during birth has been found to be associated with an increased degree of neonatal acidaemia (Szenci *et al.*, 1988; Murray *et al.*, 2015b), with one study finding that the severity of traction applied during assistance was a predictor of neonatal venous pH (Murray *et al.*, 2015b). In most cases the duration of traction applied in the present study was short (< 1 min); therefore, although previous studies (Sorge *et al.*, 2009; Homerosky *et al.*, 2017a) do not report the duration of traction it is possible that contrasting results are due to differences in the traction duration. This hypothesis is supported by Szenci *et al.* (1988) who found that acidaemic calves (blood pH < 7.0) had been subject to traction for a mean duration of approximately 3 min, whereas calves with blood pH ≥ 7.0 had experienced traction for 1.4 min or less (Szenci *et al.*, 1988).

Neonatal plasma TP concentration was not affected by assistance status. By contrast, other studies have found that assisted birth is associated with an increased risk of FPT (MacFarlane *et al.*, 2014; Homerosky *et al.*, 2017b; Pearson *et al.*, 2019b; Bragg *et al.*, 2020; Renaud *et al.*, 2020), but the aetiology of how assisted birth affects passive transfer in calves remains unclear. It has been suggested that neonatal acidosis impairs intestinal IgG absorption, but few studies have measured plasma L-lactate concentration or blood pH whilst also standardising (and optimising) colostrum consumption. Boyd (1989) and Besser *et al.* (1990) both found that IgG absorption is impaired in calves experiencing hypercapnia at birth. However, it is difficult to conclude that this finding correlates with FPT because, although colostrum consumption was standardised in both studies, Boyd (1989) fed calves a volume of colostrum that might in itself be expected to result in FPT (only 2 L) and in both studies IgG concentration was measured at 12 h after birth (Boyd, 1989; Besser *et al.*, 1990) — too early for PT to be accurately assessed. Hypoxia has also been shown to affect the absorption of IgG (Tyler *et al.*, 1991); however, calves in the study reported by Tyler *et al.* (1991) were maintained in a hypoxic environment (10.5% oxygen) for 24 h and it was during this time that the effect on IgG absorption was observed. After hypoxic calves were exposed to normal air (21% oxygen), the effect was no longer observed. Whilst this study identified an interesting effect of hypoxia on the intestinal absorption of IgG, it is unlikely to be reflective of the degree of hypoxia experienced by calves during birth. Nevertheless, these findings have been inferred to suggest that lactic acidosis at birth may be the reason assisted birth is associated with FPT in calves (MacFarlane *et al.*, 2015; Renaud *et al.*, 2020). However, Boyd (1989) and Besser *et al.* (1990) do not report the effects on recognised measures of FPT and the findings of Tyler *et al.* (1991) suggest that the adverse effect of hypoxia on IgG absorption is quickly reversed when hypoxia is resolved; therefore, the direct effects of lactic acidosis on PT in calves are currently uncertain. More recently, Homerosky *et al.* (2017b) reported that beef calves experiencing lactic acidosis at birth were less likely to voluntarily consume colostrum within the first 4 h of life (as detected by observations of calf suckling). Although the volume of colostrum consumed could not be measured or standardised, this finding was still associated with an increased risk of FPT in affected calves (Homerosky *et al.*, 2017b). It is possible that the reported increased risk of FPT in calves experiencing assisted birth is due to a combination of failure to consume an adequate volume of colostrum and impaired IgG absorption. In the current study, the minimum volume of colostrum consumed by each calf was standardised and neonatal plasma L-lactate concentration at birth was not negatively correlated with neonatal plasma TP concentration at 48 h after birth, as would be expected if neonatal acidosis adversely affected intestinal absorption of IgG. Additionally, when calves were categorised into either high or low plasma L-lactate concentration groups, no difference in plasma TP concentration at 48 h old was identified. Furthermore, plasma TP concentration in both high and low L-lactate groups exceeded the minimum cut-point recently suggested to be optimal for minimising disease incidence and maximising survival to weaning (≥ 6.2 g/dL) (Lombard *et al.*, 2020). These results suggest that, when colostrum management is optimised, calves that have an elevated plasma L-lactate concentration at birth are not automatically at increased risk of FPT. This is an important finding because it indicates that optimising colostrum management on farm can compensate for any impairment in intestinal IgG absorption that may occur in calves experiencing birth-related lactic acidosis and thus contribute to improved health of affected calves.

6.4.2.2 Effects of ketoprofen treatment

At 48 h after birth, neonatal plasma cortisol concentration of ketoprofen treated calves was lower than saline treated calves (irrespective of assistance status) — no differences were identified at other sampling time points. As all calves were managed in the same way from birth it is unlikely that saline treated calves were exposed to a new stressor in the first 2 d of life that did not affect ketoprofen treated calves. Knowles *et al.* (2000) found that neonatal plasma cortisol concentration declined after birth, but did not reach a plateau until calves were approximately three weeks of age. By contrast, Hoyer *et al.* (1990) found that by 48 h after birth neonatal plasma cortisol concentration was the same for all calves, irrespective of birth experience. The findings of the current study suggest that, although administration of ketoprofen at birth does not affect neonatal plasma cortisol concentration in the first 24 h, the return to baseline cortisol concentration is more rapid in calves treated with ketoprofen, regardless of birth assistance. A possible explanation for this novel finding could be the development of late onset post-birth pain, although it is unknown whether this occurs in neonates of any species. Late onset muscular pain termed ‘delayed onset muscle soreness’ (DOMS) occurring 24 to 72 h after strenuous exercise is well described in humans (e.g. Abraham, 1977; Schwane *et al.*, 1983) but has been ignored in the veterinary literature. One author has suggested that, as animals have similar muscle structure to humans, it would be reasonable (in the absence of evidence to the contrary) to assume this phenomenon does occur in animals (Steiss, 2002) but data to support this view are absent. It is possible that birth is a strenuous event causing delayed onset skeletal muscle pain similar to DOMS that develops after the first 24 h of life and is ameliorated by ketoprofen analgesia. This hypothesis is further supported by the findings of the study presented in Chapter 3, where calves treated with ketoprofen exhibited differences in early-life behaviour (up to 48 h old) consistent with reduced pain and improved welfare. Although ketoprofen has been shown to inhibit inflammatory mediators for up to 24 h (Landoni *et al.*, 1995), there is some evidence that the duration of clinical analgesia is more prolonged (Kantor, 1986; Whay *et al.*, 2005b). Therefore it is plausible that the development of pain in calves after 24 h old could be ameliorated by ketoprofen administered immediately after birth, thereby reducing any pain-related stress experienced 24 to 48 h after birth. In humans, DOMS has been shown to be associated with a preceding increase in plasma CK concentration (Schwane *et al.*, 1983). In the current study, neonatal plasma CK concentration of all calves was increased at 0 and 24 h old (irrespective of assistance or treatment status), further supporting the hypothesis that birth may be associated with delayed muscle pain similar to DOMS.

Ketoprofen treated calves had a lower plasma TP concentration at 48 h after birth than saline treated calves. This was an unexpected finding that had not previously been reported, although Clark *et al.* (2020) found meloxicam treatment was associated with lower plasma IgG concentration at 12 h after birth. By contrast, Pearson *et al.* (2019a) found that meloxicam administration did not have any effect on serum IgG concentration at 24 h after birth. It is unknown whether ketoprofen directly affects neonatal immunoglobulin concentration but its administration has been associated with a reduced plasma concentration of proteins associated with an inflammatory response (such as fibrinogen and haptoglobin) in postpartum cows (Kovacevic *et al.*, 2019) and also following calf castration (Earley *et al.*, 2002). Accordingly, it is possible that ketoprofen supressed a post-birth inflammatory response occurring in calves. Interestingly, neonatal plasma cortisol concentration at 48 h after birth was also lower in ketoprofen treated calves. If calves experienced an inflammatory response at 48 h post-birth that was suppressed by ongoing anti-inflammatory effects of ketoprofen, it is plausible that this contributed to the decreased plasma cortisol concentration identified in this group of calves. Data obtained from subsequent analysis of plasma IgG concentration 48 h after birth (Katharine Denholm *et al.*, unpublished) were not affected by ketoprofen treatment. Whilst more detailed analysis of individual plasma proteins is needed to investigate this further, the absence of an effect on plasma IgG concentration suggests that the ketoprofen effects on plasma TP observed at 48 h after birth is due to an effect on other plasma proteins (e.g. fibrinogen) and not related to passive transfer of IgG. Moreover, it is important to note that, at 48 h after birth, the plasma TP concentration of both ketoprofen and saline treated calves exceeded 5.6 g/dL — the suggested cut-point indicative of FPT when plasma TP concentration is measured (MacFarlane *et al.*, 2014). The effect of ketoprofen treatment on neonatal plasma TP when calves are 48 h old therefore has limited clinical significance from the perspective of FPT; however, veterinary surgeons may wish to consider performing additional testing to confirm a diagnosis of FPT based on plasma TP concentration if there is a history of NSAID administration to affected calves. The possibility of subclinical inflammation occurring in 48 h old calves (irrespective of birth experience) merits further study, a view further supported by the similar effect of ketoprofen treatment on neonatal plasma cortisol concentration at the same sample point (suggestive of the presence of a stressor in 48 h old calves).

6.4.2.3 Effects of time

Irrespective of assistance or treatment status neonatal plasma CK concentration was elevated at birth and remained at a similar concentration before declining after 24 h, a pattern similar to results reported by Knowles *et al.* (2000) and Egli *et al.* (1998). Neither previous study controlled for the effect of assisted birth, although the authors of both studies postulated that the high serum CK concentration observed at birth was likely to be due to birth trauma (Egli *et al.*, 1998; Knowles *et al.*, 2000). This hypothesis is supported by the results of the present study, and it is possible that all calves experience some muscle damage during birth, irrespective of how traumatic the parturition event is perceived to be by farmers or veterinary surgeons. Newborn calves are often managed similarly to older pre-weaned calves, but these results suggest that very young calves may have additional needs that should be met to optimise their welfare. For example, the depth of bedding provided for calves in the first week of life may need to be greater than that provided to older calves to provide additional cushioning and enhance the comfort of calves with birth-related muscle injury.

Neonatal plasma L-lactate concentration was highest immediately after birth and declined with increasing age — as expected. Previously it has been established that a degree of mixed metabolic and respiratory acidosis at birth is common in calves (Varga *et al.*, 1998) but self-resolution is typically observed within the first hour of life (Varga *et al.*, 1998, 2001). Healthy ruminants typically have blood lactate concentration between 0.5 mmol/L and 2.0 mmol/L (Omole *et al.*, 2001). The normal RI for plasma L-lactate concentration in calves immediately after birth has not been established; however, a blood pH of ≥ 7.2 has been suggested to be normal (Szenci *et al.*, 1988), which Homerosky *et al.* (2017a) found is correlated to a plasma L-lactate concentration of approximately 7.5 mmol/L. This is very similar to Bleul *et al.* (2013) who found that normal calves had a blood L-lactate concentration of 7.3 mmol/L at birth compared to hypoxic calves that had a L-lactate concentration exceeding 13 mmol/L. Although neonatal plasma L-lactate concentration was highest at birth, mean values did not exceed 7.5 mmol/L in the present study (regardless of assistance or treatment status). This result suggests that (irrespective of assistance status) most study calves did not experience an abnormal degree of lactic acidosis at birth. However, the observed range of individual results was wide (1.2 mmol/L to 19.7 mmol/L), indicating a lot of individual variation — consistent with the findings of Sorge *et al.* (2009).

Neonatal plasma TP concentration was affected in a predictable fashion by the time of sampling. Calves are born virtually agammaglobulinaemic (Weaver *et al.*, 2000); plasma TP concentration at birth is low and increases following colostrum consumption (and subsequent gastrointestinal absorption of immunoglobulins). However, plasma TP concentration in calves at birth reflects the presence of other plasma proteins; therefore, the mean plasma TP concentration at birth of 4.8 g/dL observed in this study is to be expected. This finding is similar to results reported by Tóthová *et al.* (2016) who reported a similar neonatal serum TP concentration at birth (41.1 g/L) and a similar pattern of change over time.

6.4.2.4 Effects of dam lactation and colostrum consumption

Similar to cows, plasma CK concentration of calves was negatively affected by dam lactation number. The effects of dam lactation on the plasma CK concentration of their calves have not previously been studied, but it is probable that the increased pelvic space afforded by multiparity results in calves born to older dams being subject to a lower degree of compression during birth (and consequently less foetal muscle injury).

Plasma TP concentration was not affected by the volume of colostrum consumption. This was an unexpected finding as the importance of ensuring calves consume an adequate volume of colostrum soon after birth is well described (Stott *et al.*, 1979b; Fischer *et al.*, 2018). Colostrum management is optimised on the study farm and ensuring all calves consume at least 4.0 L of colostrum within the first 4 h of life is prioritised; as such, a ceiling effect probably contributed to this finding. Only 5.7% of calves had plasma TP concentration (at 48 h) suggestive of FPT. This is an unusual finding as a FPT prevalence exceeding 15% is consistently reported (Beam *et al.*, 2009; MacFarlane *et al.*, 2015; Cuttance *et al.*, 2017b; Bragg *et al.*, 2020; Renaud *et al.*, 2020), although a wide between-herd variation of 5% to over 80% has been described (MacFarlane *et al.*, 2014; Cuttance *et al.*, 2017b). The results of this study should not be taken to indicate that the volume of colostrum consumption is unimportant. Rather, the data support both prior work and industry recommendations in demonstrating that an optimised colostrum management protocol can minimise the prevalence of FPT in calves.

6.5 Conclusion

The 2 x 2 design of this study has allowed investigation of the individual effects of assisted parturition and ketoprofen treatment (and their interactions) on some important biochemical analytes in both cows and their calves. Plasma cortisol concentration of assisted cows was higher than unassisted cows, but a similar effect was not observed in assisted calves. This result suggests that cows might be more sensitive to the effects of human intervention at parturition than their calves, although the effect was short term and was not observed beyond the first 24 h postpartum. Furthermore, assisted parturition did not affect any of the other biochemical analytes measured in either cows or their calves. These results suggest that appropriately provided parturition assistance of mild to moderate severity is a similar experience to unassisted parturition and does not result in longer term activation of the hypothalamic-pituitary-adrenal axis, or more severe muscle injury, than unassisted parturition. This key result challenges the accepted view that injury and poorer postpartum welfare are inevitable consequences of parturition assistance and suggests that appropriate obstetric training can lead to improved welfare outcomes when parturition assistance is required — a finding that should be given high consideration when designing future agricultural and veterinary training programmes.

Chapter 7 Effects of parturition assistance and ketoprofen treatment on measures of productivity

7.1 Introduction

Whilst the effects of assisted parturition and dystocia on maternal health and production are well studied, the findings are inconsistent. Some existing studies have found assisted parturition is associated with a negative effect on subsequent lactation milk yield (Barrier *et al.*, 2011; Eaglen *et al.*, 2011; Atashi *et al.*, 2012; Hossein-Zadeh, 2014; Newby *et al.*, 2017), whereas in other studies no effect was identified (Thompson *et al.*, 1983; Tenhagen *et al.*, 2007). Differences in study design and measured parameters make direct comparisons across studies difficult. For example, Atashi *et al.* (2012) found that assisted parturition (defined as grade ≥ 3 on a 5 point scale) had a negative effect on milk yield whereas Tenhagen *et al.* (2007) found dystocia had no effect, but these studies cannot be directly compared as they analysed different measures of milk production (cumulative 305 d yield and monthly yield respectively).

In contrast to milk production, the reported effects of assisted parturition on postpartum disease risk, reproductive performance, and survival in the subsequent lactation are more conclusive. Assisted parturition is associated with an increased risk of developing postpartum disease (McArt *et al.*, 2013; Vergara *et al.*, 2014; Kovács *et al.*, 2016; Mahnani *et al.*, 2021) and leaving the herd in the subsequent lactation (Thompson *et al.*, 1983; Tenhagen *et al.*, 2007; Hayes *et al.*, 2012). Additionally, although measures of reproductive performance differ between studies, the effects of assisted parturition on subsequent reproductive performance are consistently reported to be negative (Thompson *et al.*, 1983; Tenhagen *et al.*, 2007; Richards *et al.*, 2009; Hayes *et al.*, 2012).

Whilst it is well known that assisted birth is associated with an increased risk of early-life mortality (Arthur *et al.*, 2000; Lombard *et al.*, 2007; Kovács *et al.*, 2016; Norquay *et al.*, 2020), data pertaining to the effects of assisted birth on future production of calves are scant. A single study investigating the effects of early life experiences on future milking performance found that assisted birth negatively affects first lactation milk and protein yield (Heinrichs *et al.*, 2011). The same study also found that increasing severity of assistance was associated with greater negative effects (Heinrichs *et al.*, 2011), but the effects of birth experience on measures of future performance other than milk production (e.g. reproductive performance) were not studied. These results were supported by Eaglen *et al.* (2011) who found veterinary-assisted birth was associated with reduced first lactation milk production, with less intense birth assistance having less deleterious effects. In contrast to Heinrichs *et al.* (2011), Eaglen *et al.* (2011) analysed reproductive data, finding that assisted birth did not affect first lactation reproductive performance. Barrier *et al.* (2012c) investigated the effects of birth assistance on calf reproductive performance before first parturition, also finding that assisted birth had no effect.

Recent studies have found that negative foetal or neonatal experiences may have lifelong effects that have the potential to affect future success as productive dairy cows. For example, lack of early social contact has been shown to be associated with learning deficits in dairy calves (Meagher *et al.*, 2015), *in utero* heat stress has negative effects on first lactation milk production (Monteiro *et al.*, 2016), and intrauterine nutritional stress has negative effects on ovarian reserve and cardiovascular development (Mossa *et al.*, 2013). Up to 50% of calves are thought to be born to assisted parturition (Mee, 2008) therefore it is perhaps surprising that so little attention has been given to the potential effects of this common management intervention on parameters of future production, beyond neonatal mortality. As female Holstein calves represent the future of a dairy herd, understanding how early life experiences may affect future success as a productive dairy cow is crucial.

The first week postpartum is associated with an inflammatory response that occurs in all cows and is likely to be a normal response to parturition (Farney *et al.*, 2013a; Vailati Riboni *et al.*, 2015). Cows that experience postpartum disease have been found to have a greater degree of inflammatory response than healthy cows (Humblet *et al.*, 2006; Qu *et al.*, 2014), therefore modifying postpartum inflammation may reduce the risk of developing postpartum disease. Non-steroidal anti-inflammatory drugs (NSAIDs) are a widely-used class of drugs that effectively block some inflammatory pathways, reducing both pain and inflammation (Vane, 1971). Accordingly, it has been hypothesised that NSAID administration in the peripartum period may reduce the risk of postpartum disease through their anti-inflammatory effects, but the available data do not conclusively support this. Postpartum NSAID administration has been shown to reduce serum haptoglobin concentration (Carpenter *et al.*, 2016; Pascottini *et al.*, 2020) and increase the hepatic expression of pyruvate dehydrogenase kinase isozyme 4 (PDK4) (Vailati Riboni *et al.*, 2015), but studies investigating the effects of postpartum NSAID administration on development of clinically diagnosed postpartum disease have produced conflicting findings. Additionally, in recent years it has become apparent that — although NSAIDs all share a common mechanism of action — the postpartum pharmaco-clinical effects of different NSAIDs may differ. Waelchli *et al.* (1999) published the first report of flunixin being associated with an increased risk of retained foetal membranes (RFM) (compared to a placebo drug) but only included cows experiencing caesarean section. Nearly 20 years later Newby *et al.* (2017) studied the effects of administering flunixin to cows experiencing normal (unassisted) parturition and found that, compared to saline, peripartum administration of flunixin was associated with not only an increased risk of RFM but also an increased risk of stillbirth, leading the authors to conclude that the use of flunixin in the periparturient period cannot be recommended (Newby *et al.*, 2017). By contrast, peripartum administration of the NSAIDs meloxicam, ketoprofen and acetylsalicylic acid has not been found to have such deleterious effects (Richards *et al.*, 2009; Newby *et al.*, 2014b; Swartz *et al.*, 2018; Barragan *et al.*, 2020c). Results from studies investigating the effects of peripartum NSAID administration on the incidence of other postpartum diseases have been inconsistent. A single study found that a smaller proportion of acetylsalicylic acid treated cows developed clinical metritis than untreated cows (Barragan *et al.*, 2021), but several other studies have found that peripartum NSAID administration provides no protective effect on other parturition related conditions including LDA, metritis, SCK, and endometritis (Richards *et al.*, 2009; Swartz *et al.*, 2018; Barragan *et al.*, 2020c; Pascottini *et al.*, 2020).

The effects of postpartum NSAID administration on reproductive performance and risk of culling in the subsequent lactation have rarely been studied. Barragan *et al.* (2021) found that acetylsalicylic acid treated cows tended to conceive 18 d earlier than untreated cows. Similarly, Bertoni *et al.* (2004) found acetylsalicylic acid-treated cows conceived sooner, and required fewer insemination attempts, than placebo-treated cows. Bertoni *et al.* (2004) are unclear regarding the statistical significance of these results, but they are supported by data reported by Barragan *et al.* (2020a) who also found that acetylsalicylic acid treated cows conceived sooner in the subsequent lactation (needing fewer insemination attempts) than placebo treated cows. The same study found that acetylsalicylic acid treatment did not affect culling rate when compared to placebo treatment (Barragan *et al.*, 2020a). It is possible that other NSAIDs have similar effects but, to date, no studies have explored this and further work is warranted.

Results of studies investigating longer term effects of peripartum NSAID administration on milk production in the subsequent lactation are more promising. A number of studies have found that NSAID treated cows subsequently have a greater whole lactation or 305 d yield compared to untreated or placebo treated cows (Farney *et al.*, 2013b; Stilwell *et al.*, 2014; Carpenter *et al.*, 2016). By contrast, studies analysing early lactation milk yield are inconsistent with some studies identifying a beneficial effect of NSAID treatment (Bertoni *et al.*, 2004; Swartz *et al.*, 2018; Barragan *et al.*, 2020a) whereas others have not found any effect (Richards *et al.*, 2009; Shwartz *et al.*, 2009; Newby *et al.*, 2013a, 2017; Mainau *et al.*, 2014; Meier *et al.*, 2014). The effects of peripartum NSAID treatment on both early and whole lactation milk production are rarely reported together. A single study found that sodium salicylate treated cows in their 3rd lactation or older had higher whole lactation milk yield than placebo treated animals but early lactation milk yield was unaffected (Farney *et al.*, 2013b). The same study found that milk production of younger cows was not affected by sodium salicylate treatment (Farney *et al.*, 2013b). It is uncertain if there are heightened benefits of NSAID administration on the subsequent production of assisted cows (compared to unassisted cows) as most studies that include assisted parturition categorise animals by treatment (Richards *et al.*, 2009; Newby *et al.*, 2017) or do not include an unassisted control group (Newby *et al.*, 2013a). Additionally, the results of the few studies that include parturition assistance and analgesic intervention in a factorial design are conflicting. Swartz *et al.* (2018) found that whilst meloxicam treated cows experiencing normal parturition had higher daily milk yield than placebo treated cows, milk yield of cows experiencing dystocia was not affected (Swartz *et al.*, 2018). By contrast, Barragan *et al.*

(2020a) found that the daily milk yield of acetylsalicylic acid treated cows that had experienced dystocia was nearly 5 kg/d higher than placebo treated dystocia cows. It is possible that meloxicam and acetylsalicylic acid have differing effects that are contributing to the contrasting results of these two studies, but the different definitions of dystocia employed by each study also need to be taken into consideration. Swartz *et al.* (2018) defined dystocia as parturition lasting 70 min or more (and eutocia as lasting < 70 min), an unconventional definition that the authors concede limits the comparison of their results with other studies. By contrast, Barragan *et al.* (2020a) defined dystocia based on the degree of assistance provided — a more conventional approach aligned with how most farmers and veterinary surgeons would define 'difficult calving' in practice. Nevertheless, whilst the results presented by Barragan *et al.* (2020a) may be more comparative to other studies, a single study performed on an organic farm is not representative of the wider dairy industry and highlights the need for further work.

