Reproduction in post-parturient cattle and effects of prostaglandin injection

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Summary

The objectives of this study were to investigate the effect of prostaglandin on reproductive performance, endocrinology and uterine involution in post-partum beef cows and commercial dairy cows and in aborted dairy cows.

The first experiment was conducted over the course of three years using spring-calving Hereford Friesian beef cows. In the first year six cows and six heifers received no treatment. In the second and third years cows were injected with prostaglandin (10mg) either 7, 14, 21 and 28 days (n=7), or 14 and 28 days (n=7) *post-partum*. An additional group (n=7) was injected with saline 7, 14, 21 and 28 days *post-partum*. Ovarian activity was monitored by measuring plasma progesterone concentration in blood samples collected twice weekly and the animals were also observed closely for signs of oestrous behaviour. Additional blood samples were obtained from an indwelling jugular vein cannula at 15 minute intervals, for two hours before and four hours after prostaglandin or saline injection, during the third year (3 animals/group) for analysis of plasma LH and oestradiol-17β concentration. During the second year, the dimensions of the uterus and cervix were measured by ultrasonography at weekly intervals from parturition (4 animals/group). Cows and calves were turned out to pasture approximately eight to nine weeks *post-partum* and were run with a Charollais bull fitted with a chin ball-marker. Service dates were recorded.

The interval from calving to first oestrus was longer in cows which received two injections of prostaglandin than in cows which received four injections of prostaglandin or saline (78.8 ± 6.2 versus 67.8 ± 3.4 or 74.0 ± 7.6; mean ± SEM).

Conception rates and interval from calving to conception (determined by plasma progesterone concentration and oestrous records) were variable between groups but were unrelated to prostaglandin treatment. The interval from calving to first oestrus was significantly longer in heifers than cows (105.7 ± 4.5 versus 82.8 ± 3.0).

Plasma progesterone concentrations generally remained basal (≦ 0.5 ng/ml) until turn out. A rise in plasma progesterone concentration, which was not maintained for the duration of a normal cyclical corpus luteum, was recorded in most of the cows before first observed oestrus *post-partum*. The remaining cows had a prolonged period of anoestrus characterized by basal plasma progesterone concentration before first oestrus. Basal plasma LH concentration (≦ 0.5 ng/ml) were observed throughout the intensive bleeding period and no significant effect of prostaglandin was observed. Plasma oestradiol-17β concentrations in the same samples were generally below the detection limit of the assay (2 pg/ml).

Some parameters of uterine size, particularly the thickness of the uterine body wall, involuted faster in control cows than in PGF2α treated cows and it also appeared that uterine involution proceeded less regularly in the latter.

In a second experiment eight pregnant Friesian dairy cows were injected with prostaglandin (10mg) and all subsequently aborted; time from injection to abortion generally increasing with the length of pregnancy (determined by measurement of fetal crown-rump length). Four cows were injected with prostaglandin (10mg) approximately 5 and 12 days after abortion. Four cows received saline at comparable times. Blood samples were obtained daily from the day of prostaglandin injection to induce abortion and analysed for plasma progesterone concentration. Additional

intensive blood samples were obtained at 15 minute intervals for two hours before and four hours after prostaglandin or saline injection and plasma was analysed for LH and oestradiol-17β concentration. Palpation of the ovaries *per rectum* was performed regularly for the first 20 days after abortion.

Time from abortion to first oestrus was generally proportional to the length of the pregnancy. Plasma progesterone concentration illustrated a similar trend, namely that ovulation and corpus luteum formation generally occurred earlier in early pregnant cows. Oestrus occurred sooner in two cows which possessed corpora lutea at the time of prostaglandin treatment compared to two cows in the control group which had corpora lutea when injected with saline (13.0 ± 1.06 versus 18.5 ± 2.0 days). Plasma LH concentrations were basal (≦ 1.0 ng/ml) before and after prostaglandin or saline injection. Plasma oestradiol concentration generally remained below the detection limit of the assay (≦ 2 pg/ml) in the same samples. The experiment failed to demonstrate any effect of prostaglandin on short-term LH and oestradiol concentrations, the only advantage of prostaglandin injection being induction of luteolysis and the subsequent shortening of the interval from abortion to oestrus in cows with corpora lutea.

In a third experiment the effect of prostaglandin on reproductive performance, endocrine activity and uterine involution were studied in commercial Friesian dairy cows. Twenty one dairy cows which calved in late winter were used. One group (n=11) were injected with prostaglandin (10mg) 14 days *post-partum* while another group (n=10) received saline at this time. Blood samples were collected every two days from two days before injection to eight days after injection for analysis of plasma progesterone concentration. Additional half-hourly blood samples were collected

starting half an hour before and continuing until two hours after prostaglandin or saline injection. Careful oestrus detection and insemination records were kept. The progress of uterine involution was assesed by measurement of the uterine horns and the cervix *per-rectum* once on the day of prostaglandin or saline injection (14 days *post-partum*) and again 10 days later.

The interval from calving to first oestrus did not differ between treated and control cows, being approximately 80 and 81 days respectively. First service conception rate tended to be poorer in the prostaglandin treated compared to the saline treated cows (50% versus 78%) and the prostaglandin treated cows also required more services per conception (1.6 compared to 1.3).

Plasma progesterone concentrations were generally basal but showed that one cow in the control group and one in the PGF2α treated group had ovulated by the time of treatment at day 14 *post-partum* and injection of prostaglandin did not alter the pattern of progesterone concentrations, which remained basal in most cows. Plasma LH concentration was low in samples collected before and after prostaglandin and saline injection and there was no response to treatment. Plasma oestradiol-17β concentration was undetectable ($\leqq$ 2pg/ml) in approximately half of these samples and variable, reaching a maximum concentration of 8 - 10 pg/ml in the remaining samples. Again there was no detectable effect of prostaglandin injection on plasma oestradiol concentration. The rate of uterine involution was not different between treated and control cows 10 days after treatment.

Taken together these results show that prostaglandin injection into post-parturient cattle does not elicit any significant alteration in the activity of the pituitary-ovarian endocrine axis; nor does it enhance the rate of uterine involution.

Furthermore, reproductive cyclic activity did not resume earlier in treated cows so routine prostaglandin administration cannot be recommended.

Chapter I

Reproduction in the cow

Introduction

Most domesticated breeds of cattle are polyoestrous and breed all year round but there is some evidence of a seasonal effect on reproduction. Calves born during the period of increasing day length reached puberty approximately two months earlier than those born in decreasing day length (Roy *et al.*, 1980). Also spring calving beef and dairy cows had a longer period between calving and first ovulation than autumn calving cows (Bulman and Lamming, 1978; King and Hurnik, 1980; Peters, 1980; Peters and Riley, 1982a).

After puberty regular sequences of reproductive events occur in the female which are known as oestrous cycles. The oestrous or ovarian cycle is the interval between two periods of sexual receptivity or oestrus and varies from 18 - 24 days. The average length of the cycle is 20 and 21 days in heifers and cows respectively (Hansel, 1959). Compared to most farm animals, cows have a short duration of oestrus averaging only 18h (Asdell, 1964). The oestrous cycle comprises a follicular phase and a luteal phase. During the former, one or more follicles develop in the ovary while, during the latter the corpus luteum develops and achieves maximum activity before undergoing degeneration. Both of these phases are the result of complex hormonal interactions. The major hormones involved in these reproductive processes are pituitary gonadotrophins and ovarian steroids. The major gonadotrophins are follicle stimulating hormone (FSH) and luteinizing hormone (LH) and the main ovarian steroids are oestrogens and progesterone.

Growth of the Graafian follicle during the follicular phase and the accompanying increase in oestrogen synthesis and secretion are stimulated by both FSH and LH. This phase is terminated when the follicle ruptures and releases the ovum into the oviduct. This process is known as ovulation and usually occurs

spontaneously in the cow between 10 and 12h after the end of oestrus (Hansel, 1959). Following ovulation the cavity of the ruptured follicle is invaded by proliferating granulosa and theca interna cells which differentiate to form the corpus luteum under the influence of LH. The secretion of progesterone by the corpus luteum dominates the cycle from day 4 until day 17. In the non-pregnant cow the corpus luteum is destroyed by 17 days after oestrus and this permits the next cycle to be initiated. The duration of oestrus and the timing of ovulation with respect to oestrus do however vary in beef and dairy breeds. The beef cow has a longer duration of oestrus (20h) and ovulates later (31h after the end of oestrus) compared to 15 and 29h respectively for comparable times in the dairy cows (Hafez, 1980). Oestrous cycles cease after fertilization of the ovum and establishment of pregnancy.

The length of gestation varies between different breeds and types of cattle but is on average 280 days (Williamson and Payne, 1978). There is a period of acyclicity or anoestrus *post-partum*, the length of which varies between beef and dairy breeds. Thus dairy cows resume oestrus cycles sooner *post-partum* than beef cows. Bulman and Lamming (1978) observed that 95% of dairy cows had resumed ovarian cycles by 50 days *post-partum* where as only 40% of suckled beef cows had done so by this time (Peters and Riley, 1982a). Delayed onset of ovarian cyclicity after calving may also be caused by several other factors e.g. age, breed and nutrition.

Endocrinology of reproduction

Reproduction is controlled through the interactions of the nervous system and the endocrine glands. The nervous system itself performs an endocrine function through various neuro-endocrine glands. Examples of such glands which play a

role in reproduction are the hypothalamus, pineal, pituitary and adrenal cortex. The involvement of the hypothalamus and the pituitary gland is central to the control of reproductive processes.

The hypothalamus has a close association with the pituitary through a unique vascular connection which was demonstrated by the work of Harris (1949; 1950). Arterial blood flows into the pituitary stalk and median eminence by way of the superior and inferior hypophyseal arteries and forms a capillary plexus within the median eminence. From this so called primary plexus, blood passes to the anterior pituitary through the hypothalamo-hypophyseal portal vessels and into a secondary capillary plexus within the anterior pituitary gland. There is also a retrograde flow of venous blood to the hypothalamus (Bergland and Page, 1978). Both of these vascular arrangements probably play a role in the feedback control of the hypothalamo-pituitary axis as was proposed by Sawyer and Kawakami (1961).

It has been shown that the hypothalamus controls pituitary gonadotrophin secretion through a releasing hormone. This was isolated and purified and shown to evoke both LH and FSH release *in vivo* (Kastin *et al.*, 1969) and *in vitro* (Mittler *et al.*, 1970) and hence is now commonly called gonadotrophin releasing hormone (GnRH).

Release of both LH and FSH can be induced by administration of purified or synthetic GnRH Schams *et al.* (1979) and Foster *et al.* (1980) induced the release of both LH and FSH by injecting 100 to 500μg GnRH in cattle. Similar results were also reported by Riley *et al.* (1981) and Walters *et al.* (1982) after repeated intravenous injections of smaller doses (0.5 to 50μg). Conversely, gonadotrophin output can be reduced by neutralizing endogenous GnRH activity by administration of specific antibodies for the neurohormone (Fraser *et al.*, 1984) or after active immunization against GnRH (Jeffcoate and Keeling, 1984)).

The secretory pattern of LH and FSH, which can be measured by taking frequent samples of jugular blood, is found to be pulsatile - pulses occurring at approximately hourly intervals hence the term circhoral pulses. A similar pattern of pulses in the secretion of GnRH has been measured in portal blood (Levine *et al.*, 1982). These pulses tend to be well synchronized with pulses of gonadotrophins, further evidence for the role of the hypothalamus in regulating gondotrophin secretion, and thereby gonadal and steroidal activity (Sarkar *et al.*, 1976). However, the secretion and synthesis of FSH and LH by the pituitary are both critically dependent on GnRH secretion from the hypothalamus. It is believed that the regulation of gonadotrophin secretion may be carried out in two ways:- by varying the frequency or amplitude of the circhoral pulses of GnRH delivered to the anterior pituitary, or by modulating the response or sensitivity of the pituitary to GnRH (Clarke and Cummins, 1985). Both methods of gonadotrophin regulation probably exist in normal physiological conditions, the former being chiefly concerned with regulating reproduction through the action of the central nervous system while the latter is particularly involved in the control of ovarian cycle (Johnson and Everitt, 1980). Ovarian steroids also influence the secretion of pituitary gonadotrophins as their concentrations in plasma fluctuate during the course of ovarian cycle Goodman and Karsch (1980). Peters (1985) emphasised the role of steroids on the release and secretion of pituitary gonadotrophins during the bovine oestrous cycle after noting that the response of FSH and LH to GnRH varied during the follicular and luteal phases of the cycle.

The endocrine changes which occur during the bovine oestrous cycle have been studied extensively in recent years (e.g. Webb *et al*, 1980; Baird and McNeilly, 1981; Walters and Schallenberger, 1984; Schallenberger *et al.*, 1984). During the follicular phase there is an increase in LH and FSH pulse frequency

which stimulates the development of a large, preovulatory follicle which in turn secretes increasing amounts of oestradiol. Oestradiol is secreted in a pulsatile fashion and each pulse is closely related to a pulse of LH. This positive feedback relationship between LH and oestradiol results in an increase in the amplitude and frequency of both LH and oestradiol pulses. Ultimately surges in the plasma concentrations of LH and oestradiol are seen which cause ovulation. During the luteal phase FSH is secreted at a higher frequency than LH and each pulse of LH results in a pulse of progesterone. It is also evident from the above studies that LH appears to be more sensitive to steroid feedback than FSH. Thus during the late luteal phase progesterone concentration decreases thus eliminating the negative feedback suppression of LH secretion and allowing LH pulse frequency to exceed FSH pulse frequency.

FSH initiates growth of the follicle to the antral stage and stimulates the establishment of LH receptors in granulosa and theca cells (Baird and McNeilly, 1981; Hansel and Convey, 1983). FSH itself binds principally to the granulosa cell and is capable of initiating its luteinization. It is believed that FSH may stimulate progesterone secretion from the corpus luteum as Walters *et al.* (1984) observed a striking relationship between pulsatile FSH secretion and progesterone secretion during the bovine luteal phase in which pulses of FSH were followed by or were concommitant with pulses of progesterone. A surge of FSH in plasma coincides with the preovulatory surge of LH at oestrus (Akbar *et al.*, 1974; Schams and Schallenberger, 1976). In addition a less distinct secondary peak or surge of FSH was observed by Dobson (1978) and Webb *et al.* (1980) approximately 24h later. This may result from either the low level of oestradiol at this time or the low level of inhibin, both of which are a consequence of follicular rupture at ovulation (Henderson and Franchimont, 1981). The pulsatile pattern of FSH secretion (Schallenberger and Petterson, 1982) and additional peaks of FSH have also been

reported to occur at intervals throughout the oestrous cycle and are apparently related to waves of ovarian follicular development (Schams *et al.*, 1977).

LH is considered to play a major role in stimulating maturation and ovulation of the Graafian follicle and the subsequent formation and maintenance of the corpus luteum (Peters, 1985). LH appears to be the principal ovulatory hormone although FSH may also be involved in ovulation. Jones and Nalbandov (1972) successfully induced ovulation by injecting ovine LH and FSH either separately or in combination both *in vitro* and *in vivo*. Although both LH and FSH could individually cause ovulation, synergism of the two hormones was apparent. Thus in the presence of physiological concentrations of both LH and FSH, ovulation occurred most readily (Nalbandov and Bahr, 1974). These authors have suggested that the preovulatory surge of LH and FSH should be regarded as an 'ovulation-inducing hormone complex'. Gospodarowiez and Gospodarowiez (1975) studied bovine luteal cells *in vitro* and suggested that LH was required for maintenance of luteal cell structure and function. Plasma LH concentrations during the luteal phase have been reported to be about 1.2 - 1.4ng/ml (Swanson and Hafs, 1971; Wettemann *et al.*, 1973), and are thus low. A large preovulatory peak or surge occurs during the follicular phase which coincides roughly with the onset of oestrus (Henricks *et al.*, 1970; Sprague *et al.*, 1971; Swanson and Hafs, 1971; Hansel *et al.*, 1973). LH concentrations of 5-60ng/ml have been observed during the surge which precedes ovulation by 24 to 30h (Swanson and Hafs, 1971; Schams *et al.*, 1977). Rahe *et al.* (1980) demonstrated an episodic or pulsatile pattern of LH release throughout the ovarian cycle and categorized the changes according to the stage of the ovarian cycle as follows:-

1) luteal phase - high amplitude, low frequency (one pulse / 4 hour)
2) during luteolysis and the early follicular phase - lower amplitude, increasing frequency, culminating in the preovulatory surge.
3) post-preovulatory surge - low amplitude, high frequency (1pulse/h).

In addition to the LH surge seen during oestrus, mid-luteal LH episodes have also been detected in cows. These may be related to waves of follicular growth (Karg, 1972). Pituitary FSH and LH content decreases sharply between 0 and 18h after the initiation of oestrus, i.e. concurrently with the pre-ovulatory gonadotrophin surge (Walters and Schallenberger, 1984). There is also a decrease in GnRH content of the hypothalamus at this time (Foster *et al.*, 1974).

Functionally, the most important ovarian steroids are oestradiol - 17β and progesterone which are secreted from growing follicles and corpora lutea respectively. The fluid of the largest antral follicles contains the highest concentration of oestradiol - 17β (Henderson *et al.*,1982). High intrafollicular oestradiol concentrations help follicular development and further increases oestradiol secretion. Oestradiol and progesterone play an essential role in many aspects of the control of the oestrous cycle. Thus they have profound effects in maintaining the reproductive tract, secondary sexual characteristics and accessory glands and they are involved in controlling hypothalamo-pituitary function. Increased LH and FSH secretion can be induced by administering exogenous oestradiol to ovariectomized, lutectomized or post partum acyclic cows (Cummins *et al.*, 1972; Hobson and Hansel, 1972; Short *et al.*, 1973; Peters, 1984a). Conversely, the stimulatory effect of oestradiol has been blocked by the use of anti-oestrogenic agents (Spies and Niswender, 1972). It is apparent from the work of Kesner *et al.* (1981) that oestradiol stimulates the pituitary surges of LH and FSH, firstly by increasing pituitary sensitivity to GnRH and then by increasing GnRH release. Plasma oestradiol - 17β concentrations are low for most of the oestrous cycle, but rise during the preovulatory period reaching a peak at, or just prior to, the onset of oestrus (Glencross *et al.*, 1973; 1981). Increasing plasma oestradiol concentrations during the follicular phase are correlated with the increasing LH

concentrations. The highest oestradiol concentrations generally occur 6 to 8h before the onset of the LH surge (Walters and Schallenberger, 1984) but may then decrease until 1h before the onset of LH surge before increasing again suddenly with the LH surge (Peters, 1985). Glencross *et al.* (1981) demonstrated that peak oestrous behaviour coincided with maximal plasma oestradiol concentration. In an earlier study, Henricks *et al.*(1971) has shown that oestrogen concentrations rise to a peak and then drop sharply 2 - 5 hours after the onset of oestrus. Umezu *et al.* (1981) found a close temporal relationship between the onset of mating behaviour by the bull and the LH surge in the cow and suggested that oestradiol concentrations, oestrus behaviour and the LH surge are closely related.

Progesterone is secreted almost entirely by the corpus luteum and its concentration remains basal during the follicular phase, i.e. 1 - 4 days prior to the onset of oestrus (Stabenfeldt *et al.*, 1969; Henricks *et al.*, 1971; Robertson, 1972; Dobson *et al.*, 1973 and Lemon *et al.*, 1975). Henricks *et al.* (1970) showed that plasma progesterone concentrations were minimal during the day before oestrus and stayed low during oestrus while there was a large preovulatory surge of LH which itself returned to basal levels soon after oestrus and ovulation (Hansel *et al.*, 1973; Schams *et al.*, 1977). During the luteal phase, progesterone concentrations were elevated and reached a peak between days 8 and 16, before decreasing sharply to basal concentration before the next oestrus and ovulation (Hafs and Armstrong, 1968; Pope *et al.*, 1969; Robertson, 1972). Henricks *et al.* (1971) found that oestrogen concentrations, which were low during the luteal phase, rose during the 4 days before oestrus, coincident with declining plasma progesterone concentrations. Frequent blood sampling shows that progesterone is also secreted in a pulsatile manner with pulses showing good correlation with FSH (Walters *et al.*, 1984) and pulses of FSH were followed by, or were concomitant with, a pulse of

progesterone. Studies of rat and pig granulosa cells *in vitro* have shown that FSH is capable of stimulating progesterone secretion (Thanki and Channing, 1978; Dorrington and Armstrong, 1979; Adashi *et al.*, 1981). Similar results were observed when bovine luteal cells were perfused with FSH (Romanoff, 1966). Specific FSH receptors have been observed in the bovine corpus luteum (Manns and Niswender, 1983).

Pituitary LH and FSH secretion is controlled by the hypothalamus and mediated by alterations in GnRH secretion (Schally *et al.*, 1971) . However, gonadal steroids also play an important role in control of gonadotrophin secretion as they influence the functions of GnRH within the hypothalamo-hypophysial axis. Normal basal plasma LH and FSH concentrations can be restored in a gonadectomized individual by replacement of relevant steroids. This indicates a primary function of gonadal steroids in control of gonadotrophin secretion. This dynamic equilibrium between plasma gonadotrophin and gonadal steroids was first realised by Moore and Price (1932). It has become apparent that there are two modes of gonadotrophin secretion during the oestrus cycle, basal or tonic secretion and surge secretion which operate conjointly (Goding *et al.*, 1970; Scaramuzzi *et al.*, 1971). Tonic secretion is maintained by a steroid-mediated negative feedback system while the characteristic pre-ovulatory gonadotrophin surge is facilitated by a positive feedback effect exerted by oestradiol (Scaramuzzi *et al.*, 1971; Brown *et al.*, 1972; Dickman and Malven, 1973; Karsch *et al.*, 1973; Karsch and Foster, 1975).

Since the early work demonstrating the ovarian influence on gonadotrophin secretion, attempts have been made to define the individual roles of oestrogens and progesterone in tonic gonadotrophin secretion, using replacement therapy. Progesterone alone was totally ineffective in suppressing gonadotrophin concentrations when administered to ovariectomized rats (McPherson *et al.*, 1974)

ewes (Diekman and Malven, 1973) and cows (Padmanabhan and Convey, 1981). In contrast injection of oestradiol lowered LH and FSH concentration in the ovariectomized rat (McCann *et al.*, 1972), and ewe (Scaramuzzi *et al.*, 1971) but stimulated gonadotrophin secretion in cows (Hobson and Hansel, 1972; Short *et al.*, 1973; Peters, 1984a). Mean LH concentrations are inversely proportional to circulating progesterone concentrations throughout the oestrous cycle in the ewe (Hauger *et al.*, 1977) and in the cow (Walters *et al.*, 1982a). Conversely, a positive correlation was observed between plasma LH and oestradiol during the majority of the oestrous cycle in the ewe (Hauger *et al.*, 1977) and in the cow (Fonseca *et al.*,1977). It is evident from these findings that there is a positive correlation between oestradiol and tonic LH release while progesterone is correlated negatively with LH but positively with FSH (Walters *et al.*, 1982a). Furthermore, recent evidence suggests that oestradiol stimulates the surge release of LH (Kesner *et al.*, 1981) and progesterone inhibits the release of LH (Roche and Ireland, 1981), apparently by reducing the LH pulse frequency (Ireland and Roche, 1982; Riley, 1982).

Prostaglandins are unstable, hydroxylated fatty acid derivatives of prostanoic acid; have a short-half-life of about 8 minutes, and are regarded as local hormones (Walpole, 1975). They are released from a wide variety of tissues including uterine endometrium and have many diverse functions. There are approximately 15 series of naturally occuring prostaglandins of which only the F and the E series are known to play a significant role in reproduction.

Prostaglandin F2α (PGF2α) is the most important endogenous prostaglandin of the F series and is believed to affect uterine function. PGF2α is the uterine substance responsible for causing involution of the corpus luteum (ie.luteolysis) and its synthetic analogues are used as potent luteolytic agents in the cow

(Schallenberger *et al.*,1984) and other domestic animals (Hansel and Schechter, 1972; Hearnshaw *et al.*, 1974). The release of PGF2α from the endometrium appears to be dependent on a period of exposure to oestradiol - 17β during the follicular phase, followed by exposure to progesterone during the luteal phase of the cycle (McCracken *et al.*,1981). Peripheral plasma concentrations of prostaglandins are low for most of the oestrous cycle with a pronounced pattern of increased release occurring at the time of luteal regression (Kindahl *et al.*, 1981). There is evidence that PGF2α is released in a pulsatile manner over a period of 2-3 days. Pulses last for several hours and occur at regular intervals, with regression of the corpus luteum occurring within 24-48 hours after the first appreciable release of PGF2α (Peterson *et al.*, 1975; Kindahl *et al.*, 1976). The synthesis and release of PGF2α is probably stimulated by oestrogens and maintained by progesterone. Prostaglandin secretion continues until the output of progesterone from the regressing corpus luteum has reached basal levels (Kindahl *et al.*, 1979; 1980; 1981).

The oxytocic action of the corpus luteum was first discovered by Ott and Scott (1910) when they stimulated increased release of milk after injecting corpus luteum extracts into lactating goats. However, corpus luteum extracts failed to induce milk 'let down' in woman (Schafer, 1913) or cows (Gavin, 1913). This contradictory evidence led to confusion over possible progestagenic and oxytocic actions of the corpus luteum on the mammary gland (Hammond, 1913) and led to subsequent cessation of work on this topic until quite recently. Fields *et al.* (1980) rediscovered the oxytocic action of the bovine corpus luteum when they identified a factor which was capable of causing contraction of the mouse uterus. Similar results were obtained when ovine corpus luteum extracts were tested (Wathes and Swann, 1982). Further studies revealed the presence of an oxytocin (OT) - like peptide in bovine (Wathes *et al.*, 1983a, b) and ovine corpus luteum (Flint and

Sheldrick, 1983; Fields *et al.*,1983). The ability of corpus luteum extracts to stimulate uterine contraction could be prevented by preincubation of the extract with a monoclonal antibody to OT. Both bovine and ovine corpus luteum contain microgram quantities of OT (Wathes and Swann, 1982; Wathes *et al.*, 1983a, b; Fields *et al.*, 1983) the amount of which increases from a low value just after ovulation to a peak in the mid-luteal phase. Peripheral plasma concentrations of OT during the oestrous cycle in the ewe fluctuate from a minimum at oestrus to a maximum in the mid luteal phase and thus follow the pattern of changes in plasma progesterone concentrations during the oestrous cycle (Webb *et al.*, 1981; Sheldrick and Flint., 1981; Mitchell *et al.*, 1982; Schams *et al.*, 1982; Flint and Sheldrick, 1983) However, the luteal OT concentration drops before the drop in plasma progesterone concentrations at the end of the luteal phase thus indicating that the amount of production or release of OT does not exactly parallel that of progesterone (Schams *et al.*, 1983a). Also OT concentrations fall about 15 days after mating in pregnant ewes, while progesterone concentrations remain high at this time (Webb *et al.*, 1981; Sheldrick and Flint, 1981; 1983). The OT concentrations during the oestrous cycle in the cow are lower than those seen in the ewe but follow the same trend, with the highest values being found in the early and mid luteal phases (Schams, 1983). Walters *et al.* (1984) demonstrated pulsatile secretion of OT into the ovarian vein and observed a relationship between pulses of OT and pulses of progesterone. Walters *et al.* (1984) also observed that ovarian OT and progesterone are secreted concomitantly during the mid-luteal phase of the oestrous cycle which suggests a common mechanism of release. However, the same relationship between progesterone and OT cannot be demonstrated early in the luteal phase (Walters *et al.*, 1984). A relationship between ovarian OT and prostaglandin has been demonstrated. Thus the pulsatile secretion of OT is apparently stimulated by endogenous PGF2α and exogenous prostaglandin causes the release of OT from the ovary (Flint and Sheldrick,1983; Schallenberger *et al*, 1984). In addition, episodes of plasma prostaglandin metabolite secretion were

associated with episodes of ovarian oxytocin secretion (Flint and Sheldrick, 1983). Towards the end of the ruminant oestrous cycle, luteal regression is caused by the episodic release of PGF2α from the uterus (Flint and Hillier, 1976). Increased production of PGF2α is thought to be stimulated by progesterone as the uterus is exposed to progesterone for the duration of the luteal phase before luteolysis begins (McCracken *et al.*, 1981). It has been suggested that the episodic release of PGF2α may be caused by secretion of OT by the corpus luteum. There is now much evidence to support the involvement of OT in the luteolytic process. For example:-

1) OT is luteolytic when administered to cattle (Armstrong and Hansel, 1959), goats (Cooke and Knifton, 1981) and in sheep (Milne, 1963; Dobrowolski, 1973) at certain stages of the oestrous cycle;
2) surge release of OT (Flint and Sheldrick, 1983) and its precursors oxytocin-neurophysin (Fairclough *et al.*, 1980) occurs in parallel to uterine PGF2α release during luteal regression;
3) the corpus luteum secretes both oxytocin (Flint and Sheldrick, 1982) and its associated neurophysin (Watkins *et al.*, 1984) in response to PGF2α injection and OT stimulated uterine PGF2α release (Sharma and Fitzpatrick, 1974), and;

4) immunization of ewes against OT delays luteolysis and extends the interoestrous interval (Sheldrick *et al.*, 1980; Schams *et al.*, 1983a).

It has also been suggested that OT may contribute to the maintenance of the corpus luteum during pregnancy (Sheldrick and Flint, 1983).

Inhibin is a peptide which can be isolated from both testicular and ovarian extracts (DeJong and Sharpe, 1976). In the female it appears to be secreted by the

granulosa cells (Franchimont *et al.*, 1979) and selectively suppresses FSH release (Main *et al.*, 1979). There is also evidence that inhibin may affect ovarian function directly by inhibiting FSH binding to granulosa cells (Sato *et al.*, 1982).

It is apparent from the simultaneous measurement of several hormones that the oestrous cycle is dependent on the interactions of gonadotrophins and ovarian steroids (Schams *et al.*, 1977; Dobson, 1978; Walters and Schallenberger, 1984). During luteolysis or towards the end of the cycle i.e. from days 14-17, luteolysis occurs and progesterone concentrations decline to baseline (Robertson 1972; Lamon, 1973; Christensen *et al.*, 1974 and Lemon *et al.*, 1975). This permits tonic gonadotrophin secretion to increase, thus stimulating oestrogen secretion from developing follicles (Karsch *et al.*, 1979). Increasing circulating oestrogen concentrations further stimulate the secretion of LH and FSH from the pituitary via increased GnRH secretion and/ or altered pituitary sensitivity to GnRH and oestradiol secretion from the growing Graafian follicle is consequently increased further still. A detailed investigation of the interrelationship between LH, oestradiol and progesterone in the ewe has shown that LH and oestradiol only rise after progesterone concentrations begin to fall about 2 days before the pre-ovulatory LH peak (Karsch *et al.*, 1979). A rise in circulating LH concentration occurs which is followed by increased secretion of oestradiol and initiation of the surge release of LH that occurs during the early hours of oestrus (Cummins *et al.*, 1972; Wettemann *et al.*, 1973; Mori *et al.*, 1974; and Lemon *et al.*, 1975). While progesterone concentrations increase after ovulation, plasma LH concentration decreases and remains basal for the rest of the luteal phase except for some small pulses of LH accompanied by similar episodic increases in oestrogen concentrations (Baird and Scarammuzzi, 1976). FSH concentrations increase during the follicular phase in parallel to the LH concentrations and an FSH surge accompanies the preovulatory surge release of LH. However, separate FSH pulses were also reported by Schallenberger *et al.* (1984) and Walters *et al.* (1984) and a close

association between FSH and progesterone pulses during the luteal phase was demonstrated by Schallenberger *et al.* (1983) at a time when pulsatile LH secretion was completely absent. Unlike LH concentrations, FSH concentrations were not affected by increased concentrations of progesterone during the luteal phase (Padmanavan and Convey, 1981; Walters *et al.*, 1982a). A different pattern of FSH and LH secretion can therefore be observed during most of the oestrous cycle, the exception being the simultaneous FSH and LH peaks which occur during the immediate preovulatory period. Progesterone concentrations begin to rise from about day 4 of the cycle to a peak between days 8 and 16, then decrease to baseline before the next oestrus and ovulation (Hafs and Armstrong, 1968; Robertson, 1972). Increased progesterone concentrations during the luteal phase reduce the LH pulse frequency (Ireland and Roche, 1982; Riley, 1982) and inhibit the release of LH by a negative feedback mechanism (Convey *et al.*, 1977; Roche and Ireland, 1981). Rahe *et al.* (1980) have suggested that changing concentrations of ovarian steroids, progesterone in particular, are responsible for changes in the characteristics of LH secretion.

Endocrinology of the post-parturient cow

A brief review of the hormonal changes which occur *pre-* and *post-partum* is necessary in order to understand the mechanisms which control the onset of cyclicity in the post-parturient cow. During the last few days of pregnancy, oestrogen concentrations increase progressively reaching a maximum at parturition. Progesterone concentrations remain relatively steady and are about 2.0ng/ml on the day before parturition (Henricks *et al.*, 1972 ; Humphrey *et al.*, 1983). The concentration of both oestrogens and progesterone remain low until the resumption of cyclic activity *post-partum* (Donaldson *et al.*, 1970 ; Robertson, 1972). FSH concentrations are low during the last 10 days *pre-partum* (Lamming *et al.*, 1981) and rise slightly after parturition but do not change subsequently until 30 days

post-partum (Peters and Lamming, 1984). Plasma LH concentration is also low at parturition (Goodale *et al.*, 1978) but tends to increase more gradually during the post-partum period at least for the first 20 days (Peters and Lamming, 1984).

Plasma FSH and LH concentrations are low immediately *post-partum* and there appears to be an absence of episodic release. In milked cows FSH concentration rises by day 5 *post-partum* with no major changes thereafter, while LH concentrations increase by day 10 (Lamming *et al.*, 1981; 1982). In general it is apparent that plasma LH concentrations and pituitary sensitivity to GnRH are maximal by 12 - 15 days *post-partum* in dairy cows but this occurs later in suckling beef cows (Peters and Lamming, 1984). A pulsatile pattern of LH secretion, which has been absent in the early post-partum period in dairy cows reappears from day 10 *post-partum* (Peters *et al.*, 1981a ; Lamming *et al.*, 1982). However, in multiple-suckled Friesian dairy cows this may not occur until day 50 or later, while in beef cows in which suckling is restricted to once or twice daily, it appears to be intermediate between the two extremes (Lamming *et al.*, 1982). It has been suggested that the onset of ovarian cycles is delayed in some suckled cows due to an inhibition of pulsatile LH release (Carruthers and Hafs, 1980; Peters *et al.*, 1981b). Certainly the onset of a pulsatile pattern of LH release correlates well with the time to first ovulation *post-partum* (Riley,1982) and the development of episodic LH secretion may therefore be essential for the reestablishment of ovarian cyclicity. Thus there is a longer delay from parturition to the first oestrous cycle and a delay in initiation of episodic LH secretion in cows suckling two calves compared with cows suckling a single calf (Lamming *et al.*, 1982). During the normal oestrous cycle, there is an increase in basal LH concentration during the 3-4 days before oestrus which is followed by the preovulatory surge and ovulation. In the post partum cow, similar increases in basal plasma LH concentrations occur for 4-5 days before the first preovulatory LH surge (Webb *et al.*, 1980).

Changes in plasma progesterone concentrations during the post-partum period have been described. The luteal phase of the first cycle *post-partum* is shorter in duration (10 days), than that of the normal luteal phase (Bulman and Lamming, 1978; Peters and Riley, 1982a). Such a short luteal phase may be attributed to a non-ovulated luteinized follicle (Tribble *et al.*, 1973) or to a normal corpus luteum that has insufficient LH receptors to respond maximally to the normal luteotrophic stimulus (Schams *et al.*, 1979). The secretion of ovarian steroids during this first short cycle probably primes the hypothalamo-pituitary unit and helps to restore normal ovarian cyclic function and normal pituitary sensitivity to GnRH (Foster *et al.*, 1980). An artificial short luteal phase was induced in acyclic cows by administering GnRH and progestagen (Lamming and Bulman, 1976; Webb *et al.*, 1977) and it was followed by a normal cycle. However, this type of short cycle is not observed in all cows and is probably not a prerequisite for the establishment of normal cyclicity *post-partum* (Peters and Lamming, 1984).

Plasma oestradiol concentrations are low after parturition (Humphrey *et al.*, 1983) but a sustained rise in oestradiol concentrations was obtained after injection of GnRH from 3 - 8 days *post-partum* (Peters *et al.*, 1985). This suggested that even during the early post-partum period, the ovary is sensitive to GnRH-induced gonadotrophin release and quite capable of responding by secreting substantial amounts of oestradiol. In dairy cows it has been shown that the stimulatory effect of exogenous estradiol-17β on the circulating LH concentration is absent shortly after parturition but is restored by day 15 (Schallenberger *et al.*, 1982). A more prolonged inhibition of the positive feedback mechanism of oestradiol has been demonstrated in beef cows (Peters, 1984b).

The pattern of hormonal changes observed during the first full oestrous cycles

post-partum is similar to that seen during oestrous cycles at other times. Thus plasma progesterone concentrations increase rapidly 4 - 5 days after oestrus and remain elevated for 10 - 12 days and then begin to decrease 4 days before oestrus. Elevated oestrogen concentrations 2 - 3 days prior to oestrus probably trigger the preovulatory LH release. The raised plasma FSH concentrations which have been observed during the early post partum period (Dobson,1978; Webb *et al.*, 1980; Peters *et al.*, 1981a), at a time when LH concentrations remain low (Lamming *et al.*, 1982), suggest that FSH plays a purely permissive role in the onset of ovarian cycles *post-partum*. On the other hand, the negative correlation between the length of the acyclic period and the mean plasma FSH concentration,suggests that FSH has only a limited effect on the onset of ovarian cyclicity *post-partum* or is not influenced by ovarian hormones (Riley, 1982).

The plasma concentration of PGF2α increases at parturition and remains elevated for several weeks (Lindell *et al.*, 1982; Kindahl *et al.*, 1982; Madej *et al.*, 1984). It has been shown that plasma concentration of the principal prostaglandin F2α metabolite, 13-14dihydro-15keto-PGF2α (PGFM) normally returns to low levels before the first ovulation *post-partum* (Kindahl *et al.*, 1984). Increased PGFM concentrations for a longer period *post-partum* may have an inhibitory effect on ovarian activity. Schallenberger *et al.* (1984) have suggested that the utero-ovarian axis exerts an inhibitory effect on pituitary LH secretion during the early post-partum period since hysterectomy results in a rapid increase in gonadotrophin concentrations.

