Uterine involution in Holstein-Friesian cows genetically selected for high or low mature body weights

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Abstract

The main objective in dairy herds is to produce milk as economically and efficiently as possible. To achieve maximum production, cows must calve regularly; hence the fertility of the herd affects the productivity of the farm dramatically. High fertility requires early uterine involution, early onset of oestrous cycles postpartum and optimal oestrus detection and conception rates (Pelssier 1976). The modern high producing, lactating dairy cow in North America is subfertile (Thatcher et al., 2006) as managed under current production systems. However, whether such subfertility occurs in high-producing cows under our pastoral system in New Zealand is not fully known. However, recent movement of genetic material across the globe, in the form of semen or frozen embryos might suggest that New Zealand herds could be drifting in a similar direction in subfertility.

The main objective of the present study was to investigate the effect of genetics on uterine involution in the context of a pastoral system by comparing two strains Holstein/Friesian cows that had been genetically selected for high (H) or low (L) mature body weight. Involution was studied through the following measurements: (i) cervical diameter, as assessed by measurement *per rectum*, (ii) plasma concentrations of the prostaglandin $F_{2\alpha}$ metabolite, 15-keto-13,14-dihydro-prostaglandin F2α (PGFM), (iii) urinary concentrations of the collagen breakdown product pyridinoline and (iv) bacteriology of the cervical canal.

From the results, it was concluded that, whilst H cows exhibited similar physical involution characteristics to those of L cows ($P>0.5$), they had higher levels of post-calving bacterial contamination ($P<0.05$). On Days 4, 7 and 10 post partum, the anaerobic bacterial load was significantly ($P<0.05$) greater in H than L cows. On Day 10, both strains peaked with mean

anaerobic bacterial counts of 23.4 x 10^5 cfu and 0.41 x 10^5 in H and L cows, respectively. Similarly, on Days 7 and 10, the total bacterial load (aerobic plus anaerobic) was also greater ($P<0.05$) in H than L cows. On Day 7 mean total bacterial counts were 2.27 x 10^6 cfu in H cows. Peak numbers of bacteria in H cows were attained on Day 10 (3.39 x 10^6 cfu). Values in L cows were maximal on Day 7 (1.18 x 10^6 cfu).

PGFM Concentrations in L cows were significantly ($P<0.05$) higher than in H cows on Days 17, 28 and 35. Although total pyridoline and deoxypyridoline /creatinine ratio concentrations differed between strains and times, the strain.time interaction term was not significant. However, on Day 11, values in L cows tended ($P=0.07$) to be higher than in H cows.

Simple correlations established that different parameters e.g cervical diameter; PGFM, PYD and bacterial contamination are highly correlated and moving simultaneously together and are not independent of each other. Accordingly, the relationships between actual and predicted intervals between calving and; first oestrus, first insemination and conception, were calculated based on principle component and partial regression analyses of parameters of uterine involution. Indices calculated from these parameters as predictors of reproductive outcomes, were significantly correlated with intervals between calving and first oestrus ($P<0.05$) and calving and first insemination ($P<0.01$), but not significant with conception rate ($P>0.5$).

Taken together, these data show that uterine involution is impaired in H compared to L strain cows, inasmuch as there is a greater degree of bacterial contamination and a more sluggish pattern of PFGM secretion in H cows. Collagen remodelling may also be

attenuated in H than L cows, although differences between strains only tended towards significance.

More work need to be carried out to further investigate the reason for these differences whether it is genetically based or higher production is negatively affecting the above mentioned traits at a cellular level.

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CHAPTER I

Literature Review

1.1 Introduction

The main objective in dairy herds is to produce milk as economically and efficiently as possible. To achieve maximum production cows need to calve each year; hence the fertility of the herd affects the productivity of the farm dramatically, in terms of increased costs: lost milk, increased number of services, extra animals culled, reduced number of replacement heifers produced per cow and other factors which lead to a reduced profit for the farmer (Nash et al., 1980, Etherington et al., 1984, Leslie et al., 1984, Foote and Riek 1999, Royal et al., 2000,).

To ensure that productivity is not impaired by reproductive performance, the interval from calving to conception should not be increased beyond 80-85 days (Macmillan et al., 1990). A significant component of the interval between calvings is dependent on the process of uterine involution, which occurs during the early post-partum period. During this remarkable process, the size of the uterus decreases in size by some 90% compared to its post-calving size. Any interference at any stage is considered critical in causing an increase in the calving interval as, until involution has occurred, the cow is unable to conceive again.

In the context of declining fertility in the modern-day high-yielding dairy cows and the aim to improve such a reproductive performance. There is a need to understand the biochemical and physiological principles controlling reproductive and lactational processes under our pastoral system. The objective of this study was to shed some light and examine part of the physiological period that appears to be limiting reproductive performance, mainly the uterine involution.

The main objective of the present study was to investigate the effect of genetics on uterine involution in the context of a pastoral system by comparing two strains Holstein/Friesian cows that had been genetically selected for high or low mature body weight. The heavy strain (high mature weight) cows contained approximately 75% US Holstein genetics, and the light strain (low mature weight) contained approximately 15% of such genetics (García, 1998, Laborde et al., 1998, Thiengtham et al., 2004). The study focused on comparing the two strains in the postpartum period by examining a number of parameters involved in uterine involution. Those parameters included cervical diameter, as assessed by measurement per rectum; plasma concentrations of the prostaglandin F2α metabolite, 15-keto-13,14-dihydro-prostaglandin F2α (PGFM); urinary concentrations of the collagen breakdown product pyridinoline and bacteriology of the cervical canal.

1.2 Uterine involution

After parturition uterus resembles a big flabby sac, which weighs about 9 kg and is about a metre long. It goes from this enormous size to a small structure weighing about 0.9 kg and with a diameter of less than 5 cm (Gier and Marion 1968). This remarkable process encompasses reorganization as well as a reduction in size. Remarkably, this phenomenon is still not well understood, especially how it goes through such rapid, yet well-organized, atrophy in such a very short time span.

Reduction in size of the uterus requires myometrial peristaltic contraction (Guillbault et al., 1984), loss of oedema fluid, reduction in the size of the smooth muscle fibres, as well as loss of tissue (Madej et al., 1984) and tissue repair (Guillbault et al., 1981). An important characteristic of involution is the elimination of by-products of the break-down of collagen, which is a major structural portion of the endometrium and myometrium that is broken down during the period of reduction of uterine muscle mass (Hrkens 1954, Mountford and Perez-Tamayo, 1961, Woessner 1962).

1.3 Management of New Zealand cows

In New Zealand, Ireland and parts of Australia, a pasture-based grazing system is the predominant method of managing dairy cows. Grass is the main feed of the dairy cow in this system, in order to maintain costs and, hence, maximise profit in an environment in which relatively low prices are achieved for dairy products. Well managed pasture can provide a highly digestible, highly palatable forage, with a high N content that can support milk production at 25 kg/day under optimal conditions (Journet and Demarquilly, 1979).

One of the main features of New Zealand's dairy system is that over 90% of the herds have a single seasonally-concentrated late-winter/early-spring calving pattern, such that about 50% of the cows within the herd calve within a period of 14-28 days. This marked degree of seasonality is the consequence of a management decision to concentrate calving into the late winter period, in order to synchronise the seasonal changes in pasture growth rate with the seasonal changes of herds' requirements (Holmes et al., 1987, Macmillan 1998). Reproductive performance is an important factor that determines production efficiency in this system, since cows have to conceive by 85 days postpartum to maintain a calving interval of 365 days (Macmillan et al., 1990).

The optimum timing of breeding depends on the relative contributions of stocking rate and per head performance. Stocking rate can best be maximised by synchronising the time of parturition with the onset of spring pasture growth. Earlier calving may lead to possible feed deficits in late winter and the need to reduce stocking rate, whereas later calving may allow additional stock to be carried, but can lead to pasture quality problems and reduced per head performance. Calving dairy cows prior to onset of spring growth ensures that the time of maximum feed requirements for lactation coincides with greatest feed supply, thereby ensuring maximum conversion of pasture into milk before restrictions of reduced feed quality occur in summer (McCall and Smith, 1998). Importantly, McDougall et al. (1995) reported a positive relationship between daily milk yields and conception rates to the first service for cows fed pasture alone or pasture supplemented with grass silage.

In New Zealand there are few autumn calving cows. Fulkerson et al. (1987) reported a lower conception rates and higher non-pregnant rates for the autumn-calving cows in a four-year comparison of autumn versus spring calving systems.

1.4 Post partum period

During involution, the weight and size of the uterus decreases gradually under the influence of vascular changes and regular contractions (Gier and Marion, 1968, Schirar and Martinate, 1982). Rectal palpation is the straightforward way of evaluation of the uterus and cervix after calving, allowing assessment to be made of uterine size, uterine tone, cervical size and position. Rectal examination was one of the earliest methods used by researchers to give an indication of uterine involution. Using this method, Rasbech (1950) suggested that the period required for the completion of uterine involution is 20 days. However, using the same method, both Marion et al. (1968), and Britt et al. (1974) suggested that the period required for complete uterine involution is 45 days.

In fact, it is clear that estimates of the average interval from calving to clinically completed involution vary widely; from 18-25 days (Rasbech, 1950), around 30 days (Morrow et al., 1966; Moller, 1970, Lindell, 1982) and up to 40–50 days (Britt et al., 1974). During the first four to ten days postpartum, uterine involution is slow (Morrow et al., 1966), although the weight of the uterus decreases by 70% from the time of calving to Day 10 postpartum (Riesen, 1968). Between Days 10 and 14 postpartum, the reduction in uterine size is rapid (Morrow et al., 1969), coinciding with a reduction in the diameter of the previously pregnant horn to about 7-12 cm. On Day 30 postpartum, the previously pregnant horn was only 100 g heavier, and its diameter 1 cm greater, than the non-pregnant horn (Wagner and Hansel, 1969). An ultrasonographic study of involution over the period between Days 8 and 43 (Okano and Tomizuka, 1987) gave similar results and also led to the conclusion that the process was completed by Day 40 postpartum.

Post-calving uterine and cervical diameter in Holstein heifers may be affected by genotype of the conceptus they bore during pregnancy (Guilbault et al., 1985). In multiparous cows the cervical diameter remained larger than that in the primiparous animals at 8 weeks postpartum (Miettinen, 1990).

Uterine infections negatively influence the rate of uterine and cervical involution. Diameters of uterine horns and cervix were greater in cows with uterine infections than in cows without infections (Del Vecchio et al., 1994). Where bacterial endometritis occurs, there is a rise in the concentrations of $PGF_{2\alpha}$ (as assessed through its stable metabolite, 13,14-dihydro, 15-keto $PGF_{2\alpha}$: PGFM) which is significantly correlated to the duration of uterine and cervical involution (Konigsson et al., 2002). A further important factor determining the rate of involution is the resumption of oestrous cycles after the post partum anoestrus period. The minimum interval from parturition to first ovulation has been reported to be around 14 days, with first oestrus occurring between 32.6 ± 18.6 days (Morrow et al., 1969). The first ovulation usually occurs in the ovary contralateral to preovulatory gravid horn (Morrow et al., 1969). The first ovulation is preceded by one or several follicular waves. It has been suggested that, even though $PGF_{2\alpha}$ initiates pre-partum luteolysis and onset of parturition, as well completing the process of luteolysis after calving, post-partum prostaglandin release may prevent onset of cyclicity (Kindahl et al., 1992). That is to say, as long as $PGF_{2\alpha}$ concentrations are elevated, ovulations do not occur. When PFGM concentrations return to baseline, ovulations can occur (Kindahl et al., 2004).

On the other hand, there is a physiological delay to involution in all ovulating animals during the time of oestrus, with a slight increase of the cervix and uterine size (Bekana et al., 1994). Nonetheless, GnRH given to cows on Days 13 to 14 postpartum that were characterised as undergoing slow involution of the reproductive system, but with no

other clinical problems, may assist in promoting a more rapid return to normal reproductive function (Foote and Riek, 1999).

1.5 Ovarian activity

Many authors have reviewed the relationship between postpartum ovarian activity and uterine involution. Some support the idea of the correlation between ovarian activity and uterine involution but others (e.g. Oxenreider, 1968) did not. Buch et al. (1955) suggested that there is a positive correlation between ovarian activity and uterine activity. It was also reported that completion of uterine involution is associated with the occurrence of first ovulation followed by a normal luteal phase length (Madej et al., 1984, Young et al., 1984).

1.6 Endocrinology of the post partum period

It has been suggested that the endocrine changes of the purperal period in the dairy cow are associated with the completion of uterine involution and affect the bacterial dyanmics of the uterus (Olson et al., 1984, Fredriksson, et al., 1985, Kehrli, et al., 1989).

Prior to calving, there are very high oestrogen concentrations; these, in conjunction with the high concentrations of progesterone, suppress LH and FSH release, which leads to suppressed follicular waves before calving (Ginther et al., 1996). Parturition is followed by early resumption of FSH release and follicular development (Spicer and Echternkamp, 1986). The dominant follicle of the first wave is smaller and the oestrogen concentrations are lower than in those of subsequent waves (Stagg et al., 1995). Medium sized follicles (5-10mm in diameter) can be detected by Day 5 postpartum (Callahan et al., 1971), and dominant follicles are first present in the ovary

at between Days 10 and 12 (Kamimura et al., 1994) or Days 15 and 27 (Savio 1990, Kesler et al., 1980) postpartum. The fate of this first dominant follicle is determined by subsequent LH pattern of secretion. An LH pulse frequency of 3.5 –4.5 /6 h (Beam and Butler, 1997) causes ovulation of the first dominant follicle, whilst pulse frequencies of 1.7 to 2.2 / 6 h result in its atresia.

1.6.1 Role of Hypothalamo-Pitutary Axis

The hypothalamus integrates information from internal positive and negative endocrine feedback and from external stimuli, such as photoperiod, the presence of male cues and nutrition, thereby regulating pituitary gonadotrophin secretion.

In recent years, the development of new techniques and the elucidation of neuroendocrine mechanisms have contributed to our broad understanding of hypothalamo-pituitary reproductive endocrine axis. The secretory activity of the anterior pituitary is regulated by several neuropeptides that are released from nerve endings in the hypothalamic median eminence and carried to the target cells by the hypophyseal portal system. The discovery, isolation and synthesis of these hypophysiotropic hypothalamic peptides have made possible an analysis of their mechanism of action in specific target cells at the cellular and molecular levels. Much information is now available on the characteristics of pituitary GnRH receptor (Reeves, 1980, Conn, 1981) and the modulation of its level and activity by sex steroids and GnRH itself (Catt et al., 1985, Herbison, 1995). At parturition, the fall in both progesterone and oestrogen concentrations at calving results in the removal of the negative feedback on the hypothalamo-pituitary axis and permits return of normal secretory patterns and signals the start of cyclicity (Peters and Lamming, 1986).

1.6.2 GnRH and LH secretion

*1.6.2.*1 During regular oestrous cycles

There is a precise relationship between GnRH frequency and LH pulse amplitude, which has been elucidated in animal models. When exogenous pulses of GnRH pulses were given it was found that increasing pulse frequency led to decreases in LH pulse amplitude (Wildt et al., 1981). Luteinizing hormone (LH) is secreted in a pulsatile manner during the oestrogen-dominated follicular phase of the cycle, when LH pulses are more frequent and of lower amplitude than during the progesterone-dominated luteal phase (Rahe et al., 1980). The amplitude of GnRH pulses in hypophseal portal blood decrease during the transition from the luteal to the follicular phase. The increase in plasma LH pulse frequency that occurs in the transition from the luteal to the follicular phase of the oestrous cycle is thought to reflect the removal of a negative feedback influence of progesterone (Dielemann et al., 1986) on the hypothalamic GnRH pulse generator (Chenault et al., 1975).

As the follicular phase of the oestrous cycle advances, the growth of ovarian follicles causes a progressive rise in plasma concentrations of oestradiol (Schallenberger et al., 1984), reaching peak values (~45 pmol/l) coinciding with the onset of oestrus (Dielemann, 1986). An early study showed that responsiveness of the pituitary to GnRH increased at the time of the LH surge (Convey et al., 1976). LH and FSH are released coincidentally at or near the onset of oestrus in cows (Akbar et al., 1974) such that the increase in oestradiol that occurs during the preovulatory period is clearly the stimulus that triggers the gonadotrophin surge. Thus, chemical or immunological inhibition of oestradiol secretion at proestrus inhibits occurrence of the LH surge in cattle (Martin et al., 1978), whereas exogenous oestradiol induces a preovulatory LH surge (Beck et al.,

1977). A decrease in progesterone is apparently also a requisite for the ability of oestradiol to cause the gonadotrophin surge (Hansel and Convey, 1983).