Data regarding the effects of post-birth NSAID administration on measures of calf production are limited, with daily weight gain being the only calf production outcome that has been studied. Pearson *et al.* (2019b) found that meloxicam-treated assisted beef calves had an increased daily liveweight gain in the first 7 to 10 d of life (compared to placebo treated assisted calves), but this effect was not observed when calves were older than 10 d. Similar findings have been reported in Holstein dairy calves, with Murray *et al.* (2016) finding that post-birth meloxicam did not affect average daily weight gain to weaning (compared to a placebo drug), although pre-weaning milk consumption of meloxicam treated calves was increased. The effects of post-birth NSAID analgesia on future (i.e. adult) calf production parameters have not been studied and are currently unknown.

7.1.1 Study aims and hypotheses

The current study aimed to determine the effects of assisted parturition and immediate postpartum administration of ketoprofen on measures of maternal milk production, reproductive performance, health status and survival in the subsequent lactation. A second aim was to investigate the effects of assisted birth and immediate post-birth administration of ketoprofen on neonatal growth, health status, reproductive performance, survival, and milk production until completion of calves' first lactation. Three hypotheses were tested:

1. Assisted parturition has negative effects on maternal health and production in the subsequent lactation that are related to parturient pain and inflammation and as such may be ameliorated by administration of ketoprofen.

2. Assisted birth has negative effects on neonatal health and production that may be ameliorated by post-birth administration of ketoprofen.

3. Negative effects of assisted birth and beneficial effects of ketoprofen on measures of neonatal production will persist into the first lactation of affected calves.

7.2 Materials and methods

7.2.1 Animal management and ethical approval

UK Home Office approval was obtained as described in Chapter 2, Section 2.1. Farm management and animal husbandry were as described in Chapter 2, Section 2.2. Study design, animal recruitment, treatment protocols and collection of animal metadata were as described in Chapter 3, Section 3.2 and Chapter 4, Section 4.2. Video footage of all animals was obtained for the wider study as described in Chapters 3 and 4. Sequential blood samples were obtained from all animals in the first week after parturition as described in Chapter 6, Section 6.2.2.

7.2.2 Data collection

All analysed data except for calf body weight and cow body condition score (BCS) were obtained (with permission) from farm records (DairyComp 305, Valley Agricultural Software, Tulare, CA, USA). Maternal data were collected from the date of parturition until the end of the subsequent lactation (defined as the date of the next parturition event), or until the date that cows left the herd — whichever was sooner. Neonatal data were collected from the date of birth until the end of the first lactation (defined as the date of the second parturition event) or until the date that calves left the herd, whichever was sooner. Analysed measures of production at each life stage are presented in Table 7.1.

Data from animals recruited as calves is referred to in this chapter as ‘neonatal’ data up to the date of first parturition irrespective of the age at which data were collected. After first parturition these data are referred to as ‘first lactation’ data. This terminology is used to differentiate these data from data collected from cows (maternal data).

7.2.2.1 Cow body condition score and calf birth weight

Cow BCS was assessed by the author at parturition (BCS1) and again at 60 d (± 8 d) postpartum (BCS2) using the Penn-State BCS assessment method (AHDB, 2020), a 5-point scale with 0.25-point graduations. The change in BCS in the first 2 months postpartum was calculated using the formula:

$$\textit{Change in BCS} = BCS2 - BCS1$$

Calf birthweight was measured and recorded by farm staff within 3 h of birth. Calves were weighed again at 64 d (± 4 d) by the author with assistance from farm staff and veterinary students from the University of Glasgow School of Veterinary Medicine. The 2nd weight measurement was timed to take place in the week that calves were weaned, allowing the growth rate to weaning to be calculated. The same weigh scale was used for both measurements. Nulliparous animals are weighed on farm at 12 months old when they are moved into the housing used for breeding animals (due to the facilities available for restraint) — these data were obtained from farm records to enable calculation of growth rate between weaning and breeding.

Table 7.1: Production outcomes analysed at each life stage for recruited cows and calves

Analysed outcome type	Maternal subsequent lactation performance	Neonatal future performance	
		Birth to first parturition	First lactation
Milking performance[1]	Final 305ME	-	Final 305ME
	Total lactation milk yield	-	Total lactation milk yield
	Total lactation fat yield	-	Total lactation fat yield
	Total lactation protein yield	-	Total lactation protein yield
	Week four milk yield (W4MK)	-	
Reproductive performance[2]	Number of times bred	Age at first detected oestrus	Number of times bred
	Calving to conception interval (CCI)	Oestrus not detected (Y/N) (before 13 months old)	Calving to conception interval (CCI)
	Time to first service	Age at first service	
	Bred before 75 DIM (Y/N)	Age at conception	
	Conceived before 100 DIM (Y/N)	Conceived before 450 days old (Y/N)	
	Conceived before 150 DIM (Y/N)	Number of times bred	
	Conceived before 200 DIM (Y/N)	First parturition at/before 24 months old (Y/N)	
	Conceived overall (Y/N)	Age at 1st parturition	
Health outcomes	Postpartum disease (Y/N)	Growth rate to weaning[3]	-
	Mastitis occurrence during subsequent lactation (Y/N)	Growth rate between weaning and breeding[3]	-
	Lameness occurrence during subsequent lactation (Y/N)	Bovine respiratory disease (BRD) (Y/N)	-
Survival	Left the herd during the subsequent lactation (Y/N)	Mortality to weaning[4]	Left the herd during the first lactation (Y/N)
		Left the herd before first parturition (Y/N)[4]	

305ME = 305 d mature equivalent. DIM = days in milk. – indicates outcome types not analysed for this life stage

1. All parameters measured in kg
2. Continuous data measured in days (d)
3. Measured in kg/d
4. Descriptive analysis only

7.2.2.2 Measures of milk production

Milk production data (milk, fat, and protein yield) are recorded monthly by National Milk Records (NMR) (www.nmr.co.uk). These data are used by the farm management software (DairyComp 305) to calculate cumulative whole lactation yields of milk, fat, and protein. Additionally, NMR test data are used to calculate milk yield at four weeks postpartum (W4MK) (a measure of how successfully cows transition into lactation) and 305 d mature equivalent yield (305ME) in DairyComp. The 305 d mature equivalent yield is a predictive measure of milk production standardised to a 305 d lactation length for a 3rd lactation cow, thus allowing direct comparison between animals of different ages and different stages in lactation. The calculation of 305ME is based on production that has already occurred (during the relevant lactation) up to 305 days in milk (DIM), therefore 305ME changes as the lactation progresses. For the purposes of analysis, the final 305ME available for the analysed lactation was used; this value is based on the final test data for the respective lactation and (for cows that complete the lactation) most accurately represents actual performance. Measures of milking performance analysed in the current study are presented in Table 7.2.

Table 7.2: Analysed measures of milk production

Variable	Definition
Whole lactation yield	Cumulative total milk production from the date of parturition to the date of drying off.
Total fat yield	Cumulative total quantity of milk fat produced from the date of parturition to the date of drying off.
Total protein yield	Cumulative total quantity of milk protein produced from the date of parturition to the date of drying off.
305ME	305 d mature equivalent predicted milk yield. A measure standardised to the 305 d yield of a 3rd lactation cow.
W4MK	Daily milk yield at four weeks (28 d) after parturition. An indicator of how successfully a cow has transitioned into lactation.

All variables measured in kg. 305ME = 305 d mature equivalent. W4MK = week four milk

7.2.2.3 Measures of reproductive performance

Farm breeding protocols are described in Appendix 2. Insemination date, pregnancy diagnosis (date and result), pregnancy loss (date) and parturition (date, calf sex and twins) data are recorded by farm staff in DairyComp 305 (Valley Agricultural Software, Tulare, CA, USA) and were exported into Microsoft Excel for analysis. Raw data were used to calculate measures of reproductive performance [Table 7.1]. Additionally, some continuous measures were converted to binary outcomes with more clinical or commercial relevance. For example, age at first parturition (d) was analysed as a continuous variable but was also analysed in binary format as 'first parturition before/after 24 months old' because, although exact age at first parturition is of interest, 24 months has been shown to be the age at first parturition where longevity and economic returns are optimised (Ettema *et al.*, 2004; Cooke *et al.*, 2013; Sherwin *et al.*, 2016; Boothby *et al.*, 2020). Thus, achievement of this target (i.e. first parturition at ≤ 24 months) is of greater clinical and commercial interest than the exact age at first parturition.

For the purposes of analysis 'conception' was defined as an animal having a pregnancy positively diagnosed by a veterinary surgeon at 28 to 35 d post insemination. Animals that were recorded negative (for pregnancy) or did not have any record of pregnancy diagnosis after insemination were classified as 'not conceived'.

7.2.2.4 Health status and survival data

Health data were obtained from farm and veterinary records where available. Due to availability of records and known diseases on the study farm, analysis of health status focused on postpartum disease (RFM, metritis, LDA or SCK), mastitis and lameness in adult cows and bovine respiratory disease (BRD) in calves. Records of disease that were not one of these diagnoses were grouped into a single 'other' category. Animals were recorded as having experienced disease (yes/no) if at least one record was made — multiple diagnoses of the same disease were not recorded separately. Postpartum diseases were diagnosed by a veterinary surgeon in the first week postpartum as described in Chapter 2, Section 2.2.1 and Appendix 2. Records of lameness, mastitis and BRD were made if diagnosed by experienced farm staff (or a veterinary surgeon where appropriate).

The date and reason animals leave the herd is recorded on farm and were obtained for analysis. For the purposes of analysis, animals that died with no recorded diagnosis were assumed have experienced illness causing death and were recorded has having experienced (non-specified) illness.

7.2.3 Statistical analysis

Farm recorded data were exported from farm management software (DairyComp 305) into Microsoft Excel (Excel 2013, Microsoft, CA, USA) for further analysis. Calf birth weight and cow BCS data were manually entered into relevant spreadsheets. Data were summarised in three Excel spreadsheets (a single spreadsheet for maternal data and two spreadsheets for neonatal data [birth to first parturition and first lactation]) (Excel 2013, Microsoft, USA) and exported to Minitab (Minitab v.18, Minitab Inc., State College PA, USA) for statistical analysis.

Individual postpartum diseases and reasons for leaving the herd were analysed descriptively only. Due to the small numbers of animals affected, each of these datasets were collapsed into a single (binary) group ('postpartum disease: yes/no' and 'left the herd before end of lactation: yes/no') for further analysis.

Anderson Darling analysis and visual appraisal of histograms were performed to assess continuous data for normality. Statistical models were selected based on the appropriate model type for the analysed outcome variable [Table 7.3].

Table 7.3: Statistical models used for analysis of each productivity outcome variable type

Outcome variable type	Statistical model type
Continuous	General linear model
Count	Poisson regression (log link)
Binary	Binary logistic regression (logit link)
Timed (right censored)	Kaplan-Meier survival analysis

For general linear models (GLM) and Poisson regression models the goodness-of-fit statistics produced by Minitab for each model (S-value, R-squared, adjusted R-squared, AICc) were inspected for all analyses to assess how well each model fitted the data and to compare the fit of different models for the same outcome variable. For binary logistic regression models, Hosmer-Lemeshow statistics were inspected to assess goodness-of-fit. For all models, residual plots were visually appraised to verify that the assumptions of each model were met. Data transformations ($\log_{10}$) and weighted regression were applied where appropriate to ensure model assumptions were met. Where appropriate, correlation statistics were analysed to check for collinearity, excluding one variable if $r \geq 0.7$. Variance inflation factor (VIF) statistics were also inspected for each model to check for multicollinearity. Calf weight data (birthweight and growth rates) were moderately correlated and were analysed separately.

Assistance status, treatment status and assistance x treatment interaction were included as fixed effects in all models. Additional fixed and covariate factors were included in all models based on clinical knowledge of expected effect or knowledge of farm management and are presented in Table 7.4.

Backwards stepwise elimination was performed for all models, sequentially removing factors and interaction terms if $p > 0.1$ with the exception of assistance and treatment status which were included in every model. The threshold of statistical significance was defined as $p < 0.05$ and a tendency towards statistical significance was considered if $p \geq 0.05$ and $p < 0.08$. A weak tendency was considered if $p \geq 0.08$ and $p < 0.09$.

For the purposes of analysis of milking performance, cow lactation was categorised into three groups (one, two and three-plus) to align with industry standards for analysing milk production parameters — an approach chosen to maximise the wider application and relevance of results. For reproductive, health, and survival analyses cow lactation number was included in each model as the absolute value.

All recruited calves were included in analysis of birth to parturition data. To minimise the amount of missing data points, calves that did not enter their first lactation were excluded from analysis of first lactation data.

Table 7.4: Fixed and covariate factors included in statistical models of maternal and neonatal productivity

Measure of production	Analysed dataset	Fixed effects[1]	Covariates[2]
Milk production	Maternal and neonatal first lactation	Lactation length (d)[3] Lactation number[3] Postpartum disease (Y/N)	BCS change Growth rate to weaning (kg/d)
Reproductive performance	Neonatal before first parturition	Dam lactation 'Developed BRD' (Y/N)	Growth rate to weaning (kg/d) Growth rate between weaning and breeding (kg/d)
	Maternal and neonatal first lactation	Lactation number Postpartum disease (Y/N)	W4MK (kg)
Survival in the herd	Neonatal before first parturition	'Developed BRD' (Y/N)	Growth rate to weaning (kg/d) Growth rate between weaning and breeding (kg/d)
	Neonatal first lactation	First parturition at/before 24 months (Y/N) Illness (Y/N)	1st test yield (kg) 2nd test yield (kg)
	Maternal	Postpartum disease (Y/N) Lameness (Y/N) Mastitis (Y/N)	1st test somatic cell count (x 10^3/mL) BCS change 2nd test yield (kg) Time to first service (d)
Calf growth rate	Neonatal before first parturition	Dam lactation 'Developed BRD' (Y/N) Season of birth	Birthweight (kg)
Health status	Neonatal before first parturition	Dam lactation	Amount of colostrum consumed (L) Growth rate to weaning (kg/d)
	Maternal — lameness (Y/N)	Lactation	BCS change
	Maternal — mastitis (Y/N)	Parity[4]	W4MK 1st test somatic cell count (x 10^3/mL)

BCS = body condition score. BRD = bovine respiratory disease. W4MK = milk yield at 4 weeks postpartum.

1. Assistance status, treatment status and their interaction included as fixed effects in all models
2. Correlated variables (growth rate to weaning and between weaning and breeding and first and second test milk yield) were included individually in separate models and the final model was selected based on best fit.
3. Included in all models of milk production except for 305ME as these are accounted for in the calculation of 305ME. Lactation number also not included in analysis of first lactation milking performance due all animals being in lactation one.
4. Lactation number replaced with parity (primiparous/multiparous) for analysis of mastitis due to the small dataset (n = 8 and n = 17 for neonatal and maternal data respectively).

7.2.3.1 Measures of neonatal reproductive performance (birth to breeding and first lactation)

Due to farm breeding protocols, age at conception (calves) could not be below 12 months and, as such, did not meet the assumptions of GLM. To manage this, the age at first conception was analysed as the age (d) over 365. These data were also logarithmically transformed to meet model assumptions. Age at first parturition and age at first insemination could not be transformed to meet the assumptions of GLM, thus were analysed in binary format relative to breeding targets (first service ≤ 13 months [Y/N] and first parturition ≤ 24 months [Y/N]). All available calves had at least one insemination attempt (both before first parturition and in the first lactation) thus, to meet the assumptions of Poisson regression, number of times bred was analysed as number of extra insemination attempts required over and above one (i.e. $n - 1$).

Effects of BRD on risk of insemination by 13 months could not be analysed using multivariate binomial logistic regression due to quasi-complete separation of data points. For completeness univariate analysis (Chi-squared test) was performed to investigate this association.

Due to an error on farm, one animal was inseminated very early in the first lactation (7 d postpartum). As this animal was not reflective of the wider population (on the study farm or the dairy industry in general) these data were excluded from first lactation reproductive analysis.

7.2.3.2 Measures of maternal reproductive performance

Due to the use of OvSynch in farm breeding protocols [Appendix 2], the time at first insemination (d) exhibited a binomial distribution that could not be transformed. To manage this, time to first insemination was converted to a binary variable of ‘bred before 75 DIM’ [Y/N] — a threshold selected to align with the timing of introduction of synchronisation protocols (relative to parturition). As cows are only inseminated to observed oestrus before 75 DIM, this threshold also acted as an indirect indication of observation of oestrous behaviour.

7.3 Results

7.3.1 Study population

In total, 94 cows and 95 calves were recruited; study population data are presented in Chapters 3 (Section 3.3.1) and 4 (Section 4.3.1). Five cows and four calves were excluded prior to analysis (see Chapter 6, Section 6.3.1). Duration of maternal data collection ranged from 23 d to 492 d. Duration of neonatal data collection ranged from 97 d to 1247 d (3 y 5 m).

Eighty-nine cows were included in the final analysis, parity distribution and parturition related data were as described in Chapter 6, Section 6.3.1. Ninety-one calves were included in the final analysis up to first parturition, 74 calves were included in the analysis of first lactation performance.

7.3.2 Neonatal production from birth to first parturition

7.3.2.1 Descriptive results

Mean birthweight was 43.0 kg (range 27.6 to 62.8 kg) and mean growth rate to weaning was 0.6 kg/d (range 0.28 to 1.04 kg/d). Mean weights at weaning and breeding were 80.3 kg (range 49.2 to 113 kg) and 368 kg (range 275 to 451 kg) respectively. Mean growth rate between weaning and breeding was 0.81 kg/d (range 0.53 to 1.04 kg/d).

Three calves (3.3.%) died between birth and first parturition at 97, 228 and 748 days old (unassisted birth = 2; assisted birth = 1); no calves died before weaning. Fourteen animals (15.4%) were sold before first parturition and 21 records of disease affecting 20 calves (22.0%) (mean age 124 d; range 9 to 748 d) were available. The most common illness recorded was BRD (*n* = 13; 61.9% of sick calf records). Other diseases recorded were umbilical hernia (*n* = 5; 23.8%) and lameness (*n* = 1; 4.8%).

Eighty-nine calves (97.8%) reached breeding age (12 months) and were inseminated at least once. The mean number of insemination attempts was 1.7 (range 1 to 8); 55 calves (61.8%) conceived to the first insemination whereas two calves (2.2%) did not conceive at all and were culled. Further descriptive statistics of reproductive performance are presented in Table 7.5. Seventeen calves (18.7%) left the herd before first parturition; three (17.6%) died and 14 (82.4%) were sold.

Table 7.5: Summary statistics (mean, standard error, standard deviation, and range) of neonatal reproductive performance before first parturition

Measure of reproductive performance	Descriptive statistics					
	n	Mean	SE	SD	Min.	Max.
Age at first recorded oestrus	89	387.9	3.23	26.4	321.0	480.0
Age at conception	87	429.4	4.00	37.3	382.0	583.0
Age at first parturition	74	705.5	4.06	35.0	664.0	846.0

All variables measured in days (d). n = number of records. SE = standard error (of the mean). SD = standard deviation. Min. = minimum. Max. = maximum

7.3.2.2 Effects of birth assistance and ketoprofen treatment

Ketoprofen treated assisted calves had a higher growth rate to weaning than calves in the other three interaction groups ($p = 0.025$) [Table 7.6].

Table 7.6: Mean (± SE) daily growth rate (kg/d) between birth and weaning for calves in each assistance x treatment status group

Group	*n*	Growth rate (kg/d)		*p*-value
		Mean	SE	
AT	16	0.68	0.03	0.025
AP	20	0.58	0.03	
UT	17	0.57	0.02	
UP	17	0.59	0.03	

Raw data presented. AT = assisted ketoprofen group; AP = assisted saline group; UT = unassisted ketoprofen group; UP = unassisted saline group. Total n = 70 due to missing data.

Assisted calves had a higher growth rate between weaning and breeding than unassisted calves ($p = 0.008$) [Figure 7.1], but there was no interaction effect on growth rate after weaning.

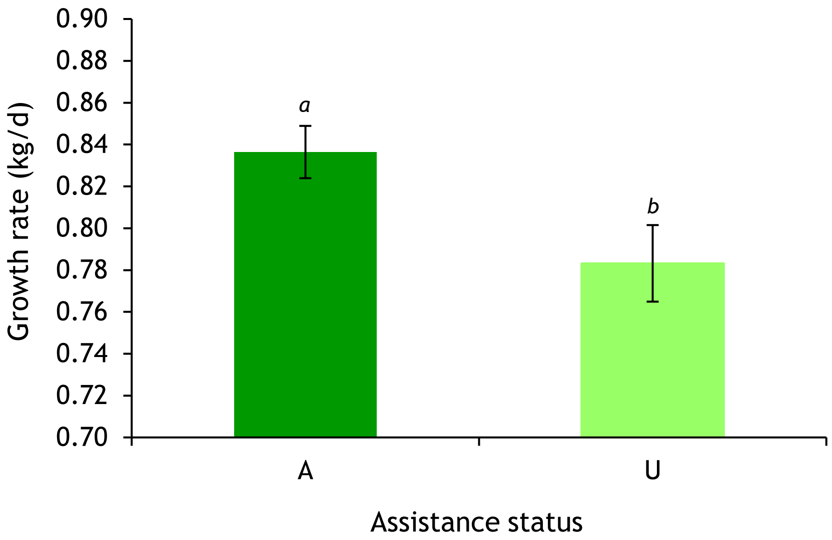


Figure 7.1: Mean (± SE) growth rate between weaning and breeding (kg/d) for calves subject to assisted and unassisted birth

Different letters indicate significant differences (p = 0.008). A = assisted calves (n = 37). U = unassisted calves (n = 33). Raw data presented.

Kaplan-Meier survival analysis indicated that, of the calves that left the herd before first parturition, assisted calves tended to be younger than unassisted calves (744 vs. 750 d; $p = 0.071$), but this finding was not replicated in multivariate analysis. Multivariate analysis found there was a weak tendency for ketoprofen treated calves to have lower odds of leaving the herd before first parturition than saline treated calves, irrespective of assistance status (OR 0.34; 95% CI 0.10, 1.16; $p = 0.084$). Neonatal reproductive performance was not affected by birth assistance or ketoprofen treatment.

7.3.2.3 Effects of birthweight and growth rate

Calf birthweight was positively associated with growth rate to weaning and growth rate between weaning and breeding ($p = 0.001$ and $p = 0.032$ respectively) [Figure 7.2]. Growth rate to weaning was negatively related to the odds of developing BRD with each 0.1 kg/d increase in growth rate to weaning being associated with a 63% reduction in the odds of developing BRD (OR 0.37; 95% CI 0.17, 0.78; $p = 0.009$).

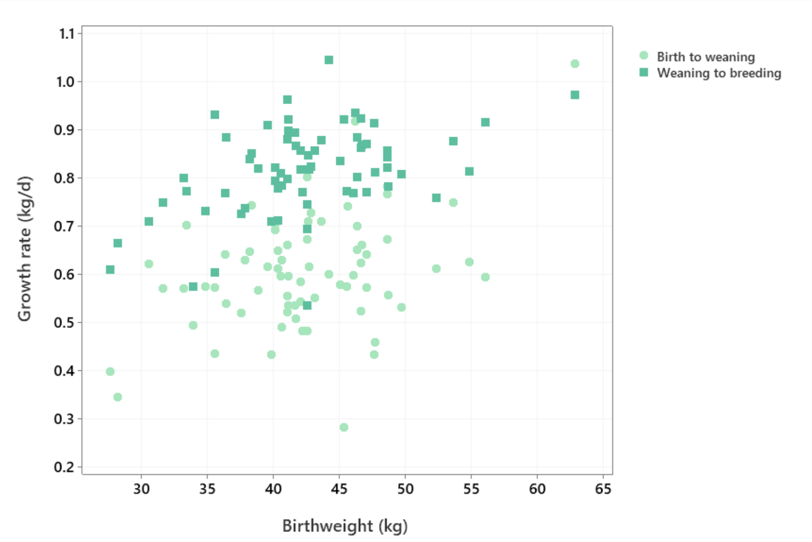


Figure 7.2: Relationship between calf birthweight (kg) and average daily growth rate (kg/d) for the life stages birth to weaning and weaning to breeding
Birth to weaning (light green) n = 70; p = 0.001.
Weaning to breeding (dark green) n = 72; p = 0.032

Age at first observed oestrus was negatively affected by growth rate to weaning with each 0.1 kg/d increase in growth rate (birth to weaning) being associated with a 10.2 d decrease in age at first observed oestrus (*p* = 0.004). Accordingly the odds of a calf being inseminated by 13 months were also affected by growth rate to weaning, with each 0.1 kg/d increase in growth rate being associated with nearly two times the odds of being inseminated by 13 months old (OR 1.70; 95% CI 1.01 to 2.85; *p* = 0.045).