A recent field study has suggested that the administration of a single dose of cloprostenol, an analogue of PGF2α, during the post-partum period may shorten the time to the onset of ovarian activity (Etherington, 1984). The mechanism of action of prostaglandin in this regard is not known. There is still some uncertainty about the precise mechanism whereby prostaglandins induce luteolysis (Baird,

1978). Pharris et al. (1970) suggested that luteolysis was caused by a restriction of blood flow to the corpus luteum but later Henderson and McNatty (1975) suggested that PFG2α specifically interferes with the coupling of LH and adenyl cyclase on the luteal cell membrane. More recently Wakeling and Green (1981) provided support for the latter suggestion when they showed a decrease in cyclic AMP synthesis which preceeded the decrease in both progesterone secretion and the number of LH receptors sites. Nevertheless, it is possible that PFG2α can induce the release of gonadotrophins as has been demonstrated in the bull (Hafs *et al.*, 1977) and hence may play a direct role in ovarian cyclic activity.

Factors affecting post-parturient reproduction

Many factors are considered to influence the resumption of normal ovarian activity *post-partum*. These include age, breed, season of calving, number of lactations, milk yeild, suckling versus milking, nutritional status, photoperiod, management and disease incidence at calving.

Age apparently influenced the resumption of ovarian cyclicity *post-partum* as indicated by Bulman and Lamming (1978) who showed older cows resumed cyclicity earlier than younger ones. Knight and Nicoll (1978) studied the effect of age and breed on post-partum reproduction and found that 2 year old cows had a longer post calving interval (PCI) than mature cows, 90 days and 63 days respectively. The effect of age is not clear cut though since some other reports show that the interval to first oestrus is shorter for middle aged cows and increases in older animals (Morrow *et al.*, 1969; De Kruif, 1975). There is also an effect of age on the pregnancy rate after the first insemination *post-partum* (Van Dienten, 1964; 1968; De Kruif, 1975), pregnancy rate being lower in those animals which have calved for the first time. There is also evidence that the pregnancy rate after

the first insemination *post-partum* may be lower in animals over 7 years of age.

In general terms dairy breeds have a shorter PCI than beef breeds. Thus it has been observed that Friesian and Friesian crossbred cows have a shorter PCI than Angus cows ie.59, 65 and 89 days respectively (Knight and Nicoll, 1978).

Season of calving apparently influences the time taken to resume ovarian cycles following parturition (Bulman and Lamming, 1978). Spring calving cows have a longer post partum anoestrus than autumn or winter calving cows (Peters and Riley, 1982a). Both dairy and beef cows calving in spring have been reported to undergo longer periods between calving and first ovulation (Bulman and Lamming 1978; King and Hurnik, 1980). The effect of season on conception rate following artificial insemination is variable. The conception rate was shown to be higher in spring than in autumn calving cows (Mercier and Salisbury, 1947; De Kruif, 1975) and Hewett (1968) found that spring and autumn calving cows required < 3 and > 3 inseminations per conception respectively.The poorer conception rates obtained in the winter months are probably attributable to a number of factors, which include fewer hours of daylight, less obvious signs of oestrus and less efficient detection of oestrus (Anderson, 1966; Deas, 1971; Roine, 1973; De Kruif, 1975) but most of the authors reported that the seasonal variation in PCI was primarily related to nutritional management. Peters and Riley (1982a) disagreed since they were able to show a strong effect of season even after adjusting for the effect of body weight at calving.

An association between high milk yield and reduced fertility has been reported (Carman, 1955). A correlation between milk yield and time to first oestrus or ovulation has been reported by several workers (e.g. Marion and Gier, 1968; Spalding *et al.*, 1975) but Smith and Legates (1962) and Bulman and Lamming

(1978) failed to observe any such relationship. However, the latter authors did observe a greater tendency for high yielding cows to stop cycling spontaneously. Whitemore *et al.* (1974) and Eley *et al.* (1981) observed a longer acyclic period in cows selected for high milk yield relative to control animals. These authors also showed a more gradual decline in PGF2α metabolite concentrations in high yielding cows during the post partum period and speculated that this might be the reason for the longer delay to first ovulation. Cystic ovarian disease has also been reported to be more common in high yielding cows (Morrow *et al.*, 1969; Roberts, 1971).

Suckling delays the onset of post-partum ovarian activity from less than 15 days to in excess of 100 days in some cases (Lamming *et al.*, 1981; 1982; Peters *et al.*, 1981b). Many studies have shown that the occurrence of ovulation and/or the onset of oestrous behaviour is delayed in both dairy and beef cows that suckle calves as compared to milked animals (Graves *et al.*, 1968; Oxenreider, 1968; Carruthers and Hafs, 1980). Milked dairy cows resumed ovarian cycles, as determined by milk progesterone measurements, after 24.0 $\pm$ 0.6 days *post-partum* (Bulman and Lamming, 1978). This compares to a figure of 56.9 $\pm$ 2.5 days (Peters and Riley, 1982a) for suckled beef cows.

It has been reported that the duration of post-partum anoestrus also varies with the suckling intensity. Thus cows suckling one calf have a shorter acyclic period than cows suckling two calves (Wetteman *et al.*, 1978; Randel, 1981). But Peters and Riley (1982a) observed no difference in the length of post-partum anoestrus in cows with single or twin calves nor was any difference observed between cows in which suckling was restricted to twice daily and cows whose calves were allowed to suckle *ad-libitum.*

Poor nutrition is an important cause of anoestrus (Lamming, 1966; Morrow,

1970; McClure, 1972). Wiltbank *et al.* (1964) and Dunn *et al.* (1969) demonstrated a positive relationship between dietary energy and the post-partum acyclic period and were able to reduce the length of anoestrus by providing increased dietary energy. Good nutritional status during late gestation seems to be more important than the nutritional status *post-partum.* A significant negative correlation between body weight at calving and the duration of the post calving acyclic period in beef cows was found by Peters and Riley (1982a) whereas bodyweight changes *post-partum* had no effect. Similarly an early return to cyclical activity could be achieved by increasing the supply of dietary energy given to pregnant beef cows (Dunn *et al.*, 1969; Wiltbank, 1970; Corah *et al.*, 1975). Nutritional status is not only an important factor in determining the length of the post-partum anoestrum but it is also important at other stages of reproduction. Body condition appears to be critical in determining the conception rate at mating. Much research effort has been directed to determine the relationship between nutritional status and reproduction. Positive relationships between nutritional status, body condition and fertility in both dairy and beef cows have been reported (King, 1968; Wiltbank, 1970; Youdan and King, 1977; Kilkenny, 1979; Somerville *et al.*, 1979). Hill *et al.* (1970) observed that low energy intake at parturition and thereafter reduces the conception rate. The latter authors and Donaldson *et al.* (1970) also found that lower plasma progesterone concentrations during gestation could be associated with low energy intake. Corah *et al.* (1974) disagreed however as they did not find any significant effect of nutrition on circulating progesterone or oestradiol concentrations either prior to or following parturition. However, dietary energy is considered to be one of the most important factors in influencing reproduction in cattle. Figures recorded by the Meat and Livestock Commision show a strong relationship between the calving interval and the body condition score of beef cows at mating; very thin and very fat cows tending to have a longer calving interval (Allen, 1979).

Fertility is also affected by deficiencies of other specific nutrients, particularly vitamins and minerals (Roberts, 1971). It is believed that the dietary precursor of vitamin A, β -carotene, improves bovine fertility (Lothammer, 1978) possibly by improving conception rate (Jackson, 1981).

Thibault *et al.* (1966) suggested that photoperiod rather than nutrition was the most important factor influencing post-partum reproduction in cattle. Peters and Riley (1982b) have suggested that a vestigial sensitivity to photoperiod may be present in the domestic cow. They observed a negative correlation between daily photoperiod during late pregnancy and the onset of ovarian cycles *post-partum* since this pattern in feral cattle would predispose toward calving during late spring and early summer, the optimal time for food supply. They also observed that cows calving between November 1 and 30 April have a significantly longer acyclic period than those which calve between May 1 and October 31st, perhaps due to the greater availability of food in the latter period which enhances ovarian activity. There was a highly significant negative correlation between photoperiod one month before parturition and the length of the acyclic period. Finally, they concluded that both the nutritional status and the photoperiod may be important in determining the length of the acyclic period in beef cows. Roy *et al.* (1980) reported that lunar phase and the season of the year affected the timing of puberty and first conception in dairy heifers. Live weight at 182 days of age and the phase of the moon were observed to influence the time of oestrus. Also animals born during increasing day length reached puberty as much as 2 months earlier than those born during decreasing day length.

Good management is absolutely necessary to maintain or improve the productivity of cattle. King *et al.* (1976) described the effect of various housing

systems on ovarian function and oestrus in dairy cows, finding that the interval from parturition to oestrus was shorter in cows in free-stall rather than tied-stall housing. Management conditions vary throughout the year and De Kruif (1977) found that the type of housing affected the proportion of cows in a herd in anoestrus, anoestrus being more common if animals were tied in stalls with no freedom of movement. The percentage of cows in which oestrus was detected at their 1st, 2nd and 3rd ovulation was higher in the free-stall housing. Terqui *et al.* (1982) also reported that loose-housed beef cows resume ovarian activity sooner than tethered cows. They also observed that the earlier the cows were put out to pasture, the earlier cyclic activity was restored.

Weaning is another important factor which is involved in the onset of oestrous cycles *post-partum*. Thus if calves are weaned soon after birth, the cows exhibit oestrus earlier than those cows whose calves are weaned later (Smith and Vincent, 1972; Laster *et al.*, 1973) and the post-partum interval in the cow could be decreased by short term calf-removal (Smith *et al.*, 1979). It has been observed that hormonal treatments have had limited success in shortening the post-partum interval in suckling cows (Miksch *et al.*, 1978). Suckling delays first oestrus and ovulation (Short *et al.*, 1972) probably by suppressing secretion of LH (Walters *et al.*, 1982b). However, hormone treatment combined with calf removal has been shown to induce oestrus in anoestrous cows (Smith *et al.*, 1979) and early weaning alone, or in conjunction with hormone treatment, has also been used to shorten the post-partum interval in cows (Smith and Vincent, 1972). Short-term weaning combined with progestogen synchronization has improved conception rates in cows with medium body condition (Kiser *et al.*, 1979). So, it has been observed that early weaning combined with hormonal treatment of cows in poor or medium body condition shortens the post-partum interval and increases the conception rate. On the other hand De Silva *et al.* (1984) used cows in good body condition and failed to advance the onset of oestrous cycles by using early calf-

removal and prostaglandin treatment.

Dystocia and other difficulties encountered at parturition may prolong post-partum anoestrus and decrease conception rates (Laster *et al.*, 1973). Venereal infections caused by *Vibrio foetus* and *Trichomonas foetus* were once considered important causes of herd infertility problems in many countries (Roberts, 1971; Winter, 1973). The incidence of such infections has been markedly reduced in most Western countries, largely as a result of improved veterinary measures and the now widespread use of artificial insemination. These infections continue to be important however in those countries where veterinary services and the standards of animal husbandry are poor. Another infection which may cause considerable losses and which is encountered in most of the developing countries is *Brucella abortus*. A large number of other infections may also give rise to abortion (e.g. leptospirosis, bovine viral diarrhoea) and retained placenta and subsequent metritis and infertility (Williams *et al.*, 1977).

Abortion means the expulsion of non-viable or dead fetuses of recognisable size, before term (Roberts, 1971). Abortion induced by disease often hampers subsequent reproductive function in cattle. In the cow, abortion before the fourth month of pregnancy is seldom followed by placental retention but this is not the case after the fifth month of pregnancy. Abortion is of great economic importance since not only is the fetus lost but also a prolonged period of uterine disease and sterility may follow and if the abortion was caused by an infectious organism, the rest of the herd may also become infected. A retained placenta predisposes the uterus to infection and subsequent delay in re-establishment of ovarian cyclicity (Roberts, 1971). Initial placental retention may predispose a cow to similar problems following subsequent pregnancies (Erb *et al.*, 1958) or to delayed uterine involution and chronic endometritis (Buch *et al.*, 1955). Moller *et al.* (1976)

reported that the occurrence of repeat breeding (defined as requiring more than 3 inseminations before conception) was higher in cows that had a retained placenta. Retention may result in permanent sterility, due to pyometra, perimetritis or other similar diseases (Roberts, 1971). Septic metritis following parturition, may occur with or without retention of the placenta and may impair the future breeding ability of the animal (Roberts, 1971).

Cows with persistent uterine infections *post-partum* have a longer duration of elevated plasma prostaglandin concentrations. It is likely that the bacterial infections may stimulate increased uterine prostaglandin production. This may ultimately stimulate the uterine muscles and aid uterine involution and recovery from infection (Fredriksson *et al.*, 1985). Thatcher *et al.* (1982) found a relationship between endogenous concentrations of prostaglandin and the rate of uterine involution, the higher the prostaglandin concentrations, the earlier involution was completed. Prolonged uterine contraction has been induced in the bovine myometrium *in vitro* after a single injection of PGF2α (Patil *et al.*, 1980). Prostaglandins have been used for the treatment of uterine infection on the basis that administration in the early post-partum period reduces subclinical uterine infection and hastens the re-establishment of a suitable uterine environment for fertilization and pregnancy (Ott and Gustafsson, 1981).

It has been shown that the amount and duration of prostaglandin secretion is closely related with the extent of uterine involution and the level of post-partum anoestrus. Lindell *et al.* (1980) demonstrated this effect after inducing abortion by injecting cloprostenol into heifers at different stages of pregnancy varying from 39 - 146 days. They observed that animals which aborted after at least 100 days of pregnancy released considerably more PGF2α than those which aborted after less than 80 days of pregnancy. In normal post-parturient cows, ovulation seldom

occurs before circulating prostaglandin levels return to basal concentrations (Kindahl *et al.*, 1982; 1984). However, longer periods of elevated concentrations of the prostaglandin metabolite PGFM have been associated with both a faster rate of uterine involution and a stimulatory effect on follicular and luteal components of the ovary (Thatcher *et al.*, 1982). The timing of the first ovulation to be followed by a luteal phase of normal duration *post-partum* was positively correlated with the time needed for completion of uterine involution (Madej *et al.*, 1984). The incidence of placental retention was higher following induction of parturition than following normal birth (Wiltbank *et al.*, 1984). It appears from the study of Chew *et al.* (1979) that retained fetal membranes following induced parturition may result from a hormonal imbalance. Parturition is normally associated with increased plasma oestrogen concentration and decreasing plasma progesterone concentration (Echternkamp and Hansel, 1973), while induced parturition tends to be associated with lower oestradiol-17β concentration and higher progesterone concentrations (Chew *et al.*, 1979).

Endocrine disorders are another cause of reproductive failure both *pre-* and *post-partum.* Most hormonal disturbances which are associated with infertility in cows are secondary to other basic factors e.g. nutrition, while some disturbances may be due to physiological imbalances or may follow injections of exogenous steroids or other hormones. Examples of common conditions resulting from endocrine disorders are cystic ovaries, absence of oestrus and repeat breeding due to failure of ovulation or fertilization or early embryonic death.

Ovarian cysts are follicle-like ovarian structures that are 2.5cm or larger and persist for 10 days or more in the absence of a corpus luteum (Youngquist, 1986). There are three classes of ovarian cyst -

i) The thin walled follicular cyst,

ii) The thick walled luteinized cyst and,

iii) The cystic corpora lutea.

Follicular and luteinized cysts are anovulatory whereas cystic corpora lutea develop after ovulation. Classes (i) and (ii) are pathological whereas (iii) is non pathological. A deficiency of pituitary LH or insufficient LH release from the pituitary are thought to be the main causes of cystic ovaries (Youngquist, 1986). As LH is considered to be the major luteotrophic hormone, abnormally low concentrations result in failure of the follicle to ovulate and to develop into a normal corpus luteum. A follicular cyst is an anovulatory follicle which persists for 10 or more days and is associated with continuous or frequent oestrus or by anoestrous. Luteinized cysts are partially luteinized structures which persist for a longer period and are characterized by anoestrus. As far as reproductive management is concerned, corpora lutea are not pathological since ovulation of contemporary follicles occurs normally (McEntee, 1958; Morrow, 1969). Most cows with cystic corpora lutea usually maintain pregnancy to term despite slightly lower levels of progesterone (McEntee, 1958). Failure of oestrus or ovulation may occur in the presence of a cystic corpus luteum due to an abnormal pattern of secretion of LH as was noted in the case of follicular and luteinized cysts. Failure of oestrus and ovulation or delayed ovulation may be caused by delayed release of LH from the pituitary. This affects ovarian steroid secretion and results in infertility in cows and heifers (Roberts, 1971). Van Rinsburg and De Vos (1962) indicated that in addition to being caused by ovarian abnormalities, delayed ovulation may be caused by malnutrition during the winter months and by some hereditary defects in certain breeds.

Influence of uterine involution in post-parturient reproduction

Complete uterine involution after parturition is necessary before a cow can

concieve again (Peters, 1984a). The time required to complete uterine involution varies with the breed, being shorter in dairy cattle (24 - 52 days) than beef cattle (38 - 56 days; Morrow *et al.*, 1969), probably due to suckling which appears to stimulate involution (Reissen *et al.*, 1968; Wagner and Hansel, 1969). The criteria that have been used to determine uterine involution vary between researchers but include for example :-

1) return of the uterus to a normal location in the pelvic cavity,

2) normal and approximately equal size of the horns, and

3) normal uterine tone and consistency (Morrow et al.,1969).

The period of time required to complete uterine involution depends on various other factors. Thus Menge *et al.* (1962) reported that a short period of uterine involution was associated with low milk production and that involution was delayed with increased milk production. They also observed that uterine involution was delayed in cows which were heavier at parturition. Foote *et al.* (1960) observed that the period required to complete uterine involution was significantly longer after the second calving and Casida *et al.* (1968) showed that the primiparous uterus usually involutes faster than the multiparous uterus. Buch *et al.* (1955) reported that the mean interval from a normal parturition to complete uterine involution was longer in winter than in summer (51 vs 42 days) and in other seasons it was in between the two periods. There is a relationship between the rate of uterine involution and normality of parturition, a longer priod of time being required for uterine involution following an abnormal parturition. Morrow *et al.* (1969) reported that a mild uterine infection often delayed uterine involution by 7 - 10 days beyond the 12 - 14 days normally required in dairy cows and cows with retained placentae, such as is frequently caused by brucellosis, often required 30 - 60 days for complete involution. Involution may also be retarded or stopped by pyometra.

The onset of the first oestrus may be postponed if uterine involution is not complete by 3 - 4 weeks *post-partum* (Albrechtsen, 1917) and a relationship between the period required to complete involution and the timing of first oestrus has been reported (Buch *et al.*, 1955; Higaki *et al.*, 1959; Foote *et al.*,1960). But Menge *et al.* (1962) reported that the process of uterine involution appears to be largely independent of ovarian activity as complete involution was not significantly associated with either the interval to first oestrus or the occurrence of cystic ovaries.

Eley *et al.* (1981) have recently demonstrated a correlation between the duration of elevated plasma concentrations of prostaglandin metabolite and the time required for complete uterine involution. Prostaglandin is secreted from the uterus and plays an important role in uterine involution (Lindell *et al.*,1982; Lindell and Kindahl, 1982; Kindahl *et al.*, 1982; 1984). In the cow, concentrations of the principal metabolite of PGF2α, PGFM, were high at parturition and remained elevated for 7 - 23 days (Lindell *et al.*, 1982). The length of the period during which elevated PGFM concentrations were observed coincided with the period required for completion of uterine involution. The pre-partum rise in circulating PGFM concentrations was initiated after progesterone concentrations began to fall from the high levels maintained throughout pregnancy. Progesterone levels remained low during the immediate post-partum period until the PGFM concentrations returned to basal levels (Lindell *et al.*, 1982). They also observed that prostaglandin release was prolonged after cases of complicated parturition which were associated with a longer period of uterine involution. It is believed that PGF2α has a beneficial effect on uterine involution. Furthermore, it has been observed that plasma PGFM concentrations invariably return to baseline before any rise in progesterone concentrations can be detected, indicating that uterine involution is usually more or less complete before the first ovulation *post-partum* (Peters, 1984a). So it appears from various studies that completion of uterine

involution is required before the commencement of ovarian activity *post-partum*.. However, studies of the relationship between conception rate and uterine involution in Hereford and Angus cattle indicated that the degree of uterine involution did not influence conception rate (Foote *et al.*, 1960). Perkins and Kidder (1963) also reported similar findings and indicated that conception rate was not affected by the involutionary state of the uterus at the time of breeding. They suggested that the length of the post-partum interval prior to breeding was of greater importance in obtaining satisfactory conception than was the involutionary state of the uterus as judged by rectal palpation.

From the foregoing discussion it may be concluded that reproduction in the cow is a complex process which involves complex interactions between the nervous system and the endocrine glands. The hypothalmus, the pituitary gland and the reproductive tract play the major roles in controlling reproduction. The hormones involved in reproduction are mainly the pituitary gonadotrophins and the ovarian steroids. The secretion of the gonadotrophic hormones, which is controlled by a hypothalmic hormone, GnRH, regulates gonadal steroid synthesis which in turn regulates gonadotrophin secretion by a feedback mechanism acting on the hypothalamus and the pituitary gland. There are some additional hormones such as, prostaglandin, OT and inhibin which are also involved in reproductive processes. Several other factors e.g. age, breed, season, management, nutrition, suckling or milking, certain diseases and the rate of uterine involution *post-partum* are also considered to influence reproduction in the cow.

A variety of hormone preparations is now available to treat disorders of reproductive function and to improve the management of reproductive processes in farm animals. The precise mechanism of action of these agents and the sequence of endocrine events which follow their use, however, is often poorly understood.

Prostaglandins are widely used to synchronise oestrous cycles, to induce parturition/abortion and to treat certain ovarian disorders in farm animals. In addition there is evidence that prostaglandins can improve conception rate in cattle *post-partum.*

The purpose of this project was to monitor reproductive events in cows after the use of prostaglandin. Response to treatment was monitored by analysis of plasma hormone concentrations, by observation of mating behaviour and by the use of ultrasonic imaging of the reproductive tract.

Chapter II

Radioimmunoassay

Introduction

Radioimmunoassay is now a widely accepted technique and is being used in many different clinical situations in veterinary science. The great advantage of radioimmunoassay over other techniques is its ability to measure small quantities of substances in large numbers of samples of biological fluids or tissue for example blood plasma etc. Measurement of hormone concentrations in plasma gives information relating to the reproductive state of the animal e.g. pregnancy, anoestrus and stage of the ovarian cycle which can be of great value to the dairyman and veterinarian. Radioimmunoassay is also a very useful tool with which to investigate responses to drugs and artificial endocrine challenges, as well as the normal hormonal interactions within the animal. In this study considerable emphasis has been placed on the development and application of radioimmunoassay for LH, progesterone and oestradiol-17β to study reproductive performance and ovarian activity in post-parturient cows.

Materials and methods.

A) General

Neutral glass rimless tubes were used for ether extraction of steroid hormones from plasma (100 x 15mm) and for drying down and radioimmunoassay of the ether extracts (75 x 10mm, Samco). Tubes were re-used after radioactive decontamination (Decon. 90, 2%; Decon Labs Ltd, Hove, U.K.) followed by soaking in biological detergent (Ariel, Proctor & Gamble, Newcastle-upon-Tyne, U.K.) and then routine laboratory glassware washing procedures. Polystyrene tubes (62 x 10mm, LP3; Luckham Plastics Ltd, Burgess Hill, U.K.) were used for the LH radioimmunoassay.

Unless specified, all chemical reagents were obtained from Sigma, Poole, U.K. Precipitating antisera for use in the double-antibody radioimmunoassays were obtained from the Scottish Antibody Production Unit (SAPU), Carluke, U.K.

Phosphate buffered saline (PBS, 0.05M) containing bovine serum albumin (BSA, 0.25% w/v; Sigma grade V) or gelatine (0.1% w/v; Davis, Leamington Spa, U.K.) was used throughout for preparation and dilution of reagents and was prepared as follows:-

Solution A. 0.5M NaH_2PO_4 $2H_20$ was prepared by dissolving 7.89g in 100ml distilled water.

Solution B. 0.5M Na_2HPO_4 was prepared by dissolving 14.2g in 200ml distilled water.

Approximately 30ml of solution A was titrated into solution B until the pH was 7.5. 1.75g diNaEDTA and 15.7g NaCl were added to 175ml to make 0.5M PBS which was diluted to 1.75 L with distilled water to give 0.05M PBS. Immediately before use, either 0.25% BSA was added to the PBS for use in the progesterone assay or 0.1% gelatine was added for use in the LH or oestradiol assays.

B) Radioimmunoassay of LH.

Plasma LH concentrations were determined using a direct double antibody radioimmunoassay using rabbit anti-ovine LH serum (GDN #15) provided by Prof. G. D. Niswender, Colorado State University, Colorado, USA. Its characteristics have been described (Niswender *et al.*, 1969) and it has been used to quantify LH in a number of mammalian species (Millar and Aehnelt, 1977). Highly purified ovine LH for use as tracer (fraction LER-1056-C2) was provided by Prof. L. E. Reichert, Albany Medical College of Union University, Albany, New York, USA. Radioiodination was performed using chloramine T as follows:-

Five micrograms LH was dissolved in 0.05M PB (20µl). A further 20µl PB and 1mCi ^{125}I as sodium iodide (Radiochemical Centre, Amersham, Bucks) in 20µl 0.05M PB were added to the reaction tube. Chloramine T (50µg/20µl 0.05 PB) was added and allowed to react for exactly two minutes on ice. The reaction was terminated by adding sodium metabisulphite (100µg/200µl 0.05M PB) and 1% BSA (1ml) and KI (2mg/200µl in PBS) were then added. The reactants were separated on a 20cm x 1cm column of Sephadex G50 eluted with PBS + 0.1% gelatine. Fractions of eluate (1ml) were collected and their radioactivity was roughly assessed using the bench radiation monitor to check the separation procedure. Subsequently a 10µl aliquot from each fraction was counted. During assay development, fractions containing labelled LH were divided into smaller aliquots (0.2ml), diluted with PBS + 0.1% gelatine (2ml) and stored frozen. Fractions were incubated with LH antibody, according to the standard assay procedure described below, to determine which fraction possessed optimum LH immunoactivity. Having thus identified suitable ^{125}I-labelled LH fractions,

corresponding fractions from subsequent iodinations were similarly diluted and stored frozen.

The assay standard was USDA-bLH-B-5. It was used in doubling concentrations from 0.25 to 32ng/ml. Sample aliquots or standards (0.1ml) were incubated in polystyrene tubes at 4°C overnight with first antibody (0.2ml/tube, initial dilution 1:70,000). Iodinated LH (approximately 12 to 15,000 cpm/0.1ml) was added and the tubes were incubated at 4°C for 24h. A 1:20 dilution of donkey anti-rabbit serum containing 1:200 normal rabbit serum (0.2ml) was added to all tubes except tubes for total counts and the reagents were incubated for at least 8h. The precipitate containing the bound tracer was counted after dilution of the contents with assay dilutent (1ml), centrifugation at 4°C for 15 min at 2,000g and aspiration of the supernatant.

C) Radioimmunoassay of oestradiol.

Plasma oestradiol-17β concentration was measured by a double antibody radioimmunoassay of ether extracts. The primary antibody was provided by Dr. B. A. Morris, University of Surrey, U.K. and was raised in sheep against 6-carboxymethyloxime-oestradiol-17β BSA. Aqueous solutions of standards (0.4ml) ranging from 15.5 to 500 pg oestradiol/ml and sample (0.4 ml) were extracted with 3 ml diethyl ether (Analar grade, May & Baker, Dagenham, U.K.) by vortex mixing for 5 min on a multivortexer (Alpha Laboratories, 40 Parham Drive, Eastleigh). The ether phase was decanted into glass tubes, after prior freezing of the aqueous phase in a bath of methanol and dry ice, and evaporated to dryness under air. ^{125}I- histamine 6-CMO- oestradiol-17β was provided by Dr. C. E. Gray, The Royal Infirmary, Glasgow, and was prepared by iodination of histamine using chloramine T followed by conjugation to 17β-oestradiol-6-CMO. The iodinated oestradiol fraction was extracted and purified by thin layer

chromatography as described by Hunter *et al.* (1975). Iodinated oestradiol (0.2ml, containing approximately 10,000cpm) and primary antibody (0.2ml, initial dilution 1:4 x 10^6 giving approximately 40% binding of tracer) were added to the dried ether extracts and the tubes were incubated for a minimum of 2h at room temperature. Second antibody (a 1:15 dilution of donkey anti-sheep/goat serum containing normal goat serum (1:150) was added and the tubes were incubated overnight at 4^oC. The supernatant was diluted by addition of assay diluent (1ml) and aspirated after centrifugation at 2,000 g for 15 min and the tubes containing the antibody bound oestradiol were counted.

D) Radioimmunoassay of progesterone.

Plasma progesterone concentrations were determined by radioimmunoassay of duplicate diethyl ether extracts of sample or aqueous solutions of progesterone standard (range 0 to 10 ng/ml). The first antibody (Y29) was provided by Dr. B. Cook, The Royal Infirmary, Glasgow and was raised in sheep, against 11α-hydroxy-progesterone hemisuccinate BSA and used at an initial dilution of 1:20,000 to give approximately 30 - 35% binding of labelled progesterone.

Plasma samples and standards (200µl) were extracted with diethyl ether as described for the oestradiol assay. Iodinated progesterone tracer was provided by Dr. C. E. Gray, The Royal Infirmary, Glasgow, U.K. It was prepared using progesterone 11α -glucuronyl tyramine, which was iodinated using chloramine T, and purified by solvent extraction and thin layer chromatography (Corrie *et al.*, 1975). Iodinated progesterone (200µl, approximately 10,000 cpm) and primary antibody (200µl) were added to the dried ether extracts and incubated at 37^oC for 45 min. Second antibody (a 1:20 dilution of donkey anti sheep/goat serum containing normal goat serum (1:200))was added and tubes were incubated at 4^oC

for 8h or overnight. The supernatant was diluted by addition of diluent (1ml) and aspirated after centrifugation at 2,000 g for 15 min and the tubes were counted.

E) ^{125}I - Counting and data reduction.

All assays employed ^{125}I-labelled tracers. Tubes containing the antibody-bound tracer were counted in a Packard Auto-Gamma Minaxi 5000 series counter equipped with a complete data reduction facility. Log hormone concentration or dose was plotted on the x-axis versus counts bound over total counts bound x 100% on the y-axis. Counts were automatically corrected for non-specific binding and replicates were averaged.

Results.

A) LH Radioimmunoassay.

i) Iodination

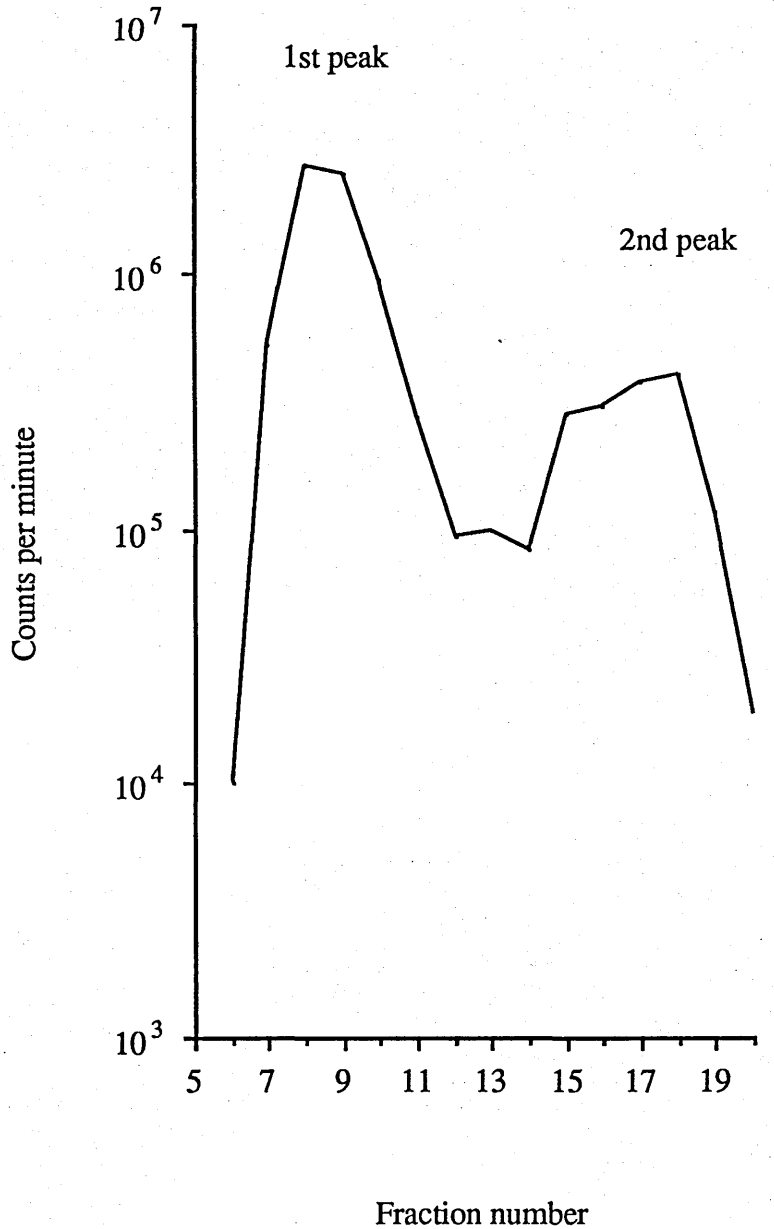
Two peaks of radioactivity were eluted from the Sephadex G50 column and a typical elution pattern of radioactivity is shown in Figure 2.1. The radioactivity of 10µl fractions eluted from the column, in counts per minute (CPM), and LH - like immunoactivity (percentage binding of each fraction by a 1:70,000 dilution of antibody, i.e. total binding (% TB) and percentage non specific binding (% NSB) are shown in Table 2.1. The percentage displacement of each fraction when coincubated with antibody (1:70,000 dilution) and either 1ng/ml or 8ng/ml LH is also shown.

Table 2.1

Radioactivity and LH-like immunoactivity of fractions eluted from the column following LH radioiodination (see text for explanation).

				% Displacement from 0	
FRACTION	CPM	%NSB	%TB	1ng/ml	8ng/ml
6	9876	3.4	35.8	92.5	53.6
7	538772	2.5	32.2	93.1	61.9
8	2756380	3.7	41.2	86.9	53.7
9	2544610	5.0	35.3	91.9	57.6
10	961396	6.9	33.7	89.3	57.9
14	83372	5.5	51.5	104.3	109.7
15	285740	4.7	52.5	99.5	105.2
16	306100	7.0	56.9	93.9	93.7
17	380976	6.9	51.3	106.8	104.3
18	406016	4.6	55.4	99.0	101.5

Figure 2.1: Elution pattern of 125I- radioactivity from a sephadex G 50 column iodination of LH


10^7
1st peak
2nd peak
10^6
10^5
10^4
10^3
Counts per minute
5
7
9
11
13
15
17
19
Fraction number

The highest LH antibody binding was observed in fraction 8. This fraction also gave the lowest NSB. Fractions 6 - 10 had good antibody binding, low NSB and also showed a good displacement when coincubated with samples containing low and high LH concentrations. Fractions 14 - 18 from the second peak of radioactivity also possessed good antibody binding characteristics but possessed high NSB properties and showed little displacement in the presence of low and high LH concentrations (Table 2.1).

ii) Assay performance

The mean standard curve obtained in 7 LH assays is shown in Figure 2.2a. The curve shows good displacement of tracer with increasing LH standard concentrations. The sensitivity of the plasma LH assay, determined from twice the standard deviation of the total binding values (i.e. the zero standard), was 0.2ng/ml (Table 2.2).

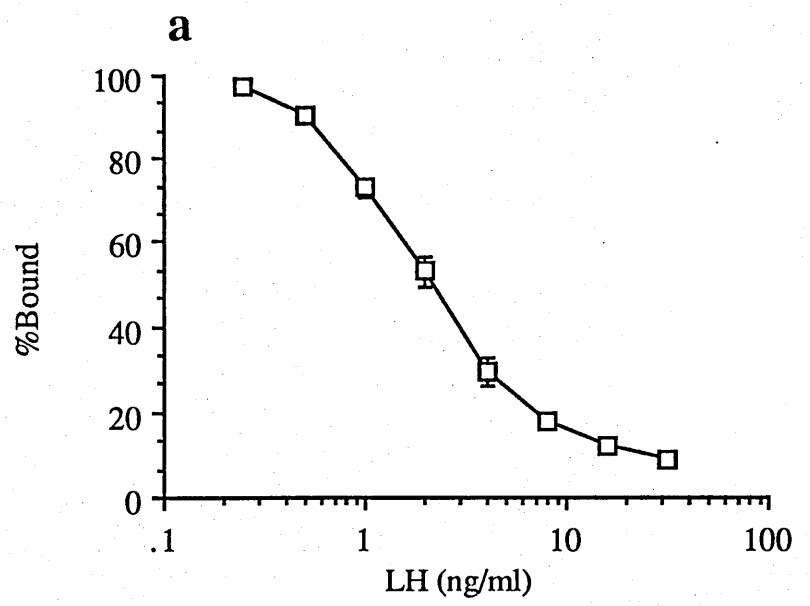
Table 2.2

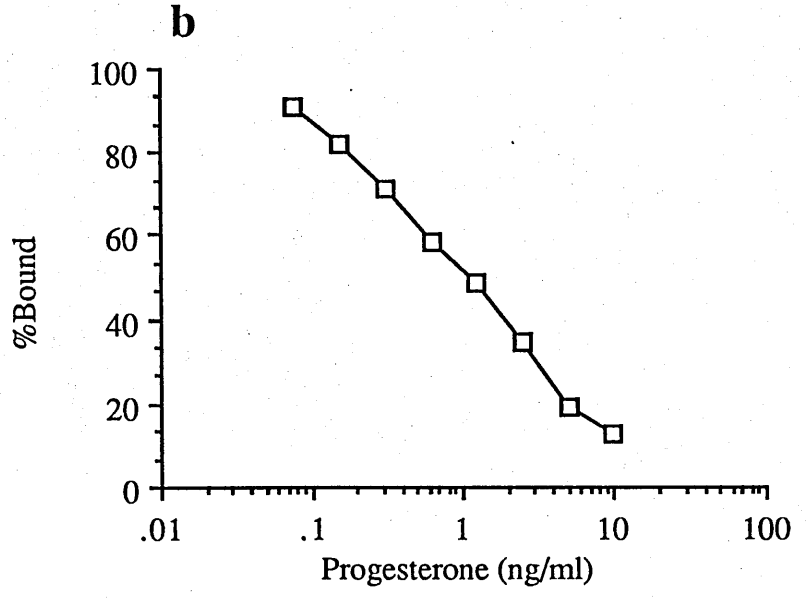
Reliability criteria of radioimmunoassay procedures.