1.6.2.2 During the postpartum period

Pregnancy has an inhibitory effect on the sensitivity of the pituitary to hypothalamic GnRH (Schallenberger et al., 1978). The sensitivity of the hypophysis to GnRH and LH plasma concentrations gradually increases again after calving. It was found that after administration of exogenous GnRH, the release of LH is lower during the first 10 days postpartum than later during the postpartum period, with a progressive rise in both the pituitary content and the ability of GnRH to elicit its secretion over the first 2 to 3 weeks after calving (Kesler et al., 1977). The frequency of pulsatile releases of GnRH increases from 0–0.25 per hour to 0.25–1.25 per hour (Gauthier et al., 1982; Humphery et al., 1983) over the same period of time. Re-establishment of a pulsatile pattern of LH secretion is one of the key factors needed before pre-ovulatory follicular growth and ovulation can be restored after calving (Lamming et al, 1981, Schallenberger et al., 1982). Circulating concentrations of LH in the early postpartum period range between 0.2 and 1.0 µg/ml (Edgerton and Hafs, 1973); The resumption of the pattern of pulsatile LH in dairy cattle occurs between 13 and 19 days postpartum (Peters et al., 1981), in a pattern which is similar to that occurring at puberty or during the transition from anoestrus to the breeding season in seasonal breeders (Bronson, 1988, Thatcher and Hansen, 1993). The frequency of LH pulses increases before the preovulatory LH surge and before ovulation (Bergfeld et al., 1996, Imakawa et al., 1986, Sanchez et al., 1995). Hence, resumption of pulsatile LH secretion is a prerequisite for the cyclicity of dairy cows postpartum (Humphrey et al., 1983, Peters and Riley, 1982). Britt et al. (1974)

reported that a subcutaneous implant of GnRH on Day 14 postpartum caused, through its ability to stimulate the release of LH, ovulation in 100% of lactating Holstein cows.

1.6.3 GnRH and FSH secretion

1.6.3.1 During regular oestrous cycles

The regulation of FSH secretion is different from that of LH secretion. A surge of FSH occurs concurrently with the preovulatory LH surge and a second rise in concentrations takes place 24 hours later (Dobson, 1987). FSH concentrations are typically 66 ± 8 ng/ml during the follicular and 78 ± 8 ng/ml during the luteal phase (Akbar et al., 1974). There is a high degree of concordance between LH and FSH pulses in response to GnRH (Walters et al., 1984), although there are no strict relationship between GnRH pulses and plasma FSH concentrations. Indeed, whilst various reports have demonstrated that GnRH input is required to maintain FSH secretion, it is clear that it regulates FSH in a different way to that of LH. Thus, Martin et al. (1986) suggested that whereas LH secretion is the acute regulation of GnRH, the control of FSH secretion by GnRH occurs over longer time-frame. It seems more probable that GnRH promotes FSH synthesis rather than release, and the release from the gland occurs by passive means. Some support for this comes from rat models (Cull, 1986), although there is evidence that GnRH actively stimulates FSH secretion at the time of LH surge.

GnRH provides the stimulus for FSH secretion, while ovarian steroids and inhibin control its secretion through the negative feedback effect (McNeilly 1988). Indeed, as inhibin modulates FSH secretion at the pituitary (rather than hypothalamus) level, perhaps the different secretion patterns of the two gonadotrophins are a reflection of this. Inhibin certainly has a major impact upon FSH release, whilst a recent study has also shown that the number of large follicles on the day of oestrus and the number of

subsequent ovulations could be increased by the administration of inhibin antiserum and were correlated with persistence of increased FSH concentrations (Akagi et al., 1997).

In general, FSH concentrations are maximal at the time of the LH surge, as there is a FSH surge concident with the LH surge (Dobson et al., 1978). A second, smaller FSH surge follows ~24 h later. The latter is thought to be responsible for recruitment of follicles into the first follicular wave. Concentrations of FSH decline as follicular products, notably oestradiol and inhibin cause negative feedback on the hypothalamus and pituitary. As LH concentrations are unable to escape from the negative feedback effects of progesterone at that stage of the cycle, first wave follicles become atretic as FSH concentrations decline to a level below which they can no longer support steroidogenesis. With the disappearance of the first follicular wave, FSH concentrations are freed from the negative feedback effects of follicular secretions, so rise. Growth of a new wave of follicles is thereby initiated, and the cycle repeats itself. The emergence of each new wave is stimulated by a transient (1–2 day) increase in FSH both in cyclic (Adams et al., 1992, Sunderland et al., 1994) and anoestrous cows (Stagg et al., 1998). After the surge reaches a peak, concentrations of FSH decline over several days while the follicles grow from about 4.0–8.5mm (Adams et al., 1992, Ginther et al., 1997). On average, these follicles grow at a similar rate and then the group or cohort of growing follicles partitions into a single dominant and a group of subordinate follicles.

1.6.3.2 FSH during the postpartum period

Concentrations of FSH are generally low at the time of calving, but increase over the next few days, which is followed by the emergence of a follicular wave and subsequent selection of a dominant follicle (Beam and Butler, 1997, Crowe et al., 1998). However,

there is a lot of variation in the pattern and maximum concentrations of the FSH hormone that are present in the early post partum period (Manns et al., 1983, Peters et al., 1981). This is because after parturition the negative feedback on the hypothalamo-pituitary axis is removed by the removal of the foetal placenta and the demise of the CL; whereas anterior pituitary FSH content does not change (Moss et al., 1985, Nett et al., 1988) or may even decrease at that time (Labhsetwer et al., 1964, Saiduddin et al., 1967). On the other hand, it has been reported that the pituitary FSH content is higher 18 hours after calving than on Day 265 of gestation (Labhsetwar et al., 1964) and higher on Day 1 postpartum than on Days 10, 20 and 30 postpartum (Saiduddin et al., 1967). Yet again, others have reported that concentrations of FSH rise from Day 20 to 45 postpartum (Beam and Butler, 1997).

The initiation of FSH secretion occurs in the presence of relatively low concentrations of GnRH (Lamming et al., 1982), whereas pulsatile secretion of LH requires rather higher concentrations / pulse amplitudes. Schallenberger et al. (1985) suggested that there is a pulsatile pattern of FSH release, and that FSH pulses appear around Day 4 postpartum, with a characteristic fluctuation similar to those in cyclic cows (Crowe et al., 1998, Williams, et al., 1983). However, this work has not been replicated by other workers. It is clear, however, that FSH concentrations during postpartum period are not related to any other endocrine events (Webb et al., 1980).

The emergence of each new wave is stimulated by a transient (1–2 day) increase in FSH in anoestrous cows (Stagg et al., 1998). Perhaps the role of FSH during the early days postpartum is primarily permissive or facilitating (Peters and Lamming, 1984), because it induces the formation of LH receptors on granulosa cells under influence of oestrogen (Richards et al., 1976). Hence, Removal of the dominant follicle or antral cohort

follicles, leads to a transient FSH increase and new wave emergence within 1–2 days (Bergfelt et al., 1994).

1.6.4 Progesterone

During the first 100 days of pregnancy, the sole source of progesterone is the corpus luteum. From 100-120 days, until ~Day 275, this progesterone is augmented from non-luteal sources such as the placenta and/or adrenal. For the final stages of pregnancy, the source of progesterone is again the corpus luteum. In the period immediately prior to parturition, two distinct phases of progesterone concentrations can be seen. The first is due to the utilization of progesterone by the foeto-placental unit with approximately 20% reduction of the concentrations that are present 1-4 weeks before parturition. During the second phase (luteolysis) there is a more abrupt decrease during the last 2–3 days prior to parturition (Edqvist et al., 1978). Similar patterns can be seen when dexamethasone is used to induce parturition (Königsson et al., 2001). After the demise of the CL at parturition progesterone concentrations decrease sharply (Liptrap and Leslie, 1984) to values of less than 0.5 ng/ml (Edqvist, 1973). In a study by Janszen et al. (1990) it was found that the withdrawal of progesterone is a prerequisite for the uterus to proceed with contraction during parturition.

Undetectable circulating progesterone concentrations postpartum indicate absence of an ovulation or of a functional CL (Peters et al., 1984, Yavas et al., 1999). Curiously, however, there are some reports of increased plasma progesterone concentrations before the first post partum oestrus (Henricks et al., 1972, Robertson 1972, Van de Wiel et al., 1979, Carstairs et al., 1980), which have been assumed to be of ovarian origin. Similarly, it has also been reported that such a transient elevation of plasma progesterone occurs in dairy and in beef cows in the absence of any detectable corpus

luteum, prior to the resumption of a normal ovarian cycles (Donald et al., 1970, Robertson, 1972, Corah et al., 1974, Rawlings, 1980). The significance of this phenomenon is not understood. There are lower plasma progesterone concentrations during the first than the second postpartum luteal phase (Schams et al., 1978, Kindahl 1981).

During pregnancy, it seems that the progesterone production reflects the activity site of production and not the well being of the calf. In cases of foetal death relatively early in gestation (after inoculation with bovine virus diarrhoea virus: BVDV), no changes in progesterone concentrations were recorded until abortion had occurred and the animals resumed oestrous cycles (Carlsson et al., 1989). Progesterone injections significantly prolonged the time needed for the uterus to be fully involuted in both ovarectomised and non-ovarectomised cows (Marion et al., 1968).

Acute progesterone administration induces turnover of persistent follicles and may increase fertility when oestrus is synchronized with melengestrol acetate (Anderson and Day, 1994). Similarly, Treatment with progesterone inserts for 5 or 7 days with, $PGF_{2\alpha}$ at the time of insert removal and 1 mg estradiol benzoate 30 h later induced high degree of synchrony of oestrus and ovulation necessary for fixed-time insemination (Bridges et al., 1999).

1.6.5 Oestrogen

Oestrogen concentrations are high during late pregnancy (Dobson and Dean, 1974); reaching peak values 24 - 48 hours before calving (Stellflug et al., 1978). Immediately after the calving period, oestrogen concentrations decrease sharply to very low values (Sasseret et al., 1979, Wagner and Sachs, 1981). Subsequently, oestradiol-17β concentrations start increasing after Day 9 postpartum, as follicular development

resumes and as the developing dominant follicles start producing oestradiol-17β (Morrow et al., 1969, Chang et al., 1981). Increased oestrogen secretion from a dominant follicle prior to first ovulation induces a positive feedback on LH secretion triggering the LH surge leading to ovulation and resumption of ovulatory cycle (Kessler et al., 1977).

Oestrogen treatment has been found to hasten the rate of uterine involution (Roberts 1971). Not everyone agrees with Roberts, however. For example, Marion et al. (1968) reported that exogenous oestradiol-17β did not influence the rate of uterine involution. Likewise, (Wagner et al., 2001) also found that prophylactic administration of oestradiol cypionate during the early postparturient period in dairy cows had no beneficial effects on reproductive efficiency and a negative effect on calving to conception interval. Treatment with oestradiol initiated after Day 26 postpartum increased the proportion of cows that ovulated during the experimental period, but no differences were seen in the average days postpartum when cows were first determined to have ovulated (Garcia-Winder et al., 1988).

1.6.6 Prostaglandin $F_{2\alpha}$

The uterus is the main source of $PGF_{2\alpha}$ (Moor 1968, Lindell, 1981). Among the archidonic acid metabolites, $PGF_{2\alpha}$ has been the most extensively studied during the puerperal period in the cow (Edqvist et al., 1978, Eley et al., 1981, Lindell et al., 1982, Lindell et al., 1983, Lewis et al., 1984). Prior to parturition (24 - 48 h) a rapid increase in uterine production of $PGF_{2\alpha}$ occurs (Fairclough et al., 1975) associated with the rapidly changing oestrogen: progesterone ratio and development of oxytocin receptors that occurs at that time. Subsequently, normal uterine involution is associated with massive synthesis and release of $PGF_{2\alpha}$ (Guilbault et al., 1984), whilst abnormal uterine

involution is associated with prolonged duration of high $PGF_{2\alpha}$ concentrations (Lindell et al., 1982). There is an association between uterine size and plasma PGFM concentrations after calving, PGFM concentrations are slightly increased in animals from which uterine discharge occurs (Wilson, 1984).

Lindell and Kindahl (1983) reported that $PGF_{2\alpha}$ has a direct ecbolic effect upon the uterus, increasing uterine muscle tone and, hence, involution rate. There is a positive correlation between the production of $PGF_{2\alpha}$ and the time needed for normal uterine involution to be completed (Kindahl et al., 1984). However, some studies call into question whether high concentrations of $PGF_{2\alpha}$ are essential for the uterine involution (Guilbault et al., 1987). Indeed, others have suggested that the primary role of $PGF_{2\alpha}$ might be in stimulating the resumption of ovarian activity, and that its role in enhancing uterine involution is secondary (Guilbault et al., 1987, Johnson et al., 1992, Villeneuve 1988).

It has also been suggested that $PGF_{2\alpha}$ secretion during the puerperal period in the dairy cow will affect the bacterial dynamics in utero (Fredriksson et al., 1985). Certainly, abnormal or delayed uterine involution is associated with elevated concentrations of $PGF_{2\alpha}$ (Lindell et al., 1982, Kindahl et al., 1984). Bekana et al. (1996) indicated that a prolonged elevation of PGFM concentrations is due to an increased frequency of uterine infections. Raised concentrations of PGFM is considered to be an indication of inflammatory process of the uterus and the degree of endometrial damage and repair in cows with retained foetal membranes (Kindahl, 1984).

Treatment of postpartum cows with $PGF_{2\alpha}$ has a positive effect on uterine involution, either directly upon the uterus itself, or indirectly. For example, Gustaffson et al. (1976) reported a reduced incidence of pyometra or endometritis following $PGF_{2\alpha}$ administration. McClary et al. (1989) reported reduced open days in cows with retained

foetal membranes or metritis after $PGF_{2\alpha}$ treatment. Similarly, Bonnet et al. (1990) found that treating cows with the $PGF_{2\alpha}$ analogue cloprestenol on Day 25 postpartum reduced the incidence of vaginal discharge, decreased the diameter of the uterine horns, reduced inflammation and fibrosis in the endometrium and lessened the chance of *Arcanobacterium pyogenes* contamination in a biopsy taken on Day 40 postpartum. Nakao et al. (1997) also reported that administration of an exogenous $PGF_{2\alpha}$ improved the postpartum reproductive performance of cows with abnormal puerperium.

Early postpartum artificial suppression of PG synthesis did not alter uterine involution, but reduced ovarian activity. Thus, replacement therapy with infusions of $PGF_{2\alpha}$ enhanced the activity of the ovary ipsilateral to the previously gravid uterine horn, and was associated with greater progesterone release for the first 60 days post partum (Guilbaut et al., 1985). The time required for expulsion of the foetal membranes and for uterine involution was shorter in cows treated with $PGF_{2\alpha}$ than in untreated cows (Lindell and Kindahl, 1983, Momont and Seguin, 1985).

Despite the importance attached to $PGF_{2\alpha}$, some studies suggested that it is not utertonic in the puerperal cow. In an experiment by Thompson et al. (1987), plasma PGFM concentrations were significantly elevated for the first 5 days postpartum in cows with endometritis (ranging from 4.0 to 5.0 ng/ml) compared to normal controls (approximately 1.0 ng/ml). Beyond 5 days postpartum, plasma PGFM concentrations were not significantly different between the two groups, decreasing to approximately 0.4 ng/ml in both groups by Day 13. Time to uterine involution was not different between groups (less than 30 days). In other words, uterine infections in cows during the puerperium were associated with elevated circulating PGFM concentrations. Consequently, the authors concluded that $PGF_{2\alpha}$ is not uterotonic in the puerperal cow, nor is there a benefit from therapeutic use of $PGF_{2\alpha}$ to evacuate the uterus.

In another trial, PGFM concentrations were lower 2 weeks after calving in cows with placental retention, either complicated by endometritis or uncomplicated, compared with healthy animals. By the 4th week, values were lower in the healthy cows than in the others. It was concluded that these variations seemed to be related to different rates of repair of uterine damage and uterine involution (Watson, 1984). Moreover, it has been reported that inhibition of $PGF_{2\alpha}$ production with flunixin meglumin does not affect the rate of uterine involution (Thun et al., 1993).