Age at conception was negatively affected by growth rate between weaning and breeding with each 0.1 kg/d increase in growth rate being associated with a 1.41 d decrease in the age of conception (over 365 d) (*p* = 0.029). Accordingly, the odds of a calf being inseminated by 13 months were also affected by growth between weaning and breeding with each 0.1 kg/d increase in growth rate being associated with three times the odds of being inseminated by 13 months old (OR 3.12; 95% CI 1.50 to 6.51; *p* = 0.002).

7.3.2.4 Effects of health status, dam lactation, and birth season

A diagnosis of BRD was associated with an increased risk of leaving the herd before first parturition (OR 7.53; 95% CI 2.03, 27.9; *p* = 0.003). None of the calves that had BRD were inseminated by 13 months — Chi-squared analysis indicated this was significant (χ^2 [1, n = 91] = 9.42, *p* = 0.002). Calves diagnosed with BRD had nearly four times higher odds of oestrus not being detected than calves not diagnosed with BRD (OR 3.76; 95% CI 1.04, 13.6; *p* = 0.043). Additionally, there was a tendency for BRD to negatively affect the age at conception (over 365 d) (*p* = 0.065).

Each increase in dam lactation was associated with a nearly two-fold increase in the odds of developing BRD (OR 1.85; 95% CI 1.10, 3.13; *p* = 0.021). Dam lactation was analysed as a binary variable (primiparous and multiparous) to explore this finding further: calves born to primiparous dams tended to have reduced odds of developing BRD (OR 0.24; 95% CI 0.05, 1.11; *p* = 0.068).

Calves born in the spring (March to May) had a lower growth rate between weaning and first insemination than calves born in other seasons (*p* = 0.004).

7.3.3 Neonatal production in the first lactation

7.3.3.1 Descriptive results

Seventy-four calves entered their first lactation at mean age of 707.6 d (23.3 months) (range 664 d [21.8 months] to 858 d [28.2 months]). Mean first lactation length (exclusive of the dry period) was 343.5 d (range 204 to 510 d). Summary statistics of measures of neonatal first lactation milking performance are presented in Table 7.7.

Table 7.7: Summary statistics (mean, standard error, standard deviation, and range) of neonatal milk production in the first lactation

Milking performance parameter	Descriptive results				
	Mean	SE	SD	Min.	Max.
Whole lactation milk yield	11743	285.0	2448	5960	21530
Whole lactation fat yield	462.3	13.2	113.8	225.0	854.0
Whole lactation protein yield	376.5	8.8	75.6	181.0	630.0
305ME	12318	235.0	2025	4740	18070

n = 74. All variables measured in kg. n = number of records. SE = standard error (of the mean). SD = standard deviation. Min. = minimum. Max. = maximum

Most animals (94.6%) did not require parturition assistance (at first parturition); four (5.4%) were assisted. Mean first lactation calving to conception interval (CCI) was 104.8 d (range 43 to 215 d) with the median number of insemination attempts being 2 (range 1 to 7).

Thirteen (17.6%) calves did not complete their first lactation — one (7.7.%) died and 12 (92.3%) were sold. The most common reason for sale was poor reproductive performance (*n* = 10; 83.3%). Illness in the first lactation was recorded for 23 calves (31.1%). The most common diagnosis recorded was lameness (*n* = 13; 56.5%), followed by mastitis (*n* = 8; 34.8%), BRD (*n* = 1; 4.3%) and postpartum disease (RFM with concurrent metritis) (*n* = 1; 4.3%).

7.3.3.2 Effects of birth assistance and ketoprofen treatment

There was a tendency for first lactation milk fat production of assisted (at birth) calves to be higher than unassisted calves (*p* = 0.063). Assisted calves (at birth) had a longer first lactation CCI than unassisted calves (*p* = 0.027) and had on average 0.88 more insemination attempts than unassisted calves (*p* = 0.001). Additionally, calves whose first parturition was assisted tended to have a longer CCI (*p* = 0.069). Assisted calves (at birth) had eight times the odds of not completing the first lactation (OR = 7.95; 95% CI 1.31 to 48.4; *p* = 0.024) compared to unassisted calves.

Ketoprofen treatment did not affect any measures of first lactation performance of calves.

7.3.3.3 Effects of growth rate, dam lactation, age at first parturition and early lactation milk yield

Growth rate to weaning and growth rate between weaning to breeding both tended to be positively related to 305ME (*p* = 0.065 and *p* = 0.073 respectively). Growth rate to weaning was negatively associated with first lactation CCI with each 0.1 kg/d increase in growth rate to weaning being associated with a 0.88 d decrease in CCI (*p* = 0.012). Accordingly, growth rate to weaning was also negatively associated with number of times bred with each 0.1 kg/d increase being associated with a 0.43 decrease in the number insemination attempts (*p* = 0.001). Additionally, each increase in dam lactation was associated with a 0.22 increase in the number of insemination attempts (*p* = 0.015).

Age at first parturition of > 24 months was associated with almost seven times greater odds of not completing the first lactation than calves that calved for the first time at ≤ 24 months (OR = 6.812; 95% CI = 1.21 to 38.3; *p* = 0.029). This finding was further analysed by absolute age at first parturition — each 1 d increase in age at first parturition was associated with 1.02 increased odds of not completing the first lactation (OR = 1.02; 95% CI = 1.00 to 1.04; $p = 0.021$).

Each 1 kg increase in second test milk yield was associated with 1.16 odds of completing the first lactation (OR = 1.16; 95% CI = 1.02 to 1.31; $p = 0.016$). First test milk yield tended to positively affect completion of the first lactation (OR = 1.11; 95% CI = 0.99 to 1.25; $p = 0.080$).

7.3.4 Maternal production in the subsequent lactation

7.3.4.1 Descriptive results

Fourteen cows (15.7%) were diagnosed with postpartum disease; five (35.7%) were diagnosed with more than one postpartum disease concurrently. Although only 11 assisted cows (23%) were diagnosed with postpartum disease, assisted cows accounted for the majority (78.6%) of postpartum disease diagnoses. The most commonly diagnosed postpartum disease was metritis ($n = 13$), followed by RFM ($n = 7$) and SCK ($n = 1$). Seventeen cows (19.1%) were diagnosed with mastitis in the subsequent lactation; four (23.5%) were diagnosed before 90 DIM. Twelve cows (13.5%) were recorded as lame and six cows (6.7%) were recorded as experiencing disease other than lameness, mastitis, or postpartum disease in the subsequent lactation.

Thirty-one cows (34.8%) left the herd before the end of the subsequent lactation at a mean time of 239.6 DIM (range 23 to 499 DIM); 24 (74.2%) were assisted. Three cows (9.7%) died and 29 (90.3%) were sold (the purchaser [e.g. abattoir, another farmer] was unavailable for analysis). The most common reason for sale was poor fertility (48.3%) followed by low productivity (13.8%), mastitis (13.8%), pregnancy loss (13.8%) and illness/injury (6.9%).

BCS1 and BCS2 data were available for 84 and 80 cows respectively. Median BCS1 and BCS2 were both 3.0 (BCS1 range 2.0 to 4.5; BCS2 range 2.0 to 4.0). Forty cows (44.9%) lost body condition in the first 60 d postpartum (median −0.5; range −0.25 to −1.75). The remaining 40 cows either gained body condition ($n = 26$; median +0.25, range +0.25 to +1.25) or BCS was unchanged ($n = 14$).

Summary statistics of maternal milking performance are presented in Table 7.8. Summary statistics and lactation curves for each assistance x treatment group are presented in Appendix 7.

Table 7.8: Summary statistics (mean, standard error, standard deviation, and range) of maternal milk production in the subsequent lactation

Milking performance parameter	Descriptive results				
	Mean	SE	SD	Min.	Max.
Whole lactation milk yield	11380	425	3962	1590	20860
Whole lactation fat yield	399.7	16.1	150.2	50.0	774.0
Whole lactation protein yield	355.4	13.7	127.3	51.0	641.0
W4MK	40.1	1.31	12.33	10.0	68.0
305ME	12314	210	1973	8520	18080

n = 89. All variables measured in kg. W4MK = week four milk yield. 305ME = 305 d mature equivalent milk yield. SE = standard error (of the mean). SD = standard deviation. Min. = minimum. Max. = maximum

One cow died before the end of the voluntary waiting period (VWP) (i.e. the opportunity to breed this cow was lost) and was excluded from analysis of reproductive performance. Of the remaining 88 cows, 83 (94.3%) were inseminated at least once in the subsequent lactation (median two inseminations [range 0 to 7]). Sixty-nine cows (82.1%) conceived, of which 57 (82.6%) subsequently produced a calf. Of the 12 cows (17.4%) that conceived but did not produce a recorded calf, six (50%) aborted, four (33.3%) were sold before parturition and two (16.7%) died. Further summary statistics of maternal reproductive performance are presented in Table 7.9.

Table 7.9: Summary statistics (mean, standard error, standard deviation, and range) of maternal reproductive performance

Reproductive performance parameter	*n*	Descriptive results				
		Mean	SE	SD	Min.	Max.
Time to 1st service (d)	83	68.87	1.73	15.78	45	104
Calving to conception interval (d)	69	101.3	5.22	43.32	46	213
Calving interval (d)	57	387.1	5.86	44.28	322	492

SE = standard error (of the mean). SD = standard deviation. Min. = minimum. Max. = maximum

7.3.4.2 Effects of ketoprofen treatment

Ketoprofen treated cows had higher 305ME than saline treated cows regardless of assistance status (*p* = 0.011) [Figure 7.3].

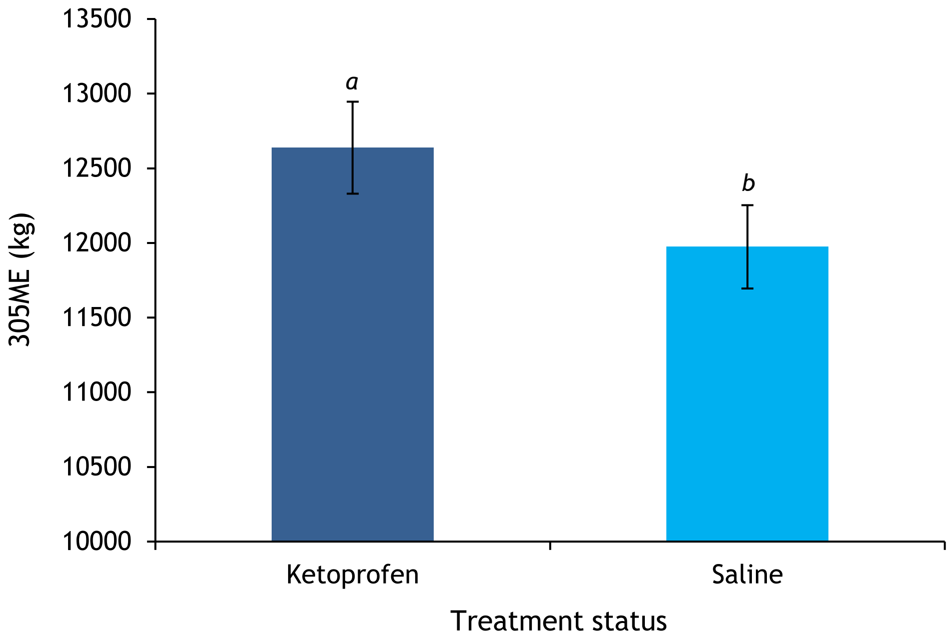


Figure 7.3: Mean (± SE) 305ME of cows treated with ketoprofen and saline
Different letters indicate significant differences (p = 0.011). Ketoprofen treated cows represented by dark blue bar (n = 45). Saline treated cows represented by light blue bar (n = 44). 305ME = 305 d mature equivalent. Raw data presented.

Irrespective of assistance status ketoprofen treated cows tended to conceive more quickly (median 22 d; *p* = 0.056) and to conceive overall (*p* = 0.056) than saline treated cows. An effect of treatment status on oestrous detection was observed with 72% of ketoprofen treated cows being inseminated before 75 DIM (i.e. to observed oestrus) compared to 57% of saline treated cows, but this was not statistically significant.

Kaplan-Meier analysis indicated that a greater proportion of ketoprofen treated cows had conceived by 100 DIM (65.7% vs. 48.6%) but this was not significant. Of the four assistance x treatment status interaction groups, only the two ketoprofen treatment groups achieved the target of ≥ 50% of cows conceived by 100 DIM with the highest proportion of cows conceiving in the ketoprofen assisted group; however, this was not a significant difference to other groups. When assisted cows were analysed separately, Kaplan-Meier analysis identified a tendency for a greater proportion of ketoprofen treated cows to conceive by 200 DIM (*p* = 0.050) [Figure 7.4]

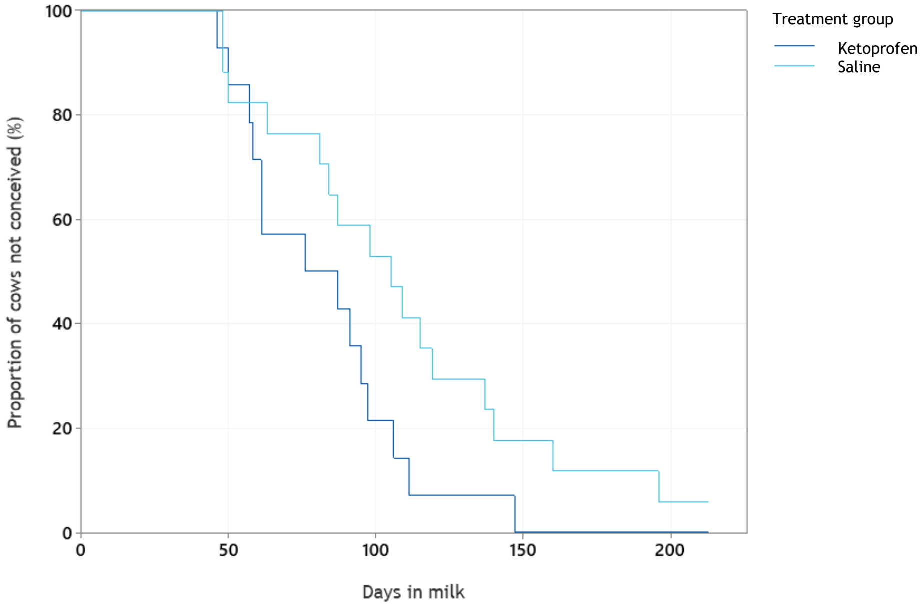


Figure 7.4: Kaplan-Meier survival analysis of percentage of assisted cows in each treatment status group conceived by 200 DIM
Log-rank statistic p = 0.050. Ketoprofen treated cows n = 17. Saline treated cows n = 14. Right censored at 200 DIM. Cows that left the herd not included in analysis. Voluntary waiting period = 50 d.

Logistic regression analysis found that overall, the likelihood of developing disease or leaving the herd during the subsequent lactation was not affected by ketoprofen treatment. However, when assisted cows were analysed alone, Kaplan-Meier survival analysis indicated that ketoprofen treated assisted cows left the herd at a numerically later stage than saline treated assisted cows (268 vs. 169 d respectively), although this was not significant.

7.3.4.3 Effects of parturition assistance

Overall assistance status did not affect 305ME but ketoprofen treated unassisted cows tended to have higher 305ME than cows in the other three interaction groups (p = 0.073) [Figure 7.5].

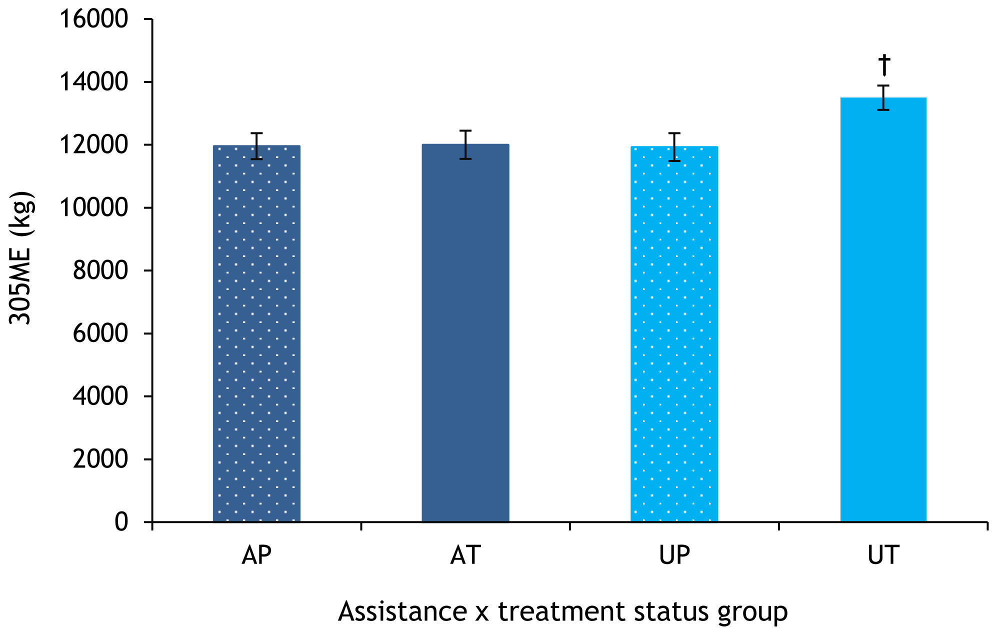


Figure 7.5: Mean (± SE) 305ME of cows in each assistance x treatment status group
† *Indicates tendency towards significant difference (p = 0.073). AP = assisted saline (n = 21). AT = assisted ketoprofen (n = 22). UP = unassisted saline (n = 18). UT = unassisted ketoprofen (n = 21). Assisted cows represented by dark blue bars. Unassisted cows represented by light blue bars. Ketoprofen treated cows represented by solid bars. Saline treated cows represented by patterned bars 305ME = 305 d mature equivalent. Raw data presented.*

Assisted cows were less likely to be in calf by 150 and 200 DIM (*p* = 0.017 and *p* = 0.011 respectively) and less likely to conceive overall (*p* = 0.005) than unassisted cows, irrespective of treatment status [Table 7.10].

Table 7.10: Odds ratios of conception by 100, 150 and 200 days in milk, and overall, for assisted cows relative to unassisted cows
Bold font indicates significant result.

Variable	Odds ratio	95% CI	*p*-value
Conceived by 100 DIM	0.57	0.16, 2.06	0.394
Conceived by 150 DIM	**0.12**	**0.02, 0.69**	**0.017**
Conceived by 200 DIM	**0.07**	**0.01, 0.54**	**0.011**
Conceived overall (at any stage)*	**0.02**	**0.002, 0.32**	**0.005**

DIM = days in milk. 95% CI = 95% confidence interval (of odds ratio). Assisted n = 46. Unassisted n = 42.
**Assistance x treatment status interaction not included in statistical model*

Irrespective of treatment status, assisted cows were more likely to be diagnosed with postpartum disease (*p* = 0.031) and leave the herd before completing the subsequent lactation (i.e. before another parturition event) than unassisted cows (*p* = 0.007) [Table 7.11].

Table 7.11: Odds ratios for health and survival outcomes of assisted cows relative to unassisted cows

Bold font indicates significant result.

Health measure	Odds ratio	95% CI	*p*-value
Postpartum disease	**5.05**	**1.16, 22.0**	**0.031**
Lameness	1.38	0.37, 5.09	0.632
Mastitis	0.94	0.32, 2.74	0.911
Leave herd before completing lactation	**4.53**	**1.53, 13.5**	**0.007**

95% CI = 95% confidence interval. Assisted n = 47. Unassisted n = 42.

7.3.4.4 Effects of lactation number, health status, and milk production

Lactation number was negatively related to the odds of conception (OR 0.52, 95% CI 0.31, 0.87; $p = 0.012$). Cows with higher early lactation milk production tended to be less likely to have been inseminated by 75 DIM (OR 0.96, 95%CI 0.92 to 1.00; $p = 0.069$).

Calving to conception interval was shorter in cows that were diagnosed with postpartum disease than healthy cows ($p = 0.026$). By contrast, CCI of cows that were diagnosed with mastitis at any point during the lactation was longer than CCI of cows not diagnosed with mastitis ($p = 0.022$).

Lactation number was negatively associated with BCS change with each increase in lactation number being associated with a 0.16 point loss of BCS in the first eight weeks of lactation ($p < 0.001$). By contrast, lactation number was positively associated with the likelihood of developing postpartum disease ($p = 0.026$), lameness ($p = 0.009$), and the likelihood of leaving the herd before the end of the lactation ($p = 0.019$) [Table 7.12].

Table 7.12: Odds ratios for the effect of each increase in lactation number on maternal health and survival outcomes

Bold font indicates significant result.

Health measure	Odds ratio	95% CI	*p*-value
Postpartum disease	**1.50**	**1.05, 2.17**	**0.026**
Lameness	**1.63**	**1.13, 2.34**	**0.009**
Mastitis	1.22	0.88, 1.70	0.229
Leave herd before completing lactation	**1.58**	**1.08, 2.34**	**0.019**

n = 89. 95%CI = 95% confidence interval.

7.3.4.5 Subsequent milk production: effects of lactation duration and lactation number

Whole lactation milk yield was affected by duration of lactation with each 1 d increase in lactation length being associated with an increase in whole lactation yield of 39.7 kg ($p < 0.001$). Total fat yield and total protein yield were similarly affected with each 1 d increase in lactation length associated with an increase in total fat yield of 1.36 kg ($p < 0.001$) and an increase in total protein yield of 1.30 kg ($p < 0.001$).

Cumulative milk and protein production of primiparous cows was lower than older animals ($p < 0.001$ and $p = 0.007$ respectively). Whole lactation fat production was unaffected by age. Similarly, W4MK was higher in older cows than cows in lactation one ($p < 0.001$) [Table 7.13].

Table 7.13: Differences in whole lactation milk yield (kg), fat yield (kg) and protein yield (kg) for cows in each lactation group

Bold font indicates significant result.

Milk production variable	Lactation group	Coefficient	SE	95% CI	*p*-value
Whole lactation milk yield	1	Ref	-	-	-
	2	**1285**	**378**	**533, 2037**	**0.001**
	≥ 3	**2691**	**296**	**2101, 3280**	**< 0.001**
Whole lactation fat yield	1	Ref	-	-	-
	2	−0.90	24.6	−49.9, 48.1	0.971
	≥ 3	18.2	29.2	−40.0, 76.4	0.535
Whole lactation protein yield	1	Ref	-	-	-
	2	**35.6**	**12.9**	**10.0, 61.2**	**0.007**
	≥ 3	**50.7**	**15.0**	**20.9, 80.5**	**0.001**
W4MK	1	Ref	-	-	-
	2	**15.1**	**2.38**	**10.3, 19.8**	**< 0.001**
	≥ 3	**21.6**	**1.79**	**18.1, 25.2**	**< 0.001**

Reference level = lactation 1 (n = 50). Each milk production outcome variable analysed individually. Lactation 2: n = 12. Lactation ≥ 3: n = 27. All variables measured in kg. n = number of cows in each lactation group. SE = standard error (of coefficient). 95% CI = 95% confidence interval (of coefficient). W4MK = week four milk yield. Ref = reference level.

7.3.5 Overall summary of results

Effects of parturition assistance and ketoprofen treatment on neonatal and maternal production identified using multivariate analysis are summarised in Tables 7.14 and 7.15.