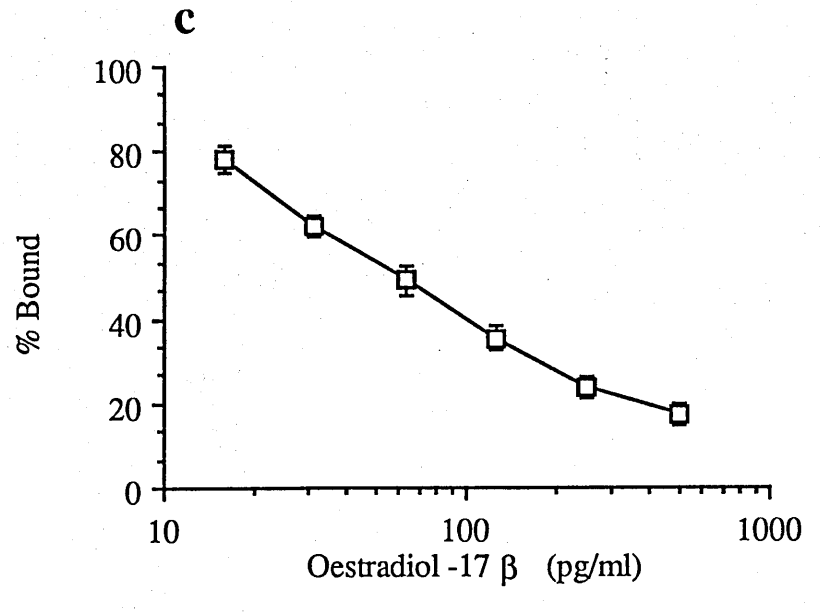
		Coefficient of variation (%)			
		Intra-assay		Inter-assay	
Assay	Sensitivity	low	high	low	high
LH	0.2ng/ml	5.6	5.7	4.3	4.5
Oestradiol	2pg/ml	8.9	6.7	11.5	7.0
Progesterone	0.04ng/ml	9.2	8.4	12.7	10.8

The intra and inter assay coefficients of variation for two plasma pools containing low and high LH concentrations were 5.6 and 5.7% and 4.3 and 4.5% respectively (Table 2.2). The accuracy of the assay was defined by determining the recovery of

Figure 2.2 : Standard curves (mean ± SEM, n = 7) obtained in radioimmunoassays for (a) LH , (b) progesterone and (c) oestradiol- 17β.


a
100
80
60
40
20
0
%Bound
.1
1
10
100
LH (ng/ml)


b
100
80
60
40
20
0
%Bound
.01
.1
1
10
100
Progesterone (ng/ml)


c
100
80
60
40
20
0
% Bound
10
100
1000
Oestradiol -17 β (pg/ml)

different concentrations of LH added to assay buffer and bovine plasma. The results are shown in Figure 2.3a. At all concentrations of LH added (range 0.25 - 32ng/ml) recovery was well correlated (r = 0.999) and above 90%.

B) Progesterone radioimmunoassay.

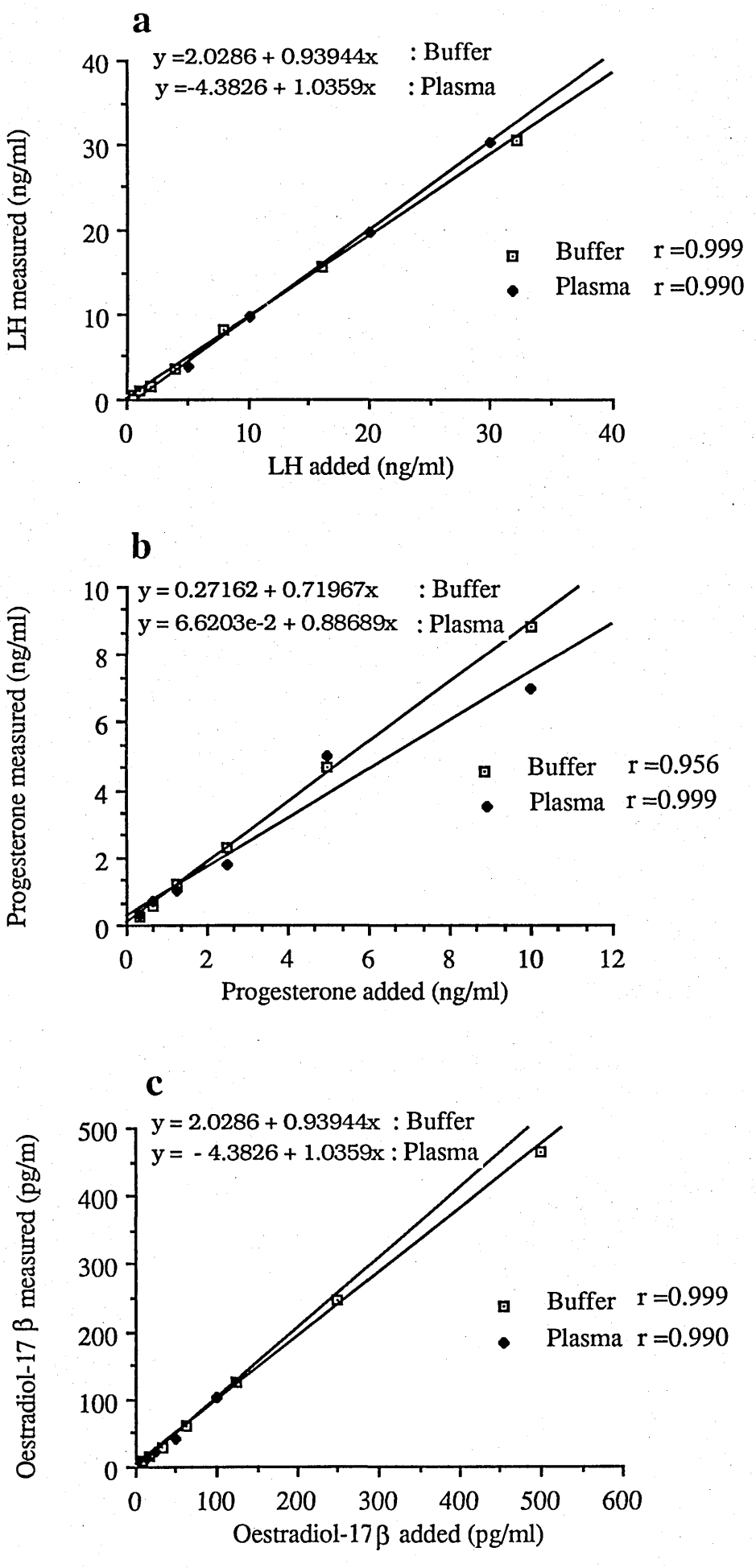
A mean standard curve obtained from 7 assays is shown in figure 2.2b. The sensitivity of the assay, calculated from twice the standard deviation of the total binding values, was 0.04ng/ml. The intra and inter assay coefficients of variation of two plasma pools containing low and high progesterone concentrations were 9.2 and 8.4% and 12.7 and 10.8% (n = 7, Table 2.2). The recovery of different concentrations of progesterone (range 0.3 - 10.0ng/ml) added to assay diluent and bovine plasma varied from 87 - 95% and 70 - 100% respectively and showed a linear correlation (r = 0.956 and 0.999, Figure 2.3b).

C) Oestradiol-17β radioimmunoassay.

A mean standard curve obtained from 7 assays is shown in figure 2.2c. The standard error of the mean (SEM) of each point in the standard curve was minimal. The sensitivity of the assay, as determined from twice the standard deviation of the total binding values, was 2pg/ml. The intra and inter assay coefficients of variation of two plasma pools containing low and high oestradiol-17β concentrations were 8.9 and 6.7% and 11.5 and 7.0% (n = 5) respectively (Table 2.2).

The recovery of different concentrations of oestradiol - 17β added to assay diluent (range 3.9 - 500pg/ml) and bovine plasma (range 12.5 - 100pg/ml) varied from 72 -111% and 84 - 102% respectively and showed a linear correlation (r = 0.999, Figure 2.3c).

Figure 2.3 : Assessment of radioimmunoassays accuracy by testing the recovery of different concentrations of (a) LH added to buffer (⊡) or plasma (◆); (b) progesterone added to buffer (⊡) or plasma (◆) and (c) oestradiol added to buffer (⊡) or plasma (◆).


a
y =2.0286 + 0.93944x : Buffer
y =-4.3826 + 1.0359x : Plasma
LH measured (ng/ml)
LH added (ng/ml)
Buffer r =0.999
Plasma r =0.990
b
y = 0.27162 + 0.71967x : Buffer
y = 6.6203e-2 + 0.88689x : Plasma
Progesterone measured (ng/ml)
Progesterone added (ng/ml)
Buffer r =0.956
Plasma r =0.999
c
y = 2.0286 + 0.93944x : Buffer
y = - 4.3826 + 1.0359x : Plasma
Oestradiol-17 β measured (pg/m)
Oestradiol-17β added (pg/ml)
Buffer r =0.999
Plasma r =0.990

The precision, accuracy and sensitivity of the oestradiol-17β assay used in these experiments were quite comparable with that used in other published reports (e.g. Niswender *et al.*, 1969; Riley *et al.*, 1981; Peters, 1984b; Okuda *et al.*, 1988).

Chapter 111

Effect of Prostaglandin on reproduction in post-partum beef cattle

Introduction

It is theoretically possible and hence economically desirable for a cow to have a calf each year. An interval of 80 to 85 days from parturition to next conception is necessary to achieve this goal (Peters and Riley, 1982a), a period longer than this resulting in substantial economic loss (Mortimer *et al.*, 1984). Optimum reproductive performance is achieved when cows come into oestrus promptly after parturition, concieve at their first service and wean a calf every twelve months (De Silva *et al.*, 1984). However, there are many factors such as nutrition, suckling as opposed to milking and endocrine disorders, which can increase the calving interval and thus reduce the productivity of cows.

The interval from parturition to first oestrus has been shown to be prolonged by low energy intake before or after parturition (Wiltbank, 1970) and by suckling (Oxenreider, 1968; Graves *et al.*, 1968; Wiltbank, 1970; Short *et al.*,1972). It is evident from various studies that the post partum anoestrus is longer in suckled than in milked cows and intensity of mammary stimulation by suckling appears to be important in prolonging the post-partum anoestrus (Short *et al.*, 1972; Wettemann *et al.*,1978). The influence of suckling can be reduced in beef cows by adequate supply of dietary energy (Wiltbank *et al.*, 1964; Dunn *et al.*, 1969). However, if suckled and non suckled cows are supplied with the same dietary energy *post-partum* to keep a similar weight, the anoestrous interval is still greater in suckled cows (Short *et al.*, 1972). Clapp (1937) and Wiltbank and Cook (1958) demonstrated a relationship between intensity of suckling or milking and the length of post-partum anoestrus and found it to be longer for cows suckled or milked four times a day than cows milked twice a day. Return to cyclic activity in cattle after parturition involves complex endocrine changes which have been reviewed by Leslie (1983).

During lactation plasma prolactin concentrations are high in cows

(Schams,1972). Suckling also causes hyperprolactinaemia in other species (Chang *et al.*, 1981). The latter authors also reported higher concentrations of basal plasma prolactin with a higher peak value in suckling beef cows than non-suckling beef cows. A high correlation between the length of post-partum anoestrus and number of prolactin peaks ($r = 0.87$, $p < 0.05$) was also demonstrated. It is believed that suckling retards the occurrence of the first oestrus and ovulation *post-partum* since an association between inhibition of oestrus and suckling has been demonstrated in post-partum ewes (Kann *et al.*, 1978) and in cows (Chang *et al.*,1981).

Smith *et al.* (1972) and Wagner and Oxenreider (1971) reported an increased corticoid secretion during milking and suckling but the latter authors reported higher serum corticoids in suckled than in non-suckled or milked dairy cows. Dunlop *et al.* (1981) demonstrated a peak of plasma cortisol concentrations in beef cows 15 minutes after the onset of suckling and a significant decrease in plasma LH within 30 minutes. Plasma LH concentrations are lower in suckled as compared to non-suckled beef cows (Carruthers *et al.*, 1980). The increased secretion of cortisol or adrenocorticotrophic hormone (ACTH) might have an inhibitory effect on the initiation of oestrous cycles through a suppression of LH (Ellicott *et al.*, 1981). Graves *et al.* (1968) and Saiduddin *et al.* (1968) reported similar concentrations of pituitary LH during the early post-partum period in suckled and non-suckled cows, whereas Short *et al.* (1972) and Carruthers *et al.* (1978) observed decreased plasma LH concentration in suckled cows during the early post-partum period. Plasma LH concentrations at this time are low and fluctuate little (Rawlings *et al.*, 1980; Williams and Ray, 1980). The occurrence of the first oestrus *post-partum* appears to be temporarily related to the initiation of episodic LH secretion 4 to 8 weeks previously (Humphrey *et al.*, 1983). Suckling suppresses secretion of LH during the early post-partum period (Walters *et al.*, 1982) since pulsatile release of

LH can be initiated by removal of the suckling stimulus (Walters *et al.*, 1982).

Plasma oestrogen concentrations decline from prepartum peaks to basal values by 2 to 4 days *post-partum* (Kesler *et al.*, 1979; Stellflug *et al.*, 1978). Arije *et al.* (1974) observed that plasma total oestrogen concentrations (Oestradiol -17β, oestrone and oestriol) ranged between 870 and 1300 pg/ml during the last 20 days prepartum, fell to 500pg/ml at parturition and decreased to 200pg/ml *post-partum* finally rising again to 500pg/ml 2 days before oestrus. Throughout most of the post-partum period, oestrogens fluctuated around 200pg/ml, with occasional peaks of 300 to 400 pg/ml. Mean serum oestradiol -17β concentrations declined rapidly from 113 ± 54 pg/ml on the day of parturition to 7 ± 3 pg/ml 6-7 days *post-partum* in beef cattle. Serum oestradiol -17β concentration subsequently rose 2-3 days before oestrus, reaching a mean of 10 ± 3 pg/ml on the day of oestrus (Humphrey *et al.*, 1983) but it has not been posible to relate oestradiol concentrations with follicular growth during the early post-partum period (Rawlings *et al.*, 1980).

Early post-partum concentrations of plasma progesterone are generally low (Erb *et al.*, 1971; Arije *et al.*, 1974). Suckling has no effect on plasma progesterone concentrations (Dunlop *et al.*, 1981), but Corah *et al.* (1974), Humphrey *et al.* (1976) and Prybil and Butler (1978) have reported a transient rise of plasma progesterone for 4 to 5 days before first oestrus whereas Echternkamp and Hansel (1973), Kesler *et al.* (1979) and Stevenson and Britt (1979) have failed to observe any preovulatory rise in plasma progesterone concentration in dairy cows.

It is evident that large follicles are present soon after parturition (Marion and Gier, 1968; Wagner and Hansel, 1969; Stevenson *et al.*,1983) and there is a gradual increase in follicular size as Wagner and Hansel (1969) reported average follicular diameters at 7, 14 and 30 days *Post-partum* in beef cattle to 9.6, 11.3 and

13.0mm respectively. The interval from parturition to the development of the first ovarian follicle of at least 8.6mm is about 16 days (Challahan *et al.*, 1971; Oxenreider and Wagner, 1971; Wagner and Oxenreider, 1971; Kesler *et al.*,1979). However, inhibitory factors, possibly of uterine origin, appear to limit the earlier development of follicles during post-partum anoestrus (Rexford and Casida, 1975). This inhibitory effect is reported to be maintained during the first 20 days *post-partum* and suppresses follicular development and ovulation (Saiduddin *et al.*, 1967).

Dufour and Roy (1985) reported that the corpus luteum of the previous pregnancy has a local carry-over effect on the rate of growth of the antral follicles after parturition since initial follicular development and the first ovulation occurred in the ovary opposite to that containing the corpus luteum of pregnancy but the time to first ovulation was not prolonged.

Involution of the uterus must occur soon after parturition and should proceed spontaneously (Kiracofe, 1980). However, the interval from parturition to completion of involution depends on several conditions such as the incidence of infection (Boyd, 1925), parity (Foote *et al.*, 1960) and the endocrine situation (Lindell *et al.*, 1982). The interval from parturition to the completion of uterine involution in beef cattle varies from 35 to 56 days (Morrow *et al.*, 1969). There is no definite index of uterine involution but Morrow *et al.* (1969) included several criteria such as return of the uterus to its normal location in the pelvic cavity, normal and approximately equal size of the uterine horns and normal uterine tone and consistency. The diameter of the previously pregnant horn and the extent of reduction in the diameter of the cervix have also been used as indicators of the degree of the involution process by Oltenacu *et al.* (1983), who also suggested that cows with medium to large cervices had a lower conception rate and a higher

interval from parturition to conception in comparison with cows with small cervices. There is an apparent relationship between completion of uterine involution and returned to ovarian cyclicity *post-partum* (Madej *et al.*, 1984).

The uterus plays an important role in governing the length of the oestrous cycle since hysterectomy of the guinea pig during the luteal phase prolonged the life span of the corpus luteum (Loebs, 1923). Loebs (1923) also suggested that the uterus may produce a luteolytic substance. It is now clear from more recent work that such a substance does indeed exist in several species and it has been termed uterine luteolysin (Cooper, 1974). There is good reason to believe that, in sheep at least, this luteolysin is prostaglandin F2 α (PGF2α) (McCracken *et al.*, 1972).

During the early post-partum period, the bovine uterus produces large amounts of PGF2α (Guilbault *et al.*. 1984) as indicated by high peripheral concentrations of the principal metabolite, 15-Keto-13, 14-dihydro-Prostaglandin F2α (PGFM) (Edqvist *et al.*, 1978; Eley *et al.*, 1981; Lindell *et al.*, 1982; Guilbault *et al.*, 1984; Madej *et al.*, 1984). Increased plasma concentration of PGF2α occurs concomitantly with, or just prior to, parturition in the cow and remains elevated for 7-23 days *post partum* (Edqvist *et al.*, 1978; Lindell *et al.*, 1982).

Basal plasma PGFM concentrations ranged from 25 to 100 pg/ml until day 18-19 of the cycle but several peaks of 250 pg/ml were also detected (Kindahl *et al.*, 1976; Peterson *et al.*, 1975). One to five hour episodes of pulsatile PGF2α release from the uterus for a period of 2-3 days have also been reported (Nancarrow *et al.*, 1973; Kindahl *et al.*,1976; 1979). Kindahl *et al.* (1979) compared such pulsatile patterns of PGF2α release with those seen during luteolysis and suggested that the pattern of prostaglandin secretion may be important in the process of uterine

involution. It is not clearly understood what mechanism is involved in the initiation of prostaglandin release during the oestrous cycle but experimental evidence suggests that oestrogens from the growing follicles might be involved (Horton and Poyser, 1976). The steroid output from the ovary seems to be important, as prostaglandin release can be initiated after exogenous administration of oestradiol in oophorectomized sheep given a period of progesterone priming (Caldwell *et al.*, 1972; Roberts *et al.*,1975). It is conceivable that prostaglandin is involved in the release of progesterone since the action of prostaglandin results in an inhibition of progesterone secretion (Kindahl *et al.*,1979) and in post-partum cattle progesterone concentrations remained low until the prostaglandin concentrations reached base line (Lindell *et al.*,1982). It also remains to be acertained whether both progesterone and oestrogens are necessary for the maintenance of the prolonged release of prostaglandin which is observed during the luteolysis at the end of the luteal phase of the oestrous cycle but it is assumed that prostaglandin synthesis and release is triggered by oestrogens secreted from developing follicles and, once started, is maintained by both oestrogens and progesterone or perhaps by progesterone alone. Finally, when progesterone concentrations have fallen to baseline, prostaglandin release is terminated (Peterson *et al.*, 1975; Kindahl *et al.*, 1979). In one experimental attempt to control the release of prostaglandin it was shown that insertion of a progesterone implant, subcutaneously on day 12 of the oestrous cycle in heifers, did not change the PGF2α plasma concentration nor the magnitude duration of PGF2α release, and luteolysis occurred normally with a concomitant decline in progesterone concentrations (Kindahl *et al.*, 1979). Henderson and McNatty (1975) presumed that the preovulatory LH surge might protect the early corpus luteum from PGF2α induced luteolysis. This hypothesis was defied by Mencio *et al.* (1977) who showed that prolonged infusion of LH around day 10-12 of the cycle, when the corpus luteum was supposed to be resistant to PGF2α , could not prevent PGF2α induced luteolysis and a subsequent decrease in plasma progesterone concentration,

nor alter plasma PGF2α concentration.

The increased release of PGF2α , which is observed for 2-3 weeks post partum correlates well with the time needed for uterine involution, (i.e. 16-23 days, Lindell *et al.*, 1982). A massive dose of exogenous PGF2α can accelerate uterine involution shortening it to a period of 20 days (Lindell and Kindahl, 1983), which is comparatively less than the time required for completion of involution seen in earlier investigations in untreated cows (29.4 days, Casida and Wisnicky, 1959; 29.6 days, Lindell *et al.*, 1982; 27.0 days, Larsson *et al.*, 1982 and 24.7 days, Madej *et al.*, 1984). As far as the mode of action of PGF2α on uterine involution is concerned, it had been suggested that uterine synthesis of PGF2α might increase uterine muscle tone (Lindell *et al.*, 1982). In addition to its effect on smooth muscle contractility further evidence for this effect comes from work which has shown that a single intramuscular injection of PGF2α induced prolonged uterine contraction in sheep (Edqvist *et al.*, 1975) and in cows (Patil *et al.*, 1980) with a consequent increase in uterine tone.

Prostaglandin F2α has been extensively used for oestrus synchronization since the discovery of its powerful luteolytic effects (Rowson *et al.*, 1972; Louis *et al.*, 1972 and Lauderdale, 1972). The efficiency of oestrus synchronization with a single injection of prostaglandin is limited by its inability to cause luteolysis when administered within 5 days of previous oestrus (Cooper, 1974; Beal *et al.*, 1980). Generally, the aim of oestrus synchronization involves regulation of cyclicity but the animals must be capable of responding to treatment. Prostaglandin synchronizes oestrus by causing premature regression of the corpus luteum and therefore will not be effective unless treated animals are cycling (Roche, 1976). To overcome this limitation a double injection regime using synthetic PGF2α or its analogue cloprostenol, with injections given at an interval of 11 days and followed

by timed double insemination has been practised (Peters *et al.*, 1980). Such treatments proved satisfactory in increasing conception rate in cycling heifers (Cooper, 1974; Hafs *et al.*, 1975) but poorer results were obtained in adult cows (Waters and Ball, 1978). The latter authors suggested that this might be because some animals were still in a state of post-partum anoestrus at the time of Prostaglandin injection thus rendering the treatment ineffective.

It is evident from the preceding review that a longer duration (Lindell *et al.*, 1982; Madej *et al.*, 1984) and higher magnitude (Lewis *et al.*, 1984; Guilbault *et al.*, 1985) of plasma PGFM concentrations can be related to faster uterine involution, which in turn may favour earlier resumption of ovarian activity and reduce the calving interval in beef cattle. Exogenous administration of PGF2α has also been reported to hasten uterine involution (Lindell and Kindahl, 1983), improve fertility (Smith *et al.*, 1984), and increase conception rate (Young *et al.*, 1984) in post-partum dairy cattle.

Early post-partum endocrine and ovarian changes leading to the resumption of ovarian cycles in suckled beef cows are not well understood. Therefore, the objectives of this experiment were to determine the effects of exogenous PGF2α on uterine involution and on the post parturient endocrinology of suckled beef cows.

Materials and methods.

Animals

Twenty seven Friesian Hereford beef cows, ranging from 4 - 11 years of age, and 6 heifers were assigned to this experiment which was undertaken at the Cochno Farm Experimental station Glasgow University, Veterinary School. The animals

were selected on the basis of calving dates. Calving occurred between 21st February and 24th March, 1986; between 7th and 18th March, 1987 and between 23rd Februrary and 7th March 1988. The animals were housed in open sheds in the winter months and fed hay and concentrates in accordance with MLC requirements. Cows and calves were put out to pasture on May 12, 1986; May 15, 1987 and May 6, 1988. The calves born in 1986 and 1987 ran with the cows and were able to suckle *ad_libitum*. Suckling was restricted to twice daily (i.e. 0800 and 1600h) in six out of nine cows used in 1988 until the cows and calves were turned out.

Six cows and 6 heifers were used in 1986 (Group A1 and Group A2) respectively. In 1987, 12 cows were divided into 3 groups of 4 (Group B1, B2 and B3). In 1988, 9 cows were divided into 3 groups of 3 (Group C1, C2 and C3). Group B1 and C1 received 4 injections of 2ml (5mg/ml) dinoprost tromethamine i.m. (Lutalyse, Upjohn Ltd., Crawley, West Sussex) at 7 day intervals starting 7 days after parturition. Group B2 and C2 received 2 injections of 2ml (5mg/ml) dinoprost tromethamine 14 and 28 days after calving. Groups A1, A2, B3 and C3 were not treated. The animals were observed closely twice a day for signs of oestrus during the last 2 - 3 weeks of the housed period and whilst at pasture. A charollais bull of proven fertility, fitted with a chin ball marker was placed with the cows at pasture during the last week of May and cows were checked twice daily for signs of oestrus until confirmed pregnant.

Blood sampling procedures

A single blood sample (10ml) was collected from all the animals twice weekly from the tail vein using evacuated heparinized tubes and 20G x 1 inch needles (Vacutainer system, Becton and Dickinson).

Multiple blood samples were taken every 15 min for 6 hours starting 2 hours before Prostaglandin injection, using an indwelling jugular vein cannula which was inserted in the following way:- the area around the jugular vein was swabbed with a solution of chlorhexidine. A 14G x 1.5 inch hypodermic needle (Monoject, St. Louis, MO) was inserted into the vein percutaneously and a sterile heparinized polythene tube (O.D. = 1.27mm, I.D. = 0.86mm, Portex) was threaded through the needle and into the vein to a depth of about 15cm. The needle was then removed and a sterile blunt 20G needle was inserted into the free end of the polythene tube which was sealed with a stopper. The patency of the cannula was tested and it was flushed out first with saline and then with saline containing heparin (2ml, 25 i.u./ml, Sigma, Poole, Dorset). The cannula was secured in place with a gummed bandage. The sampling sequence involved firstly the removal of waste saline and diluted blood from the cannula. A blood sample (8ml) was then taken and any blood remaining in the cannula was flushed back into the vein with saline. Finally, the cannula was refilled with heparinized saline to prevent the formation of a blood clot and then stoppered securely. The blood samples were transferred into glass tubes containing a drop of heparinized saline (250 i.u./ml). The plasma was harvested after centrifugation at 3,000 g for 15 min and transferred into 7ml polystyrene tubes (Luckham Plastics Ltd.) and stored at -20^{o}C

Ultrasonography

The ovaries and reproductive tracts of all the cows in group B were examined by ultrasound at weekly intervals *post-partum* starting during the first week *post-partum* and continuing for approximately 6 weeks. All cows were examined on one further occasion at 11 weeks *post-partum.*

The ultrasound examinations were carried out using a real time B-mode two

dimensional ultrasound scanner (Concept one, Dynamic Imaging, Livingstone, Scotland). Three types of rectal transducer of different frequencies were used in the examination. A 3-5 MHz linear array transducer was used from days 20 - 30 and a 7.5 MHz linear array transducer was used for the rest of the period. Examinations were carried out per rectum. The transducer was lubricated and passed through the anal sphincter in the palm of the operator's hand and laid in close proximity to the dorsal surface of the uterine tract. Scanning was performed according to the following protocol: right ovary, left ovary, right horn, left horn, uterine body and cervix. Diameter and area of the uterine horns, and thickness of the wall of the uterine body and horns and of the cervix were recorded. Linear measurements were made using a light pen measurement caliper applied directly to the freeze frame image displayed on the scanner screen. Measurements taken during the early stages of the post partum period were calculated from the centimeter scale which was displayed at the edge of the screen. Video recordings of the examinations were kept which provided the additional facility of play back recall. Permanent records were made on polaroid film. The dimensions of the uterine horns, the body of the uterus and the cervix were measured in the laboratory by analysis of the videotape.

Sample analysis

Plasma progesterone concentrations were determined in the twice weekly blood samples and plasma from serial blood samples collected from the cannulae were assayed for LH, and oestradiol-17β concentrations as described in chapter II

Statistical analysis

Differences in the intervals from calving to first oestrus and calving to conception and the number of services per conception were tested for statistical

significance using Student's t-test. Two way analysis of variance was used to assess the effects of prostaglandin treatment and the number of post-partum days on uterine involution as described by Shil and Debnath (1985). When significant variation was observed, the interactions between groups were compared to find the level of statistical significance as described by Campbell (1979). The variation in mean plasma LH concentrations after prostaglandin injections were expressed as a percentage of mean pre-injection values.

Results

Oestrus was not detected in any of the 6 heifers or 6 cows in Group A before they were turned out to pasture i.e. 49 - 70 days *post-partum*. First oestrus in the heifers (Group A1) ranged from 95 to 119 days *post-partum*. The mean interval from calving to first service (first oestrus) in the heifers and cows was 105.7 ± 4.5 and 82.8 ± 3.0 days respectively (mean ±SEM). This difference is statistically significant ($p<0.01$; Table 3.1). Conception rate at this first service was 100% in both heifers and cows.

Table 3.1

Interval (mean ± SEM) and range (parentheses) from calving to first oestrus in beef cows injected with prostaglandin either 7,14,21 and 28 days (Group B1 and C1) of 14 and 28 days *post-partum* (Group B2 and C2) and in untreated heifers (Group A1) and cows (Group A2,B3 and C3)

A1 (n=6)	**A2** (n=6)	**B1** (n=4)	**B2** (n=4)	**B3** (n=4)	**C1** (n=3)	**C2** (n=3)	**C3** (n=3)
105.7[a] ±4.5 (95-119)	82.8 ±3.0 (73-94)	67.8 ±3.4 (58-72)	78.8 ±6.2 (71-98)	74.0 ±7.6 (60-97)	87.3 ±0.6 (86-90)	105.06[b] ±5.6 (98-116)	96.0 ±5.0 (86-102)

[a], Group A1 significantly different from Group A2 ($p<0.01$).
[b], Group C2 significantly different from Group C1 ($p<0.02$).

The interval from calving to first oestrus in the cows in Group B1 (one injection of dinoprost 7, 14, 21 and 28 days *post-partum)*, Group B2 (one injection of dinoprost 14 and 28 days *post-partum*) and the untreated control cows (Group B3) was 67.8 ± 3.4; 78.8 ± 6.2 and 74.0 ± 7.6 days respectively (mean ± SEM; Table 3.1). Most of the cows in Group B first came into oestrus soon after turn out, the exception being one cow from each of Group B2 and B3 which did not come to oestrus until 97 days *post-partum.*

The interval from calving to first oestrus in Group C1, C2 and C3 was 87.3 ± 0.6; 105.0 ± 5.6 and 96.0 ± 5.0 days respectively (mean ± SEM, Table 3.1). The difference between Group C1 and C2 was significant ($P<0.05$). There was a longer interval from calving to conception in Group C3 compared to Groups C1 and C2 (see Table 3.2).

Table 3.2

Reproductive performance in cows injected with PGF2α either 7, 14, 21 and 28 days (Group C1) or 14 and 28 days (Group C2) *post-partum*, or with saline 7, 14, 21 and 28 days (Group C3) *post-partum.*

Group	Interval (mean ± SEM) from calving to first service. (days)	Interval (mean ± SEM) from calving to first conception. (days)	First service conception rate. (%)	Number (mean ± SEM) services per conception.
C1 (3)	87.3±0.6	95.0±7.1	66.6	1.3±0.3
C2 (3)	105.0±5.6 **b**	105.0±5.6	100	1.0±0.0
C3 (3)	96.0±5.0	110.0±4.9	33	1.6±0.3

b, Group C2 significantly different from Group C1 ($p<0.05$).
Figures in parenthesis indicate the number of cows in each group.

Mean plasma progesterone concentrations measured in twice weekly blood

samples in cows and heifers in Group A are shown in Figure 3.1a and b respectively. Progesterone levels were basal and varied from 0.1 - 0.4 ng/ml in the heifers. Plasma progesterone concentrations remained low in 5/6 cows. Slightly elevated plasma progesterone concentrations (i.e. up to 1 ng/ml) were however seen in the cows between 15 and 30 days *post-partum*. Elevated plasma progesterone conentrations (i.e. up to 2.4ng/ml) were seen in one cow between 34 and 48 days *post-partum* (Figure 3.1a). Plasma progesterone concentrations measured in twice weekly samples in the cows in Group B are shown in Figure 3.2. Plasma progesterone concentrations were generally basal in all the cows before they were put out to pasture except for a transient rise immediately before turn out in all cows in Group B1 and in one cow in each of Group B2 and B3. After turn out, many of the remaining cows in Group B2 and B3 also had transiently elevated plasma progesterone concentrations before first oestrus. The first service conception rate based on continually elevated plasma progesterone concentrations and failure to return to oestrus was 100% in all cows in Group B.

Plasma progesterone concentrations measured in the cows in Group C are shown in Figure 3.3. Plasma progesterone concentrations remained basal (<0.5ng/ml) in most of the cows before they were put out to pasture. Progesterone rose to 4.0ng/ml 8 - 12 days before turn out in two cows from Group C1. Two cows in Group C2 and one cow in Group C3 had a prolonged period with low plasma progesterone concentrations which extended until 86 - 102 days *post-partum*. Seven out of 9 cows in Group C (3 in C1, 2 in each of Groups C2 and C3) had one or two phases of prolonged elevated plasma progesterone concentrations before first oestrus. Six cows (2/3 in C1, 3/3 in C2 and 1/3 in C3) conceived at their first oestrus, as indicated by plasma progesterone concentrations which remained elevated. The remaining three cows (one in Group C1 and 2 in C3) returned to oestrus and were served again before becoming pregnant.

Figure 3.1 : Mean plasma progesterone concentrations in post-partum beef cows (section a, n = 5) and beef heifers (section b, n = 6). Section a , plasma progesterone concentration in cow 22 over the same period *post-partum* .

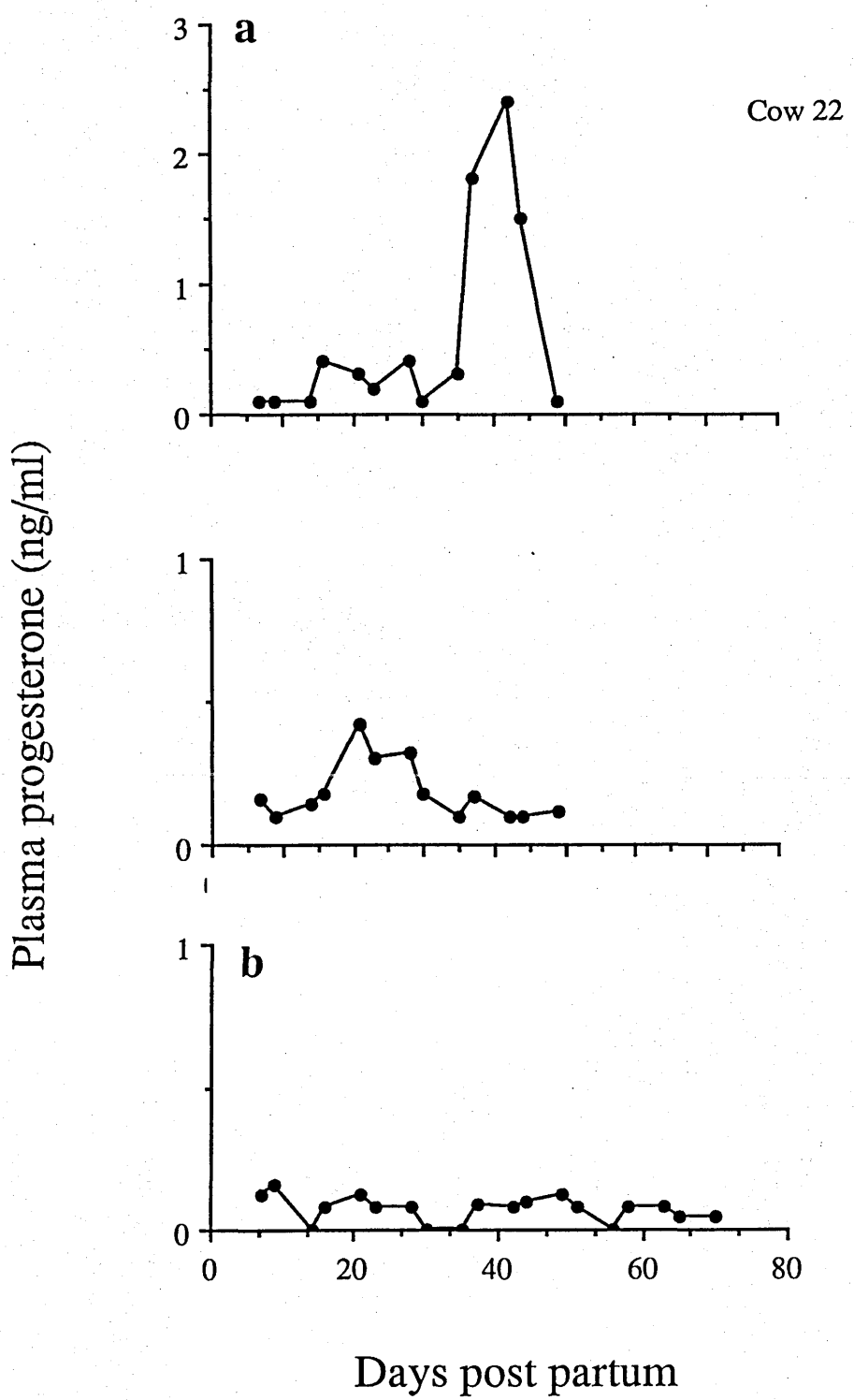

a
Cow 22
3
2
1
0
1
0
1
b
0
0
20
40
60
80
Plasma progesterone (ng/ml)
Days post partum

Figure 3.2 : Plasma progesterone profiles in cows injected with PGF2α 7, 14, 21, and 28 days *post-partum* (Group B1 , panel a) or 14 and 28 days *post-partum* (Group B2 , panel b) or with saline 7, 14, 21, and 28 days *post-partum* (Group B3 , panel c). **P**=Turned out to pasture. ↓ = day of oestrus and service.