Prostaglandins other than $PGF_{2\alpha}$ may affect the uterus. Slama et al. (1991) gave Holstein cows intrauterine infusions of 16,16-dimethyl PGE_2 ($dmPGE_2$) or saline alone (control) twice daily for 7 days, starting from 10 days after calving. Rectal palpation was performed twice weekly to assess utero-ovarian morphological changes. Blood samples were collected before, during and after the treatment for the assay of plasma PGFM, PGE and progesterone concentrations. Endometrial secretions were taken for bacterial culture. Treatment with $dmPGE_2$ affected many of the macroscopic characters relating to uterine involution, such as the tone, length and weight of the uterus, as well as increasing the incidence and severity of uterine infections with *A. pyogenes*. Moreover, intrauterine infusion of PGE_2 has been associated with an inhibition of lymphoblastic transformation, and with decreased concentration of immunoglobulins in uterine secretions. The persistence of uterine infections could therefore be due to the maintenance of relatively high concentrations of PGE_2. It was concluded that PGE_2 impedes uterine involution in the cow (Slama et al., 1991).

1.6.7 Prolactin

Serum prolactin concentrations increase dramatically around the time of parturition (Edgerton and Hafs, 1973), with higher concentrations present in cows that have been

selected for high milk production than in lower-producing animals (Eley et al., 1981). Suckling is associated with the release of prolactin (Akers et al., 1981) due to inguinal contact (Stevenson et al., 1994). Therefore, once the initial post partum period of prolactin secretion that is responsible for initiation of copious lactation is completed, prolactin release is not thereafter associated with milking in dairy cows (Carruthers et al., 1980). It is, however, associated with suckling, so concentrations remain high in sucked cows for a considerable period after calving (Smith et al., 1981).

Endogenous prolactin does not inhibit the resumption of ovarian function following parturition in the beef cows (Weiss et al., 1981). However, injection of prolactin (Forrest et al., 1980) or bromocryptine (Williams and Ray, 1980), an inhibitor of prolactin release, influences neither the variations of LH or FSH concentrations nor the resumption of cyclic activity after calving in cattle. Furthermore, there is no evidence of any effect of prolactin on the rate of uterine involution in cattle.

1.7 Bacteriology of the post partum uterus

Quantitative and qualitative nature of the uterine flora after calving is probably a reflection of the environment at and immediately after calving (Markusfeld, 1984). Different opinions regarding the bacterial species within the uterus have been expressed, but all seem to agree that cows acquire postpartum uterine infection (Johannes and Clarck 1968; Studar and Morrow, 1978, Bull and Butter, 1981). It has been reported that bacterial contamination is almost absent immediately after calving, but rapidly increases thereafter in the favourable conditions of the post partum uterine environment (Kudlac et al., 1970). Others, have suggested that bacterial contamination is frequently present during the first week postpartum and decreases rapidly during the period of the elimination of the lochia (Griffin et al., 1974, Olson et al., 1984). Proportions of animals

having significant bacterial contamination decrease from 93% on Day 15 to 9% on Days 46-60 (Elliot et al., 1968). The increase in bacterial load immediately after calving may cause clinical signs of uterine infection, with consequential adverse effects on the process of uterine involution by delaying uterine involution for the period over which uterine infection is present (Elliot et al., 1968).

There have been many attempts to identify the main organisms which cause post partum uterine infection. Many organisms have been isolated, including *A. pyogenes, Streptococus* spp, *Escherichia coli* and *Staphylococci*, alone or in combination (Griffin et al., 1974, Studer and Morrow, 1978). In most reports it was found that purulent discharge is mainly correlated to *A. pyogenes*, whilst the presence of this organism is also the main cause of delayed conception (Fivaz and Swanpoel, 1978).

Understanding of the significance of anaerobic bacteria has increased dramatically since researchers in both human and veterinary medicine have established the presence of obligate anaerobes in various infections, including those occurring during the involution of the postpartum uterus (Dow and Jones, 1987; Hofstad 1989). Thus, it is now considered that anaerobes, as well, play a significant role in the aetiology of uterine infections (Elliot et al., 1968, Studder and Morrow, 1978, Bekana 1996). For example, recent studies have shown that *A. pyogenes,* together with obligate Gram-negative pathogens (predominantly *Bacteriodes levii*, other *Bacteriodes* species and *Fusobacterium necrophorum*), probably affect the postpartum uterus synergistically (Ruder et al., 1981a, Fredriksson et al., 1985, Bekana et al., 1994b). For example, a combination of *A pyogenes*, *E. coli and Eubacteria* spp. causes purulent discharges (Joubert et al., 1982).

The significance of these anaerobes stems from their ability to produce the toxins, enzymes and other virulence determinants that are responsible for their pathogenicity. For example, *Fusobacterium* is known to produce an endotoxin that destroys leukocytes (Dow and Jones, 1987, Hofstad, 1989). Endotoxins also facilitate tissue invasion by *A. pyogenes*, which, in turn, results in the production of growth enhancement factors for *F. necrophorum*. Such endotoxins may possibly act as a growth stimulating-factor for the species of *Bacteroides*, whose unusually potent lipopolysaccharide molecules may account for some of the clinical signs associated with acute metritis (Price and McCallum, 1986, Markusfeld, 1993). It has also been reported that *A. pyogenes*, in combination with *B. levii* and *F. necrophorum*, are the main pathogens which are associated with the prolonged $PGF_{2\alpha}$ release that occurs in cows with retained foetal membranes (Bekana et al., 1996).

On the other hand, some bacterial contamination might exert a positive effect on the uterine involution. Eduvie et al. (1984) indicated that postpartum uterine bacterial contamination by nonspecific bacteria did not affect the process or the duration of uterine involution. The endometrium is the main tissue in the uterus responsible for the production of $PGF_{2\alpha}$ (Wlodaver et al., 1976) and inflammation or bacterial endotoxins act as potent agents in triggering the release of $PGF_{2\alpha}$ (Roberts et al., 1975). Hence, the finding of Elliot et al. (1968) that 93% of uteri are contaminated during the first 15 days after calving, suggests that the presence of these bacteria may contribute to the generation of the high concentrations of $PGF_{2\alpha}$ that are associated with the period of uterine involution. Moreover, Fredriksson (1984) found that endotoxins from Gram-negative bacteria are very important triggers of $PGF_{2\alpha}$ in cows. It was also reported that more prolonged elevation of $PGF_{2\alpha}$ is positively correlated to the length of uterine involution in cows with uterine infection (Fredriksson et al., 1985). Taken together,

these studies show that the relationship between $PGF_{2\alpha}$ release and the resumption of oestrous cycles is complicated, although it is clear that the presence of infection and the onset of oestrous cycle affect, and are affected by, patterns of $PGF_{2\alpha}$ secretion.

A recent clinical trial on the effect of uterine infection on uterine involution and ovarian activity in dairy cows (Mateus et al., 2002), confirmed that infection significantly retarded uterine involution assessed by the uterine body diameter and a score of intrauterine fluid volume. Even so, by the sixth week postpartum, cows with normal puerperium (controls) and cows that showed mild puerperal endometritis had similar uterine body diameter and intrauterine fluid volume, indicating spontaneous recovery within the postpartum voluntary waiting period. However, in cows with severe puerperal endometritis, although uterine body diameter had regressed to pregravid size, intrauterine fluid volume remained significantly higher than in control cows and those with mild endometritis. This was taken to indicate that chronic endometritis had become established and that there was consequential impairment of the process of involution. Cows with mild or severe endometritis had a significantly higher prevalence and persistence of pathogenic bacteria (*E. coli, A. pyogenes*, Gram negative anaerobes) than controls. The presence of *A. pyogenes* was associated with Gram negative anaerobes in 74% of isolations.

In the same study (Mateus et al., 2002), ovarian activity, as measured by ultrasound scanning of the ovaries, and plasma progesterone (P4) concentrations, was more likely to be abnormal (in terms of prolonged anoestrus, prolonged luteal phases and/or ovarian cysts) in cows with severe endometritis than in controls. Likewise, the presence of a purulent vaginal discharge significantly decreased the proportion of animals with a CL in the ovary contralateral to the previously gravid uterine horn but not in the ipsilateral ovary (Sheldon et al., 2000). Perhaps the significance of this finding is to be understood

in terms of recent studies which have showed that endocrine and bacterial factors may modulate synthesis of leukotriene B_4; an autocoid that has been postulated to have a significant role in delaying uterine involution in cattle (Salama et al., 1993). Whatever the mechanism, however, taken together, these findings provide strong evidence for the mpact of bacterial contamination upon fertility.

1.8 Factors influencing uterine involution

There are several factors that can exert an influence on the process of involution of the bovine uterus, such as calving problems, infection, genetics, milk production, parity, and season.

1.8.1 Abnormalities of parturition

Various studies have revealed that there is strong relationship between abnormalities of parturition, such as dystocia, prolapse of the uterus, the presence of a dead calf or abortion, and prolonged intervals from parturition to the completion of involution of the uterus (Buch et al., 1955, Oltenacu et al., 1983, Morton, 2000). Delayed uterine involution after dystocia is further associated with abnormal ovarian cyclicity and prolonged intervals to the next pregnancy (Dobson et al., 2001). Likewise, Zain et al. (1995) found that puerperal disorders had the most severe effect on the uterine involution followed by parity and season of calving. This is probably due to residual inflammation of the uterus as a result of such infections. At least, Marion et al. (1968) noted that, following retention of foetal membranes, leucocytic infiltration in the uterine wall occurred in a large number of animals, and that such cells remained present until 70 to 90 days postpartum (i.e. for 20 to 30 days beyond the normal period of histological involution of the uterus), suggesting that this may indeed be the case.

However, Marion et al. (1968) also concluded that it was probably an inhibition of uterine motility which was the primary factor which caused delayed involution in affected animals.

The hormonal profiles associated with retained foetal membranes have been widely studied. In general, these show that cows with retained foetal membranes have higher prepartum concentrations of progesterone, and lower concentrations of oestradiol-17β and prolactin than normal cows. The abnormal ratio of progesterone to oestradiol-17β is taken to indicate an asynchrony of hormonal mechanism(s) that normally synchronises birth and release of the foetal membranes (Chew et al., 1977). The extent to which these abnormal hormonal profiles influence the subsequent process of involution is not clearly understood, however, as it is difficult to isolate the effects of the hormonal environment *per se* from those of the physical presence, and consequential infection, of the retained membranes within the uterus. Moreover, there is increasingly strong evidence (Miyoshi et al., 2002) that abnormalities of the immune system contribute to the pathogenesis of retention of the foetal membranes; again, there is little knowledge about how this may impinge upon the ability of the uterus to undergo involution.

Interestingly, in a study of animals with dystocia and/or retained foetal membranes (Bekana et al., 1996) showed that clinically affected cows showed a massive release of $PGF_{2\alpha}$ after parturition (as indicated by a rise of plasma PGFM concentrations), which was significantly higher than in cows with a normal puerperium. However, the duration of elevated plasma PGFM concentrations in the cows with abnormal puerperium was shorter than in normal cows. The most important result of this study was that normal cows showing a relatively longer duration of elevated plasma PGFM concentrations needed a shorter period ($P<0.01$) for postpartum uterine involution to take place than

did the cows with a shorter period of PGFM elevation, but that no such relationship was observed in cows with abnormal puerperium. The administration of the exogenous $PGF_{2\alpha}$ is an effective means improving the postpartum reproductive performance of cows with abnormal puerperium (Kindahl et al. 1992, Nakao et al., 1997, Kindahl et al. 2004) and, in field trials, administration of a long-acting $PGF_{2\alpha}$ analogue, fenprostalene, on Days 7 to 10 or 14 to 28 postpartum facilitated uterine involution and improved reproductive performance.

Some other studies have looked at the effect of treatments of postparturient problems upon uterine involution. In a study by Konigsson et al. (2001), the effect of oxytetracycline and flunixin upon retained foetal membranes was examined, with the finding that early treatment of retained foetal membranes with oxytetracycline did not shorten the duration of either uterine involution or uterine infection, but it did slow down the detachment process of the retained membranes. On the other hand, after placental shedding had occurred, oxytetracycline treatment did shorten the duration of uterine infection, but otherwise did not affect the process of involution.

1.8.2 Infection

The severity, duration and nature of the post partum infection cause variable effects upon the involution process. Most cows develop a degree of uterine contamination in the post partum period. The prevalence of postpartum uterine infections during the first two weeks in cows with normal calving has been variously estimated to range from about 50% (Fredriksson et al., 1985) to 85-90% (Hussain et al., 1990). The risk of uterine infection in cows with abnormal calving, for example, retained foetal membranes (Bekana et al., 1994b) or dystocia (Markusfeld, 1993) effectively increases to 100%. The significance of this infection is that any degree of uterine infection

prolongs the time taken for the uterus to become fully involuted (Bostedet, 1984, Steffan et al., 1984). Reduced bodily defense mechanisms, alone or in conjugation with uterine bacterial infection, may retard epithelial regeneration and uterine involution (Elliot et al., 1968). Lynn et al. (1966), in an attempt to isolate the effect of bacterial infection on the uterine involution, gave cows an intrauterine inoculation of bacteria, with the result there was a strong correlation between the presence of bacterial infection and the interval to completion of involution. Many other authors have reported that uterine involution is delayed by chronic infections, such as metritis or pyometra, in the postpartum period (Sandals et al., 1979, Dohoo, 1983, Fonseca et al., 1983, Fredriksson et al., 1985).

1.8.3 Milk production

There is growing concern in many parts of the world that fertility of dairy cattle is reducing as milk yields increase (Dobson et al., 2001). The onset and attainment of peak of milk production in the postpartum period is considered a stress factor that affects reproductive efficiency in the dairy cow (Collick et al., 1989, Butler et al., 1981). Furthermore, it has been shown that the risk of reproductive problems, such as post partum metritis and ovarian cysts, increases with increasing milk production (Grohn et al., 1994, Butler and Smith, 1989, Saloniemi et al., 1986).

It is generally recognised that beef cattle have more rapid uterine involution than dairy cattle (Wagner and Hansel, 1969) and that the rate of uterine involution is faster in suckled than non-suckled cows (Riesen, 1968). Hence, the effect of suckling can not be ignored. It has also been found that morphological involution of the uterus was inhibited when suckling was restricted, but that surface epithelium height was influenced by ovarian function regardless of uterine involution (Izaike et al., 1989). Likewise, Okano

and Fukuhara (1980) compared the rate of involution in suckled and early weaned cows and concluded that early weaning delayed uterine involution.

Izaike et al. (1989), in their study of Japanese black cows, examined the effects of various factors on the duration of uterine involution, including, parity, milk yield and suckling frequency. The relative importance of parity, milk yield and suckling was 75.2, 21.7 and 3.1% respectively. According to this model, increased parity and milk yield lengthen the duration of uterine involution, while increased suckling frequency shortens it.

1.8.4 Breed differences

It has been demonstrated that uterine involution is faster in beef than dairy cows (Wagner and Hansel, 1969). In a study to determine the effect of genotype of conceptus on maternal responses, using sires of different breeds it was found that the genotype of the conceptus influenced various responses of the dam such as gestation length, frequency of dystocia, postpartum milk production, and postpartum function (uterine and ovarian responses) (Thatcher et al., 1980).

In a study to determine the interval between calving and first ovulation in a high yielding cows it was found that this interval was 27.9 days in high yielding cows compared to 18.0 days in other cows (Saiduddin et al., 1967). Two groups of 10 Holstein cows were chosen by pairs from a 20-year genetic selection project that used either breed average or breed high sires chosen only for predicted differences in milk production. Milk production (305-day mature equivalent) was 10,814 kg and 6,912 kg for the high and average groups of cows. Days to first visual oestrus and number of ovulations before first visual oestrus were greater for the high versus the average group

(66 vs. 43 d and 1.6 vs. 0.7 ovulations). No differences were significant between groups for the interval from parturition to uterine involution or for days to first ovulation. Energy balance was less for the high group during Weeks 1, 2, 10, and 11. Plasma glucose concentration was lowest during Week 2 for both groups, and non-esterified fatty acids and β-hydroxybutyrate were greatest for both groups during Week 1 and 2. Liver glycogen content was lower at Day 15 postpartum for the high group, and liver triglyceride content was greater on Day 30 for the high group. The data for reproductive functions support the concept that high milk production is antagonistic to expression of oestrous behaviour, but not to reactivation of ovarian function (Harrison et al., 1990).

In a study of the postpartum period in Swedish Red and White Breed, the Swedish Friesian Breed, crosses between these two breeds, and the Swedish Jersey Breed, the first, second and third ovulations had significant differences between parities and breeds. However, complete uterine involution was achieved at between 41 and 50 days post partum, independently of breed (Garcia and Larsson, 1982). Likewise, the period of involution was similar in Finnish Ayrshire and Finnish Friesian cows, although it was affected by diet and parity (Heinonen et al., 1988).

