Comparison of three reproductive management strategies for lactating dairy cows using detection of oestrus or synchronisation of ovulation and Fixed-Timed Artificial Insemination

Richard Vazquez, DVM

Submitted in fulfilment of the requirement for the
Degree of Master of Veterinary Medicine

Scottish Centre for Production Animal Health and Food Safety
School of Veterinary Medicine
College of Medical, Veterinary & Life Sciences
University of Glasgow

January 2022

Abstract

The most common dairy cattle reproductive management strategies combine oestrus detection with hormonal protocols for synchronisation of ovulation for breeding. However, approximately 50% of oestrus cycles are missed in commercial dairy farms due to human error and poor expression of oestrus behaviour. Furthermore, around 20% of cows experience prolonged postpartum anovulation. Synchronisation protocols may have variable synchronisation rates, producing suboptimal pregnancies per artificial insemination (P/AI). The aim of this study was to compare the reproductive performance of three commercial reproductive management strategies in lactating dairy cows: a combination of oestrus detection (OD) followed by ovulation synchronisation protocol for fixed timed artificial insemination (FTAI) either using Ovsynch or PRID-synch, or Double Ovsynch at FTAI. Cows (n = 1681) were randomly assigned to one of three different reproductive strategies at calving: Oestrus detection - Ovsynch (OD-Ov), Oestrus detection - PRIDsynch (OD-PR) and Double Ovsynch (DO). Cows enrolled in OD-Ov, and OD-PR were eligible to be inseminated after observed oestrus between 50 and 70±3 days in milk (DIM). Cows in which oestrus was not detected between 50 and 70±3 DIM (OD-Ov, n = 541; OD-PR, n = 562) received their respective hormonal treatments at 70±3 DIM. In these two groups, cows that returned to oestrus within the period for first FTAI (<83DIM) had more than one opportunity for AI. Cows enrolled in DO were subjected to FTAI only. Postpartum disorders were recorded between 1 and 7 DIM; and lameness, mastitis and bovine respiratory disease were recorded until first AI. Body condition score (BCS) was recoded at calving, 43±3 and 70±3 DIM. Ovarian monitoring was performed by transrectal ultrasonography (US) at 43±3 and 50±3 DIM, and at 70±3 and 77±3 DIM only for synchronised cows. Effects of treatments were assessed with multivariable statistical methods relevant for each outcome variable. Pregnancy 32±3 d after first AI was similar among treatment groups (OD-Ov = 43.2%, OD-PR = 41.6%; DO = 45.7%) and proportion of cows pregnant by 83 DIM was also similar among treatment groups (OD-Ov = 46.5%, OD-PR = 46.6%; DO = 45.7%). Farm, parity, BCS at 43±3 DIM and breeding sire were associated with reproductive performance. Pregnancy loss (PL) was significantly higher in the OD-Ov (9.7%) than in the OD-PR (4.1%). In conclusion, no difference in reproductive performance among reproductive strategies was observed in this study, suggesting that reproductive performance is influenced by farm-specific factors such as oestrus detection rate and P/AI, and overall cow health.

Table of Contents

List of Tables

List of Figures

Acknowledgement

Firstly, I would like to thank the farmers that participated in this trial as the completion of this study could not have been possible without their collaboration, expertise and friendliness.

I would like to acknowledge and give my warmest thanks to my supervisor Lorenzo Viora who made this work possible. His guidance and advice carried me through all the stages of my Combined 4-year Residency and Masters Programme in Bovine Health Management. I would also like to give special thanks to my supervisor, Katie Denholm, who helped and supported me to step into the exciting world of statistics, for her brilliant comments and suggestions, and her kindness.

I am extremely grateful to all my colleagues and friends of the Scottish Centre for Production Animal Health & Food Safety. Working with you has been a great experience and a privilege. I am deeply grateful for the occasion I had to join this team.

Many other people cooperated with their expertise to the realisation of this research. I am pleased to remember here Dr. Monika Mihm-Carmichael, Theo Pepler and John Cook for their help.

A heartfelt thank is to my wife and family, which have always been of tremendous and prompt support.

Finally, thanks to Ceva-Sante for funding this project.

Author's Declaration

I declare that this dissertation is the result of my work, except where explicit reference is made to other people's contributions. It has not been submitted for any other degree at the University of Glasgow or other institution.

Name: Richard Vazquez

Signature:

Abbreviations

AAM: Automated activity monitoring

AI: artificial insemination

BHB: β-hydroxybutyrate

BRD: bovine respiratory disease

ICI: inter-calving interval

CI: confidence interval

CL: corpus luteum

CR: conception rate

DA: displaced abomasum

DIM: days in milk

DMI: dry matter intake

DNB: do not breed

DO: Double Ovsynch

DOPN: days open

DPR: daughter pregnancy rate

E2: oestradiol-17β

EIA: enzyme immunoassays

FSH: follicle-stimulating hormone

FTAI: fixed time artificial insemination

G1: first gonadotropin-releasing hormone injection of the breeding protocol

G2: last gonadotropin-releasing hormone injection of the breeding protocol

GnRH: gonadotropin-releasing hormone

IFN: interferon -tau

Kg: kilograms

KPI: key performance indicators

LBF: Liver blood flow

LH: luteinising hormone

min/h/d: minutes/hours/days

MK30: cumulative milk by 30 days after calving

NEB: negative energy balance

NEFAs: non-esterified fatty acids

OD: oestrus detection

OD-Ov: Oestrus detection - Ovsynch

OD-PR: Oestrus detection - PRIDsynch

OR: odds ratio

P4: progesterone

P/AI: pregnancies per artificial insemination

PD: pregnancy diagnosis

PG1: first prostaglandin F2α hormone injection of the breeding protocol

PG2: second prostaglandin F2α hormone injection of the breeding protocol

PGF2α: prostaglandin F2α

PL: pregnancy loss

PR: 21-day pregnancy rate

PRID: progesterone releasing intravaginal device

RIA: radioimmunoassay

RP: retained placenta

SR: submission rate

UK: United Kingdom

US: ultrasound

US$: United States dollars

VWP: voluntary waiting period

W4MK: fourth week yield

Chapter 1 General Introduction and Literature Review

1.1 Introduction

The dairy industry is one of the most important agricultural sectors in the United Kingdom (UK). In 2020, around 15.5 billion litres of milk were produced with an average price of 28.6 pence per litre, with a total income for the UK economy of £4,441 million in current market prices. Dairy industry contributions reached 29% of the total livestock economic output of the UK in 2020 (DEFRA, 2020). Dairy farm profitability is influenced by four main factors: i) economic environment (i.e. milk price, feed costs, fuel costs, etc.); ii) production (i.e. land and labour); iii) management of resources (i.e. facilities, crop production); and iv) productive efficiency (i.e. milk production, reproductive performance, feed conversion, etc.) (March et al., 2017).

Reproductive management has an important influence on farm profitability (Louca & Legates, 1968; Giordano et al., 2011). Interventions to improve fertility have a positive effect on milk production (Pecsok, McGilliard, & Nebel, 1994), reduce the number of cows culled because of failure to conceive (Giordano et al., 2011) and increase the proportion of female replacements born (Giordano et al., 2012a).

In UK dairy farms, it is generally accepted that a 365 to 395-day inter-calving interval (ICI) is necessary to achieve optimal performance and profitability (Esslemont, 2003; Hudson et al., 2012). The cost of a longer ICI is estimated to be around £1.98 and £2.08 for every day ICI increases over 395 and 426 days, respectively (Hudson et al., 2012). Nevertheless, it is essential to achieve as high submission rates (SR) and pregnancies per artificial insemination (P/AI) by 100 days in milk (DIM) as possible. Reduction of the calving to conception interval from 150 days to 100 days has been shown to increase overall herd milk production, reduce the ICI by 63 days, and increase income over feed costs by 7% (Ribeiro et al., 2012). Since 2010, national herd ICI decreased from around 417 to 400 days, with 21% of service intervals greater than 50 days (suggesting approximately 2+ missed oestrus cycles). Twenty-five per cent of these farms detected less than 31% of service returns at the first available oestrus, and the median submission rate in 2020 was 40% (Hudson et al., 2010; Hanks & Kossaibati, 2020). This suggests that oestrus detection (OD) failure is a major limiting factor for reproductive performance in dairy herds. Colazo et al. (2014) reported that oestrus detection failure could be as high as 50% in North American dairy herds, depending on animals, farm staff and environment (Colazo & Mapletoft, 2014). In addition, 20% of cows in which oestrus is not detected are non-cycling animals (Opsomer et al., 2000; Walsh et al., 2007a; Bamber et al., 2009). Farm profitability, therefore, could be improved by reducing the ICI and culling due to poor fertility by increasing SR and P/AI, through minimising human error and stimulating cyclicity in anovular cows (Pursley, Mee, & Wiltbank, 1995).

Nowadays, ovulation synchronisation protocols such as Ovsynch and its modifications are available to maximize 21-d pregnancy rates (PR) by submitting non-pregnant cows to fixed timed AI (FTAI) either not seen in oestrus or even without the need for oestrus detection. Ovsynch protocol manipulates ovarian activity, resulting in similar pregnancies per artificial insemination (P/AI) compared with cows inseminated after detection of natural oestrus. However, this hormonal protocol synchronisation rates may be variable regarding the stage of the oestrus cycle the cows are when starting Ovsynch (Pursley et al., 1995; Vasconcelos et al., 1999). Inclusion of a progesterone releasing intravaginal device (PRID) during the Ovsynch protocol (PRIDsynch) has been shown to increase conception rates, by improving synchronisation rates and embryo survival, particularly in anovular cows (Chebel et al., 2010; Bilby et al., 2013; Bisinotto et al., 2015b). Further modifications to Ovsynch, such as Double Ovsynch (DO), have been designed to improve fertility for FTAI protocols, presynchronise the follicular wave, optimise ovulation and re-establish cyclicity in anovular cows (Souza et al., 2008; Herlihy et al., 2012; Carvalho et al., 2018). Double Ovsynch has also been shown to influence calving to first service interval, resulting in equivalent (Denis-Robichaud et al., 2017; Stangaferro et al., 2018), or increased (Souza et al., 2008; Herlihy et al., 2012; Dirandeh, Roodbari, & Colazo, 2015) P/AI compared with other reproductive strategies combining OD and FTAI, especially in primiparous cows.

1.2 Bovine oestrus cycle

After puberty, the cow starts a period of reproductive cyclicity that continues throughout most of her reproductive life. Dynamic changes in ovarian structures and hormonal environments take place in 21-day period cycles until the cow becomes pregnant. During these cycles, there is follicular growth, a CL formation after ovulation of the dominant follicle, and regression of subordinate follicles.

One of the most physiological challenging points in a heifer's life is puberty, which can be defined as when animals become capable of reproducing themselves (Robinson & Shelton, 1977). In heifers, puberty is triggered when the hypothalamic-pituitary-gonadal axis loses its sensitivity to the negative feedback effects of oestradiol-17β (E2), allowing a surge of luteinising hormone (LH) to occur. The onset of puberty is variable in time and is highly dependent on genetic and environmental factors (i.e. nutrition, management) (Moran, Quirke, & Roche, 1989; Macdonald et al., 2007; Handcock et al., 2021; Meier et al., 2021) For example, it has been reported that in seasonal calving systems around 15% of the heifers fail to reach puberty at the time of breeding (15 months of age), and this delayed onset of puberty is usually explained by poor growth in heifers during the rearing period (Handcock et al., 2021; Meier et al., 2021)

The oestrus cycle begins with standing heat (Day 0) triggered by a high concentration of E2 produced by the preovulatory follicle in a low P4 environment (Figure 1-1). The hypothalamus response to exposure to high concentrations of E2 (for a sufficient time and in the absence of P4) triggers typical signs of oestrus behaviour in the cow, such as standing when mounted, mounting other animals, and increased physical activity. Simultaneously, the hypothalamus secretes a surge of GnRH that will induce the following hormonal secretions by the pituitary gland: i) LH, causing ovulation of the dominant follicle 24 to 32h later, reducing E2 concentration to basal levels; ii) follicle-stimulating hormone (FSH), initiating a new follicular wave emergence (Sartori, Pursley, & Wiltbank, 2017)

After ovulation, evacuation of follicular fluid, granulosa cells, and oocyte occur (Peter et al., 2009). The vascularisation and luteinisation process of the remaining granulosa and theca cells of the former follicle will form a functional CL (Adams & Singh, 2015). While the functional CL grows, circulating P4 concentrations increase until it reaches its maximum diameter (Day 7) and remains elevated until CL regression occurs (Day 16). Any dominant follicle growing under a high P4 environment will fail to ovulate and become atretic, allowing a new surge in FSH, initiating the next follicular wave (Day 10). High P4 environments are necessary to prepare the uterus for an embryo and maintenance of pregnancy. However, there will be maternal recognition only if the conceptus has interferon -tau secretion (IFN) (Day 16). Otherwise, the luteolytic process will start with the pulsatile release of PGF2α from the uterus, and a new oestrus cycle will start 4–6 days after (Adams & Singh, 2015).

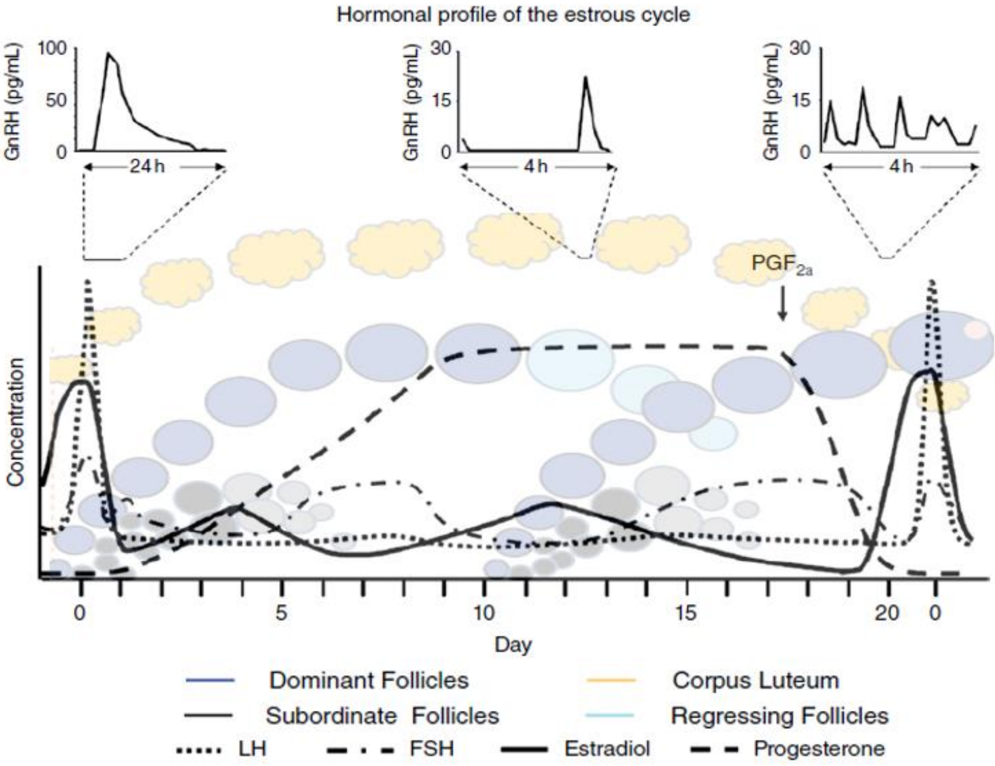


Figure 1-1. Hormonal modulation of the bovine oestrus cycle.

Follicular dynamics during two-wave oestrous cycle and interactions among hormones that contribute to modulate the oestrus cycle at the central level. Day 0 of the oestrus cycle is when oestrus behaviour was observed, followed by ovulation of the dominant follicle and the creation of a corpus luteum and starting the luteal phase. Progesterone concentrations increase and remain elevated until day 18, when prostaglandin F2α (PGF2α) is released by the uterus, causing luteolysis. After a sharp decrease in progesterone concentrations, there is a marked increase of oestradiol concentrations produced by the dominant follicle triggers oestrus behaviour again. The preovulatory surge of gonadotropin-releasing hormone (GnRH) occurs under low progesterone and high oestradiol environment (graph on top left). High progesterone concentrations during the luteal phase inhibits the frequency of pulsatile release of GnRH (graph on top, in middle), resulting in pulsatile release of luteinising hormone (LH). During the follicular phase, the absence of progesterone leads to increased frequency of GnRH (graph on top right) and LH pulses, which enhances follicular maturation and ovarian steroidogenesis. Initiation and progression of follicular waves are associated to fluctuations in circulating concentrations of follicle-stimulating hormone (FSH) and oestradiol. Figure adapted from Adams & Singh and Amstalden & Williams (Adams & Singh, 2015; Amstalden & Williams, 2015).

1.3 Oestrus detection

After AI technology was introduced in the agricultural sector, submitting cows to AI after oestrus behaviour became the most common reproductive management strategy in cattle; and despite the development of hormonal treatments for oestrus cycle manipulation, it is still preferable on many farms to AI cows after oestrus detection (Ferguson & Skidmore, 2013).

1.3.1 Oestrus behaviour

Expression of oestrus behaviour is triggered by elevated concentrations of E2 secreted by the preovulatory follicle in a low P4 environment (Adams & Singh, 2015). Traditionally, oestrus detection is performed by visual observation of primary and secondary behavioural signs of oestrus (Van Vliet & Van Eerdenburg, 1996):

1.3.1.1 Primary signs:

a. Standing to be mounted is the most characteristic behavioural sign for determining when a cow is in oestrus and considered sexually receptive for AI. Ovulation often occurs 30±5 hours after the onset of standing behaviour and can be used as a predictor to determine the time of ovulation (J. B. Roelofs et al., 2005).
b. Mounting behaviour often starts approximately 9.6 hours before the onset of standing oestrus and continues until 18.4 hours after the end of this primary sign (Yoshida & Nakao, 2005), with a peak of mounting events between 6 and 3 hours after standing oestrus(Sveberg et al., 2013).

1.3.1.2 Secondary signs

c. Chin-resting/chin-rubbing, sniffing/licking another cow's anogenital region (vulva), and orientation have been associated with oestrus behaviour (Van Vliet & Van Eerdenburg, 1996). However, these signs can also be considered as part of the animal's social behaviour. In a study performed by Roelofs et al. (2005), it was reported that 87% and 46% of the animals displayed sniffing and chin resting when not in oestrus, respectively.

d. Vaginal discharges are less reliable but may be more than an indication when observed often in one observation period or in successive periods.

1.3.2 Oestrus detection

Van Vliet and Van Eerdenburg (1996) developed a scoring system for oestrus detection consisting of attributing a score to the different primary and secondary behavioural signs of oestrus, recommending a total threshold score of 50 points for diagnosis of oestrus, see (Table 1-1). This scoring system standardised oestrus detection. To achieve the threshold of 50 points at least one primary sign of oestrus must be identified. When implementing the scoring system on farms for two observation periods of 30 min daily, Van Vliet and Van Eerdenburg (1996) reported SR of over 70% with 100% accuracy and identified after feeding and before and after milking as the optimum times for oestrus detection. In addition, in another study performed by the same authors, where oestrus detection was performed every 2 hours, 12 times a day for a duration of 30 min, they found that the cows had the highest oestrus expression 2 hours after milking and the lowest score during the milking process (Van Eerdenburg, Loeffler, & van Vliet, 1996). Furthermore, an association between the number of points and time of ovulation has been reported, where cows with higher oestrus detection scores ovulated sooner than cows with lower scores (65.6% vs 34.4%) (Van Eerdenburg et al., 2002).

Table 1-1. Scoring system for oestrus detection (Van Vliet & Van Eerdenburg, 1996).

Signs of oestrus	Score
Mounting signs (primary)	
Mounted by another cow but not standing	10
Mounting (or attempting to mount) other cows	35
Mounting head end of another cow	45
Standing to be mounted	100
Other signs (secondary)	
Mucous vaginal discharge	3
Cajoling	3
Restlessness	5
Sniffing the vulvas of other cows	10
Chin-resting on other cows	15

Oestrus detection limitations

Although scoring systems for oestrus detection have shown to be accurate; as herd size increases, visual observation of individual cows becomes complicated as this task demands more time, more highly trained staff and consistency combined with a good environment for the cow to display oestrus (Lucy, 2001; Colazo & Mapletoft, 2014; Reith & Hoy, 2018). Overall, approximately 50% of oestrus cycles are missed in commercial dairy farms (Van Vliet & Van Eerdenburg, 1996; Colazo & Mapletoft, 2014). Thus, a large proportion of cows are not submitted to AI, resulting in suboptimal PR. It is estimated that 90% of reasons for low submission rates are attributable to management and environment, whereas 10% are attributable to cow-specific factors (Diskin & Sreenan, 2000).

In pastured-based Dairy systems, high SR and P/AI are critical to achieving a compact calving season, and therefore, better production output. Generally, OD expression is better when grazing, as they interact freely and the surface underfoot is not hard and slippery, so females in oestrus can express sexual behaviour (Palmer et al., 2010). However, similarly to cattle housed all year round, lack of appropriate staff training in recognising cow's oestrus signs and reduced frequency and timing of observations may result in poor reproductive performance in pasture-based systems. In addition, difficulties in recognising the animals in oestrus due to poor identification, poor lighting in housing, herd size and accessibility and size of the paddocks may have an additional negative impact on SR (Van Vliet & Van Eerdenburg, 1996; Hudson et al., 2012; Roche et al., 2017).

Other environmental factors that have been identified as affecting SR due to poor oestrus expression are (Diskin & Sreenan, 2000; López-Gatius et al., 2005; Hudson et al., 2012):

- insufficient loafing area
- poor housing layout
- overstocking (bedding area, water provision and feed space)
- slippery floor surface
- poor cattle movement (excessive pressure)
- narrow passageways for moving cows to and from milking
- heat stress due to poor ventilation

The main cow-specific factors limiting oestrus detection are oestrus intensity, length, and anovulation. A decrease in the duration of oestrus based on standing mounts have been reported from 8 to 9 h to <6 h in cows producing over 40 kg of milk per day (At-Taras & Spahr, 2001; Lopez, Satter, & Wiltbank, 2004). Therefore, daily milk production has a clear effect on oestrus duration.

Good recording systems are key to improving the accuracy of oestrus detection. Reproductive data entry and analysis should be easy to perform and include at least i) animal number; ii) calving date; iii) pre-breeding heat dates; iv) first and repeat service dates and sire used on each date and inseminator code; v) date and the result of pregnancy diagnosis and, (vi) date of expected calving (Diskin & Sreenan, 2000).