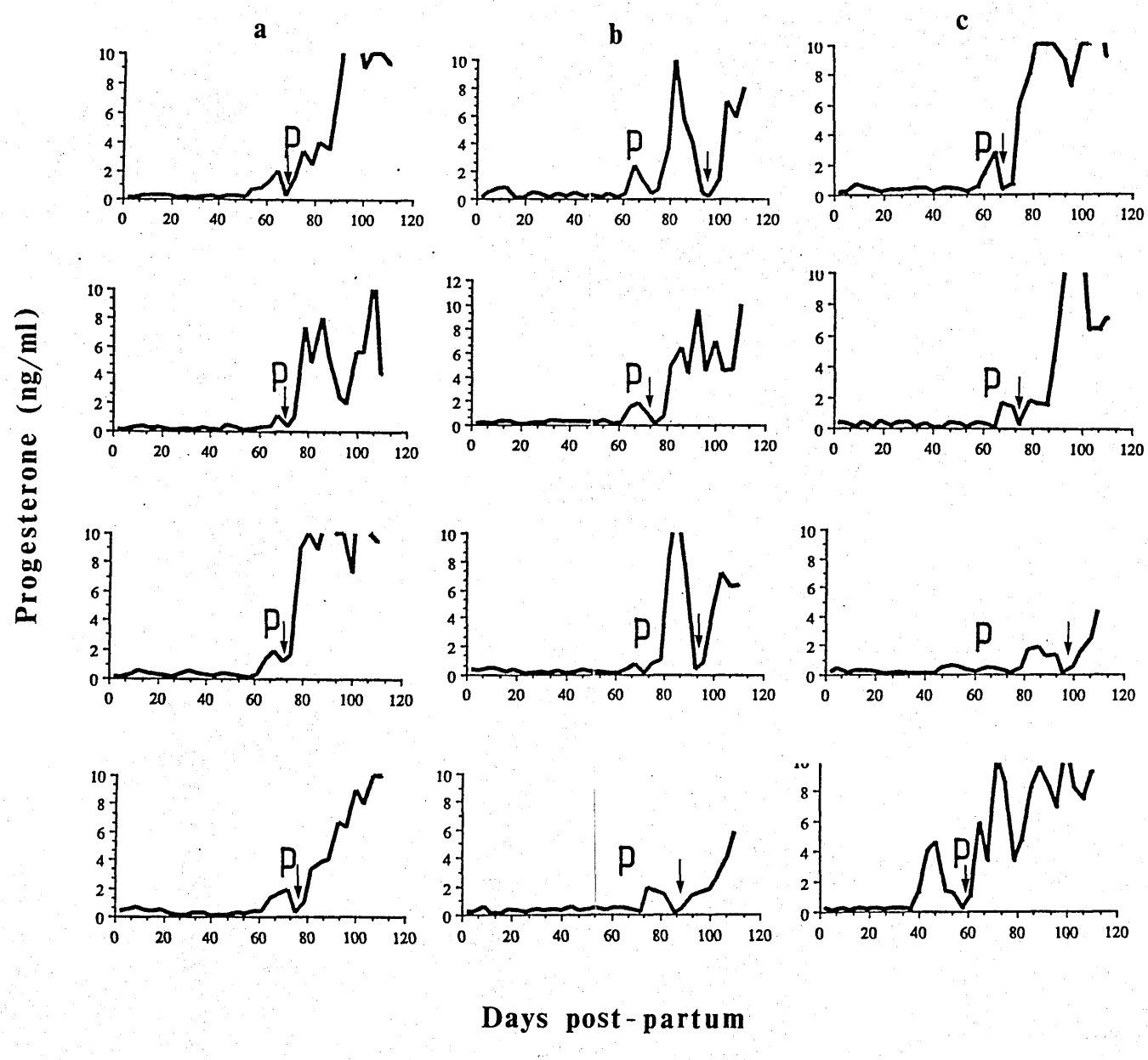
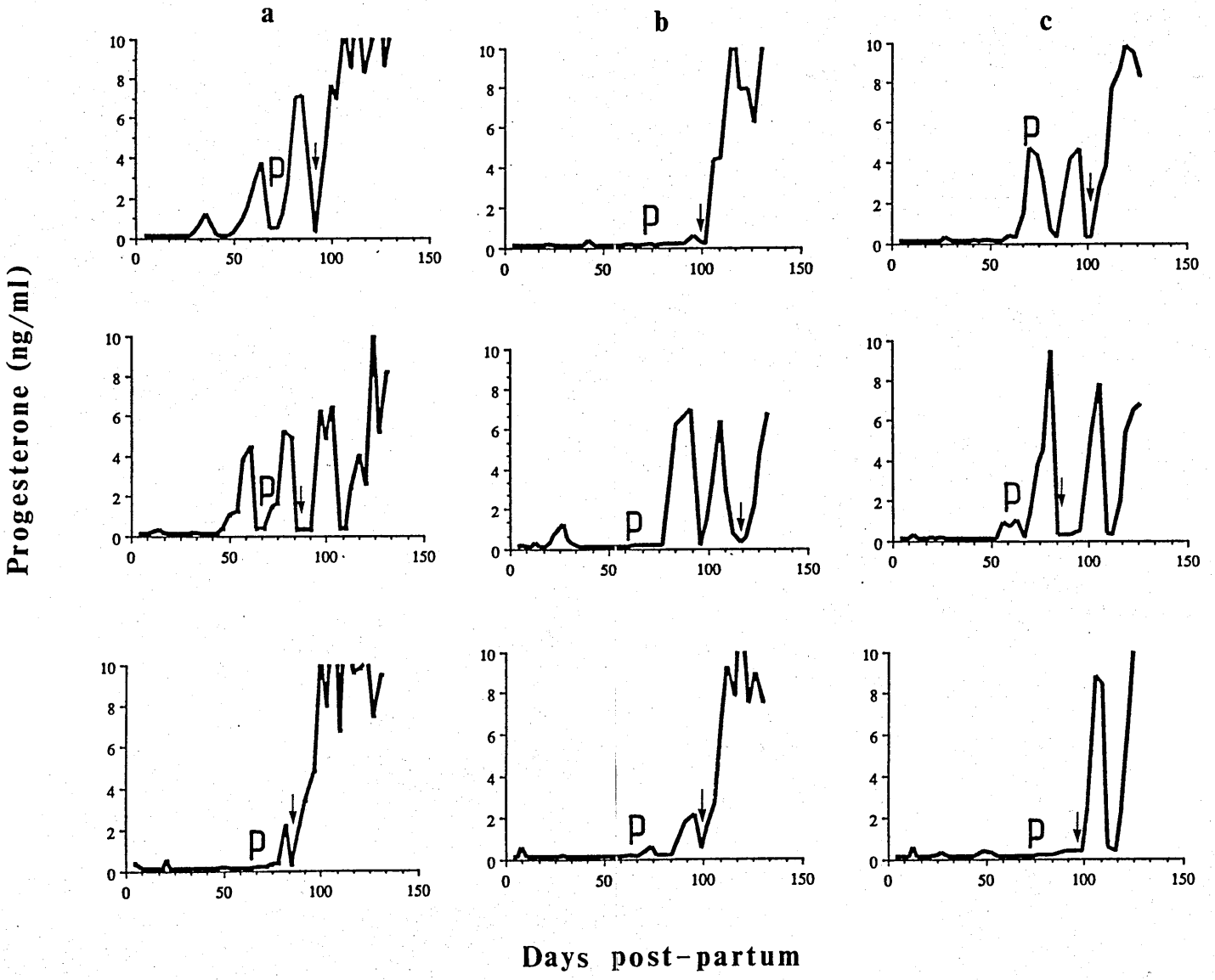

a
b
c
P
Progesterone (ng/ml)
Days post-partum

Figure 3.3 : Plasma progesterone profiles in cows injected with PGF2α 7 , 14 , 21 & 28 days *post-partum* (Group C1, panel a) or 14 and 28 days *post-partum* (Group C2, panel b) or with saline 7, 14, 21 and 28 days *post-partum* (Group C3, panel c).

P = turned out to pasture. ↓ = day of oestrus and service.


a
b
c
P
Progesterone (ng/ml)
Days post-partum
0
2
4
6
8
10
50
100
150

The mean intervals from calving to first and second ovulation as evaluated from the rise of plasma progesterone concentrations on or above 0.5ng/ml for 10 - 15 day period were shorter in Group B1 compared to B2 and B3. The difference in first and second ovulation between Group B1 and B2 were significant ($P<0.05$ and $P<0.01$ respectively, Table 3.3). Two cows in Group B2 ovulated a third time and had a cycle of normal duration. Similarly in Group C cows, the interval from calving to first and second ovulation was shorter in Group C1, than Group C2 and C3. The differences between C1 and C2 were again significant ($P<0.05$; $P<0.01$, Table 3.4).

Table 3.3

Age, interval from calving to ovulation* and oestrus cycle length in cows injected with either PGF2α 7,14,21 and 28 days or 14 and 28 days *post-partum* (Group B1 and B2 respectively) or with saline 7, 14, 21 and 28 days (Group B3) *post-partum.*

Group	Animal number	Age (years)	Interval (days) from calving to ovulation. first	second	third	Oestrous cycle length (days) first	second
B1	1	4	51	68	-	18	-
	2	11	54	72	-	19	-
	3	6	58	73	-	16	-
	4	9	52	-	-	-	-
Mean ±			53.8	71.0	-	17.7	-
SEM			± 1.5	±1.3		±0.9	
B2	5	7	61	74	94	14	20
	6	5	57	73	-	17	-
	7	7	70	90	-	20	-
	8	4	72	86	96	14	20
Mean ±			65.0b	80.8a	95.0	16.3	20.0
SEM			± 3.6	± 4.3	± 0.7	± 1.4	± 0
B3	9	5	54	68	-	14	-
	10	4	75	95	-	20	-
	11	6	38	59	-	22	-
	12	6	62	74	-	12	-
Mean ±			57.3	74.0	-	17.0	-
SEM			± 7.7	± 7.6		± 2.4	-

* Ovulation was assumed to have occurred if plasma progesterone concentration rose above 0.5 ng/ml and remained elevated for at least 10 days.

a, Group B2 significantly different from Group B1 ($P<0.01$)

b, Group B2 significantly different from Group B1 ($p<0.02$)

Table 3.4

Age, interval from calving to ovulation* and oestrous cycle length in cows injected with either PGF2α 7, 14, 21 and 28 days or 14 and 28 days *post-partum* (Group C1 and C2, respectively or with saline 7, 14, 21 and 28 days *post-partum* (Group C3).

Group	Animal number	Age (years)	Interval (days) from calving to ovulation			Oestrous cycle length (days)	
			first	second	third	first	second
C1	1	7	57	72	92	15	20
	2	10	47	64	86	17	22
	3	4	69	88	-	19	-
Mean ±			57.7	74.7	89.0	17.0	21.0
SEM			± 6.4	± 7.1	± 2.9	± 1.2	± 1.0
C2	4	4	101	-	-	-	-
	5	6	73	91	112	18	21
	6	5	86	98	-	12	-
Mean ±			86.7**b**	94.5**a**		15.0	
SEM			± 8.0	± 3.5		± 2.9	
C3	7	5	63	82	102	19	20
	8	4	67	86	110	19	24
	9	4	99	118	-	19	-
Mean ±			76.3	95.3	106	19.0	22.0
SEM			± 11.4	± 11.4	± 4.0	± 0	± 2.0

* Ovulation was assumed to have occurred if plasma progesterone concentration rose above 0.5 ng/ml and remained elevated for at least 10 days.

a, Group C2 significantly different from Group C1 ($P<0.01$)

b, Group C2 significantly different from Group C1 ($P<0.05$)

Most cows in Group C (2 in each of Group C1 and C3, one in C2) had a third ovulation. The length of oestrous cycles in prostaglandin treated cows was shorter than in untreated control cows.

The diameters of the uterine horns; the thickness of the endometrium; the cross-sectional area of the uterine horns, both ipsilateral and contralateral to the previously gravid horn; uterine body wall thickness; and the diameters of the cervical canal and the entire cervix, decreased with increasing number of days *post-partum*, as shown in Figure 3.4 and 3.5. The diameters of both the ipsilateral and contralateral horns, the cross-sectional area of the contralateral uterine horns and the diameter of the cervix, tended to regress faster in control cows (Group C3) than in prostaglandin treated cows (Group C1 and C2), although this difference was not statistically significant (Tables 3.5 and 3.6 respectively).

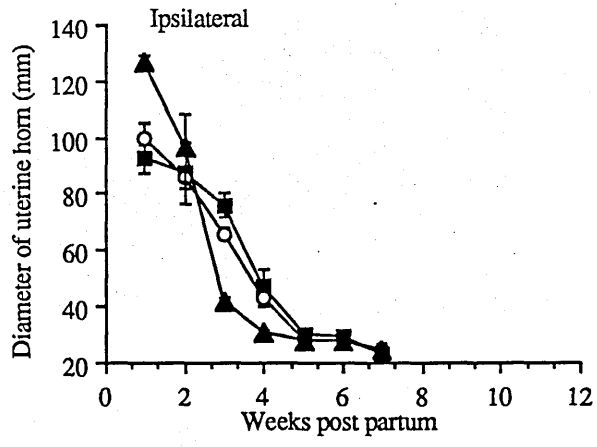
Table 3.5

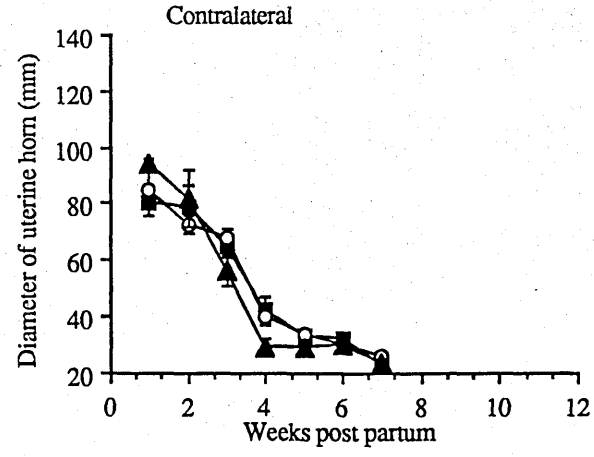
F-values obtained by two way analysis of variance of uterine horn dimensions with respect to treatment (PGF2α or saline) and time elapsed *post-partum*.

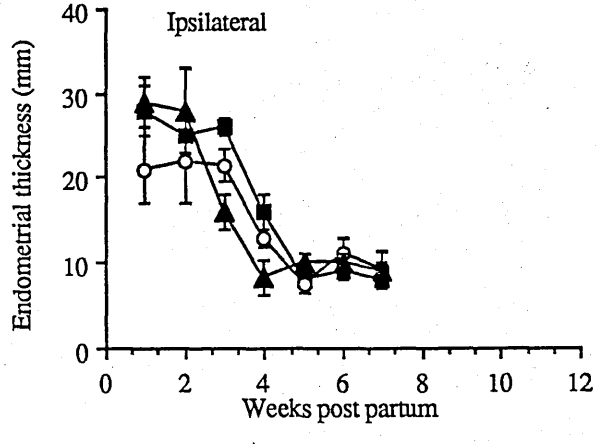
	Ipsilateral uterine horn			Contralateral uterine horn		
	diameter	endometrial thickness.	cross sectional area.	diameter	endometrial thickness.	cross sectional area.
Treatments	0.04	0.80	4.30 *	0.17	2.15	3.60
Weeks *pp.*	25.80 ***	10.14 **	18.90 ***	52.80 ***	2.66	26.80 ***

* = Significant at 5%
** = Significant at 1%
*** = Significant at 0.1%

Figure 3.4 : Changes in diameter of the uterine horns and in the thickness of the endometrium in cows injected with PGF2α 7, 14, 21, and 28 days (Group B1, ■) or 14 and 28 days (Group B2, ○) or with saline 7, 14, 21and 28 days *post-partum* (Group B3, ▲). Measurements from both ipsilateral and contralateral uterine horns are shown. (Mean ± SEM).


Ipsilateral
Diameter of uterine horn (mm)
140
120
100
80
60
40
20
0
2
4
6
8
10
12
Weeks post partum


Contralateral
Diameter of uterine horn (mm)
140
120
100
80
60
40
20
0
2
4
6
8
10
12
Weeks post partum


Ipsilateral
Endometrial thickness (mm)
40
30
20
10
0
0
2
4
6
8
10
12
Weeks post partum

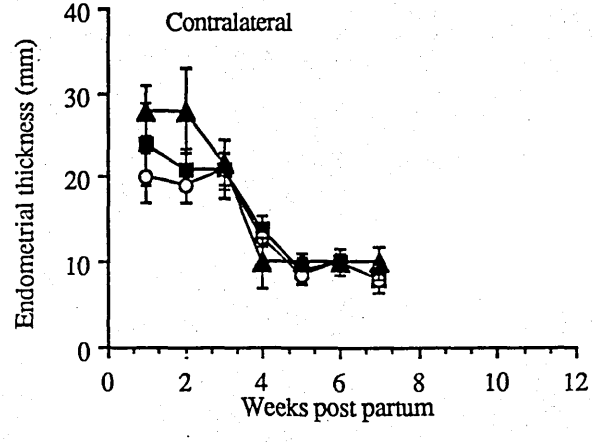

Contralateral
Endometrial thickness (mm)
40
30
20
10
0
0
2
4
6
8
10
12
Weeks post partum

Figure 3.5 : Changes in cross-sectional area of the ipsilateral and contralateral uterine horns, thickness of the uterine body wall and diameter of the cervix in cows injected with PGF2α 7, 14, 21, and 28 days (Group B1, ■) or 14 and 28 days (Group B2, ○) or with saline 7, 14, 21 and 28 days *post-partum* (Group B3, ▲)

Ipsilateral Horn
Cross sectional area of uterine horn (cm 2)
Weeks post partum
0
10
20
30
40
50
60
0
2
4
6
8
10
12

Uterine body wall thickness (mm)
Weeks post partum
20
30
40
50
60
70
80
0
2
4
6
8
10
12

Contralateral Horn
Cross sectional area of uterine horn (cm2)
Weeks post partum
0
10
20
30
40
50
60
0
2
4
6
8
10
12

Diameter of cervix (mm)
Weeks post partum
10
20
30
40
50
60
70
0
2
4
6
8
10
12

Table 3.6

F-values obtained by two way analysis of variance of both uterine body wall thickness and cervical diameter with respect to treatment (PGF2α or saline) and time elapsed *post-partum.*

	Uterine body wall thickness	Cervical diameter	
		entire cervix	canal
Treatment	6.60 *	3.99	2.69
Weeks *post-partum*	53.50 ***	130.83 ***	61.97 ***

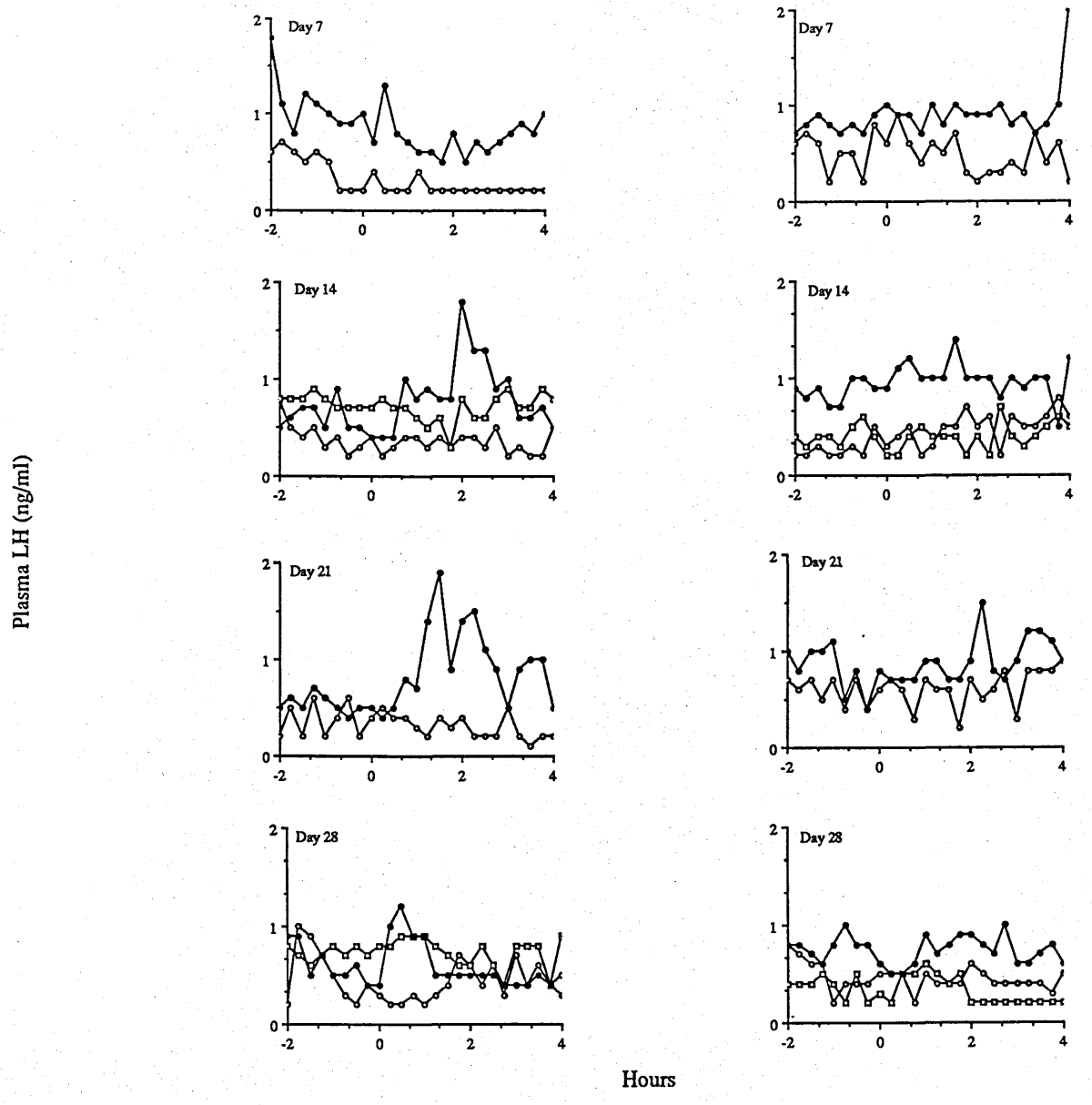
* = Significant at 5%
*** = Significant at 0.1%

The cross sectional area of the ipsilateral uterine horn and the thickness of the uterine body decreased faster in the control cows than in the prostaglandin treated cows ($P<0.5$, Table 3.5 and 3.6 respectively).

The decrease in size of all the reproductive tract parameters was significantly correlated with increasing number of days *post-partum* for all three groups of cows (see Table 3.5 and 3.6).

Plasma LH concentrations for Group C cows measured in samples collected at 15 min intervals for 2h before and 4h after prostaglandin (Groups C1, C2) or saline (Group C3) injection are shown in Figure 3.6. Plasma LH concentrations were basal for all the cows during the entire sampling period. A slight non-significant decrease in plasma LH concentration was observed in the prostaglandin treated cows compared to control cows, as shown by expressing the mean LH concentration in post injection samples as a percentage of mean pre-injection values

Figure 3.6 : Plasma LH concentration in a representative cow (left-hand panel) and group means (right-hand panel) from Group C1 (PGF2α injected on days 7, 14, 21, and 28 days *post-partum,* ○) ; Group C2 (PGF2α injected on days 14 and 28 days *post-partum,* □) and Group C3 (saline injected on days 7, 14, 21 and 28 days post-partum,●) in samples collected at 15 min intervals for 2 hours before and 4 hours after injection.


Day 7
Day 7
Day 14
Day 14
Day 21
Day 21
Day 28
Day 28
Plasma LH (ng/ml)
Hours

(Table 3.7).

Table 3.7

Mean LH concentrations in cows injected with prostaglandin (Groups C1 and C2) or saline (Group C3) in samples collected at 15 min intervals for 2 hours before injection and 4 hours after injection.

	Mean LH concentrations in ng/ml		Change %
Group	Pre-injection	Post-injection	
C1 (n=3)	0.46+0.03	0.44 +0.04	95.7
C2 (n=3)	1.09 +0.04	1.08 +0.05	99.1
C3 (n=3)	0.65 +0.06	0.75 +0.03	114.9

The plasma oestradiol-17β concentrations in Group C cows were undetectable in the majority of blood samples collected during the intensive sampling periods, either 7, 14, 21 and 28 days (Group C1, C3) or 14 and 28 days (Group C2) *post-partum.*

Discussion

In 1986, the pattern of plasma progesterone concentration was studied in beef cattle during the post-partum period. Blood sampling was continued until 49 - 70 days *post-partum* at which time the animals were put out to pasture. None of the cows or heifers exhibited oestrus and plasma progesterone concentrations generally remained basal until turn out. Elevated plasma progesterone concentrations were seen in only one cow between 34 and 49 days *post-partum* but she did not exhibit oestrus until day 73. It is apparent from this study that the interval from calving to first oestrus is longer in heifers than cows (105 vs 85 day). A similar result has been reported by Knight and Nicoll (1978). It is also evident from the basal plasma progesterone concentrations that ovulation and subsequent development of functional corpora lutea occurred in only one cow towards the end of the study period.

The remaining animals were therefore anoestrous for a minimum of 49-70 days. A number of other workers (eg. Dunn and Kaltenbach, 1980; Carruthers and Hafs, 1980; Lamming *et al.*, 1981; 1982; Peters *et al.*, 1981a) have similarly demonstrated the long duration of anoestrus in the suckled beef cow.

In addition to the well known ability of prostaglandins to synchronize oestrus in cattle (Jackson *et al.*, 1979; Beal, 1983) by causing premature luteolysis (Cooper, 1974; Hansel and Beal, 1979; Beal *et al.*, 1980), a single injection of the prostaglandin analogue, dinoprost tromethamine given 14-28 days *post-partum* has been reported to increase conception rate significantly (Young *et al.*, 1984) and to shorten the interval from calving to first oestrus (Young and Anderson,1986). The experiments conducted in 1987 and 1988 were designed to further investigate the possibility of a direct effect of prostaglandin to enhance the rate of uterine

involution and perhaps to stimulate ovarian activity.

Uterine dimensions and other characteristics such as consistency and tone and position of the uterus in the pelvic cavity, have been regarded as suitable criteria with which to judge the progress of uterine involution. It appeared from the study of Young and Anderson (1986) that prostaglandin shortened the interval from calving to first oestrus and from calving to conception in cows without a functional corpus luteum as indicated by low plasma progesterone concentrations (< 0.5ng/ml). A direct effect of prostaglandin on the reproductive endocrine axis, a function which has not yet been attributed to prostaglandin, was thus postulated (Etherington, 1984; Young and Anderson,1986).

Ovarian activity was judged by evaluating the intervals from calving to oestrus and ovulation and from calving to conception and the number of sevices required before pregnancy was confirmed. These evaluations were based on plasma progesterone concentrations, rectal palpation and ultrasound recordings of the reproductive tract, records of oestrus and frequent blood sampling to investigate LH and oestradiol-17β concentrations.

The mean interval from calving to first oestrus in prostaglandin treated cows was not significantly different from saline treated control cows in the 1987 experiment. On the other hand in the 1988 experiment the interval between parturition and first oestrus was shorter in Group C1 (4 prostaglandin injections) compared to the control Group C3 ($P< 0.1$). It therefore appears that prostaglandin may shorten the interval from calving to first oestrus when given at weekly intervals after calving. Young and Anderson (1986) also reported that prostaglandin shortened the interval to first oestrus but they used one prostaglandin injection given between 14 and 28 days *post-partum.*

The results from the present study differ from those obtained by Revah *et al.* (1988) in which there was no effect of prostaglandin-treatment on reproductive performance in Mexican dairy cows. Similarly Stevenson and Call (1988) have failed to find any improvement of reproductive performance in cows after the use of prostaglandin during the post-partum period.

The mechanism of action of prostaglandin treatment in shortening post-partum anoestrus in the cow has not yet been elucidated. The present results would seem to rule out a stimulatory effect on the pituitary-ovarian endocrine axis but such a pharmacological dose of prostaglandin may act directly on the uterus to accelerate involution as indicated by Lindell and Kindahl (1983). However, it may also be argued that the results obtained in the present study can be accounted for by the variation in age of the cows rather than an effect of prostaglandin. Thus most of the cows in the control groups and in the groups given two prostaglandin injections were younger than cows given 4 prostaglandin injections (Table 3.3).

It appears from the results that the length of the first post-partum oestrous cycles were shorter in treated cows than in control cows. A high incidence of short oestrus cycles *post-partum* has been reported in dairy cows (Macmillan and Watson, 1971; Eger *et al.*, 1988) beef cows (Odde *et al.*, 1980) and ewes (Land, 1971) but this is considered to be a normal physiological phenomenon. It has been suggested that the length of post-partum anoestrus decreases with decreased suckling intensity (Randel, 1981). So one effect that prostaglandin treatment *post-partum* might have had is to shorten the interval between calving and first oestrus. This tended to hold true for Groups B1 and C1 which received four injections of prostaglandin, although the effect was not statistically significant (see Table 3.1). However other parameters (eg. age, nutrition or housing) are also determinants of the length of the post-partum anoestrus. The interval from calving to first service in

cows in Group C1 (4 prostaglandin injections) was significantly shorter than the equivalent intervals in Group C3 (control) and C2 (two prostaglandin injections, see Table 3.4). This result is difficult to justify in view of the results of Young and Anderson (1986) who reported that a single injection of prostaglandin administered between 14 and 28 days *post-partum* shortened the interval to first service.

However, the interval from calving to conception was shorter in Group C2 (2 prostaglandin injections) and first service conception rate was 100%, better than was seen in control cows or cows given 4 prostaglandin injections. However, these results need corroborating in view of the small number of cows involved.

During the normal bovine oestrous cycle the luteal phase extends from days 1 to 17 and high plasma progesterone concentrations are observed from days 3-4 to 17-18 (Hafs and Armstrong, 1968; Pope *et al.*, 1969; Robertson, 1972). A shorter period of elevated plasma progesterone was observed before first observed oestrus in the majority of the cows in both treated and control groups in 1987 (Group B) and in 1988 (Group C). A similar rise in serum progesterone has been observed in suckled beef cows before the first observed oestrus (Kiracofe, 1980; Wettemann, 1980) but such a feature is not universally seen (e.g. Odde *et al.*, 1980). Indeed some cows in the present study did not display elevated plasma progesterone concentrations before first oestrus but had a more prolonged period of anoestrus (ie.low plasma progesterone concentration) instead (Figure 3.2; 3.3). The source of this pre oestrous rise in plasma progesterone has been reported to be a corpus luteum that forms after ovulation which is unaccompanied by oestrus (Castenson *et al.*, 1976; Kesler *et al.*, 1980).

The transient nature of these early luteal structures may be due to improper luteinization. Possible reasons to explain such luteal insufficiency after the first

post-partum ovulation include: i) lack of sufficient luteotrophin, ii) lack of luteotrophin receptors, iii) presence of a luteolytic agent (Odde *et al.*, 1980). The presence of a corpus luteum was not investigated in the present study. Corah *et al.* (1974) also suggested that progesterone may be produced from luteinized follicles. A similar increase in progesterone was observed before first oestrus in prepubertal heifers (Berardinelli *et al.*, 1979) which was assumed to be secreted by non palpable luteal tissue embedded in the ovary. It has also been demonstrated that ovarian follicles undergoing atresia in post-partum beef cattle can become luteinized (Wagner and Hansel, 1969; McKenzie and Kenney, 1973) and capable of secreting progesterone in response to gonadotrophin treatment (D'Amato *et al.*, 1979). The frequent occurrence of at least one transient rise in plasma progesterone in cows used in the present study, however, supports the view that exposure to progesterone may be necessary for the development of normal cyclic reproductive activity (Donaldson *et al.*, 1970).

From the foregoing discussion it can be seen that there is considerable evidence to suggest that ovulation, followed by luteal function, can occur in the absence of oestrus during the post-partum period. In the present study the occurrence of ovulation was not investigated directly (ie. by rectal palpation or ultrasonography) but was assumed to have occurred if plasma progesterone concentrations rose more than 0.5 ng/ml and remained elevated for at least 4 consecutive blood samples (10-15 days duration). Subsequent ovulations were similarly implied if plasma progesterone concentrations rose again at a later date after first dropping to basal levels (< 0.5ng/ml). On this basis the mean intervals from calving to first and second ovulations were shorter in the cows in Group B1 (4 prostaglandin injections)than in cows in groups B2 and B3 but the differences were not significant. Similarly the intervals from calving to first and second ovulations in Group C1 (4 prostaglandin injections) were shorter than Groups C2

(2 prostaglandin injections) and C3 (Saline injected) cows. Again, the differences were not statistically significant.

Uterine involution appeared to be adversely affected by prostaglandin treatment as the rate of involution was faster in control cows than in prostaglandin treated cows. Reports of similar experiments in the literature offer conflicting evidence. For example Lindell and Kindahl (1983) demonstrated that exogenous PGF2α promoted uterine involution but more recently Guilbault *et al.* (1987) demonstrated no difference in decrease in cervical and uterine horn diameter in cows receiving Flunixin Meglumine, a prostaglandin synthetase inhibitor and cows receiving PGF2α and or saline, early in the post-partum period (days 1-10), a time when endogenous prostaglandin production is supposed to be maximal. Additionally, in the latter study, ovarian activity was reported to be reduced by partial suppression of prostaglandin synthesis early in the post-partum period. However, the slower rate of decrease in diameter of the uterine body wall and in the area of the ipsilateral uterine horn in prostaglandin treated cows in the present study supports the view that such treatment does not enhance uterine involution. In fact it would seem that exogenous prostaglandin interferes with uterine involution. This might be caused either by antagonism of endogenous prostaglandin synthesis /release or by an action on the uterus, either directly (eg. affecting motility or uterine vasculature) or indirectly (eg. interfering with endogenous prostaglandin actions). The work of Guilbault *et al.* (1987) confuses the issue further since they showed that inhibition of endogenous prostaglandin synthesis did not reduce the rate of uterine involution, whereas results published by Kindahl's group (eg Lindell and Kindahl, 1983) emphasise the importance of prostaglandins in promoting uterine involution.

The finding of consistently low plasma LH concentrations observed in this study differs from the results of Riley *et al.* (1981) who reported development of a

distinct pattern of pulsatile LH release by day 25 *post-partum* in suckled beef cows. However, the mean plasma LH concentration in the latter study (0.89 ± 0.02 ng/ml) agrees quite well with results from the control cows in this study (0.82 ± 0.15ng/ml.).

The absence of a pulsatile pattern of LH release in the present study may be attributable to the sampling frequency (i.e. once every 15 minutes) which is perhaps too slow for detailed analysis of LH release patterns, but which was considered adequate to detect any effect of prostaglandin on basal plasma LH concentration. Basal plasma LH concentration and a conspicuous absence of LH surges were demonstrated in the present study after every prostaglandin and saline injection. This rules out the possibility that injection of prostaglandin can initiate ovarian activity by causing LH release during the first four weeks of the post-partum period. This conclusion agrees with the view of Peters (1988) that injection of prostaglandin has no consistent effect on LH patterns. Peters (1988) did show however that prostaglandin injection appeared to depress LH pulse amplitude in some cases. Plama LH concentration is suppressed by suckling during the early post-partum period and does not increase during the first four weeks after calving (Kesler *et al.*, 1980). Complete removal of the suckling stimulus not only stimulates ovarian activity but also increases the proportion of cows exhibiting oestrus concurrently with the first ovulation *post-partum* (Smith *et al.*, 1979).

Several factors and combinations of conditions are known to influence post-partum reproduction as reviewed in chapter I. The failure of prostaglandin to improve reproductive performance by enhancing uterine involution and advancing ovarian cyclicity can probably be attributed to continued suckling which may have delayed ovarian cyclicity, as has been suggested by several authors. Despite this, however, Peters and Riley (1982a) observed no difference in the length of post-

partum anoestrus in cows which were suckled only twice daily compared to cows which suckled *ad libitum*. However, it is reasonable to assume that suckling is one of the factors responsible for prolonging the post-partum acyclic period.

It is apparent from the results obtained by ultrasound scanning, from detailed hormonal analysis of blood samples collected at the time of prostaglandin injections and from studying the progesterone profiles of the cows that no consistent effect of PGF2α could be detected either on ovarian activity and endocrinology or on uterine involution, at least within a 28 day period *post-partum* when the study was conducted.

Chapter IV

Effects of prostaglandin administration on reproductive endocrinology and uterine involution in dairy cattle.

Introduction

Induction of premature parturition in cattle has several potential advantages such as termination of gestation for clinical reasons; shortening of gestation to take advantage of available fodder; easier management of calving; prevention of late calving and termination of unwanted pregnancies (Adams, 1969; Day, 1977a, b; Prakash and Madan, 1985). However induction of pre-term parturition has been associated with placental retention (McDonald *et al.*, 1954; Chew *et al.*, 1978). Several other factors are also associated with placental retention such as bacterial infections, poor nutrition and genetic pre-disposition (Leslie, 1983).

There are several methods available for induction of parturition but different formulations of synthetic glucocorticoids such as dexamethasone, flumethasone and betamethasone are most popular. All these formulations have been used to terminate pregnancy in cattle, sheep and goats (Adams and Wagner, 1970; Jochle, 1971; Carroll, 1974; MacDiarmid, 1983). However, the incidence of placental retention following induction of parturition generally has been high (Wiltbank *et al.*, 1984; Prakash and Madan, 1985). The use of oestrogens in combination with dexamethasone or flumethasone reduced the occurrence of placental retention in a study by Garverick *et al.* (1974) whereas (Kesler *et al.*, 1976; Prakash and Madan, 1985) reported that additional oestrogens gave variable results.

Normally expulsion of fetal membranes occurs spontaneously after parturition and depends on the balance between plasma progesterone and oestradiol-17β concentrations. It has been suggested that altered hormone ratios may be responsible for placental retention (Chew *et al.*, 1979a). Thus Agthe and Kolm (1975) and Chew *et al.* (1977; 1978; 1979 a, b) demonstrated that plasma progesterone values were elevated while oestrogen values were depressed in cows in which retention of the placenta occurred compared to problem free cows,

regardless of whether the calving occurred spontaneously or after glucocorticoid induction. The incidence of placental retention is tenfold lower when plasma progesterone and oestradiol-17β concentrations were intermediate (4 - 8 ng/ml) and high (>99 pg/ml) respectively than when progesterone and oestradiol-17β concentrations were low (Chew *et al.*, 1979a).

A well balanced co-ordinated endocrine environment is necessary for normal reproductive functions *post-partum*. This chiefly involves integration of synthesis and release of gonadotrophin releasing hormone (GnRH) from the hypothalamus; FSH, LH and prolactin from the anterior pituitary; PGF2α from the uterus and the steriods, oestradiol and progesterone, from the ovaries. It has been demonstrated that increased amounts of oestrogens are synthesized before parturition from the feto-placental units (Convey, 1974) whereas progesterone levels decline rapidly during the last 48 hours before parturition. The plasma concentrations of both oestradiol and progesterone remain very low throughout the early post-partum period (Hoffman *et al.*, 1973; Keslar *et al.*, 1977) even though ovarian follicular activity is initiated by 5 - 10 day *post-partum* (Marion and Gier, 1968; Erb *et al.*, 1981). Plasma FSH concentrations fluctuate between 30 - 70 ng/ml and are somewhat independent of other hormonal changes. Plasma FSH concentrations increase slightly or remain constant during parturition and the early post-partum period (Dobson, 1978) suggesting that FSH secretion is unlikely to be a limiting factor in the onset of ovarian activity *post-partum* (Carruthers *et al.*, 1980).