Table 7.14: Summarised results of multivariate analysis of effects of birth assistance, ketoprofen treatment and assistance x treatment interactions on measures of neonatal production before first parturition and in the first lactation

Measure of production	Parturition assistance	Ketoprofen treatment	Assistance x treatment interaction	Summarised result
Growth rate to weaning			*	AT > other groups
Growth rate between weaning and breeding	*			A > U
Total lactation fat yield	†			A > U
Number of times bred (first lactation)	**			A > U
First lactation calving to conception interval	**			A > U
Left the herd during the first lactation (Y/N)	*			A > U

** p < 0.05. ** p < 0.01. *** p < 0.001. † = tendency (p > 0.05 and p ≤ 0.08). Exact p-values reported in text. A = assisted calves. U = unassisted calves. AT = assisted ketoprofen group. Absence of notation indicates not significant/no tendency (p > 0.08). Production measures not affected by assistance or treatment status not presented. All data except for growth rates are first lactation data.*

Table 7.15: Summarised results of multivariate analysis of effects of parturition assistance, ketoprofen treatment and assistance x treatment interactions on maternal production in the subsequent lactation

Measure of production	Parturition assistance	Ketoprofen treatment	Assistance x treatment interaction	Summarised result
Final 305ME		*	†	T > P UT > other groups (†)
Calving to conception interval		†		T > P
Conceived before 150DIM (Y/N)	*			A < U
Conceived before 200DIM (Y/N)	*			A < U
Conceived overall (Y/N)	**	†		A < U T > P (†)
Postpartum disease (Y/N)	*			A > U
Left the herd during the subsequent lactation (Y/N)	*			A > U

*DIM = days in milk. * p < 0.05. ** p < 0.01. *** p < 0.001. † = tendency (p > 0.05 and p ≤ 0.08). Exact p-values reported in text. A = assisted. U = unassisted. T = ketoprofen treated. P = saline treated. UT = unassisted ketoprofen group. Absence of notation indicates not significant result (p > 0.08). Production measures not affected by assistance or treatment status not presented.*

7.4 Discussion

The 2 x 2 factorial design and longitudinal nature of this study has allowed the longer-term effects of parturition assistance and postpartum ketoprofen analgesia on the health and production of cows and their calves to be investigated. Previous studies investigating the interaction effects of analgesia and parturition assistance have only assessed maternal production in early lactation (Swartz *et al.*, 2018; Barragan *et al.*, 2020a, 2021), whereas studies investigating the effects of postpartum analgesia on whole lactation production parameters have analysed the effects of blanket treatment of all cows, regardless of parturition experience (Farney *et al.*, 2013b; Stilwell *et al.*, 2014; Carpenter *et al.*, 2016). As such, the design of the current study is novel and has provided additional information on the longer-term production effects of parturition assistance and analgesia that, when applied to the whole herd, have potentially large economic benefits.

Ketoprofen analgesia did not affect any measures of calf first lactation performance, a finding that, although not supporting our hypothesis, was perhaps to be expected given the duration of time elapsed between the timing of ketoprofen administration and collection of data (> 2 y). By contrast, the results support the hypothesised negative effects of birth assistance, with birth assistance being negatively associated with measures of productivity both before first calving and extending into the first lactation. It is known that late gestational heat stress *in utero* affects future production of calves (Dahl *et al.*, 2016; Monteiro *et al.*, 2016), and that early gestational nutritional stress affects ovarian reserve and cardiovascular development (Mossa *et al.*, 2013); therefore, it is possible that stress related to birth pain and inflammation contributes to similar long term effects.

7.4.1 Measures of neonatal production

7.4.1.1 Growth rate and survival

Overall, growth rate to weaning was similar to that previously reported in UK Holstein calves (Atkinson, 2015). Ketoprofen treated assisted calves had higher growth rate to weaning than calves in the other three interaction groups, suggesting assisted calves experience enhanced pre-weaning benefits of ketoprofen treatment. This is an interesting finding that contrasts with previous studies that did not identify a similar effect of meloxicam treatment (Murray *et al.*, 2016; Pearson *et al.*, 2019a, 2019b; Clark *et al.*, 2020). Previous studies have not employed a 2 x 2 design — either only including assisted calves (Pearson *et al.*, 2019a, 2019b), or investigating blanket analgesia treatment (Murray *et al.*, 2016; Clark *et al.*, 2020) — thus assistance x treatment interaction effects could not be determined. As such, caution needs to be applied when making comparisons between previous work and the results presented here. Some previous work has found that growth is not affected by birth assistance (Arthur *et al.*, 2000; Villettaz Robichaud *et al.*, 2017b). Accordingly, there may be limited capacity for analgesia to affect pre-weaning growth rate of assisted calves but more studies using a 2 x 2 factorial design to enable study of birth assistance x treatment interaction effects are needed. Additionally, previous studies (Murray *et al.*, 2016; Pearson *et al.*, 2019a, 2019b; Clark *et al.*, 2020) have all investigated administration of meloxicam which may have different effects on growth rate than ketoprofen. Meloxicam administration at the time of dehorning has been associated with increased growth rate of older calves (Coetzee *et al.*, 2012), therefore it is unlikely that meloxicam has a negative effect on growth, but effects on newborn calves are currently unknown. Growth rate is an important production outcome for beef calves with economic implications, and higher pre-weaning growth rates of dairy calves have been associated with improved odds of survival to first lactation (Van De Stroet *et al.*, 2016). Furthermore, in the current study higher daily weight gain was associated with improved reproductive performance and reduced BRD incidence. As such, the observed ketoprofen effect on daily growth rate of assisted calves is an important finding with wider implications for calf welfare and production; further study to explore the effects of different NSAIDs as well as the effects of ketoprofen in beef calves is merited.

Cumulative mortality rate before first parturition was 3.4%; whilst this figure is much lower than previously reported (approximately 10%) (Hyde *et al.*, 2020), it should be noted that perinatal mortality was not included. Just one calf born to assistance died before first parturition, but only live-born calves were included thus the effects of assisted birth on perinatal mortality could not be analysed. Nonetheless, the findings are in contrast to previous studies that have consistently found that birth assistance is associated with an increased risk of mortality beyond the first few days of life (Wells *et al.*, 1996; Lombard *et al.*, 2007; Henderson *et* *al.*, 2011; Barrier *et al.*, 2012c). Only mild to moderate farmer-provided birth assistance was included in the current study and it is probable that more severe birth assistance has a greater effect on calf mortality. This hypothesis is supported by Barrier *et al.* (2012c) who found that 'high' birth difficulty was associated with a greater hazard ratio of calf mortality than 'moderate' birth difficulty, although 'high' and 'moderate' birth difficulty were not defined. Although the findings of the present study appear to be in contrast to the established view that birth assistance is associated with increased calf mortality, the small dataset and inability to apply inferential statistical analysis to mortality data mean a cautious interpretation of these results is recommended as it is unlikely that the low mortality rate of assisted calves in the current study is reflected in the wider population.

7.4.1.2 Reproductive performance

Interestingly, whilst reproductive performance before first parturition was not affected by birth experience, birth assistance was associated with a negative effect on first lactation reproductive performance. Similarly, previous studies have also found that reproductive performance before first parturition is not affected by birth assistance (Heinrichs *et al.*, 2005; Barrier *et al.*, 2012c), but — in contrast to this study — first lactation reproductive performance of adult calves has not previously been found to be affected (Eaglen *et al.*, 2011). Unlike previous studies, the current study has analysed data from the same calves at each life stage (i.e. before first parturition and in the first lactation), demonstrating that birth assistance has long term adverse associations with reproductive performance. Prior to this study, it might have been expected that effects of birth assistance would have similar effects at different life stages of the same calves. It is possible that the effects of birth assistance are delayed but, given the duration of time elapsed between birth and first lactation breeding (> 2 y), this seems unlikely. An alternative explanation for this finding may relate to the reduced fertility of (high-yielding) lactating dairy cows compared to nulliparous cows. Oltenacu (1991) found reproductive performance of primiparous animals was poorer than nulliparous animals of similar genetic merit and it is possible that heightened fertility of calves before first parturition (compared to in first lactation) has compensated for the adverse effects of birth assistance. If this is the case, the possibility that birth assistance only affects reproductive performance of high yielding dairy cows needs to be considered. The hypothesis that birth assistance may have different effects on the reproductive performance of different bovine production types (e.g. beef, lower-producing dairy breeds) merits further study.

Ketoprofen treatment did not affect reproductive performance suggesting that, whilst birth assistance has an adverse effect on first lactation reproductive performance, this is not related to inflammation or pain in the first few days of life. No previous studies have investigated the effects of post-birth analgesia on future reproductive performance of calves therefore no data are available to substantiate this hypothesis.

7.4.1.3 First lactation milk production

In contrast to previous studies (Eaglen *et al.*, 2011; Heinrichs *et al.*, 2011), the current study did not identify any effects of birth assistance on first lactation protein or total milk yield. Differences in the degree of birth assistance studied may explain the differences in findings: previous studies have included calves born to veterinary assistance and caesarean section (Eaglen *et al.*, 2011; Heinrichs *et al.*, 2011) whereas the present study only included farmer-assisted calves. It is possible that first lactation milk protein and total milk yield are only affected by severe birth assistance — Eaglen *et al.* (2011) found veterinary assistance had the greatest negative effect on first lactation milk production. As milk production is the primary economic concern of most dairy farming businesses, this is an important finding that demonstrates the far-reaching effects of parturition management decisions and further illustrates the importance of judicious birth assistance. Assisted birth was associated with a tendency for increased first lactation fat production — a result supported by Eaglen *et al.* (2011), who found that moderately assisted calves had higher first lactation fat yield than unassisted calves, although veterinary assistance was associated with reduced fat yield. By contrast, Heinrichs *et al.* (2011) found first lactation fat production was not affected by birth assistance score. Fat is the most variable component in milk (Bauman *et al.*, 2006), and is affected by factors including nutrition, parity, season and farm type (conventional vs. organic) (Bauman *et al.*, 2006; Ellis *et al.*, 2006; Schwendel *et al.*, 2015; Mele *et al.*, 2016). Thus, it is possible that milk fat is more sensitive to the effects of mild to moderate birth assistance than other milk production parameters. Nevertheless, reasons for the mild positive effect of birth assistance on first lactation milk fat production identified in this study are unclear. Currently, this is a novel finding and — given the wider negative effects of birth assistance on calf production — should be interpreted with caution so as to avoid propagating the misperception that birth assistance is a positive intervention that should be applied routinely.

Milk production genomic data (e.g. maternal and paternal predictive transmitting ability) were not included in analyses for practical reasons. Due to farm breeding policies, genetic variation within the animals recruited to the study is considered likely to be small and as such the results are unlikely to be affected. However, this may limit the application of results to other farms. Previous studies also do not include genetic data (Eaglen *et al.*, 2011; Heinrichs *et al.*, 2011), therefore it is currently unknown how milk production genotype interacts with birth experience. However, the results of the present study are similar to previous studies suggesting that interactions between genetics and birth assistance may be limited.

7.4.2 Measures of maternal production

7.4.2.1 Milk production

Ketoprofen treated cows in the current study had a higher 305ME than saline treated cows, a similar finding to Farney *et al.* (2013b) and Stilwell *et al.* (2014) who identified a positive effect of NSAID treatment on 305 d milk yield. Huzzey *et al.* (2015) found that biomarkers of postpartum inflammation were negatively associated with 305ME and it is possible that the positive effects of NSAID treatment on milk production identified by the current and previous studies are due to anti-inflammatory effects of NSAIDs. Interestingly, a similar positive effect of ketoprofen on whole lactation yield was not observed — a contrast to previous work (Carpenter *et al.*, 2016). Whilst it is possible that the difference in findings may be explained by treatment with different NSAIDs, this hypothesis is not supported by Carpenter *et al.* (2016) who investigated the effects of meloxicam and sodium salicylate and found that both NSAIDs were associated with an increased whole lactation yield (compared to a placebo drug). Alternatively, inclusion of primiparous animals in the current study (in contrast to Carpenter *et* *al.* (2016) who only included cows in lactation ≥ 2) may also explain differences in findings — it is possible that postpartum analgesia has a greater effect on milk production of older animals. This hypothesis is supported by the results of 305ME analysis (a measure standardised to a 3rd lactation cow) in the current study.

Milk components were not affected by ketoprofen treatment. This finding is consistent with the results of Barragan *et al.* (2020a) who measured production for the first 30 d postpartum, but contrasts with results of other studies that found NSAID administration had a positive effect on longer term measures of milk fat and protein production (Farney *et al.*, 2013b; Carpenter *et al.*, 2016; Swartz *et al.*, 2018). Differences in study design may explain differences in results as previous studies include multiparous animals only (Farney *et al.*, 2013b; Carpenter *et al.*, 2016) or analysed daily production in the first half of lactation (Swartz *et al.*, 2018), whereas the present study analysed whole lactation production of primiparous and multiparous animals. It is possible that multiparous animals experience heightened whole lactation milk production effects of NSAID analgesia, and that the beneficial effects of NSAID analgesia do not persist throughout lactation.

A tendency for unassisted cows to experience enhanced benefits of analgesia compared to assisted cows (with respect to 305ME) was observed. By contrast, Barragan *et al.* (2020a) found a tendency for assisted cows to experience heightened benefits of analgesia (on milk production). Whilst these findings appear to be conflicting, it is difficult to make comparisons between the two studies as the current study analysed 305ME whereas Barragan *et al.* (2020a) analysed daily milk production for up to 30 d postpartum. It is possible that assisted cows experience short term milk production benefits of NSAID analgesia, whereas the beneficial effects of NSAID treatment of unassisted cows are longer-term. This hypothesis is supported by Swartz *et al.* (2018) who found that meloxicam treated eutocia cows had a higher daily milk production (for 15 weeks postpartum) than saline treated eutocia cows or meloxicam treated dystocia cows. However, Swartz *et al.* (2018) employed different definitions of dystocia and eutocia to most studies ('eutocia' = parturition duration ≤ 70 min and 'dystocia' = parturition duration > 70 min), thus care needs to be taken when making direct comparisons with the current study as the parturition experience of cows in each assistance status group is likely to differ.

Parturition assistance did not affect milk production in the current study, a finding similar to existing studies (Thompson *et al.*, 1983; Tenhagen *et al.*, 2007; Eaglen *et al.*, 2011). Previous studies have found that milk production earlier in lactation is negatively affected by parturition assistance (Thompson *et al.*, 1983; Tenhagen *et al.*, 2007; Eaglen *et al.*, 2011) and — although W4MK was not affected by parturition assistance here — it is possible that early negative effects of parturition assistance are compensated for later in lactation.

7.4.2.2 Reproductive performance

Assisted cows had reduced odds of conceiving by 150DIM and 200DIM (and overall), but risk of conception in earlier lactation (< 100DIM) was not affected. This finding is consistent with the results of Hayes *et al.* (2012) who found that overall in-calf rate (%) and 200 d in-calf rate (%) were negatively impacted by parturition assistance whereas there was no effect on 100 d in-calf rate (%). Hayes *et al.* (2012) concluded that their findings were related to the greater proportion of assisted cows that were removed from the herd before 100 DIM (8.8% vs. 5.7% of unassisted cows). Different methods of analysis mean that it cannot be determined whether this is a factor affecting the results presented here. Moreover, only six cows left the herd before 100 DIM in the current study; therefore, although assisted cows accounted for a greater proportion of these animals (n = 4; 66.7%), data are too limited to enable accurate conclusions to be drawn.

Irrespective of assistance status, ketoprofen treated cows tended to conceive 22 d sooner than saline treated cows — equivalent to one oestrous cycle (Remnant *et al.*, 2018). This finding is similar to results reported by Barragan *et al.* (2020a) who found acetylsalicylic acid treated cows tended to conceive 18 d earlier than saline treated cows. Persistent postpartum inflammation has been found to negatively affect ovarian function (Cheong *et al.*, 2017) and it is likely that the anti-inflammatory effect of ketoprofen has contributed to this finding. As assisted parturition has been associated with a delayed resumption of ovarian cyclicity (Opsomer *et al.*, 2000; Richards *et al.*, 2009), it might be expected that assisted cows would experience heightened reproductive benefits of ketoprofen analgesia (compared to unassisted cows). This was not supported by the results of the current study although, when assisted cows were analysed as a separate group, ketoprofen treatment tended to improve reproductive performance. Poor reproductive performance was the primary reason for removal of cows from the study herd, a finding consistent with previous studies (Seegers *et al.*, 1998; Hadley *et al.*, 2006; Orpin *et al.*, 2010; Kerslake *et al.*, 2018). Thus, the tendency for ketoprofen to have a beneficial effect on reproductive performance may have the potential to improve retention of cattle in dairy herds.

7.4.2.3 Health and survival

The proportion of assisted cows diagnosed with postpartum disease (23%) was similar to the proportion of appropriately assisted cows diagnosed with RFM in a previous study (25%; Kovács *et al.*, 2016). Nonetheless, consistent with previous studies (McArt *et al.*, 2013; Vergara *et al.*, 2014; Kovács *et al.*, 2016), assisted cows were more likely to be diagnosed with postpartum disease than unassisted cows — supporting the argument that even judiciously applied parturition assistance is associated with development of postpartum disease. The diseases diagnosed in the current study (metritis, RFM, SCK) are associated with postpartum inflammation (Qu *et al.*, 2014), and, as postpartum NSAID administration is associated with a reduction in postpartum inflammatory biomarkers (Carpenter *et al.*, 2016; Pascottini *et al.*, 2019, 2020), ketoprofen treatment might be expected to have a beneficial effect on disease incidence, but this was not observed. Similarly, previous studies of alternative NSAIDs have also failed to identify a beneficial NSAID effect (Swartz *et al.*, 2018; Barragan *et al.*, 2020a), with studies of flunixin identifying a negative effect (Waelchli *et al.*, 1999; Newby *et al.*, 2017) — suggesting that postpartum inflammation may not be the most important determinant in the development of clinical postpartum disease. A single study has investigated the effects of ketoprofen on postpartum disease, finding that overall there was no effect but when diseases were analysed individually ketoprofen tended to have a protective effect for the development of RFM (Richards *et al.*, 2009). A limitation of the current study that could not be avoided is the low incidence of postpartum disease and inability to accurately perform detailed analysis of individual diseases. Given the adverse effects of RFM on future productivity (Fourichon *et al.*, 2000; Han *et al.*, 2005), further work would be of interest to further explore the potentially protective effects of ketoprofen on RFM.

Parturition assistance was associated with an increased risk of leaving the herd before the end of the subsequent lactation, a finding consistent with previous studies (Thompson *et al.*, 1983; Dematawewa *et al.*, 1997; López de Maturana *et al.*, 2007; Tenhagen *et al.*, 2007; Bonneville-Hébert *et al.*, 2011; Hayes *et al.*, 2012). Ketoprofen treatment did not affect maternal survival, but most cows (65.2%) completed the lactation. It is possible that study of a larger number of cattle may identify effects of ketoprofen on maternal survival that were not identified by the current study and further investigation is merited.

7.4.3 Conclusions

As hypothesised, assisted parturition was negatively associated with measures of maternal and neonatal productivity (irrespective of treatment status). Additionally, negative effects of birth assistance were observed in the first lactation of affected calves — more than 2 y after birth. This finding is supported by previous work (Eaglen *et al.*, 2011; Heinrichs *et al.*, 2011) and suggests that the adverse effects of assisted birth are difficult to reverse. Furthermore, parturition assistance was provided judiciously on the study farm suggesting that whilst careful assistance is important for promoting welfare [see Chapter 6], the beneficial effects on productivity are few — supporting the view that parturition assistance should be provided judiciously. Irrespective of assistance status, ketoprofen treatment was positively associated with measures of productivity, although few interaction effects were observed, suggesting that the potential for ketoprofen to ameliorate the negative effects of assistance is limited. The measures of productivity analysed are related to economic performance and accordingly, these results suggest that farmer-provided assistance may have negative economic consequences. However, it is important that these are balanced against the potential consequences of failing to provide assistance (e.g. cow/calf death or injury) and parturition assistance should not be avoided at all costs as there will be occasions when the cow is unlikely to achieve a successful parturition unaided (e.g. malpresentation or uterine torsion). By contrast, ketoprofen administration is a safe and widely available intervention that is likely to lead to economic gains far exceeding the cost of provision. Accordingly it can be recommended that farmers and veterinary surgeons consider inclusion of ketoprofen administration into routine parturition management of cows.

Chapter 8 General Discussion

8.1 Introduction

Parturition assistance is a common event affecting up to 50% of cattle (Egan *et al.*, 2001; Hansen *et al.*, 2004; Kovács *et al.*, 2016) but detailed studies investigating the welfare impacts of assisted parturition have rarely been performed. Although dystocia is considered by farmers and veterinary surgeons to be painful (Whay *et al.*, 2005a; Huxley *et al.*, 2007; Laven *et al.*, 2009; Remnant *et al.*, 2017), data to support this belief were limited prior to the studies described in this thesis and it was also unknown if normal (unassisted) parturition is painful. Additionally, previous studies investigating birth related pain experienced by newborn calves are few (Murray *et al.*, 2016; Pearson *et al.*, 2019b, 2019a; Clark *et al.*, 2020) and do not employ a 2 x 2 factorial design to study the effects of both birth assistance and analgesia treatment. The studies presented in this thesis have contributed to our understanding of these topics, primarily by testing the following overall hypothesis:

> *'Parturition is a painful event for cows and their calves and as such, immediate postpartum administration of ketoprofen will be associated with improvements in health, welfare and production outcomes of cows and calves. Assisted animals will experience heightened benefits of postpartum analgesia.'*

Detailed behavioural, biochemical and production data analysis produced a comprehensive characterisation of the effects of assisted parturition and analgesia treatment on the welfare, productivity, and physiological status of cows and calves. Overall, the results demonstrate that assisted parturition has negative health and welfare impacts on cows and calves and that a single administration of ketoprofen analgesia can ameliorate some of these adverse effects — supporting the original hypothesis.

8.2 Study design

The 2 x 2 factorial design employed in the studies described in Chapters 3, 4, 6 and 7 is considered optimal for the investigation of animal pain (Weary *et al.*,

2006) and allowed the number of animals recruited to be minimised whilst yielding meaningful results. Nevertheless, this is an uncommon approach to the investigation of bovine parturition-related pain. For instance, just two studies have utilised this type of design to investigate the effects of assisted parturition and analgesia on the behaviour and productivity of adult cattle (Swartz *et al.*, 2018; Barragan *et al.*, 2020a) and no studies employ a factorial design to investigate the effects of birth assistance and analgesia on newborn calves. Whilst this study design is robust, the low prevalence of assisted parturition on the study farm and time-matched nature of recruitment of unassisted and assisted animals meant that animal recruitment took nearly a year and as such this design was impractical for use in the later studies [Chapter 5]. The valuable dataset obtained and presented in Chapters 3 and 4 showed that all cows and calves benefited from analgesia treatment and that assistance x treatment interaction effects on animal behaviour were minimal, leading to the enhanced focus on analgesia treatment in Chapter 5.

8.2.1 Choice of analgesic treatment

Ketoprofen was chosen for use in all studies described in this thesis as, although not licensed for use in newborn calves (National Office of Animal Health, 2020), it has been safely administered to calves as young as three days old (Merial Animal Health Ltd., 2007). Data regarding the pharmacokinetics of ketoprofen in younger calves (i.e. < 3 d) are not available, but ketoprofen pharmacokinetics in babies and young children is similar to adults (Kokki *et al.*, 2000). A previous study found that the pharmacokinetics of ketoprofen was altered in newborn foals (< 24 h old), concluding that the dose rate of ketoprofen administered to young foals could be safely increased by up to 50% (relative to the recommended adult dose), although longer dose intervals would be needed to avoid toxicity if there were repeated administration (Wilcke *et al.*, 1998). Thus, it was considered that administration of a single dose of ketoprofen to newborn calves at the recommended dose rate was unlikely to result in adverse side effects. The duration of ketoprofen efficacy in newborn calves is unknown and may be different to that observed in adult cattle, thus analyses were performed in 12 h time periods to account for this and identify potential effects of time. A further consideration in very young calves is the possibility that NSAID administration might have an adverse effect on renal development. Whilst cyclo-oxygenase-2 (COX-2) selective inhibitors have been shown to impede the normal renal development of mice, cyclo-oxygenase-1 (COX-1) inhibition has not been found to affect renal development (Kömhoff *et al.*, 2000); however, this has not been studied in calves. Although the effect of NSAIDs on bovine renal development is unknown, the immature renal function described in the neonates of many species has not been identified in calves (Dalton, 1968). As such, the maturity of the neonatal bovine kidney is likely to offer a protective effect against potential adverse effects of NSAID administration on renal development and the risk of developmental abnormalities occurring was considered to be low. Neonatal data were collected until calves completed their first lactation [see Chapter 7] and many are still in the herd at the time of writing (2021). During this time, abnormal clinical signs that might be consistent with impaired renal development (e.g. poor growth (Philbey *et al.*, 2009)) have not been identified. Additionally, analysis of neonatal urine specific gravity (an early indicator of renal dysfunction) did not identify acute adverse effects of ketoprofen administration [data presented in Appendix 5]. Together, this work did not identify adverse effects of ketoprofen, confirming that it is a suitable analgesic for use in healthy newborn calves. Similarly, Pearson *et al.* (2019b) did not identify any adverse renal effects in calves treated with meloxicam (an alternative NSAID) at birth. No analgesic medications are licensed for use in calves under one week of age in the UK and as such, administration is performed in accordance with the prescribing cascade for veterinary medicines (Veterinary Medicines Directorate, 2015) and should be under the direction of a veterinary surgeon. Recruited calves were all clinically healthy and adequate consumption of colostrum was ensured. Extra caution should be applied when considering administration of ketoprofen to unhealthy newborn calves, or to calves that may be dehydrated, as the safety of ketoprofen treatment of such calves is unknown.