The use of tail paint or chalk to identify cows in oestrus is a cheap, effective, and simple method which can be used in conjunction with or to aid good heat observation. However, these are not substitutes for time spent observing cows, and animals need to be checked at least once a day to monitor changes in the paint after the animal has been mounted (Rivera, Lopez, & Fricke, 2004). Adhesive devices placed at the head of the tail have also been developed to increase the reliability of 'paint based' tools (i.e., Kamar®) (Hudson et al., 2012), but these also rely on user competence (for accurate placement) and good observation.

Increased physical activity and mounting have been associated with oestrus behaviour, facilitating automated monitoring (Giordano & Fricke, 2017). There are several automated activity monitoring (AAM) systems available on the market and most of them are designed to be placed on the cows ankle or neck; however, some devices have been designed to be placed on the rump and ears of the animals (Giordano & Fricke, 2017). The main parameters recorded by AAM for oestrus identification are the number of steps (pedometers), neck movements (accelerometers) and duration of cow activity. In addition, some sensors continuously measure health parameters such as temperature, lying time, lying bouts, feeding time and rumination, since these physiological measurements change during oestrus (Dolecheck et al., 2015). It has been reported that between 51% and 87% of oestrus events may be accurately detected using pedometers, and when more than one animal formed a 'sexually active group', detection percentages increased to 95% (Judith B. Roelofs et al., 2005). A similar study reported 90% of oestrus events were detected by accelerometers, with a false positive rate of 17% (Aungier et al., 2015). AAM may thus be deemed a reliable technology to improve oestrus detection; however, the accuracy of all these sensors may be reduced by the same managerial, environmental, and cow-specific factors affecting oestrus detection and expression in visual oestrus detection.

1.4 Postpartum oestrus cycle alterations

1.4.1 Hormonal metabolism in high producing dairy cows

Changes in circulating hormones concentrations can affect the oestrus cycle physiology and animal behaviour. Although cows with higher milk production have larger preovulatory follicles and CL, they have lower circulating E2 and P4 concentrations and are more likely to have double ovulations (Lopez, Satter, & Wiltbank, 2004; Lopez et al., 2005).

Elevated feed intakes in lactating dairy cows have been shown to result in higher liver blood flow (LBF), increasing steroid metabolism. In one particular study, Sangsritavong et al. (2002) found that the LBF was higher in lactating cows (1,561 ±47 L/h) when compared with nonlactating cows (747 ±57 L/h) of similar size and age. In both groups, LBF was increased immediately after feeding; however, E2 and P4 metabolism were greater in lactating than nonlactating cows (Sangsritavong et al., 2002). Therefore, in high producing dairy cows, high LBF and consequently increased steroid hormone metabolism could cause alterations in follicular dynamics (i.e., larger follicles, multiple ovulations) and changes in the length and intensity of the cow oestrous behaviour (Figure 1-2) (Lopez, Satter, & Wiltbank, 2004; Lopez et al., 2005; Wiltbank et al., 2014).

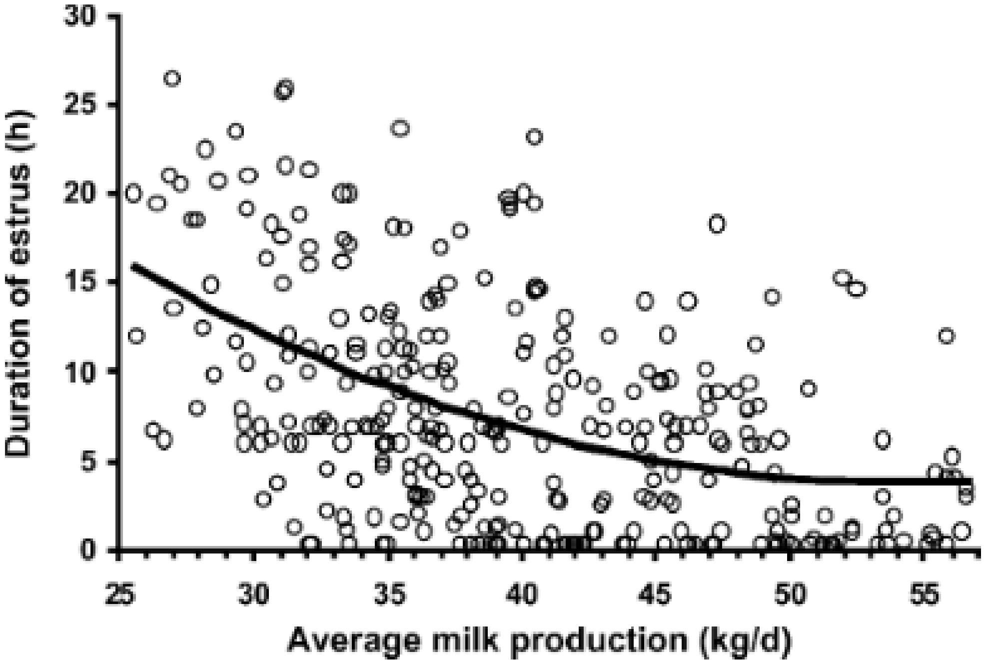


Figure 1-2. Relationship between level of milk production and duration of oestrus.

Correlation between duration of oestrus (h) and the average milk production (Kg/d) during the 10 days preceding the day of oestrus, determined by standing activity recorded by the radiotelemetry system. Figure adapted from Lopez et al. (2004)

1.4.2 Prolonged postpartum anovulation

In the majority of the cows, the first postpartum ovulation occurs between 27 - 33 DIM (Ferguson, 1996; Darwash, Lamming, & Wooliamns, 1997; McCoy et al., 2006). The prevalence of postpartum anovulation in dairy cows is variable and has been reported to be as high as 60, 40, and 30% at 21, 49, and 60 DIM, respectively (Beam & Butler, 1997; Walsh et al., 2007a; Galvão et al., 2010).

The prevalence of prolonged postpartum anovulation in dairy herds has been identified as one of the major detrimental influences on dairy cattle fertility regardless of the first service reproductive strategy (i.e., at detected oestrus or FTAI) (Gümen, Guenther, & Wiltbank, 2003; Santos, Rutigliano, & Filho, 2009). Failure of cows to ovulate reduces the opportunity for these animals to be inseminated and decreases P/AI in the wider dairy herd. In most studies where anovulation was evaluated, primiparous cows have been reported to have a higher prevalence of anovulation than multiparous cows (Opsomer et al., 2000; Gümen, Guenther, & Wiltbank, 2003; Souza et al., 2008; Santos, Rutigliano, & Filho, 2009; Monteiro et al., 2020); however, other studies have reported a higher prevalence of anovulation in multiparous cows (Herlihy et al., 2012), or no association between parity and anovulation (Lopez et al., 2005).

Primiparous cows may have a higher prevalence of anovulation because of their higher energy requirements for growth and milk production. In these animals, a small change in body condition score (BCS) could still indicate extensive mobilisation of reserves, and consequent delayed first postpartum ovulation (Monteiro et al., 2020). Furthermore, it has been reported that primiparous cows may have a lower feed intake capacity than multiparous cows, resulting in a delay in re-establishing energy balance post calving. (Remond et al., 1991). It has been hypothesised that hypothalamic sensitivity to signals from the splanchnic tissues to re-establish normal GnRH and LH pulsatility differs in cows that are growing compared with fully grown animals under negative energy balance (NEB) (Monteiro et al., 2020).

The main risk factors for anovulation reported in the literature are diseases such as retained placenta (RP), metritis, hypocalcaemia, mastitis and NEB (Ribeiro et al., 2013; Monteiro et al., 2020), farm environment (Santos, Rutigliano, & Filho, 2009), and low BCS (Lopez, Satter, & Wiltbank, 2004).

Anovular cows have been classified into four categories based on the ovarian structures phenotype and circulating E2 concentrations (Table 1-3) (Wiltbank, Gümen, & Sartori, 2002):

1. Anovulation with follicle growth only to emergence is a rare condition reported in Zebu (*Bos indicus*), where cattle are exposed to extreme postpartum malnutrition (Ruiz-Cortés & Olivera-Angel, 1999). It is suspected that this condition may result from a relative deficiency in FSH (Wiltbank, Gümen, & Sartori, 2002).
2. Anovulation with preovulatory size follicles (4-14 mm) has been reported to be common in cows with low BCS (Gümen, Guenther, & Wiltbank, 2003). In this type of anovulation, the dominant follicle produces small amounts of E2, inhibiting the GnRH pulse centre in the hypothalamus. Therefore, GnRH pulses are inhibited, leading to inadequate numbers LH pulses, limiting follicular growth (Wiltbank, Gümen, & Sartori, 2002).
3. The most common anovular phenotype are follicles of ovulatory size or larger (15 to 25 mm) (Table 1-3; Figure 1-3), but not big enough to be considered follicular cysts (Gümen, Guenther, & Wiltbank, 2003; Monteiro et al., 2020). In this group of animals, there is a high concentration of E2 produced by the follicles but failure to induce a GnRH surge; therefore, there is no LH surge, and no ovulation occurs. These cows may or not show signs of oestrus (Wiltbank, Gümen, & Sartori, 2002).
4. Finally, follicular cysts are caused by similar pathways to the previous anovular phenotype. However, in this category, the follicles are ≥25 mm diameter and in the absence of a CL. This phenotype is reported between 10 to 20% in anovular cows (Kesler & Garverick, 1982; Gümen, Guenther, & Wiltbank, 2003; Colazo et al., 2015; Monteiro et al., 2020).

Table 1-2. Incidence of type of anovulation in US dairy farms

Follicle diamter[1]	Number[2]	Percentage
4-8 mm	1	0.7
9-14 mm	27	19.3
15-25 mm	85	60.7
>25 mm	27	19.3
Total	140	100

[1]Follicle diameter was evaluated 3 to 6 times (55 to 95 DIM) using weekly transrectal ultrasonography of the ovaries

[2]Based in 24% anovular cows (140/583 dairy cows examined) in two studies (Wiltbank, Gümen, & Sartori, 2002; Gümen, Guenther, & Wiltbank, 2003)

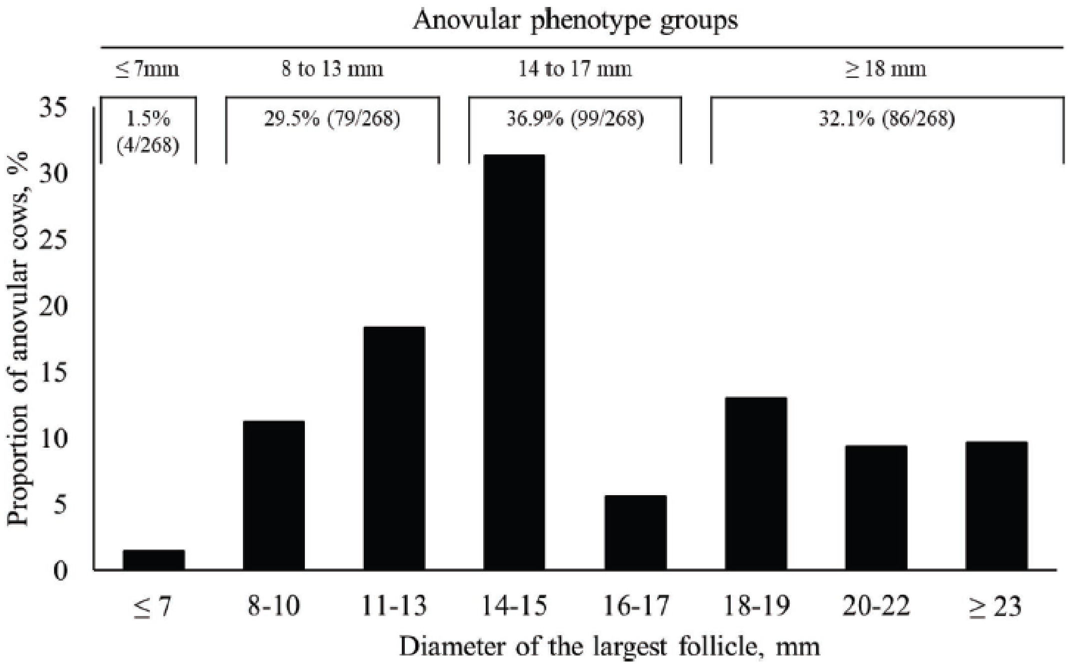


Figure 1-3. Distribution of anovular phenotype groups

Distribution of diameter of the largest follicle on the ovary from cows with anovular condition (n = 268) from a total of 942 lactating dairy cows evaluated in a randomised controlled trial (28.5% prevalence of anovulation) (Monteiro et al., 2020).

1.4.3 Ovarian dysfunction after first ovulation

Ovarian dysfunction after first ovulation has been reported in different scenarios: i) after a short oestrus cycle (premature CL regression); ii) due to delayed CL regression (persistent CL); iii) and due to prolonged inter-luteal intervals after ovulation (Sartori, Pursley, & Wiltbank, 2017).

Premature secretion of PGF2α from the uterus can occur during the postpartum period when the mechanisms responsible for CL maintenance are impaired, resulting in short oestrus cycles. The CL regresses after becoming responsive to PGF2α around day 7 of the oestrus cycle, and the cow shows oestrus behaviour 3 to 4 d later (day 10 – 11 of the oestrus cycle) (Sartori, Pursley, & Wiltbank, 2017). Persistent CL is usually associated with uterine diseases; therefore, it is more commonly observed in cows starting ovarian activity <25 d postpartum (before the uterine disease is resolved) when compared with cows that first ovulated between 25 and 45 d after parturition (Ball & McEwan, 1998). Uterine infection (UI), lack of uterine involution or other uterine abnormalities could be responsible for alterations to uterine secretions and compartmental transport of PGF2α from the uterus to the ovary through the utero-ovarian plexus at the time of luteolysis, failing to cause CL regression (Sartori, Pursley, & Wiltbank, 2017).

1.4.4 Ovarian dysfunction after first AI

Identifying non-pregnant cows and submitting them to new insemination as soon as possible is crucial for high-producing dairy cows' reproductive performance. Non-pregnant cows should return to standing oestrus within 20–25 days after AI. Non-pregnant cows that do not return to oestrus can only be identified when pregnancy diagnosis is performed, and those that are assumed to be pregnant but are actually anovular are commonly known as "phantom cows" (Fricke, 2002; Cuttance & Mason, 2015; Jaśkowski et al., 2019). Performing early pregnancy diagnosis by US combined with resynchronisation protocols improves PR by increasing SR (Fricke, 2002). The main reason identified for cows not returning to expected oestrus are early and late embryonic losses (<42 days pregnancy) (McDougall, Rhodes, & Verkerk, 2005; Bowyer-Smyth, Malmo, & Macmillan, 2008). Embryo survival may be reduced in cows receiving AI early after parturition (higher prevalence of intra-uterine infection), in periods of NEB (Cuttance & Mason, 2015) and when suffering diseases such as mastitis (McDougall, Rhodes, & Verkerk, 2005). Furthermore, in cases of embryonic death, the embryo tissue debris may cause alterations in the uterine environment affecting the normal luteolytic mechanism, contributing to the persistence of the corpus luteum, delaying the submission of the cow for further AI (Cavalieri et al., 2003). Much like prolonged postpartum anovulation, the risk factors for "phantom cows" seems to be linked to farm-specific conditions (Cuttance & Mason, 2015).

1.4.5 Identifying the anovular cow

Two different methods can be used to identify cows with prolonged postpartum anovulation: i) sequential ultrasound (US) exams; ii) circulating P4 measurements in serum and milk (Wiltbank, Gümen, & Sartori, 2002). For proper identification of anovular cows on both methods, at least two evaluations 7 to 14 days apart are needed. A summary of the prevalence of anovulation, evaluation method and DIM when the evaluation is performed (i.e., US or circulating P4 measurement) adapted from Bamber (2009), is shown in Table 1-3.

Radioimmunoassay (RIA) in serum has been defined as the reference test (‘gold standard’) for measuring P4 concentrations in cattle. Cows with serum P4≥1.0 ng/mL in at least 1 of 2 samples were classified as ovular, whereas cows with serum P4<1.0 ng/mL in both samples were classified as anovular (Silva, Sterry, & Fricke, 2007; Bicalho et al., 2008). However, due to the obvious practical limitations of blood sampling every cow (to identify anovular cows) in a commercial farm environment, other methods such as transrectal US and measuring P4 concentrations in milk have been developed.

Since the introduction of the US technologies in dairy cattle reproduction, it is possible to identify anovular cows with this practical and not as invasive technique as blood sampling. When using the US method, a cow is considered anovular if there is no CL present on any ovaries in 2 US examinations (Wiltbank, Gümen, & Sartori, 2002). Previous studies have reported a 0.66 statistical agreement (kappa) between the serum P4 RIA and US methods to identify anovular cows (Silva, Sterry, & Fricke, 2007), with sensitivity (Se) and specificity (Sp) over 80% (Table 1-4).

Progesterone concentrations in milk and serum have also been successful in identifying the presence of a CL in ovular and pregnant cows. Milk P4 enzyme immunoassays (EIA) has been found to be highly correlated with serum P4 RIA ($r = 0.9$), and with high sensitivity and specificity detecting ovular cows (Table 1-5) (Chang & Estergreen, 1983). There are different kits for measuring P4 concentrations in milk available commercially that use different thresholds, but for the one mentioned in this review, a threshold of P4<4 ng/ml milk concentration was used to identify anovular cows (Chang & Estergreen, 1983).

Table 1-3. Prevalence of anovulation, evaluation method and DIM of evaluation

Number of farms[1]	Cows (no.)	Overall anovulation %	Method[2]	DIM of evaluation	Reference
20	1,682	10.9	Progesterone	0 to 100 (3 times/week)	(Lamming & Darwash, 1998)
6	334	21.5	Progesterone	15, 22, 29, 36, 42 and 49	(Opsomer et al., 2000)
3	705	16.2	Progesterone	1 (47 to 64)	(Cartmill et al., 2001)
1	316	20	Ultrasound	50 and 57	(Gümen, Guenther, & Wiltbank, 2003)
1[3]	1,682	18.1	Progesterone	51 and 65 41 and 56 51 and 63 44 and 58	(Cerri et al., 2004) (Galvão et al., 2004) (Santos et al., 2004a) (Santos et al., 2004b)
1[3]	811	25.6	Progesterone	51 and 65 41 and 56 51 and 63	(Cerri et al., 2004) (Galvão et al., 2004) (Juchem et al., 2010)
1	232	7.3	Progesterone	37 and 51	(Bruno et al., 2009)
1	976	41.7	Progesterone	49	(Chebel et al., 2006)
1	234	24.8	Ultrasound	53 and 65	(Sterry, Welle, & Fricke, 2006)
1	466	30	Progesterone	53 and 65	(Silva, Sterry, & Fricke, 2007)
18	1,341	19.5	Progesterone	46 and 60	(Walsh et al., 2007a)
6	2,178	17	Progesterone	21, 35, 49 and 63	(Dubuc et al., 2012)
2	849	27	Progesterone	35 and 49	(Denis-Robichaud et al., 2017)
1	463	28.3	Progesterone	26 and 33	(Stangaferro et al., 2018)
1	942	29	Ultrasound	35 and 49	(Monteiro et al., 2020)

[1]Number of farms included in the study

[2]Milk or serum progesterone

[3]Different studies within the same herd measured anovulation at different times.

Table 1-4. Sensitivity and specificity for ultrasound and milk P4 EIA for detecting anovular cows

Method	Se (%)	Sp (%)	Reference
Ultrasound[1]	85.7	87.7	(Silva, Sterry, & Fricke, 2007).
Milk P4	80.5	96.6	(Chang & Estergreen, 1983)

[1]Based in 1 US scanning

[2]Based in 2 consecutive milk samples

1.5 Measuring reproductive performance in dairy herds

Standard parameters for measuring reproductive performance are ‘days to first service’, ‘days to conception’, ICI, ‘services per conception’, ‘100-day in-calf rate’, CR, SR, and PR (Smith, Oultram, & Dobson, 2014). It is challenging to find an international consensus on how to evaluate reproductive performance on dairy farms since there are discrepancies in terminology and definitions of the fertility key performance indicators (KPI) between countries; however, PR has been accepted as one of the best indicators of fertility. Knowing the fertility performance after close monitoring, identifying problems, and providing a prompt solution to them as soon as possible is important to enhance the farm profitability.
Pregnancy rate provides a close to date view of the farm fertility performance; however, it can only be updated knowing the outcome of the AI after pregnancy diagnosis by transrectal US (~4 weeks after service) (Ginther, 1998).

Pregnancy rate is defined as the proportion of cows pregnant of the total number of cows eligible to be served (i.e., a cow that has finished the voluntary waiting period (VWP), not pregnant and not classified as not to be bred by the farmer) in a 3-week period. Therefore, PR is a combined measure of SR and CR, which are calculated considering changes in the herd dynamics, such as cows that died, were culled, or were classified as not to be bred during the 3-week period under evaluation. It is important to realise that high PR does not necessarily mean higher economic benefits, and it should be interpreted with care. Although it may be assumed that greater PR would place most cows in the more productive part of the lactation curves and result in greater feed efficiency (Ferguson & Galligan, 1999); actual income depends on the shape and persistency of lactation curves (Cabrera, 2014). Therefore, optimum PR is strongly linked to farm economic conditions, such as milk price, milk yield, feed and fuel costs, cull cow value, calf value, replacement heifer cost, reproductive management cost, culling rate, transition cow cost, among other economic drivers (Figure 1-4).