In a recent study, cows of high and low genetic merit were fed one of two isonitrogenous (19.3% crude protein), isoenergetic (11.3 MJME) diets that differed in concentration of rumen-degradable protein. Cows with higher dry matter intake were more likely to show signs of oestrus at first ovulation and to become pregnant by Day 150 of lactation. Cows of high genetic merit were less likely to show signs of oestrus at first ovulation. It was concluded that that continued selection for increased production of milk and a more negative nutrient balance during early lactation may reduce the reproductive performance particularly for cows fed high concentrations of rumen degradable protein. On the other hand, there was no direct correlation between genetic

merit and fertility but cows of higher genetic merit would be at a greater risk of prolonged interval from calving to first ovulation due to nutritional effects rather than due genetic merit or breed per se. (Westwood et al., 2002).

1.8.5 Parity

Uterine involution is delayed by rising parity (Buch et al., 1955 Morrow et al., 1966, Marion et al., 1968 Izaike, et al., 1988), such that most studies have found that younger cows have faster involution than older cows (Rasbech, 1950). However, Bastidas et al. (1984) reported that the rate of uterine involution was faster in pluriparous than primiparous cows. There is also a positive relation between the number of parities and the size of the involuted uterus (Tennant and Pedicord, 1958).The effect of parity on the cervical and uterine involution was studied in Finnish dairy cows (Miettinen, 1990). The cows were examined by rectal palpation during the first 8 weeks postpartum, so that the diameters of the cervix and uterine horns in the parous uterine horn could be compared with the non-parous horn. Significant differences between the parous and non-parous uterine horns were obtained until 21 days post partum. Thereafter, involution still continued, although the diameters for the horns were not equal until 5 weeks after parturition. A decline of cervical diameter continued until 30 days post partum. Parity had no significant effect on the rate of uterine or cervical involution; however, in multiparous cows the cervical diameter still remained larger than that in the primiparous animals at 8 weeks postpartum.

1.8.6 Season

It has been reported that uterine involution is faster in summer and spring than in winter (Buch et al., 1955, Marion and Gier, 1968, Shrestha, 1978). In another study (Bastidas

et al., 1984), the rate of uterine involution was faster in cows calving during the rainy season than the dry season. In New Zealand, where the feeding system depends mainly on grazing, feed supply is affected by season, which in turn affects reproductive performance. Macrae et al. (1985) suggested that reproductive performance might be suppressed due to seasonal differences in maintaining adequate herbage allowances (e.g. during the winter, when pasture growth rates are low) or to limitations upon its nutritive value (e.g. autumn pastures).

It has been suggested that genuine seasonal effects related to light and photoperiod may occur (Peters and Riley, 1982, Hansen and Hauser, 1984). In cattle, sexual behaviour is stimulated by a long photoperiod, and is inhibited by a short photoperiod. Exogenous melatonin prolongs postpartum interval in spring calving cows (Sharpe et al., 1986). Moreover, most wild species of bovidae are seasonal breeders and, although cattle have been selected against seasonality (Yavas and Walton, 2000), beef cows remain relatively highly sensitive to photoperiod. For example, autumn calving beef cows exposed to long days during summer resume cycling earlier than spring-calving cows (Montgomery et al., 1985). Spring-calving cows display greater number of follicles and larger follicles than autumn-calving cows (Lammoglia et al., 1996).

In a study using path analysis to determine the interrelationships between ambient temperature, age at calving, postpartum reproductive events and reproductive performance in dairy cows (Etherington et al., 1985), winter months were associated with an increase in the incidence of retained foetal membranes, the percentage of cows with abnormal postpartum vaginal discharges and a delay in uterine involution. In addition, cows that calved during the winter had longer intervals to first oestrus, first service and conception, than did cows calving during the summer. Cows calving during the warmest months were seen in oestrus an average of 24 days sooner, received first

service 42 days sooner and conceived 27 days sooner than cows calving during the coldest months of the year.

On the other hand, some workers have found no significant correlation between season and the rate of uterine involution (Morrow et al., 1966, Johanns et al., 1967, Morrow et al., 1969a).

1.8.7 Nutrition

The fertility of the lactating dairy cow is compromised by her energy status during the postpartum period. This in turn will be influenced by her genetic merit, yield and general nutrition. Specific components of the diet can alter reproductive processes, although the mechanisms whereby dietary manipulation alters reproductive performance are generally not precisely understood.

Many attempts have been made to study the effects of different dietary constituents, such as vitamins, minerals and proteins, on the uterine involution process. Wathes et al. (1998) showed that the level of undegradable protein and the composition of dietary fats are closely related to fertility. Ruder et al. (1981) suggested that uterine involution can be delayed by restricting the amount of crude protein intake, probably as a result of increasing susceptibility of the uterus to infection. Similarly, Miettinen (1990) showed that low dietary energy levels in the early puerperium caused a delay in uterine involution and in the onset of ovarian activity. It has also been reported that reducing the severity of negative energy balance in the postpartum period is associated with early resumption of postpartum reproductive function (Butler and Smith, 1989). On the other hand, Houghton et al. (1990) found that low energy intakes before calving had a more deleterious effect on subsequent postpartum reproduction than did low energy post-

calving, whilst Menge et al. (1962) found that within same strain, as the weight of the cow at parturition increased, so did the time needed for the uterus to be fully involuted. Miettinen (1990), looked at the effect of two types of feeding on the involution of the genital tract and on the fertility. Cows were divided into a hay-urea group and a silage group and were examined by rectal palpation three times a week for 8 weeks postpartum. The cows in the silage group had a significantly longer time in uterine involution, a lower fertility rate at first insemination and a longer interval from calving to conception than those in the hay-urea group.

In a further study, Holstein cows were given diets containing moderate or high levels of crude protein from 30 days before calving until Day 120 of lactation. Increasing dietary crude protein did not influence the number of days to complete uterine involution (Santos et al., 1999). However, the incidence of uterine infections at Day 40 postpartum was greater for cows fed a diet that was deficient in protein than in cows fed a diet that was adequate in protein (Ruder et al., 1981).

Dietary supplement of a combination of vitamin D and calcium enhanced uterine involution better than when each of these supplements is given alone (Ward et al., 1971). In another study, where cows were given vitamins A, D_3, E by intramuscular injection weekly, for two weeks before and after calving, the time to complete uterine involution was significantly reduced (El-Naggar, 1977). Supplementation with β-carotene did not affect uterine involution; neither did it improve the fertility of Holstein cows or milk yield (Akordor et al., 1986). In another experiment, cows were given a single injection of a selenium salt 3 weeks before calving, some were given additional vitamin E in the feed during the dry period, and some were given both vitamin E and selenium. In cows that developed endometritis the rate of uterine involution was slightly faster in those that had received selenium (Harrison et al., 1986). In another study, 18

pregnant Friesian heifers were treated with intraruminal selenium pellets, oral α-tocopherol or selenium + α-tocopherol. Six control animals received no treatment. However, all treatments failed to accelerate uterine involution, hasten resumption of postpartum ovarian activity or reduce the incidence of clinical postpartum abnormalities (Wichtel et al., 1996).

1.9 Collagen remodelling

During pregnancy the uterus has to increase in size to accommodate the developing foetus. At parturition the cow's uterus is ten times bigger than normal. After calving, during the postpartum period, the uterus decreases in size to approach its normal size, during which 10 kg of uterus formed during pregnancy are resorbed within a few days. Post-partum involution of the bovine uterus can be monitored biochemically by measuring the total urinary excretion of hydroxyproline and the collagen cross-link, pyridinoline. In a study by Harkness and Harkness (1954), on a rat model, it was found that changes in uterine weight during and after pregnancy coincided with deposition and resorption of collagen. Cattle uterine tissue contains 0.13 ± 0.04 residues of pyridinoline per mole of collagen and negligible (less than 0.005 residues/mole) deoxypyridinoline (Kaidi et al., 1991). The pyridinoline/creatinine ratio was found to be a clearer and more reliable indicator of uterine resorption than the hydroxyproline/creatinine ratio (Kaidi et al., 1991). Kaidi et al. (1991) also found that maximal excretion of pyridinoline occurred at Day 6 and returned to baseline values by 2 - 3 weeks after parturition. An electron microscope study during the period of peak resorption revealed macrophage-like cells apparently engulfing or containing collagenous material, indicating phagocytic removal of the connective tissue (Kaidi et al., 1991)

1.9.1 Structure of collagen

Collagen is the most abundant protein in all mammals. It has supportive and structural functions in different body structures such as bone and connective tissue. Collagens are polymers composed of repetitive groups of monomers; these monomers are called tropocollagen. These collagen molecules consist of three polypeptide chains, termed α chains, each of which consists of a sequence of three amino acids, with every third residue being glycine. Hence, the structure of collagen is GL-X-Y, where X and Y are frequently proline and hydroxyproline respectively (Bailey and Etherington, 1980).

Cross-links of mature Type I collagen are the pyridinium cross-links, pyridinoline (PYD) and deoxypyridinoline (DPD). PYD and DPD are formed by the enzymatic action of lysyl oxidase on the amino acid lysine; they are released into the circulation during the resorption process. Pyridinium cross-links are excreted unmetabolized in urine and are unaffected by diet, making them suitable for monitoring resorption of the bovine uterus during Post-partum involution by measuring the total urinary excretion of PYD and DPD.

1.9.2 Collagen breakdown

In previously mentioned study by Harkness and Harkness (1954) on rat uterus, it was found that there was a total reduction in collagen content of the uterine horn from an average of 29.2 mg immediately after delivery to 3.1 mg 5 days postpartum.

Collagen breakdown may occur in one of two main ways (Woessner, 1980). The first involves cleavage of collagen fibrils due to the action of specific collagenase enzymes which cause fibre destruction. The second pathway requires the digestion of collagen fibrils by phagocytic cells. It is probable that partial degradation by collagenase is a

prequisite before such phagocytosis by macrophages. Phagocytosed collagen fibrils will be digested inside the lysosomal system of the cell at acid pH by cathepsin B (Burleigh, 1977) and collagenolytic cathepsin (cathepsin N) (Etherington, 1977).

1.9.3 Detection of collagen

Collagens exhibit a characteristic amino acid profile due to the high content of glycine and proline or hydroxyproline. The presence of the amino acid hydroxyproline in collagen (14% in fibrous collagens) is an identifying feature, because this amino acid occurs only in a few other proteins: the other proteins with a significant hydroxyproline content being elastin (1.6%) and to a lesser extent the serum complement protein C1q (Robins, 1982). Hence, assessing hydroxyproline content is quite specific for collagen as can be used as an identification tag to quantify the amount of collagenous protein (Etherington and Sims, 1981).

1.9.4 Urinary collagen degradation products

The major routes of excretion of collagen metabolites are via lung and kidneys. About 75% of the peptides released by the degradation of collagen are hydrolysed to their constituent amino acids and catabolised to carbon dioxide to be excreted by the lung. The remaining 25% of the peptides released are excreted in the urine. Hydroxyproline appears to be mostly excreted in the urine, presumably, because of its resistance to peptidase activity (Weiss and Klein, 1969).

Pyridinium (PYD) cross-links are used as trace catabolites for the process of collagen degradation. Pyridinium and its closely related derivative, 3-hydroxypyridinium (together termed pyridinoline) are considered to be accurate indicators of collagen

degradation (Fujimoto et al., 1978). Deoxypyridinoline (DPD), which is derived from a lysyl residue and appears to be specifically derived from bone collagen (Robins and Duncan, 1987). These compounds have intrinsic fluorescence, which has enabled the development of selective and sensitive chromatographic assays for their quantification in urine samples (Black and Duncan, 1987).

Antibody technology was employed to produce a monoclonal antibody that demonstrates specificity for pyridinium cross-links and this lead to the development of enzyme immunoassay kits for the quantification of pyridinium cross-links. The specificity of the monoclonal antibody used in the assay kits allows for simple, convenient, reproducible and direct quantification of PYD and DPD (Gomez et al., 1996).

1.10 Summary

In seasonal calving dairy herds, once a year calving is generally accepted as optimal to derive maximum economic benefit. This requires early uterine involution, early onset of oestrous cycles postpartum and optimal oestrous detection and conception rates (Pelssier 1976). The modern high producing, lactating dairy cow in North America is subfertile (Thatcher et al., 2006) as managed under current production systems. However, whether such subfertility occurs in high-producing cows under our pastoral system in New Zealand is not fully known. In recent years the movement of genetic material across the globe has enabled the world to gain access to such materials either in the form of semen or frozen embryos. This might suggest that New Zealand herds might be drifting in a similar direction in subfertility. Reasons for the decline in fertility are multifactorial and not associated only with an increase in milk production (Thatcher et al., 2006). Epidemiological studies indicate that other factors such as reproductive

diseases (i.e., retained placenta, metritis and ovarian cysts) were relatively more important than milk yield on influencing reproductive performance. However, it is clear that lactation, as a physiological process, is associated with a lower reproductive rate compared to that of non-lactating heifers (Lucy, 2001). Herds experiencing low pregnancy rates may be encountering an array of possible cow and management inefficiencies such as reduced health and compromised immune function, poor oestrus expression and/or detection, extended anovulatory periods, low conception rates and increased early- and late-embryonic mortality. Impediments to optimal reproductive performance are exacerbated under stressful environmental conditions, such as underfeeding in the New Zealand pastoral system.

In the context of declining fertility in the modern-day high-yielding dairy cows and the aim to improve such a reproductive performance. There is a need to understand the biochemical and physiological principles controlling reproductive and lactational processes under our pastoral system. The objective of this study was to shed some light and examine part of the physiological period that appears to be limiting reproductive performance, mainly the uterine involution.

Slow recovery of reproductive competence during the post-partum period is a major limitation to the success of subsequent reproductive management programs that are implemented for inseminations beginning at the start of the voluntary waiting period. It was proposed initially that early and frequent occurrences of oestrus following calving were associated with increased reproductive performance due to a more optimal restoration of the uterine environment. A certain sequential occurrence of events from the time of parturition to the time of first service is needed to be optimal. Therefore, a uterus to ovarian pathway, i.e., completion of physical involution and clearance of the uterus should occur in a short period of time post-partum without the occurrence of

ovulations. After completion of uterine involution, sequential ovulations would be a goal towards normal fertility.

During the first 4 weeks post-partum, the immune system of the cow is challenged severely (Goff and Horst, 1997). Most cows develop a mild non-pathological bacterial contamination during the early puerperal phase of the post-partum period (Lewis, 1997), and uterine fluids (lochia) are usually voided during the first 2 weeks post-partum. The uterine immune system seems to up-regulate during the periparturient period and remains so until progesterone from the first post-partum ovulation down-regulates the uterine immune system. Onset of endometritis has been associated with an increase in progesterone concentrations (Seals et al., 2000). Cows that developed endometritis had prolonged PGFM concentrations during the early post-partum period (i.e. 0 – 14 days post-partum), perhaps altering the local immunity function that ultimately compromises the ability of the uterus to involute in due time.

The main objective of the present study is to investigate the effect of genetics on uterine involution in the context of a pastoral system by comparing two strains Holstein/Friesian cows that had been genetically selected for high or low mature body weight. The heavy strain (high mature weight) cows contained approximately 75% US Holstein genetics, and the light strain (low mature weight) contained approximately 15% of such genetics (García, 1998, Laborde et al., 1998, Thiengtham et al., 2004). The study focused on comparing the two strains in the postpartum period by examining some parameters involved in uterine involution.

CHAPTER II

Materials and Methods

2.1 Introduction

The process of post partum uterine involution was studied in two strains of Holstein-Friesian cattle, which had been genetically selected for heavy (H) or light (L) mature bodyweight (García-Muñiz et al., 1998, Laborde et al., 1998b). Involution was studied through the following measurements

- Cervical diameter, as assessed by measurement *per rectum*

  The diameter of the previously pregnant uterine horn commonly is regarded as the main indicator of involution of the genital tract (Madej et al., 1984, Young et al., 1984). However, for many cows the uterus cannot be palpated easily during the first three weeks postpartum so diameter of the cervix is a more useful indicator of the involution process (Fonseca et al., 1983). In general, cervical measurement has been considered by many authors (Tennenat and Peddicord, 1968, Miller et al., 1980, Oltenacu et al., 1983) as an indicator for the reproductive performance of the individual cow. It has been reported that the involution of the uterus closely paralleled involution of the cervix in different breeds (Fonseca et al., 1983).