Ketoprofen is licensed for use in postpartum cattle and has zero milk withdrawal period (National Office of Animal Health, 2020), thus minimising the impact of its administration to the commercial interests of the study farm. Previous reports have identified adverse effects related to administration of the NSAID flunixin to peri-partum cows (increased risk of RFM and stillbirth) (Waelchli *et al.*, 1999; Newby *et al.*, 2017), but similar effects have not been identified for other NSAIDs, including ketoprofen. The results presented in Chapter 7 support the hypothesis that ketoprofen does not have negative clinical effects on postpartum cattle and is an appropriate analgesic choice for parturition-related pain. Adverse effects of ketoprofen were almost absent in the studies presented in this thesis with just a single negative effect being identified: elevated maternal plasma CK concentration at 24 and 48 h postpartum [see Chapter 6]. It is known that intramuscular injection is associated with localised muscle injury (Lefebvre *et al.*, 1996) and a placebo group was included in the study design to account for this; however, NSAIDs are known to be irritant, and ketoprofen administered by intramuscular injection has been shown to cause a greater degree of muscle injury than saline administered by the same route (albeit to a lesser extent than other NSAIDs) (Pyörälä *et al.*, 1999). It is likely that the CK elevation reported here was related to localised tissue irritation and myocyte damage at the site of injection. Intravenous administration of treatments may have avoided this effect; however, this route of administration is less practical and more invasive than intramuscular administration and as such was not considered appropriate. Additionally, intravenous administration is less likely to be adopted by farmers, potentially limiting the wider application of results. An alternative approach that might be adopted in future studies could be to administer ketoprofen at multiple sites, thus injecting smaller volumes at any single site. As higher NSAID volumes have a greater irritant effect (Pyörälä *et al.*, 1999), this approach may reduce the negative effect of ketoprofen on plasma CK concentration; however, this has not been studied and it is possible that two injections of smaller volume may have similar effects to administration of a single larger volume. Furthermore, use of multiple injection sites may be more stressful for the animal, reduce the availability of unaffected muscle if subsequent injections are needed, may increase the likelihood of injection-site infections, and (if the animal is destined for the food chain) may result in reduced carcase value. As such, careful consideration of all possible implications is needed before multiple intramuscular injections are used. It should be noted that ketoprofen does not appear to cause pain at the injection site (Pyörälä *et al.*, 1999) therefore, unless plasma CK concentration is the outcome of interest, a single dose of ketoprofen administered by deep intramuscular injection is unlikely to adversely affect the results of a study or the welfare of animals.

8.2.2 General limitations

All of the studies presented here were performed on a single farm where animal management and husbandry were the same for all animals. Accordingly, several important factors (e.g. nutrition, breed) were controlled for but it is unknown if similar results would be obtained on farms where management is less optimised. This is particularly evident in Chapter 6 where, in contrast to the known relationship between colostrum volume and FPT (Stott *et al.*, 1979a; Renaud *et al.*, 2020), no relationship between volume of colostrum consumed and neonatal plasma TP concentration was identified. This probably reflects the optimised colostrum management on the study farm and is unlikely to be repeated on farms where colostrum provision is less well managed. Therefore, extrapolation of the results of the studies presented in this thesis to other farms should be cautious. The analgesic and anti-inflammatory effects of ketoprofen mean it is likely that all cows and calves will experience some welfare benefits of ketoprofen analgesia around parturition, but the impact of between-farm variation on the welfare benefits of ketoprofen administration is currently unknown. By contrast, between-farm variation of measures of production is likely to be high and it is possible that similar production benefits might not be observed on other farms.

Visual observations of behaviour were performed for the studies reported in Chapters 3 and 4 but technical failure of video recording equipment resulted in exclusion of up to 20% of recruited animals. Power failure on farm and downloading issues were the primary causes of lack of availability of video footage and this study highlights a limitation to video recording that is rarely discussed. The CCTV system used was chosen, in part, because it was designed for outdoor use and as such was robust to factors that could not be controlled (e.g. adverse weather conditions) but, nonetheless, it was reliant on mains electrical supply. A back up power supply is available on farm but even short interruptions in electrical power caused the DVR clock to reset, resulting in an inaccurate timestamp on video footage. Prior to starting data collection, problems with power supply were not expected and the large number of animals excluded due to technical failure was not anticipated. Future studies should take power supply into account when considering the type of video recording equipment used as careful and regular inspection of equipment is required to identify problems quickly and minimise loss of data. Such challenges highlight important differences in the extent of environmental control between commercial premises and dedicated research facilities.

The study presented in Chapter 5 monitored behaviour using accelerometers that were powered using internal batteries and the proportion of animals excluded due to technical failure was much lower (approximately 3%). Additionally, due to the more immediate nature of data collection (i.e. data were downloaded immediately after device removal whereas processing of video footage took much longer), all subjects affected by technical failure were able to be replaced within the allocated data collection time, maintaining the *a priori* planned sample size (80). Time budgets constructed from data generated by remote-worn devices are less detailed than those constructed from visual observations — most notably the range of behaviours monitored is more limited. However, data are collected continuously and can be collected for long periods of time where visual observations would be impractical. Additionally, the findings presented here suggest that remote devices might be more reliable than video footage in field research. As such, if detailed behavioural time budgets (e.g. different lying postures) are not required, remote monitoring devices offer a valuable alternative to visual observations that is more robust for use in environments such as farms, where equipment can be expected to have to endure a number of external challenges (e.g. adverse weather, dust/soiling, animal attention). Both visual observations and remote monitoring devices (such as accelerometers) have important complementary roles in behavioural research. Thorough consideration of the research question at the design stage and careful evaluation of the ability of different data collection techniques to address the research question(s), as well as consideration of how data might be analysed, should inform selection of behavioural observation methods.

8.2.3 Animal recruitment

Primiparous dams were over-represented in the studies reported in Chapters 3, 4, 6 and 7, accounting for 53.2% of recruited adult animals. This reflects the herd management on the farm: primiparous animals account for approximately 40% of the study herd and are the only animals that are bred using sex-sorted semen. Due to the breeding management protocols used, approximately 85% of primiparous animals in the herd produce female Holstein calves. As only female

Holstein calves were recruited in the studies reported here (and the eligibility of the calf determined the eligibility of the cow) this resulted in a moderate over-representation of primiparous animals. Nevertheless, assistance status was not affected by parity [Appendix 3]. This is in contrast to previous work that has suggested that primiparity is a risk factor for both dystocia and assisted parturition (Johanson *et al.*, 2003; Mee *et al.*, 2011; Atashi *et al.*, 2012). The studies in this thesis were not designed to assess risk factors for assisted parturition and, as such, did not intend to replicate the prevalence of assisted parturition or birth in the wider population; rather, the study design was balanced for assistance status. Additionally, factors known to be associated with an increased likelihood of parturition assistance (such as male calf (Berger *et al.*, 1992; Johanson *et al.*, 2003), non-Holstein breed (Hansen *et al.*, 2004)) were controlled; therefore, the primary foetal influence on assistance status was size (as measured by birthweight). Increasing birthweight has been shown to be a risk factor for dystocia in cattle (Johanson *et al.*, 2003; Atashi *et al.*, 2012) and calves born to multiparous dams in these studies were 3.7 kg heavier than calves born to primiparous dams. As such, it is likely that absence of a parity association with assisted parturition in these studies is related to both farm breeding policies and study design.

8.3 Effects of parturition assistance and ketoprofen treatment

The studies presented in this thesis suggest that overall, assisted parturition did not result in greater tissue trauma or stress (for cows or calves) than unassisted parturition — challenging the commonly held belief that increased stress (relative to unassisted parturition) and iatrogenic tissue injury are inevitable when parturition/birth are assisted. Parturition assistance on the study farm is provided judiciously by experienced personnel and parturition management is similar to optimised protocols suggested by Schuenemann *et al.* (2011). Accordingly, it cannot be certain that similar results would be obtained on farms where parturition management is less optimised; it is possible that assisted parturition provided less judiciously, or by less experienced staff, may result in additional tissue damage not seen here. Although the results of biochemical analysis suggested that assisted parturition was not more physically traumatic than unassisted parturition (for cows or calves), parturition assistance was nonetheless found to have negative impacts on the welfare and productivity of cows and calves, irrespective of ketoprofen treatment. It is likely that parturition assistance is associated with additional challenges for cows and calves that cannot be pharmacologically manipulated or detected using the methods presented in these studies; a hypothesis supported by the results presented in Chapter 3 that identified behavioural effects of birth assistance consistent with increased fatigue of affected calves. All but one study [Chapter 5] investigated the same cohort of animals, revealing the scale of the impact of parturition assistance on both cows and calves. When data obtained by previous studies [see Chapter 1] are appraised as a collective body of work, wide-ranging negative effects of parturition assistance are implied; however, different animals managed in different environments are studied and accordingly this finding has not previously been demonstrated so clearly using the same animals within a single study.

Although few cows were diagnosed with postpartum disease, assisted cows were over-represented, a finding consistent with previous studies (McArt *et al.*, 2013; Vergara *et al.*, 2014; Kovács *et al.*, 2016). Assisted parturition has been identified as a risk factor for disease in the first 30 d postpartum (Vergara *et al.*, 2014); however, the interactions between parturition-related metabolic changes and parturition-associated inflammation mean that the relationship between parturition assistance and postpartum disease is likely to be complex. It is possible that cows that are more predisposed to needing parturition assistance are also more predisposed to developing postpartum disease. For example, path analysis (a statistical method of examining relationships between variables (Streiner, 2005)) has shown that subclinical hypocalcaemia is associated with an increased risk of dystocia and that both dystocia and subclinical hypocalcaemia are associated with an increased risk of developing postpartum disease (Curtis *et al.*, 1983; Erb *et al.*, 1985; Correa *et al.*, 1993). The transition period is a time of physiological challenge for cattle and, despite being subject to much research, is still not fully understood. It is probable that, for some cows, parturition assistance and dystocia form part of a wider complex of 'transition problems' but dystocia (or assisted parturition) may be the first indicator of this seen by a farmer (or veterinary surgeon). Therefore, it is important from a clinical perspective to recognise that assisted cows are more likely to develop postpartum disease (irrespective of the underlying aetiology) to enable targeted monitoring of these cows and prompt treatment as needed, thus minimising the adverse welfare and production impacts related to postpartum diseases.

Although parturition assistance was graded, assisted animals were grouped together into a single ‘assisted’ group for analysis. This approach was adopted for practical reasons as it was not possible in the time available to recruit enough animals subject to each assistance grade (2 and 3) for meaningful analysis to be performed. However, as a result it is not possible to differentiate the effects of different severities of farmer-provided assistance. Some studies have found that individual assistance grade is correlated with the risk of adverse events such as postpartum disease (Mahnani *et al.*, 2021) and stillbirth (Norquay *et al.*, 2020), and has a negative correlation with future production (Heinrichs *et al.*, 2011), but the relationship between parturition assistance and pain is less clear. Dystocia is thought to be more painful than unassisted birth (Whay *et al.*, 2005a; Huxley *et al.*, 2007; Laven *et al.*, 2009; Remnant *et al.*, 2017) but data to support this belief are limited. The results presented here suggest that, whilst all types of (*per vaginum*) parturition and birth are associated with some pain for cows and calves, farmer-provided assistance need not be more painful than unassisted parturition. Furthermore, as grade 3 assistance was predominant (accounting for 68.9% of assistance events across all studies), it is possible that the method of application of parturition assistance has a greater impact on pain experienced by cows and calves than the reason for assistance (i.e. appropriately provided correction of dystocia may be less painful than less severe but inappropriately provided [e.g. very early intervention or poor technique] assistance). Although a factorial design is considered optimal for detecting animal pain (Weary *et al.*, 2006), this approach is reliant on known analgesic efficacy (i.e. absence of a response to analgesia does not necessarily indicate absence of pain but may indicate poor drug efficacy). Additionally, individual variation in responses to analgesia also make it difficult to determine the degree of pain experienced. In recent years the use of facial expression (in the form of ‘grimace scales’) has been gaining traction for detecting animal pain (reviewed by Mogil *et al.*, 2020), with a scale for adult cattle developed in 2015 (Gleerup *et al.*, 2015). Due to camera positioning and the quality of video footage, it was not possible to analyse the facial expressions of animals in these studies and as such grimace scoring was not performed but would be of interest for future studies. Alternatively, substance P (a biomarker of nociception) offers another possibility for assessing bovine pain that may be more practical for studies performed on commercial farms or studies involving large numbers of animals. Just a single study has measured substance P in the postpartum period (Barragan *et al.*, 2020b) and grimace scores have not been evaluated for postpartum cattle (or calves). It is possible that these more recent developments in the assessment of bovine pain may have the ability to detect more nuanced pain responses of cows and calves experiencing assisted parturition which in turn may be able to aid optimisation of parturition assistance, but further study is needed.

Ketoprofen treatment was found to have beneficial effects on welfare and productivity of cows and calves, although — contrary to the hypothesis — most results indicated that all cows and calves experienced similar benefits of analgesia (i.e. assisted animals did not experience enhanced analgesic benefits). This is an interesting finding that has not previously been reported and suggests that unassisted parturition and birth is associated with pain for both cows and calves. This hypothesis is supported by parallel work performed at the University of Glasgow that found some stillborn unassisted calves had subclinical injuries identifiable at post-mortem examination that would likely have caused pain had the calf survived (Norquay *et al.*, unpublished). A further important implication of this finding is that, when assistance is applied judiciously, the degree of pain experience by assisted and unassisted animals is likely to be similar. However, it does need to be considered that similar results would be expected if no pain (rather than similar degrees of pain) was experienced by animals in these studies and, accordingly, placebo-treated animals were included to control for this possibility. Whilst the studies presented here suggest that parturition in general is painful for cows and calves, few assistance x treatment status interaction effects were identified. This suggests that pain and inflammation are not primary factors contributing to the negative impact of parturition assistance (albeit when parturition assistance is optimised); thus, the hypothesis that assisted animals would experience greater analgesic benefits (than unassisted animals) was not supported. Further study is warranted to explore alternative challenges resulting from parturition assistance; however, it needs to be considered that the number of animals in each assistance x treatment interaction group was relatively small (approximately 20) and it is possible that there was not enough power to detect small effect sizes, particularly in infrequent outcomes such as play behaviour. Nevertheless, similar studies of 194 cows (Swartz *et al.*, 2018) and 464 cows (Barragan *et al.*, 2020b, 2020a) also did not identify any assistance x treatment interaction effects on biomarkers of inflammation (Barragan *et al.*, 2020b), cow activity (Swartz *et al.*, 2018; Barragan *et al.*, 2020a) or measures of productivity (Swartz *et al.*, 2018; Barragan *et al.*, 2020a), in line with the findings of the studies in this thesis. Furthermore, a distinction needs to be made between statistical and biological/clinical significance when interpreting the results of studies such as these, as small effect sizes — whilst of academic interest — may not translate to meaningful differences in a farm setting (e.g. a treatment effect on milk production needs to be large enough to compensate for the cost of the analgesia to be meaningful for most commercial dairy farms). Accordingly, the value of detection of small effect sizes may not justify recruiting larger numbers of animals to a research study. Unlike measures of production or biochemical analytes, it is more difficult to determine a meaningful difference in behavioural time budgets of cattle and calves as few studies employ detailed behavioural analysis (as performed in Chapters 3 and 4) and 'normal' time budgets are poorly defined. As such, it may be beneficial for future behavioural studies to have increased power to detect small assistance x treatment interaction effect sizes as even small differences in behavioural observations might be meaningful; however, the available data suggest that larger studies are only likely to be valuable for studies employing detailed behavioural analysis, not for studies of production outcomes or biomarkers.

8.4 Wider application of results

The studies presented here were designed to allow the results to be applicable to an 'on farm' situation. Aspects of the study design that reflect the wider dairy industry included the focus on farmer-provided assistance, the recruitment of a commercial dairy farm for data collection, and the use of an inexpensive, licensed analgesic drug widely available to UK farmers and veterinary surgeons for use in postpartum cattle. The results of these studies indicate that a single dose of ketoprofen can be safely administered to cows and their calves immediately after parturition and this results in wide-ranging beneficial effects for welfare and productivity. Furthermore, the results suggest that all cows and calves experience beneficial effects of ketoprofen treatment, not just cows and calves subject to farmer-assisted parturition. As such, immediate postpartum administration of ketoprofen can be recommended for use on dairy farms as part of an optimised parturition management protocol. Some of the results may also be applicable to the beef sector (e.g. welfare improvements and growth rate of calves) but as beef calves were not studied, it cannot be certain that similar findings would be obtained. Accordingly, recommendations for administration of postpartum ketoprofen to beef cattle need to be cautious.

A recent study of Canadian farmers found that whilst almost 90% agreed that dystocia is painful, a much smaller proportion provide analgesia to affected cows (33%) and calves (28%) (Moggy *et al.*, 2017). This discrepancy between the recognition of pain and the use of analgesia is a common finding in surveys of both farmers and veterinary surgeons (Huxley *et al.*, 2007; Thomsen *et al.*, 2012; Remnant *et al.*, 2017), with reported barriers for analgesic use including cost and logistics of administration, lack of awareness of drug availability and the perception that an injection is more painful than the issue being treated (Whay *et al.*, 2005a; Huxley *et al.*, 2006, 2007; Moggy *et al.*, 2017; Remnant *et al.*, 2017). Farmers commonly report that their veterinary surgeon is an important influence but often describe limited discussion around analgesic usage and express frustration with poor communication regarding product availability (Huxley *et al.*, 2007; Moggy *et al.*, 2017). Furthermore, farmer access to analgesic drugs typically requires veterinary involvement, either for prescribing (e.g. UK) or administering (e.g. Finland (Hokkanen *et al.*, 2015) and Brazil (Andrighetto Canozzi *et al.*, 2020)). Therefore, it is clear that veterinary input is vital for the uptake of analgesia provision on farms but is currently limiting adoption of routine pain-relief on some farms – particularly around parturition. Reasons for this are unclear but some studies have found that veterinary surgeons believe farmers are not prepared to bear the cost of analgesia (Huxley *et al.*, 2006, 2007; Remnant *et al.*, 2017), even though the available data suggest this perception may be misplaced (Huxley *et al.*, 2007). Even if cost is a barrier to uptake of postpartum analgesia, the production gains identified in Chapter 7 (the value of which exceed the cost of a single dose of ketoprofen) may offer an incentive basis on which veterinary surgeons can discuss the value of postpartum analgesia with their clients. Inclusion of parturition management in a herd health plan offers an ideal opportunity for discussion between farmers and veterinary surgeons but many farms do not seek veterinary input for herd health planning (Bell *et al.*, 2006), an area where there is scope for improvement.

Ketoprofen is licensed for use in adult cattle (in the UK and Europe), is straightforward to administer and has zero milk withdrawal period (National Office of Animal Health, 2020). Farmers often report relating personal experiences of pain to that of their cattle, with the experience of childbirth often mentioned (Moggy *et al.*, 2017). Accordingly, this ability to relate to cows' experiences, coupled with the availability of ketoprofen, may facilitate adoption of ketoprofen administration to cows into routine parturition management on farms. By contrast, it is difficult to relate to the experiences of newborn calves as adults cannot remember their own birth (termed 'infantile amnesia') (Josselyn *et al.*, 2012; Alberini *et al.*, 2017). Additionally, the use of NSAIDs in newborn calves is 'off licence' in the UK and needs to be directed by a veterinary surgeon. These factors might both prove to be greater barriers for routine administration of ketoprofen to newborn calves. Additionally, some farmers hold the perception that pain experienced by very young calves is less than older animals (Moggy *et al.*, 2017) and accordingly, there may be more reluctance to adopt the practice of routinely providing pain relief to newborn calves (compared to treating dams), particularly if dystocia did not occur. The results of the present studies indicate that calves (both assisted and unassisted) experience pain for up to 48 h after birth, but it is not possible to compare the magnitude of pain experienced to that of cows and as such this finding may not be enough to incentivise the use of post-birth analgesia. By contrast, the production gains associated with post-birth ketoprofen treatment identified in Chapter 7 may offer a stronger incentive for farmers to provide pain relief to newborn calves.

An important finding of the studies presented in this thesis is that tissue injury associated with assisted parturition is not inevitably greater than that associated with unassisted parturition. These results support the idea that measures should be adopted to minimise dystocia prevalence (e.g. through breeding decisions) and that parturition assistance should only be provided when necessary (i.e. to prevent death or severe injury to cow or calf). Previous studies have found the prevalence of parturition assistance consistently exceeds dystocia prevalence — suggesting that parturition assistance is routinely provided to cattle in the absence of dystocia, and possibly unnecessarily. As such, the welfare and productivity of many cows and calves may be being compromised by this apparently routine management practice — an area that could be addressed by farmers seeking to optimise welfare and productivity of their herd. However, although minimising the prevalence of parturition assistance should be the overall aim of parturition management protocols, assisted parturition is not totally avoidable (e.g. in cases of foetal malpresentation) and appropriately provided assistance can be beneficial in some cases. These studies were not designed to investigate optimal parturition techniques, but the finding that judiciously provided farmer-assisted parturition need not be more physically traumatic than unassisted parturition is an important one that highlights the value of appropriate obstetrical training of farmers and veterinary surgeons. Parturition management should focus on prompt assistance for cows that require it (i.e. cows experiencing dystocia) but if dystocia is not present, a more cautious approach can be adopted. Previous studies have found intervening too early or too late to assist calves that are presented normally is associated with poorer outcomes, with a delay (in provision of assistance) of approximately 1 h being ideal (Schuenemann *et al.*, 2011; Villettaz Robichaud *et al.*, 2017a). It is likely that this short delay allows further relaxation of maternal soft tissues to facilitate safer delivery of the calf; a hypothesis supported by Kovács *et al.* (2016) who reported vulvovaginal lacerations in 80% of cows subject to early assistance (28.4 ± 13.5 min after appearance of foetal hooves) compared to 18.8% of cows subject to delayed assistance (73.1 ± 5.2 min after appearance of foetal hooves). One study found that over 40% of farmers intervene with parturition assistance less than 1 h after observing foetal hooves (Egan *et al.*, 2001), with some farmers waiting for as little as 5 min (Villettaz Robichaud *et al.*, 2016). Additionally, over 25% of farmers report assisting every calving (Villettaz Robichaud *et al.*, 2016). Whilst it is recognised that not all farms have the staff availability to provide 24 h monitoring of parturient cattle (such as the study farm), the findings of Egan *et al.* (2001) and Villettaz Robichaud *et al.* (2016) nevertheless represent an area where improvements could be made. Judicious, appropriately timed, parturition assistance needs to be encouraged on farms and the results of these studies suggest that achieving this will be associated with improved welfare in the postpartum period and also meaningful production gains for both cows and calves.

8.5 Future work

Whilst the studies presented in this thesis have aided the understanding of pain experienced by cows and calves immediately after parturition and identified welfare and production effects of assisted parturition, several areas that merit further study have also been identified.

The studies reported in Chapters 4 and 5 identified parity effects on cow behaviour consistent with improved welfare of multiparous animals. Similarly, the studies reported in Chapters 3 and 5 identified effects of dam parity on calf behaviour consistent with improved welfare of calves born to multiparous dams. These are interesting findings, and it was hypothesised that the smaller size and lack of parturition experience of younger dams resulted in increased tissue trauma being present in both cows and their calves. This was supported by the results of the study presented in Chapter 6 that found plasma CK concentration (a biomarker of muscle injury) was highest in calves born to primiparous dams. Parity x treatment status interaction effects were not investigated in the current studies and as such it cannot be certain that primiparous cows (or calves born to primiparous dams) experience more parturition-related pain (irrespective of assistance status). This finding merits more study in the future and dam parity should be considered when assessing postpartum welfare of dairy cattle and calves. Additionally, given the beneficial effects of analgesia on measures of maternal production [Chapter 7], it is possible that primiparous and multiparous animals may experience different production outcomes associated with analgesia administration — a hypothesis that deserves further study.