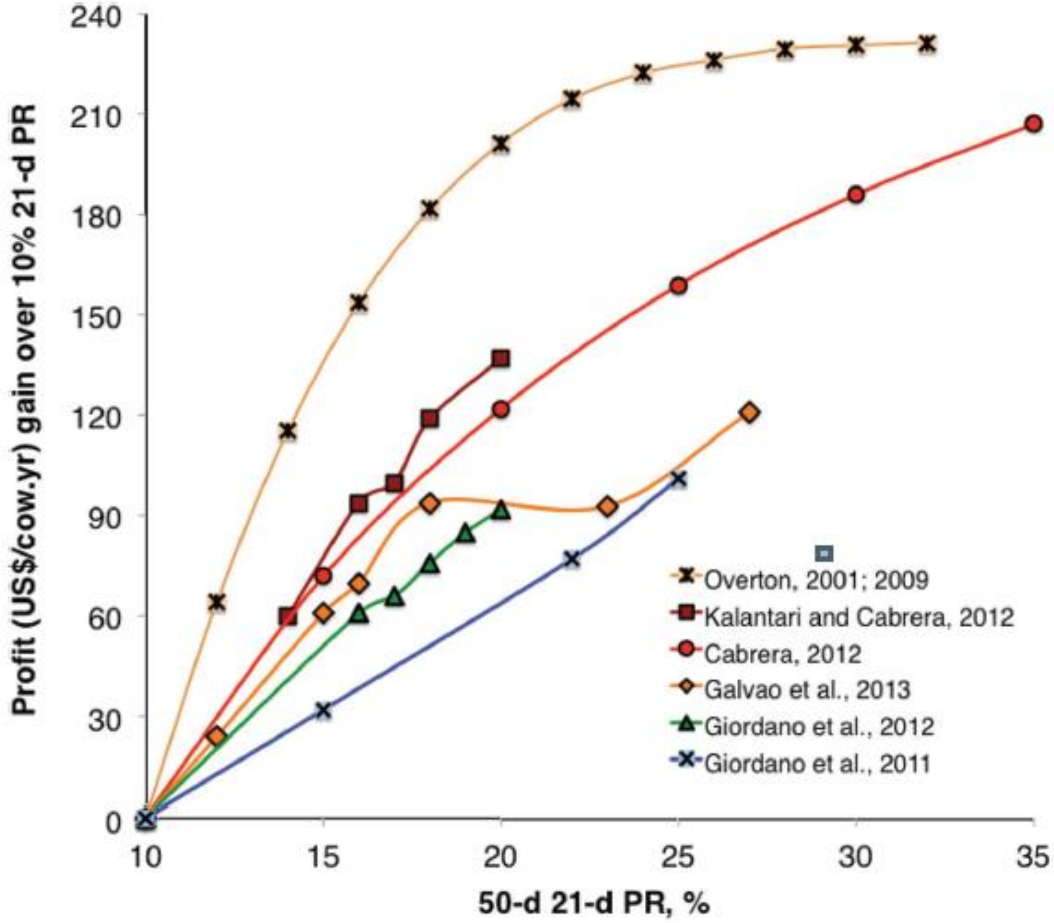


Figure 1-4. Profit gain of improving pregnancy rate in dairy cows

The profit gain in United States dollars (US$) per cow per year of improving pregnancy rate (PR) at 50-day voluntary waiting period (VWP), reproductive efficiency reported by different studies. Adapted from Overton and Cabrera (Overton & Cabrera, 2017).

1.6 Dairy cattle reproductive strategies

1.6.1 Manipulation of the oestrus cycle

Improved understanding of reproductive tract structure and endocrinological functionality of the oestrus cycle, in conjunction with the production of synthetic versions of GnRH, PGF2α and P4, has resulted in the unique opportunity to manipulate follicular surge – development – ovulation, and lifespan of the CL. It is now possible to either shorten or extend the length of the oestrus cycle to AI the cows at a prearranged time. The relatively low heritability of fertility traits (<10%) (VanRaden & Cole, 2014), and the continuous decline in the efficiency of oestrus detection (Colazo & Mapletoft, 2014) have played an important role in the increased use of synchronisation protocols for FTAI, resulting in rapid increases in fertility performance in high-producing dairy cows.

1.6.2 Double prostaglandin injection

Early attempts at oestrus synchronisation were performed with prostaglandin-based breeding protocols, using the reduction of the lifespan of the CL, after an injection of PGF2α, as a predictor of ovulation in cattle (Thatcher & Chenault, 1976). It has been reported that administration of PGF2α to cows results in a decrease in serum progesterone (Louis, Hafs, & Seguin, 1973) and return to oestrus approximately 3 days after PGF2α administration (Rowson, Tervit, & Brand, 1972). This treatment is not unilaterally successful and will not work in the presence of newly formed CL that has not developed PGF2α receptors to undergo luteolysis (day 1 to 4 of the oestrus cycle) (Cooper, 1974; Thatcher & Chenault, 1976), in the presence of an old CL that is already regressing (> day 16 of the oestrus cycle) and in the absence of a CL. Thus, it was suggested to administer an additional PGF2α 11-12 days after the first dose to increase the proportion of animals with a responsive CL at the second injection, improving synchronisation rates (Inskeep, 1973; Lauderdale, 1975). Despite these improvements, this synchronisation protocol was not designed to restart cyclicity in anovular cows and submission to AI was still highly dependent on excellent OD. It has been reported that CR after FTAI in PGF2α-based synchronisation protocols is reduced compared with animals receiving AI at detected oestrus due to variations in the time of ovulation after

PGF2α administration, failing to achieve the synchronisation of ovulation with the FTAI (Macmillan, Day, & Smith, 1980; Heuwieser et al., 1997).

1.6.3 Addition of other hormones to double Prostaglandin injections

Further attempts at synchronising oestrus were focused on controlling the CL lifespan and the follicular wave, including combinations of hormones such as GnRH, PGF2α, E2 and P4. For instance, Lucy et al. (1986) evaluated the reproductive performance of dairy cows subjected to three treatments using timed insemination after PGF2α and GnRH: cows receiving AI after standing oestrus; cows synchronised with 2 injections of PGF2α 11 days apart and receiving AI 80 h after second PGF2α injection; and cows synchronised with 2 injections of PGF2α 11 d apart, GnRH 72 h later and AI 8 h later. These methods for FTAI resulted in low CR, failing to provide evidence for the utility of oestrous synchronisation for improving reproductive performance, and attributing poor reproductive performance to reduced P4 concentrations in serum during the luteal phase after GnRH treatment (indicating poor synchronisation rates) (Lucy, Stevenson, & Call, 1986). However, this poor synchronisation rate using GnRH may have been expected, as in early synchronisation of ovulation experiments it was believed that GnRH could be used to control the CL lifespan (Milvae, Murphy, & Hansel, 1984).

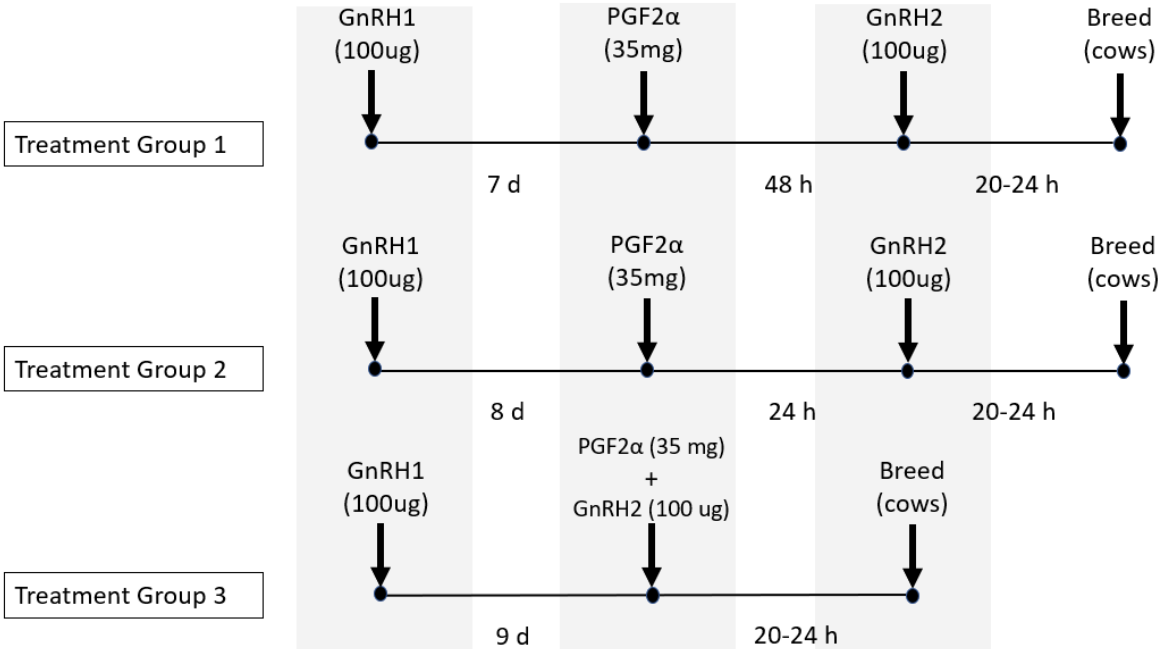
MacMillan and Thatcher (1991) further investigated the effect of GnRH on ovarian structures by conducting a series of trials to investigate the effects of treatment with GnRH (by injection on Days 11, 12, or 13 of the oestrous cycle) on the diameter and number of ovarian follicles, measured by transrectal ultrasonography. Administration of GnRH on day 12 of the oestrus cycle caused ovulation of the dominant follicle and altered the normal wave pattern of the follicle development (Macmillan & Thatcher, 1991), piquing interest in the potential use of GnRH to control follicular waves and facilitating the development of the Ovsynch protocol by Pursley et al. (1995).

1.6.4 Ovsynch protocol

Ovsynch was designed to synchronise ovulation and mitigate dependence on oestrus detection for AI (natural or PGF2α - induced) (Pursley, Mee, & Wiltbank, 1995). The first synchronisation regime tested in dairy cows and heifers, comparing different timings and combining GnRH and PGF2α injections in common use today was performed by Pursley (1995). In this study, animals (n=66) were allocated to 3 different treatment groups:1) GnRH1 - 7 d PGF2α - 48 h GnRH2 - 20 to 24 h AI; 2) GnRH1 - 8 d PGF2α - 24 h GnRH2 - 20 to 24 h AI; 3) GnRH1 - 9 d PGF2α + GnRH2 - 20 to 24 h AI (Figure 1-5). To ensure the same age follicle at breeding, Group 2 and 3 animals were treated with PGF2 α 8 and 9 d after GnRH1 injection with the GnRH2 injection given 24 and 0 h from PGF2α, respectively. The authors reported greater CR in treatment groups 1 and 2 when compared with treatment group 3, (55 and 46 % vs 11%, respectively), establishing a 7-d period between the first GnRH injection and the PGF2α injection; and 48 h period between PGF2α and the last GnRH injection as the optimum timing for synchronisation of ovulation in lactating dairy cows (Pursley, Mee, & Wiltbank, 1995). In addition, a study was performed to determine the optimal time for FTAI following the Ovsynch protocol, concluding that greater P/AI are obtained when FTAI is performed 16 h (45%) after the second GnRH injection compared to 8 (41%) or 24 h (41%) (Richard Pursley, Silcox, & Wiltbank, 1998).

Two years after the development of the Ovsynch protocol, it was evaluated in commercial farm conditions, including a larger number of animals (n = 333). Cows inseminated after standing oestrus and Ovsynch resulted in similar pregnancies per artificial insemination (39 vs 37%, respectively). However, animals in the Ovsynch group had a higher number of cows pregnant by 100 DIM and shorter calving to first AI interval (Pursley, Kosorok, & Wiltbank, 1997). These results have been corroborated by several other studies, where cows that received AI after Ovsynch failed to achieve significantly better CR than cows receiving AI after standing oestrus in most of the reports were overall lower by around 5 percentage points. In the studies summarised in Table 1-6 (Rabiee, Lean, & Stevenson, 2005), the overall mean CR (± SD) of the cows served at OD and Ovsynch in some studies was 36.3±13.3 and 31.2±10.4%, respectively. Failure to synchronise the cows when starting the Ovsynch protocol at random stages of the oestrus cycle was identified as responsible for the reduced performance of Ovsynch compared with AI to detected oestrus. Cows failing to ovulate to the first GnRH injection (Pursley et al., 1995; Vasconcelos et al., 1999) and premature CL regression with spontaneous oestrus before the Ovsynch protocol is completed have been identified as the main causes for reduced CR observed (Moreira et al., 2000). Further work identified that initiation of Ovsynch between day 5 and 12 of the oestrous cycle optimised synchronisation and conception rates in lactating dairy cows (Vasconcelos et al., 1999; Moreira et al., 2001; Cartmill et al., 2001;El-Zarkouny et al., 2004)

The economic performance of Ovsynch relative to PGF2α-based protocols (at 11-day intervals) is highly dependent on individual farm oestrous detection efficiency. Herds with low oestrous detection efficiency will achieve the greatest return from the 100% SR in Ovsynch (Pursley, Kosorok, & Wiltbank, 1997) and will struggle to get good returns from PGF2α-based protocols. Farmers also often find it simpler to schedule reproductive treatments and AI than perform multiple daily oestrous detection sessions, especially in large dairy herds (Lucy, 2001).



GnRH1: Ovulation of the present follicle and recruiting a new follicular wave
PGF2α: Corpora lutea regression
GnRH2: Ovulation of the present follicle

Figure 1-5. Ovsynch protocol variations tested by Pursley (1995)

Schematic representation of the timing and hypothetical action for each of the injections used to synchronise ovulation in 66 lactating dairy cows to determine the flexibility in the timing of the second injection of gonadotropin-releasing hormone (GnRH2) with respect to the prostaglandin F2α (PGF2α) injection during the development of the Ovsynch. Treatment group 1: GnRH1 - 7 d PGF2α - 48 h GnRH2 - 20 to 24 h AI. Treatment group 2: GnRH1 - 8 d PGF2α - 24 h GnRH2 - 20 to 24 h AI. Treatment group 3: GnRH1 - 9 d PGF2α + GnRH2 - 20 to 24 h AI (Pursley, Mee, & Wiltbank, 1995).

Table 1-5. Comparison of conception rate Ovsynch vs standing oestrus

Trial design	Cows (no.)	Treatment groups[1]		Submission method[2]		Conception rate		Reference
		Control	Ovsynch	Control	Ovsynch	Control	Ovsynch	
Cows and heifers	130	2×PGF2α (14 d apart)	G-P-G 0-7-9	AI at detected oestrus	FTAI 18-19 h later	47.1	35.3	(Stevenson et al., 1996)
Cows and heifers	333	Spontaneous oestrus (visual monitoring and occasional use of PGF2α and GnRH	G-P-G 0-7-9	AI at detected oestrus	FTAI 16-20 h later	39.2	37.1	(Pursley, Kosorok, & Wiltbank, 1997)
Cows	310	3×PGF2α (14 d apart)	G-P-G 0-7-9	AI at detected oestrus and FTAI after 3rd PGF2α (72-80 h)	FTAI 16-20 h later	38.9	37.8	(Pursley et al., 1997)
Heifers	155	3×PGF2α (14 d apart)	G-P-G 0-7-9	AI at detected oestrus and FTAI after 3rd PGF2α (72-80 h)	FTAI 16-20 h later	74.4	35.1	
Cows	304	1×PGF2α	G-P-G 0-7-9	AI at detected oestrus	FTAI 16 h later	22.9	13.2	(De la Sota et al., 1998)
Cows Experiment 1	353	Spontaneous oestrus (visual monitoring and use of K-mar)	G-P-G 0-7-9	AI at detected oestrus or a day after	FTAI 15-25 h later	12.5	13.6	
Cows Experiment 2	70	Spontaneous oestrus (visual monitoring and use of K-mar)	G-P-G 0-7-9	AI at detected oestrus or a day after	FTAI 15-25 h later	8.6	11.4	(Aréchiga et al., 1998)
Cows Experiment 3	192	Spontaneous oestrus (visual monitoring and use of K-mar)	G-P-G 0-7-9	AI at detected oestrus or a day after	FFTAI 15-25 h later	16	14.9	
Cows Herd A	216	2×PGF2α (14 d apart) Spontaneous oestrus (visual monitoring)	G-P-G 0-7-9	AI at detected oestrus	FTAI 16-20 h later	15.9	13.3	(Keister et al., 1999)
Cows Herd B	145	2×PGF2α (14 d apart) Spontaneous oestrus (visual monitoring)	G-P-G 0-7-9	AI at detected oestrus	FTAI 16-20 h later	28.2	12.2	
Cows	169	2×PGF2α (14 d apart)	G-P-G 0-7-9	AI at detected oestrus	FTAI 16-20 h later	52.2	35.6	(Stevenson, Kobayashi, & Thompson, 1999)
Cows	440	Spontaneous oestrus (visual monitoring)	G-P-G 0-7-9	AI at detected oestrus	FTAI 12-20 h later	40	50	(Mawhinney, Biggadike, & Drew. B., 1999)
Cows Experiment 1	180	1 or 2×PGF2α (13 d apart)	G-P-G 0-7-9	AI at detected oestrus after 1st PGF2α and FTAI after 2nd PGF2α (72-96 h)	FTAI 20-24 h later	32.5	36.1	(Mialot et al., 1999)
Cows Experiment 2	168	1 or 2×PGF2α (13 d apart)	G-P-G 0-7-9	AI at detected oestrus after 1st and 2nd PGF2α (72-96 h)	At oestrus on day 0 or FTAI 20-24 h later	53.3	53.7	
Cows	840	2×PGF2α (14 d apart)	G-P-G 0-7-9	AI at detected oestrus	FTAI 16-20 h later	56.5	38.1	(Jemmeson, 2000)
Cows	450	2×PGF2α (14 d apart)	G-P-G 0-7-9	AI at detected oestrus	FTAI 16-18 h later	45.6	30.1	(Jobst et al., 2000)
Cows	288	2×PGF2α (14 d apart)	G-P-G-hCG 0-7-9-14	AI at detected oestrus	FTAI 22 h later	40.3	36.2	(Tallam et al., 2001)
Cows	466	G-P 0-7	G-P-G 0-7-9	AI at detected oestrus	FTAI 16-19 h later	34	33.7	(Cartmill et al., 2001)
Cows	90	2×PGF2α (14 d apart)	G-P-G 0-7-9	AI at detected oestrus after 2nd PGF2α (72-96 h)	FTAI 16-20 h later	16.7	36.7	(Alnimer et al., 2002)
Cows	228	Tail paint	G-P-G 0-7-9	AI basted on removed tail-paint	FTAI 12-18 h later	47.3	43.1	(Cordoba & Fricke, 2001)
Cows	683	2×PGF2α (11 d apart)	G-P-G 0-7-9	AI at detected oestrus	FTAI 17-21 h later	41.1	37.6	(Lean et al., 2003)
Overall (SD)[3]	6,210					36.3 (±13.3)	31.2 (±10.4)	

[1]GnRH: gonadotropin-releasing hormone
[1]PGF2α: prostaglandin F2α
[1]Treatments: G-P-G = GnRH–PGF2*α*–GnRH; and 0–7–9 = days the injections were given
[2]FTAI = Timed artificial insemination
[3]SD: Standard deviation

1.6.5 Improving synchronisation and conception rates to Ovsynch

1.6.5.1 Progesterone supplementation during Ovsynch - PRIDsynch

Subluteal P4 (Serum P4<1.0 ng/mL) concentrations during follicular growth have been recognised as a determining factor for fertility in high-producing dairy cows submitted to AI after a synchronisation protocol (Wiltbank et al., 2014). Cows in prolonged postpartum anovulation, cycling cows lacking a CL at the initiation of the synchronisation protocol, and high-producing dairy cows with high steroids metabolism will have subluteal concentrations of circulating P4 at the initiation of the synchronisation protocol (Sangsritavong et al., 2002; Sartori et al., 2004; Stevenson et al., 2008; Bisinotto, Chebel, & Santos, 2010). These animals may benefit from the inclusion of progesterone releasing intravaginal device (PRID) between the first injection of GnRH and the PGF2α injection of the Ovsynch protocol (Figure 1-6) (Chebel et al., 2010; Bilby et al., 2013; Bisinotto et al., 2015).

A meta-analysis performed by Bisinotto et al. (2015), including 8,285 P4 supplemented Ovsynch treated cows and 8,398 untreated controls (25 randomised controlled studies), showed an increase (~4,6 %) in P/AI at 32 d and 60 d post AI. High P4 levels during follicular growth have been associated with better oocyte quality, subsequent fertilisation and embryo development (Cerri et al., 2011; Rivera et al., 2011). Cows with low P4 before AI were also found to have premature uterine PGF2α secretion, which could be associated with a reduction in fertility (Cerri et al., 2011).

It has been shown that hypothalamic unresponsiveness of anovular dairy cows is effectively restored by a 7-day P4 supplementation (Gümen & Wiltbank, 2005). Progesterone supplementation decreased basal LH and LH pulse frequency resulting in regression of dominant follicles and initiation of a new follicular wave (Calder et al., 1999; Gümen & Wiltbank, 2005). After removal of the PRID negative feedback effects on the pituitary and hypothalamus, a GnRH/LH surge re-establishes cyclicity (Lucy, McDougall, & Nation, 2004; Gümen & Wiltbank, 2005). Moreover, PRIDsynch improves synchronisation rates by preventing premature ovulation before prearranged FTAI (Stevenson et al., 2006; Colazo et al., 2013).

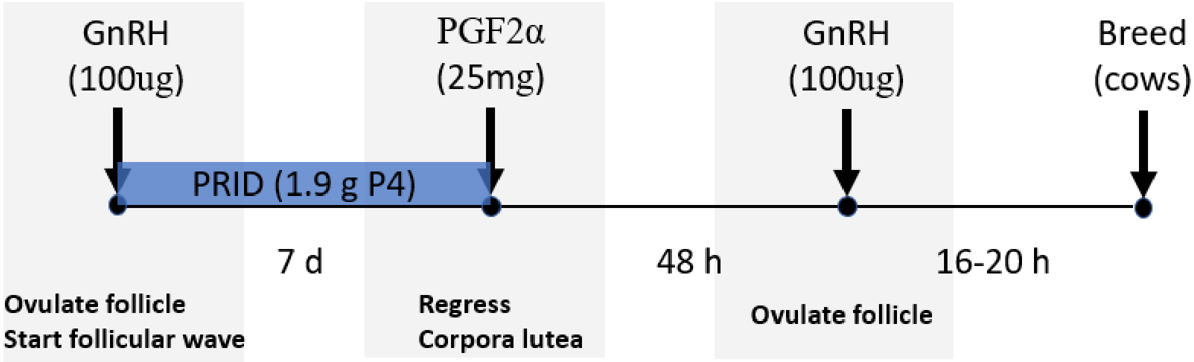


Figure 1-6. PRIDsynch protocol

Schematic representation of the timing and hypothetical action for each of the injections used to synchronise ovulation with PRIDsynch in 313 Holstein lactating dairy cows to determine the effect of progesterone supplementation during Ovsynch in fertility (GnRH + PRID - 7 d PGF2α + PRID removal - 48 h GnRH - 16 to 20 h AI), in an experiment performed by Stevenson (2006)

1.6.5.2 Presynchronisation with PGF2α before Ovsynch - *Presynch-Ovsynch*

Follicular wave synchronisation followed by timed AI is more efficacious when cows are between days 5 and 12 of the oestrous cycle (Vasconcelos et al., 1999; Moreira et al., 2001; Cartmill et al., 2001;El-Zarkouny et al., 2004). A presynchronisation strategy in which cows are treated with 2 injections of PGF2α 14 days apart before the Ovsynch protocol can be employed to start Ovsynch within this optimum response period (Figure 1-7) (Thatcher et al., 2002). However, presynchronisation with PGF2α is not effective in anovular cows.