- Plasma concentrations of the prostaglandin F2α metabolite, 15-keto-13,14-dihydro-prostaglandin F2α (PGFM).

  A delayed pattern of PGFM elevation has been significantly correlated to duration of uterine infection and cervical involution. A correlation between prostaglandin release, the final cervical involution and the end of infection was found. This suggests a link between uterine endocrinology, bacteriology and involution in

cows with bacterial infection with aerobic as well as anaerobic bacteria (Konigsson et al., 2002).

- Urinary concentrations of the collagen breakdown product pyridinoline.

  Post-partum involution of the bovine uterus can be monitored bio-chemically by measuring the total urinary excretion of hydroxyproline and the collagen cross-link, pyridinoline (Kaidi et al., 1991).

- Bacteriology of the cervical canal

  Postpartum bacterial endometritis is a very common cause of infertility in cows (Hussein and Daniel, 1992). It is the primary cause of economic losses in the cattle industry because it prolongs the time to first oestrus; delays in uterine involution increases the number of services per conception and prolongs the calving interval (Erb et al., 1985).

2.2 Animals and Management

Estimates of the genetic composition of the cows (based upon pedigree information), were that the H strain animals contained an average of 75% North American Holstein, and the L strain 15% (García, 1998; Laborde et al., 1998; Thiengtham et al., 2004). Twelve animals of each strain were selected for the study. Groups were balanced by calving date and age. One of the animals selected for the H group was culled at the start of the experiment and was replaced by a parity/calving date matched cow. All of the animals had had uncomplicated calvings and had established normal lactations. No animals calving for the first time or of parity ≥6 were used.

2.3 Grazing and pasture management

The cows were managed in one herd and were rotationally grazed on mixed ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture, receiving generous allowances of pasture that were designed to permit the cows to eat to appetite (Laborde et al., 1998b). The cows received supplementary maize silage during early lactation when pasture quality or quantity was inadequate (August to September). Average stocking rate was 2.6 animals/Ha. Average pasture covers, as assessed by rising plate meter, were 2199 kgDM/Ha and 2019 kgDM/Ha in August and September, respectively.

2.4 General reproductive management

Both groups of cows were spring-calving, with a planned start of calving of 29th July and a mean calving date (whole herd) of 16th August. Breeding started in late October (24th October), with cows inseminated following observed oestrus over the first 4 weeks of the mating period. All insemination dates, as well as the dates of all heats that were observed between the time of calving and the start of the mating period were recorded. At the end of the AI mating period (25th November), bulls were run with the cows for a further 4 weeks (bulls removed 23rd December). All cows were pregnancy tested by palpation *per rectum* in January.

Milk production data, bodyweights and body condition scores were recorded fortnightly over the trial period.

2.5 Sampling regimen

Samples were collected twice weekly for the first 4 weeks after calving and weekly thereafter for a two further weeks. Actual sampling days were:

Day 3 or 4, Day 7, Day 10 or 11, Day 14, Day 17or 18, Day 21, Day 24 or 25, Days 28, 35 and 42). On each occasion, the following samples were collected

1) Blood samples (lithium heparin anticoagulant) were collected by caudal venepuncture for measurement of concentrations of 13,14-dihydro,15-keto prostaglandin $F_{2\alpha}$ (PGFM). Plasma was separated by centrifugation at 1000 g for 10 min, after which it was stored at –20°C until assayed.

2) Mid-stream urine samples were collected (during spontaneous or induced urination, tickling the perinial area), for the measurement of the collagen break-down product pyridinoline. Samples were stored at -70°C until assayed.

3) Cervical swabs were collected for bacteriology. Firstly, the tail was tied to one side; the perennial area and the vulva were thoroughly washed with antiseptic soap and well rinsed with clean warm water. The perennial area was dried using disposable clean paper towel. An assistant then parted the vulval lips and a disposable guarded swab (Kalayjian Industries, Inc, California) composed of an outer sheath with the rod inside it, with a sterile cotton tip was inserted into the cervix. The tip of the guarded swab was guided through the genital tract by the fingertip through the rectal wall, once the tip was in the cervix the rod was pushed out of the sheath and rotated until the swab was well soaked. The guarded swab was then retracted again into the outer protective tube and withdrawn from the genital tract. Using sterile scissors the cervical swabs were cut off into a transport medium (Thyoglocolate broth, Fort Richard Laboratories, Auckland, New Zealand) then transported to the bacteriology laboratory within a couple of hours for culturing.

4) The cervix and uterus were palpated *per rectum*. Uterine tone and the diameter of the cervix were estimated by hand, using abattoir specimens as a means of calibrating the estimates that were made.

2.6 Radioimmunoassay of PGFM concentrations

Concentrations of PGFM were determined using the radioimmunoassay described by Keelan and Mitchell (1998). Using duplicate 100 µl aliquots, the limit of sensitivity was 6.5 pg/ml and the intra-assay coefficient of variation was 15%. All samples were measured in a single assay.

Samples were thawed to room temperature before assay and subsequently mixed well. Once the samples had been thawed samples were gently inverted 4-5 times to mix them.

Table 2.1 Reagents (µl) used in PGFM radioimmunoassay

Tube	Buffer	Standards and samples	PG-free plasma	1st AB*	Tracer	Charcoal	Scintillant
TC					100		
NSB	100		100		100	750	3.5 ml
B0	100		100	100	100	750	3.5 ml
Standards		100	100	100	100	750	3.5 ml
Unknown samples	100	100		100	100	750	3.5 ml

*) 1^{st} *Antibody*

Day1

1. Standards were prepared and 100 µl aliquots were dispensed into polypropylene test tubes (12 x 75 mm) in triplicate. Buffer (100 µl) was added to the BO and

NSB tubes in triplicate. PGFM-free plasma (100 µl) was added to all standard tubes.

2. Aliquots of samples (100 µl) were dispensed into duplicate into labelled tubes and 100 µl of buffer wa added to all samples tubes.
3. To all tubes except the NSBs 100 µl of antiserum (1: 20,000) was added. Buffer (100 µl) of was e added to the NSBs. All tubes were vortex and incubate for 4 hours at 4°C.
4. To all, 100 µl of radiolabelled PGFM ([5,6,8,9,11,12,14-^{3}H] PGFM; Amersham International PLC) (~5000 cm per tube: tracer) were added and vortex mixed. Tubes were covered and incubated at 4°C overnight. Also tracer was added to 2 scintillation vials to prepare the TCs.

Day 2

1. Centrifuge was turned on and let to cool to 4°C.
2. The working strength of charcoal was prepared and kept stirring on ice.
3. Keeping the assay tubes in an ice bath, 750 µl of charcoal was added to each tube. The rack was then shaken to mix the tubes.
4. The tubes were placed into the pre-cooled centrifuge and after 15 minutes they were centrifuged for 15 minutes at 3000 rpm at 4°C.
5. The tubes were removed then placed in ice bath.
6. The supernatant was decanted into scintillation vials and 3.5 ml of scintillant was added (Starscint, Medtec products Ltd., Auckland).
7. Vials were counted in β-counter (5 min per tube).

2.7 Microbiology

After vortex mixing, 100 µl aliquots of transport medium were placed in 900 µl of Schaedler's broth and a series of ten fold serial dilutions were made out of each sample (10^{-1} through to10^{-4}), vortexing after each serial dilution. From each dilution, 100 µl aliquots were plated on solid media blood-agar plate for aerobic incubation and on a Fastidious Anaerobe Agar-plate for anaerobic incubation (Fort Richard Laboratories) with the first plate being inoculated directly from the sample, followed by the rest of the serial dilutions (taking precautions to avoid any contamination). Using a sterile bent glass rod the inoculum was rapidly spread over the entire agar surface until dried. The aerobic plates were incubated for 72 hours at 35°C. Anaerobic plates were placed in an anaerobic jar together with an anaerobic, envelope (AnaeroGen, Oxoid Limited, UK) and an indicator (monitoring if there was any oxygen leak into the anaerobic jar). These plates were incubated without disturbing for 5 days at 35°C. Counting was undertaken for the bacterial colonies on the incubated plates. Number of colonies was calculated as Colony Forming Units (CFU) in 1 ml of sample. Species of bacteria that were present were not identified.

2.8 Measurement of collagen breakdown products (pyridoline and deoxypyridoline)

Type 1 collagen is given structural rigidity by cross-links of pyridinium, pyridoline and deoxypyridoline. Measurements of urinary concentrations of pyridoline and deoxypyridoline are widely used in medical practice as markers for collagen resorption, as they are excreted unchanged in the urine. Their use as markers of uterine collagen breakdown in cattle has also previously been demonstrated (Kaidi et al., 1991).

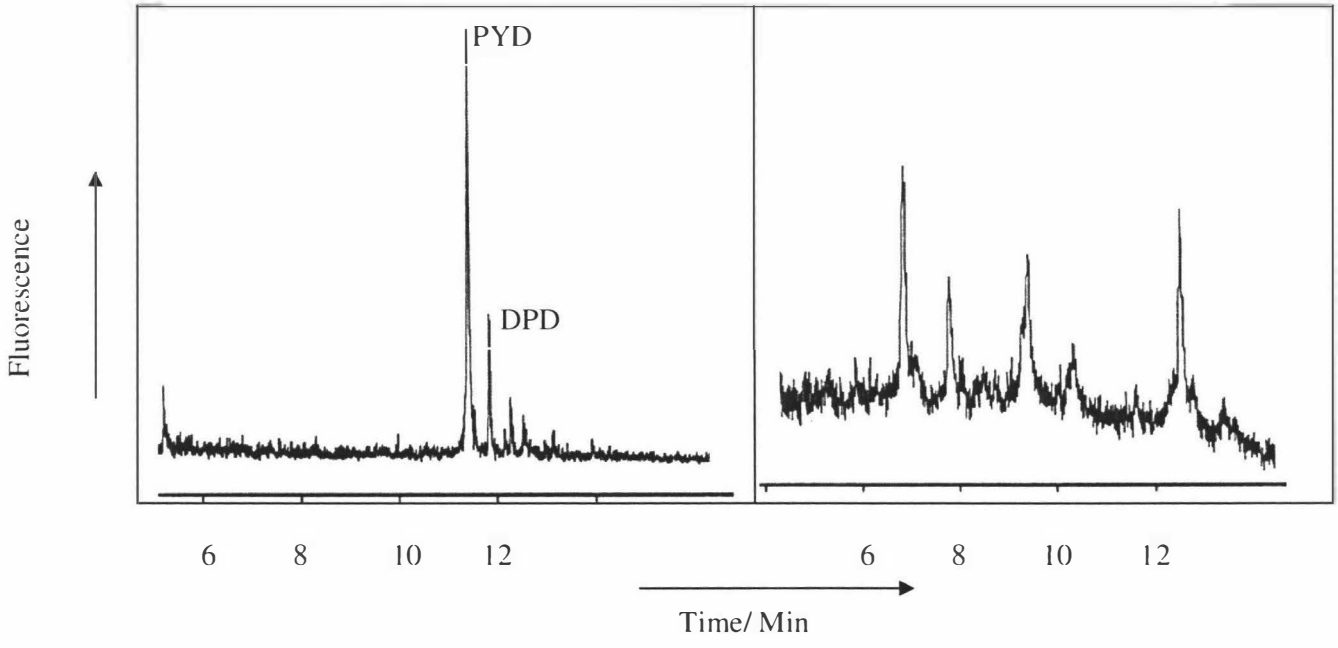
2.8.1 HPLC

Initially, it was decided to use an HPLC method to measure pyridoline and deoxypyridoline concentrations. Samples were thawed at room temperature before assay and subsequently mixed well. A standard was used in every run to of the assay to assure the validity of the results. On the first day, 3 ml of each sample was transferred into glass tubes which were tolerant to high temperature and high pressure. One ml of I M HCl (BDH Laboratory Supplies, UK) was added to all tubes. Tubes were then sealed and boiled at a temperature of 116°C for 20 hours. On the second day, standards were prepared as aliquots of 400 µl of 100 mM sodium formate adjusted to pH 5 with HFBA (Sigma Chemical Company, Poole, UK) into clear screw-cap vials (12 x 32 mm Alltech, Columbia, USA) containing 40 µl of 1 M heptaflurobutyric acid. Standards were diluted at 10, 15 and 20 µl respectively. Hydrolysates were transferred to centrifuge tubes and centrifuged at 13,000 g for 10 min to remove any precipitant prior to extraction.

Hydrloysates were then diluted 10 fold with 25 mM disodium tetraborate, pH 9.3, giving a final pH of 6. Separation cartridges using strata screen c cartidges 150 mg/ 3ml (Screen-C, Phenomenex NZ, Auckland) were conditioned with 2 ml of 5 mM sodium formate, pH 2.75, prior to application of 2.5 ml dilute hydrloysate at 0.5 ml/min. This was followed by washing with 6 ml of 5 mM sodium formate, pH 2.75 containing 40 % methanol. Then was followed by another washing step by 2 ml of 5 mM sodium formate, pH 2.75 at 3 ml/min and drying by sucking air through the cartridges for 30 seconds. Cross-links were eluted at 0.5 ml/min with 400 µl of 100 mM sodium formate adjusted to pH 5 with HFBA. Aliquots of 200 µl were injected (Sample injector model 231-401, Gilson, France) into the HPLC column

(150mm x 4.6-mm (i.d.) Techsphere 5 µm ODS column, HPLC Technologies Ltd, Hertfordshire, UK). Detection was by fluorescence detector (Shimadzu Fluorescence detector RF-10A XL) (Ex. 295nm, Em. 400 nm).After considerable effort to stabilise this assay, it did not prove possible to do so to a satisfactory standard for a large-scale trial in which a few hundred samples had to be processed and validated. The inter-assay as well as the intra-assay validation failed many times to be within acceptable limits. Normal results of the standards showed elution of a double peak (PYD/DPD) at a retention time of approximately 11.8 and 12 minutes respectively (Figure 2.1a). But such peaks unfortunately also eluted at varying interval ranging between 8 to 20 minutes, in addition to some irrelevant peaks spiking, and a lot of noise at base levels probably due to some impurities at such time periods making accurate interpretation of the results, although a standard was run prior to every run, quite a tedious and a complicated job rendering it to be unreliable method (Figure 2.1b).

Figure 2.1 Differences in elution time between (a) standard, and an example of a (b) sample where PYD/DPD eluted at a different time with multiple peaks before and after the expected elution time of PYD/DPD.



2.8.2 ELISA

Hence, concentrations of pyridoline and deoxypyridoline were measured using a commercially available ELISA kit (Metra 8010 PYD; Quidel Corpopration, San Diego), as described by Gomez et al. (1996). The assay does not differentiate between pyridoline and deoxypyridoline, so results are for total pyridoline and deoxypyridoline contents. The kit was validated for use with bovine urine by demonstrating parallelism between kit standards and samples of urine to which serial dilutions of pyridoline and deoxypyridoline (Pyd/Dpd HPLC Calibrator; Quidel Corporation, SanDiego) had been added. This procedure did not require prior processing of urine samples. Samples were only filtered prior to use in the procedure. And all steps were performed in one day. Procedure was as follows:

1. Samples, standards and controls were diluted, 1:10 with assay buffer (50µl sample + 450 µl assay buffer).
2. Diluted standard, control or sample (50µl) was added to each well of the coated strips. This step was completed within 30 min.
3. Enzyme conjugate was prepared within 2 h of use. Each required vial of enzyme conjugate was reconstituted with 7 ml cold (2-8°C) assay buffer. Reconstituted enzyme conjugate was stored at 2-8°C until use.
4. Reconstituted enzyme conjugate (100 µl) was added to each well. Strips were covered with tape cover provided. Incubated for 3 h (±10 min) at 2-8°C. This incubation was carried out in the dark.
5. Working substrate solution was prepared within 1 h of use. One substrate tablet was put into each required bottle of substrate buffer at 20-28°C. 30-60 minutes

were allowed for tablet(s) to dissolve. Bottle was shaken vigorously to completely mix.

6. A washing step required the amount of 1X wash buffer to be prepared by diluting 10X wash buffer 1:10 with deionized water. At least 250 µl of 1X wash buffer were added to each well and manually inverted/emptied. Process was repeated two more times for a total of three washes. Strips were vigorously blotted dry on paper towels after the last wash. While strips are inverted, bottom of strips were carefully wiped with a lint-free paper towel to ensure that the bottom of the strips were clean.
7. Working substrate solution (150 µl) was added to each well, then incubated for 60 minutes (±5 minutes) at 20-28 ^{o}C.
8. Stop solution (100 µl) was added to each well. Stop solution was added in the same pattern and time intervals as the substrate solution addition.
9. The optical density was read at 405 nm. It was ensured that no large bubbles were present in the wells and that the bottom of the strips were clean. Strips were read within 15 min of stop solution addition.
10. In order to adjust for urinary concentration, ELISA results were corrected for urinary creatinine concentrations. These were measured by New Zealand Veterinary Pathology laboratory (NZVP), Massey University, Palmerston north, New Zealand, using standard colorimetric methods. Values for the ELISA PYD results were divided by the corresponding urinary creatinine value for normalization. PYD and DPD are expressed as molar ratios with the creatinine concentration.