During the oestrous cycle basal plasma LH concentrations are interrupted by pulses, each of which comprises a rapid increase followed by a more gradual return to basal levels (Foster *et al.*, 1980; Rahe *et al.*, 1980). The frequency of pulsatile LH release increases markedly just prior to ovulation and this results in a marked

increase in LH concentrations. This is known as the preovulatory LH surge. A similar LH release pattern is found prior to the first ovulation in post-partum cows (Schallenberger *et al.*, 1978; Carruthers and Hafs, 1980; Foster *et al.*,1980; Webb *et al.*, 1980). So pulsatile LH secretion may be required to initiate oestrous cycles *post-partum*. Certainly pulsatile LH release and ovulation can be induced in beef cows during post partum anoestrus by repeated i.v. injections of small doses (0.5 - 5.0 ug)of GnRH (Riley *et al.*, 1981; Walters *et al.*, 1982). This approach did not however always induce ovulation (Edwards *et al.*, 1983).

The classical endocrinological techniques of ablation and replacement therapy clearly demonstrate the importance of the ovarian steroids, oestradiol and progesterone in regulating LH secretion in cattle (Cummins *et al.*, 1972; Hobson and Hansel, 1972; Short *et al.*, 1973; Convey *et al.*, 1977; Kesner *et al.*, 1981). Oestradiol can exert both negative and positive feedback effects on LH secretion (Scaramuzzi and Baird, 1977; Walters *et al.*, 1984). The latter is likely to be of great importance in the initiation of cyclic activity *post-partum* (Peters, 1984b). Injection of oestradiol-17β into sexually mature female cattle can induce LH release (Rahe *et al.*, 1980) and, in a review of the subject, Leslie (1983) concluded that the interactions between oestradiol and LH to cause a preovulatory-type LH surge depend primarily on the circulating oestradiol-17β concentration. In late pregnancy there is a gradual increase in oestrogen concentrations to values markedly higher than those seen during the oestrous cycle and this may have an inhibitory effect on pituitary LH synthesis and release.

This situation differs from the naturally stimulatory effects of oestradiol and LH seen during preovulatory period and during late proestrus of the normal cycle when plasma progesterone concentrations are very low (Leslie, 1983). Oestrogen levels are high during the last 40 to 60 days prepartum until 6 days *post-partum* and this inhibits the pituitary LH surge mechanism during the early post-partum

period (Kesler *et al.*, 1977; Fernandes *et al.*, 1978). It has also been postulated that the previously gravid uterine horn and/or the ovary bearing the corpus luteum of the previous pregnancy is involved in inhibition of follicular development (Nalbandov and Casida, 1940; Rexford and Casida, 1975) and this inhibitory effect is maintained during the early postpartum period and reduces the frequency of ovulation from the ovary ipsilateral to the previously gravid uterine horn (Saiduddin *et al.*, 1967).

Plasma oestradiol-17β concentrations fall after parturition and fluctuate considerably prior to the first preovulatory oestradiol-17β peak (Pope, 1982) which induces the LH surge by positive feedback effects on both the hypothalamus and the pituitary gland (Kesner *et al.*, 1981). Several attempts to investigate the response of the pituitary to exogenous GnRH during the pre and post-partum period have been made and these demonstrate that LH release in response to GnRH injection is substantially reduced from 35 days before until 8 - 10 days after parturition (Kesler *et al.*, 1977; Fernandes *et al.*, 1978; Schallenberger *et al.*, 1978). Full responsiveness returns by around day 10 *post-partum.* However, a full response could be produced as early as 2 days *post-partum* by using a combination of oestradiol, progesterone and then GnRH (Azzazi *et al.*, 1980).

Plasma concentrations of progesterone remain high during pregnancy, except perhaps for a small fall between 60 and 90 days after conception (Robertson, 1972). Progesterone appears to suppress the hypothalamus but not the pituitary (Pope *et al.*, 1968) since the pituitary can fully respond to exogenous GnRH throughout pregnancy (Schallenberger *et al.*, 1978) and follicular activity, which requires gonadotrophin support, is apparent in both ovaries irrespective of the presence of the corpus luteum of pregnancy (Ramirez-Godinez *et al.*, 1987). Plasma progesterone concentration must be low to allow the rise of oestradiol-17β

concentration, through gonadotrophin induced follicular activity, prior to ovulation in cycling cows and the first oestrous cycle in post-partum cows (Short *et al.*, 1979).

The size of ovarian follicles at parturition varies from 0.16 - 0.68 mm in diameter and whereas follicles smaller than 0.29mm decrease in size, follicles larger than 0.29mm increase in size with time from parturition (Dufour and Roy, 1985). Scaramuzzi *et al.* (1980) demonstrated that an interval of 22 days was required for a follicle to grow from 0.4mm to 10mm in diameter. Garverick *et al.* (1980) suggested that a follicle larger than 10mm is required for the first post-partum ovulation since they induced ovulation after injecting GnRH at this critical stage of follicular development. The first ovulation has been reported to occur between 10-25 days *post-partum* (Morrow *et al.*, 1966; Marion and Gier, 1968) and 50% of dairy cows exhibit a short oestrous cycle during the early post-partum period (Morrow, 1969; Schams *et al.*, 1978). The oestrous cycle following the first ovulation may be shortened due to premature regression of the corpus luteum Morrow *et al.*, 1969) perhaps due to insufficient release of LH during the preovulatory LH surge, which fails to complete luteinization (Echternkamp and Hansel, 1973). Such short cycles may be important in the establishment of normal oestrous cycles during the post-partum period since they provide a short period of exposure to progesterone which primes reproductive endocrine processes (Short *et al.*, 1979).

Plasma PGF2α concentrations increase sharply at parturition and are associated with the marked decrease in plasma progesterone concentrations (Fairclough *et al.*, 1975; Edqvist *et al.*, 1978). Prostaglandin induced abortion is also associated with a sudden drop in progesterone concentrations and this drop is considered to affect the luteolytic action of PGF2α released immediately after

abortion (Lindell *et al.*, 1980) . As the PGF2α release continues for about 10 - 20 days after calving, any change in action of PGF2α at this time seems to be related to the time course of uterine involution (Lindell *et al.*, 1982). Prolonged uterine involution is associated with an extended period of elevated plasma PGF2α concentration (Edqvist *et al.*, 1978). It has been reported that uterine involution is delayed significantly (Marion *et al.*, 1968) and that resumption of cyclic ovarian activity *post-partum* progresses more slowly in cows with periparturient diseases or complications, such as retention of the placenta and metritis, than in cows which undergo normal trouble-free delivery (Morrow *et al.*, 1966). Thus in the former group the uterus was significantly larger at 10 - 20 days *post-partum* and did not return to a non gravid size until approximately day 30; although as discussed in chapter I, age and parity are important determinants of the size of the uterine horns (Buch *et al.*, 1955; Morrow *et al.*, 1966; Morrow, 1969). It is assumed that uterine infection immediately after parturition may reduce PGF2α release and consequently delay involution (Stabenfeldt *et al.*, 1980). Certainly abnormal delay in uterine involution *post-partum* has been associated with inadequate endogenous production of prostaglandin during the immediate post calving period (Lindell *et al.*, 1982) and repeated administration of synthetic prostaglandins for 3 - 13 days shortened the involution time (Lindell and Kindahl, 1983).

A breeding programme for cattle in which abortion has been induced should consider their subsequent pattern of reproduction. Lindell *et al.* (1980) reported a marked difference between cows in which abortion had been induced before 80 days of pregnancy and cows which aborted after 100 days of pregnancy. They demonstrated that cows aborted before 80 days came into oestrus within three days and subsequently had normal oestrous cycles whereas cows aborted after 100 days of pregnancy took longer to return to oestrus and subsequently had a short oestrous cycle before normal cycles resumed.

PGF2α seems to be useful in reducing the incidence of placental retention. It has been shown to induce profound (Zerobin *et al.*, 1971) and prolonged myometrial contractions in humans (Karim *et al.*, 1971), sheep (Edqvist *et al.*, 1975) and cattle (Patil *et al.*, 1980) and its metabolites have been seen to rise during spontaneous parturition in cows (Fairclough *et al.*, 1975; Hunter *et al.*, 1977; Edqvist *et al.*, 1981). PGF2α and its analogues are widely used as therapeutic agents in pyometra (Fazeli *et al.*, 1980) and in chronic metritis, (Jackson, 1977; Duncanson, 1980) for synchronization of oestrus (Hafs and Manns, 1975; Olson, 1980; Lucas *et al.*, 1983), for the treatment of cows not detected in oestrus (O'Farrell and Hartigan, 1984) and for the improvement of fertility (Macmillan *et al.*, 1980; Young and Anderson, 1986). The latter authors concluded that increased fertility might be the result of a direct effect of the prostaglandin on the endocrinology of the cow. However, the physiological role of endogenous PGF2α and its role as an agent for the improvement of reproductive performance is not clearly understood. The present study was undertaken to investigate the possibility of a direct effect of exogenous prostaglandin on the endocrine function of the anterior pituitary gland and the ovaries and also on uterine involution.

Materials and Methods.

Experiment 1.

Eight Friesian dairy cows were bought in at various stages of pregnancy (46 - 124 days) over the period from October, 1986 to December, 1987. They were housed tied in stalls and were fed hay and concentrates to maintain requirements. Pregnancy was confirmed by rectal palpation.

Each cow was injected with 2ml (500μg) of prostaglandin analogue (cloprostenol, Estrumate, I.C.I.) in order to induce abortion. After abortion, stage of pregnancy was determined by measurement of the crown-rump length of the aborted fetus and by reference to a normogram (Evans and Stack, 1973). The condition of the ovaries and the uterus of each cow were assessed daily by rectal palpation for aproximately 20 days after abortion. The cows were released into a yard and observed for signs of standing oestrus at aproximately 8.00 a.m. and 4.00 p.m. each day.

The animals were divided randomly into two groups of four. Group P1 (Prostaglandin treated) received 2 injections of 2 ml cloprostenol (500 μg) 3 to 5 days apart starting approximately one week after abortion. Group S1 (saline control) received saline (2 ml) at a comparable time after abortion.

Daily blood samples were taken starting before prostaglandin injection was given to induce abortion. Blood samples were also collected as described in Chapter III at 15 min intervals for 2 hours before and 4 hours after each prostaglandin or saline injection during the post-abortion period.

Daily blood samples were analysed for plasma progesterone concentration by RIA as described in chapter II. Blood samples collected during the intensive sampling periods were analysed for oestradiol-17β and LH as described in chapter II.

Experiment 2.

In another separate experiment 21 Friesian dairy cows from a commercial dairy farm were used. The cows calved normally during the period from 27 January to 16 February 1988. The dairy farm was visited regularly by D. B. Anderson from the University of Glasgow Veterinary Practice, Whitelees Road, Lanark.

The cows were divided randomly into 2 groups. Each cow in Group P2 (n=11) received 2ml (500μg) of the prostaglandin analogue, cloprostenol, 14 days *post-partum*. Cows in Group S2 (n=10) received 2ml of physiological saline at a comparable time. One cow in each group was excluded later due to E.coli mastitis. Blood samples were taken every 2 days for 10 days, starting 2 days before prostaglandin/saline injection (Day 0=Day of injection) and were used for analysis of progesterone concentration by RIA (see chapter II).

Blood samples were also taken at 30 min intervals starting 30min before and continuing for 2h after prostaglandin/saline injection. Plasma from these blood samples was analysed for LH and oestradiol-17β concentration by RIA (see chapter II).

The interval from calving to first service was recorded and first service conception rate was calculated from the subsequent return data. The dimensions of the cervix and the uterine horns were measured on day 0 and day 10 by rectal palpation.

Results.

Experiment 1.

Each cow aborted after prostaglandin injection. A bloody discharge was seen at the vulva only in cows 4,7 and 8 which were estimated to be between 115 and 138 days pregnant. The other cows which were at earlier stages of pregnancy, aborted without a bloody discharge. The time taken from injection to abortion varied from 68 to 168 hours as shown in Table 4.1 and tended to be longer the more advanced the pregnancy.

Table 4.1

Summary of clinical observations in cows following prostaglandin - induced abortion. Cows 1 - 4 (Group P1) received additional injections of prostaglandin (PG) approximately 5 and 10 days after abortion. Cows 5 -8 (GroupS1) received injections of saline at comparable times.

Animal number.	Stage of pregnancy. (Days)	Interval from PG injection to abortion. (Hours)	Interval from abortion to first observed oestrus. (Days)	Interval from abortion to first palpable corpus. (Days)
Group P1				
1	46	72	12	14
2	70	96	15	18
3	99	168	19	-
4	124	168	23	-
Group S1				
5	73	68	21	9
6	100	96	15	-
7	115	120	16	13
8	138	144	13	-

Cow 1 and 2 in Group P1 (prostaglandin treated) and cow 5 in Group S1 (saline

treated) were less than 100 days pregnant and required less time to abort. One cow in Group P1 (no.3) and another in Group S1 (no.6) which were 99 and 100 days pregnant respectively, took 168 and 96 hr to abort. The other cows were more than 100 days pregnant and aborted between 120 and 168 hr after prostaglandin injection.

Rectal palpation of the ovaries was performed on a regular basis for the first 20 days post-abortion. Pertinent observations are recorded on Figures 4.1 and 4.2. A corpus luteum was palpated prior to oestrus in two cows in Group S1 (cows 5 and 7) and after oestrus in two cows in Group P1 (cows 1 and 2). The interval from abortion to first oestrus varied from 12 to 23 days, as shown in Table 4.1, and tended to be shorter the shorter the pregnancy.

Changes in plasma progesterone concentrations after abortion are shown in Figure 4.1 and 4.2. Plasma progesterone concentrations in the pregnant cows before prostaglandin injection varied from 4-6 ng/ml. Progesterone dropped to less than 2ng/ml within approximately 2 days of prostaglandin injection and to less than 0.5ng/ml on the day of abortion in all the cows. Plasma progesterone concentrations remained basal after abortion for a length of time which generally increased with increasing length of pregnancy. The only real exception to this was in cow 7 (Group S1, 115 days pregnant, Fig 4.2) in which plasma progesterone concentration became elevated in daily samples starting approximately 5 days *post partum*. In many instances elevations in plasma progesterone concentration coincided with detection of a corpus luteum by rectal palpation. Injections of PGF2α or saline coincided with elevated plasma progesterone concentration in two cows in Group P1 (cows 1 and 2) and two cows in Group S1 (cows 5 and 7), but only in the latter group were corpora lutea palpated. PGF2α injections caused rapid lowering of plasma progesterone concentration to baseline and this was associated

Figure 4.1 : Plasma progesterone concentration in cows in Group P1 after injection of prostaglandin to induce abortion(Day 0 = day of abortion) and following abortion, when each cow was injected twice (↓) with PGF2α

NPS = No palpable structure found on either ovary by rectal palpation.
CL = A corpus luteum found on one ovary by rectal palpation.
PUS = Pus recorded in the vagina.
Oe = Cow observed in oestrus.

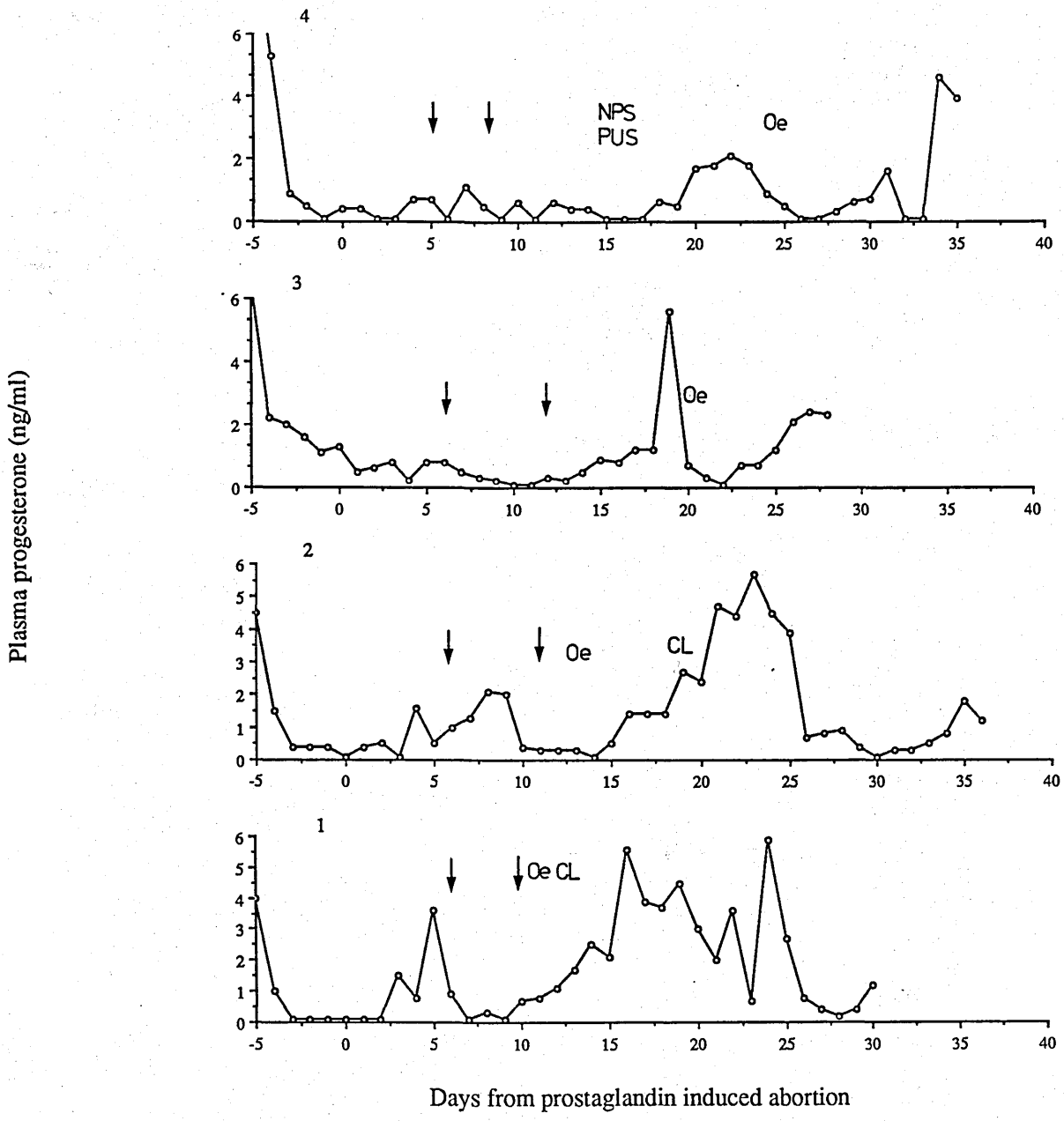
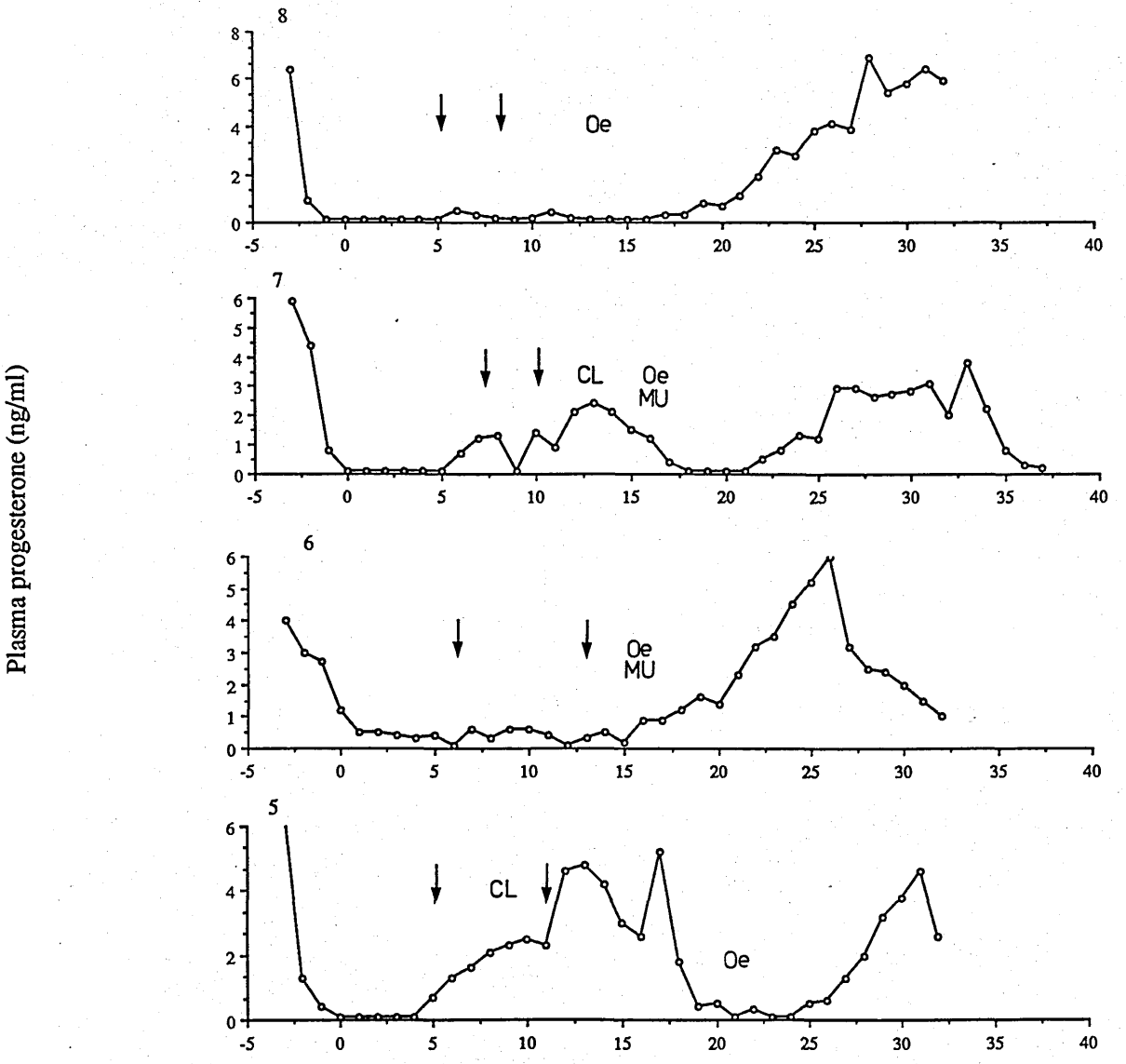

4
NPS
PUS
Oe
3
Oe
2
Oe
CL
1
Oe CL
Plasma progesterone (ng/ml)
Days from prostaglandin induced abortion

Figure 4.2 : Plasma progesterone concentration in cows in Group S1 after injection of prostaglandin to induce abortion (Day 0 = day of abortion) and following abortion , when each cow was injected twice (↓) with saline.

MU = Mucus recorded in the vagina .
CL = A corpus luteum found on one ovary by rectal palpation.
Oe = Cow observed in oestrus.


8
Oe
7
CL
Oe
MU
6
Oe
MU
5
CL
Oe
Plasma progesterone (ng/ml)
Days from prostaglandin induced abortion

with earlier oestrous behaviour in these cows (see Fig 4.1 and 4.2).

Plasma LH concentrations in a representative cow from Group P1 and Group S1 during each 6 hour sampling period were basal as shown in Figure 4.3 and no difference was seen between Groups P1 and S1. There was no effect of prostaglandin injection on plasma LH concentration during the 4 hours of post-injection sampling. Mean ± S.E.M. of LH concentrations in pre- and post-injection samples were 0.94 ± 0.04 and 0.93 ± 0.04 compared to 1.57 ± 0.07 and 1.53 ± 0.05 ng/ml for Groups P1 and S1 respectively.

Experiment 2.

Various aspects of the reproductive performance of dairy cows injected once with either PGF2α or saline 14 days *post-partum* are shown in Table 4.2.

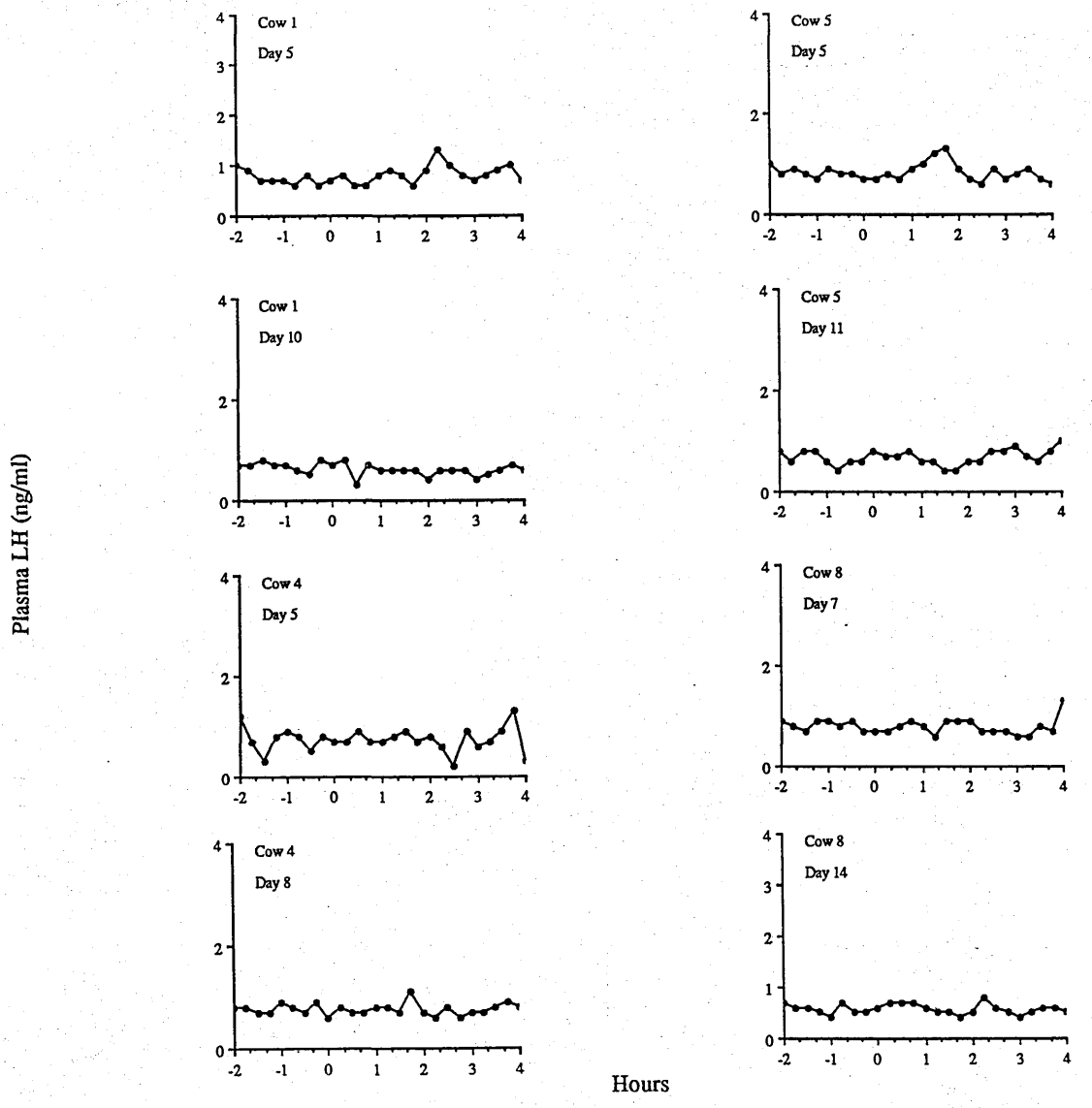
Table 4.2

Interval (mean ± SD) and range from calving to first service, first service conception rate and the number of services per conception in dairy cows injected with either PGF2α (Group P2) or saline (Group S2) 14 days *post-partum.*

	GroupP2	Group S2
Calving to first service interval (days)	80.6±13.4	81.1±22.3
Range (days)	67-101	57-128
First service conception rate (%)	50	78
Number of service per conception	1.6	1.3

The mean interval from calving to first service in prostaglandin treated (Group P2) and saline treated control (Group S2) cows was 80.6 ± 13.4 (range 67-101) and 81.1 ± 22.3 (range 57-128) days respectively. This difference is not statistically significant. The first service conception rate in treated and control cows was 50%

Figure 4.3 : Plasma LH concentration in blood samples collected at 15 min intervals for 2 hours before and 4 hours after injection of either prostaglandin (cows 1 and 4) or saline (cows 5 and 8) on two separate occassions after abortion (day 0).


Cow 1
Day 5
Cow 5
Day 5
Cow 1
Day 10
Cow 5
Day 11
Cow 4
Day 5
Cow 8
Day 7
Cow 4
Day 8
Cow 8
Day 14
Plasma LH (ng/ml)
Hours

(5/10) and 78% (7/9) respectively and control cows required less services per conception than the treated cows 1.3 vs 1.6 (Table 4.2).

The mean plasma LH concentrations in half hourly blood samples collected for 2 hours after prostaglandin or saline injection did not differ in Groups P2 and S2, being 0.5 ± 0.03 vs 0.5 ± 0.03 respectively. Plasma oestradiol-17β concentrations in the post-partum dairy cows at the time of saline or prostaglandin injection are shown in Figure 4.5. Oestradiol-17β concentration in approximately half of the samples was at or below the detection limits of the assay. Oestradiol concentration in the remainder of the samples was variable with a maximum around 8-10 pg/ml. There was no relationship between the timing of saline or prostaglandin injection and changes in plasma oestradiol concentration.

An increase in plasma progesterone concentration was observed eight days after PGF2α injection in one cow in Group P2 as shown in Figure 4.4. Elevated plasma progesterone concentrations were seen in one cow in Group S2 throughout the sampling period and in another cow in this group by two days after saline injection (Figure 4.4). Basal plasma progesterone concentrations were present throughout the sampling period i.e. from days 12 to 20 post-partum in all the remaining cows in Groups P2 and S2.

The dimensions of the uterine horns and cervix of the cows in Groups S2 and P2 are shown in Table 4.3

Table 4.3

Effect of PGF2α or saline injected 14 days *post-partum* on uterine involution assessed from measurements of cervical length and diameter and uterine horn diameter, on the day of and 10 days after injection (mean ± SD).

Treatment		Cervix length	Cervix diameter	Uterine horn diameter (mm) left	Uterine horn diameter (mm) right
PGF2α	Day 0	86±2.5	55±1.6	59±5.1	76±5.3
	Day 10	61±1.6	40±1.2	28±1.5	35±1.6
	% change	64	66	47	46
Saline	Day 0	80±2.5	52±1.7	63±5.1	49±2.8
	Day 10	47±1.5	35±1.2	25±0.4	25±0.5
	% change	59	67	40	51

The length and diameter of the cervix had decreased and the uterine horns were smaller after 10 days in both groups but there was no effect of prostaglandin treatment on these parameters.

Figure 4.4 : Plasma progesterone concentrations in PGF2α treated cows (Group P2, left- hand panel) and saline treated cows (S2, right-hand panel) starting two days before continuing for eight days after injection. Eight cows in Group P2 & 9 cows in Group S2 had low progesterone concentrations throughout the sampling period. This is illustrated in one representative cow from each group.

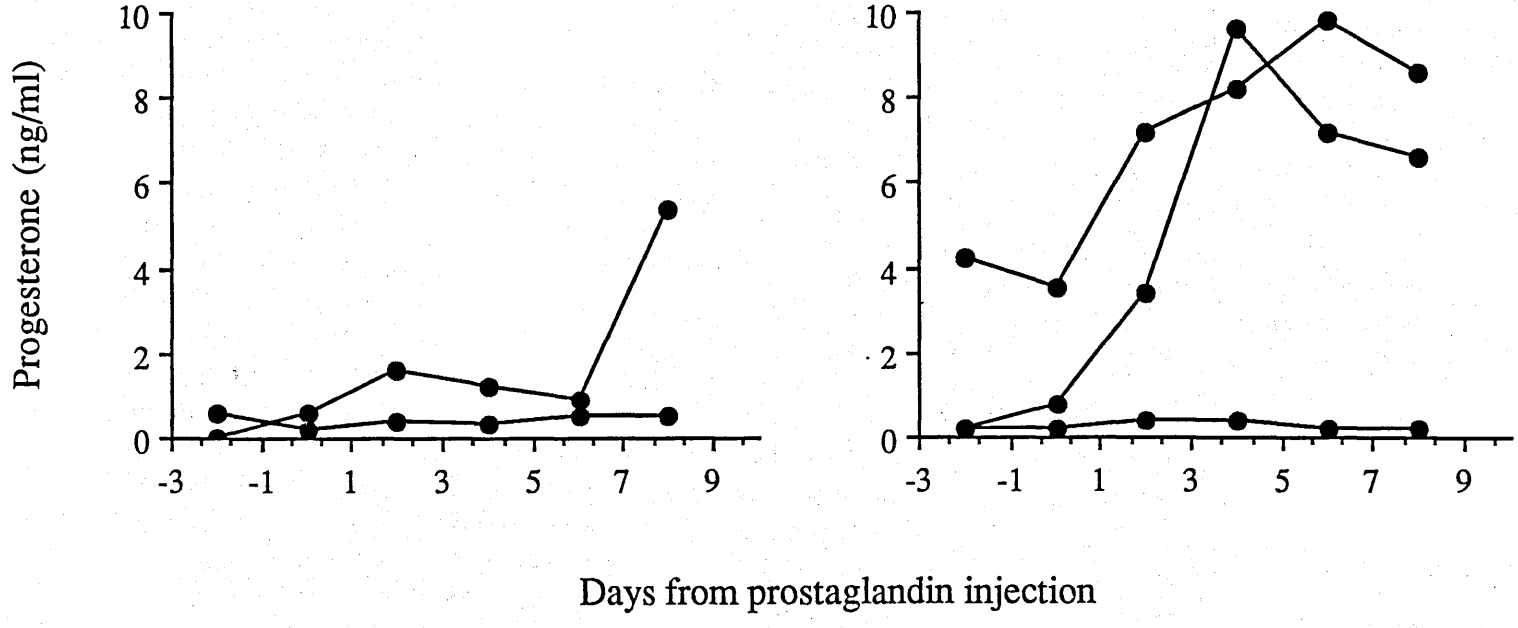
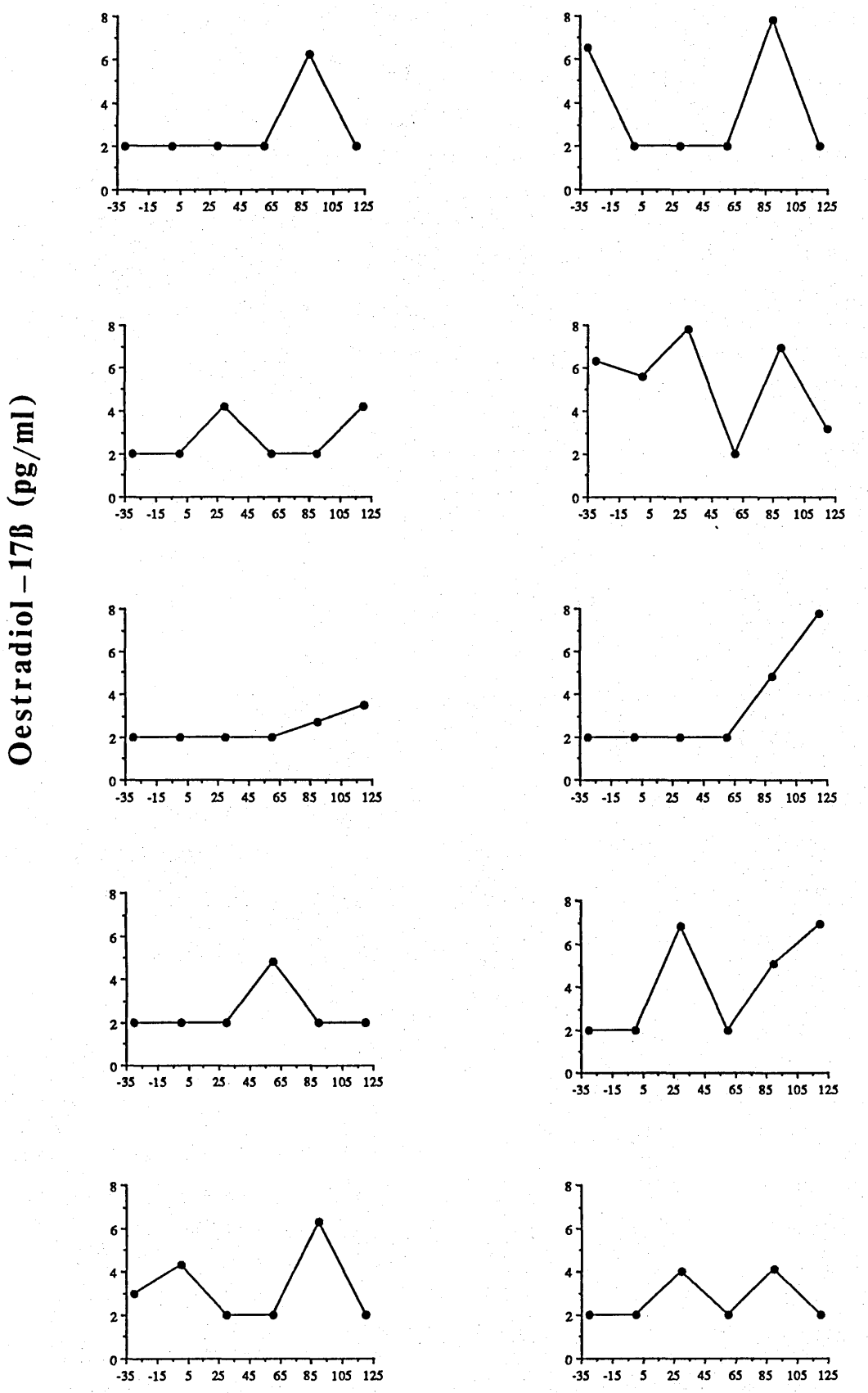
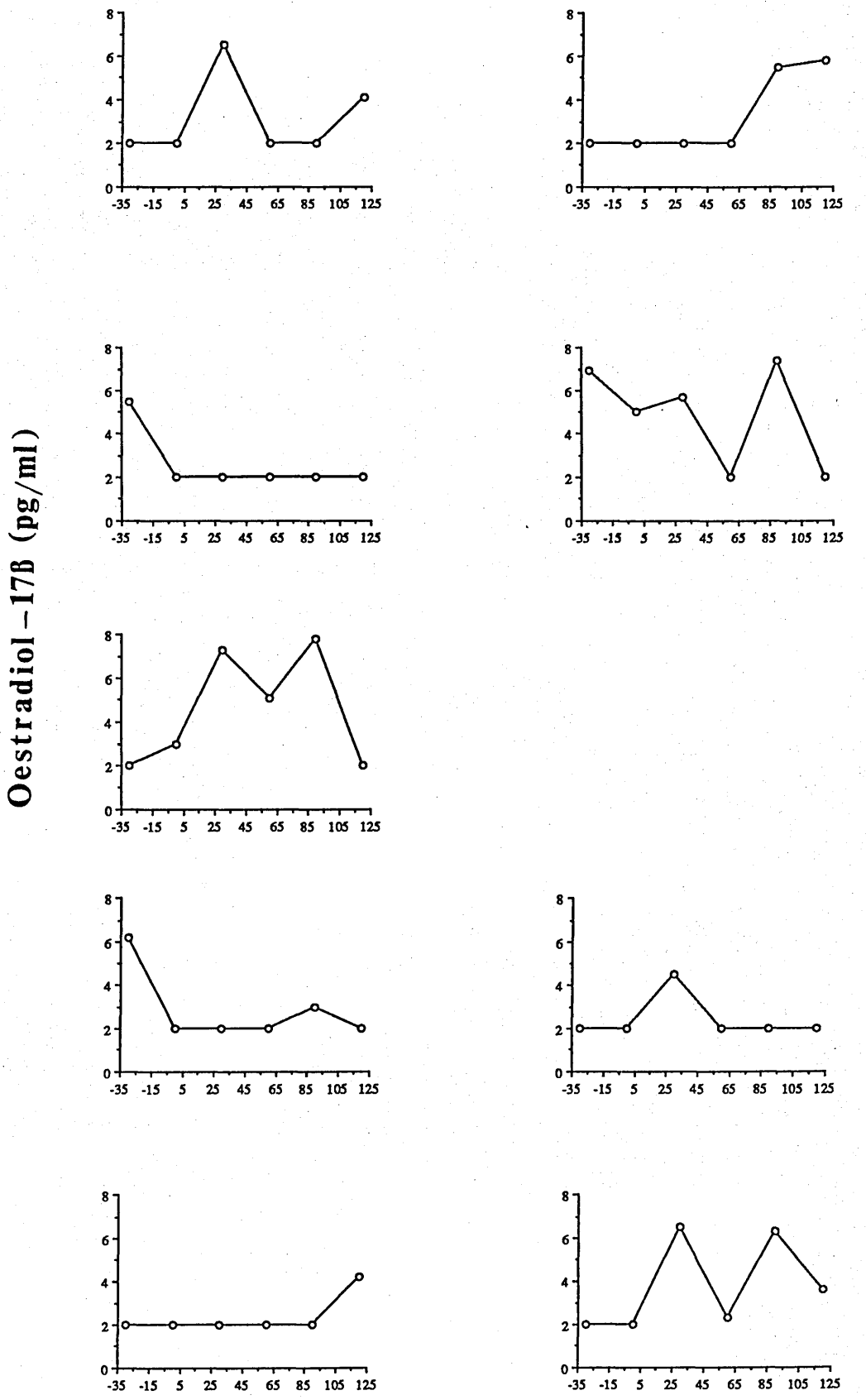

Progesterone (ng/ml)
10
8
6
4
2
0
-3
-1
1
3
5
7
9
Days from prostaglandin injection

Figure 4.5 : Plasma oestradiol- 17β concentrations in samples collected 30 minutes before to 2 hours after injection of either PGF2α (●, n = 10) or saline (○ , n = 9) into post-parturient dairy cows (0 = time of injection).