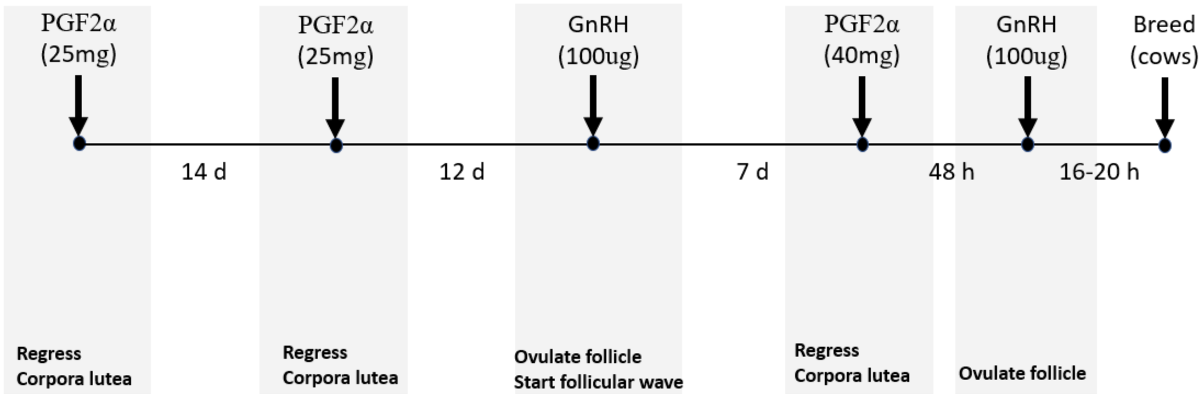


Figure 1-7. Presynch-Ovsynch protocol

Schematic representation of the timing and hypothetical action for each of the injections used to synchronise ovulation with Presynch-Ovsynch in 269 Holstein lactating dairy cows to determine the effect of presynchronisation with 2 doses of prostaglandin F2α (PGF2α) 14 days apart before starting Ovsynch 12 after in fertility (PGF2α - 14 d PGF2α - 12 d GnRH - 7 d PGF2α - 48 h GnRH - 16 to 20 h AI), in an experiment performed by Moreira (2001)

1.6.5.3 Presynchronisation with PGF2α and GnRH before Ovsynch - Double Ovsynch

Another presynchronisation strategy is the Double Ovsynch (DO) protocol, which consists of 2 consecutive Ovsynch protocols 7 days apart, commonly known as presychronisation Ovsynch and breeding Ovsynch (Figure 1-8) (Souza et al., 2008). Souza et al. (2008) reported that DO resulted in higher P/AI when compared to Presynch-Ovsynch protocol in primiparous (65,2% s 45,2%; P = 0.02) but not multiparous cows (37.5% vs 39.3%; P = 0.58). These results were corroborated by more recent studies (Herlihy et al., 2012; Borchardt et al., 2017). The observed improved fertility was associated with increased probability of a dominant follicle ovulating following the third GnRH injection and elevated circulating progesterone concentrations prior to PGF2α administration (Wiltbank et al., 2012). Double Ovsynch has also been shown to effectively induce ovulation in anovular cows, increasing the proportion of cows starting the second Ovsynch in the presence of a CL, so restoring the fertility of anovular cows (Ribeiro et al., 2011; Wiltbank & Pursley, 2014). This benefit of the DO protocol could partially explain the positive association between this protocol and primiparous cows, as anovulation has been reported to be more common in primiparous than multiparous cows (29.6% and 19.1%, respectively) (Bamber et al., 2009). However, these data should not be generalised as other studies have reported a higher prevalence of anovulation in multiparous cows (Herlihy et al., 2012) or no difference by parity (Lopez et al., 2005). Furthermore, when using DO protocol as the reproductive strategy for first postpartum FTAI, the VWP is usually longer than in reproductive strategies allowing AI after detected oestrus. Therefore, the animals will have more time to recover after parturition, resolving anovulation, improving BCS and uterine health at the moment of AI, especially primiparous cows (Stangaferro et al., 2018).

In herds with good OD, the benefits conferred by the DO protocol have shown to be insufficient to generate enough P/AI at first service to economically compensate the delay of the first postpartum AI. Cows with shorter VWP will have earlier opportunities for re-insemination and, consequently, a greater proportion of cows will become pregnant in a shorter time period (Denis-Robichaud et al., 2017; Stangaferro et al., 2018; Stangaferro, Wijma, & Giordano, 2019).

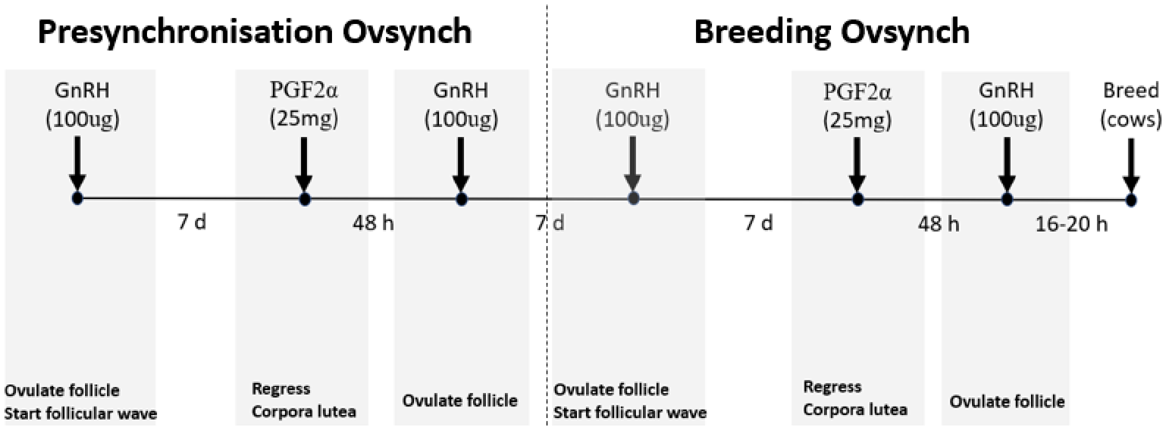


Figure 1-8. Double-Ovsynch protocol

Schematic representation of the timing and hypothetical action for each of the injections used to synchronise ovulation with Double Ovsynch (DO) in 157 Holstein lactating dairy cows to determine the effect of using an Ovsynch for presynchronisation 7 days before the breeding Ovsynch in fertility (GnRH - 7 d PGF2α - 48 h GnRH - 7 d GnRH - 7 d PGF2α - 48 h GnRH - 16 to 20 h AI), in an experiment performed by Souza (2008)

1.6.5.4 Effect of an additional PGF2α treatment during the Ovsynch

Incomplete luteal regression after treatment with a single dose of PGF2α during an Ovsynch protocol has been reported to decrease fertility to FTAI. Lack of complete regression of the CL to a single PGF2α treatment has been observed in 12 to 21% of cows submitted to FTAI after Ovsynch treatment (Brusveen, Souza, & Wiltbank, 2009; Carvalho et al., 2015; Wiltbank et al., 2015; Heidari et al., 2017; Barletta et al., 2018).
Wiltbank et al. (2015) reported an increase of CL regression from 83% with 1 PGF2α to 97% with 2 PGF2α within Ovsynch-type protocols. Moreover, they also observed that administering 2 PGF2α increased 4.6% and 23% the proportion of pregnant primiparous and multiparous cows, respectively; and an overall tendency for improvement of P/AI of 3.2% in multiparous cows (1 PGF2α = 34.4 vs 2 PGF2α = 37.6%). This work concluded that a second PGF2α treatment in Ovsynch-type protocols may be beneficial to enhance fertility, particularly in multiparous cows (Wiltbank et al., 2015). More recent studies provided further evidence that an additional treatment of PGF2α given on a subsequent day after the first PGF2α enhanced fertility, increasing in 4.6% to 5.6% additional P/AI (Borchardt et al., 2018, 2021); however, no interaction treatment by parity was found (Borchardt et al., 2021).

One of the biggest drawbacks of administering an extra PGF2α to cows is the requirement for extra labour needed for animal handling and the additional costs of the hormones. However, a stochastic simulation performed by Borchardt et al. (2021), including studies performed in the US and Europe, has shown that an additional treatment with PGF2α given on a subsequent day after the first PGF2α was more profitable in 95% of the scenarios simulated because of the associated increase in fertility, particularly in multiparous cows (Borchardt et al., 2021).

1.6.6 Adverse reactions and side effects of hormonal treatments

Reproductive drugs are chemical agents that may produce adverse effects in animals and humans.

Dinoprost Tromethamine and Gonadorelin Diacetate Tetrahydrate have been rarely linked to cases with anaphylactic reactions that require immediate medical attention in cattle (Papich, 2021). Like other injectable drugs, reproductive drugs may cause localised tissue damage and post-injection bacterial infections (Fajt et al., 2011)

Dinoprost Tromethamine in particular may cause increased smooth muscle tone, resulting in diarrhoea, abdominal discomfort, bronchoconstriction and increase in blood pressure. In addition, induction of abortion with this drug may cause retained placenta (Papich, 2021).

Although progesterone release intravaginal device (PRID) placement have been associated with vaginitis, there no documented evidence of a generalised pathologic response to intravaginal device or reduction of the likelihood of pregnancy (Chenault et al., 2003; Villarroel et al., 2004; Walsh et al., 2008). However, assessing the impact of this treatment in the vaginal mucosa may be difficult as the animals could have purulent vaginal discharges due to uterine infection that has not been identified before PRID placement (Dubuc et al., 2010)

In humans, precautions for Dinoprost Tromethamine and Gonadorelin Diacetate Tetrahydrate include risks to pregnant women. People with respiratory problems also should not handle Dinoprost as it may cause bronchospasms (Papich, 2021). These reactions are well documented in the human medical literature.

1.7 Factors affecting reproductive efficiency in dairy herds

Factors affecting reproductive strategies based on FTAI and oestrus detection were reviewed in the previous sections. However, many factors can affect dairy herds reproductive performance regardless of the hormonal manipulations in place.

The success of fertility in lactating dairy herds is determined by multiple factors such as genetic selection, cow health, transition period management and nutrition (Cardoso Consentini, Wiltbank, & Sartori, 2021). Figure 1–9 summarises and provides some examples of associations between physiological and external factors that contribute to the reproductive performance in lactating dairy herds using FTAI and oestrus detection proposed by Cardoso Consentini et al. (2021).

1.7.1 Genetic selection

In dairy cows, despite the heritability of reproductive traits being low, genetic selection for health, fertility, and longevity is a priority in genetic selection programs. Selection for fertility depends on bull and cow fertility. The primary trait for selection in the cow is the daughter pregnancy rate (DPR), which is computed from the reported days open (DOPN) data for each cow (VanRaden, 2004). An increase of 1% on DPR is equivalent to a reduction of approximately 4 DOPN; and the bull DPR is measured as the proportion of his daughters that exceed or fall short of the PR on a given farm. Previous studies have shown that the selection of cows and heifers for fertility can (and should) be utilised by all dairy herds regardless of their chosen reproductive manipulation strategy (Sitko et al., 2019; Lima et al., 2020).

Reduction in cow's fertility is a consequence of single-minded selection for milk production, but with the introduction of simple genetic indices for fertility and good management practices, things seem to be improving (Fleming et al., 2019). Management practices may influence the expression of the current phenotypes used in genetic evaluations (i.e., inter-calving interval, non-return rate, calving first insemination interval, DOPN and CR). Therefore, new phenotypes such as progesterone-based phenotypes (i.e., luteal activity, anovulation) and oestrous expression and activity traits (i.e., length and strength of heat signs) can be evaluated as they more adjusted to the cow’s physiology and less affected by management practices (Fleming et al., 2019). In addition, Veerkamp and Beerda (2007) have highlighted the importance of improving fertility through genetic selection as hormone usage in food production animals is dwindling due to the negative public perception of the excessive use of hormones for milk production (Pieper, Doherr, & Heuwieser, 2016).

1.7.2 Nutrition

High-producing dairy cows’ diet must be balanced to provide the required nutrients for maintenance, milk production and for optimum reproduction. Any vitamins, minerals, or nutrient deficiency could have a detrimental effect on reproductive performance. After parturition, the onset of milk production and the change of nutrition produce a NEB physiological state in the cow. Animals undergoing a severe NEB could experience alterations of the follicular patterns (Butler, 2003); postpartum immune system impairment; development of uterine disorders (LeBlanc, 2014), and poor embryo development after fertilisation (Leroy et al., 2008b, 2008a), thereby affecting fertility. Body condition score and BCS changes are widely used as a proxy measure for energy balance. Therefore, poor BCS and severe BCS loss after calving can also have a detrimental effect on fertility (Santos, Rutigliano, & Filho, 2009; Carvalho et al., 2014). However, if there are BCS changes between 2 measurements (i.e., dry off and calving), NEB may have already happened. Therefore, monitoring metabolic markers such as NEFAs and BHB during the transition period (from the mid-late dry period to early lactation) and protein-to-fat ratio during lactation can be more accurate to identify and prevent NEB in dairy herds (Grieve et al., 1986; Duffield et al., 1997; Hayton, Husband, & Vecqueray, 2012)

1.7.3 Postpartum uterine disease and other diseases

Bacterial contamination of the uterus between 14 and 21 DIM is common in most cows (Sheldon, 2004). Around 10 to 20% of animals develop metritis after parturition (between 3 and 9 DIM); and approximately 10% of all animals have purulent vaginal discharges or clinical endometritis after a 50-day VWP, decreasing to less than 2% by 70 DIM (Sheldon, 2004). Although uterine disorders may occur in isolation, affected animals could present with more than one disease. Factors such as stillbirths, twins, dystocia, RP and NEB could contribute to the onset and severity of uterine disorders (Hussain, Daniel, & O'Boyle, 1990; Bicalho et al., 2010; LeBlanc, 2014). In addition, other non-uterine diseases such as lameness, mastitis, bovine respiratory disease (BRD) and digestive diseases have also been linked to reduced reproductive performance (Monteiro et al., 2020).

1.7.4 Heat stress, overstocking and poor group management

In modern high-producing dairy herds, three of the major circumstances influencing the cows' behaviour are heat stress, poor stall design and comfort, and overstocking (Cook et al., 2007; Galama et al., 2020).

The negative impact of heat stress on reproduction is extensively reported in the literature, with a reduction of P/AI from 20 to 50% in hot seasons compared to cooler months of the year (Schüller, Burfeind, & Heuwieser, 2016). Increased temperatures reduce the intensity of external oestrus signs and the size of the dominant follicle (Schüller, Michaelis, & Heuwieser, 2017).

Overstocking cows results in reduced feed intake and less social interactions before parturition leading to severe NEB, which has been identified as a predisposing factor for metritis (Huzzey et al., 2007). In addition, transition cows movements, regrouping and mixing primiparous and multiparous animals are important stressful events to consider in traditional close-up pen management where the cows are moved in and out on a weekly basis compared with all-in-all-out systems (Lobeck-Luchterhand et al., 2014). Affecting the cow's behaviour may also affect their physiology, contributing to an increased risk of postpartum diseases (Chebel et al., 2016).

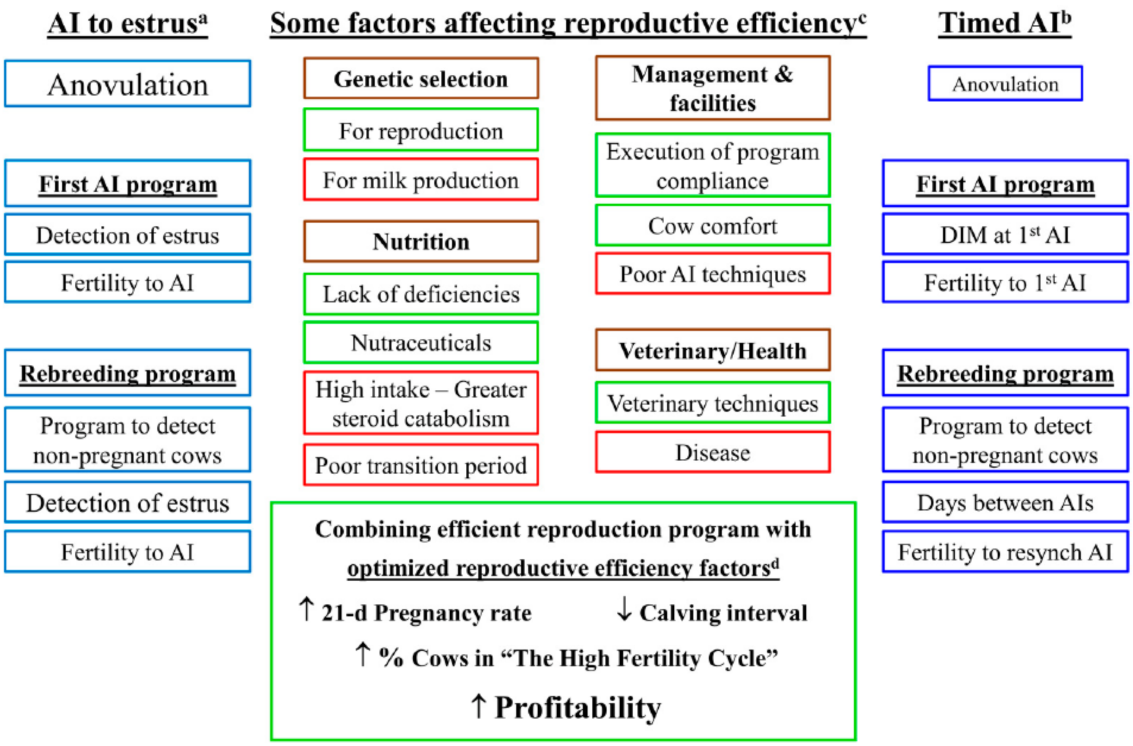


Figure 1-9. Factors affecting reproductive efficiency

Schematic representation of the key reproductive factors that directly affect a reproductive management program using artificial insemination (AI) to oestrus (left a) or timed AI (right b). Some of the key factors affecting reproductive efficiency in either or both programs are shown (center c). Factors shown in red squares tend to decrease fertility, whereas factors shown in green rectangles tend to increase fertility. (Bottom d). Days in milk (DIM). (Cardoso Consentini, Wiltbank, & Sartori, 2021).

1.8 Public perception of hormone usage for dairy cattle reproduction

Nowadays, there is a growing societal concern on excessive use of medications, including hormonal treatments, and public demands on food producers to extend the longevity of dairy cows (von Keyserlingk et al., 2013). There is a potential impact of consumer opinion regarding reproductive technologies and hormonal manipulation of the bovine oestrus cycle, as they have occurred without much public consultation (Pieper, Doherr, & Heuwieser, 2016). Therefore, public opinion plays an important role in determining future reproductive management strategies (von Keyserlingk et al., 2013).

In a study performed in Germany by Pieper et al. (2016), where a commercial market institute interviewed 1,646 participants, it was found that most of the people did not agree with the use of any reproductive technologies for milk production (i.e., hormone treatments to increase fertility (65%) embryo transfer (58%), cloning (81%), even the use of sexed semen (53%)). They also found that around 50% of the participants did not have basic knowledge about milk production. The authors concluded that educating and providing information to the public could help them to understand dairy cows' reproductive management practices (Pieper, Doherr, & Heuwieser, 2016).

In another study performed in the UK, veterinary practitioners were asked, "Does the use of fertility drugs to get dairy cows served give you any cause for concern?" and responses were divided but not far from the results obtained in the German study (yes = 52% (48) yes vs no = 48%) (Higgins et al., 2013). When 'positive respondents' were asked to describe their concerns, the main answers were: i) the use of hormones is not addressing the causes of poor fertility; ii) its effect on genetic selection for infertility within the herd (i.e. the production of replacements from treated cows); iii) negative public opinion; and iv) health and welfare issues per se (Higgins et al., 2013). However, over 80% of the veterinarians agreed that the targeted use of hormones for reproductive management in dairy cows improved fertility and farm businesses profitability (Higgins et al., 2013).

To reduce the excessive use of medications and hormonal treatments and extend dairy cow's longevity are growing public expectations and demands, farmers, veterinary practitioners, along with veterinary scientists nutritionist and geneticists may need to develop strategies to improve the fertility of the high-producing dairy cows less with blanket hormone strategies and more with tailored treatments to individual cows or groups relying mainly on genetic selection and improvements of the overall farm management (Oltenacu & Algers, 2005; Veerkamp & Beerda, 2007).

1.9 Objective and hypotheses

Although there is extensive work evaluating dairy cattle reproductive strategies in the US, to the author's knowledge, there are not large trials comparing reproductive strategies involving oestrus detection and FTAI with Double Ovsynch in the UK, where dairy farming conditions in terms of feeding, climate and economics are considerably different.

Therefore, the objective of this work was to assess the relative efficacy of three commercially relevant reproductive management strategies in two Scottish dairy farms, with regard to pregnancy in lactating dairy cows. The protocols were i) AI after detection of natural oestrus followed if required with Ovsynch (OD-Ov); ii) AI after detection of natural oestrus followed if required with PRIDsynch (OD-PR); iii) Double Ovsynch (DO).

The primary hypothesis was that DO would result in greater P/AI at the time of first postpartum AI when compared with protocols reliant on oestrous detection followed by a hormonal synchronisation (OD-Ov, OD-PR).

A secondary hypothesis was that those strategies allowing FTAI after OD (OD-Ov and OD-PR) where cows have more than one opportunity to be inseminated would result in a higher proportion of cows pregnant by 83 DIM than cows that received only the FTAI protocol (DO).

Chapter 2 Material and Methods

2.1 Experimental design

The trial involved 1,681, lactating Holstein cows and was conducted between October 2018 and February 2020 from two commercial Scottish dairy farms. Both farms were serviced by the Scottish Centre for Production, Animal Health and Food Safety, University of Glasgow. This study received ethical approval from the University of Glasgow (Faculty Ethics and Welfare Committee), license number 44a/18.

Cows on both farms were milked three times a day and housed in free-stall sheds, separated into high and low yielding multiparous cows groups. Primiparous and multiparous animals were housed separately. During the study, Farm 1 and Farm 2 were milking 765 and 580 cows on average, respectively, and mean farm 305d mature-equivalent milk production of 13,279 and 10770 kg, respectively. Cows were fed once a day in a single rail feedline barrier with a total mixed ration (TMR) based on grass silage, cereals and a concentrate-mineral mix, meeting or exceeding the requirements for high-level milk production (NRC, 2001), with ad libitum access to water. Other characteristics of the enrolled farms are shown in Table 2-1.