For practical reasons, the studies presented in this thesis all investigated the effects of a single dose of ketoprofen, but in the UK ketoprofen is licensed for administration (to cattle) of up to three (consecutive) daily doses (National Office of Animal Health, 2020). Additionally, alternative NSAIDs are available (Hudson *et al.*, 2008; National Office of Animal Health, 2020) and — although several NSAIDs have been studied individually — just a single study has compared the effects of more than one NSAID in postpartum cattle, finding that meloxicam and sodium salicylate had similar beneficial effects on measures of milk production (Carpenter *et al.*, 2016). Carpenter *et al.* (2016) investigated analgesia effects on measures of productivity and biomarkers of inflammation but did not conduct behavioural analyses, or measure biomarkers more traditionally associated with welfare (e.g. cortisol). Additionally, only multiparous animals were studied, and parturition assistance was not accounted for. It would be of interest to compare the effects of different NSAIDs on measures of welfare, and also include younger animals and calves. Investigation of different dose regimes would also be valuable in the future as this would aid determination of the optimal analgesic protocol for postpartum cattle and newborn calves. However, future work should also take into consideration NSAID availability and national veterinary medicines legislation (for example, ketoprofen is not licensed for use in food producing animals in the USA) and as such, country- or region-specific optimised postpartum analgesic protocols may need to be developed.

Although just a small number of studies have investigated the effects of assistance status and analgesia on cows and their calves, analysis of the available data and development of evidence-based recommendations for farmers and veterinary surgeons is currently challenging because dystocia and parturition assistance are not consistently defined [Appendix 1]. It would be beneficial for future researchers and industry stakeholders to collaborate and establish a more standardised bovine parturition grading system as well as an agreed definition of ‘dystocia’. This approach would result in improved consistency and enable more accurate comparisons between studies. It would also aid farmers in recording parturition assistance on farm in a consistent manner that allows benchmarking comparisons to be made. A similar approach has already been successfully applied to bovine lameness in the UK (AHDB, 2021) and has been widely adopted within the dairy industry, as well as by researchers (Afonso *et al.*, 2020). Thus, this type of standardised approach is already familiar to farmers and other stakeholders within the UK dairy industry which may aid adoption of similar methodology for monitoring and recording parturition assistance.

Data presented in Chapter 5 identified an association between the step count and the number of lying bouts in the last 48 h of gestation and subsequent parturition experience (assisted/unassisted). Basic CART analysis showed promise for identification of thresholds for prediction of unassisted parturition, but the prediction of assisted parturition was less promising. This study was primarily designed to investigate the effects of postpartum analgesia and the combined sample size (n = 80) was selected for this purpose. As such, the number of assisted cows in this study was small (*n* = 23) and caution should be applied when interpreting the pre-partum analysis presented in Chapter 5. Nevertheless, these analyses have demonstrated that CART is an appropriate statistical methodology to apply to these data (i.e. ‘proof of concept’) — an approach not previously attempted. It would be beneficial for future work to build on this and refine the findings of this study. Of particular interest is establishment of a robust threshold for identification of cows likely to need assistance, although identification of cows unlikely to need assistance is also valuable. It would also be of interest to analyse data obtained from different farms and different breeds of cattle to understand variability in these metrics and improve the wider application of results.

Although preliminary, the results presented in Chapter 5 offer exciting possibilities for future research as increasingly sophisticated algorithms may be developed for identifying cows likely to need assistance and possibly also predicting the time of parturition. In the future it is possible that animal-worn devices will be able to combine these two outcomes to identify cows more likely to need assistance as well as providing an estimate of the timing of parturition. This would allow farmers to provide tailored parturition management to individual cows and allocate resources efficiently (e.g. move high risk cows to individual parturition pens to enable closer monitoring), contributing to further improvements in parturient cow welfare.

8.6 Conclusions

When consolidated, the results presented in this thesis indicate that ketoprofen treatment immediately after parturition is beneficial to cows and calves. Identified positive impacts were wide-ranging — encompassing welfare and productivity — and differed between cows and calves. Protecting and improving the welfare of animals used for food production (as well as other activities) is strongly supported by consumers and industry stakeholders and as such, farm animal welfare is under increasing scrutiny. Accordingly, the primary focus of this thesis was investigation of the welfare impacts of parturition and the potential for analgesia to generate improvements in postpartum welfare of cows and calves, a previously neglected area of study. Nevertheless, the commercial aspect of dairy farming should not be forgotten, and cattle productivity is of high importance. A single dose of ketoprofen administered immediately after parturition was validated as a simple, accessible tool for improving welfare and productivity of cows and calves. Whilst welfare improvement is arguably the moral imperative, the accompanying production gains identified offer an important additional incentive for uptake of this practice on farms.

Although some of the results of these studies are more likely to be applicable to high-performing dairy farms, the effects of parturition assistance and postpartum ketoprofen treatment identified are still relevant to the wider dairy industry. The results highlight that farmers (and veterinary surgeons) should be encouraged to apply parturition assistance judiciously and it would be beneficial for appropriate obstetrical training to be provided as part of the curricula in agricultural and veterinary training institutions. Collectively, the findings presented here provide a robust basis on which inclusion of ketoprofen administration in parturition and newborn calf management protocols can be recommended to dairy farmers and veterinary surgeons seeking to optimise the welfare and productivity of Holstein cows and calves managed in a housed dairy system.

Appendix 1

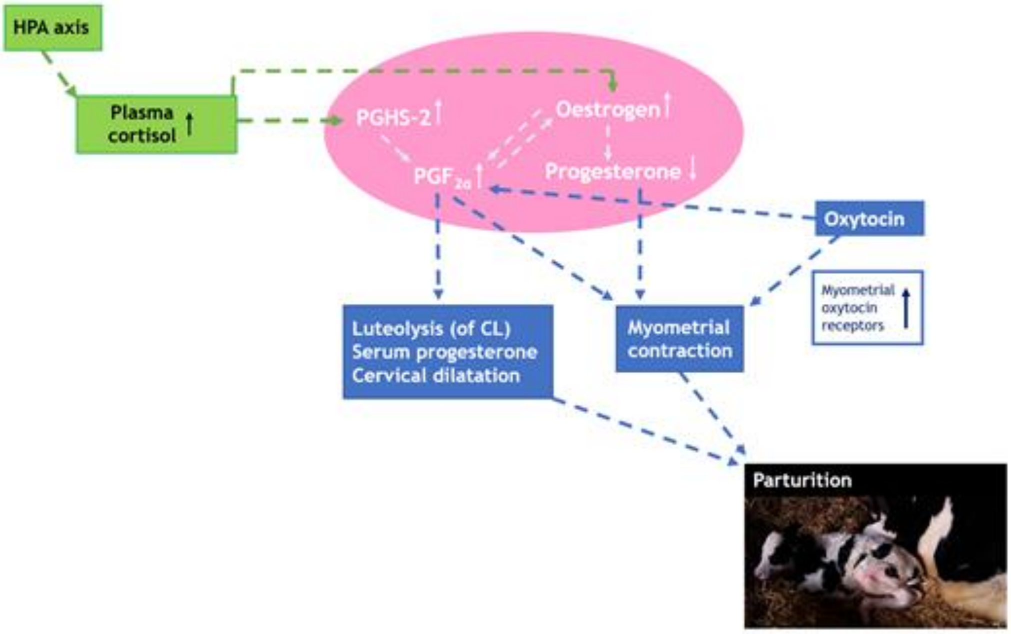


Figure A1.1: Simplified diagram illustrating pathways involved in the initiation of bovine parturition

Green = changes occurring in the foetus. Pink = changes occurring in the placental trophoblasts. Blue = changes occurring in the dam. PGHS-2 = prostaglandin synthase-2. $PGF_{2\alpha}$ *= Prostaglandin-*$F_{2\alpha}$. *CL = corpus luteum.* (Fuchs *et al.*, 1992; Challis *et al.*, 2000, 2005; Shenavai *et al.*, 2012) ↑ *= increase.* ↓ *= decrease. Broken arrows indicate (simplified) relationships within pathway.*

Table A1.1: Examples of scoring systems used to define parturition assistance of cattle

Author(s)	Score	Definition
Atashi *et al.* (2012, 2021) & Dematawewa and Berger (1997)	1	No assistance
	2	Slight assistance
	3	Needed assisted
	4	Considerable force needed
	5	Extreme difficulty
Barragan *et al.* (2020b, 2020a, 2020c)	1	Unassisted
	2	Manual assistance provided by one person
	3	Manual assistance provided by ≥ 2 people
	4	Mechanical traction applied
	5	Surgical extraction
Barrier *et al.* (2013b)	N	No assistance required
	FN	Farmer assistance — normally presented calf
	FM	Farmer assistance — malpresented calf
	V	Veterinary assistance
Bellows and Lammoglia (2000) & Nix *et al.* (1998)	1	No assistance required
	2	Minor manual assistance
	3	Heavy traction using a mechanical calving aid
	4	Caesarean section performed
Heinrichs and Heinrichs (2011)	1	No assistance required
	2	Mild traction applied
	3	Substantial traction applied/mechanical extraction/caesarean section performed
Homerosky *et al.* (2017b, 2017a)	Easy assist	Manual assistance
	Difficult assist	Assistance requiring a mechanical calving aid or traction by ≥ 3 personnel
	Caesarean section	Caesarean section performed (following veterinary examination)
Kovács *et al.* (2017b, 2017a)	Eutocia	Unassisted parturition duration ≤ 2 h/brief manual traction by one person
	Dystocia	Unassisted parturition duration > 2 h/traction provided by ≥ 2 people (± mechanical calving aid)
Murray *et al.* (2015b)	1	Unassisted birth
	2	Easily assisted birth
	3	Substantial force needed to extract calf
Pearson *et al.* (2019b, 2019a)	Easy assist	Manual traction provided by one person
	Difficult assist	Manual traction provided by ≥ 2 people/mechanical calving aid used/caesarean section performed[1]
Proudfoot *et al.* (2009)	1	No assistance required
	2	Traction provided by one person
	3	Traction provided by ≥ 2 people or confirmed dystocia
Swartz *et al.* (2018)[2]	Eutocia	Parturition of duration ≤ 70 min
	Dystocia	Parturition of duration > 70 min
Vannucchi *et al.* (2015b, 2015a)	Eutocia	Normal (unassisted) parturition with stage II parturition < 2 h
	Dystocia	Parturition assistance required due to foetal malpresentation or foeto-maternal disproportion and stage II parturition ≥ 2 h
	Inertia	Parturition assistance required due to uterine inertia. Stage II parturition ≥ 2 h with no sign of progress (normally presented calf)

1. Caesarean section not included in Pearson *et al.* (2019b)
2. Study used an ordinal system (1 to 3) to describe assistance but defined dystocia by parturition duration for the purposes of analysis

Table A1.2: Examples of modified Apgar scoring systems used to assess neonatal vigour in veterinary species
bpm = beats per minute

Author	Neonate tested	Parameters measured	Abnormal result [0]	Intermediate result [1]	Normal result [2]
Cruz *et al.* (2017)	Foals	Heart rate	Absent	< 60 bpm	≥ 60 bpm
		Mucous membrane colour	Cyanotic	Pale pink	Pink
		Muscle tone	Limp extremities	Some limb flexion	Sternal recumbency
		Nasal stimulation	No response	Grimace	Cough or sneeze
		Respiration	Absent	Slow, irregular	≥ 60/min, regular
Flora *et al.* (2020)	Lambs	Mucous membrane colour	Cyanotic	Pale	Pink
		Response to nose stimulation	No response	Moves head slightly	Shakes head
		Response to rump stimulation	No movement	Moves but does not attempt to stand	Attempts to stand
Gillingham *et al.* (2018)[1]	Calves	Meconium staining	Completely covered with meconium	Mild staining of tail/perineum	No staining
		Tongue/head appearance	Tongue protruding and swollen	Tongue protrusion, no swelling	Normal
		Calf movement	Remains in sternal recumbency for up to 3 h	Attempts to stand within 1.5 h but standing not achieved	Standing within 30 min
Homerosky *et al.* (2017a)[2]	Calves	Corneal reflex	Incomplete blink	-	Complete blink
		Meconium staining	Present	-	Absent
		Mucous membrane colour	Red, blue, white	-	Pink
		Nasal stimulation	No/minimal movement	-	Active head shaking
		Suckle reflex	Weak	-	Strong
		Tongue appearance	Protruding or swollen	-	Normal size
		Tongue withdrawal	Incomplete	-	Complete

1. Based on Murray *et al.* (2015a) [Table A1.3].

2. Intermediate and abnormal results grouped together into a single ‘abnormal’ category for binary (normal/abnormal) analysis.

Table A1.2 continued

Study	Neonate tested	Parameters measured	Abnormal result [0]	Intermediate result [1]	Normal result [2]
Revermann *et al.* (2018)	Piglets	Movement in first 15 s	No movement	Some movement	Much movement
		Latency to first teat contact	> 30 min	10 to 30 min	< 10 min
		Latency to stand	> 5 min	1 to 5 min	< 1 min
		Meconium staining	Much	Some	None
		Respiration	First breath in > 15 s, irregular thereafter	First breath in > 15 s, regular thereafter	First breath in < 15 s
		Skin colour	Blue	Pale	Pink
		Umbilical cord condition	Ruptured < 15 cm	Ruptured ≥ 15 cm	Not ruptured
Sorge *et al.* (2009)	Calves	Interdigital reflex	No reaction	Weak limb retraction	Strong, immediate limb retraction
		Mucous membrane colour	White	Pale pink/cyanotic	Pink
		Reaction to cold water dripped onto head	No reaction	Reduced/delayed reaction	Active head shaking
		Respiration	Absent	Irregular	Regular
Veronesi *et al.* (2009)	Puppies	Heart rate	< 180 bpm	180 to 220 bpm	> 220 bpm
		Motility	Flaccid	Some movement	Active movement
		Mucous membrane colour	Cyanotic	Pale	Pink
		Reflex irritability	Absent	Grimace	Vigorous
		Respiration	< 6/min and/or silent	6 to 15/min and/or mild crying	> 15/min and/or loud crying

Table A1.3: VIGOR score[1] developed by Murray *et al.* (2015a) for assessing newborn calf vigour (Modified from Murray *et al.*, 2015a)

VIGOR category	Measure assessed	Score			
		0 [normal]	1	2	3 [abnormal]
Visual appearance	Meconium staining	No staining	Mild staining of tail/perineum	Moderate staining extending to body	Severe staining completely covering body
	Tongue/head appearance	Normal	Tongue protrusion, no swelling	Tongue protruding and swollen	Tongue protruding. Head and tongue swollen
Initiation of movement	Calf movement	Standing within 30 min	Attempts to stand within 1.5 h but standing not achieved	Remains in sternal recumbency for up to 3 h	Still in lateral recumbency > 3 h after birth
General responsiveness	Response to nasal stimulation	Vigorous head shake	Moves head away	Twitching but does not move head	No response
	Response to tongue pinch	Tongue withdrawal	Attempts tongue withdrawal	Moves tongue but does not attempt withdrawal	No response
	Corneal reflex[2]	Immediately blinks and retracts globe	Slow blink	No response	NA
Oxygenation	Mucous membrane colour	Bright pink	Pale pink	Brick red	White/blue
	Tongue length[3]	< 50 mm	50 to 61 mm	> 62 mm	NA
Rates	Heart rate[4]	80 to 100	> 100	< 80	NA
	Respiration rate[4]	24 to 36	approximately 24	> 36	NA

1. Developed for Holstein dairy calves. Scored in opposite direction to other veterinary vigour scores (i.e. low composite score = good vigour; high composite score = poor vigour)
2. Response to gentle touch of cornea
3. Measured from the lips within 5 min of birth
4. Per minute

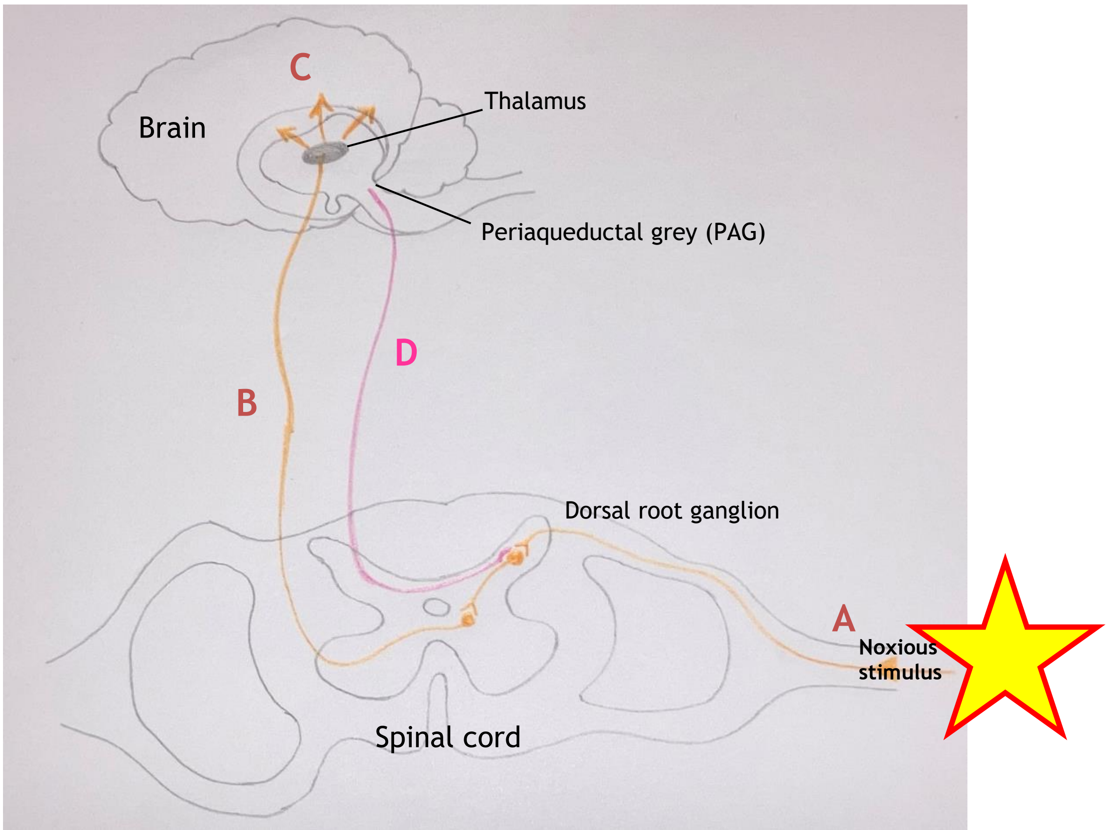


Figure A1.2: Simplified diagram of the basic pain pathway
Orange indicates ascending pathway. Pink indicates descending (modulatory) pathway. A = transduction. B = transmission. C = perception. D = modulation.

Appendix 2

Farm breeding protocols

All breeding on the study farm is performed using artificial insemination with Holstein or beef breed semen. Holstein bull selection is made based on calving ease, production predictive transmitting ability (PTA) data, proven performance, and genetic relationship to breeding female animals. Sex-selected Holstein semen is used to inseminate nulliparous animals only; conventional Holstein semen is used to inseminate primiparous and multiparous cows. Aberdeen Angus bull semen is used to inseminate nulliparous animals – individual bulls are primarily selected based on calving ease. Beef bull semen used to inseminate lactating animals is a composite of three breeds selected for enhanced conception rate (Genus ABS Fertility Plus). Bulls used on the farm are updated annually and bull selection is performed by technicians at Genus ABS with some input from the farmer.

Twenty-eight to 35 d after insemination, ultrasound examination of all animals is performed by a veterinary surgeon from the University of Glasgow School of Veterinary Medicine. If pregnancy is confirmed a second ultrasound examination is performed at 60 to 67 d post-insemination to confirm pregnancy has continued and detect possible foetal abnormalities. If the first ultrasound examination does not detect pregnancy any pathology that might be preventing conception (e.g. cysts, pyometra) is treated (if present) and animals are bred again when appropriate.

Nulliparous animals

Breeding starts at 13 months old with the aim to achieve conception by 15 months old (450 d) and first parturition at 22 to 24 months old. Nulliparous animals are bred using the Genus ABS Reproductive Management System (RMS) (Genus ABS, 2021) — insemination is performed by a single RMS technician. Nulliparous animals are bred to oestrus (aided by RMS) until they are 15 months old. If oestrus is not detected by the RMS technician, animals are examined by a veterinary surgeon. Nulliparous animals that have a healthy reproductive tract and a corpus luteum (CL) ≥ 2 cm in diameter present on one, or both, ovaries (diagnosed ultrasonographically) are treated with Cloprostenol, a prostaglandin-2α analogue (Estrumate, MSD Animal Health, Milton Keynes, Bucks., UK) and inseminated at the subsequent oestrus. All (non-pregnant) nulliparous animals ≥ 15 months old, and animals < 15 month old with a healthy reproductive tract but no detectable CL (or a CL <2 cm in diameter), are treated with the CIDRSynch protocol [Figure A2.1] and inseminated at a fixed time at the end of the programme. Reproductive abnormalities are treated if possible but if treatment cannot be performed (e.g. freemartinism) or is unsuccessful the affected animal is sold.

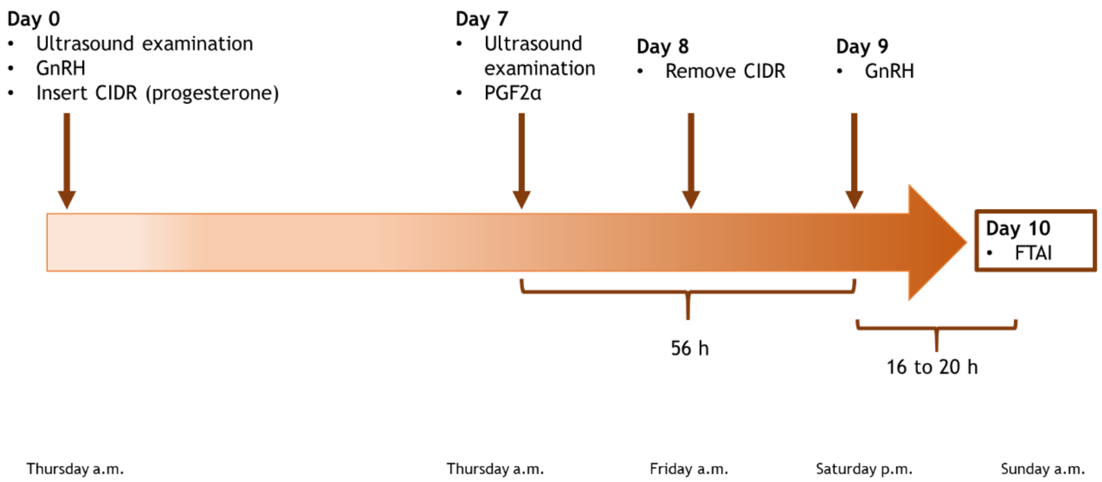


Figure A2.1: CIDRSynch protocol used on the study farm
GnRH = Gonadotropin-releasing hormone. CIDR = Controlled Internal Drug Release (intravaginal progesterone releasing device). PGF2α = Prostaglandin-F2α (analogue used). FTAI = fixed time artificial insemination. Days of the week indicate farm schedule.

Nulliparous animals are initially inseminated with sex-selected Holstein semen. If the animal has not conceived after three insemination attempts, conventional beef breed (Aberdeen Angus) semen is used. Nulliparous animals that have not conceived by 17 months old are sold.

Primiparous and multiparous animals

Lactating animals are all inseminated by the head and deputy head stockman who are trained and certified to inseminate cattle. A 50 d voluntary waiting period (VWP) is observed after parturition during which cows are not bred. After the VWP has elapsed, animals are bred to observed oestrus until 75 DIM — oestrus is observed visually by senior farm staff with the aid of pedometers. All cows that are not in calf by 75 DIM are bred to the standard OvSynch synchronisation protocol [Figure A2.2]. Cows not in calf by 150 DIM are bred to a CIDRSynch protocol [Figure A2.1]. The threshold to remove animals from the herd due to failure to conceive is set at 200 DIM for multiparous cows and 250 DIM for primiparous cows. These thresholds have been selected based on economic and production factors.