The limit of sensitivity was 7.5 nmol/ml and the intra-assay coefficient of variation was 9% while the inter-assay coefficient of variation was 11%.

2.9 Analysis of data

Data were subjected to repeated measures analyses of variance, with respect to strain and time (i.e. days after calving) using a repeated-measures model, in which individual cows were nested within strain (Genstat 5, Lawes Agricultural Trust, Rothamsted, UK). Data for total pyridoline and deoxypyridoline concentrations and for PGFM concentrations were normalised by $\log_e$ transformation prior to analysis. Microbiology data approximated to a Poisson distribution, so were subjected to restricted maximum likelihood procedures after logit transformation, using a linear mixed model (ASREML, VSN International Ltd., Hemel Hempstead, UK; Gilmour *et al.*, 1998) that included effects of strain, time (Days 3 to 13 only) and in which cows were nested within strain. Simple correlations established that different parameters e.g cervical diameter; PGFM, PYD and bacterial contamination are highly correlated, moving simultaneously together and, hence, are not independent of each other. Consequently data were subjected to linear and non-linear regression analysis.

In order to determine whether reproductive outcomes were affected by the various indicators of uterine involution (size, PGFM concentrations, pyridinoline concentrations, microbiological contamination), data from both groups (H and L) were combined. Because data sets were highly correlated (e.g PGFM on Day 10 and microbiology on Day 10), principal component analysis was undertaken. From the results of this analysis, eigenvectors 1 and 2 (which accounted for ~75% of the variance) were used to construct a partial regression analysis of the identified variables upon reproductive outcome. Partial regression analysis was undertaken using $\log_e$ transformed microbiology data, but other variables were untransformed. A stepwise regression analysis was used, which iterated the components of the regression until no further reduction in residual variance (residual mean squares) was

achieved. Analysis of variance was then performed on the entire model and its individual components, to determine whether a significant proportion of the variance in the reproductive outcomes data could be explained by the predictive model.

2.10 Ethics Approval

All procedures using experimental animals were approved by the Massey University Animal Ethics Committee.

CHAPTER III

Results

Basic reproductive outcomes data from the cows in the two strains are given in Table 3.1

Table 3.1 Reproductive outcomes

Cow	Calving date	Calving to 1st heat (d)	Calving to 1st AI (d)	Calving to Conception (d)	Services per conception
Heavy Strain					
25	22-Aug	73	73	DNC	*
61	10-Aug	86	86	DNC	*
65	16-Aug	30	71	119	4
66	15-Aug	50	74	74	1
67	15-Aug	56	74	74	1
76	11-Aug	29	90	90	1
91	18-Aug	68	68	88	2
99	19-Aug	24	67	67	1
104	3-Aug	61	98	DNC	*
127	10-Aug	30	91	110	2
160	5-Aug	27	90	112	2
157	3-Aug	29	94	94	1
Mean (s.e.m)	**12-Aug ± 1.7d**	**46.9 ± 5.7**	**81.3 ± 3.0**	**92.0 ± 16.5**	**(median) 2**
Light strain					
13	5-Aug	38	99	99	1
17	17-Aug	43	84	108	2
19	23-Aug	54	74	74	1
28	2-Sep	24	58	58	1
48	7-Sep	71	71	89	2
50	26-Aug	39	85	85	1
55	4-Aug	13	93	93	1
73	16-Aug	24	78	78	1
111	3-Aug	47	86	107	2
119	13-Sep	17	44	65	2
141	7-Aug	28	93	93	1
145	25-Aug	48	67	67	1
152	5-Aug	32	93	93	1
Mean (s.e.m)	**18-Aug ± 3.7d**	**36.8 ± 4.5**	**78.8 ± 4.1**	**85.3 ± 14.4**	**(median) 1**

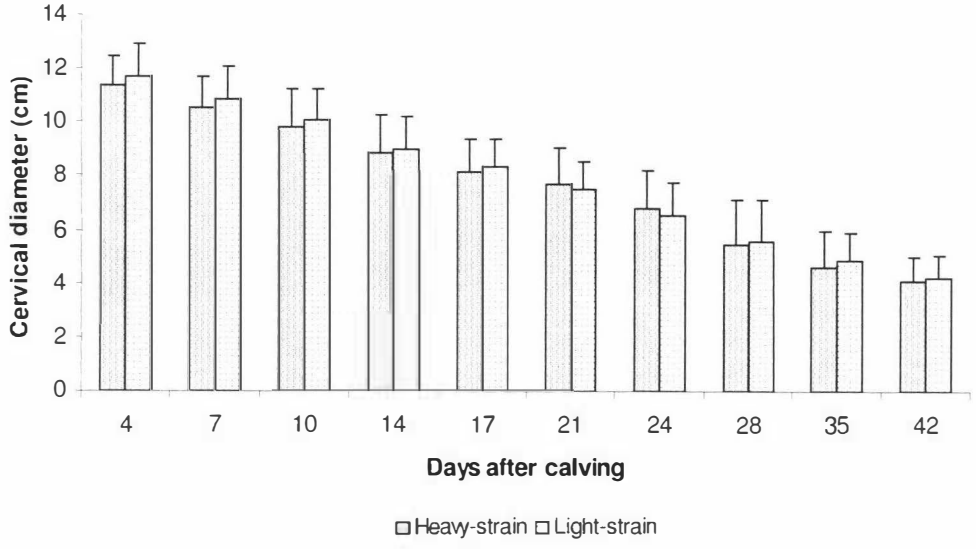
*) Cow did not conceive. DNC: Did Not Conceive

3.1 Cervical diameter

Uterine involution was considered complete when the uterus was in normal position in the pelvic cavity, the uterine horns were of equal or almost equal size. The cervix was easily palpable immediately after parturition and accurate measurements were possible. However, the uterus itself was difficult to palpate during the first two to three weeks postpartum. Hence, measurements were only made of the uterine horns.

There were no significant differences between the H and L strain cows, but there was significant ($P<0.01$) change in cervical diameter over time (Figure 3.1). The diameter on Day 4 post-partum ranged from 9.5 to 13 cm, with mean value of 11.4 ± 1.1 cm and 11.7 ± 1.2 cm for H and L cows. By Day 7 post-partum the diameter ranged from 8 to 12.5 cm (mean ± sem: H: 10.5 ± 1.14 cm, L: 10.8 ± 1.20 cm) and by Day 14 values had decreased to between 7.5 and 11.5 cm (H: 8.8 ±1.44 cm, L: 9.0 ± 1.2 cm). Thereafter, dimensions continued to decrease: on Day 21 the range was 6 to 10 cm (H: 7.7 ± 1.3 cm, L: 7.5 cm ± 1.0 cm), on Day 28 the range was 4.0 to 8.5 cm (H: 5.5 ± 1.7 cm, L: 5.6 ± 1.5 cm), on Day 35 the range was 3 to 7 cm (H: 4.6 ± 1.4 cm, L: 4.9 ± 1.0 cm) and on Day 42 post-partum the diameter ranged from 3 to 5.5 cm (H: 4.1 ± 0.9 cm, L 4.0 ± 0.8 cm).

Figure 3.1 Changes of cervical diameter with time after calving. Differences between days were significant ($P<0.01$), but there were no differences between strains or between strains and different days.



3.2 Microbiology

A total of 28 samples from the 130 collected from L cows, and 31 samples from the 120 collected from H cows, gave positive microbiological cultures. The remainder (76.4 % of the total number of samples) failed to yield microbiological cultures.

Positive cultures had been found in 85% of H strain and 79% of L strain cows by Day 10 post partum. The remaining cows did not yield positive cultures at any time (Figure 3.4). The sum of the aerobic plus the anaerobic bacterial count was taken to equate to the total bacterial contamination.

3.2.1 Aerobic bacterial contamination

Total aerobic bacterial counts were higher in H than L cows ($P<0.05$) and there was a significant ($P<0.05$) effect of time on the numbers of bacteria isolated from each strain (Figure 3.2a).

Aerobic bacterial counts peaked on Day 7 in both strains, with values in H cows (16.6×10^5 total cfu) being significantly ($P<0.05$) greater than in L stain cows (11.6×10^5 total cfu). Likewise, on Day 10, there were significantly ($P<0.05$) more organisms isolated from H cows (10.5×10^5 total cfu) than in L cows (1.5×10^5 total cfu). Thereafter, total counts decreased dramatically, with values in both strains being <100,000 cfu from Day 17 onwards.

3.2.2 Anaerobic bacterial contamination

Heavy strain cows had significantly ($P<0.05$) higher levels of anaerobic contamination than did L cows (Figure 3.2b), notably between Days 4 and 14 postpartum. Numbers of anaerobic bacteria were maximal in both strains on Day 10; however, mean values in H cows were 23.4×10^5 cfu, whereas in L cows, the figure was only 0.41×10^5 cfu ($P<0.01$).

In both strains anaerobic contamination had declined to low levels by Day 17, remaining low thereafter.

3.2.3 Total bacterial contamination

Total bacterial contamination, in terms of total cfu (Figure 3.3) and the proportion of cows from which positive cultures were obtained (Figure 3.4) followed the same pattern as that of aerobic and anaerobic organisms. Differences between days

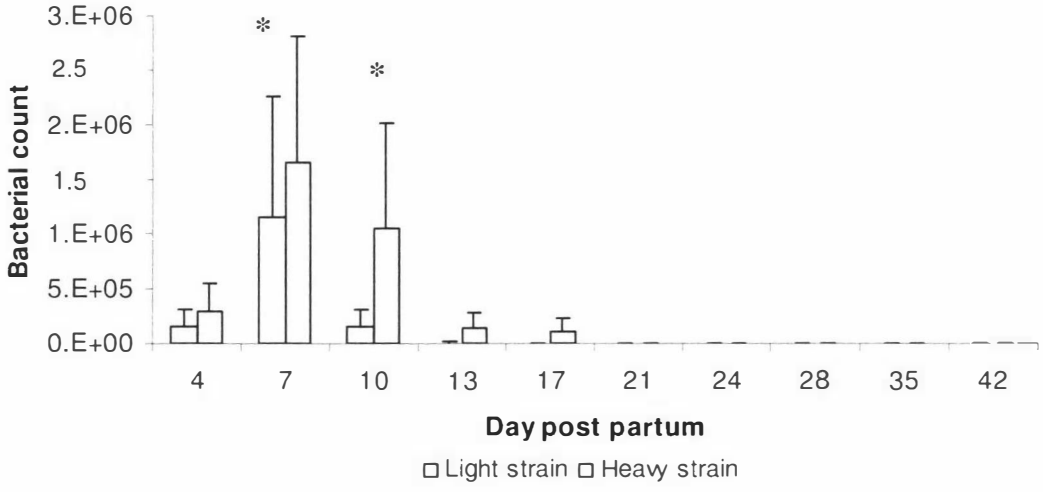
($P<0.01$), strains ($P<0.01$) and the numbers of cfus in the two strains over time ($P<0.05$) were significant.

By the end of the first week (Day 7) mean values had reached 2.27×10^6 cfu in H cows. Peak numbers of bacteria in H cows were attained on Day 10 (3.39×10^6 cfu) Values in L cows were maximal on Day 7 (1.18×10^6 cfu); a figure that was significantly ($P<0.05$) lower than in H cows. By Day 10, total contamination in L cows had declined from this peak (1.96×10^5 cfu); a difference from H cows that was also significant ($P<0.05$). Mean total bacteria counts in L cows had declined to <100,000 cfu before Day 10, whereas they remained about this figure in H cows until Day 17.

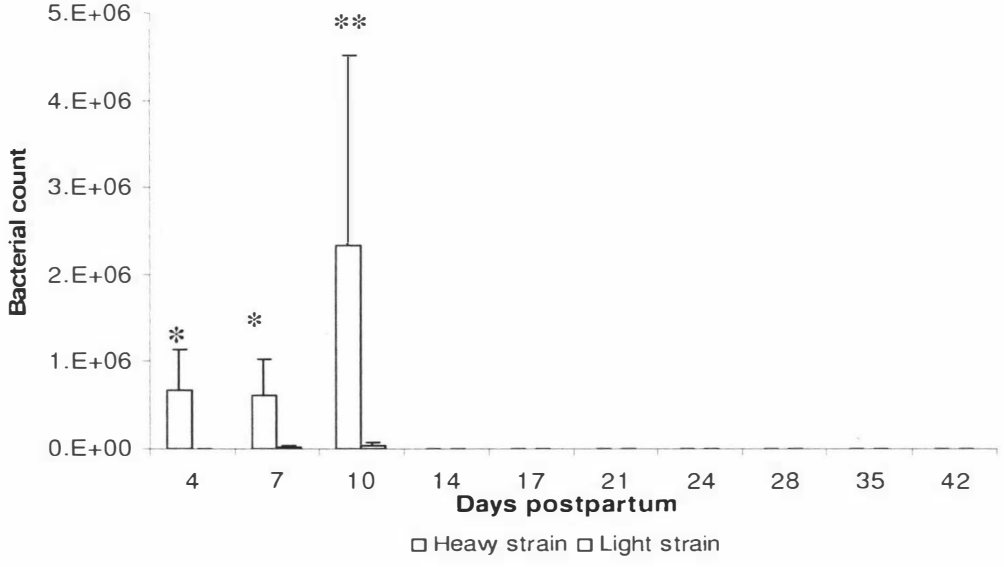
The proportion of cows that yielded positive bacterial cultures (i.e. had postpartum uterine contamination) is shown in Figure 3.4. This was similar between the two strains. Proportions increased from ~40% of animals on Day 4 to 61.5% of L cows and 66.6% of H cows on Day 7, which was the day on which the peak numbers were recorded. Thereafter, numbers declined at a similar rate in the two strains, such that by Day 14 ~30% of animals were infected, and by Day 21 only individual animals were still infected.