Table 2-1. Enrolled farms characteristics

Farm	Farm 1	Farm 2
Land	900 acres rented 400 owned Soil type – medium to heavy	200 acres rented 450 acres owned Soil type – medium to heavy
Cropping	700 acres winter wheat, spring beans, rape, and spring barley 600 acres of grass silage (4 to 5 cuts/year)	100 acres of spring wheat 400 acres of grass silage (3 to 4 cuts/year)
Buildings	Accommodation for 830 cows - 60 sand cubicles for dry cows - 50 fresh calved/late dry cow straw yard - 720 milking cow cubicles Slurry storage - 2 ½ months storage Parlour - 50-point Westfalia rotary	Accommodation for 586 cows - 100 milking cows on straw - 66 Dry cows/pre calvers on straw - 420 milking cow cubicles Slurry storage - 3 months storage Parlour - 24/48 Delaval swing over
Milking group pens	Primiparous High yielders Mid yielders	Primiparous High yielders Mid yielders Low yielders
Stocking	900 cows (milking and dry cows) 408 young-stock	650 cows (milking and dry cows) 400 young-stock
305d mature-equivalent milk production	13,279 kg	10770 kg
Reproductive performance	Submission rate: 56% Conception rate: 39% 21-day pregnancy rate: 22% Pregnant by 100 DIM: 51%	Submission rate: 59% Conception rate: 41% 21-day pregnancy rate: 24% Pregnant by 100 DIM: 52%

2.1.1 Reproductive strategies

All cows were blocked by parity (primiparous and multiparous) and randomly assigned to one of three different synchronization protocols at calving: combination of oestrus detection and Ovsynch (OD-Ov), combination of oestrus detection and PRIDsynch (OD-PR), and Double Ovsynch (DO). Cows enrolled in OD-Ov, and OD-PR were eligible to be inseminated by detected oestrus between 50 and 70±3 DIM (Figure 2-1). Visual OD was performed 3 times a day by trained farm personnel (for 30 minutes each time), and cows were artificially inseminated 16 h after observed oestrus. No heat detection aids such as pedometers or tail paint were used.

If cows in the OD-Ov group (n=541) were not detected in oestrus between 50 and 70±3 DIM, they were subjected to the following hormone protocol: GnRH (G1) at 70±3 DIM, PGF2α (PG1) 7 d later, PGF2α (PG2) 24 h later and GnRH (G2) 32 h later, followed by FTAI 16 h later. If cows in the OD-PR group (n=562) were not detected in oestrus between 50 and 70±3 DIM, they were subjected to the following hormone protocol: GnRH (G1) + PRID at 70±3 DIM, PGF2α (PG1) 7 d later, PGF2α (PG2) + PRID removal 24 h later and GnRH (G2) 32 h later, followed by FTAI 16 h later. In these two groups, cows that received AI at detected oestrus (within 50 and 70±3 DIM) and returned to oestrus before 83DIM were reinseminated, having more than one opportunity for AI, but not following any hormone protocol for FTAI. Cows enrolled in the DO group (n=578), received GnRH at 53±3 DIM, 7 d later PGF2α, GnRH 3 d later, GnRH (G1) 7 d later, PGF2α (PG1) 7 d later, PGF2α (PG2) 24 h later and GnRH (G2) 32 h later, followed by FTAI 16 h later with no opportunity for mating to observed oestrus.

All inseminations were performed by one certified technician on Farm 1 and two certified technicians on Farm 2, using conventional frozen-thawed commercial semen from Holstein and beef sires selected by following the farm's standard commercial breeding program. For all synchronization of ovulation protocols, GnRH treatments consisted of 100 µg of Gonadorelin diacetate administered intramuscularly (Ovarelin®, Ceva-Sante, Libourne, France), whereas PGF2α treatments consisted of 25 mg of Dinoprost Thromethamine Sodium administered intramuscularly (Enzaprost®, Ceva-Sante, Libourne, France).

Progesterone supplementation consisted of 1.55g progesterone, administered via a progesterone intravaginal device (Prid® Delta, Ceva-Sante, Libourne, France).

Cows were excluded from the study if they were sold; classified as do not breed (DNB) by the farmers; died before AI; received AI before the end of the VWP or during the protocols, and if they had missing data. Animals with uterine infection were also excluded as the farm's treatment protocols included PGF2α, which may have acted as a pre-synchronization and influenced the trial results.

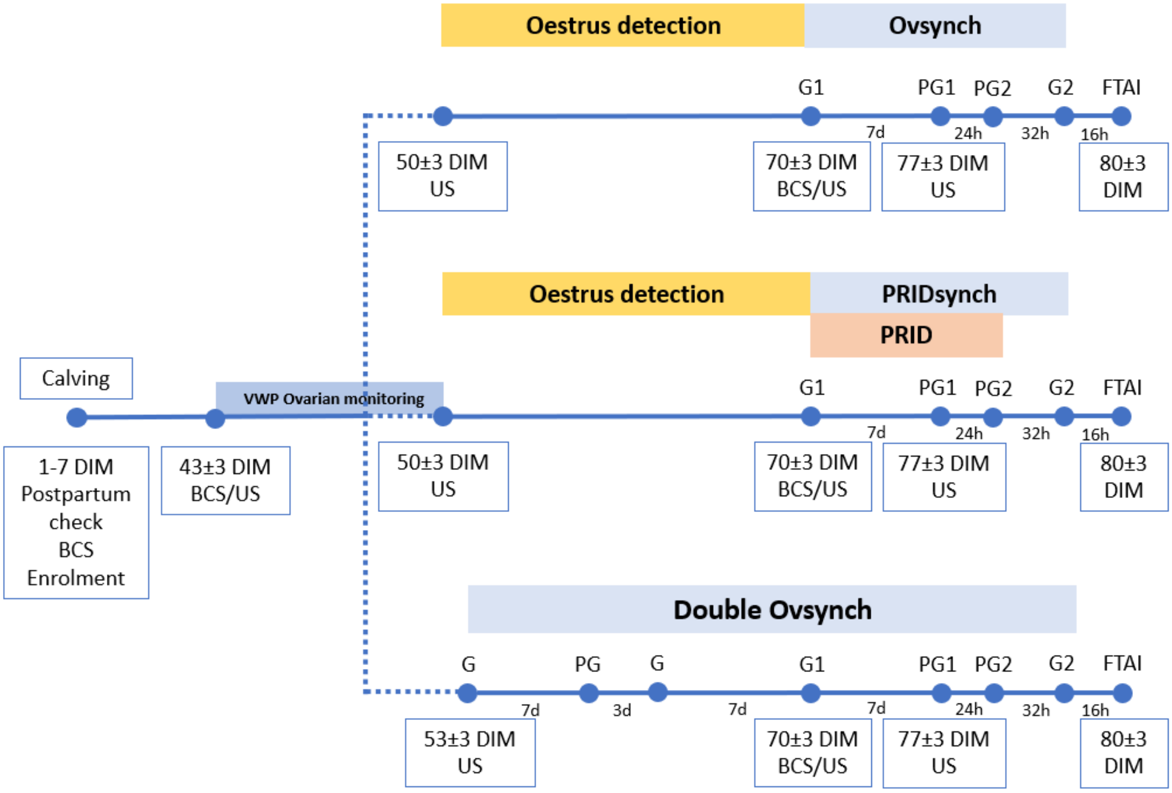


Figure 2-1. Schematic representation of the reproductive strategies

Schematic representation of the synchronization protocols, postpartum check, BCS and ultrasound (US) evaluations. Cows in Oestrus detection - Ovsynch (OD-Ov) and Oestrus detection - PRIDsynch (OD-PR) were allowed to be inseminated at standing heat between 50 and 70±3 DIM, and if not detected in oestrus, enrolled in a synchronisation protocol at 70±3 DIM and received an AI at 80±3 DIM. Cows in Double Ovsynch (DO) started the protocol at 53±3 DIM and received an AI at 80±3 DIM. Pregnancy diagnoses were performed 32±3 d and 63±3 d after AI (oestrus detection or FTAI).

2.1.2 Body Condition Scoring and milk production

Animals were body condition scored at calving and at 43±3 DIM using a scale of 1 to 5 and 0.25 increments (Figure 2-2) (Edmonson et al., 1989). Cows were classified as BCS ≤2.5 or BCS >2.5 (Carvalho et al., 2014), and BCS 'loss' was attributed when ≥0.5 points were lost between measurements. Fourth-week yield (W4MK) and cumulative milk by 30 days (MK30) after calving data were retrieved from the dairy management software (DairyComp305) and were considered as covariates in all the regression models.

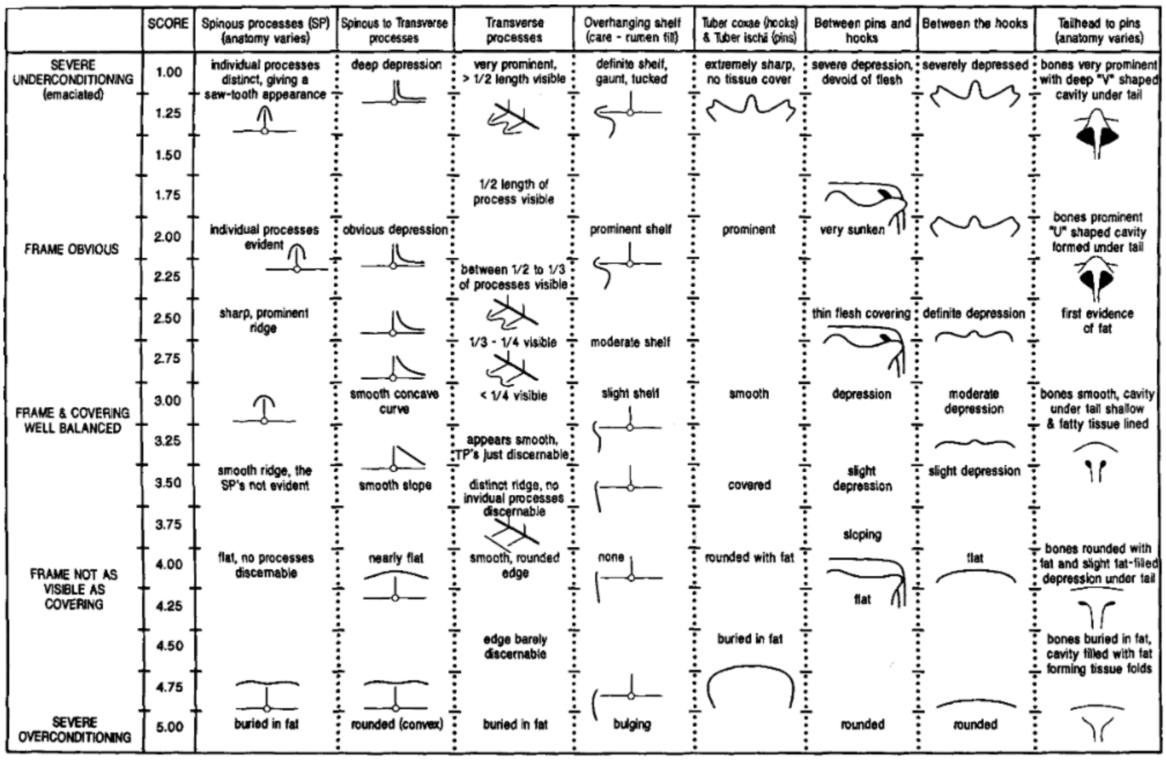


Figure 2-2. Body condition scoring chart for Holstein cows (Edmonson et al., 1989)

Changes in conformation with body condition change for eight body locations identified as important in body condition scoring suggested by Edmonson (1989).

2.1.3 Post-partum disease, ovarian cyclicity and uterine health monitoring

All animals were examined by Glasgow University veterinary clinicians between 1 and 7 DIM for post-partum disorders. Subclinical ketosis was diagnosed using a commercial urine-dip test strip (KetoStix®, Bayer Diagnostics Europe Ltd., Dublin, Ireland), and a positive result was defined as coloured ≥ trace on Ketostix strips (Figure 2-3) (Carrier et al., 2004). Retained placenta was recorded as failure to expel foetal membranes for greater than 12h after parturition. Metritis was characterized by watery red-brown foul-smelling odour post-natal vulval discharges, with or without pyrexia (Sheldon et al., 2006). Displaced abomasum (DA) was recorded when a ping was detected by flicking the cow's abdominal wall while simultaneously listening with a stethoscope in the area between the 9^{th} and 12^{th} ribs above and below an imaginary line extending from the hip to the elbow on each side of the animal on the abdominal wall. Clinical hypocalcaemia was recorded by the farm staff when a cow displayed clinical signs that included muscle weakness, nervousness, muscle shaking, cold ears, eventually leading to recumbency.

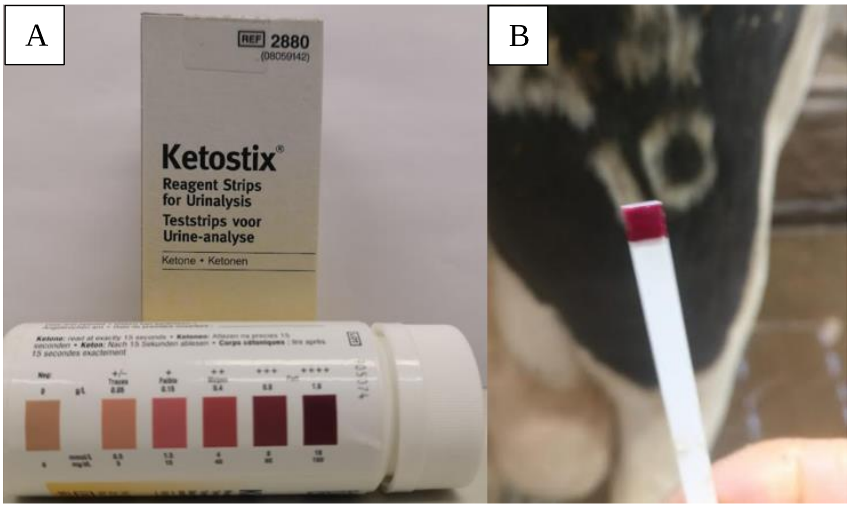


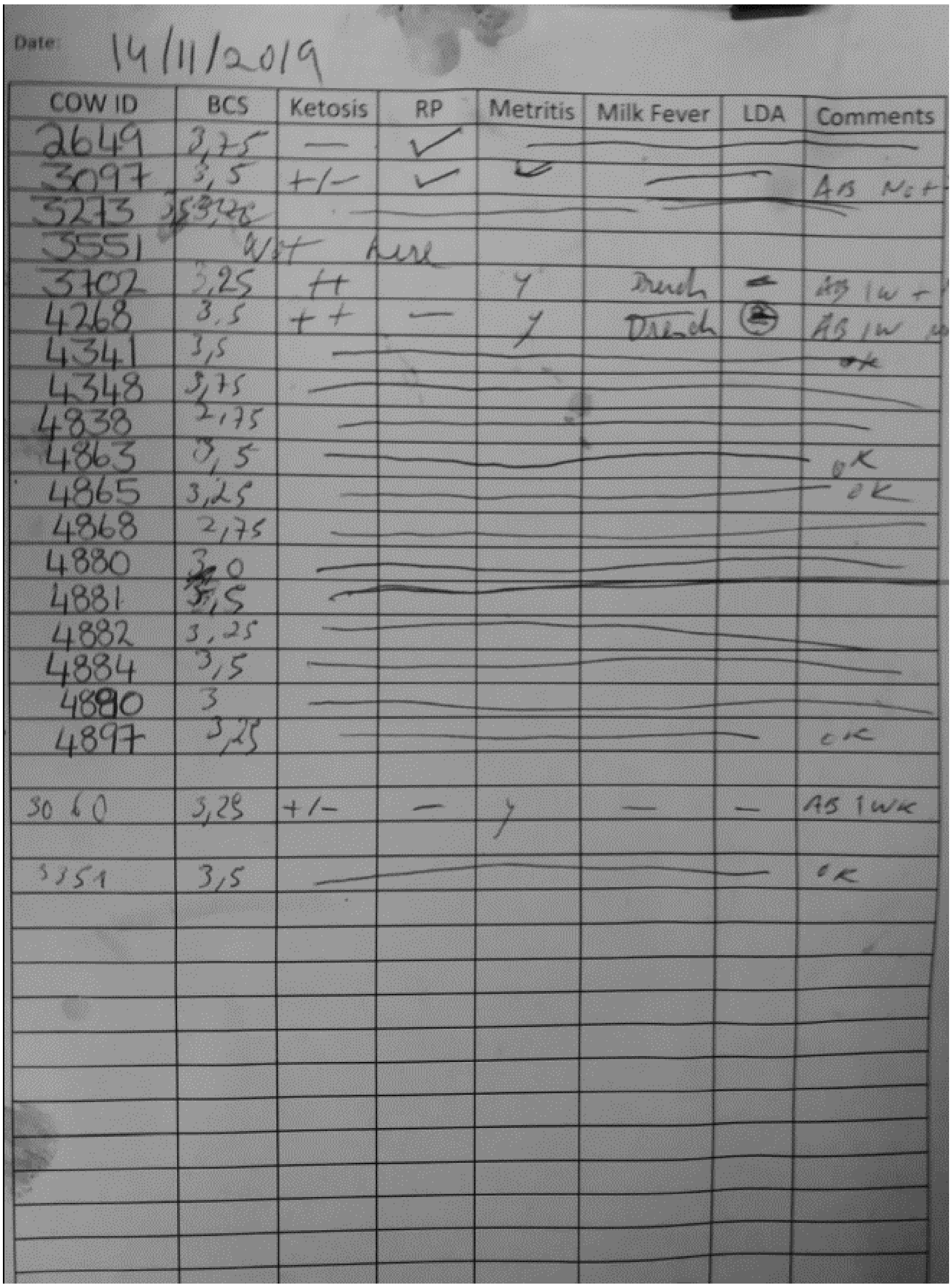
Figure 2-3. KetoStix®, Bayer Diagnostics Europe Ltd., Dublin, Ireland

KetoStix® strips, Bayer Diagnostics Europe Ltd., Dublin, Ireland, used for diagnosis of subclinical ketosis in the trial (A); and the observed result of +++ in one cow tested for ketosis in urine in one of the cows included in the trial (B).

Diagnoses of lameness, mastitis and BRD were also recorded until first AI. Lameness was recorded in cows showing uneven weight bearing on a limb that was immediately identifiable and/or obviously shortened strides (mobility score ≥2; Table 2-2) (Whay et al., 2003). Clinical mastitis was defined as inflammatory changes to the mammary gland or milk visually detected in one or more quarters. Bovine respiratory disease was recorded when the animals showed signs such as pyrexia, increased respiratory rate, coughing, purulent nasal discharges and the presence of abnormal lung sounds at auscultation. If any disease was detected, animals were monitored weekly until they were considered healthy by Glasgow University veterinary clinicians. Data were collected on the farm on paper datasheets and were collated and transferred to a spreadsheet (Microsoft Excel) (Figure 2-4).

Table 2-2. Mobility score system suggested by Whay (2003)

Score	Description of the cow behaviour
0	Sound
1	Abnormal locomotion/perhaps tender-footed
2	Lame
3	Severely lame



Date: 14/11/2019

COW ID	BCS	Ketosis	RP	Metritis	Milk Fever	LDA	Comments
2649	3,75	—	✓				
3097	3,5	+/–	✓				AB Not
3273	3,5						
3551	Not here						
3702	3,25	++		y	Drench	–	AB 1w
4268	3,5	++	—	y	Drench		AB 1w
4341	3,5						ok
4348	3,75						
4838	2,75						
4863	3,5						ok
4865	3,25						ok
4868	2,75						
4880	3,0						
4881	3,5						
4882	3,25						
4884	3,5						
4890	3						
4897	3,25						ok
3060	3,25	+/–	—	y	—	—	AB 1wk
3351	3,5						ok

Figure 2-4. Postpartum diseases data collection sheet

On-farm data recording of body condition score (BCS) and postpartum diseases such as ketosis, metritis, retained placenta (RP), milk fever and left and right displaced abomasum (LDA) between 1 and 7 DIM in 1030 cows included in the trial.

Anovulation and uterine health were monitored by transrectal US with a portable device equipped with a 7.5-MHz linear transducer (Easy-Scan II, BCF Technology Ltd., Livingston, UK) at 43±3 DIM and 50±3 DIM. All synchronized cows were also examined at the time of injections G1 and PG1. Presence and size (using the Easy-Scan wrist screen grid) of follicles and CL were recorded for both ovaries at each examination (Figure 2-6). Cows were categorized into ovular or anovular, based on the presence or absence of a CL respectively at one or two of the US evaluations (Gümen & Wiltbank, 2005). Uterine infection was defined as the presence of echogenic intrauterine fluid at US (Figure 2-5) (Kasimanickam et al., 2004).