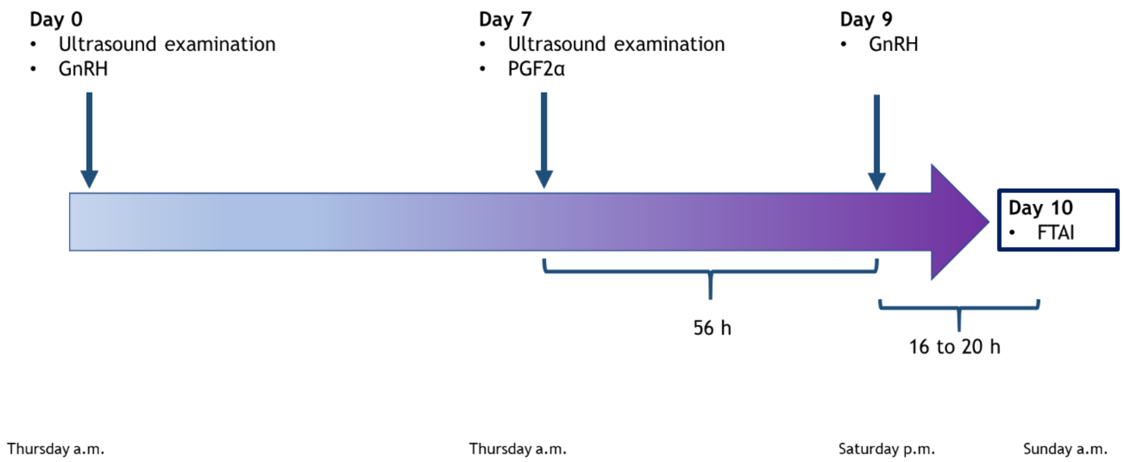


Figure A2.2: OvSynch protocol used on the study farm
GnRH = Gonadotropin-releasing hormone. PGF2α = Prostaglandin-F2α (analogue used). FTAI = fixed time artificial insemination. Days of the week indicate farm schedule.

Farm protocols for diagnosing and treating postpartum disease

Metritis

Diagnostic criteria (Sheldon *et al.*, 2006):

- **Puerperal metritis:** Presence of foetid, reddish brown, vaginal discharge of watery consistency within 21 d of parturition [Figure A2.3]. Affected animals are usually (but not always) pyrexic and may be systemically unwell; pyrexia is defined as T ≥ 39.5°C.

- **Clinical metritis:** Presence of a purulent vaginal discharge within 21 d of parturition in the absence of pyrexia; affected animals are systemically well.

Treatment:

Protocols for the treatment of metritis were changed between 2016 and 2020 due to changes in regulation regarding the use of flunixin in food producing animals and changes in recommended best practices regarding antimicrobial use in food producing animals.

2016: Tylosin once daily for 5 to 7 d by deep intramuscular injection at manufacturer's recommended dose rate. Flunixin* also administered for 3 d if animal is pyrexic (T ≥ 39.5 °C).

2020: Penicillin/streptomycin for 7 d by deep intramuscular injection at manufacturer's recommended dose rate. Meloxicam* also administered as a single dose if animal is pyrexic (T ≥ 39.5 °C).

*NSAIDs are only provided to pyrexic animals; animals that are normothermic in the presence of metritis are treated with parenteral antibiotic therapy only.

Figure A2.3: Reddish-brown, watery postpartum vaginal discharge typical of puerperal metritis.

Retained foetal membranes (RFM)

Diagnostic criteria: The failure to expel placental membranes before 24 h postpartum (Sheldon *et al.*, 2008) [Figure A2.4].

Treatment: Manual removal of membranes with minimum traction if possible — membranes that do not come away with minimal traction are not removed. If the animal is diagnosed with concurrent metritis, treatment is as described above.

Figure A2.4: Placental membranes still present more than 24 h after parturition

Subclinical ketosis (SCK)

Diagnostic criteria: Ketone positive urine sample as measured using commercially available urine testing strips (Ketostix, Bayer, Leverkusen, Germany). The presence of subclinical ketosis is defined as ++ and above on the urine testing strip [Figure A2.5].

Treatment: 250 mL propylene glycol administered orally twice daily for 3 d followed by once daily for two more days.

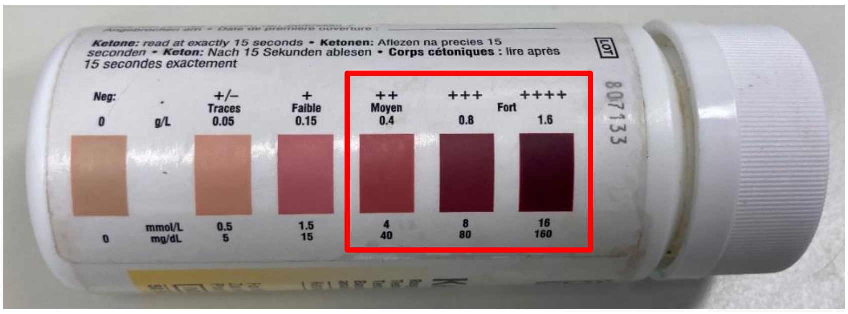


Figure A2.5: Urinalysis guide for interpreting results of the Bayer Ketostix urine test
Reagent colours inside red box indicate subclinical ketosis (SCK)

Left displaced abomasum (LDA)

Diagnostic criteria: Almost pathognomonic high-pitched metallic ‘ping’ audible when left abdomen concurrently auscultated and percussed.

Treatment: Surgical correction. The right-sided omentopexy LDA correction technique is performed in most cases however the choice of surgical approach is both case and surgeon dependent.

Pre-partum diet composition

Table A2.1: Representative composition of pre-partum diet fed in the last 3 weeks of gestation for cows studied in Chapters 4, 6 and 7

Dietary component	Amount fed[1] (kg/cow/d)
Wholecrop	10.8
Straw (wheat)	1.43
Bruised barley[2]	0.65
Wheat[3]	1.50
Oatfeed	2.63
Biochlor[4]	0.65
Soya	1.06
Oilseed rape	0.71
Soya hulls	0.20
Mineral mix	0.10
Limestone	0.25
Magnesium chloride	0.08
Acid buffer	0.10
Water	8.50

Minor modifications to pre-partum diet made four times during the study, mean composition of four diets presented.

1. Weight (kg) as fed
2. Fed September to December 2016 instead of wheat
3. Fed March to September 2016 instead of barley
4. Acidogenic supplement (Arm & Hammer, USA. Supplied by Almins, Edinburgh, UK) 48.6% CP; 7.38 MJ/kg; −337.9 mEq/100g DCAD

Table A2.2: Representative nutritional profile of pre-partum diet fed in the last 3 weeks of gestation for cows studied in Chapters 4, 6 and 7

Nutrient	(% DM)
Forage[1]	48.0
Concentrate	52.0
Crude protein	15.4
Rumen bypass protein	3.65
Starch	16.1
Sugar	4.03
Neutral Detergent Fibre	42.7
Oil	2.73
Calcium	1.47
Phosphorus	0.33
Magnesium	0.61
Potassium	1.05
Sodium	0.10
Chloride[2]	105.3
Metabolisable energy[3]	95.8

Minor modifications to pre-partum diet made four times during the study, mean nutritional profile of four diets presented.

1. 88% wholecrop, 12% wheat straw as fed
2. Measured in g/d
3. Measured in MJ/d

Appendix 3

Table A3.1: Individual neonatal calf behaviours and combined categories used for analysis

Individual behaviour	Combined behavioural category	
Sternal recumbency, head up	Lying (sternal recumbency)	Lying total
Sternal recumbency, head down		
Sternal recumbency, head position unknown		
Lateral recumbency, head up	Lying (lateral recumbency)	
Lateral recumbency, head down		
Lateral recumbency, head position unknown*		
Unidentified lying posture, head up*	Lying (unknown body posture)	
Unidentified lying posture, head down*		
Unidentified lying posture, head position unknown*		
Attempting to stand	Active total	
Standing		
Walking		
Play		
Feeding and drinking directed behaviours	Secondary total	
Self-grooming		
Grooming others*		
Investigatory behaviours		
Other social behaviour		
Other secondary behaviour*		
Not visible	Not visible	

* *Rare behaviours not analysed individually*

Table A3.2: Individual neonatal calf lying behaviours and the combined head position category used for analysis.

Individual lying behaviours	Head position category
Sternal recumbency, head up	Total head up
Lateral recumbency, head up	
Unidentified lying posture, head up*	
Sternal recumbency, head down	Total head down
Lateral recumbency, head down	
Unidentified lying posture, head down*	
Sternal recumbency, head position unknown	Total head unknown
Lateral recumbency, head position unknown	
Unidentified lying posture, head position unknown*	

**Rare behaviours not analysed individually*

Examples of calf lying behaviours

Identifying marks (ear tags) obscured in all images to preserve farm anonymity

Figure A3.1: Calf lying in sternal recumbency with the head held up in an alert position (NU)

Figure A3.2: Calf lying in sternal recumbency with the head held down in a rested position (NH)

a ***b***

Figure A3.3: Calves lying in lateral recumbency with the head held down in a rested position (RH). *a = example of head resting on the body. b = example of head resting on the ground.*

Figure A3.4: Calf lying in lateral recumbency with the head held up in an alert position (RU)

Univariate analysis of birth-related metadata

Table A3.3: Chi-squared analysis of associations between assistance status (assisted/unassisted) and categorical birth-related data. *n = 95.*

Variable	Degrees of freedom	χ^2 statistic	*p*-value
Time of birth[1]	2	2.80	0.247
Date of birth[2]	3	6.74	0.081
Dam parity[3]	2	0.99	0.610
Consumption of extra colostrum (Y/N)[4]	1	0.006	0.939

1. Categorised as morning (06:00 to 13:59), afternoon (14:00 to 21:59) and night (22:00 to 05:59) for Chi-squared analysis.
2. Categorised by season (i.e. spring, summer, autumn, winter) for Chi-squared analysis.
3. Categorised as 1, 2 and ≥ 3 for Chi-squared analysis.
4. Y = > 4.5 L consumed (*n* = 61); N = ≤ 4.5 L consumed (*n* = 34).

Table A3.4: Two-sample t-test analysis of associations between assistance status (assisted/unassisted) and continuous birth-related data.

Variable	Assistance status	*n*	Mean	SE	*p*-value
Birthweight (kg)	Assisted[1]	46	44.3	5.94	0.022
	Unassisted	47	41.4	5.92	
Amount of colostrum ingested (L)	Assisted	48	4.89	0.07	0.295
	Unassisted	47	4.76	0.09	

1. Assisted *n* = 46 due to missing data for two calves as a result of weigh scale failure

Table A3.5: Chi-squared analysis of associations between treatment status (ketoprofen/saline) and categorical birth-related data. *n = 94[1].*

Variable	Degrees of freedom	χ^2 statistic	*p*-value
Time of birth[2]	2	0.350	0.839
Date of birth[3]	3	1.537	0.674
Dam parity[4]	2	0.387	0.824
Consumption of extra colostrum (Y/N)[5]	1	0.184	0.668
Assistance status (A/U)[6]	1	0.170	0.680

1. Treatment not recorded for one calf (excluded from analysis).
2. Categorised as morning (06:00 to 13:59), afternoon (14:00 to 21:59) and night (22:00 to 05:59) for Chi-squared analysis.
3. Categorised by season (i.e. spring, summer, autumn, winter) for Chi-squared analysis.
4. Categorised as 1, 2 and ≥ 3 for Chi-squared analysis.
5. Y = > 4.5 L consumed (*n* = 61); N = ≤ 4.5 L consumed (*n* = 34).
6. A = assisted (*n* = 48). U = unassisted (*n* = 46).

Table A3.6: Two sample t-test analysis of associations between treatment status (ketoprofen/saline) and continuous birth-related data. *n = 94[1].*

Variable	Treatment status	*n*	Mean	SE	*p*-value
Birthweight[2] (kg)	Ketoprofen	46	43.4	0.83	0.357
	Saline	46	42.2	0.96	
Amount of colostrum ingested (L)	Ketoprofen	47	4.79	0.08	0.564
	Saline	47	4.86	0.09	

1. Treatment not recorded for one calf (excluded from analysis).
2. *n* = 46 in each treatment group due to missing data for two calves as a result of weigh scale failure

Appendix 4

Categorisation of cow postpartum behaviours

Table A4.1: Individual postpartum cow behaviours and combined categories used for analysis

Individual behaviour	Combined behavioural category	
Sternal recumbency, head up Sternal recumbency, head down Sternal recumbency, head position unknown	Lying - sternal recumbency	Lying total
Lateral recumbency, head up Lateral recumbency, head down Lateral recumbency, head position unknown*	Lying - lateral recumbency	
Unidentified lying posture, head up* Unidentified lying posture, head down* Unidentified lying posture, head position unknown	Lying - unknown body posture	
Standing Walking	Active total	
Feeding directed behaviours Drinking directed behaviours Self-grooming Grooming others Investigating calf (1st 12 h only) Allowing calf to suckle (1st 12 h only) Other social behaviour Other secondary behaviour*	Secondary total	
Not visible	Not visible	

**Rare behaviours that could not be modelled and were not analysed individually*

Table A4.2: Individual lying behaviours and the combined head position category used for analysis

Individual lying behaviours	Head position category
Sternal recumbency, head up Lateral recumbency, head up Unidentified lying posture, head up*	Total head up
Sternal recumbency, head down Lateral recumbency, head down Unidentified lying posture, head down*	Total head down
Sternal recumbency, head position unknown Lateral recumbency, head position unknown* Unidentified lying posture, head position unknown	Total head unknown

**Rare behaviours that could not be modelled and were not analysed individually*

Positioning of cameras in the parturition and postpartum pens

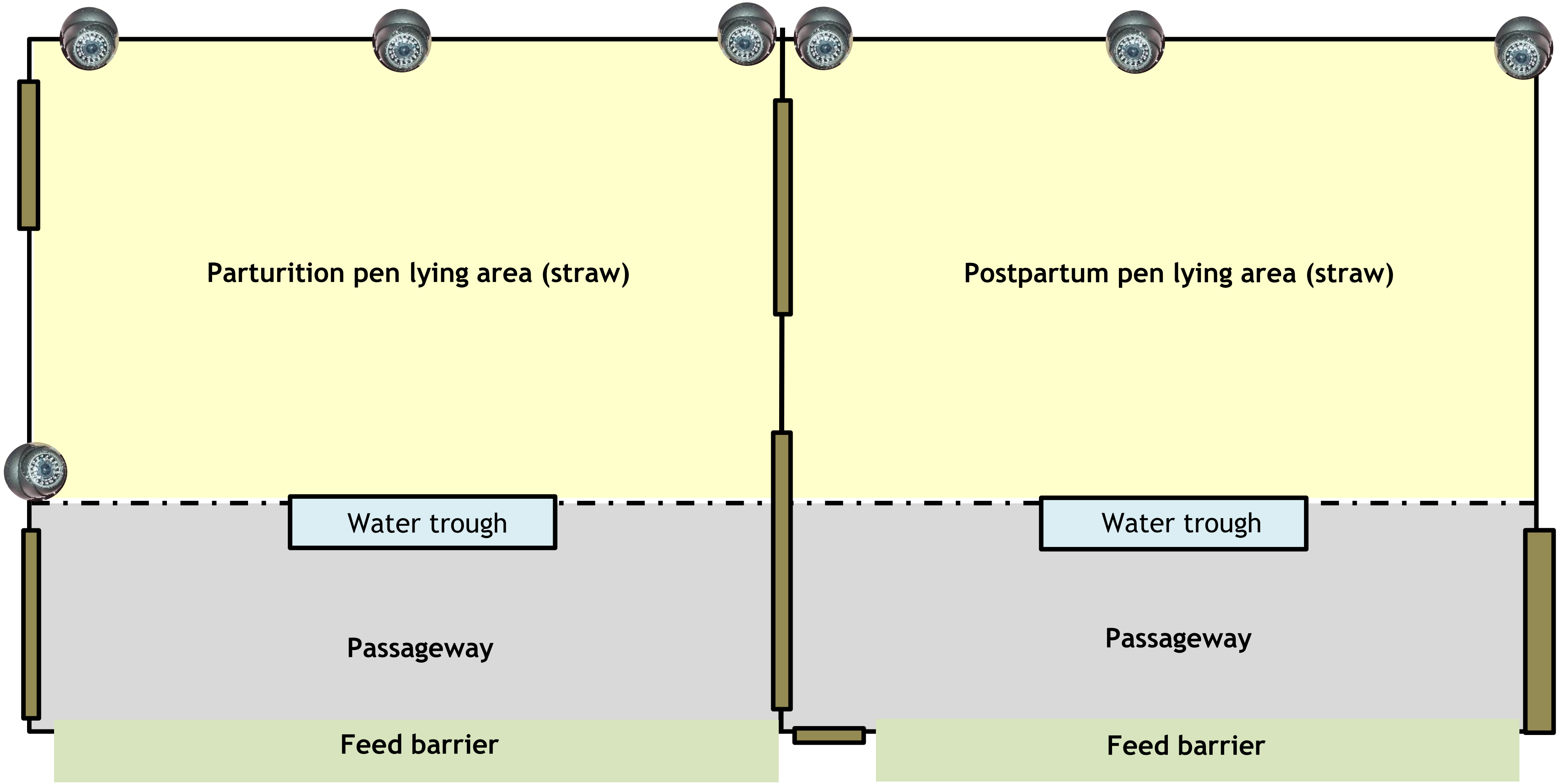


Figure A4.1: Schematic diagram illustrating the layout of the parturition and postpartum pens and camera positioning (not to scale)

● = camera position

▬ = gate position

— · — · — = kerb position

Examples of maternal lying behaviours

Identifying marks (ear tags and freeze brands) obscured in all images to preserve owner anonymity

Figure A4.2: Cow lying in sternal recumbency with head held up in an alert position (NU)
Gladden et al. (2021), reproduced with permission.

Figure A4.3: Cow lying in sternal recumbency with head held down in a rested position (NH)
Gladden et al. (2021), reproduced with permission.

Figure A4.4: Cow lying in lateral recumbency with head held down in a rested position (RH)
Gladden et al. (2021), reproduced with permission.

Appendix 5

Univariate analysis of relationships between parturition/birth related covariate data and treatment status of animals recruited to the study presented in Chapter 5

Table A5.1: Relationships[1] between treatment status and parturition-related variables for cows treated with ketoprofen (*n* = 40) and saline (*n* = 40)

Variable	Test statistic[2]	Degrees of freedom	*p*-value
Parity	0.065	1	0.799
Assistance status	0.061	1	0.805
Season	0.000	1	1.000
Parturition pen	0.027	1	0.870
Body condition score	1686.00	79	0.513

1. Categorical data (parity, assistance status, season, and parturition pen) analysed using Chi-square analysis. Body condition score analysed using Mann-Whitney U test.

2. Pearson Chi-square statistic presented for categorical data (parity, assistance status, season, and parturition pen) and W-value for body condition score.

Table A5.2: Relationships[1] between treatment status and birth-related variables for calves treated with ketoprofen (*n* = 40) and saline (*n* = 40)

Variable	Test statistic[2]	Degrees of freedom	*p*-value
Dam parity	1.818	1	0.178
Season	2.464	1	0.116
Birth pen	0.512	1	0.474
Birthweight (kg)	0.900	77	0.371

1. Categorical data (dam parity, season, and birth pen) analysed using Chi-square analysis. Continuous data (birthweight) analysed using two-sample t-test.

2. Pearson Chi-square statistic presented for categorical data (dam parity, season, and birth pen) and T-value for continuous data (birthweight)

Neonatal urine specific gravity results

Table A5.3: Urine specific gravity of calves treated at birth with ketoprofen (*n* = 18) and saline (*n* = 18)

Treatment group	Age at sampling (d)[1]	Urine specific gravity[2]			*p*-value[3]
		Median	Minimum	Maximum	
Saline	6	1.014	1.007	1.023	0.105
Ketoprofen	6	1.016	1.010	1.030	

1. Mean age (d) presented

2. RI = 1.004 to 1.025 (Divers, 2018)

3. Mann-Whitney U test result

Four node optimal CART tree for predicting parturition assistance using prepartum step count data

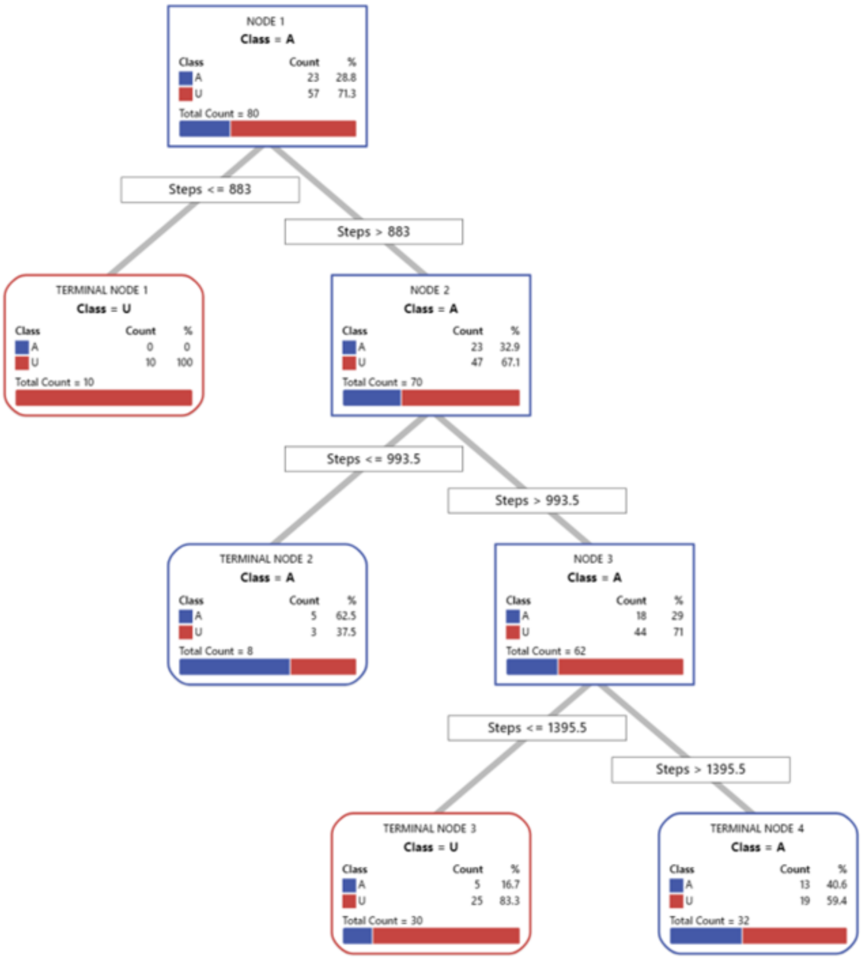


Figure A5.1: Four node Classification and Regression Analysis Tree for predicting assisted parturition based on cumulative 48 h prepartum step count data.
Blue = event (assisted [A]). Red = non-event (unassisted [U]). Optimal tree selected based on minimal misclassification cost.