Figure 3.2 Mean (± sem) numbers of colony forming units of organisms cultured from cervical swabs collected from H and L strain cows over the post partum period

(a) Aerobic bacteria

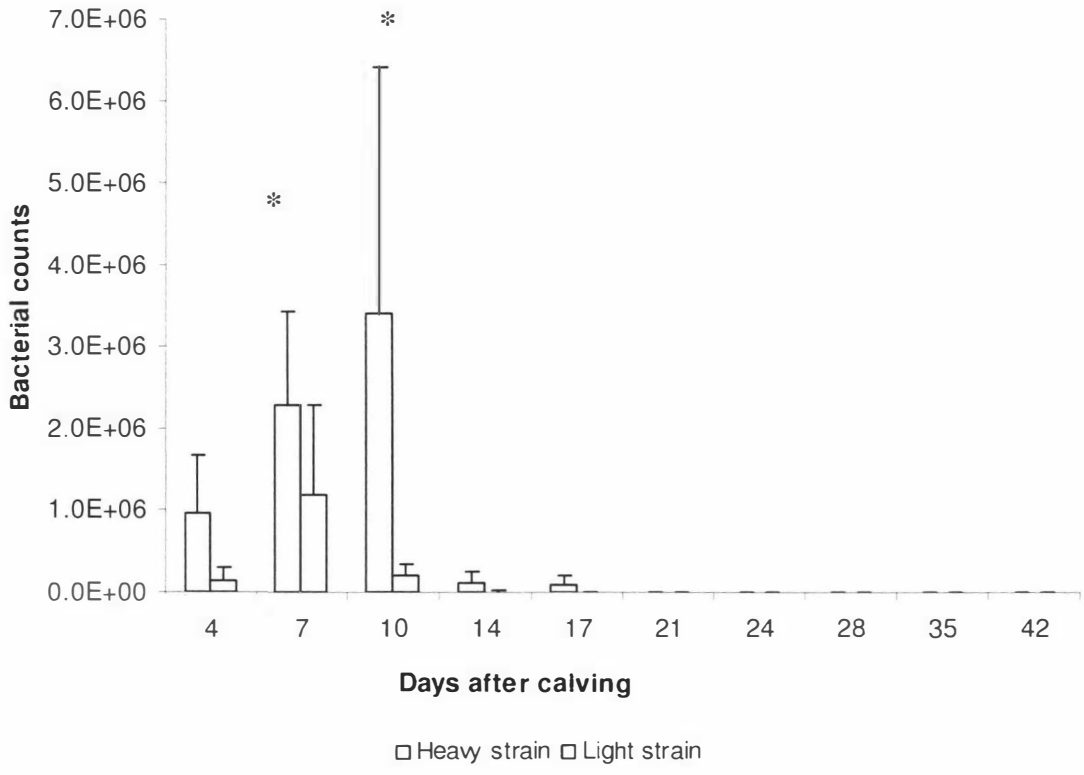


(b) Anaerobic bacteria



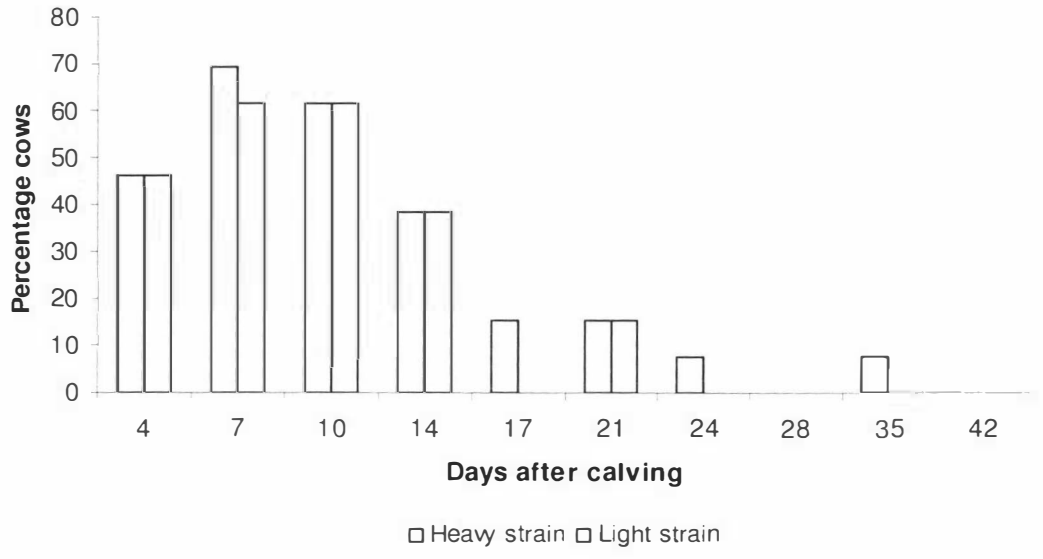
Differences between strains were significant where shown (** P<0.01, * P<0.05)

Figure 3.3 Mean (± s.e.m.) total colony forming units of aerobic and anaerobic bacteria cultured from cervical swabs of H and L strain cows over the post partum period.



Differences between strains were significantly different ($P<0.05$) where shown (*)

Figure 3.4 Proportion of H and L strain cows from which positive microbiological cultures were obtained from cervical swabs collected over the post partum period. Differences between days, but not strains, were significant ($P<0.05$).



3.3 13,14-dihydro, 15-keto prostaglandin $F_{2\alpha}$ (PGFM)

Changes in PGFM concentrations over time after parturition differed significantly (P=0.03) between H and L cows. These data are shown in Figure 3.5

Concentrations in L cows were significantly (P<0.05) higher than in H cows on Days 17, 28 and 35. They also tended to be higher on Day 10 (P=0.10). On Day 4, concentrations tended (P=0.08) to be higher in H than L cows; concentrations in H cows were also maximal on Day 4. Hence, values in H cows declined over the period of the trial from peak values on Days 4 to minimal after Day 17, whereas in L cows concentrations increased from Day 4 to Day 7, declining thereafter.

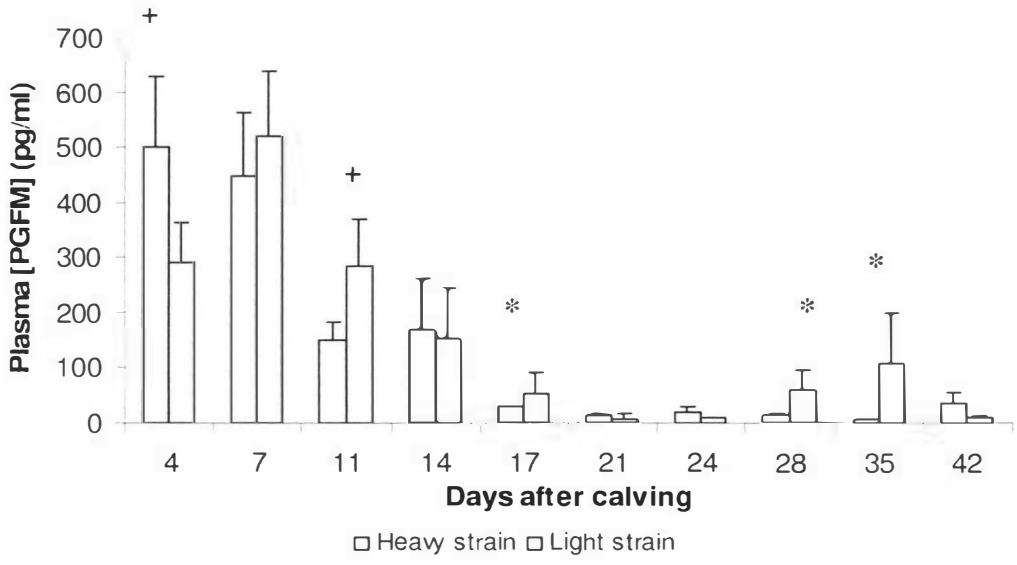
The effect of time postpartum on PGFM concentrations was significant (P<0.01). Overall mean concentrations were maximal on Day 7, declining rapidly thereafter, to reach basal values by Day 17. There was a modest increase in concentrations on Days 28 and 35, due to the higher concentrations in L cows on those days.

Individual animals' PGFM concentrations at different times post calving were highly correlated with cervical diameters (P<0.001; Figure 3.6). Concentrations on Day 7, but not those on Day 4, Day 10 or the mean of values between Days 4 and 10, were correlated (P=0.04) with the day on which cervical diameter first fell to below 7.5 cm

.

PGFM concentrations were related to $\log_e$ aerobic (P<0.001) anaerobic (P=0.03) and total bacterial P<0.001) counts (Figure 3.7), such that concentrations were higher in animals that had greater numbers of bacteria present.

Figure 3.5 Mean (± sem) peripheral PGFM concentrations in H and L strain cows over the postpartum period



Differences between means are significant ($P<0.05$) where shown (*) and approached significance ($0.05<P<0.10$) where indicated (+).

Figure 3.6 Relationship between plasma PGFM concentrations and cervical diameter in H and L cows during the post partum period. Data are for individual measurement from each cow at each time point. The correlation is significant (P<0.001).

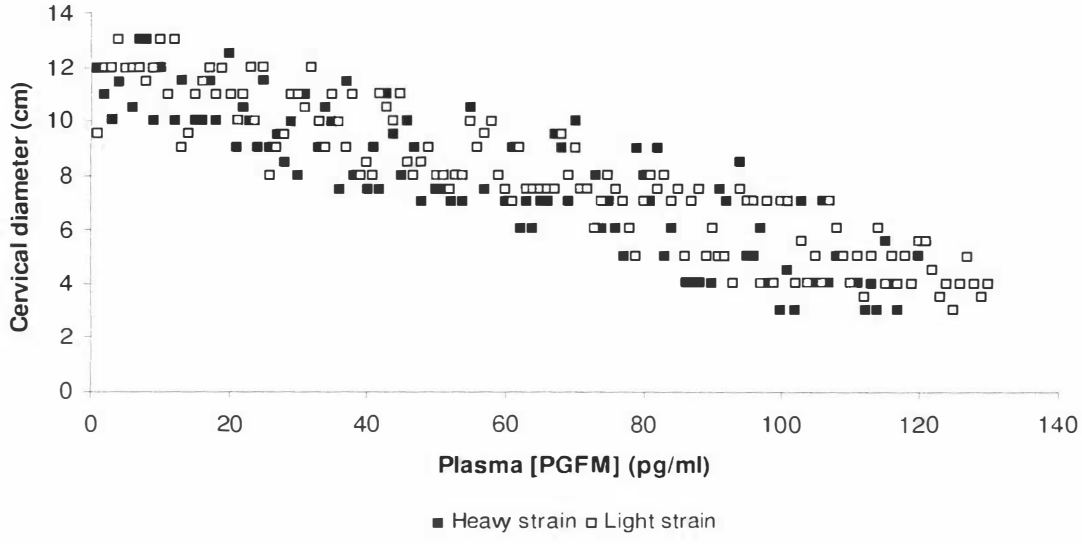
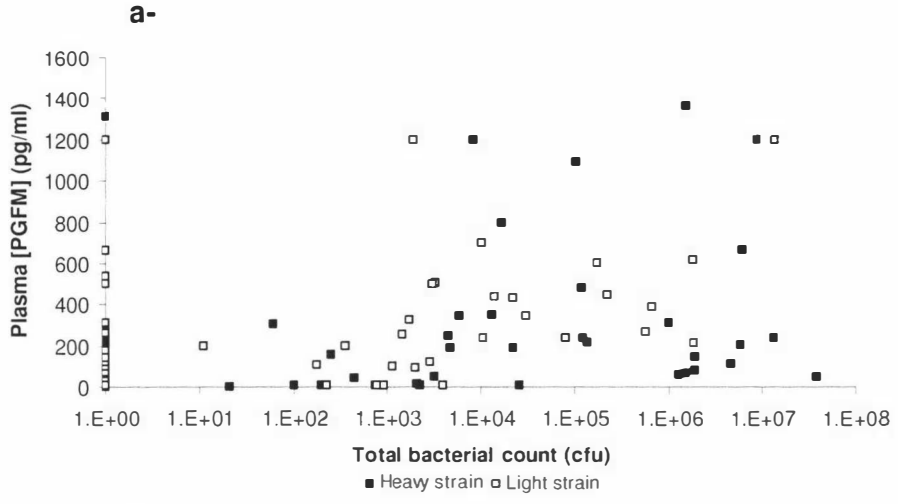
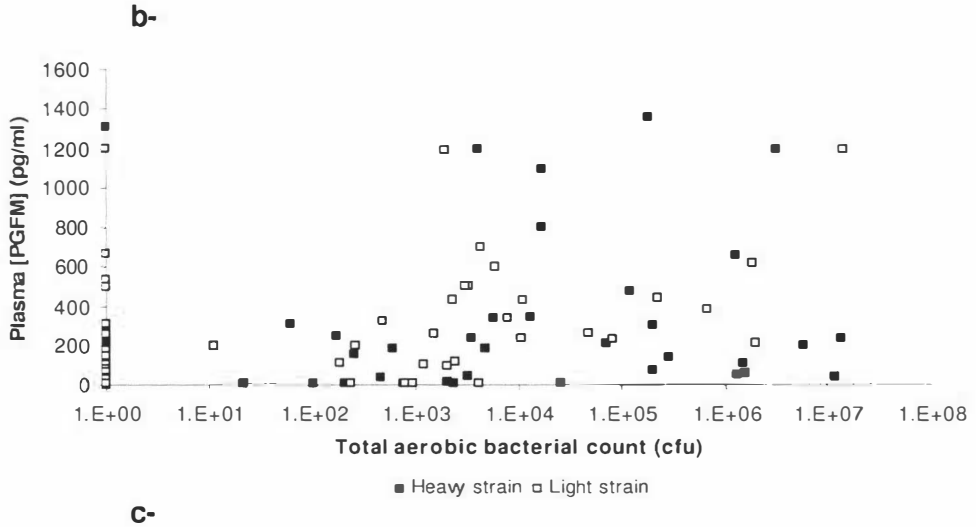
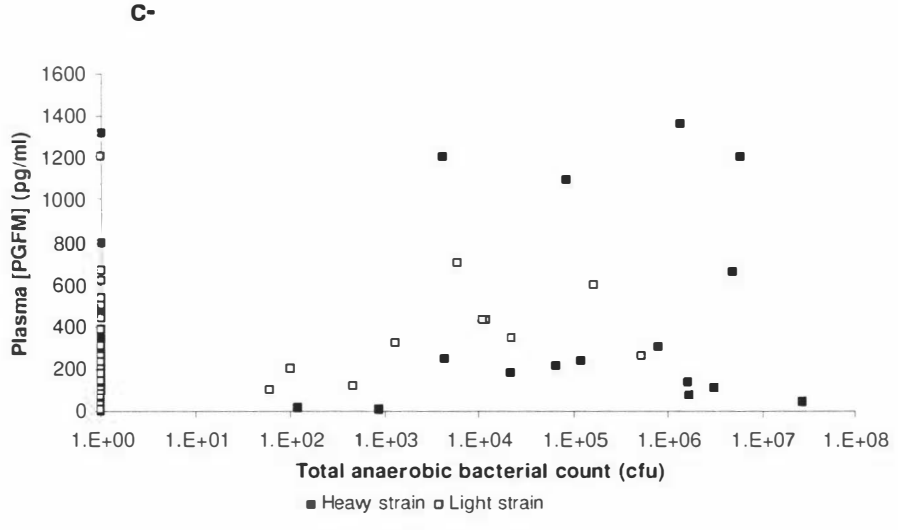


Figure 3.7 Relationship between numbers of (a) aerobic bacteria, (b) anaerobic bacteria and (c) total bacterial count and plasma concentations of PGFM in H and L cows over the post partum period.







3.4 Pyridoline and deoxypyridoline

Pyridoline and deoxypyridoline data are presented as the ratio of total pyridoline and deoxypyridoline /creatinine (nmol pyridoline + deoxypyridoline /mmol creatinine: referred hereafter as nmol Pyr). Concentrations differed between strains and times, but the strain.time interaction term was not significant (Figure 3.8). On Day 11, values in L cows tended (P=0.07) to be higher than in H cows.

Overall, concentrations increased between Day 4 (224.1 ± 16.5 nmol Pyr) and Day 7, achieving maximal values on Days 7 (379.7 ± 29.4 nmol Pyr) and 10 (371.2 ± 40.8 nmol Pyr). Thereafter concentrations decline rapidly until Day 17 (202.6 ± 14.5 nmol Pyr) and more slowly thereafter (Day 42: 127.4 ± 8.4 nmol Pyr).

Total pyridoline and deoxypyridoline /creatinine ratio was positively related to cervical diameter, although the relationship was less clear than had been the case for PGFM (Figure 3.9). The regression (cervical diameter (cm) = 0.008 nmol Pyr + 6.13) was significant ($P<0.01$).

Figure 3.8 Urinary contents of pyridoline and deoxypyridoline, as expressed as the ratio of total pyridoline and deoxypyridoline /creatinine (nmol pyridoline + deoxypyridoline /mmol creatinine) in H and L strain cows over the post partum period. Differences between time points, but not between strains, were significantly different from each other ($P<0.01$) and approached significance ($0.05<P<0.10$) where indicated (+).