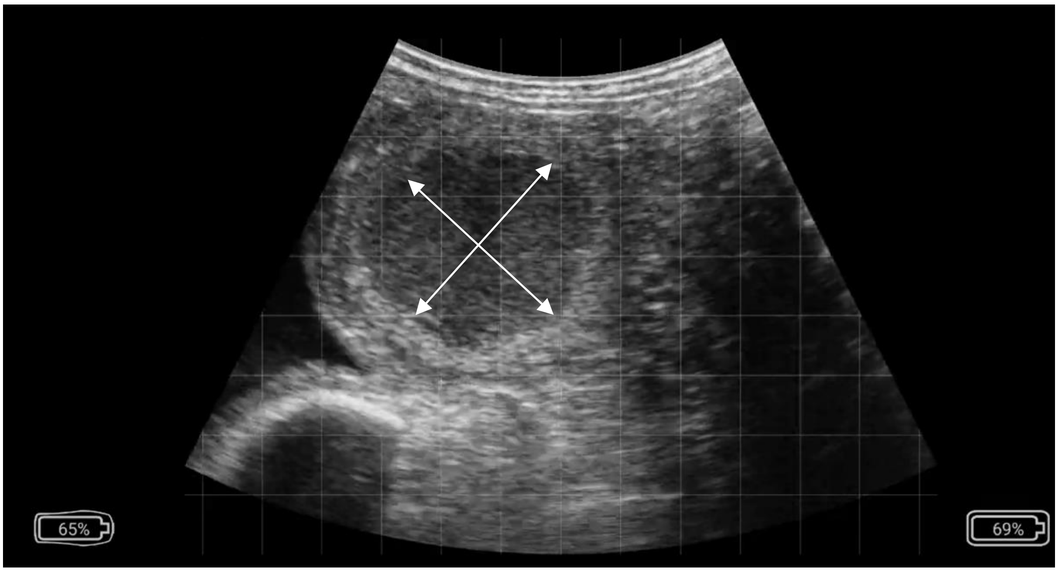


Figure 2-5. Uterine infection diagnosis

Ultrasonography of a cow diagnosed with uterine infection during the examination at 43 DIM. The figure shows a 3 cm uterine lumen filled with hyperechoic fluid (white arrows)

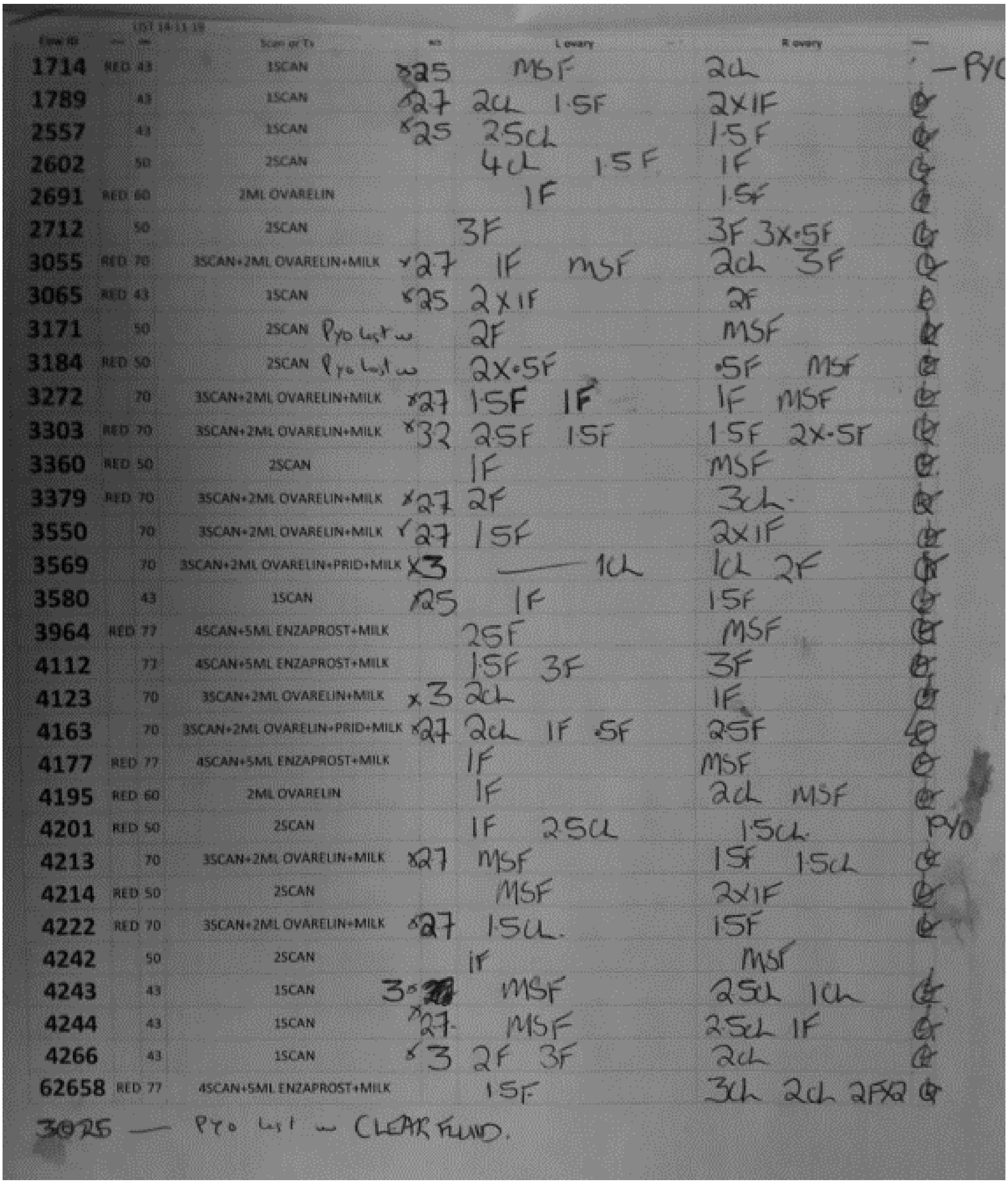


Figure 2-6. Ovarian cyclicity and uterine health data collection sheet

On-farm data recording of the ultrasonography (US) results at 43±3 (1 scan), 50±3 (2 scan), 70±3 (3 scan) and 77±3 (4 scan) DIM; results of BCS at 43±3 and 70 DIM±3. Presence and size in centimetres (using the Easy-Scan wrist screen grid) of follicles (F), corpora lutea (CL) and multiple small follicles (MSF) recorded for both ovaries at each examination. Uterine infection was also recorded (PYO) if found at any examination.

2.1.4 Pregnancy Diagnosis

Pregnancy diagnosis (PD) was performed between 29 and 35 d after AI by transrectal US using the same device as for ovarian activity and uterine health monitoring. Pregnancy diagnosis in dairy cows was based on the presence of intraluminal uterine fluid, a CL and foetal heartbeat (Fricke, 2002). Cows diagnosed as pregnant then had a second US examination between 60 and 66 d post-AI to confirm pregnancy (Figure 2-1); non-pregnant animals at this point were recorded as PL. Table 2-3 summarises the identifiable structures assessed by US in a pregnant cow at first PD (highlighted in light grey) and PD confirmation (highlighted in dark grey).

Table 2-3. Day of first detection of ultrasonographically identifiable characteristics of the bovine conceptus (Fricke, 2002)

Characteristic	First day detected	
	Mean	Range
Embryo proper	20.3	19 to 24
Heartbeat	20.9	19 to 24
Allantois	23.2	22 to 25
Spinal cord	29.1	26 to 33
Forelimb buds	29.1	28 to 31
Amnion	29.5	28 to 33
Eye orbit	30.2	29 to 33
Hindlimb buds	31.2	30 to 33
Placentomes	35.2	33 to 38
Split hooves	44.6	42 to 49
Foetal movement	44.8	42 to 50
Ribs	52.8	51 to 55

2.2 Statistical Analysis

Individual cow data were obtained from DairyComp 305 (Valley Agricultural Software, Tulare, CA, USA), and exported to Microsoft Excel (Version 2011, Microsoft Corporation). Statistical analysis was performed in R (R Core Team, 2020). The animals were blocked by parity and randomly assigned to the reproductive strategies evaluated in the trial using Microsoft Excel's RAND function (Version 2011, Microsoft Corporation).

Fisher's exact test was used to compare the distribution of cows on the categorical risk factors by reproductive strategy and farm. Binary outcome variables—pregnancy at 32±3 d after first AI; pregnancy at 63±3 d after first AI; pregnancy at 32±3 d by 83 DIM; pregnancy at 63±3 d by 83 DIM and pregnancy loss (between 32±3 d and 63±3 d)—were modelled using logistic regression. Farm and protocol were included as fixed effects in all models, regardless of their effect sizes or statistical significance. Other covariates considered were: previous calving details (abortion, twins and dead calf); BCS at calving and 43±3 DIM; BCS loss between the evaluation points; post-partum diseases (hypocalcaemia, ketosis, metritis, retained placenta, displaced abomasum); lameness; mastitis; respiratory disease; ovarian activity; season when the AI was received (Spring: Mar-May; Summer: Jun-Aug; Autumn: Sep-Nov; or Winter: Dec-Feb); sire type (Beef or Holstein); AI technician; week 4 milk yield (W4MK) and 30-day cumulative milk yield (MK30). Multivariable logistic regression models were constructed using stepwise variable selection with Akaike's Information Criterion (AIC) to optimize predictive ability and model fit. All biologically plausible interactions were tested, including protocol and farm, protocol and parity and parity and farm. Covariates and interactions between protocols and covariates were retained only if they were statistically significant ($p<0.05$) in the final models. Diagnostic checks of the final models were done with the *DHARMa* package (Hartig, 2020).

Kaplan-Meier survival curves (Therneau, 2021) were used to visualize time to pregnancy, and the three protocols were compared using pairwise log-rank tests.

Chapter 3 Results

By the end of the trial 1030 animals were available for analysis. Reasons for exclusion (OD-Ov, n=208; OD-PR, n = 204; DO, n = 239), are presented in Figure 3-1.

3.1.1 Postpartum diseases, milk yield, BCS and ovarian cyclicity

Descriptive statistics are presented in Table 3-1. The proportion of cows with DA, ketosis, mastitis, BRD and lameness were similar among reproductive strategies. There were more cows with RP and metritis in the OD-PR group compared with OD-Ov and DO groups. There was also a difference in the proportion of cows with lameness, where Farm 2 had a greater proportion of lame cows when compared with Farm 1.

Multiparous cows were more likely to be lame before AI than primiparous cows and more likely to suffer from ketosis and RP, but no effect of parity was observed for hypocalcaemia, DA, metritis, mastitis and BRD.

Due to recording issues, milk production data was available only for 896 cows. Mean W4MK yields for Farm 1 were (standard errors and number of cows in brackets): OD-Ov, 42.1 litres (+-0.83, n = 192); OD-PR, 42.2 litres (+-0.81, n = 210); DO, 42.2 litres (+-0.81, n = 201). Mean W4MK yields for Farm 2 were: OD-Ov, 36.0 litres (+-1.11, n = 93); OD-PR, 34.2 litres (+-0.92, n = 107); DO, 35.4 litres (+-1.00, n = 93) (Figure 3-2). W4MK and MK30 were strongly correlated (r = 0.94). Therefore, we included only one of these at a time in the regression models, to avoid multicollinearity. However, milk production (W4MK) was not significantly linked to pregnancy outcome in any of the regression models. There was a similar proportion of cows receiving AI after OD in OD-Ov (46.7%; 189/333) and OD-PR (53%; 190/358). Only 9.9% (n = 33/333) and 10.1% (n = 36 cows received 2 AI after OD in OD-Ov and OD-PRIDsynch, respectively.

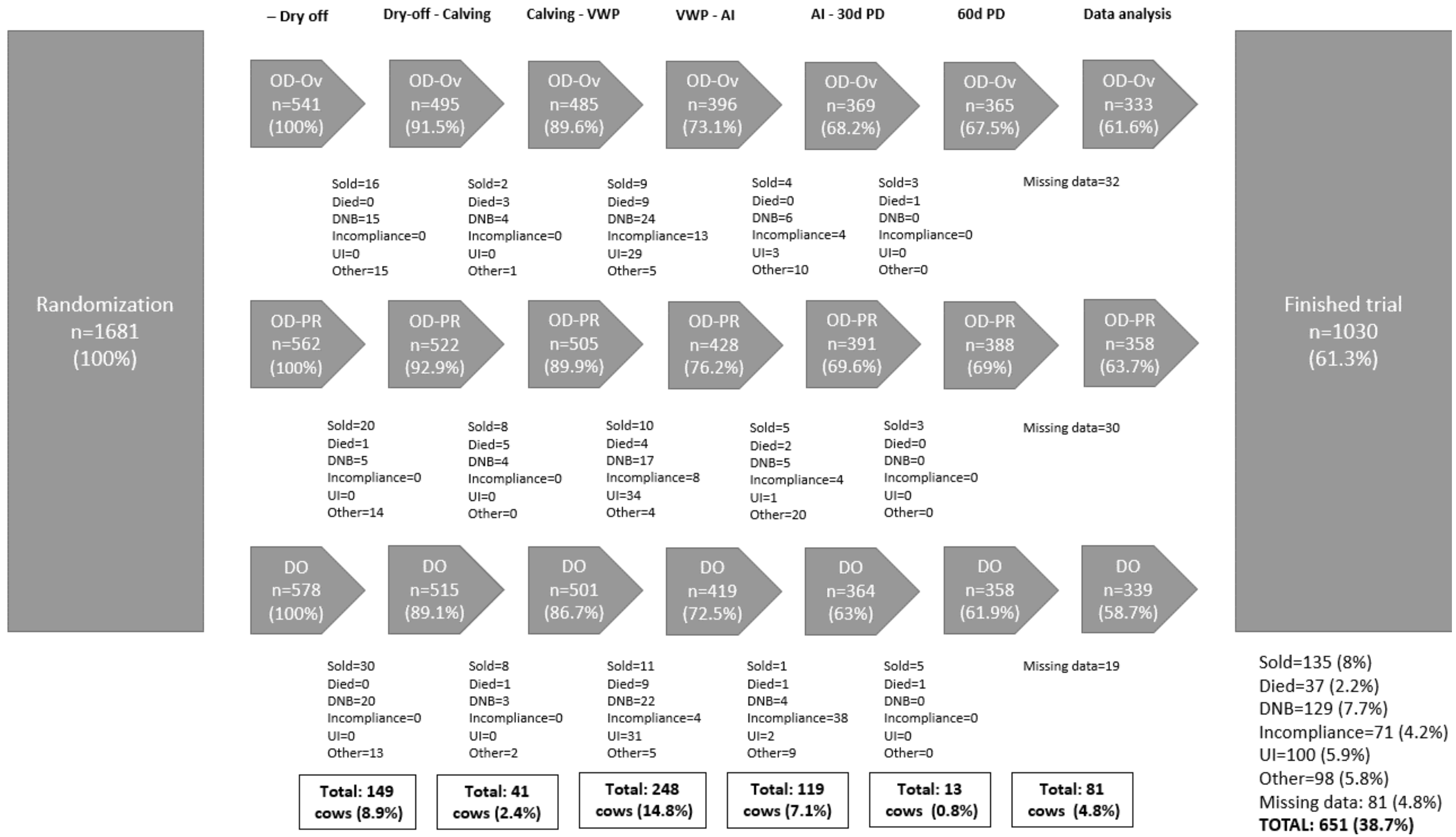


Figure 3-1. Flow of numbers of lactating dairy cows enrolled in the study by reproductive strategy

Schematic representation of the flow of numbers of lactating dairy cows enrolled in the study by reproductive strategy Oestrus detection – Ovsynch (OD-Ov), Oestrus detection – PRIDsynch (OD-PR) and Double Ovsynch (DO) showing the proportion of animals excluded because of missing data, sold, died, classified as do not breed (DNB), incompliance of protocols, uterine infection (UI) and other reasons and the moment of exclusion.

Table 3-1. Descriptive statistics comparing observations by treatment and farm

	Protocol				Farm		
	OD-Ov	OD-PR	DO		1	2	
	n = 333	n = 358	n = 339	*P*-value	n = 620	n = 410	*P*-value
Variable	(%)	(%)	(%)		(%)	(%)	
Lactation				0.88			<0.01
Primiparous	39.9	39.1	38.1		43.4	32.4	
Multiparous	60.1	60.9	61.9		56.6	67.6	
Previous calving							
Abortion	0.3	0	0	0.32	0	0.24	0.4
Twins	3.3	2.8	2.1	0.62	3.6	1.5	0.05
Dead calf	6.6[a]	2.2[b]	4.4[a]	0.02	3.7	5.47	0.22
Post-partum diseases							
Hypocalcemia	0	0.6	0.3	0.77	0.16	0.5	0.57
Ketosis 7d	7.2	5.6	6.5	0.69	6.9	5.6	0.44
DA	0.9	0.8	1.2	0.93	0.7	1.5	0.21
RP	4.2[ab]	8.4[b]	2.4[a]	<0.01	6.1	3.4	0.06
Metritis	6 [ab]	9.5 [b]	3.2 [a]	<0.01	5.3	7.8	0.12
Mastitis	2.1	3.1	2.7	0.76	2.4	2.9	0.69
BRD	0	0.3	0.9	0.22	0.3	0.5	0.65
Lameness	8.1	7.3	7.4	0.91	4.7	11.9	<0.01
Cyclicity				0.93			<0.01
Ovular	92.5	92.5	91.7		94.2	89.3	
Anovular	7.5	7.5	8.3		5.8	10.7	

[a,b] Means within a row with different superscripts differ (P < 0.05).

A greater proportion ($P < 0.01$) of primiparous cows had BCS > 2.5 than multiparous cows, both at calving (96.5% (333/345) vs. 90.8% (515/567), respectively) and at 43±3 DIM (92.3% (371/402) vs. 86.3% (542/628), respectively) (Figure 3-3). Cows at Farm 1 were more likely to have BCS > 2.5 at calving than cows at Farm 2 (99.1% (555/560) vs 83.2% (293/352), respectively) and at 43±3 DIM (95.3% (591/620) vs 78.5% (322/410), respectively) ($P < 0.01$) (Figure 3-4). However, there was no observed difference in BCS between reproductive strategies at the same time points. In addition, a greater proportion of multiparous cows (25.2%; n = 143/567) lost ≥ 0.5 BCS between calving and 43±3 DIM when compared to primiparous cows (11.6%; n = 40/345) ($P < 0.01$) (Figure 3-5).

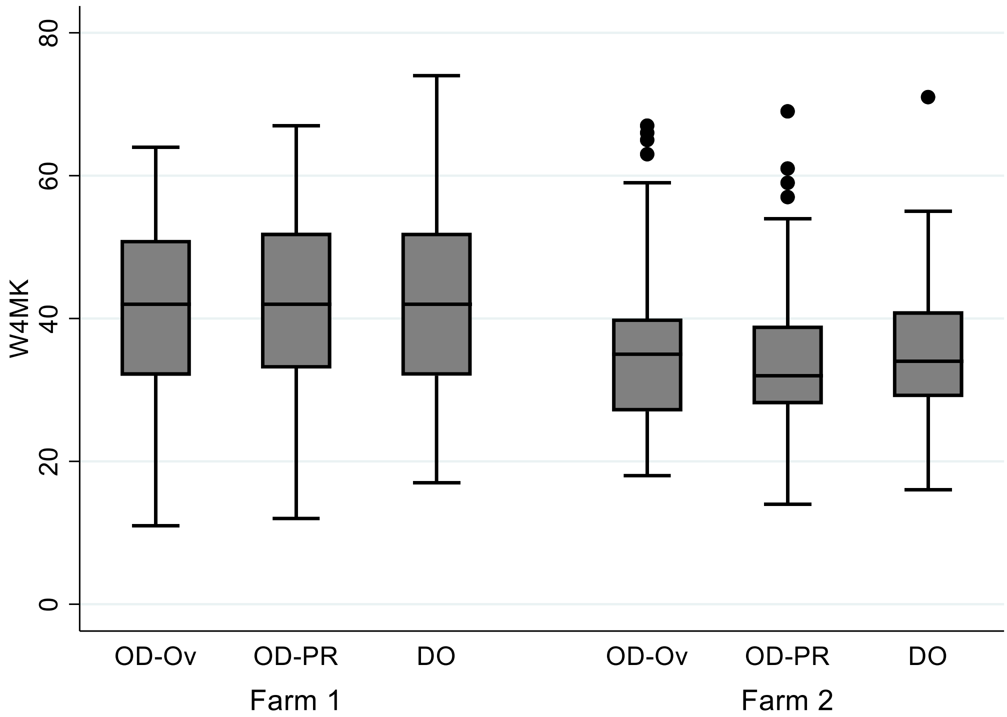


Figure 3-2. W4MK by reproductive strategy and farm

The box and whisker plots show the fourth-week milk yield (W4MK) in Oestrus detection – Ovsynch (OD-Ov), Oestrus detection – PRIDsynch (OD-PR) and Double Ovsynch (DO) reproductive strategies, stratified by farm. The horizontal dark line in each box plot is the median value for each W4MK.

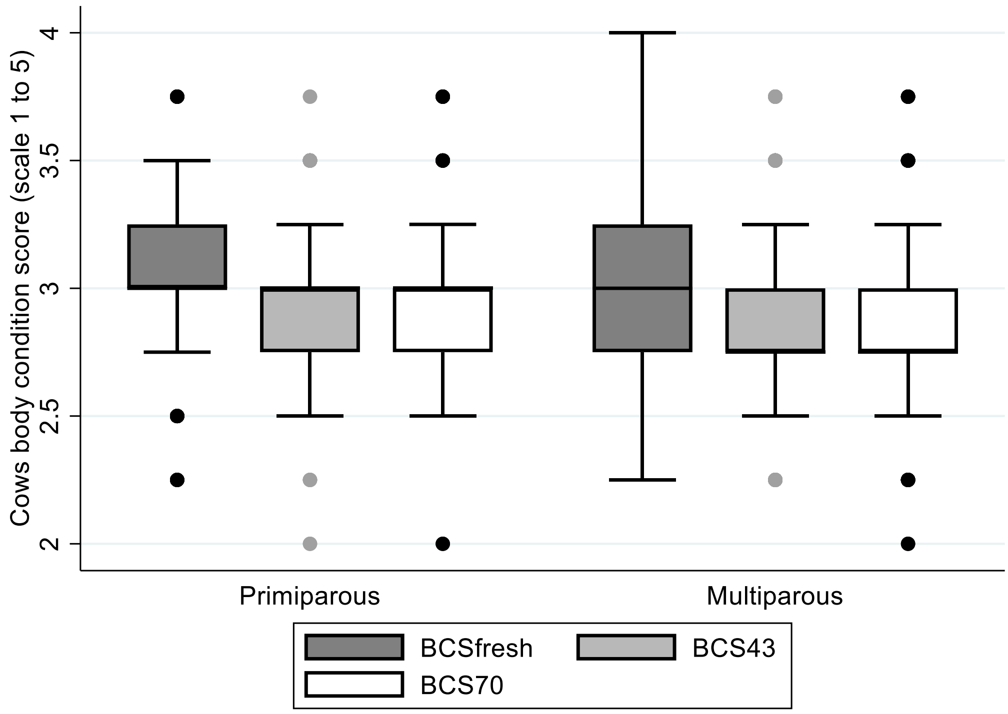


Figure 3-3. Body condition score at calving, 43±3 and 70±3 DIM by parity

The box and whisker plots show the body condition score (BCS) at calving (dark grey), 43±3 DIM (light grey) and 70±3 DIM (white), stratified by parity. The horizontal dark line in each box plot is the median value for each BCS using a scale of 1 to 5 and 0.25 increments.