Prediction of timing of parturition using IceQube generated data

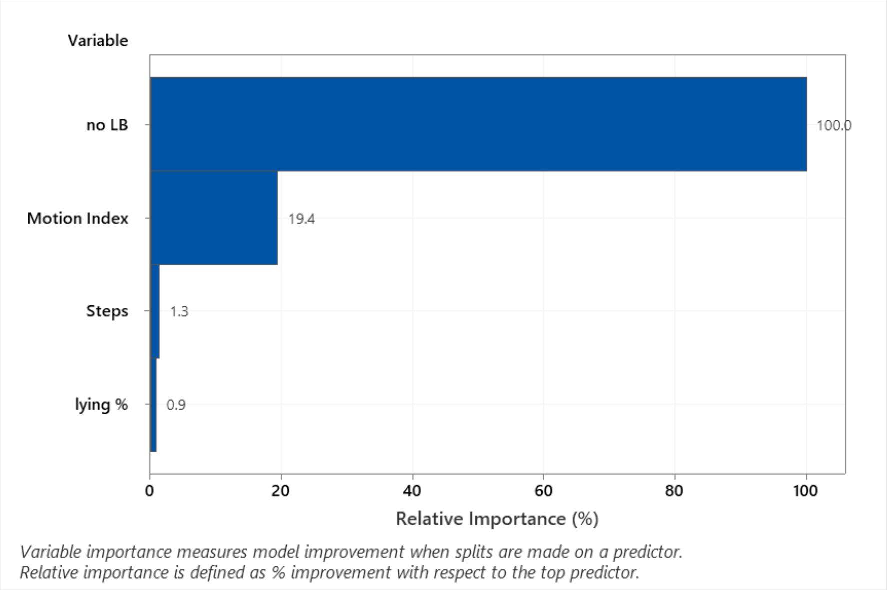


Figure A5.2: Relative importance of each IceQube generated data type for predicting the last 12 h of gestation.
Percentage of time budget engaged in standing not included due to inverse relationship with percentage of time budget engaged in lying. All percentages relative to the most important predictor (LB).

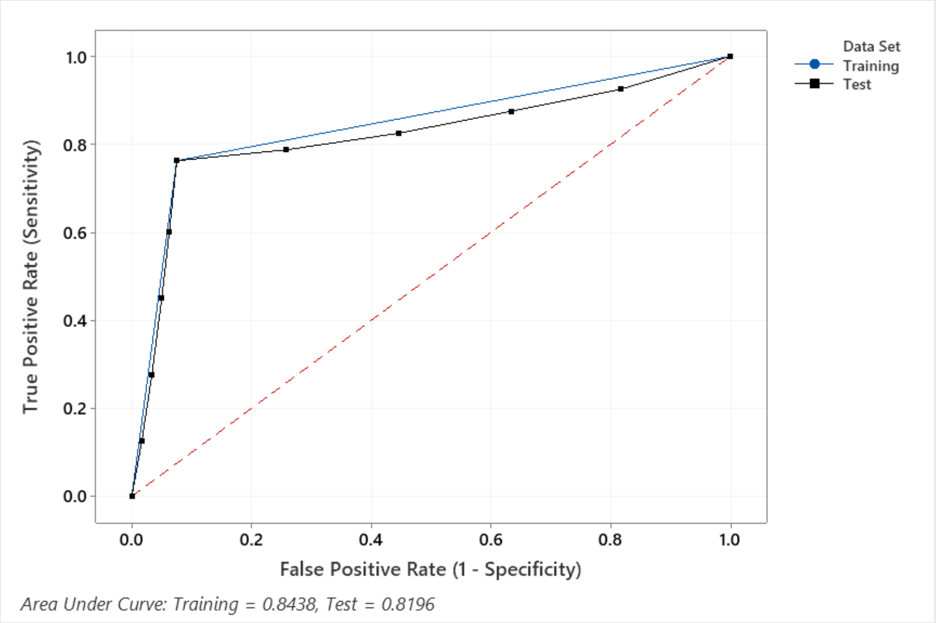


Figure A5.3: Receiver operating characteristic (ROC) curve demonstrating the sensitivity and specificity of LB > 10.5 for detecting the last 12 h of gestation as calculated from a training dataset (blue line) and when applied to a test dataset (black line) using 5-fold cross validation.

Appendix 6

Blood sample processing

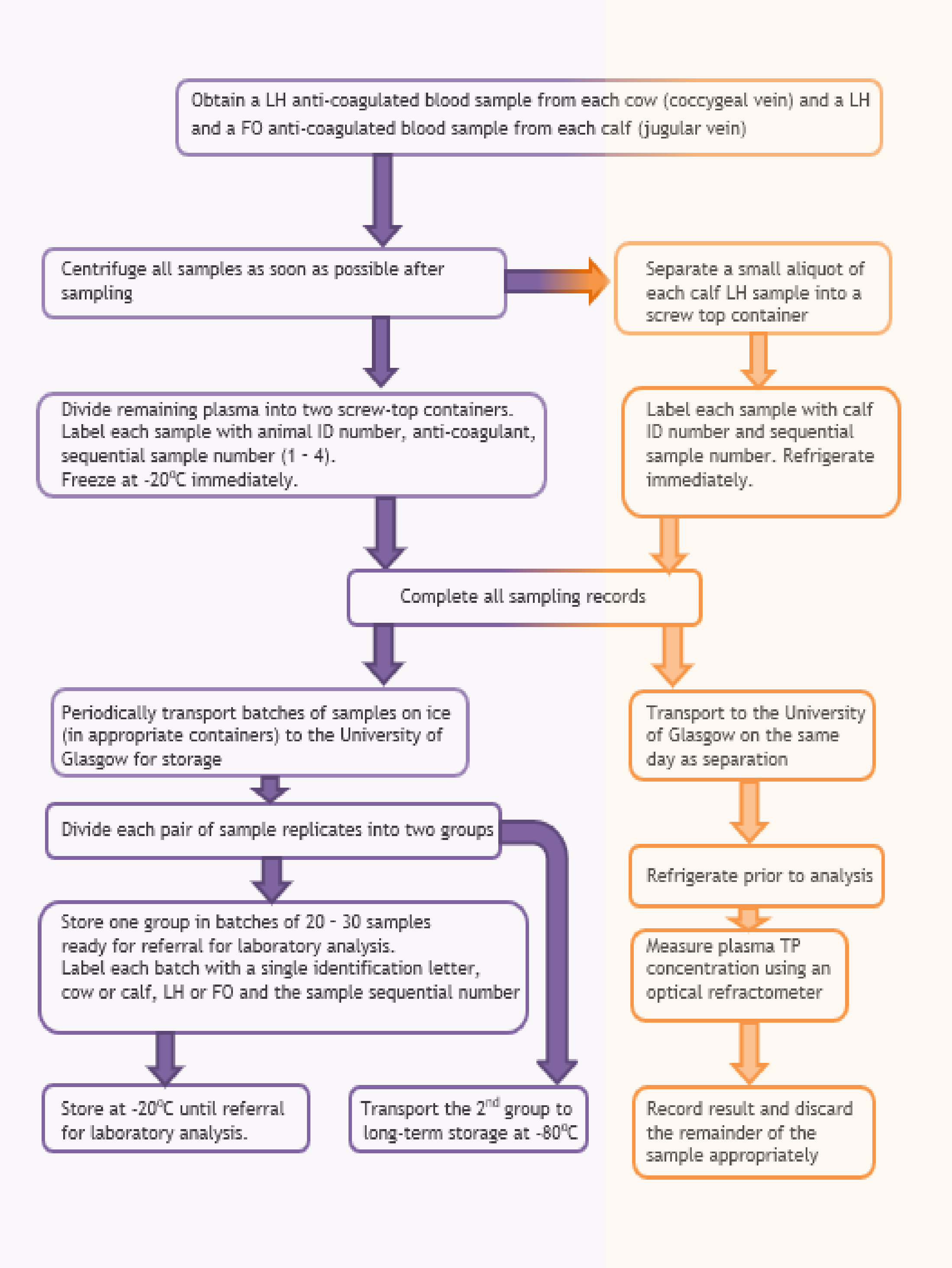


Figure A6.1: Protocol for processing blood samples
Purple indicates processing pathway for analysis of plasma cortisol, creatine kinase and L-lactate concentration. Orange indicates processing pathway for analysis of plasma total protein concentration. Detailed description of individual steps presented in Table A6.1.

Table A6.1: Details of processing and storage protocols for blood samples

Processing step	Blood sample	Timing	Processing protocol
Separation of plasma	All samples	As soon as possible after sampling (samples refrigerated until separation)	Relative Centrifugal Force of 4015 x *g* for 5 min in a benchtop centrifuge[1] kept on farm
Removal of plasma	All samples	Immediately after separation	Plastic Pasteur pipette[2]
Storage of plasma	Cow LH and calf FO samples	Immediately after separation	Divided and stored in 2 mL screw top containers[2]
	Calf LH samples	Immediately after separation	Approximately 0.2 mL separated into a plain 2 mL plastic screw-top container[2]. Remainder divided equally into two similar plastic 2 mL screw-top containers
Storage	Aliquot of calf LH plasma	Immediately after distribution into 2 mL containers[2]	Labelled with animal ID and sample date, and refrigerated until transport to the University of Glasgow for analysis
	All other samples	Immediately after distribution into 2 mL containers[2]	Labelled with the animal ID, the date of sampling, the anti-coagulant used when the sample was obtained and sampling time (0 h, 24 h, 48 h or 7 d). Stored at −20 ºC on farm until transport to the University of Glasgow where half of samples stored at −20 ºC (short-term) and the remaining half stored at −80 ºC (long-term)

LH = lithium heparin. ID = identification number.

1. VWR Collection CompactStar CS 4 laboratory centrifuge, VWR International Ltd., Lutterworth, UK
2. Henry Schein Animal Health, Dumfries, UK

Details of statistical models used for biochemical analyses

Table A6.2: Details of each final statistical model for analysis of maternal biochemical data.
X indicates factors included in final model after backward stepwise elimination if $p > 0.1$. Factors not presented were not included in any final model

	Model summary						
		Fixed effects*					Covariates†
Biochemical analyte	Adjusted R^2	Assistance status	Treatment status	Time	Assistance x time	Treatment x time	Lactation number
Cortisol	55.08%	X	X	X	X		
Creatine kinase	Not available	X	X	X			X

**Categorical predictors in binary logistic models. †Continuous predictors in binary logistic models.*

Table A6.3: Details of each final statistical model for analysis of neonatal biochemical data.
X indicates factors included in final model after backward stepwise elimination if $p > 0.1$. Factors not presented were not included in any final model.

	Model summary							
		Fixed effects					Covariates	
Biochemical analyte	Adjusted R^2	Assistance status	Treatment status	Time	Assistance x time	Treatment x time	Dam Lactation	Calf birthweight
Cortisol	63.58%	X	X	X		X		
Creatine kinase	44.05%	X	X	X			X	
L- lactate	51.69%	X	X	X			X	X
Total protein	86.76%	X	X	X		X		

Descriptive statistics (mean, standard error [SE], standard deviation [SD], minimum [min.] and maximum [max]) for each biochemical analyte (*raw data presented in all tables*).

Table A6.4: Maternal plasma cortisol concentration (nmol/L) at each sampling time point for each assistance x treatment status group

Sample time point	Assistance x treatment status group																			
	Assisted saline					Assisted ketoprofen					Unassisted saline					Unassisted ketoprofen				
	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.
0 h	77.06	8.76	42.92	6.00	177.00	92.20	10.80	51.60	12.00	241.00	58.19	9.68	43.29	16.00	185.00	55.25	5.77	27.07	18.60	140.00
24 h	24.47	3.56	16.30	6.00	63.70	18.40	2.47	11.83	6.00	48.30	16.61	2.67	11.95	6.00	49.70	16.99	2.37	10.88	6.00	45.00
48 h	18.61	2.78	12.76	6.00	61.00	17.09	2.84	13.34	6.00	58.00	21.49	3.38	14.32	6.00	60.70	19.32	3.27	14.97	6.00	61.20
7 d	19.30	4.31	20.67	6.00	77.00	15.83	2.35	11.26	6.00	38.90	22.79	8.34	36.33	6.00	167.00	24.03	5.38	25.24	6.00	93.80

Table A6.5: Maternal plasma creatine kinase concentration (IU/L) at each sampling time point for each assistance x treatment status group

Sample time point	Assistance x treatment status group																			
	Assisted saline					Assisted ketoprofen					Unassisted saline					Unassisted ketoprofen				
	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.
0 h	702.0	320.0	1569.0	72.0	7472.0	288.9	62.0	297.4	98.0	1397.0	217.5	27.7	120.9	106.0	613.0	472.0	193.0	904.0	93.0	4213.0
24 h	506.9	94.4	432.8	92.0	1322.0	593.0	61.3	294.0	204.0	1378.0	204.3	18.9	84.4	104.0	425.0	577.7	61.8	283.3	197.0	1356.0
48 h	1352.0	1116.0	4992.0	91.0	22544.0	342.2	79.9	374.9	110.0	1835.0	717.0	350.0	1526.0	82.0	5991.0	395.3	96.6	442.9	136.0	1774.0
7 d	242.7	52.3	250.7	83.0	959.0	251.2	43.6	209.1	95.0	858.0	192.5	59.7	260.2	73.0	1251.0	506.0	213.0	999.0	58.0	3985.0

Table A6.6: Neonatal plasma cortisol concentration (nmol/L) at each sampling time point for each assistance x treatment status group

Sample time point	Assistance x treatment status group																			
	Assisted saline					Assisted ketoprofen					Unassisted saline					Unassisted ketoprofen				
	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.
0 h	231.6	18.7	93.7	90.2	436.0	212.8	16.9	79.5	62.9	397.0	249.2	43.9	196.5	66.0	963.0	218.8	19.9	97.3	87.0	439.0
24 h	89.8	9.38	43.0	28.1	188.0	102.8	10.4	48.8	39.0	196.0	102.0	9.6	43.1	49.4	222.0	118.3	12.9	61.7	42.2	334.0
48 h	129.9	20.0	93.9	35.0	400.0	94.1	10.6	48.5	36.1	217.0	164.3	44.7	199.7	46.0	744.0	99.5	13.0	62.4	26.2	274.0
7 d	38.9	5.66	27.1	9.0	121.0	45.7	5.76	27.0	11.0	108.0	34.3	5.6	25.1	6.0	85.3	38.7	5.5	26.5	6.0	108.0

Table A6.7: Neonatal plasma creatine kinase concentration (IU/L) at each sampling time point for calves in each assistance x treatment status group

Sample time period	Assistance x treatment status group																			
	Assisted saline					Assisted ketoprofen					Unassisted saline					Unassisted ketoprofen				
	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.
0 h	329.8	58.0	290.0	66.0	1321.0	402.3	82.5	386.8	127.0	1911.0	378.1	59.2	264.7	104.0	1046.0	310.1	46.5	228.0	108.0	972.0
24 h	351.5	69.1	316.8	76.0	1468.0	362.1	57.4	269.3	149.0	1183.0	256.2	32.0	142.9	107.0	732.0	306.3	44.2	211.9	127.0	800.0
48 h	142.8	25.9	121.7	47.0	530.0	109.7	22.2	101.9	41.0	515.0	121.2	12.1	53.9	32.0	236.0	111.0	12.4	58.1	43.0	228.0
7 d	129.8	21.7	106.1	45.0	562.0	142.5	28.0	131.4	46.0	639.0	221.4	99.8	446.2	53.0	2102.0	130.6	28.3	138.4	46.0	720.0

Table A6.8: Neonatal plasma L-lactate concentration (mmol/L) at each sampling time point for calves in each assistance x treatment status group

Sample time point	Assistance x treatment status group																			
	Assisted saline					Assisted ketoprofen					Unassisted saline					Unassisted ketoprofen				
	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.
0 h	4.15	0.37	1.87	1.80	9.80	3.99	0.67	3.14	1.60	16.90	3.91	0.86	3.86	1.40	19.70	3.36	0.27	1.31	1.20	6.70
24 h	2.45	0.22	1.00	1.10	6.20	2.60	0.19	0.88	1.30	4.40	2.67	0.24	1.08	1.00	5.20	2.65	0.25	1.19	1.40	6.30
48 h	2.64	0.42	1.98	1.00	10.30	2.41	0.29	1.32	0.90	7.00	2.77	0.38	1.71	1.20	8.90	2.34	0.21	0.99	0.90	4.30
7 d	1.65	0.16	0.78	0.70	3.30	1.87	0.22	1.05	0.90	5.40	1.74	0.14	0.65	0.70	3.40	1.60	0.15	0.73	0.70	3.00

Table A6.9: Neonatal plasma total protein concentration (g/dL) at each sampling time point for calves in each assistance x treatment status group

Sample time point	Assistance x treatment status group																			
	Assisted saline					Assisted ketoprofen					Unassisted saline					Unassisted ketoprofen				
	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.	Mean	SE	SD	Min.	Max.
0 h	4.76	0.08	0.40	4.20	5.80	4.83	0.08	0.39	4.20	5.80	4.70	0.07	0.32	4.20	5.30	4.87	0.11	0.54	4.00	6.00
24 h	6.33	0.15	0.67	5.20	7.60	6.30	0.14	0.63	4.70	7.40	6.78	0.13	0.56	6.00	8.10	6.40	0.18	0.85	4.60	7.80
48 h	6.84	0.16	0.74	5.40	8.60	6.47	0.15	0.68	4.30	7.40	6.90	0.13	0.57	6.00	8.10	6.50	0.18	0.88	4.60	8.00
7 d	6.14	0.13	0.66	4.80	7.40	6.05	0.09	0.41	5.00	6.60	6.30	0.14	0.61	5.40	7.60	6.09	0.16	0.79	4.40	7.40

Appendix 7

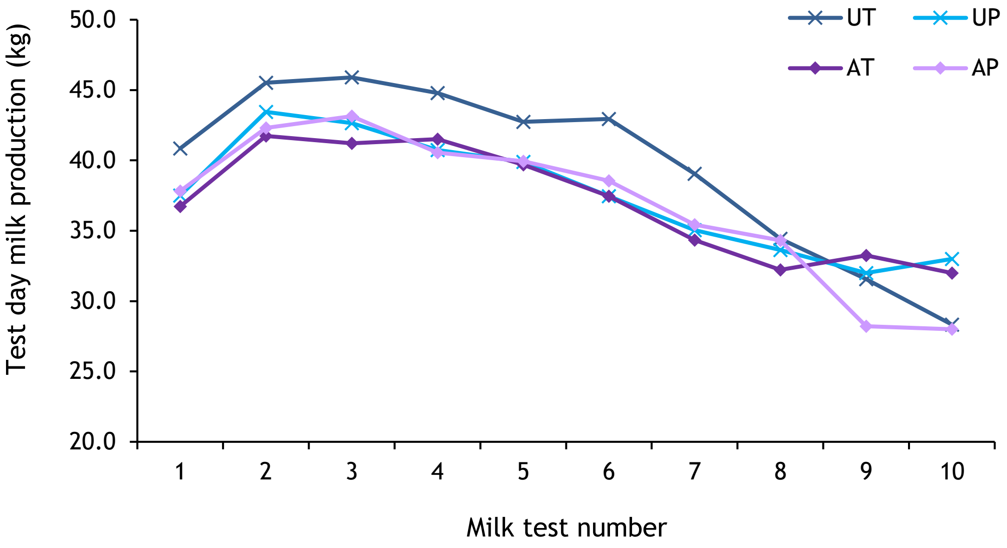


Figure A7.3: Maternal lactation curves for each assistance x treatment interaction group
n = 89. Mean production at each (monthly) test plotted. Lactation curves truncated at the 10th test due to low numbers of animals with lactation longer than this. Raw data presented. UT = unassisted ketoprofen group. UP = unassisted saline group. AT = assisted ketoprofen group. AP = assisted saline group

Table A7.1: Summary statistics of maternal milk production for each assistance x treatment status group

Milk production measure	Assistance x treatment group	Mean	SE	SD	Min.	Max.
Whole lactation milk yield	AP	9825	849.0	3892	1590	16840
	AT	11143	804.0	3683	4170	17120
	UP	12462	691.0	2933	8240	20860
	UT	12030	972.0	4453	2050	20080
Whole lactation fat yield	AP	358.3	32.5	149.0	50.0	681.0
	AT	386.0	29.1	133.3	148.0	667.0
	UP	429.3	29.6	125.5	265.0	698.0
	UT	413.0	34.8	159.3	79.0	615.0
Whole lactation protein yield	AP	305.7	27.6	126.3	51.0	550.0
	AT	343.9	25.3	115.8	136.0	535.0
	UP	395.6	23.1	97.9	265.0	641.0
	UT	376.8	31.3	143.2	60.0	611.0
W4MK	AP	41.24	2.33	10.67	26.0	61.0
	AT	37.82	3.08	14.43	13.0	62.0
	UP	39.22	2.64	11.19	15.0	64.0
	UT	43.76	2.54	11.63	28.0	68.0
305ME	AP	11956	413.0	1894	8850	17060
	AT	11999	449.0	2105	8520	16400
	UP	11926	443.0	1880	8740	16670
	UT	13496	387.0	1773	11070	18080

All variables measured in kg. AP = assisted saline group (n = 21). AT = assisted ketoprofen group (n = 22). UP = unassisted saline group (n = 18). UT = unassisted ketoprofen group (n = 21). SEM = standard error (of the mean). SD = standard deviation. Min. = minimum. Max. = maximum. W4MK = week four milk yield. 305ME = 305 d mature equivalent milk yield. Raw data presented.

Glossary

305 d yield	Total volume of milk produced in the first 305 days of lactation. This metric allows milk yield to be compared between cows of different lactation lengths.
305ME	305 day mature equivalent. A prediction of milk yield standardised to a 305 d lactation and a 3rd lactation cow. The standardisation of this figure allows comparison of milk production between animals of different ages and at different stages in lactation. For cows in the 3rd lactation and over with a lactation length greater than 305 days, 305ME and 305 d yield are equal.
Calving interval	The number of days between the date of parturition and the date of the next parturition.
Calving to conception interval	The number of days between the date of parturition and the date of conception in the subsequent lactation.
Close-up dry cow	A cow in the last three weeks of the dry period.
Colostrum	The first milk produced by a mammal after parturition. Colostrum has a high immunoglobulin content (resulting in a high protein concentration) and also has a higher concentration of fat than non-colostral milk.
Cut-point	The threshold of a clinical test that indicates the presence/absence of disease.
Days in milk	The number of days a lactating cow has been producing milk. For lactating cows, this is equivalent to the number of days since parturition.
Dietary cation anion difference (DCAD)	The ratio of cations and anions in a ration on a dry matter basis; expressed as mEq/kg dry matter.
Dry cows	Cows that are not lactating.
Dry matter	The proportion of a dietary component that remains after all of the water has been removed. Expressed as a percentage and forms the basis of dietary calculations.
Dry matter intake	The amount of food on a dry matter basis that has been consumed. This is the standard measure of the amount of food a cow has eaten: expressed as kg DM.
Dry period	The period in late pregnancy during which a cow is not lactating. Typically 6 to 8 weeks duration.
Failure of passive transfer (FPT)	Inadequate gastrointestinal absorption of colostral immunoglobulins resulting in a calf serum concentration of IgG < 10 g/dL.

Far-off dry cow	A cow in the earlier part of the dry period > 3 weeks away from parturition.
Left Displaced Abomasum	A pathological condition in which the abomasum moves across to the left side of the abdomen and elevates, adopting a position between the rumen and the abdominal body wall.
Mastitis	Inflammation of the mammary gland. Typically (but not always) as a result of bacterial infection.
Metritis	Uterine inflammation occurring within the first 3 weeks postpartum. Metritis can be further defined as either '*puerperal metritis*' or '*clinical metritis*'. See Appendix 2.
Multiparous	Having experienced parturition two or more times.
Neonate	A very young calf less than 28 d old.
Nulliparous	Having not experienced parturition at all.
Passive transfer	Absorption of immunoglobulins (from colostrum) across the gastrointestinal tract membrane into the neonatal bloodstream.
Postpartum disease	Disease occurring in the postpartum period. By convention the definition is usually restricted to diseases related to the transition period. In this thesis postpartum disease is defined as: *any one of (or a combination of) metritis, retained foetal membranes, left displaced abomasum or subclinical ketosis diagnosed up to 8 d after parturition.*
Primiparous	Having experienced parturition once.
Retained foetal membranes	Failure to expel the placenta and associated tissues within 12 h of parturition. See Appendix 2.
Subclinical ketosis	Hyperketonaemia in the absence of clinical abnormalities consistent with ketosis (e.g. neurological and cognitive deficits). In the first 14 d postpartum, this is most commonly defined as serum BHB concentration ≥ 1.2 mmol/L. Outwith the immediate postpartum period, the defined threshold is ≥ 0.6 to 0.8 mmol/L.
Total mixed ration (TMR)	A method of feeding cows where all components of the diet are mixed together and presented as a single (combined) foodstuff.
Transition period	The period encompassing the three weeks before and three weeks after parturition. During this period, the cows are transitioning from late pregnancy (i.e. non-lactating) into lactation.
Voluntary waiting period	The interval after parturition during which breeding is not attempted, even if oestrus is observed. In the studies reported in this thesis this period is defined as < 50DIM.

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