Oestradiol – 17β (pg/ml)
0
2
4
6
8
-35 -15 5 25 45 65 85 105 125
Time (min) from PGF-2∝ injection


Oestradiol-17β (pg/ml)
0
2
4
6
8
-35 -15 5 25 45 65 85 105 125
Time (min) from Saline injection

Discussion

In experiment 1 all of the cows aborted after prostaglandin injection and it appeared that cows treated later than 100 days took longer to abort than cows treated earlier than 100 days of pregnancy and abortion after 100 days of pregnancy was followed by discharge of mucopus. Lindell *et al.* (1980) also reported that in heifers induced to abort at less than 80 days of pregnancy, retention of fetal membranes did not occur, whereas in heifers aborted after 80 days of pregnancy, fetal membranes were retained and abortion was preceded by a bloody discharge. Similar variability in the appearance of a bloody discharge before abortion was seen in the present experiment and was also related to stage of pregnancy. This may be related to the firmness of the connection between fetal and maternal tissues (Kingman, 1951). Hence, in the case of abortion in the early stages of pregnancy (i.e.<100 days), these connections might be loose allowing abortion to procede without any retention of fetal membranes or discharge of blood. In later stages of pregnancy (i.e. > 100 days) placental connections are stronger and abortion results in discharge of blood and or retention of fetal membranes.

Plasma progesterone concentration dropped sharply after prostaglandin injection from 4-6 ng/ml to less than 0.5 ng/ml on the day of abortion in all cows (Figure 4.1 and 4.2). It is assumed that two of the cows from each group ovulated soon after abortion (3-5 days) as indicated by the rise in plasma progesterone concentration. Ovulation occurred somewhat later in the two other cows from each group as indicated by basal plasma progesterone concentration and failure to detect corpora lutea by rectal palpation until 15-20 days after abortion.

The interval from abortion to first oestrus in cows 1 and 2 in group P1 was probably shortened by prostaglandin treatment since the luteal phase was shorter (7

and 5 days) compared to 17 and 14 days in cows 5 and 7 respectively in group S1 (Figure 4.2). In the present study luteolysis was induced by the prostaglandin treatment as indicated by the earlier onset of oestrous cycles in cows which had corpora lutea at the time of prostaglandin injection in group P1 compared to cows in group S1 (13.0 ± 1.06 vs 18.5 ± 2.5 days respectively).

Shortened luteal phases are also common in post-partum cows before resumption of regular normal length oestrous cycles (Morrow *et al.*, 1966; 1969; King *et al.*, 1976; Samuel, 1977; Lindell *et al.*, 1980). It has been reported that short luteal phases are associated with elevated plasma concentrations of the principle PGF2α metabolite PGFM (Lindell *et al.*, 1981).

Administration of prostaglandin has been reported to release endogenous PGF2α in cycling ewes (Challis *et al.*, 1976). No such release has been seen in the bovine following the injection of cloprostenol during the first 12 - 14 days of the oestrous cycle although treatment at later stages of the cycle may release a certain amount of PGF2α, which is however still considerably less than that produced during normal luteolysis (Kindahl *et al.*, 1980). In this present study luteolysis is thought to have occurred in aborted cows following two injections of prostaglandin between 6 and 11 days after abortion but in the absence of any measurements of PGF2α or PGFM it is impossible to determine the effect of these injections on endogenous prostaglandin levels. In this connection, Lindell *et al.* (1980) and Kindahl *et al.* (1981) reported a good correlation between endogenous release of PGF2α during the post-partum period (following normal pregnancy and parturition) and completion of uterine involution, stating that involution was faster the longer the duration of elevated plasma PGF2α concentration. Similarly delayed uterine involution has been related to insufficient endogenous secretion of PGF2α during the immediate post calving period (Lindell *et al.*, 1982) while repeated

administration of PGF2α from 3 to 13 days *post-partum* effectively shortens uterine involution time (Lindell and Kindahl, 1983). The relationship between the occurrence of first oestrus *post-partum* and the necessity for completion of uterine involution has been reported (Albrechtsen, 1917; Buch *et al.*, 1955 Higaki *et al.*, 1959) but the rate of uterine involution is retarded by placental retention (Morrow *et al.*, 1966) and uterine infection (Stabenfeldt *et al.*, 1980). However, ovarian activity *post-partum* is not soley dependent on uterine involution (Foote *et al.*, 1960; Menge *et al.*, 1962; Morrow *et al.*, 1966).

It is apparent from the results of experiment 2 which was conducted in post-partum dairy cows that prostaglandin did not shorten the interval from calving to first service, or improve first service conception rate or reduce the number of services needed per conception. In fact, reproductive efficiency measured in terms of the latter two parameters was poorer in the prostaglandin treated cows (Table 4.2). These results are dissimilar to those obtained by Young and Anderson (1986) who showed an increased first service conception rate in dairy cows injected once with prostaglandin between 14 and 28 days *post partum.* The latter authors suggested that improved reproductive efficiency in prostaglandin treated cows might have resulted from a direct endocrine effect of the prostaglandin, perhaps acting somewhere on the pituitary-ovarian axis.

The plasma oestradiol-17β concentrations measured in the dairy cows 14 days *post-partum* are similar to those reported by Pope (1982) and Watson (1987). It is apparent from the results that PGF2α had no effect on plasma 0estradiol-17β concentration. However, the present results failed to show any direct effect of prostaglandin either on the pattern of LH secretion or on oestradiol-17β and progesterone concentration. This largely supports the findings of Peters (1988) who concluded that injections of prostaglandin between 10 and 21 days *post-partum* did not change the subsequent pattern of plasma progesterone concentration, and had no

consistent effect on plasma oestradiol-17β concentration but might have depressed the amplitude of pulsatile LH secretion.

It appears from the results that administration of prostaglandin does not enhance uterine involution; rather the opposite may occur, possibly due to inhibition of or interference with the action of endogenous PGF2α by prostaglandin injection.

Prostaglandins have been advocated for the improvement of fertility in cows (Day, 1977; MacMillan *et al.*, 1977; 1980; Young and Anderson, 1986) but the factors contributing to this improvement have not been identified and the underlying physiological functions of prostaglandins regarding the enhancement of uterine involution and or ovarian activity are not known. Al-Raheem and Al-Ritha (1988) have recently shown that a single injection of prostaglandin 25 days *post-partum* advanced first oestrus but Revah *et al.* (1988) were unable to improve reproductive efficiency in dairy cows injected once with prostaglandin either 30 or 40 days *post-partum*. However, these results were obtained from animals at a comparatively later stage *post-partum* and the treatments were probably aimed more at advancing oestrus by inducing luteolysis than at promoting uterine involution.

The present study shows that prostaglandin shortened the interval to first oestrus in cows with a functional corpus luteum, probably by causing premature luteolysis. There was however no other evidence of improved reproductive performance nor was there a direct effect of prostaglandin on LH or oestradiol-17β, which would seem to preclude a stimulatory effect of prostaglandin on the pituitary-ovarian axis.

Administration of prostaglandin to early post-partum cows should not be recommended from the present results unless a corpus luteum is present, in which case luteolysis would be initiated and there would be an earlier resumption of cyclicity. Similar observations have been made by Chupin *et al.* (1977) and Waters and Ball (1978).

Chapter V

General disscussion

Prostaglandins are widely used in cattle to synchronize oestrus cycles, to induce parturition or abortion, to treat uterine infection or retention of placenta and to treat certain ovarian abnormalities. In addition there is evidence that prostaglandin can improve reproductive performance in post-partum cattle. In one study, Young and Anderson (1986) suggested that the resumption of ovarian activity might be hastened by a single prostaglandin injection, which they suggested may act through a direct effect on endocrine activity. Such an effect may be possible in the cow since the secretion of LH is stimulated by exogenous prostaglandin in the bull (Hafs *et al.*, 1977; Haynes *et al.*, 1978). These might also be alternative mechanisms of action such as :-

i. a direct effect on the ovarian follicles to enhance oestradiol secretion thereby triggering events leading upto oestrus or ovulation.

ii. an effect on ovarian blood flow leading to increased availability of trophic hormones or steroid precursors to the follicle.

iii. a direct effect on the uterus to promote uterine tone and involution.

Any such effect is likely to be rapid and short-lived due to the short half life of prostaglandin in blood plasma.

The effects of injections of prostaglandin were investigated in the post-partum suckled beef cow, the post-partum commercial dairy cow and in dairy cows after termination of pregnancy by prostaglandin. The pattern of reproductive activity is markedly different in the suckled beef cow and in the dairy cow as reviewed in Chapter I, the principal difference being the longer period of anoestrus in the former. The pattern of reproductive activity after abortion in the cow would appear to depend on the duration of pregnancy before abortion, the longer the pregnancy, the more protracted the period of anoestrus before resumption of cyclicity. These three reproductive states in the cow presented different situations in which to investigate and test the effects of prostaglandin on:-

1. reproductive performance,
2. short and long term changes in reproductive hormone concentrations and
3. uterine involution.

One measure of reproductive performance in the cow is the interval from parturition to the start of the next pregnancy. This interval comprises a variable period of anoestrus followed by a period during which oestrus cycles become established. In the present study it appears that ovarian activity resumed earlier in the beef cows in 1987 compared to those in 1988 as indicated by the interval from calving to first oestrus (Table 3.1). The reason for this may be related to age as most of the cows in the 1987 group were older than the cows in 1988. There is certainly evidence in the literature which suggests that the age can influence timing of the resumption of ovarian activity *post-partum*, older cows resuming ovarian cycles earlier than the younger cows (Bulman and Lamming, 1978; Knight and Nicoll, 1978). Onset of ovarian activity *post-partum* is also influenced by the time to turnout according to Terqui *et al.* (1982) who reported that the earlier cows were turned out to grass the sooner cyclic activity resumed. This may be due to the reduction in suckling intensity which follows turnout when the calves have access to grass. There was certainly a close association between turnout and resumption of cyclicity in all three years in the present study and only one cow apparently ovulated before going out to grass (Figure 3.2 and 3.3). However, the theory that earlier turnout advances ovarian activity could not be tested in this series of experiments because of the narrow spread of turnout dates. So from the present results it is difficult to make any comment on the effect of turnout date on resumption of cyclicity since this was only about one week earlier in 1987 compared to 1988. Even so cows in 1987 resumed cyclicity earlier than cows in 1988.

The length of the acyclic period in this study was comparatively longer than some reports in the literature, for exanple, 56.9 ± 2.5 days in a study using spring calving cows turned out to pasture on 20th May (Peters and Riley, 1982a). However, it is difficult to make comparisons of this nature between herds which are probably subject to different nutrition and management practices. Ovarian activity is supposed to be delayed in suckling beef cows as compared to dairy cows (Bulman and Lamming, 1978). In the present study the occurrence of first oestrus in the beef and dairy cows was very similar (ie. approximately 80 days, see Table 3.1 and 4.2) Plasma progesterone profiles indicate that ovarian activity had resumed considerably earlier than the first recorded oestrus *post-partum* in some beef cows. Comparable plasma progesterone profiles were not obtained from dairy cows so it is not possible to determine the onset of ovarian activity in this group. These observations support the statement made by Peters and Riley (1982a) that beef cows do not necessarily undergo a longer acyclic period *post-partum* than dairy cows. From the present results it is also apparent that PGF2α treatment has no beneficial effect on the resumption of ovarian activity.

In the aborted dairy cows, it was apparent that the interval from abortion to first observed oestrus was comparatively shorter (ie. 12-23 days) but that cows which were aborted earlier in pregnancy tended to come into heat earlier than the cows which were aborted at later stages of pregnancy.

The performance of the radioimmunoassays for LH, oestradiol and progesterone was good and comparable to other published reports in terms of sensitivity, precision and accuracy (see Chapter II)

Determination of plasma progesterone concentrations in samples collected daily from the aborted cows (Group P1 and S1, Chapter IV) gave a very detailed analysis of ovarian activity and this permitted close scrutiny of ovarian response to

the injection of prostaglandin and saline. Blood samples could only be collected twice weekly from the beef cattle (Groups A, B and C, Chapter III) for practical reasons, but nevertheless, these clearly demonstrate when luteal activity occurred in these cattle. When plasma progesterone concentrations remained elevated for a week or more, ovulation and luteinization was assumed to have occurred. Unfortunately the impracticality of using ultrasound in the field prevented more detailed investigation of ovarian function at this time since some authors (eg. Corah *et al.*, 1974) have suggested that structures other than corpora lutea may be responsible for secretion of progesterone and may be the source of the short-lived episodes of induced plasma progesterone concentration seen in many of the cows. Similar transient rises in plasma progesterone concentrations prior to the first post-partum oestrus have been frequently reported (Corah *et al.*, Humphrey *et al.*, 1976; Prybil and Britt, 1978; Kesler *et al.*, 1980). This type of progesterone pattern has been reported to occur before the establishment of nomal cyclicity in post-partum beef (Humphrey *et al.*, 1976; Kesler *et al.*, 1980; Peters and Riley, 1982a) and dairy cows (Eger *et al.*, 1988). The source of this short rise in plasma progesterone concentration has been described to be follicles that luteinize but fail to ovulate because of insufficient gonadotrophic hormone secretion (Lamming *et al.*, 1981).

Oestradiol concentrations were determined in plasma collected at fifteen minute intervals for two hours before and four hours after prostaglandin or saline administration but not from daily or twice weekly samples on account of the variability in plasma oestradiol concentration (Pope, 1982). In the aborted dairy cows these intensive samples were obtained on two occasions, at the end of the first and second weeks after abortion. Most of the samples had undetectable oestradiol concentrations, despite the fact that the assay sensitivity was around 2pg/ml. Reference to plasma progesterone concentrations at this time (see figure 4.1 and

4.2) shows that the cows were either anoestrous or under progesterone influence. Comparable samples from the beef cows (Group C) were collected either 7, 14, 21 and 28 days, in case of one prostaglandin treated group and the saline treated controls, or 14 and 28 days in the case of the second prostaglandin treated group, after parturition. Reference to figure 3.2 shows that plasma progesterone concentrations at this time were basal indicating that the cows were anoestrous Pope (1982) has shown that oestradiol concentrations may be quite variable during both the post-patum anoestrous and the luteal phase but generally fall below 4-5 pg/ml. Similarly, although Watson (1987) was able to detect episodic fluctuations in oestradiol-17β concentration in acyclic cows between 21 and 27 days *post-partum*, peak concentrations were generally below 8-10 pg/ml. Plasma oestradiol concentrations also fell within this range in samples collected from the dairy cows around the time of saline & prostaglandin injection (see Fig 4.5). It seems probable therefore that in practice the sensitivity of the oestradiol assay used in the present experiment was not high enough for accurate analysis of basal plasma oestradiol concentration in the cow. Insufficient blood was collected during the sampling procedure to permit the samples to be reassayed using a larger plasma volume. However, it is felt that the present oestradiol assay was sensitive enough to have detected any increase in plasma concentration should it have occurred following prostaglandin injection. Such an increase was not detected either in the aborted cows or in the post-partum beef or dairy cows.

Short term changes in plasma LH concentration were also analysed in the post-partum beef and dairy cows and in the aborted dairy cows before and after injection of either prostaglandin or saline. Low plasma LH concentration was observed before injection at all of these treatment times. Low plasma LH concentrations have also been reported by several other workers during the early post-partum period in cows (Gonzales-padilla *et al.*, 1975; Rawlings *et al.*, 1980;

Humphrey *et al.*, 1983; Guilbault *et al.*, 1987). An absence of episodic LH secretion was demonstrated throughout the sampling period and after injection in all these treatment groups (see Figure 3.4 and 4.3) There was no evidence of any effect of prostaglandin on LH except for a slight non-significant reduction in mean LH concentration in the post injection samples in the beef cows (Table 3.7). Peters (1988) reported similar results but did find some evidence of reduced LH pulse amplitude after prostaglandin injection in post-partum beef cows. The sampling frequency of once every 15 minutes used in the present experiment was probably too slow to detect episodic LH secretion, which has been observed in cattle sampled at 10 minute intervals at equivalent times during the post-partum period (Riley *et al.*, 1981; Watson, 1987). However, it was considered adequate to monitor the response to prostaglandin injection, which would need to be substantial, it was assumed, if there was to be any lasting effect on the reproductive endocrinology of the cow.

Study of the plasma progesterone concentrations in the aborted cows (Figure 4.1 and 4.2) shows that injection of prostaglandin coincided with elevated progesterone levels in two cows which presumably indicated that corpora lutea were present. In both cases the prostaglandin appeared to exert a luteolytic effect, a property of this drug which is exploited a great deal in the management of reproduction in cattle (Louis *et al.*, 1972; Cooper, 1974). These two cows subsequently came into oestrus earlier than cows in the control group which had corpora lutea when injected with saline. The effect of the prostaglandin in shortening the interval to first oestrus cannot have been due to its stimulating the release of either LH or oestradiol since the concentrations of both hormones remained basal after injection. Young *et al.* (1984) and Young and Anderson (1986) reported that a single injection of prostaglandin given between 14 and 28 days *post-partum* improved first service conception rate and shortened the interval to first oestrus provided the cows had low plasma progesterone at the time of

injection. Luteolysis cannot therefore have been the mechanism of action of the prostaglandin in this instance and yet evidence from the present experiment would also seem to preclude an action of prostaglandin on the reproductive endocrinology of the cows. It seems unlikely, therefore, that any beneficial effect of prostaglandin on post-partum reproductive activity in the cow is due to it's stimulating activity of the pituitary-ovarian endocrine axis.

Prostaglandin F2α is also commonly used in the treatment of pyometra (Fazeli *et al.*, 1980) and chronic metritis (Jackson, 1977; Duncanson, 1980) due to its strong and prolonged myometrial effect (Patil *et al.*, 1980). For this reason, it is reasonable to believe that PGF2α has some beneficial effect on uterine involution. Lindell and Kindahl (1983) demonstrated that PGF2α promoted uterine involution if administered twice daily for 3 - 13 days but this is clearly not a practical application of prostaglandin. In the present study it appears that PGF2α has no beneficial effect on uterine involution in either beef (see Chapter III) or dairy cows (see Chapter IV) rather the opposite may be true. Thus the rate of involution of the uterus and cervix was faster in control cows than in prostaglandin treated cows. Young *et al.* (1984) and Young and Anderson (1986) found that a single injection of prostaglandin given between 14 and 28 days *post-partum* shortened the post-partum anoestrus and improved first service conception rate if the cows had low plasma progesterone concentration at the time of treatment. All the beef cows in the present study could be classified as having low plasma progesterone (i.e. were anoestrous) at the time of prostaglandin injection and yet reproductive performance was often poorer than in saline treated controls. Although no effects of prostaglandin injection on plasma LH and oestradiol concentrations were observed in this study, Peters (1988) did show slight diminution of LH pulse amplitude after prostaglandin injection. However, such an effect is likely to be quite transitory owing to the plasma clearance of prostaglandin and it is considered

unlikely that this would have any long term detrimental effects on reproductive performance. Perhaps more relevant was the finding that the pattern of uterine involution as seen from weekly ultrasonographic recordings in the beef cows was more irregular and somewhat slower in the prostaglandin treated cows. This suggests that administration of prostaglandin can interfere with the normal processes of uterine involution, possibly through an effect on endogenous prostaglandin synthesis or release. However, the twice weekly blood sampling regimen and weekly programme of ultrasound recordings was not designed to investigate this effect more fully.

Uterine involution is likely to be a major determining factor in the onset of cyclicity *post-partum* in cattle (Buch *et al.*, 1955; Morrow *et al.*, 1969). It was obvious in the aborted dairy cattle that the more advanced the pregnancy the longer the period of post-partum anoestrus and this too was probably related to the need for more extensive uterine involution and repair in the later pregnant cows. However, the role of prostaglandin in uterine involution is unclear. Guilbault *et al.* (1987) suppressed prostaglandin synthesis but concluded that this treatment did not alter the rate of uterine involution. Of interest was the finding that ovarian activity in the cows treated with prostaglandin synthetase inhibitor was delayed. This suggests that prostaglandin may be involved in the resumption of ovarian activity, possibly by promoting the demise of the corpus luteum of pregnancy (Guilbault *et al.*, 1987) or by altering blood flow to the uterus and ovary (Ford and Chenault, 1981; Spicer and Echternkamp, 1986).

In summary these experiments were undertaken to investigate the mode of action of prostaglandin in promoting reproductive efficiency in cattle as reported by Young *et al.* (1984). Reproductive performance, hormone concentrations and uterine involution were the parameters chosen for study. Reproductive

performance of beef or dairy cows was not altered by injection of prostaglandin during post-partum anoestrus, which disagrees with the favourable results obtained by Young *et al.* (1984) and Young and Anderson (1986), although they did use a larger number of animals. This discrepancy cannot be accounted for, particularly in the dairy cows which were treated exactly as described by Young and co-workers. Nevertheless no statistically significant alterations in plasma LH or oestradiol-17β concentration resulted from injection of prostaglandin into post-partum beef or dairy cows or into aborted dairy cows. Any benefits derived from the prostaglandin treatments used by Young and co-workers were therefore unlikely to have resulted from either direct stimulation of pituitary gonadotrophin release, an effect which has been demonstrated in the bull (Hafs *et al* .,1977; Haynes *et al.*, 1978); or stimulation of ovarian oestradiol secretion, which might arise by a direct effect on the follicle or by altering ovarian blood supply. Prostaglandin treatment of aborted cows did shorten the interval to first oestrus in animals which possessed a corpus luteum at the time of treatment, but this was through induction of luteolysis (Cooper, 1974) a property which was discounted in the beneficial effects of prostaglandin administration reported by Young and Anderson (1986).

The present study also suggests that the results obtained by Young and co-workers were not due to prostaglandin treatment increasing the rate of uterine involution *post-partum.* Thus uterine involution in beef cows was, if anything, delayed in prostaglandin treated animals and similar results were obtained in dairy cows, although in this case uterine and cervical dimensions were only measured once, 10 days after treatment.

Routine prostaglandin treatment of post-partum cattle was not beneficial to completion of uterine involution or resumption of cyclicity but the apparent interference by injected prostaglandin in the normal course of uterine involution deserves further investigation.

References

Adams, W. M. 1969. The effective induction of labor and parturtition in cattle. *J. Am. Vet. Med. Assoc.*, **194**: 261-265.

Adams, W. M. & Wagner, W. C. 1970. The role of corticoids in parturition. *Biol. Reprod.*, **3**: 223-228.

Adashi, E. Y., Jones, P. B. G. & Hsues, A. J. W. 1981. Synergestic effect of glucocorticoids on the stimulation of progesterone production by follicle stimulating hormone in cultured rat granulosa cells. *Endocrinology*, **109**: 1888-1894.

Agthe, O. & Kolm, H. P. 1975. Oestrogen and progesterone levels in the blood plasma of cows with normal parturition or with a retained placenta. *J. Reprod. Fert.*, **43**: 163-166.

Akbar, A. N., Reichert, L. E. Jr., Dunn, T.G., Kaltenbach, C. C. and Niswender, G. D. 1974. Serum levels of follicle stimulating hormone during the bovine estrous cycle. *J. Anim. Sci.*, **39**: 360-365.

Albrechtsen, J. 1917. Sterility of cattle and methods of treatment. *Cornell Vet.*, **7**: 57-66.

Allen, D.M. 1979. The farming picture - beef. In fertility and reproduction in cattle. Proc. Second Study Conf. Scottish Agric. Colleges, 17-21.

Al-Raheem, S. M. & Al-Ritha, A. 1988. The effect of GnRH and PGF2α (PGF) treatments on post-partum estrus in dairy cows. Proc. 11th Int. Congr. Anim. Reprod. and A. I. Dublin, Ireland. 26-30 June 1988. **I**: 422 (Abstr.)

Anderson, H. 1966. The effect of season of the year, age of the cow and size of the herd on fertility in cows. *Asbertn. Inst. Sterillitetsfokn.*, **I**: 227.

Arije, G. R., Wiltbank, J. N. & Hopwood, M. D. 1974. Hormone levels in pre- and post-parturient beef cows. *J. Anim. Sci.*, **39**: 338-347.

Armstrong, D.T. & Hansel, W. 1959. Alteration of the bovine estrus cycle with oxytocin. *J. Dairy Sci.*, **42**: 533-542.

Asdel, A. 1964. In *patterns of mammalian reproduction.* pp. 632-650. 2nd edition, Ithaca, New York. Cornell University Press.

Azzazi, F. Krause, G.F. & Garverick, H. A. 1980. Altration of GnRH induced LH release early *post-partum* in dairy cattle with estrogen and progesterone. *Ann. Meet. Am. Soc. Anim. Sci.,* Ithaca, New York. **72**: 367.

Baird, D. T. & Scaramuzzi, R. J. 1976. Changes in the secretion of ovarian steroids and pituitary luteinizing hormone in the periovulatory period in the ewe : the effect of progesterone. *J. Endocrinology*, **70**: 237-245.

Baird, D. T. 1978. In *control of ovulation,* pp. 217. eds. D. B. Crighton, N. B. Haynes, G. R. Foxcroft & G. E. Lamming, London. Butteworth.

Baird ,D. T. & McNeilly, A. S. 1981. Gonadotophic control of follicular development and function during the oestrous cycle of the ewe. *J.ReprodFert.*, Suppl. **30**: 119-133.

Ball, P. J. H. & Jackson, N. W. 1979. The effect of dairy cows inseminated on the basis of milk progesterone measurement. *Br. Vet. Jour.*, **135**: 537-540.

Beal, W. E., Milvae, R. A. & Hansel, W. 1980. Oestrous cycle length and plasma progesterone concentrations following administration of prostaglandin F2α early in the bovine oestrous cycle. *J. Reprod. Fert.*, **59**: 393-396.

Beal, W. E. 1983. A note on synchronization of oestrous in post-partum cows with PGF2α and progesterone releasing device. *Anim. prod.*, **37**: 305-308.

Beal, W. E., Good, G. A. & Peterson, L. A. 1984. Estrus synchronization and pregnancy rates in cyclic and noncyclic beef cows and heifers treated with syncromate B, Norgestromet and alfaprostol. *Theriogenology*, **22**: 59-66.

Berardinelli, J. G. Daily, R. A. , Butcher, R. L. and Inskeep, E. K. 1979. Source of progesterone prior to puberty in beef heifers. *J. Anim. Sci.*, **49**: 1276-1280.

Bergland, R. M. & Page, R. G. 1978. Can the pituitary secrete directly to the brain? (affirmative anatomical evidence). *Endocrinology*, **102**: 1325-1338.

Blockey, M. A. deB., Chamley, W. A., Cummings, L., Perry, M. & Goding, J. R. 1973. Steroid plasma levels about oestrous in the beef cows. *J. Reprod. Fert.*, **32**: 342-343.

Boyd, W. L. 1925. A study of the physiological and pathological changes occurring in the reproductive organs of the cow following parturition. *Minn. Agr. Expt. Sta. Bul.* No. 23.

Boyd, H. 1977. Anoestrous in cattle. *Vet. Rec.*, **100**: 150-153.

Brown, J. G., Peterson, D. W. & Foote W. D. 1972. Reproductive response of beef cows to exogenous progesterone and gonadotropins at various stages of *post-partum. J. Anim. Sci.*, **35**: 362-369.

Buch, N. C., Tyler, W. J. & Casida, L. E. 1955. Post-partum estrus and involution of the uterus in an experimental herd of Holstein-Freisian cows. *J. Dairy Sci.*, **38**: 73-79

Bulman, D. C. & Lamming, G. E. 1978. Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. *J. Reprod. Fert.*, **54**: 447-458..

Caldwell, B. V., Tilson, S. A., Brock, W. A. & Speroff, L. 1972. The effects of exogenous progesterone and oestradiol on prostaglandin F levels in ovariectomised ewes. *Prostaglandins*, **I**: 217 - 228.

Callahan, C. J., Erb, A. H., Surve, R. E. & Randel, R. D. 1971. Variables influencing ovarian cycles in post-partum dairy cows. *J. Anim. Sci.*, **33**: 1053 - 1059

Campbell, R. C. 1979. The normal variable in experiments and surveys. In *Statistics for Biologists*. pp. 177 - 185. 2nd ed. Cambridge University Press, Cambridge, London, New York, Melbourne.

Carman, G. M. 1955. Interrelations of milk production and breeding efficiency in dairy cows. *J. Anim. Sci.*, **14** : 753 - 759.

Carroll, E. J. 1974. Induction of parturition in farm animals. *J. Anim. Sci.*, **38**, Suppl. 1 : 1 - 9.

Carruthers, T. D., Kesner, J. S., Convey, E. M. & Hafs, H. D. 1978. Hypothalamic GnRH and pituitary function in suckled and non suckled Holstein cows. *J. Anim. Sci.*, 47, Suppl. I: 349

Carruthers, T. D. & Hafs, H. D. 1980. Suckling and four times daily milking: Influence on ovulation, estrus and serum luteinizing hormone, glucocorticoids and prolactin in post-partum Holsteins. *J. Anim. Sci.*, **50**: 919 - 925.

Carruthers, T. D., Convey, E. M., Kesner, J. H., Hafs, H.D. & Cheng, K. W. 1980. The hypothalamic-pituitary gonadotrophic axis of suckled and non suckled dairy cows post-partum. *J. Anim. Sci.*, **51**: 949 - 953.

Casida, L. E. & Wisnicky, W. 1959. Effects of diethylstillboestrol dipro pionate upon post-partum changes in the cow. *J. Anim. Sci.*, **9**: 238 - 242.

Casida, L. E., Graves, W. E., Hauser, E. R., Lauderdale, J. W., Riesen, J. W., Saiduddin, S. & Tyler, W. J. 1968. Studies on the post-partum cow. *Research Bull. of the College of Agriculture and Life Sciences,* University of Wisconsin, **270:** 1 -54.

Castenson, P. E., Sorenson, Jr., A. M., Cobos, C. R. & Fleeger, J. L. 1976. Source of post-partum P and 20β-0HP preceding estrus in heifers. *J. Anim. Sci.*, **43** : 227 (Abstr.).

Challis, J. R. G., Forster, C. S., Furr, B. J. A., Robinson, J. S. & Thorburn, G. D. 1976. Production of PGF2α in ewes following luteal regression induced with a prostaglandin analogue, estrumate (cloprostenol; I.C.I. 80996). *Prostaglandins*, **11**: 537 - 543.

Chang, C. H. , Gimenez, T. & Henricks, D. M. 1981. Modulation of reproductive hormones by suckling and exogenous gonadal hormones in young beef cows *post-partum. J. Reprod. Fert.*, **63**: 31 - 38.

Chew, B. P., Keller, R. E., Erb, R. E. & Malven, P. V. 1977. Periparturient concentrations of prolactin, progesterone and the estrogens in blood plasma of cows retaining and not retaining fetal membranes. *J. Anim. Sci.*,**44** : 1055 - 1060.

Chew, B. P., Erb, R. E., Randel, R. D. & Roquette, Jr., F. M. 1978. Effect of corticoid-induced parturition on lactation and on prepartum profiles of serum progesterone and estrogens among cows retaining and not retaining fetal membranes. *Theriogenology*, **10** : 13 - 25.

Chew, B. P., Erb, R. E., Fessler, J. F., Callahan, C. J. & Malven P. V. 1979a. Effects of ovariectomy during pregnancy and prematurely induced parturition on progesterone, estrogens and calving traits. *J. Dairy Sci.*, **62** : 557 - 566.

Chew, B. P., Erb, R. E., Claudie, Z. N., Colebrander, V. F., Malven, P. V. & D'Amico, M. R. 1977b. Variables associated with peripartum traits in dairy cows: Hormonal profiles associated with retained fetal membranes. *Theriogenology*, **12** : 245 - 253.

Christensen, D. S., Hopwood, M. L. & Wiltbank, J. N. 1974. Levels of hormones in the serum of cycling beef cows. *J. Anim. Sci.*, **38** : 577 - 583.

Chupin, D., Pelot, J. & Mauleon, P. 1977. Control of estrus and ovulation in dairy cows. *Theriogenology*, **7** : 339 - 350.

Clapp, H. 1937. A factor in breeding efficiency of dairy cattle. proc. *Anim. Soc. Anim. Prod.*, 259 - 263.

Clarke, I. J., & Cummins, J. T. 1985. Increased GnRH pulse frequency associated with estrogen- induced LH surges in ovariectomized ewes. *Endocrinology*, **116**: 2376 - 2383.

Convey, E. M. 1974. Serum hormone concentrations in ruminants during mammary growth, lactogenesis and lactation: A review. *J. Dairy Sci.*, **57**: 905 - 917.

Convey, E. M., Beck, T. W., Neitzel, R. R., Bostwick, E. F. & Hafs, H. D. 1977. Negative feedback control of bovine serum luteinizing hormone (LH) concentration of the preovulatory LH surge until resumption of luteal function. *J. Anim. Sci.*, **45** : 792 - 796.

Cooke, R. G. & Knifton, A. 1981. Oxytocin induced oestrus in the goat. *Theriogenology*, **16** : 95 - 97.

Cooper, M. J. 1974. Control of oestrous cycles of heifers with a synthetic prostraglandin analogue. *Vet. Rec.*, **95** : 200 - 203.

Corah, L. R., Queely, A. P., Dunn, T. G. & Kaltenback, C. C. 1974. Pre- and post-partum levels of progesterone and estradiol in beef heifers fed two levels of energy. *J. Anim. Sci.*, **39** : 380 - 385.

Corah, L. R., Dunn, T. G. & Kaltenback C. C. 1975. Influence of pre-partum nutrition on the reproductive performance of beef females and the performance of their progeny. *J. Anim. Sci.*, **41** : 819 - 824.

Corrie, J. E. T., Hunter, W. M. & Macpherson, J. S. 1975. A Strategy for radio-immunoassay of plasma progesterone with use of a homologous - site 1-labeled radioligand. *Clin. Chem.*, **27** : 594 - 599.

Cummins, L. J., Deblockey, M. A., Brown, J. M. & Goding, J. R. 1972. A study of LH secretion in the cow. *J. Reprod. Fert.*, **28** : 135 - 136.

D'Amato, C., Bahr, J., Stockert, B., Calvo, F. & Kesler, D. 1979. Long term perifusion of rabbit ovarian follicles. In *Ovarian Follicular and Corpus Luteum Function*. p. 235. Eds. C. P. Channing, F. M. Marsh and W. A. Sadler, Plenum Press.

Day, A. M. 1977a. Cloprostenol for termination of pregnancy in cattle. B. The induction of abortion. *New zealand Vet. Jour.*, **25** : 139 - 144.

Day, A. M. 1977 b. Cloprostenol as an aid to dairy herd management. I. Mating management. *New Zealand Vet. Jour.*, **25** : 300 - 305.

Deas, D. W. 1971. The effect of supplimentary light on winter infertility in cattle. *Vet. Rec.*, **89** : 242.

De Jong, F. H. & Sharpe, R. M. 1976. Evidence for inhibin - like activity in bovine follicular fluid. *Nature, London*, **263** : 71 - 72.

De Kruif, A. 1975. An investigation of the parameters which determine the fertility of a cattle population and of some factors which influence these parameters. *Tijdschr Diergeneesk.* **100** : 1089 - 1098. Cited from *J. Reprod. Fert.*, **54** : 515

De Kruif, A. 1977. Gen onderzock van runderen in anoestrua. (Studies on cattle in anoestrus). *Tijdschr. Diergeneesk.*, **102** : 247 - 253. from *J. Reprod. Fert.*, **54** : 457.