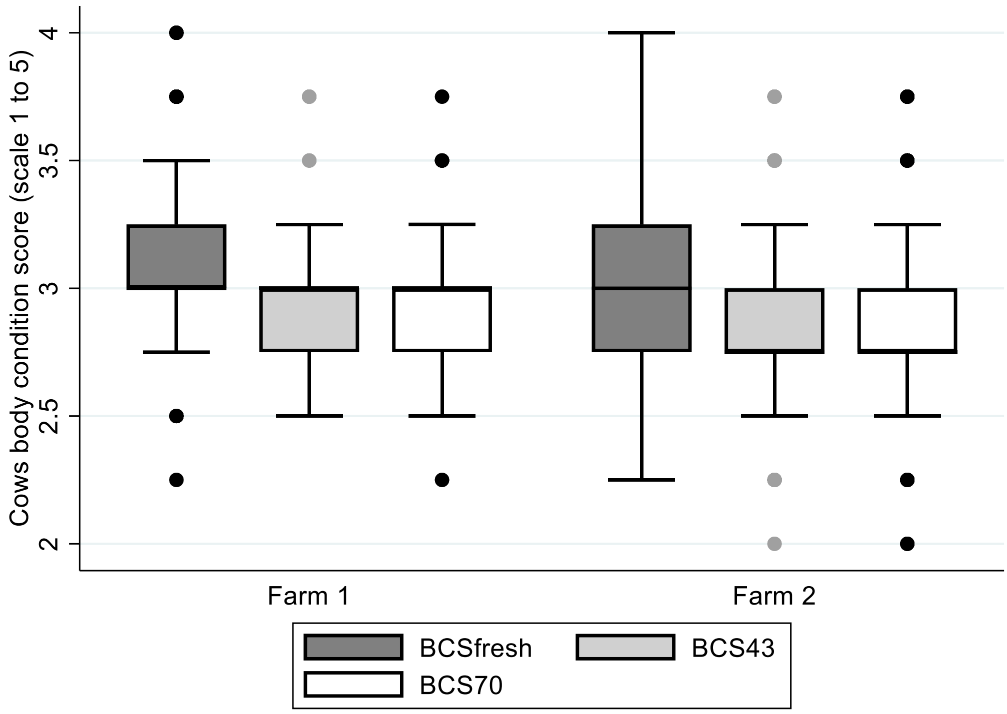


Figure 3-4. Body condition score at calving, 43±3 and 70±3 DIM by farm

The box and whisker plots show the body condition score (BCS) at calving (dark grey), 43±3 DIM (light grey) and 70±3 DIM (white), stratified by farm. The horizontal dark line in each box plot is the median value for each BCS using a scale of 1 to 5 and 0.25 increments.

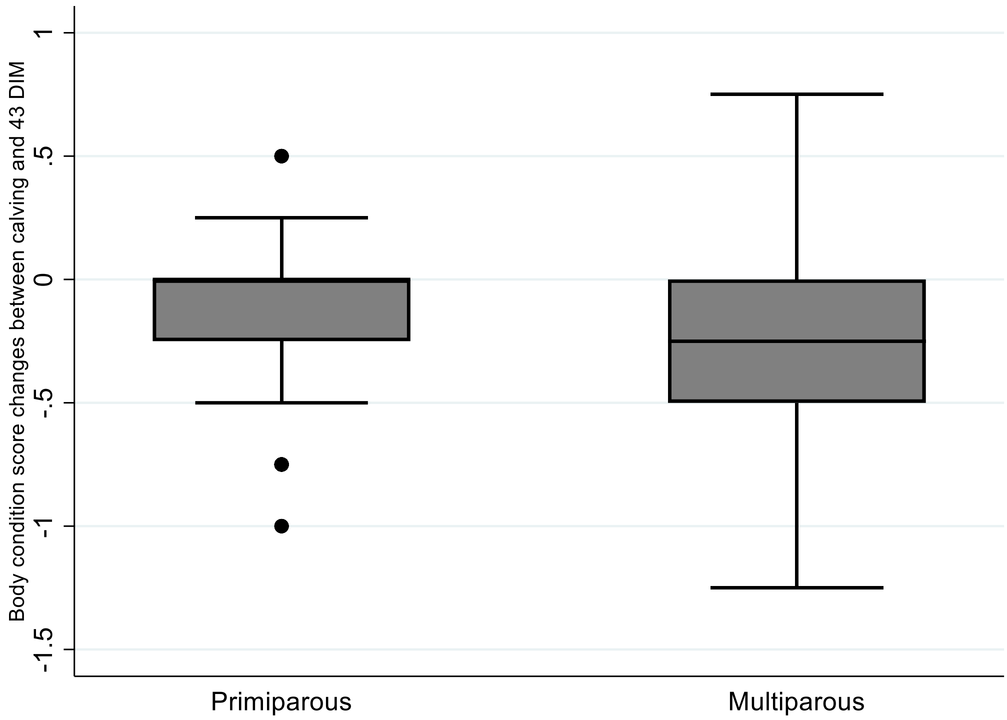


Figure 3-5. Body condition score changes between calving and 43±3 DIM by parity

The box and whisker plots show the variation of body condition score (BCS) between calving and 43±3 DIM stratified by parity. The horizontal dark line in each box plot is the median value for each BCS change, using 0.5 points of variation.

There was a difference in the proportion of anovular cows between farms (*P* < 0.01), with 5.8% (n = 36/620) and 10.7% (n = 44/410) for Farm 1 and 2, respectively. However, these differences were not associated with either parity (*P* = 0.1) or reproductive strategy (*P* = 0.93). A greater proportion of cows with BCS ≤ 2.5 (18.8%; n = 15/80) were anovular compared with animals with BCS > 2.5 at 43±3 DIM (10.7%; n = 102/950) (*P* = 0.04) (Figure 3-6). Also, anovulation was higher in the cohort of cows that lost ≥ 0.5 points of BCS between calving and 43±3 DIM (33%; n = 24/183) than cows losing 0.25 points, gained, or maintained BCS (18.9%; n = 159/840) (*P* < 0.01) (Figure 3-7). Ovular cows had higher W4MK than anovular cows (n = 947; *P* = 0.05).

A greater proportion of cows in DO have a CL at US examination at G1 (*P* < 0.01) and PG1 (*P* = 0.02) than cows enrolled in the OD-Ov and OD-PR protocols. However, there was no effect of parity or farm. There was a larger proportion of multiparous cows receiving beef sire AI compared to primiparous cows (21.7% vs 14.4%; *P* < 0.01).

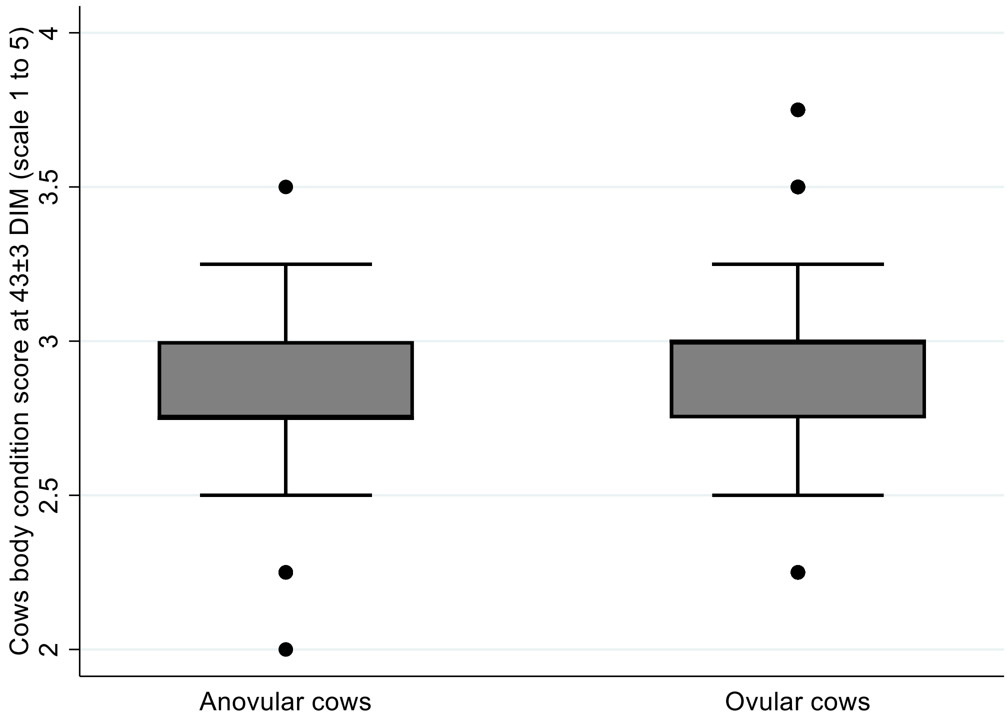


Figure 3-6. Body condition score of ovular and anovular cows at 43±3 DIM

The box and whisker plots show the body condition score (BCS) at 43±3 DIM, stratified by ovarian cyclicity status. The horizontal dark line in each box plot is the median value for each BCS, using a scale of 1 to 5 and 0.25 increments.

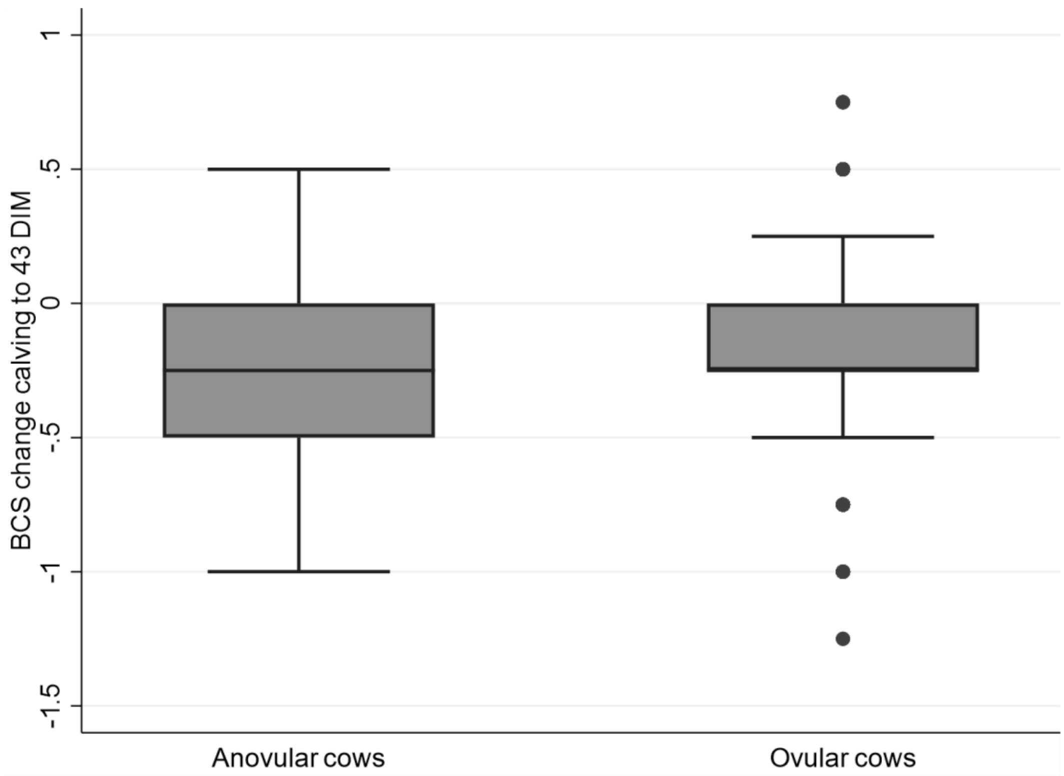


Figure 3-7. Body condition score changes between calving and 43±3 DIM in ovular and anovular cows

The box and whisker plots show the variation of body condition score (BCS) between calving and 43±3 DIM, stratified by ovarian cyclicity status (anovular and ovular). The horizontal dark line in each box plot is the median value for each BCS change using 0.5 points of variation.

3.1.2 Pregnancy per AI after first insemination

Regression coefficients for the final logistic regression models for P/AI 32±3d and 63±3 d after first AI in 1030 cows enrolled in the trial are summarised in Table 3-2. Odds ratios (OR) are also presented for each coefficient, with 95% confidence intervals for each estimate (95%CI). P/AI 32±3 d after first AI or at 63±3 days did not differ among OD-Ov, OD-PR and DO reproductive strategies. Multiparous cows had lower P/AI than primiparous cows for all reproductive strategies 32±3 d after first AI (OR = 0.49, 95% CI =0.38-0.63; $P < 0.01$) and at pregnancy confirmation (OR = 0.52, 95% CI = 0.40-0.67; $P < 0.01$).

Cows on Farm 2 were more likely to become pregnant than cows on Farm 1 (OR: 1.38; 95% CI = 1.06-1.81; $P = 0.02$). Moreover, cows with BCS > 2.5 at 43±3 DIM on both farms had greater odds of becoming pregnant to first insemination compared with cows with BCS ≤ 2.5 (OR: 1.55; 95% CI = 1.02-2.39; $P = 0.04$). There was a numerical difference in P/AI between cows that received FTAI using Ovsynch (37.5%; n = 54/144) and PRIDsynch (41.7%; n = 70/168) in OD/Ov and OD-PR strategies, respectively; however, this difference was not statistically significant ($P = 0.23$). Significant differences between farms ($P < 0.01$) and sires ($P = 0.02$) on P/AI were observed at 63±3 days.

Table 3-2. Regression coefficients for the final logistic regression models for P/AI in cows diagnosed pregnant 32±3 d and 63±3 d relative to first AI

Variable	n	Pregnancy 32±3 d (%)	Odds ratio (95% CI)	*P*-value	Pregnancy 63±3 d (%)	Odds ratio (95% CI)	*P*-value
Protocol							
OD-Ov	333	43.2	Referent		39	Referent	
OD-PR	358	41.6	0.94 (0.69-1.28)	0.71	39.9	1.06 (077-1.45)	0.72
DO	339	45.7	1.14 (0.84-1.56)	0.4	42.5	1.21 (0.88-1.66)	0.24
Farm							
1	619	41.9	Referent		39	Referent	
2	406	45.9	1.38 (1.06-1.81)	0.02	42.7	1.42 (1.08-1.86)	0.01
Parity							
Primiparous	400	54	Referent		50.2	Referent	
Multiparous	625	36.8	0.49 (0.38-0.63)	<0.01	34.2	0.52 (0.40-0.67)	<0.01
BCS 43 DIM							
≤2.5	115	35	Referent		29.9	Referent	
>2.5	910	44.6	1.55 (1.02-2.39)	0.04	41.8	1.77 (1.15-2.87)	<0.01
Sire							
Beef	194				32	Referent	
Holstein	836				42.5	1.51 (1.08-2.13)	0.02

3.1.3 Proportion of cows pregnant in cows that received AI by 83 DIM

The current study design did not allow to AI cows in DO reproductive strategy before 83 DIM, whereas in OD-Ov and OD-PR a proportion of the cows may have already received 2 AI within that period. Therefore, pregnancy by 83DIM might be a preferable measurement than P/AI for first AI as starting to serve cows after OD at 50 DIM gives them opportunity to be reinseminated at approximately 21 d intervals before 83 DIM, increasing the probability of pregnancy. Double Ovsynch was expected to have higher P/AI after first AI but fewer animals pregnant by time due to the longer VWP.

Regression coefficients for the final logistic regression models for the proportion of cows pregnant 32±3 d after first AI and 63±3 d after first AI in 1030 cows enrolled in the trial are summarised in Table 3-3. As for the previous outcome measure of P/AI 32±3 above, odds ratios (OR) are shown for each coefficient, with 95% CI for each estimate. Although cows in OD-Ov and OD-PR had more than one opportunity for insemination (unlike animals in the DO group), results of pregnancy by 83 DIM also showed no difference between reproductive strategies (P = 0.99). Sixty-nine cows received more than one insemination to oestrus detection before 83 DIM in the OD-Ov (9.9%; n = 33/333) and OD-PR (10.1%; n = 36/358) groups. The proportion of pregnant cows by 83 DIM was lower (OR = 0.48, 95% CI = 0.37-0.62; P < 0.01) for multiparous (39.5%; n = 248/628) than primiparous (56.9%; n = 229/402) cows. On Farm 2, cows were more likely to become pregnant by 83 DIM than Farm 1 (49% vs 44.5%, respectively) (OR = 1.44; 95% CI =1.10-1.89; P < 0.01). Cows with BCS > 2.5 at 43±3 DIM were more likely to be pregnant at 83 DIM compared with cows with BCS ≤ 2.5 (47.4% vs 37.6%; OR = 1.54; 95% CI = 1.02-2.35; P = 0.04).

At pregnancy confirmation, there was no difference (P=0.68) between OD-Ov, OD-PR and DO reproductive strategies, but there was an effect of parity (P < 0.01), farm (P < 0.01) and BCS 43±3 DIM (P < 0.01). The proportion of pregnant animals by 83 DIM was higher (OR = 1.64; 95% CI = 1.02-2.70; P = 0.04) for ovular (47.2%; n = 448/950) cows than their anovular (36.2%; n = 29/80) counterparts by 83 DIM (32±3 d PD). At 63±3 d PD, cows that received an AI from a Holstein (48.1%; n = 402/836) sire were more likely (P < 0.01) to be pregnant by 83 DIM compared to cows that received an AI from a beef sire (38.7%; n = 75/194).

Table 3-3. Regression coefficients for the final logistic regression models for proportion of cows diagnosed pregnant 32±3 d and 63±3 d relative to AI by 83 DIM

Variable	Pregnancy 32±3 d (%)	Odds ratio (95% CI)	*P*-value	Pregnancy 63±3 d (%)	Odds ratio (95% CI)	*P*-value
Protocol						
OD-Ov	46.5	Referent		42	Referent	
OD-PR	46.6	1.02 (0.74-1.38)	0.92	44.7	1.13 (0.84-1.55)	0.41
DO	45.7	1 (0.73-1.37)	0.99	42.5	1.06 (0.78-1.46)	0.7
Farm						
1	44.5	Referent		41.5	Referent	
2	49	1.44 (1.10-1.89)	<0.01	45.6	1.44 (1.10-1.89)	<0.01
Parity						
Primiparous	57	Referent		53.2	Referent	
Multiparous	39.5	0.48 (0.37-0.62)	<0.01	36.6	0.51 (0.40-0.67)	<0.01
BCS 43 DIM						
≤2.5	37.6	Referent		32.5	Referent	
>2.5	47.4	1.54 (1.02-2.35)	0.04	44.5	1.76 (1.15-2.72)	0.01
Sire						
Beef				33	Referent	
Holstein				45.5	1.63 (1.17-2.29)	<0.01
Cyclicity						
Anovular	36.2	Referent				
Ovular	47.2	1.64 (1.02-2.70)	0.04			

3.1.4 Time to pregnancy

Pairwise log-rank tests showed that time to pregnancy relative to AI differed significantly between OD-Ov and DO (P = 0.02). Animals in the OD-PR group tended to have a shorter time to pregnancy than the animals in DO group (P = 0.06). No significant difference in time to pregnancy was observed between the OD-Ov and OD-PR groups (P = 0.7) (Figure 3-8).

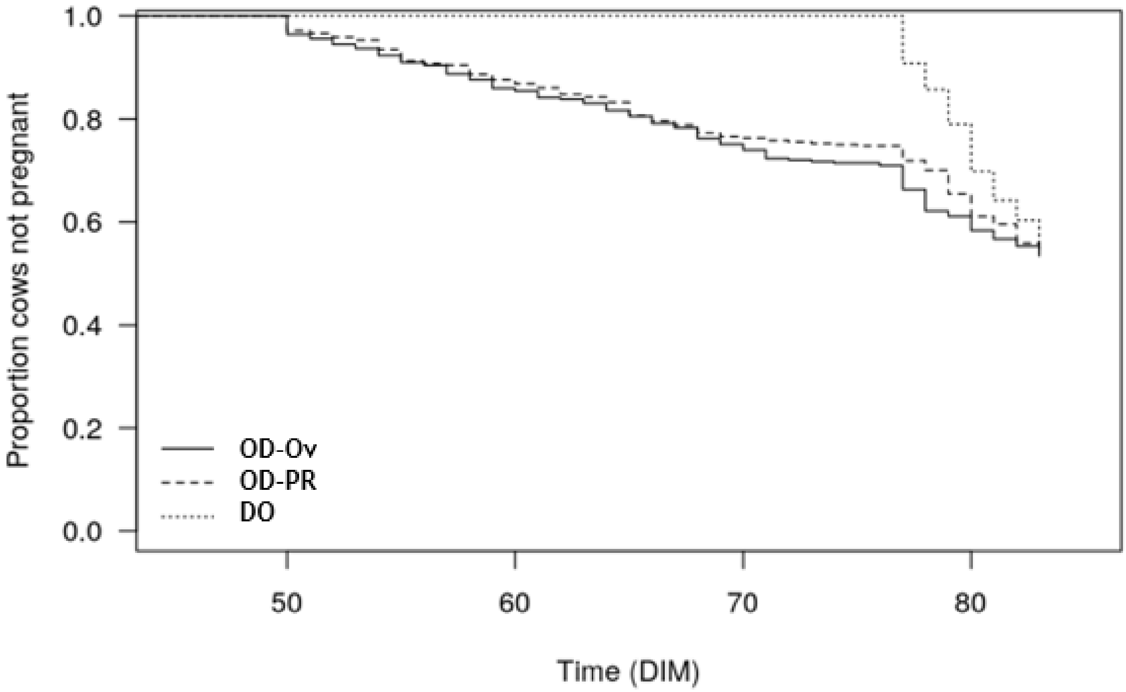


Figure 3-8. Kaplan-Meier survival curves for time to pregnancy

Time to pregnancy relative to AI in 1030 lactating dairy cows enrolled in a randomized controlled trial comparing the reproductive performance between three different reproductive strategies: oestrus detection – Ovsynch (OD-Ov), oestrus detection – PRIDsynch (OD-PR) and Double Ovsynch (DO).

3.1.5 Pregnancy loss

Regression coefficients for the final logistic regression models for PL after first AI, and by are described in Table 3-4.

Pregnancy loss was significantly higher in the OD-Ov group than in the OD-PR group after first AI (9.7 % vs 4.1%; *P* = 0.04) and by 83 DIM (9.7 % vs 4.2%; *P* = 0.02), but neither of these differed from the DO group (*P* = 0.19 and *P* = 0.35, respectively). Cows with BCS ≤ 2.5 at 43±3 DIM had greater PL than cows with BCS > 2.5 after first AI AI (OR = 0.30; 95% CI = 0.10-0.88; *P* = 0.03) and by 83 DIM (OR = 0.34; 95% CI = 0.12-0.98; *P* = 0.05). Cows with RP had greater odds of PL after first insemination (*P* = 0.01) and by 83 DIM (*P* < 0.01). In addition, cows pregnant from a beef sire were 3.13 and 2.92 times more likely to suffer PL after first AI (95% CI = 1.39-7.01; *P* < 0.01) and by 83 DIM (95% CI = 1.31-6.54; *P* < 0.01), respectively, compared with cows that received an AI from a Holstein sire. There was no effect of farm and parity on PL after first AI (*P* = 0.32 and *P* = 0.54, respectively) or by 83 DIM (*P* = 0.48 and *P* = 0.75, respectively).

Table 3-4. Regression coefficients for the final logistic regression models for pregnancy loss after first AI and by 83 DIM.