De Kruif, A. 1978. Factors influencing the fertility of a cattle population. *J. Reprod. Fert.*, **54** : 507 - 518.

De Silva, M., Dunn, T. G. & Kaltenback, C. C. 1984. Estrus response and pregnancy rates following calf removal in beef cows treated with prostaglandin F2α. *Theriogenology*, **21** : 835 - 839.

Diekman, M. A. & Malven, P. V. 1973. Effect of ovariectomy and estradiol on LH patterns in ewes. *J. Anim. Sci.*, **37** : 562 - 567.

Dobrowolski, W. 1973. Wplyw oksytocyny na cyki plciowy owcy w sezonie rozplodui ciszy seksualnej. Polskie Archwum Weterynaryjne., **16** : 649 - 654.

Dobson, H., Hopkinson, C. R. N. & Ward, W. R. 1973. Progesterone, 17β oestradiol and LH in relation to ovulation in cows. *Vet. Rec.*, **93** : 76.

Dobson, H. 1978. Plasma gonadotrophins and oestradiol during oestrus in the cow. *J. Reprod. Fert.*, **52** : 51 - 53.

Donaldson, L. E. & Hansel, W. 1968. Cystic corpora lutea and normal and cystic graafian follicles in the cow. *Australian Vet. Jour.*, **44** : 304 - 308.

Donaldson, L. E., Bassett, J. M. & Thorburn, G. D. 1970. Peripheral plasma progesterone concentrations of cows during puberty, oestrous cycle, pregnancy and lactation, and the effects of under nutrition or egogenous oxytocin on progesterone concentrations. *J. Endocrinology*, **48** : 599 - 614.

Dorrington, J. H. & Armstrong, D. T. 1979. Effects of FSH on gonadal functions. *Recent Prog. Horm. Res.*, **35** : 301 - 342.

Dufour, J. J. & Roy, G. L. 1985. Distribution of ovarian follicular populations in the dairy cow within 35 days after parturition. *J. Reprod. Fert.*, **73** : 229 - 235.

Duncanson, G. R. 1980. A four year study on a one hundred and twenty cow dairy unit with a high rate of retained placenta and subsequent endometritis. *XI Int. Congr. Diseases of Cattle.* pp. 931 - 985.

Dunlap, S. E., Kiser, T. E., Rampacek, G. B., Kraeling, R. R. & Thompson, F. N. 1981. Effect of suckling on cortisol, progesterone and LH in post-partum beef cows. *Theriogenology*, **16** : 185 - 193.

Dunn, T. G., Ingalls, J. E., Zimmerman, D. R. & Wiltbank, J. N. 1969. Reproductive performance of 2 year old Hereford and Angus Heifers as influenced by pre- and post calving energy intake. *J. Anim. Sci.*, **29** : 719 - 726.

Dunn, T. G., & Kaltenback, C. C. 1980. Nutrition and the postpartum interval in the ewe, sow and cow. *J. Anim. Sci.*, **51**, Suppl. II : 29.

Dunn, R. T. Jr., Smith, M. F., Garverick, H. A. & Fotey, C. W. 1985. Effects of 72 hour calf removal and/or gonadotropin releasing hormone on luteinizing hormone release and ovarian activity in post- partum beef cows. *Theriogenology*, **23** : 767 - 776.

Echternkamp, S. E. & Hansel, W. 1973. Concurrent changes in bovine plasma hormone levels prior to and during the first post-partum estrous cycle. *J. Anim. Sci.*, **37** : 1362 - 1375.

Echternkamp, S. E. 1978. Stimulation of estrogen and luteinizing hormone secretion in post-partum beef cows. *J. Anim. Sci.*, **47** : 521 - 531.

Edqvist, L. E., Eisarsson, S., Gustafson, B., Linde, C. & Lindell, J. O. 1975. The in-vitro and in-vivo effects of prostaglandin EI and F2α and of oxytocin on the tubular genital tract of ewes. *Int. Jour. Fert.*, **20** : 234 - 238.

Edqvist, L. E., Kindahl, H. & Stabenfeldt, G. 1978. Release of prostaglandin F2α during the bovine peripartal period. *Prostaglandins*, **16** : 111 - 119.

Edqvist, L. E. Lindell, J. O. & Kindahl, H. 1981. ProstaglandinF2α release at premature and normal term deliveries in the cow. *Acta Veterinaria Scandinavia,* Suppl. **77** : 267 - 277.

Edwards, S., Roche, J. F. & Niswender, G. D. 1983. Response of suckling beef cows to multiple low dose injections of GnRH with or without progesterone treatment. *J. Reprod. Fert.*, **69** : 65 - 72.

Edwards, S. 1985. The effect of short term calf removal on pusatile LH secretion in the post-partum beef cow. *Theriogenology*, **23** : 777 - 785.

Eger, S., Shemesh, M., Schinler, H., Amir, S. & Foote, R. H. 1988. Characterization of short luteal cycles in the early *post-partum* period and their relation to reproductive performance of dairy cows. *Anim. Reprod. Sci.*, **16** : 215 - 224.

Eley, D. S., Thatcher, W. W., Head, H. H., Collier, R. J., Wilcox, C. J. & Call, E. P. 1981. Perparturient and post-parturient endocrine changes of conceptus and maternal units in Jersey cows bred for milk yield. *J. Dairy Sci.*, **64** : 312 - 320.

Elias, K. A., Kelch, R. P. Lipner, H. & Blake, C. A. 1982. Relationship between basal gonadotrophin secretion rates and serum gonadotrophin concentrations in proestrous rats. *Biol. Reprod.*, **27** : 1159 - 1168.

Ellicott, A. R., Henricks, D. M., Gimenez, T. & Kiser, T. E. 1981. Suckling induced cortisol secretion in beef cows. *Theriogenology*, **16** : 469 - 475.

England, B. G., Hauser, E. R. & Casida, L. E. 1973. Some effects of unilateral ovariectomy in the post-partum beef cow. *J. Anim. Sci.*, **36** : 45 - 50.

Erb, R. E., Hinze, P. M., Gildow, E. M. & Morrison, R. A. 1958. Retained fetal membranes - The effect on prolificacy of dairy cattle. *J. Am. Vet. Med. Assoc.*, **133** : 489 - 496.

Erb, R. E., Surve, A. H., Callahan, C. J., Randel, R. D. & Gaverick, H. A. 1971. Reproductive steroids in the bovine. VIII. changes *post-partum. J. Anim. Sci.*, **33** - 1060.

Erb, R. E. D'Amaco, F., Chew, B. P., Malven, P. V. & Zamet, C. N. 1981. Variables associated with perpartum traits in dairy cows. VII. Hormonal profiles associated with dystocia. *J. Anim. Sci.*, **52** : 346 - 358.

Etherington, W. G. 1984. The post-partum cow. Physiology, uterine involution and hormonal therapy. In *Reproduction Management in Food Animals*. pp. 16 - 21. Veterinary learning system co. Inc. Princeton Junction, New Jersey.

Evans, H. E. & Sack, W. O. 1973. Prenatal Development of Domestic and Laboratory Mammals : Growth Curves, External Features and Selected References. *Anat., Histol., Embryol.*, **2** : 11 - 45.

Fairclough, R. J., Hunter, J. T. & Welch, R. A. S. 1975. Peripheral plasma progesterone and utero-ovarian prostaglandin F concentrations in the cow around parturition. *Prostaglandins*, **9** : 901 - 914.

Fairclough, R. J., Moore, L. G., McGowan, L. T., Peterson, A. J., Smith, J. F., Tervit, H. R. & Watkins, W. B. 1980. Temporal relationships between plasma concentrations of 13, 14- dihydro- 15- keto- prostaglandin F and neurophysin I/II around luteolysis in sheep. *Prostaglandins*, **20** : 199 - 208.

Fazeli, M. H., Ball, L. & Olson, J. D. 1980. Comparison of treatment of pyometra with estradiol cyprionate or cloprostenol followed by infusion or noninfusion with nitrofurazone. *Theriogenolgy*, **14** : 338 - 347.

Fernandes, L. C., Thatcher, W. W., Cox, C. J. W. & Call, E. P. 1978. LH release to GnRH during the post-partum period of dairy cows. *J. Anim. Sci.*, **46** : 443 - 448.

Fields, M. J., Fields, P. A., Castro-Hermandez, A. & Larkin, L. H. 1980. Evidence for relaxin in copora lutea of late pregnant cows. *Endocrinology*, **107** : 869 - 876.

Fields, P. A., Eldrige, R. K., Fuchs, A. R., Roberts, R. F. & Fields, M. J. 1983. Human placental and bovine corpora luteal oxytocin. *Endocrinology*, **112** : 1544 - 1546.

Flint, A. P. F. & Hillier, K. 1976. Prostaglandins and reproduction processes in female sheep and goats. In *Prostaglandin and Reproduction.* pp. 271 - 308. Ed. S. M. M. Karim, Lancaster, M. T. P. Press Ltd.

Flint, A. P. F. & Sheldrick, E. L. 1982. Ovarian secretion of oxytocin is stimulated by prostaglandin. *Nature*, **297** : 587 - 588.

Flint, A. P. F & Sheldrick, E. L. 1983. Evidence for a systemic role for ovarian oxytocin in luteal regression in sheep. *J. Reprod. Fert.*, **67** : 215 - 225.

Flores, A. G. 1972. A study of calving interval. *Diss. Abstr. B.*, **32** : 4320. Cited from *J. Reprod. Fert.*, 1978. **54** : 515.

Fonseca, F. A., Kosugiyma, M. A., Britt, J. H. & Ritchis, H. D. 1977. Ovulation, oestrous cycles and endocrine responses after GnRH in suckled cows. *Proc. 69th Annual Meeting, Amer, Soc. Anim. Sci.*, (Abstr.)p. 158.

Foote, W.D. & Hauser, E. R. and Casida, L. E. 1960. Some causes of variation in post-partum reproductive activity in Hereford cows. *J. Anim. Sci.*, **19** : 238 - 241.

Foote, W. D., & Hunter, J. E. 1964. Post-partum intervals of beef cows treated with progesterone and estrogen. *J. Anim. Sci.*, **23** : 517 - 520.

Foote, W. D. & Peterson, D. W. 1968. Relationships between side of pregnancy and side of subsequent ovarian activities in beef and dairy cattle. *J. Reprod. Fert.*, **16** : 415 - 421.

Ford, S. P. & Chenault, J. R. 1981. Blood flow to the corpus luteum-bearing ovary and ipsilateral uterine horn of cows during the oestrus cycle and early pregnancy. *J. Reprod. Fert.*, **62** : 555 - 562.

Foster, J. P., Holland, D. T., Jeffcoate, S. L. & Crighton, D. B. 1974. Simultaneous determination by radioimmunoassay of luteinizing hormone and luteinizing hormone releasing hormone at different stages of the oestrous cycle in the sheep. *J. Endocrinology*, **61** : IXiii.

Foster, J. P., Lamming, G. E. & Peters, A. R. 1980. Short term relationship between plasma LH, FSH and progesterone concentration in post-partum dairy cows and the effect of GnRH injection. *J. Reprod. Fert.*, **59** : 321 - 327.

Foulkes, J. A., Cookson, A. D. & Sauer, M. J. 1982. AI in cattle based on daily microtitreplate EIA of progesterone in whole milk. *Br. Vet. Jour.*, **138** : 515 - 521.

Franchimont, P., Verstraelen, J. P., Hazee- Hagelstein, M. T., Renard, Ch., Demoulin, A., Brouguignon, J. P. & Hustin, J. 1979. Inhibin : From Concept to Reality. *Vitamins and Hormones*, **37** : 243 - 302.

Fraser, H. M., McNeilly, A. S. & Popkin, R. M. 1984. Passive immunization against LH-RH : Elucidation of the role of LH - RH in controlling LH and FSH secretion and LH-RH receptors. In *Immunological Aspects of Reproduction in Mammals*. pp. 399. Ed. D. B. Crighton, Butterworths, London, Boston, Durban, Singapore, Sydney, Toronto, Wellington.

Fredriksson, G., Kindahl, H., Sandstedt, K. & Edqvist, L. E. 1985. Intrauterine bacterial findings and release of PGF2α in the post-partum dairy cow. *Zbl. Vet. Med. A.*, **32** : 368 - 380.

Garverick, H. A., Day, B. N., Mather, E. C. Gomez, L. & Thompson, G. B. 1974. Use of estrogen with dexamethasone for inducing parturition in beef cattle. *J. Anim. Sci.*, **38** : 584 - 590.

Garverick, H. A., Elmore, R. G., Vallaincourt, D. H. & Sharp, A. J. 1980. Ovarian response to gonadotrophin releasing hormone in post-partum dairy cows. *Am. J. Vet. Res.*, **41** : 1582 - 1585.

Gavin, W. 1913. On the effects of administration of extracts of pituitary body and corpus luteum to milch cows. *Queensland J. Exp. Physiol.*, **6** : 13 - 16.

Glencross, R. G., Munro, I. B., Senior, B. E. & Pope, G. S. 1973. Concentrations of oestradiol-17β, oestrone and progesterone in jugular venour plasma of cows during the oestrous cycle and in early pregnancy. *Acta Endocrinol Copenhagen*, **73** : 374 - 384.

Glencross, R. G., Esslemont, R. J., Bryant, M. J. & Pope, G. S 1981. Relationships between the Incidence of Pre-ovulatory Behaviour and Concentrations of Oestradiol-17β and Progesterone in Bovine Plasma. *Applied Anim. Ethol.*, **7** : 141 - 148.

Goding, J. R., Blockey, M. A., deB., Brown, J. M., Catt, K. J. & Cummins, I. A. 1970. the role of oestrogen in the control of oestrous cycle in the ewe. *J. Reprod. Fert.*, **21** : 368 - 369.

Goodale, W. S., Garverick, H. A., Kesler, D. J., Bierschwal, C. J., Elmore, R. G. & Youngqvist, R. S. 1978. Transitory changes of hormones in plasma of post-partum dairy cows. *J. Dairy Sci.*, **61** : 740 - 746

Goodman, R. L., & Karsch, F. J. 1980. Pulsatile secretion of luteinizing hormone : differential suppression by ovarian steroids. *Endocrinology*, **107** : 1286 - 1290.

Gonzalez- Padilla, E., Wiltbank, J. N. & Niswender, G. D. 1975. Puberty in beef heifers. I. The interrelationship between pituitary, hypothalamic and ovarian hormones. *J. Anim. Sci.*, **40** : 1091 - 1095.

Gospodarowicz, D. & Gospodarowicz G. 1975. The morphological transformation and inhibition of growth of bovine luteal cells in tissue culture induced by luteinizing hormone and dibutyl cyclic AMP. *Endocrinology*, **96** : 458 - 467.

Graves, W. E., Lauderdale, J. W., Hauser, E. R. & Casida, L. E. 1968. Relation of post-partum interval to pituitary gonadotropins, ovarian follicular development and fertility in beef cows. *Wisconsin Res. Bull.*, **270** : 23 - 28.

Greenstein, J. S. 1960. Experimental induction of cystic corpora lutea in dairy cattle. *Internat. J. Fert.*, **6** : 79 - 83.

Guilbau lt, L. A., Thatcher, W. W., Drost, M. & Hopkins, S. M. 1984. Source of F series prostaglandins during the early post-partum period in cattle. *Biol. Reprod.*, **31** : 879 - 887

Guilbault, L. A., Thatcher, W. W., Collier, R. J. & Wilcox, C. J. 1985. Perparturient endocrine changes of conceptus and maternal units in Holstein heifers bearing genetically different conceptuses. *J. Anim. Sci.*, **61** : 1505 - 1515.

Guilbau lt, L. A., Thatcher, W. W., Drost, M. & Haibel, G. K. 1987. Influence of a physiological infusion of prostaglandin F2α into postpartum cows with partially suppressed endogenous production of prostaglandins. I. Uterine and ovarian morphological responses. *Theriogenology*, **27** : 931 - 946.

Hafez, E. S. E. 1980. In *Reproduction in Farm Animals*. pp 339. 4th Ed. Lea and Febiger, Philadelphia.

Hafs, H. D. & Armstrong, D. T. 1968. Corpus luteum growth and progesterone synthesis during the bovine oestrous cycle. *J. Anim. Sci.*, **27** : 134 - 141.

Hafs, H. D. & Manns, J. G. 1975. Onset of oestrus and fertility of dairy heaifers and suckled beef cows treated with prostaglandin F2α. *Anim. Prod.*, **21** : 13 - 20.

Hafs, H. D., Manns, J. G. & Drew, B. 1975. Onset of oestrus after prostaglandin F2α in cattle. *Vet. Rec.*, **96** : 134 - 135.

Hafs, H. D., Kiser, T. E., Haynes, N. B., Kesner, J. S. & Stellflug, J. 1977. Release of pituitary hormones, cortisol, testosterone and insulin in response to prostaglandin F2α given during intracarotid infusion of somatostatin in bulls. *J. Anim. Sci.*, **44** : 1061 - 1066.

Hammond, J. 1913. The effect of pituitary extract on the secretion of milk. *Queensland J. Exp. Physiol.*, **6** : 311 - 338.

Hammond, J. & Sanders, H. G. 1923. Some factors affecting milk yield. *J. Anim. Sci.*, **13** : 74 - 78.

Hansel, W. 1959. The oestrous cycle of the cow. In *Reproduction in Domestic Animals. pp* 223 Vol. **I.** Eds. H. H. Cole and P. T. Cupps. Academic Press, London.

Hansel, W. 1964. Sterility due to permanent corpus luteum and cystic degeneration.: Some observations on bovine cystic corpora lutea. *5th Int. Congress on Anim. Reprod. and Arti. Insemination,* Trento, Italy.

Hansel, W. & Schechter, R. E. 1972. Biotechnical procedures for control of the oestrous cycles of domestic animals. *Proc. 7th Congr. Anim. Reprod. A. I.* (Munich) **I** : 75 - 96.

Hansel, W., Concannon, P. W. & Lukazewska, J. H. 1973. Corpora lutea of the large domestic animals. *Biol. Reprod.*, **8** : 222 - 245.

Hansel, W. & Beal, W. E. 1979. Ovulation control in cattle. In *Beltsville Symposium in Agricultural Research. III. Animal Reproduction.* pp 91 - 110. Ed. H. W. Hawk. Wiley, New York.

Hansel, W. & Convey, E. M. 1983. Physiology of the oestrous cycle. *J. Anim. Sci.*, **57**, Suppl. 2 : 404 - 424.

Harris, G. W. 1949. Regeneration of the hypophyseal portal vessels. *Nature,* **163** : 70.

Harris, G. W. 1950. Oestrous rhythm, pseudopregnancy and the pituitary stalk in the rat. *J. Physiol.*, III **: 347** - 360.

Hauger, R. L., Karsch, F. J. & Foster, D. L. 1977. A new concept for control of the oestrous cycle of the ewe based on temporal relations between LH, oestradiol and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion. *Endocrinology,* **101** : 807 - 817.

Haynes, N. B., Collier, R. J., Kiser, T. E. & Hafs, H. D. 1978. Effect of prostaglandin E2 and F2α on serum luteinizing hormone, testosterone and prolactin in bulls. *J. Anim. Sci.*, **47** : 923 - 926.

Hearnshaw, H., Restall, B. J., Nancarrow, C. D. & Mattner, P. E. 1974. Synchronization of oestrus in cattle, sheep and goats using a prostaglandin anaolgue. *Proc. Aust. Soc. Anim. Prod.*, **10 :** 242 - 245.

Henderson, K. M. & McNatty, K. P. 1975. A biochemical hypothesis to explain the mechanism of luteal regression. *Prostaglandins*, **9** : 779 - 797.

Henderson, K. M. & Franchimont, P. 1981. Regulation of inhibin production by bovine ovarian cells in vitro. *J. Reprod. Fert.*, **63** : 431 - 442.

Henderson, K. M., McNeilly, A. S. & Swanston, I. A. 1982. Gonadotrophin and steroid concentrations in bovine follicular fluid and their relationship to follicle size. *J. Reprod. Fert.*, **65** : 467 - 473.

Henricks, D. M., Dickey, J. F. & Niswender, G. d. 1970. Serum Luteinizing hormone and plasma progesterone levels during the oestrous cycle and early pregnancy in cows. *Biol Reprod.*, **2** : 346 - 351.

Henricks, D. M., Dickey, J. F. & Hill, J. R. 1971. Plasma estrogen and progesterone levels in cows prior to and during estrus. *Endocrinology,* **89** : 1350 - 1355.

Henricks, D. M., Dickey, J. F., Hill, J. R. & Jonston, W. E. 1972. Plasma estrogen and progesterone levels after mating, and during late pregnancy and post-partum in cows. *Endocrinology,* **90** : 1336 - 1342.

Herman, H. A., & Edmondson, J. H. 1950. Factors A affecting interval between parturition and first oestrus in dairy cattle. *Montana Agr. Expt. Stn. Bul. No.* **462.**

Hewett, C. D. 1968. A survey of the incidence of the repeat breeder cow in Sweden with reference to herd size, season, age and milk yield. *Br. Vet. Jour.*, **124** : 342 - 352.

Higaki, S., Suga, T. & Awai, Y. 1959. Postparturient uterine involution in dairy cows. II. Artificial accelerating effects on the uterine involution in the post-parturient cows. *Bul. Nat. Inst. Agri. Sci.*, (Japan) Series G. No. **18** : 85 - 89.

Hill, J. R., Lamond, D. R., Henricks, D. M., Dickey, J. F. & Niswender, G. D. 1970. Effect of under nutrition on ovarian function and fertifility in beef heifer. *Biol Reprod.*, **2** : 78 - 84.

Hobson, W. C. & Hansel, W. 1972. Plasma LH levels after ovariectomy, corpus luteum removal and estradiol administration in cattle. *Endocrinology*, **91** : 185 - 190.

Hoffman, B., Schams, D., Bopp, R., Ender, M. L., Giiminez, T. & Karg, H. 1973. Changes of Prostaglandi, totalestrogens and progesterone at parturition in cow. *Acta Endocr.*, **73** : 385 - 395.

Horton, E. W. & Poyser, N. L. 1976. Uterine luteolytic hormone. A physiological role for prostraglandin F2α. *Physiol. Rev.*, **56** : 595 - 601.

Humphrey, W. D., Koritnik, D. R., Kaltenback, C. C., Dunn, T. G. & Niswender, G. D. 1976. Progesterone and LH in post-partum suckled beef cows. *J. Anim. Sci.*, **43** : 290 (Abstr.).

Humphrey, W. D., Kaltenbach, C. C., Dunn, T. G., Koritnik, D. R. & Niswender, G. D. 1983. Characterization of hormonal pattern in the beef cows during post-partum anoestrus. *J. Anim. Sci.*, **56** : 445 - 453.

Hunter, W. M., Nars, P. W. & Rutherford, F. F. 1975. In *Steroidimmunoassay*. Eds. E. H. D. Cameron, S. J. Hillier and K. Griffiths. Fifther Tenovus Workshop, Cardiff. Alpha Omega Publications. pp. 141 - 152.

Hunter, J. T., Fairclough, R. J., Peterson, A. J. & Welch, R. A. S. 1977. Foetal and maternal hormonal changes preceding normal bovine parturition. *Act Endocr.*, **84** : 653 - 662.

Imakawa, K., Day, M. L., Zalesky, D. D., Garcia-Winder, M., Kittok, R. J. & Kinder, J. E. 1986. Regulation of pulsatile LH secretion by ovarian steroids in the heifers. *J. Anim. Sci.*, **63** : 162 - 168.

Ingraham, R. H., Pool, S. H. & Godke, R. A. 1984. Ovarian function in crossbreed heifers treated with dexamethasone. *Theriogenology*, **21** : 875 - 886.

Ireland, J. J. & Roche, J. F. 1982. Effects of progesterone on basal LH and episodic LH and FSH secretion in heifers. *J. Reprod. Fert.*, **64** : 295 - 302.

Jackson, P. S. 1977. Treatment of chronic post-partum endometritis in cattle with cloprostenol. *Vet. Rec.*, **101** : 441 - 443.

Jackson, P. S., Johnson, C. T., Bulman, D. C. & Holdsworth, R. J. 1979. A study of cloprostenol- induced oestrus and spontaneous oestrus by means of the milk progesterone assay. *Br. Vet. Jour.*, **135** : 578 - 580.

Jackson, P. S. 1981. A note on a possible association between plasma B-carotene levels and conception rate in a group of winter housed dairy cattle. *Anim. Prod.*, **32** : 109 - 111.

Jeffcoate, I. A. & Keeling, B. J. 1984. Active immunization against LH-RH in the female. In *Immunological Aspects of Reproduction in Mammals.* pp. 363. Ed. D. B. Crighton, Butterworths, London, Boston.

Jóchle, W. 1971. Corticoid induced parturition in domestic animals: Mechanism of action and economic importance. *Folia Veterinaria Latin, Roma.*, **I** : 229 - 248.

Johnson, M. H. & Everitt, B. J. 1988. In *Essential Reproduction.* Blackwell, Oxford.

Jones, E. E. & Nalbandov, A. V. 1972. Effects of intra follicular injection of gonadotrophins on ovulation or Luteinization of ovarian follicle. *Biol. Reprod.*, **7** : 87 - 93.

Kann, G. Martinet, J. & Schirar, A. 1978. Hypothalamic- pituitary control during lactation in sheep. In *Control of ovulation.* pp. 319 - 333. Eds. D. B. Crighton, N. B. Haynes,. G. R. Foxcroft and G. E. Lamming. Butterworth, London.

Karg, H. 1972. Recent results concerning cyclic effects of pituitary gonadotrophins. **7th Internat. congr. Anim. Reprod. and A. I., Munich**, Vol. **I** : 45 - 56.

Karim, S. M. M., Hillier, K., Somers, K. & Trussel, R. R. 1971. The effects of prostaglandin E2 and F2α administered by different routes on uterine activity and the cardiovascular system in pregnant and non-pregnant women. *J. Obst. Cynaecol. British Commonwealth,* **78** : 172 - 179.

Karsch, F. J., Dierschke, d. J., Weick, R. F., Yamaji, T., Hotchkiss, J. & Knobil, E. 1973. Positive and negative feedback control by oestrogen of luteinizing hormone secretion in the Rhesus monkey. *Endocrinology,* **92** : 799 - 804.

Karsch, F. J. & Foster, D. L. 1975. Sexual differentiation of the mechanism controlling the preovulatory discharge of luteinizing hormone in sheep. *Endocrinology,* **97** : 373 - 379.

Karsch, F. J., Foster, D. L., Legan, S. J., Ryan, K. D. & Peter, G. K. 1979. Control of the preovulatory endocrine events in the ewe : inter-relationship of oestradiol, progesterone and luteinizing hormone. *Endocrinology*, **105** : 421 - 426.

Karsch, F. J., Cummins, J. T., Thomas G. B. & Clarke, I. J. 1987. Steroid feedback inhibition of pulsatile secretion of gonadotropin-releasing hormone in the ewe. *Biol. Reprod.*, **36** : 1207 - 1218.

Kastin, A. J., Schally, A. V., Gual, C., Midgley, A. R., Bowers, C. Y. & Diaz- Infante, A. 1969. Stimulation of LH release in men and women by a LH releasing hormone purified from porcine hypothalami. *J. Clin. Endo. & Metabolism*, **29** : 1046 - 1050.

Kesler, D. J., Garverick, H. A., Youngqvist, R. S., Elmore, R. G. & Bierschwal, C. J. 1977. Effect of days *post-partum* and endogenous reproductive hormones on GnRH induced LH release in dairy cows. *J. Anim. Sci.*, **46** : 797 - 806.

Kesler, D. J., Garverick, H. A., Youngqvist, R. S., Elmore, R. G. & Bierschwal, C. J. 1979. Reproductive hormones associated with normal and abnormal changes in ovarian follicles in post-partum dairy cows. *J. Dairy Sci.*, **62** : 1290 - 1296.

Kesler, D. J., Toxel, T. R. & Hixon, D. L. 1980. Effects of days *post-partum* and exogenous GnRH on reproductive hormones and ovarian changes in post-partum suckled beef cows. *Theriogenology*, **13** : 287 - 296.

Kesner, J. S., Convey, E. M. & Anderson, C. R. 1981. Evidence that oestradiol induces the preovulatory LH surge in cattle by increasing pituitary sensitivity to LH-RH and then increasing LH-RH release. *Endocrinology*, **108** : 1386 - 1391.

Kilkenny, J. B. 1978. Reproductive Performance of Beef Cows. *World Review of Anim. Prod.*, **14** : 65 - 74.

Kindahl, H., Edqvist, L. E., Bane, A. & Granstrom, E. 1976a. Blood levels of progesterone and 15- keto- 13, 14- dihydro-prostaglandin F2α during normal oestrous cycle and early pregnancy in heifer. *Acta Endocrin.* (kbh.) , **82** : 134 - 149.

Kindahl, H., Edqvist, L. E., Granstrom, E. & Bane, A. 1976b. The release of prostaglandin F2α as reflected by 15- keto- 13, 14- dihydro prostaglandin F2α in the peripheral circulation during normal luteolysis in heifer. *Prostaglandins*, **11** : 871 - 878.

Kindahl, H., Lindell, J. O. & Edqvist, L. E. 1979. On the control of prostaglandin release during the bovine oestrous cycle. Effects of progesterone implants. *Prostaglandins*, **18** : 813 - 820.

Kindahl, H., Lindell, J. O. & Edqvist, L. E. 1980. Luteolysis in domestic animals. Control of PGF2α release. *Proc. 9th Internat. congr. Anim. Reprod. and A. I.*, Madrid, **2** : 17 - 26.

Kindahl, H., Lindell, J. O. & Edqvist, L. E. 1981. Release of prostaglandin F2α during the oestrous cycle. *Acta Vertinaria Scandinavica,* Suppl. **77** : 143 - 158.

Kindahl, H., Edqvist, L. E., Larsson, K. & Marmqvist, A. 1982. Influence of prostaglandins on ovarian function Post-partum. In *Factors Influencing Fertility in the Post-partum Cow.* pp. 173 - 196. Eds. H. Karg and E. Schallenberger. Martinus Nijhoff, The Hague.

Kindahl, H., Fredriksson, G., Madej, A. & Edqvist, L. E. 1984. Role of Prostaglandings in uterine involution. *Proc. 10th Internat. Congr. on Anim. Reprod. and A I.*, Vol. **IV** : PXI, 9 - 16. Illinois.

King, J. O. L. 1968. The Relationship between the Conception Rate and Changes in Body weight, Yield and SNF Content of Milk in Dairy Cows. *Vet. Rec.*, **83** : 492 - 494.

King, G. J., Hurnik, J. F. & Robertson, H. A. 1976. Ovarian function and estrus in dairy cows during early lactation. *J. Anim. Sci.*, **42** : 688 - 692.

King, G. J. & Hurnik, J. F. 1980. Reproductive function in postpartum beef cows. *Proc. 9th Internat. Congr. Reprod. and A. I.*, **3** : 39 - 43.

Kingman, H. E. 1951. Implantation of the fertilized ovum and its relation to the infertility problems in the cow. pp. 26 - 29. *Proc. Nation. Egg. Transfer Breeding Conference*, San Antonio, U.S.A.

Kiracofe, G. H. 1980. Uterine involution : Its role in regulating post-partum intervals. *J. Anim. Sci.*, **51**, Suppl. II : 16 - 28.

Kiracofe, G. H., Keay, L. E. & Odde, K. G. 1985. Synchronization of oestrus in cyclic beef heifers with the prostaglandin anologue alfaprostol. *Theriogenology*, **24** : 737 - 745.

Kiser, T. E., Dunlap, S. E. & Benyshek, L. L. 1979. The effect of calf removal on oestrous response and pregnancy of beef cows after Syncro-Mate-B treatment. *J. Anim. Sci.*, **49** : (Suppl. I), 309 (Abstr.)

Knight, T. W. & Nicoll, G. B. 1978. Factors influencing the interval from calving to first oestrus in beef cattle on North Island Hill Country. *Proc. New Zealand. Soc. Anim. Prod.*, **38** : 175 - 180.

Lamming, G. E. 1966. Nutrition and the endocrine system. *Nutritional Abstracts and Reviews*. **36** : 1 - 13.

Lamming, G. E., & Bulman, D. C. 1976. The use of milk progesterone radio-immunoassay in the diagnosis and treatment of subfertility in dairy cows. **Br. Vet. Jour., 132** : 507 -517.

Lamming, G. E., Wathes, D. C. & Peters, A. R. 1981. Endocrine patterns of the post-partum cow. *J. Reprod. Fert.*, Suppl. **30** : 155 - 170.

Lamming, G. E., Peters, A. R., Riley, G. M. & Fisher, M. W. 1982. Endocrine regulation of post-partum function. In *Factors Influencing Fertility in the Post-Partum Cow. Proc. of EEC Seminar*, 1981. Eds. H. Karg and E. Schallenberger. Martinus Nijhoff, The Hague.

Lamond, D. R. 1973. The role of the bovine practitioner in synchronization and twinning in cattle. *Bovine Practit.* **8** : 2 - 8.

Land, R. B. 1971. The incidence of oestrus during lactation in Finnish Landrace, Dorset Horn and Finn- Dorset sheep. *J. Reprod. Fert.*, **24** : 345 - 352.

Larsson, K., Kindahl, H., Malmqvist, A., Edqvist, L. E. & Jansson, L. 1982. Post-partum periodens forlopp hos forstakalvande mjolkkor. (The post-partum period in primparous dairy cows). *Proc. 14th Nordic Vet. Congr., Copenhagen,* 327 - 331.

Laster, D. B., Glimp, H. A. & Gregory, K. E. 1973. Effects of early weaning on post-partum reproduction of cows. *J. Anim. Sci.*, **36** : 734 - 740.

Lauderdale, J. W. 1972. Effects of PGF2α on pregnancy and estrous cycle of cattle. *J. Anim. Sci.*, **35** : 246 (Abstr.).

Lemon, M., Pelletier, J., Saumande, J. & Signoret, J. P. 1975. Peripheral plasma concentrations of progesterone, oestradiol - 17β and LH around oestrus in the cow. *J. Reprod. Fert.*, **43** : 137 - 140.

Leslie, K. E. 1983. The events of normal and abnormal reproductive endocrinology and uterine involution in dairy cows : A review. *Canadian* Vet. Jour., **24** : 67 - 71.

Levine, J. E., Pau, K. Y., Ramirez, U. D. & Jackson, G. L. 1982. Simultaneous measurement of luteinizing hormone releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. *Endocrinology*, **111** : 1449 - 1455.

Lewis, G. S., Thatcher, W. W., Bliss, E. L., Drost, M. & Collier, R. J. 1984. Effects of heat stress during pregnancy on post-partum reproductive changes in Holstein cows. *J. Anim. Sci.*, **58** : 174 - 186.

Liehr, R. A., Marion, G. B. & Olson, H. H. 1972. Effect of prostaglandins on cattle estrous cycles. *J. Anim. Sci.*, **35** : 247 (Abstr.).

Lindell, J. O., Kindahl, H. & Edqvist, L. E. 1980. Prostaglandin induced early abortions in the bovine : Clinical outcome and endogenous release of prostaglandinF2α and progesterone. *Anim. Reprod. Sci.*, **3** : 389 - 399.

Lindell, J. O., Kindahl, H., Jansson, L. & Edqvist, L. E. 1982. Post-partum release of prostaglandin F2α and uterine involution in the cow. *Theriogenology*, **17** : 237 - 245

Lindell, J. O. & Kindahl, H. 1983. Exogenous PGF2α Promotes uterine involution in the cow. *Acta Vetrinaria Scandinavica*, **24** : 269 - 274.

Loeb, L. 1923. The effect of extirpation of the uterus on the life and function of the corpus luteum in the Guinea pig. *Proc. Soc. Exp. Biol. Med.*, **20** : 441 - 443.

Lothammer, K. H. 1978. In *Importance of Beta- Carotene for Bovine Fertility,* pp. 5. Roche Symposium.

Louis, T. M., Hafs, H.D. & Morrow, D. A. 1972. Estrus and ovulation after uterine PGF2α in cows. *J. Anim. Sci.*, **35** : 247 (Abstr.).

Lucas, M. J., Olson, J. D., Fariana, P. W. & Ball, L. 1983. The economics of prostaglandin F2α as a replacement for detection of estrus in dairy cattle. *J. Dairy Sci.*, **66**: 1245-1252

MacDiarmid, S. C. 1983. Induction of parturition in cattle using corticoids; A review, part I. Reasons for induction, mechanism of induction, and preparations used. *Anim. Breed. Abstracts.*, **51** : 403 - 419.

Macmillan, K. L. & Watson, J. D. 1971. Short oestrous cycles in New Zealand dairy cattle. *J. Dairy Sci.*, **54** : 1526 - 1529.

Macmillan, K. L., Curnow, R. J. & Morris, G. R. 1977. Oestrus synchronization with a prostaglandin analogue. I. Systems in lactating dairy cattle.*New Zealand Vet. Jour.*, **25** : 366 - 372.

Macmillan, K. L., Day, A. M. & Smith, J. F. 1980. Onset of oestrus and fertility in lactating dairy cows injected with an analogue of prostaglandin F2α cloprostenol. *Anim. Reprod. Sci.*, **3** : 171 - 180.

Madej, A., Kindahl, H., Wyono, W., Edqvist, L. E. & Stupnicki, R. 1984. Blood levels of 15 keto- 13, 14- dihydro- prostaglandin F2α during the post-partum period in primiparous cows. *Theriogenology*, **21** : 279 - 287.

Main, S. J., Davies, R. V. & Setchell, B. P. 1979. the evidence that inhibin must exist. *J. Reprod. Fert./*, Suppl., **26** : 3 - 14.

Manns, J. G. & Niswender, G. D. 1983. FSH receptors in the bovine corpus luteum. *J. Anim. Sci.*, **57**, Suppl., I : 354, Abstr. 536

Manns, J. G., Humphrey, W. D., Flood, P. F., Mapletoft, R. J., Rawlings, N. & Cheng, K. W. 1983. Endocrine profiles and functional characteristics of corpora lutea following onset of post-partum ovarian activity in beef cows. *J. Anim. Sci.*, **63** : 331 - 347.

Marion, G. B. & Gier, H. T. 1968. Factors affecting bovine ovarian activity after parturition. *J. Anim. Sci.*, **27** : 1621 - 1626.

Marion, G. B., Norwood, J. S. & Gier, H. T. 1968. Uterus of the cow after parturition. Factors affecting regression. *Am. J. Vet. Res.*, **29** : 71 - 75.

Mauleon, P. 1974. New trends in the control of reproduction in the bovine. *Livestock Prod. Sci.*, **I** : 117 - 125.

Mencio, F. G., Murphy, B. D. & Manns, J. 1977. Failure of exogenous LH to prevent PGF2a induced luteolysis in beef cattle. *Prostaglandins*, **14** : 535 - 542.

Menge, A. C., Mares, S. E., Tyler, W. J. & Casida, L. E. 1962. Variation and association among post-partum reproduction and production characteristics in Holstein- Friesian cattle. *J. Dairy Sci.*, **45** : 233 - 241.

Mercier, E. & Salisbury, G. W. 1947. Fertility level in artificial breeding associated with season, hourse of daylight and the age of cattle. *J. Dairy Sci.*, **30** : 817 - 826.

Miksch, E. D., Lefever, D. G., Mukembo, G., Spitzer, J. C. & Wiltbank, J. 1978. Synchronization of estrus in beef cattle. II Effect of an injection of Norgestomet and estrogen in conjunction with a Norgestomet implant in heifers and cows. *Theriogenology*, **10** : 201 - 221.

Millar, R. P. & Aehnelt, C. 1977. Application of ovine luteinizing hormone (LH) radioimmunoassay in the quantitation of LH in different mammalian species. *Endocrinology*, **101** : 760 - 768.

Milne, J. A. 1963. Effects of oxytocin on the oestrous cycle of the ewe. *Aust. Vet. Jour.*, **39** : 51 - 52.

Mitchell, M. D., Kraemer, D. L., Brennecke, S. P. & Webb, R. 1982. Pulsatile release of oxytocin during the oestrous cycle, pregnancy and parturition in sheep. *Biol. Reprod.*, **27** : 1169 - 1173.

Mittler, J. C., Arimura, A. & Schally, A. V. 1970. Release and synthesis of luteinizing hormone and follicle stimulating hormone in pituitary cultures in response to hypothalamic preparation. *Proc. Soc. Exp. Biol. Med.*, **133** : 1321 - 1325.

Moller, K., Newling, P. E., Robson, H. T., Jansen, G. J., Meursingee, J. A. & Cooper, M. G. 1967. Retained Fetal Membrane in Dairy Herds in the Huntley District. *New Zealand Vet. Jour.*, **15** : 111 - 116.

Moore, C. R. & Price, D. 1932. Gonadal hormone fluctuations and the reciprocal influence between gonads and hypophysis, with its bearing on sex hormone antagonism. *Amer. Jour. Anatomy*, **50** : 13 - 71.

Mori, J. J., Masaki, T., Wakabarayashi, T., Endo, P. & Hasoda, T. 1974. Serum LH levels in cattle under various reproductive states. *Theriogenology*, **I** : 131 - 136.

Morrow, D. A., Roberts, S. J., McEntee, K. & Gray, H. G. 1966. Post-partum ovarian activity and uterine involution in dairy cattle. *J. Am. Vet. Med. Assoc.*, **149** : 1596 - 1609.

Morrow, D. A. 1969. Estrous Behaviour and Ovarian Activity in Prepuberal and Postpuberal Dairy Heifers. *J. Dairy Sci.*, **52** : 224 - 227.

Morrow, D. A., Roberts, S. J. & McEntee, K. 1969. A review of post-partum ovarian activity and involution of the uterus and cervix in cattle. *Cornell Vet.*, **59** : 134 - 154.

Morrow, D. A. 1970. Daignosis and prevention of fertility in cattle. *J. Dairy Sci.*, **53** : 961 - 969.

Mortimer, R. G., Ball, L., Olson, J. D., Huffman, E. M. & Fariana, P. W. 1984. The effect of PGF2- alpha on reproductive performance of naturally bred dairy cows with or without pyometra. *Theriogenology*, **21** : 869 - 874.

McCann, S. M., Kalra, P. S., Schneider, H. P. G., Watson, J. T, Wakabayashi, K., Fawcett, C. P. & Frulich, L. 1972. Studies on the feedback activities of gonadal steroids on gonadotropin and prolactin secretion : effects, sites and mechanism of action. In *Steroid hormones and brain function.* pp. 311 - 323. Eds. C. H. Savvyer and R. A. Gorski. University of California Press.

McClure, J. J. 1972. Blood Glucose and Female Fertility. *Vet. Rec.*, **91** :193.

McCracken, J. R., Baird, D. T., Green, K. & Samuelsson, B. 1972. ProstaglandinF2α identified as a luteolytic hormone in sheep. *Nature, New Biol.*, **238** : 129 - 134.

McCracken, J. A., Schramm, W., Barcikowski, B. & Wilson, L. 1981. The identification of prostaglandinF2α as a uterine luteolytic hormone and the hormonal control of its synthesis. *Acta Veterinaria Scandinavica* Suppl. **77** : 71 - 88.

McDonald, L. E., McNutt, S. H. & Nichols, R. E. 1954. Retained placenta. Experimental production and prevention. *Am. J. Vet. Res.*, **15** : 22 - 24.

McEntee, K. 1958. Cystic corpora lutea in cattle. *Int. J. Fert.*, **3** : 120 - 127.

McKenzie, B. E. & Kenney, R. M. 1973. Histologic features of ovarian follicles of gonadotropin-injected heifers. *Am. J. Vet. Res.*, **38** : 479 - 482.

McPherson, J. C., Costoff, A., Eldrige, J. C. & Mahesh, V. B. 1974. Effects of various progestational preparations on gonadotropin secretion in ovariectomized immature female rats. *Fertility and Sterility*, **25** : 1063 - 1070.

Nalbandov, A. V. & Casida, L. E. 1940. Gonadotropic action of pituitaries from pregnant cows. *Endocrinology*, **27** : 559 - 566.

Nalbandov, A. V. & Bahr, J. M. 1974. In *Physiology and Genetics of Reproduction,* part A. pp. 399-407. Eds. E. M. Continho & F. Fuchs. Plenum, New York.

Nancarrow, C. D. , Buckmaster, J., Camley, W., Cox, R. I., Cumming, I. A., Cummings, L., Drinan, J. P., Findlay, J. K., Goding, J. R., Bestal B. J., Schneider, W. & Thornburn, G. D. 1973. Hormonal changes around oestrus in the cow . J. *Reprod. Fert.*, **32**: 320-326.

Niswender, G. D, Reichert, Jr. L. E., Midgley, Jr., A. R. & Nalbandov, A. V. 1969. Radioimmunoassay for bovine and ovine luteinizing hormoe. *Endocrinology,* **84** : 1166 - 1173.

Odde, K. G., Ward, H. S., Kirakofe, G. H., McKee, R. M. & Kittok, R. J. 1980. Short estrous cycles and associated serum progesterone level in beef cow. *Theriogenology*, **14** : 105 - 110.

O'Farrell, K. J. & Hartigan, P. J. 1984. The treatment of non detected oestrus (NDO) in dairy cows with cloprostenol. *Irish Vet. Jour.*, **38** : 28 - 33.

Okuda, K., Gaona, W. A. & Sato, K. 1988. Effects of gonadotropin releasing hormong and prostaglandin F2a on the reproductive performance in post partum cows. *Theriogenology*, **29** : 823 - 833.

Olson, J. D. 1980. The use of prostaglandins as a replacement for heat detection in dairy cattle. *Proc. Ann. Meet. Soc. for Theriogenolgy*, pp : 180 - 197

Oltenacu, P. A., Britt, J. H., Braun, R. K. & Mellenberger, R. W. 1983. Relationship among type of parturition, type of discharge from genital tract, involution of cervix, and subsequent reproductive performance in Holstein cows. *J. Dairy Sci.*, **66** : 612 - 619.

Ott, I. & Scott, J. C. 1910. The glactogogue action of the thymus and corpus luteum. *Proc. Soc. Exp. Biol. Med.*, **8** : 49.

Ott, R. S. & Gustafsson, B. K. 1981. Therapeutic application of prostaglandins for post-partum infections. *Acta Veterinaria Scandinavica*, Suppl. **77** : 363 - 369.

Oxenreider, S. L. 1968. Effects of suckling and ovarian function on post-partum reproductive activity in beef cow. *Am. J. Vet. Res.*, **29** : 2099 - 2102.

Oxenreider, S. L. & Wagner, W. C. 1971. Effect of lactation and energy intake on post-partum ovarian activity in the cow. *J. Anim. Sci.*, **33** : 1026 - 1031.

Padmanabhan, B. & Convey, E. M. 1981. Progesterone inhibits the ability of oestradiol to increase basal and luteinizing hormone releasing hormone induced luteinizing hormone release from bovine pituitary cells in culture: Neither progesterone nor oestradiol affects follicle stimulating hormone release. *Endocrinology*, **109** : 1091 - 1096.

Patil, R. K., Sinha, S. N., Einarsson, S. & Settergren, I. 1980. The effect of prostaglandin F2α and oxytocin on bovine myometrium *in vitro*. *Nordisk Veterinaer Medicin.*, **32** : 474 - 479.

Pharris, B. B., Cornette, J. C. & Gutknecht, G. D. 1970. Vascular control of luteal steroidogenesis. *J. Reprod. Fert.*, Suppl. **10** : 97 - 103.

Pelletier, J. 1976. Influence of LH-RF on LH and FSH releases in domestic mammals. *Ann. Biol. anim. Bioch. Biophys.*, **16** : 213 - 234.

Perkins, J. L. & Kidder, H. E. 1963. Relation of uterine involution and post-partum interval to reproductive efficiency in beef cattle. *J. Anim. Sci.*, **22** : 313 - 315.

Peters, A. R. 1980. Ovarian activitiy in post-partum beef cows. *Proc 11th congr. Diseases of cattle.* pp. 801 - 804. Ed. E. Mayer, Bregman Press, Haifa.

Peters, A. R., Riley, G. M., Rahim, S. E. A. & Lowman, B. G. 1980. Milk progesterone profiles and the double injection of cloprostenol in post-partum cows. *Vet. Rec.*, **107** : 174 - 177.

Peters, A. R., Kingsley, S. R. & Riley, G. M. 1981a. Gonadotrophin response to combined progesterone and prostaglandin treatment in beef cows. *Theriogenolgy,* **16** : 259 - 269.

Peters, A. R., Lamming, G. E. & Fisher, M. W. 1981b. A comparison of plasma LH concentration in milked and suckling post-partum cows. *J. Reprod. Fert.*, **62** : 567 - 573.

Peters, A. R. & Riley, G. M. 1982a. Milk progesterone profiles and factors affecting post-partum ovarian activity in beef cows. *Anim. Prod.*, **34** : 145 - 153.

Peters, A. R. & Riley, G. M. 1982b. Is the cow a seasonal breeder? *Br. Vet. Jour.* **138** : 533 - 537.

Peters. A. R. 1984a. Reproductive activity of the cow in the post-partum period. 1. Factors affecting the length of the post-partum acyclic period. *Br. Vet. Jour.*, **140** : 71 - 84.

Peters, A. R. 1984b. Effect of oestradiol-17β on gonadotrophin secretion in post-partum beef cows. *J. Reprod. Fert.*, **72** : 473 - 478.

Peters, A. R. & Lamming, G. E. 1984. Reproductive activity of the cow in the post-partum period. II. Endocrine patterns and induction of ovulation. *Br. Vet. Jour.*, **140** : 269 - 279.

Peters, A. R. 1985. Hormonal control of the bovine oestrous cycle. I. The natural cycle. *Br. Vet. Jour.*, **141** : 564 - 575.

Peters, A. R., Pimentel, M. G. & Lamming, G. E. 1985. Hormone responses to exogenous GnRH pulses in post-partum dairy cows. *J. Reprod. Fert.*, **75** : 557 - 568.

Peters, A. R. 1988. The effect of prostaglandin F2a on hormone concentrations in post-partum dairy cows. *11th Internat. Congr. Anim. Reprod. and A.I.*, 26 - 30 June, 1988, Dublin, Ireland. **4** : 453 - 455.

Peterson, A. J., Fairclough, R. J., Payne, E. & Smith, J. F. 1975. Hormonal changes around bovine luteolysis. *Prostaglandins*, **10** : 675 - 684.

Pope, G. S., Gupta, S. K. & Munro, T. B. 1968. Progesterone levels in the systemic plasma of pregnant, cycling and ovariectomized cows. *J. Reprod. Fert.*, **20** : 369 - 381.

Pope, G. S. 1982. Oestrogens and progesterone in plasma and milk of post-partum dairy cattle. In *Factors Influencing Fertility in the Post-partum Cow.* pp. 248 - 276. Eds. H. Karg and E. Schallenberger. Martinus Nijhoff, The Hague.

Prakash, B. S. & Madan, M. L. 1985. Concentration of plasma hormone in relation to placental retention in Karan Swiss cows during and after induction of parturition with dexamethasone and stilboestrol dipropionate. *Anim. Prod.*, **40** : 1 - 9.

Prybil, M. K. & Butler, W. R. 1978. The relationship between progesterone secretion and the initiation of ovulation in post-partum beef cows. *J. Anim. Sci.*, **47,** Supple. I : 393 (abstr.).

Radford, H. M., Nancarrow, C. D. & Mattner, P. E. 1978. Ovarian function in suckling and non suckling beef cows *post-partum. J. Reprod. Fert.*, **54** : 49 - 56.

Rahe, C. H., Owens, R. E., Fleeger, J. L., Newton, H. J. & Harms, P. G. 1980. Pattern of plasma luteinizing hormone in the cyclic cow : Dependence upon the period of the cycle. *Endocrinology*, **107** : 498 - 503.

Ramirez- Godinez, J. A., Lowe, K. A. & Jimenez- Castro. 1987. Ovarian follicular population in pregnant beef cows. *J. Anim. Sci.*, **65,** Suppl. I : 370.

Randel, R. D., Short, R. E. & Bellows, R. A. 1976. Suckling effect on LH and progesterone in beef cows. *J. Anim. Sci.*, **42** : 267- 272.

Randel, R. D. 1981. Effect of once daily suckling on post-partum interval and cow calf performance of first calf Brahman Hereford heifers. *J. Anim. Sci.*, **53** : 755 - 557.

Rawlings, N. C., Weir, L., Todd, B., Manns, J. & Hyland, J. H. 1980. Someendocrine changes associated with the post-partum period of the suckling beef cows. *J. Reprod. Fert.*, **60** : 301 - 308.

Revah, I. Zarco, L., Galina, C. S. & Serratos, G. 1988. Effect of prostaglandin F2α on the ovarian activity in two dairy herds in Mexico. *Proc. 11th Internat. Congr. Anim. Reprod. and A. I.*, Dublin, Ireland, 26 - 30 June, 1988. vol. **I** : 455 (Abstr.).

Rexford, C. E. & Casida, L. E. 1975. Ovarian follicular development in cows, sows and ewes in different stages of pregnancy as affected by number of corpus luteum in the same ovary. *J. Anim. Sci.*, **41** : 1090 - 1097.

Riesen, J. W., Saiduddin, S., Tyler, W. J. & Casida, L. E. 1968. Studies on the post-partum cow. *Wisconsin Agr. Station Res. bull.*, **270** : 1 54.

Riley, G. M., Peters, A. R. & Lamming, G. E. 1981. Induction of pulsatile LH release, FSH release and ovulation in post-partum acyclic beef cows by repeated small doses of GnRH. *J. Reprod. Fert.*, **63** : 559 - 565.

Riley, G. M. 1982. *Reproductive endocrinology of the post-parturient beef cow.* PhD Thesis. University of Nottingham.

Roberts, S. J. 1971. Infertility in the cow. In *Veterinary Obstetrics and Genital Diseases*. pp. 376 - 506,. Edwards Brothers, Inc. Ann- Arbor, Michigan.

Roberts, J. S., Barcikowski, B., Wilson, L., Skarnes, R. C. & McCracken, J. A. 1975. Hormonal and related factors affecting the release of prostaglandin F2α from the uterus. *J. Ster. Biochem.*, **6** : 1091 - 1095.

Robertson, H. A. 1972. Sequential changes in plasma progesterone in the cow during the oestrous cycle, pregnancy, at parturition and *post-partum. Can. J. Anim. Sci.*, **52** : 645 - 658.

Roche, J. F. 1974. Effect of short term progesterone treatment on oestrus response and fertility in heifers. *J. Reprod. Fert.*, **40** : 433 - 440.

Roche, J. F. 1976. comparison of pregnancy rate in heifers and suckler cows after progesterone or prostaglandin treatments. *Vet. Rec.*, **99 :** 184 - 186.

Roche, J. F., Prendiville, D. J. & Davis, W. D. 1977. Calving rate following fixed time insemination after a 12 day progesterone treatment in dairy cows, beef cows and heifers. *Vet. Rec.*, **101** : 417 - 419.

Roche. J. F. & Gosling, J. P. 1977. Control of oestrus and progesterone levels in heifers given intravaginal progesterone coils and injection of progesterone and oestrogen. *J. Anim. Sci.*, **44 :** 1026 - 1029.

Roche, J. F. & Ireland, J. J. 1981. Effect of exogenous progesterone on time of occurrence of the LH surge in heifers. *J. Anim. Sci.*, **52** :580 - 586.

Roine, K. 1973. The most frequent reproductive disorders and their seasonal variation in dairy cows. *Nordisk Vet. Med.*, **25** : 242 - 247.

Romanoff, E. B. 1966. Steroidogenesis in the perfused bovine ovary. *J. Reprod. Fert.*, Suppl. **I** : 89 - 99.

Rowson, L. E. A., Tervit, R. & Brand, A. 1972. The use of prostaglandins for synchronization of estrus in cattle. J. *Reprod. Fert.*, **29** : 145 - 154.

Roy, J. H. B., Catherine, M., Gillies, M., Perfitt, W. & Stobo, I. J. F. 1980. Effect of season of the year and phase of the moon on puberty and on the occurrency of oestrus and conception in dairy heifers reared on high planes of nutrition. *Anim. Prod.*, **31** : 13 - 26.

Saiduddin, S., Riesen, J. W., Tyler, W. J. & Casida, L. E. 1967. Some carry-over effects of pregnancy on post-partum ovarian function in the cow. J. *Dairy Sci.*, **50** : 1846 - 1847.

Saiduddin, S., Quevedo, M. A. & Foote, W. D. 1968. Response of beef cows to exogenous progesterone and estradiol at various stages *post-partum. J. Anim. Sci.*, **27** : 1015 - 1020.

Samuel, C. 1977. Physiologoical and pathological characteristics of the bovine uterus *post-partum* - a review. *Malaya. Vet Jour.*, **6** : 125 - 132.

Sarkar, D. K., Chiappa, S. A., Fink, G. & Sherwood, N. M. 1976. Gonadotropin releasing hormone surge in pre-oestrus rats. *Nature*. **264** : 461 - 463.

Sato, E., Ishibashi, T. & Iritani, A. 1982. Purification action sites of a follicle stimulating hormone inhibitor from bovine follicular fluid. *J. Anim. Sci.*, **54** : 873 - 877.

Sawyer, C. H. & Kawakami, M. 1961. Interactions between the central nervous system and hormones influencing ovulation. In *Control of Ovulation.* pp. 79 - 100. Ed. C. A. Villee. Oxford. Pergamon Press.

Scaramuzzi, R. J., Tilson. S. A., Thorneycroft, I. H. & Caldwell, B. V. 1971. Action of exogenous progesterone and estrogen on behavioral estrus and luteinizing hormone levels in the ovariectomized ewe. *Endocrinology,* **88** : 1184 - 1189.

Scaramuzzi, P. J. & Baird, D. T. 1977. Pulsatile release of luteinizing hormone and the secretion of ovarian steroids in sheep during anestrus. *Endocrinology*, **101** : 1801 - 1806.

Scaramuzzi, R. J., Turnbull, K. E. & Nancarrow, C. D. 1980. Growth of Graafian follicles in cows following luteolysis induced by the prostagland F2α analogue, cloprostenol. *Australian J. Biol. Sci.*, **33** : 63 - 69.

Schafer, E. A. 1913. On the effect of pituitary and corpus luteum extracts on the mammary gland in the human subject. *Queensland J. Exp. Physiol.*, **6** : 17 - 19.

Schallenberger, E., Oerterer, U. & Hutterer, G. 1982. Neuroendocrine regulation of post-partum function. In *Factors Influencing Fertility in the Post partum Cow. Current Topics in Veterinary Medicine and Animal Science,* Vol **20),** pp 123 - 147. Eds. H. Karg & E. Schallenberger. Martinus Nijhoff, The Hague.

Schallenberger, E. & Peterson, A. J. 1982. Effect of ovariectomy on tonic gonadotrophin secretion in cyclic and post-partum dairy cows. *J. Reprod. Fert.*, **64** : 47 - 52.

Schallenberger, E., Rampp, J. & Walters, D. L. 1983. Pulsatile progesterone secretion occurrs during mid gestation in the cow even though pulsatile LH secretion is abolished. *J. Anim. Sci.*, **57** ; Suppl. I : 371, Abstr. 577.

Schallenberger, E., Schams, D., Bullermann, B., & Walters, D. L. 1984. Pulsatile secretion of gonadotropins, ovarian oxytocin during prostaglandin induced regression of the corpus luteum in the cow. *J. Reprod. Fert.*, **71** : 493 - 501.

Schally, A. V., Arimura, A., Kastin, A. J., Matsuo, H., Baba, Y., Redding, T. W., Nair, R. M. G., Debeljuk, L. & White, W. F. 1971. Gonadotrophin releasing hormone : One polypeptide regulates secretion of luteinizing hormone and follicle stimulating hormone. *Science,* **173** : 1036 - 1038.

Schams, D. 1972. Prolactin levels in bovine blood influenced by milking manipulation, genital stimulation and oxytocin stimulation with specific consideration of the seasonal variations. *Acta Endocr., Copenhagen*, **71** : 684 - 696.

Scham, D., Hoffer, F., Schallenberger, E., Hartle, M. & Karg, H. 1974. Pattern of luteinizing (LH) and follicle stimulating hormone (FSH) in bovine blood plasma after injection of a synthetic gonadotropin - releasing hormone (GnRH). *Theriogenology*, **I** : 137 - 151.

Schams, D. & Schallenberger, E. 1976. Heterologous radioimmunoassay for bovine follicle stimulating hormone and its application during the oestrous cycle in cattle. *Acta Endocr., Copenhagen*, **81** : 461 - 473.

Schams, D., Schallenberger, E., Hoffman, B. & Karg, H. 1977. The oestrous cycle of the cow : hormonal parameters and time relationship concerning oestrus, ovulation and electrical resistance of the vaginal mucus. *Acta Endocr., Copenhagen*, **86** : 180 - 192.

Schams, D., Schallenberger, E., Menzer, C., Stangt, J., Zottmeier, K., Hoffman, B. & Karg, H. 1979. Profiles of LH, FSH and progesterone in post-partum dairy cows and their relationship to the commencement of cyclic functions. *Theriogenology*, **10** : 453 - 464.

Schams, D., Lahlov - Kassi, A. & Glatzel, P. 1982. Oxytocin concentration in the peripheral blood during the oestrous cycle and after ovariectomy in two breeds of sheep with high and low facundity. *J. Endocrinology*, **92** : 9 - 13.

Schams, D., 1983. Oxytocin determination by radioimmunoassay. III. Improvement to subpicagram sensitivity and application to blood levels in cyclic cattle. *Acta Endocr., Copenhagen*, **103** : 180 - 183.

Schams, D., Prokopp, S. & Barth, D. 1983a. The effect of active and passive immunization against oxytocin on ovarian cyclicity in ewes. *Acta Endocr., Copenhagen*, **103** : 337 - 344.

Schams, D., Walters, D. L., Schallenberger, E., Bullermann, B. & Karg, H. 1983b. Ovarian oxytocin in the cow. *Acta Endocr. Copenhagen*, Suppl. **253**, Abstr. 164.

Sharma, S. C. & Fitzpatrick, R. J. 1974. Effect of oestradiol-17β and oxytocin treatment on prostaglandin alpha release in the anoestrus ewe. *Prostaglandins*, **6** : 97 - 105.

Sheldrick, E. L., Mitchell, M. D. & Flint, A. P. F. 1980. Delayed luteal regression in ewes immunized against oxytocin. *J. Reprod. Fert.*, **59** : 37 - 42.

Sheldrick, E. L. & Flint, A. P. F. 1981. Circulating concentrations of oxytocin during the oestrous cycle and early pregnancy in sheep. *Prostaglandins*, **22** : 631 - 636.

Sheldrick, E. L. & Flint, A. P. F. 1983. Regression of the corpora lutea in sheep in response to cloprostenol is not affected by loss of luteal oxytocin after hysterectomy. *J. Reprod. Fert.*, **68** : 155 - 168.

Shil, R. N. & Debnath, S. C. 1985. In *An Introduction to the theory of statistics*. pp. 264 - 299. Published by Minati Shil and Amita Debnath. Bangladesh Agricultural University Campus, Mymensingh, Bangladesh.

Short, R. V. 1962. Steroid Concentrations in Normal Follicular Fluid and Ovarian Cyst Fluid from Cow. *J. Reprod. Fert.*, **4** : 27 - 45.

Short, R. E., Bellows, R. A., Moody, E. L. & Howland, B. E. 1972. Effet of suckling and mastectomy on bovine post-partum reproduction. *J. Anim. Sci.*, **34** : 70 - 74.

Short, R. E., Howland, B. E., Randel, R. D., Christensen, D. S. & Bellows, R. A., 1973. Induced LH release in spayed cows. *J. Anim. Sci.*, **37** : 551 - 557.

Short, R. E., Randel, R. D., Staigmiller, R. B. & Bellows, R. A. 1979. Factors affecting oestrogen induced LH release in the cow. *Biol. Reprod.*, **21** : 683 - 689.

Smith, J. W. & Legates, 1962. Relation of days open and days dry to lactation, milk and fat yields. *J. Dairy Sci.*, **45** : 1192 - 1198.

Smith, L. E. & Vincent, C. K. 1972. Effects of early weaning and exogenous hormone treatment on bovine post-partum reproduction. *J. Anim. Sci.*, **35** : 1228 - 1232.

Smith, V. G., Convey, E. M. & Edgerton, L. A. 1972. Bovine serum corticoid response to milking and exteroceptive stimuli. *J. Dairy Sci.*, **55** : 1170 - 1173.

Smith, M. F., Burrell, W. C., Shipp, L. D., Sportt, L. R., Songster, W. N. & Wiltbank, J. N. 1979. Hormone treatments and use of calf removal in post-partum beef cows. *J. Anim. Sci.*, **48** : 1285 - 1294.

Smith, M. F., Lishman, Lewis, G. S., Harms, P. G., Ellersieck, M. R., Inskeep, E. K., Wiltbank, J. N. & Amoss, M. S. 1983. Pituitary and ovarian responses to gonadotropin releasing hormone, calf removal and progesterone in anestrus Beef Cows. *J. Anim. Sci.*, **57** : 418 - 424.

Smith, R. D., Pomerantz, A. J., Beal, W. E., McCann, J. P., Pilbeam, T. E. & Hansel, W. 1984. Insemination of Holstein heifers at a pre-set time after estrous cycle synchronization using progesterone and prostaglandin. *J. Anim. Sci.*, **58** : 792 - 800.

Somerville, S. H., Lowman, B. G. & Des, D. W. 1979. The effect of plane of nutrition during lactation on the reproduction of beef cows. *Vet. Rec.*, **104** : 95 - 97.

Spalding, R. W., Everett, R. W. & Foote, R. H. 1975. Fertility in New York artificially inseminated Holstein herds in dairy herd improvement. *J. Dairy Sci.*, **58** : 718 - 723.

Spicer, L. J. & Echternkamp, S. E. 1986. Ovarian follicular growth, function and turnover in cattle : a review. *J. Anim. Sci.*, **62** : 428 - 451.

Terqui, M., Chupin, D., Gauthier, D., Perex, N., Pelot, J. & Mauleon, P. 1982. Influence of management and nutrition on post-partum endocrine function and ovarian activity in cows. In *Factors Influencing Fertility in the Post-partum Cow.* pp. 384 - 408. Eds. H. Karg and E. Schallenberger. Martinus Nijhoff, The Hague, Boston, London.

Tervit, H. R., Rowson, L. E. A. & Brand, A. 1973. Synchronization of oestrus in cattle using a PGF2α analogue (ICI 79939). *J. Reprod. Fert.*, **34** : 179 - 181.

Thanki, K. H. & Channing, C. P. 1978. effects of FSH and estradiol upon progesterone secretion by porcine granulosa cells in tissue culture. *Endocrinology,* **103** : 74 - 80.

Thatcher, W. W., Guilbault, I. A., Collier, R. J., Lewis, G. S., Drost, M. Knickerbocker, J., foster, D. B. & Wilcox, C. J. 1982. The impact of ante-partum physiology on post-partum performance in cows. *Current Topics in Veterinary Medicine and Animal Science,* **20** : 3 - 25. Eds. H. Karg and E. Schallenberger. Martinus Nijhoff, The Hague.

Thibault, C., Courot, M., Martinet, Lise., Mauleon, P., Du Mensil Du Buisson, F., Ortavant, R., Pelletier, J. & Singoret, J. P. 1966. Regulation of breeding season and estrous cycles by light and external stimuli in some mammals. *J. Anim. Sci.*, **25** ; Suppl. : 119 - 142.

Tribble, R. L., Sorenson, A. M., Woodward, T. L., Connor, J. S., Beverley, J. J., & Fleeger, J. L. 1973. Serum progestins and luteinizing hormone levels in non suckled primiparous heifers. *Nature,* London, **246** : 494 - 495.

Umezu, M., Masaki, J., Sasada, H. & Ohta, M. 1981. Mating behaviour of a bull and its relationship with serum LH levels in a group of oestrous cows. *J. Reprod. Fert.*, **63** : 467 - 470.

Van Dieten, S. W. J. 1964. Fertility after stillbrith in cattle. *Proc. 5th Internat. Congr. Anim. Reprod. and A. I.*, Torento, vol. V : 177. From *J. Reprod. Fert.*, (1978) **54** : 517.

Van Dieten, S. W. J. 1968. De invloed van enige bedrijfsomstandigheden op de vruchtbaarheid van rundvee. *Tijdschr. diergeneesk*, **93** : 1279 - 1288. From *J. Reprod. Fert.*, (1978) **54** : 517.

Van Rinsburg, S. W. J. & De Vos, W. H. 1962. Ovulatory Failure in *Bovines. Onderst. J. Vet. Research,* **29** : 55 - 58.

Verne La Voie, Han, D. K., Foster, D. B. & Moody, E. L. 1981. Suckling effect on estrus and blood plasma progesterone in post-partum cows. *J. Anim. Sci.*, **52** : 802 - 812..

Wagner, J. F., Veenhuizen, E. L., Gregory, R. P. & Tonkinson, L. V. 1968. Fertility in the beef heifer following treatment with 6-chloro-6-17 Acetoxy progesterone CAP. *J. Anim. Sci.*, **27** : 1627 - 1630.

Wagner, W. C. & Hansel, W. 1969. Reproductive physiology for the post partum cow. *J Reprod. Fert.*, **18** : 493 - 500.

Wagner, W. C., Saatman, R. & Hansel, W. 1969. Reproductive physiology of post-partum cow. II Pituitary and thyroid function. *J. Reprod. Fer.*, **18** : 501 - 508.

Wagner, W. C. & Oxenreider, S. L. 1971. Endocrine physiology following parturition. *J. Anim. Sci.*, **32**; Suppl. I : I.

Wagner, W. C. & Li, P. S. 1982. Influence of adrenal corticosteroids on post-partum pituitary and ovarian function. In *Factors Influencing Fertility in the Post-partum Cow.* pp. 197 - 219. Eds. H. Karg and E. Schallenberger. Martinus Nijhoff, The Hague.

Walpole, A. L. 1975. Characteristics of Prostaglandins. *Ann. Biol. Anim. Bioch. Biophys.*, **15** : 389 - 406.

Walters, D. L., Short, R. E., Convey, E. M., Staigmiller, R. B., Dunn, T. G. & Kaltenback, C. C. 1982a. Pituitary and ovarian function in post-partum beef cow. III. Induction of oestrus, ovulation and luteal function with intermittent small dose injection of GnRH. *Biol . Reprod.*, **26** : 655 - 662.

Walters, D. L., Smith, M. F., Harms, P. G. & Wiltbank, J. N. 1982b. Effect of steroids and/or 48 hour calf removal on serum LH concentrations in anoestrus beef cows. *Theriogenology,* **18** : 349 - 356.

Walters, D. L. & Schallenberger, E. 1984. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the preovulatory phase of the oestrous cycle in the cow. *J. Reprod. Fert.*, **71** : 503 - 512.

Walters, D. L., Schams, D. & Schallenberger, E. 1984. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the luteal phase of the oestrous cycle in the cow. *J. Reprod. Fert.*, **71** : 479 - 491.

Wakeling, A. E. & Green, L. R. 1981. Corpus luteum prostaglandin receptors and luteolysis. *Acta Veterinaria Scandinavica,* Suppl. **77** : 131 - 142.

Watters, R. J. & Ball, R. 1978. Commercial ovulation and fixed time aritificial insemination in cattle. *Vet. Rec.*, **103** : 585 - 587.

Wathes, D. C. & Swann, R. 1982. Is oxytocin an ovarian hormone? *Nature, London,* **297** : 225 - 227.

Wathes, D. C., Swann, R. W., Brikett, S. D., Porter, D. G. & Pickering, B. T. 1983a. Characterization of oxytocin, vasopressin and neurophysin from the bovine corpus luteum. *Endocrinology*, **113** : 693 - 698.

Wathes, D. C., Swann, R. W., Hull, M. G. R., Drife, J. O., Porter, D. G. & Pickering, B. T. 1983b. Gonadal sources of the posterior pituiray hormones. *Progress in Brain Res.*, **60** : 513 - 520.

Watkins, W. B., Moore, L. G., Flint, A. P. F. & Sheldrick, E. L. 1984. Secretion of neurophysins by the ovary in sheep. *Peptides*, **5** : 61 - 64.

Watson, E. D. 1987. concentration of LH and oestradiol-17β in plasma of the dairy cow during the post-partum acyclic period. *Anim. Prod.*, **45** : 1 - 8.

Webb, R., Lamming, G. E., Haynes, N. B., Hafs, H. D. & Manns, J. G. 1977. Response of cyclic and post-partum suckled cows to injections of synthetic LH-RH. *J. Reprod. Fert.*, **50** : 203 - 210.

Webb, R., Lamming, G. E., Haynes, N. B. & Foxcroft, G. R. 1980. Plasma progesterone and gonadotrophin concentrations and ovarian activity in post-partum dairy cows. *J. Reprod. Fert.*, **59** : 133 - 143.

Webb, R., Mitchell, M. D., Falconer, J. & Robinson, J. S. 1981. Temporal relationship between peripheral plasma concentrations of oxytocin, progesterone and 13, 14 Dihydro- 15 keto prostaglandin F2α during the oestrous cycle and early pregnancy in the ewe. *Prostaglandins,* **22** : 443 - 454.

Welch, R. A. S., Tucker, H. A., Oxender, W. D., Porteus, S. & Kirton, K. T., 1975. Plasma prostaglandins at parturition in cows. *J. Anim. Sci.*, **41** : 386.

Wettemann, R. P., Hafs, H. D., Edgerton, L. A. & Swanson, L. V. 1973. Hormone associated with fertile and non fertile insemination at synchronized and control oestrus. *J. Anim. Sci.*, **36** : 716 - 721.

Wettemann, R. P., Turman, E. J., Wyatt, R. D. & Totusek, R. 1976. Suckling Intensity and Reproduction in Range cows. *J. Anim. Sci.*, **42** : 267 - 272.

Wettemann, R. P., Turman, E. J., Wyatt, R. D. & Tortusek, R. 1978. Influence of suckling Intensity on Reproductive Performance of Range cows. *J. Anim. Sci.*, **47** : 342 - 346.

Wettemann, R. P. 1980. Postpartum endocrine function of cattle, sheep and swine. *J. Anim. Sci.*, **51** : Suppl. II : 2.

Whitemore, H. L., Tyler, W. J. & Casida, L. E. 1974. Effects of early post partum Breeding in Dairy cattle. *J. Anim. Sci.*, **38** : 339.

Wiltbank, J. N. & Cook, A. C. 1958. The comparative reproductive performance of nursed cows and milked cows. *J. Anim. Sci.*, **17** : 640 - 648.

Wiltbank, J. N., Rowden, W. W., Ingalls, J. E. & Zimmermann, D. R. 1964. Influence of post-partum energy level on reproductive performance of Hereford cows restricted to energy intake prior to calving. *J. Anim. Sci.*, **23** : 1049 - 1053.

Wiltbank, J. N. 1970. research needs in beef cattle reproduction. *J. Anim. Sci.*, **31** : 755 - 762.

Wiltbank, J. N., Trevino, R., Villalon, A. & Crenshow, D. 1984. Incidence of retained placenta following induction of parturition with corticoids of prostaglandins. *Theriogenology*, **21** : 427 - 434.

Williams, B. M., Shreeve, B. J., Heebert, C. N. & Swize, P. W. 1977. Bovine mycotic abortion : Some epidemiological aspects. *Vet. Rec.*, **100** : 382 - 385.

Williams, G. L. & Ray, D. E. 1980. Hormonal and reproductive profiles of early post-partum beef heifers after prolactin suppression or steroid induced luteal function. *J. Anim. Sci.*, **50** : 906 - 918.

Williamson, G. & Payne, W. J. A. 1978. In *An Introduction to Animal Husbandry in the Tropics.*, pp 155. 3rd edition, Longman, London .

Winter, A. J. 1973. Vibriosis : toward an understanding of immunity in genital infections. *Cornell Vet.*, **63** : 5 - 16.

Wishart, D. F. & Young, I. M. 1974. Artificial insemination of progestin (Sc- 21009) treated cattle at predetermined times. *Vet. Rec.*, **95** : 503 - 508.

Youdan, P. G. & King, J. O. L. 1977. The effects of body weight changes on fertility during the post-partum period in dairy cows. *Br. Vet. Jour.*, **133** : 635 - 641.

Young, I. M., Anderson, D. B. & Plenderleith, R. W. J. 1984. Increased conception rate in dairy cows after early post-partum administration of prostaglandin F2α THAM. *Vet. Rec.*, **115** : 42 - 431.

Young, I. M. & Anderson, D. B. 1986. Improved reproductive performance from dairy cows with Dinoprost Tromethamine soon after calving. *Theriogenology* , **26** : 199 - 208.

Youngqvist, R. S. 1986. Cystic follicular degeration in the cow. In *Current Therapy in Theriogenology,* pp. 243. Ed. D. A. Morrow, W. B. Saunders Company, Philadelphia, London, Toronto, Mexico-City, Rio De Janeiro, Sydney, Tokyo, Hong Kong.

Zerobin, K., Jochle, W. & Steingruber, Ch. 1973. Termination of pregnancy with prostaglandin E2 (PGE2) and F2α (PGF2α) in cattle. *Prostaglandins*, **4** : 891 - 901.