Variable	n	Pregnancy loss first AI (%)	Odds ratio (95% CI)	*P*-value	n	Pregnancy loss by 83DIM (%)	Odds ratio (95% CI)	*P*-value
	446				477			
Protocol								
OD-Ov	144	9.7	Referent		155	9.7	Referent	
OD-PR	148	4.1	0.33 (0.12-0.94)	0.04	167	4.2	0.31 (0.12-0.85)	0.02
DO	154	7.1	0.55 (0.23-1.34)	0.19	155	7.1	0.57 (0.24-1.33)	0.19
Farm								
1	260	6.9	Referent		276	6.9	Referent	
2	186	7.0	0.71 (0.31-1.60)	0.41	201	7.0	0.75 (0.34-166)	0.48
RP	425	6.4	Referent		453	6.2	Referent	
	21	19.1	4.75 (1.38-16.38)	0.01	24	20.8	5.97 (1.89-18.8)	<0.01
BCS 43 DIM								
≤2.5	40	15.0	Referent		44	13.6	Referent	
>2.5	406	5.9	0.30 (0.10-0.88)	0.03	433	6.2	0.34 (0.12-0.98)	0.05
Sire								
Holstein	374	5.4	Referent		402	5.5	Referent	
Beef	72	15.3	3.13 (1.39-7.10)	<0.01	75	14.7	2.92 (1.31-6.54)	<0.01

Chapter 4 Discussion

4.1 Effect of reproductive strategy in fertility

Pregnancy per AI at first postpartum service and proportion of cows pregnant by 83 DIM did not differ between the three reproductive strategies, providing evidence against the hypotheses that cows that receive AI after DO have increased P/AI when compared with OD plus FTAI; and that OD (OD-Ov and OD-PR) would result in a higher proportion of cows pregnant by 83 DIM than cows that received only the FTAI protocol (DO).

These results disagreed with previous studies reporting higher P/AI in DO when compared with AI to detected oestrus (Souza et al., 2008; Herlihy et al., 2012; Santos et al., 2017). However, the findings of the current work agreed with studies reporting no significant difference in P/AI when comparing AI after OD (visual, AAM and after PGF2α presynchronisation) plus FTAI with FTAI only (Neves et al., 2012; Denis-Robichaud et al., 2017; Stangaferro et al., 2018)

The similar reproductive performance of OD-Ov, OD-PR and DO observed in the present study may have resulted from a combination of high submission rate and consequent high P/AI in the cows that received AI after OD between 50 and 70±3 DIM for both OD-Ov and OD-PR strategies, and similar P/AI in the animals that were synchronised with Ovsynch, PRIDsynch and DO. These results are consistent with other studies that have associated good reproductive efficiency with high SR and P/AI when oestrus detection is part of the farm reproductive management (Ferguson & Skidmore, 2013; Denis-Robichaud et al., 2017; Stangaferro et al., 2018). Oestrus detection is a major control point of reproductive efficiency as it controls the insemination rate or frequency; and, with a good insemination technique, the proportion of fertilized oocytes could reach 60% or more (Grimard et al., 2006); however, 30 to 40 days after service P/AI is reduced due to embryonic death to around 40% (ranging from 30 to 50%) (Santos et al., 2004c; Grimard et al., 2006). Therefore, reproductive efficiency is an outcome of the combination of SR and P/AI within a farm.

Although the study design allowed for re-insemination of cows that returned to oestrus before 83 DIM in OD-Ov and OD-PR strategies, there was no difference in reproductive performance among OD-Ov, ED-PR and DO at 83 DIM, similar to previous reports (Denis-Robichaud et al., 2017; Stangaferro et al., 2018). Extending the VWP, such as in DO protocol, has been shown to reduce the proportion of animals with uterine inflammation and the number of cows with BCS $\leq$ 2.5 when starting the synchronization protocol, as well as the proportion of anovular cows (Sheldon et al., 2009; Stangaferro et al., 2018). Postpartum cows experience a reduction of DMI, large nutrient demands and metabolic adaptations to cope with the onset of the lactation, resulting in a state of NEB, which can be extended in severe cases (Nydam et al., 2017). An extension of the VWP would provide the cows with more time to overcome NEB, re-establish immune function, reduce the incidence of postpartum uterine bacterial infection and re-establish cyclicity before AI (Sheldon et al., 2009; Nydam et al., 2017; Stangaferro et al., 2018). In the present study, uterine inflammation was not evaluated, and the effect of extended VWP on BCS reported by Stangaferro et al. (2018) was not observed. This may be due to the differences between study design. In the Stangaferro et al. (2018) work, BCS was measured at 88 DIM instead of 70±3 DIM, giving the cows more time to recover after calving. However, similar to the present study, an extended VWP did not result in a significant increase in P/AI so as to outperform OD-Ov and OD-PR (Stangaferro et al., 2018).

4.2 Effect of prolonged postpartum anovulation on fertility

In this study, the US assessment of the CL was carried out as a proxy for circulating P4 concentrations. Although the sensitivity and specificity of the US evaluation may be affected by the cow reproductive physiologic stages (i.e., cows undergoing luteal regression, cows in proestrus) and human error, it has been reported that this method has a high level of agreement with the gold standard (P4 concentrations determined by RIA) (Silva, Sterry, & Fricke, 2007). Moreover, due to its practicality under field conditions, using ultrasound can result in early treatment of anovular cows (Silva, Sterry, & Fricke, 2007).

Several authors have described the negative effect of anovulation in dairy cow fertility (Santos et al., 2004c; Ribeiro et al., 2016). The prevalence of prolonged post-partum anovulation is commonly reported to be around 20% in high producing dairy cows (Opsomer et al., 2000; Walsh et al., 2007a; Bamber et al., 2009), in contrast to the 7.8% anovular cows (evaluated by US at 43±3 and 50±3 DIM) found in the current study. Such low anovular prevalence was only observed in a small number of other studies (Walsh et al., 2007a; Bamber et al., 2009).

Interactions between parity and anovulation are inconsistently reported in the literature. Most of the work that evaluated anovulatory condition in dairy herds reported that anovulation is more frequently found in primiparous cows than in multiparous cows (Opsomer et al., 2000; Gümen, Guenther, & Wiltbank, 2003; Souza et al., 2008; Santos, Rutigliano, & Filho, 2009; Monteiro et al., 2020); however, in other work multiparous cows are overrepresented (Herlihy et al., 2012). Furthermore, similar to the present study, other literature reported no difference in parity (Lopez et al., 2005). The results of the current work could be partly explained by the low proportion of primiparous cows with BCS ≤ 2.5 at calving (3.5%), at 43±3 DIM (7.7%), and the fact that only 11.6% of these animals lost ≥ 0.5 BCS between calving and 43±3 DIM, suggesting an overall good energy balance in this group of cows (Monteiro et al., 2020).

Anovulation is known to be influenced by disease (Ribeiro et al., 2013; Monteiro et al., 2020), farm (Santos, Rutigliano, & Filho, 2009), and BCS (Lopez, Satter, & Wiltbank, 2004). Monteiro et al. (2020) reported a prevalence of anovulatory cows of 28.5%, with 64%

of these animals suffering one disease event and 30% suffering two or more disease events. In the present study, 24% of the animals had one disease event, and only 5% of the animals had 2 or more disease events, which could partly explain the low prevalence of anovulation. Clinical and subclinical diseases have been associated with anovulation and reduction of P/AI, and when multiple diseases affect the animals, there is an increased negative impact in fertility compared with animals presenting a single disease (Ribeiro et al., 2013; Monteiro et al., 2020). Postpartum diseases such as hypocalcaemia and ketosis can predispose the animals to metritis and endometritis due to impaired immunity, compromising fertility (Ribeiro et al., 2013). In addition, changes in blood metabolites such as NEFAs and BHB would affect the follicular fluid composition, which could impair follicular function (i.e., steroidogenesis) and oocyte quality, delaying first postpartum ovulation and reducing fertility (Leroy et al., 2008c).

Moreover, in accordance with the present results, previous studies have demonstrated that as BCS decreases, the proportion of anovular cows increases (Lopez, Satter, & Wiltbank, 2004; Santos, Rutigliano, & Filho, 2009; Monteiro et al., 2020). However, this data should be interpreted with care because BCS used as an individual factor cannot fully explain anovulation in dairy cows since many individual cow risk factors may be implicated (Bamber et al., 2009).

As reported in previous work, in this study, cows with lower milk yield were more likely to be anovular than those with greater milk yield (Santos, Rutigliano, & Filho, 2009). This could be explained by the lower milk yield found in cows could be affected with post-partum, metabolic and other diseases, some of which were “subclinical”, but nevertheless having a detrimental effect on milk production and delaying their first post-partum ovulation (Monteiro et al., 2020).

4.3 Effect of P4 supplementation (PRID-synch) and presynchronisation (Double Ovsynch) on reproductive performance

Protocols such as PRIDsynch and Double Ovsynch were developed to overcome the synchronization limitations of Ovsynch. Although the Ovsynch protocol has been successful in re-establishment of ovarian cyclicity in anovular cows, it has been reported to result in poor synchronization rates and reduced P/AI when starting at a random stage of the oestrus cycle (Galvão, Sá Filho, & Santos, 2007; Souza et al., 2008; Giordano et al., 2012b).

The PRIDsynch protocol has been shown to re-establish cyclicity in anovular cows, improving synchronization rates and uterine environment for embryo implantation and survival (i.e., changes in uterine gland morphology and vascularization, endometrial expression of proteins); minimizing premature ovulation before prearranged FTAI and maximizing P/AI (Shaham-Albalancy et al., 1997; Stevenson et al., 2006; Cerri et al., 2011; Colazo et al., 2013; Bisinotto et al., 2015)

A limitation of the present study was that it was not designed (and was therefore underpowered) to detect a statistical difference in P/AI for cows synchronized with PRIDsynch and Ovsynch. However, the numerical results found agree with other studies reporting an increase in P/AI for PRIDsynch (~4.6%) when compared with protocols without P4 supplementation (Bisinotto et al., 2015). A low prevalence of anovulation and a large proportion of cows inseminated after OD between days 50 and 70±3 DIM (54.8%) may explain these results, as PRIDsynch benefits are especially reported in reproductive strategies based on FTAI only and where the proportion of anovulatory cows is high, and heat detection is poor (Bisinotto et al., 2015).

In the current study, the proportion of cows that lost the pregnancy was similar to the reported in the literature (Bamber et al., 2009; Bisinotto et al., 2015; Stangaferro et al., 2018). As reported in previous studies, there was an effect of P4 supplementation in PL, where cows in the OD-PR had reduced PL than cows in OD-Ov (Bisinotto et al., 2015). High P4 levels during follicular growth have been associated with optimum uterine environment, oocyte quality, subsequent fertilisation and embryo development, increasing embryo survival (Shaham-Albalancy et al., 1997; Stevenson et al., 2006; Cerri et al., 2011; Colazo et al., 2013; Bisinotto et al., 2015). The lack of difference in PL between OD-PR and DO could be due to presynchronisation success in DO; whereby most cows ovulated after G1, producing a CL and consequent high P4 concentrations during the follicular growth of the synchronized follicular wave.

In the current study, animals in OD-PR group had a reduced risk of PL compared with OD-Ov, suggesting an effect of P4 supplementation on embryo survival (Cerri et al., 2009; Rivera et al., 2011; Bisinotto et al., 2015). These results may be related to the increased P4 concentration during the synchronised follicular wave provided by the PRID, increasing the oocyte quality, subsequent fertilisation and embryo development in an optimum uterine environment (Cerri et al., 2011; Rivera et al., 2011).

Double Ovsynch has demonstrated higher P/AI in first lactating animals compared with multiparous animals (Souza et al., 2008; Astiz & Fargas, 2013). Our data showed no interaction between DO and parity, which could be explained by a similar proportion of anovulatory animals in the primiparous and multiparous groups, and the administration of a two doses of PGF2α in all synchronisation protocols (Wiltbank et al., 2015; Santos et al., 2016; Borchardt et al., 2017). It has been reported that the effect of DO increasing P/AI only in primiparous cows may be related to a greater rate of CL regression after a single dose of PGF2α compared with multiparous cows (Giordano et al., 2013). Greater metabolism of PGF2α in multiparous than primiparous cows may be responsible of the reduced responsiveness to the administration of this hormone (Giordano et al., 2013). Therefore, treatment with a higher dose of PGF2α or an increased number of PGF2α treatments appears to increase CL regression, lowering P4 concentrations near AI and improving fertility (Giordano et al., 2013; Wiltbank et al., 2015; Borchardt et al., 2017).

Previous studies have also shown that presynchronization using DO increase ovulation rates and the proportion of cows with a CL present in one of the ovaries at the initiation of the breeding Ovsynch. Therefore, the response to PGF2α administration of the carried over CL and the potential new CL formed after G1 administration will be enhanced, improving synchronisation rates and fertility (Galvão & Santos, 2010; Giordano et al., 2012b; Carvalho et al., 2018). The previously reported effect of presynchronisation in DO protocols, measured by the greater proportion (~10% higher) of cows with a CL at the initiation of the breeding Ovsynch, was seen in the current study, with 89.6% of the cows bearing at least one CL in one of the ovaries at G1 administration (Souza et al., 2008; Herlihy et al., 2012; Ayres et al., 2013; Dirandeh, Roodbari, & Colazo, 2015). However, there was no effect on P/AI as previously reported, which can be explained by the low proportion of anovular cows in all treatment groups and high SR and CR in cows receiving AI at detected oestrus (Souza et al., 2008; Herlihy et al., 2012; Dirandeh, Roodbari, & Colazo, 2015).

4.4 Effect of farm, parity, and energy balance in fertility

4.4.1 Farm

A combination of farm-specific characteristics such as genetics (Bamber et al., 2009), cow health (Monteiro et al., 2020), parity (Souza et al., 2008; Santos, Rutigliano, & Filho, 2009), milk yield (Santos, Rutigliano, & Filho, 2009) and BCS (Bamber et al., 2009) may have contributed to the low anovulation prevalence observed in the current study. Other unmeasured farm management risk factors may also have contributed to the incidence of anovulatory cows over the study period.

In this study, there was no interaction between farm and reproductive strategy, as described by other authors (Denis-Robichaud et al., 2017); however, P/AI was 4% higher in Farm 2 when compared to Farm 1. The difference in P/AI between farms may be linked to unmeasured differences, including genetics, overstocking, and other farm-specific factors (i.e., management, facilities) (Santos, Rutigliano, & Filho, 2009; Denis-Robichaud et al., 2017).

4.4.2 Parity

Higher P/AI in primiparous cows when compared with multiparous cow have been reported in several controlled trials (Moreira et al., 2001; Gümen, Guenther, & Wiltbank, 2003; Silva, Sterry, & Fricke, 2007; Herlihy et al., 2012); although these differences are not yet fully understood, it is believed that postpartum diseases, including hypocalcaemia, ketosis, RP, DA; and other diseases such as mastitis, BRD and lameness, more commonly found in multiparous cows, and may have detrimental effects on fertility (Sheldon et al., 2009; Mayasari et al., 2019; Monteiro et al., 2020). In this study, primiparous cows were less likely to have ketosis, lameness and RP, suggesting that this group of animals may be under less metabolic stress and have better uterine health than multiparous cows (Butler, 2000; Sheldon et al., 2009). In addition, housing the primiparous cows separately from the multiparous cows may have facilitated their adaptation to the dairy system and improved their health (Val-Laillet et al., 2009; Østergaard, Thomsen, & Burow, 2010). For example, when primiparous and multiparous cows are housed together, due to the lower hierarchical status of primiparous animals, they have less socio-positive relationships (Val-Laillet et al., 2009), spend less time eating (Grant & Albright, 1995), and experience higher NEB (Østergaard, Thomsen, & Burow, 2010).

4.4.3 Energy balance

In the current study, milk yield was not linked to P/AI and PL, which is consistent with some previous studies (López-Gatius et al., 2002; Chebel et al., 2004; Santos, Rutigliano, & Filho, 2009). However, greater milk yields have also been associated with increased metabolism, NEB and poor reproductive performance (Lucy, 2001; Butler, 2003; Wathes et al., 2007). Previous studies have reported that high producing dairy cows have decreased circulating progesterone, having a detrimental effect on follicular development, and therefore, on embryo quality (Inskeep, 2004; Sartori et al., 2004). However, milk yield is also linked to dry matter intake (DMI) (Liefers et al., 2003), suggesting that cows with high milk production and high DMI may have adequate energy balance during early lactation. Mobilization of non-esterified fatty acids (NEFAs) and β-hydroxybutyrate (BHB) when cows are in NEB has been linked to a reduction in fertilization and embryo quality and development. (Leroy et al., 2005; Walsh et al., 2007b). Body condition score and BCS changes are widely used to assess energy balance in dairy cows and have been associated with fertility performance in several studies (Domecq et al., 1997; Santos et al., 2009; Carvalho et al., 2014). Although in the current work, the cows were scored at different time points than in previous studies, our results agree with those reporting that cows with BCS $\leq$ 2.5 near AI had decreased P/AI compared with cows with BCS > 2.5 (Santos, Rutigliano, & Filho, 2009; Carvalho et al., 2014). However, the effect of BCS loss in fertility was not observed in the current work (Carvalho et al., 2014).

4.5 Effect of sire selection on fertility

In the current study, it was observed that cows that received a beef sire AI had higher pregnancy loss and numerically lower P/AI than cows receiving Holstein sire AI. This could be because the farm's breeding program dictates that beef sires be used in cows with poor fertility performance in previous lactations (McWhorter et al., 2020). Other studies have linked pregnancy loss with sire; however, no link to a specific breed type was mentioned (Markusfeld-Nir, 1997; López-Gatius et al., 2002; Starbuck, Dailey, & Inskeep, 2004). PL and embryo survival could be related to sire genetics, which has been identified as having a greater effect on PL than maternal effects (Bamber et al., 2009). These data should be interpreted with caution but suggest that beef sires, commonly used as terminal sires, may not have been selected for their fertility traits. However, further studies on the effect of sire on fertility could provide more evidence about the genetic implications of Holstein and beef terminal sires in PL.

4.6 Economic impact of extended VWP and reproductive strategies

A limitation of this work was that the economic parameters for each reproductive strategy were not evaluated. However, several other studies have evaluated the impact of an extension of the VWP for different reproductive strategies. Some studies have found that cows with longer VWP resulted to fewer calvings, lower incidence of postpartum metabolic diseases, lower veterinary costs, less culling with fewer replacements needed, and an overall improvement in herd life, animal well-being and dairy farm profitability (Van Amburgh et al., 1997; Arbel et al., 2001); however, in the farms enrolled in these studies, synchronisation protocols were not part of their reproductive strategies.

In contrast, in a more recent experiment performed by Stangaferro et al. (2019), it was found that only primiparous cows had higher economic returns (calculated combining daily income over feed cost, replacement cost, calf value, recombinant bovine somatotropin treatment cost, reproductive cost, and other operating expenses) when the VWP was extended from 60 to 88 d in FTAI (Double Ovsynch) strategies, and also when using a combination of AI at detected oestrus at 50 DIM and FTAI at 72 ± 3 DIM (Presynch Ovsynch for first AI and Ovsynch for second and subsequent AI) (Stangaferro, Wijma, & Giordano, 2019). Another study performed by Giordano et al. (2012a) in the United States focused on comparing the economic and reproductive performance of different reproductive strategies: i) One hundred per cent FTAI (Double Ovsynch) (42% CR for first TAI and 30% for second-and-later services); ii) Combination of AI at detected oestrus and FTAI (Ovsynch), with different levels of SR (ranged from 30 to 80%) and CR (25, 30, and 35%). The modelling output was represented as the net value (NV) of each reproductive strategy that was calculated with the aggregate of sum of milk income over feed cost, replacement and mortality cost, income from new-borns, and reproductive costs (i.e., hormones, cows handling and oestrus detection costings) (Giordano et al., 2012a). They found that reproductive strategies combining AI at detected oestrus and FTAI resulting in a CR of 35% for cows receiving AI at detected oestrus had the greatest NV and reproductive performance at all levels of SR compared to FTAI only. In addition, they reported that the strategy based on 100% FTAI after DO had greater

NV and better reproductive performance than the combination of OD and FTAI when CR in cows receiving AI at detected oestrus was 25% or below (Giordano et al., 2012a).

On the other hand, other studies reported no difference in profitability for cows with different VWP lengths (Chebel & Santos, 2010; Gobikrushanth et al., 2014). Chebel and Santos, (2010) found no difference in cow profitability when comparing cows managed with a short and long (49 ± 3 DIM and 72 ± 3 DIM, respectively) VWP. Similarly, Gobikrushanth et al. (2014) observed that cows with an extension of the VWP during the spring, autumn and winter (from 57 to 63 d) and during the summer (from 64 to 121 d) did not increase the profitability of the animals evaluated.

The results obtained in the current study of high SR and CR in both strategies combining OD and FTAI, and the higher cost of the hormones in the UK market compared with the US, seemed to suggest that reproductive strategies combining OD and FTAI may yield similar reproductive and higher economic performance when compared to FTAI only. However, despite the similarities between UK and USA intensive indoor dairy systems, Giordano et al. (2012a) results cannot be fully extrapolated to this study as an economic analysis of the farms enrolled in the current experiment under UK market price conditions was not performed. A possible area of future research would be to investigate the economic performance of the reproductive strategies discussed above in a detailed manner to determine the most profitable reproductive strategy evaluating UK market price conditions.

Conclusion

The present study did not detect differences in P/AI for first AI and proportion of cows pregnant by 83 DIM when comparing reproductive strategies combining oestrus detection and Ovsynch (OD-Ov), oestrus detection and PRIDsynch (OD-PR) and Double Ovsynch (DO). Combined high SR and P/AI for cow's receiving AI at detected oestrous and FTAI, low prevalence of post-partum diseases and low prevalence of prolonged post-partum anovulation in the farms enrolled in the current study may have contributed to the similar performance of DO and OD-PR, when compared with OD-Ov. Overall fertility performance was affected by cow factors such as BCS and parity; and other unaccounted farm-specific characteristics. Lower PL found in the OD-PR and DO strategies suggested that a high P4 environment after P4 supplementation and Presynchronisation (resulting in a high proportion of cows with a CL present during follicular growth) may have had a positive effect on embryo quality and survival. Cows that received AI from a beef sire were more likely to lose pregnancy; however, this study was not designed to measure the genetic impact of the sire on fertility; therefore, these results, in particular, need to be interpreted with caution. Future studies should assess the impact of terminal sires in high producing dairy cow's fertility, particularly in relation to fertility and embryo survival; and to determine the most profitable reproductive strategy for dairy farms in the UK market price conditions.

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