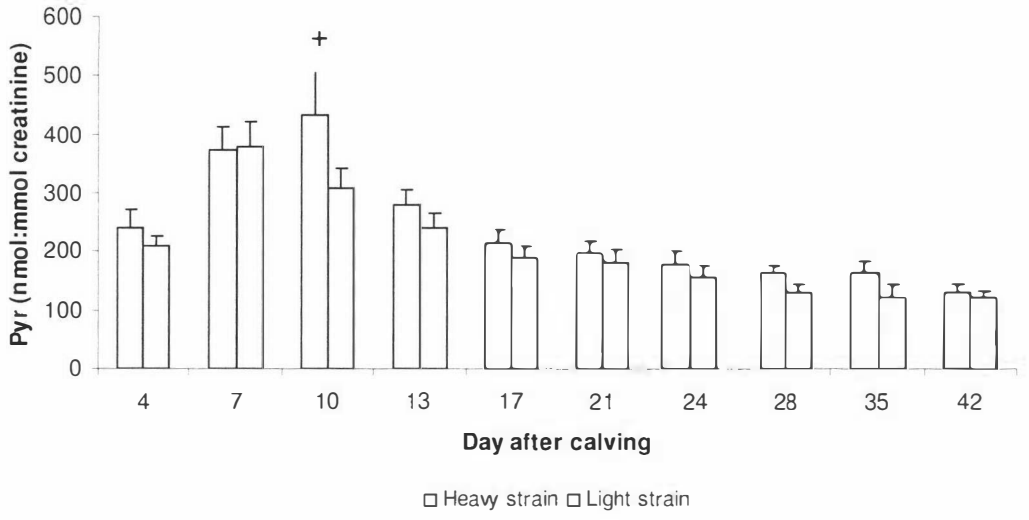
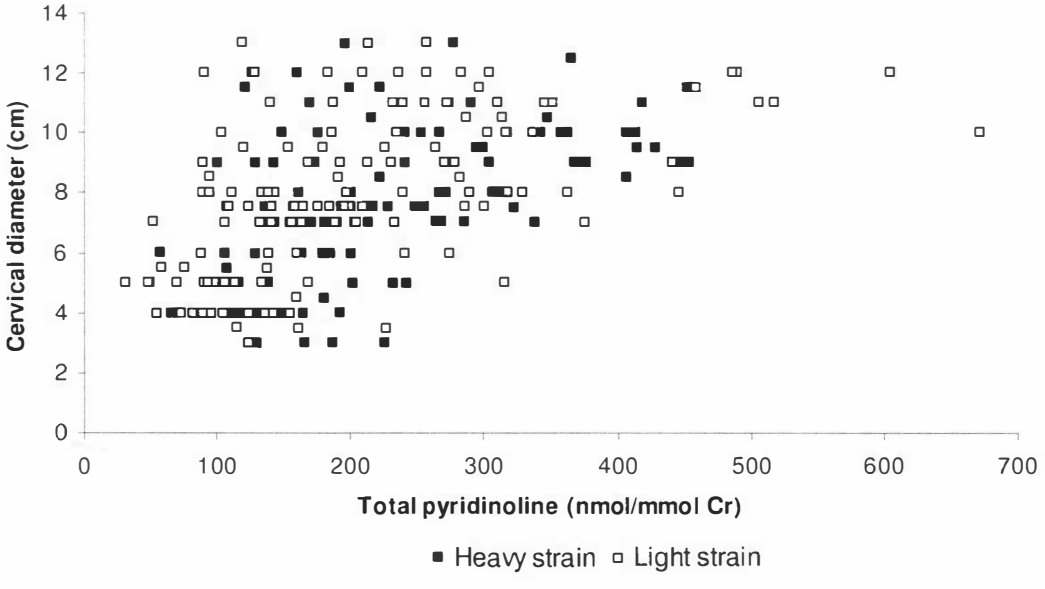


Figure 3.9 Relationship between total pyridoline and deoxypyridoline /creatinine ratio and cervical diameter in H and L cows over the post partum period. There were no differences between strains or between strains and different days.



3.5 Relationships between parameters of uterine involution and fertility outcomes.

The interval between calving and first observed oestrus was related to bacterial contamination, pyridinoline concentrations and line, as shown in Table 3.2. No measures of the concentrations of PGFM or cervical diameter were represented in the final model (i.e. their inclusion did not reduce the residual variance). Predicted values, based upon the partial regression coefficients were then calculated and compared with actual values (linear regression). These results are illustrated in Figure 3.10

Table 3.2 Relationship between uterine involution and interval between calving and first oestrus

Term	Partial regression coefficient	P value (individual term)
Constant	54.7	
Line: heavy	-9.1	0.312
Day 7aerobic cfu	1.29E-06	0.244
Day 10 aerobic cfu	0.00013	0.095
Day 10 anaerobic cfu	-5.7E-05	0.06
Day 7 [pyridinoline]d7pyr	0.0902	0.073
Day 10 [pyridinoline]	-0.0503	0.043
Day 21 [pyridinoline]	-0.1271	0.118
Overall		<0.001

Figure 3.10 Relationship between actual and predicted intervals between calving and first oestrus, based on partial regression analysis of parameters of uterine involution. The relationship ($y = 0.5236x + 22.021$; $R^2 = 0.4149$) was significant ($P<0.05$).

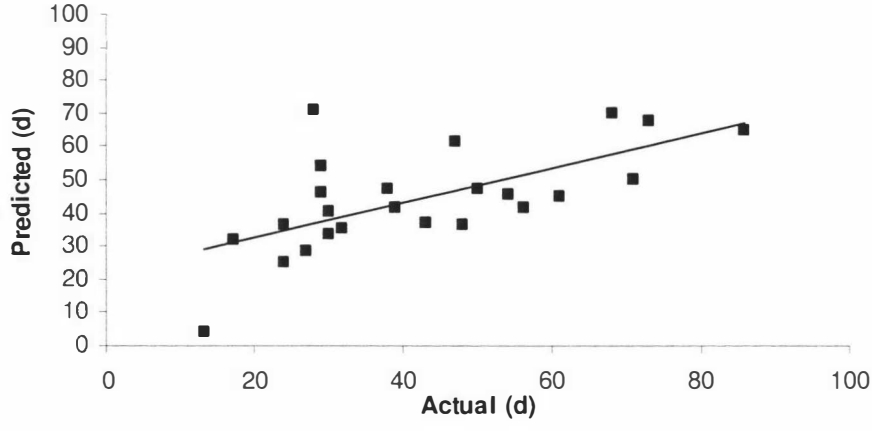
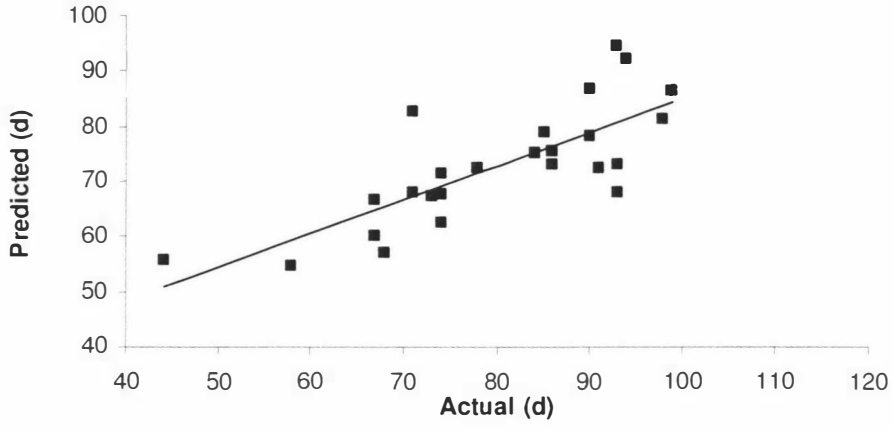


Figure 3.11 Relationship between actual and predicted intervals between calving and first insemination, based on partial regression analysis of parameters of uterine involution. The relationship ($y = 0.612x + 23.839$; $R^2 = 0.6009$) was significant ($P<0.01$).



The interval between calving and first insemination was related to concentrations of PGFM and pyridinoline, and cervical diameter, as shown in Table 3.3. No measures of bacterial contamination were represented in the final model. Predicted values were calculated and compared with actual values, as previously described. These results are illustrated in Figure 3.11.

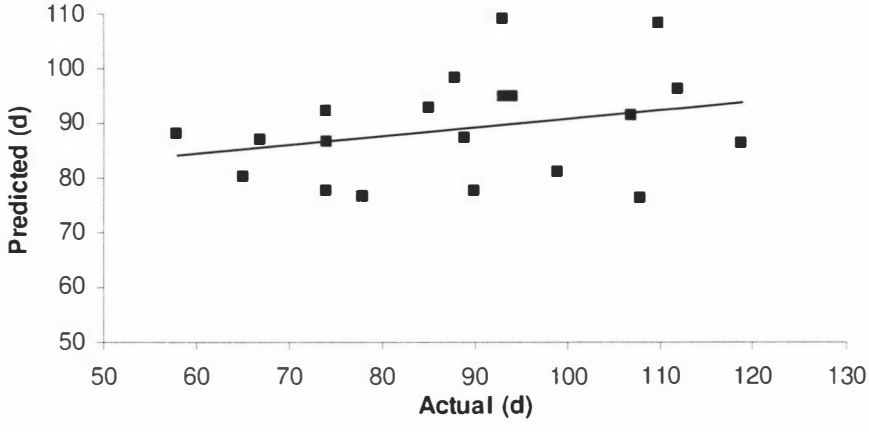
Table 3.3 Relationship between uterine involution and interval between calving and first insemination

Term	Partial regression coefficient	P value (individual term)
Constant	157.4	
Day 7 cervix diameter	-8.1	<.001
Day 7 [PGFM]	0.01136	0.039
Day 24 [PGFM]	-0.0676	0.328
Day 10 [pyridinoline]	0.0112	0.286
Overall		<0.001

Likewise, the interval between calving and conception was related to concentrations of PGFM and cervical diameter, as shown in Table 3.4. No measures of bacterial contamination or pyridinoline concentrations were represented in the final model. Predicted values were again and compared with actual. These results are illustrated in Figure 3.12.

Table 3.4 Relationship between uterine involution and interval between calving and conception		
Term	Partial regression coefficient	P value (individual term)
Constant	157.8	
d7cervix	-6.74	0.028
d7pgfm	0.01001	0.144
d24pgfm	-0.129	0.248
Overall		<0.05

Figure 3.12 Relationship between actual and predicted intervals between calving and conception, based on partial regression analysis of parameters of uterine involution. The relationship ($y = 0.1618x + 74.688$; $R^2 = 0.0822$) was not significant.



CHAPTER IV

Discussion

4.1 Introduction

During the post-partum period the cow should re-establish the normal uterine and ovarian activities. Thus, a failure to return to normal function may delay the first postpartum ovulation and cause prolongation of the uterine involution (Lindell et al., 1982).

Reproductive performance is determined by the interplay of management, environmental and biological factors. Management factors, such as heat detection, biological factors, such as the activity of the reproductive endocrine system, milk yield and age, and environmental factors, especially feed supply; interrelate to affect the reproductive outcomes of individual animals and herds. Amongst these, the environment of the uterus between the time of parturition and re-conception is a generally neglected, yet vitally important, factor that affects fertility.

During the first four weeks post-partum, the cow's immune system undergoes a significant period of challenge (Goff and Horst, 1997). Most cows develop a mild or subclinical endometritis during the early phase of the post-partum period (Lewis 1997), and uterine fluids (lochia) which contain the products of this contamination, together with the material derived from the breakdown of the residuum of the previous pregnancy, are voided during the first two weeks post-partum. The uterine immune system seems to be up- regulated during the periparturient period and remains highly active until progesterone from the first post-partum ovulation down-regulates the uterine immune system (Seals et al., 2000).

Many studies have reported a low heritability and repeatability of fertility (e.g. Dunbar and Henderson, 1953, Pou et al., 1953). However, in a study by Studer and

Morrow, (1978) a significant correlation was reported between conception rates and repeatability of fertility. The main goal of the present study was to establish if there was tangible evidence of a genetically-based component to sub-fertility that was manifested through alterations of the process of uterine involution during the post-partum period. If this were to be the case, it might help to unravel some factors causing infertility problems in the New Zealand herd. This was achieved by comparing various parameters of uterine involution in two different strains that had been genetically selected for low or high mature body weight.

4.2 Cervical Diameter

The diameter of the previously pregnant uterine horn commonly is regarded as a valid indicator of involution of the genital tract. It is of particular importance during the first three weeks postpartum, when, in many cows, the uterus cannot be palpated easily; hence, the diameter of the cervix is used as a useful indicator of the involution process. For example, Fonseca et al. (1983) reported that the involution of the uterus closely paralleled involution of the cervix across a range of different breeds Moreover, many authors have considered cervical measurement as an indicator for the subsequent reproductive performance of the individual cow (Miller et al. 1980, Oltenacu et al. 1983; Madej et al. 1984; Young et al. 1984; Del Vecchio et al. 1994).

Many attempts have been made to associate cervical and uterine measurements with fertility and subsequent reproductive performance. Oltenacu et al. (1983) reported that the critical cervical diameter of first-lactation Holstein cows three weeks postpartum is 55 mm, and that cows with a greater cervical diameter should be considered

'problem cows'. Likewise, it was suggested that for cows in second or later lactation the critical cervical size is 60 mm, and that cows with greater cervix diameter should again be considered 'problem cows'. Similarly, Miller et al. (1980) reported a lower conception rate at first service for cows with a larger diameter of the uterus.

In the present study, which investigated whether there was any difference between two genetically different strains of Holstein-Friesian cows managed under a pastoral system, cervical diameter significantly ($P<0.001$) dimished over time in both strains, as expected. However, this was independent of strain of cow, inasmuch as the rates of change of cervical diameters were identical between the two strains (Figure 3.1).

This finding of the present study is consistent with previous investigations of cervical involution. For example, it has been shown previously that cervical diameter and uterine involution differed neither between two genetically different strains within the same breed (Harrison et al., 1990) nor between breeds (Heinonen et al., 1988). Moreover, whilst some of the parameters of uterine involution that were measuered in the present study differed (or nearly differed) between strains, cervical diameter was identical.

4.3 Bacterial contamination

Non-specific uterine infections reduce the reproductive efficiency of cows. Most cows develop a degree of contamination of the uterus in the post-partum period (Sreenan and Diskin, 1993; Lewi,s 1997). In most instances, these organisms are cleared from the uterus by 3 to 4 weeks post-partum (Sreenan and Diskin, 1986). However, in a proportion of animals this contamination develops into infection, where colonisation and multiplication of organisms occurs within the endometrium and, sometimes, deeper layers of the uterine wall (Sheldon et al., 2006). Whether contamination

develops into infection is multifactorial in aetiology, being associated with several factors such as dystocia, premature calving, retention of foetal membranes and metabolic disorders, which render these cows more likely to develop infection rather than eliminating the bacterial contamination (Sandals et al., 1979, Fonseca et al., 1983, Dohoo, 1983, Olson et al, 1984, Fredriksson et al., 1985, Lewis, 1997).

Hence, in cows in which this clearance of uterine contamination does not occur, there is the potential for spontaneous uterine infection (endometritis) to occur. This condition may be recognized by the presence of a purulent cervical or vulval discharge, or by detection of the presence of enlarged uterine horns and/or intrauterine fluid upon palpation per rectum. In many instances, these cows have had at least one postpartum ovulation, inasmuch as they have a corpus luteum; which, if it fails to regress, renders the animal to be at risk for the development of chronic endometritis or pyometra. Sometimes, the presence of a vulvar discharge or intrauterine fluid may not be obvious clinically, in which case, the condition is referred to as subclinical endometritis. Chronic endometritis can only be diagnosed by demonstrating chronic inflammation of the endometrium in uterine biopsy specimens, collection of swabs or flushings for microbiology and cytology, or by visualisation of fluid in the lumen of the uterus using ultrasonography. Sheldon et al. (2005) proposed that the diagnosis of endometritis is best made on the presence of neutrophils in cervical fluids.

Postpartum endometritis is a major cause of infertility in the dairy cow. Clinical endometritis is clearly associated with subfertility (Morton, 2000) and there is good evidence (Tennant et al., 1968; Hussein and Daniel, 1992) that chronic or subclinical endometritis has similar effects. Indeed, Manspeaker et al. (1983) and Gonzalez (1984) associated infertility with the degree of endometrial damage present in uterine biopsies, such that mild chronic endometritis is significant contributor to the 'repeat

breeder' syndrome. Such infertility can be manifested in an increased number of days from calving to conception, and a low conception rate (Erb et al., 1985). Studies of repeat breeders suggest that the reasons are a subnormal fertilisation rate and grossly abnormal early embryonic death rates, probably resulting from induced dysfunction of the endometrium (Maurer and Echternkamp, 1985, Gustafsson and Larsson, 1985, Tanabe et al., 1985).

The initial uterine defence against bacterial infection is phagocytosis, primarily by neutrophils, with subsequent digestion of bacteria by uterine leukocytes (Vandeplassche and Bouters, 1983). The immunosuppressive action of progesterone is well-recognised, which can affect the uterine defence mechanisms and predispose establishment of endometritis and pyometra (Olson et al., 1984). One of the other factors that may determine the effectiveness of this response is uterine PGE_2 secretion. Uterine tissues are able to synthesize different types of prostaglandins, in particular PGE_2. The inflammatory reaction, which is associated with uterine involution, could be responsible for the synthesis and liberation of other immunoactive eicosanoides (PGE_2), as has been shown for ulcerative colitis in man (Slama et al., 1991). Uterine fluid PGE_2 concentrations have been related to the degree of endometritis (Weems et al., 1983), probably as, in utero, PGE_2 has a negative effect on Polymorph nuclear cells activity (Morkok et al., 1985), which is the major effector of the defence mechanisms of the uterus against bacterial infections during the puerperal period (Kehrli et al., 1989). Moreover, intrauterine infusion of PGE_2 during the puerperal period has been associated with an inhibition of lymphoblastic transformation and with decreased concentrations of immunoglobulins in uterine secretions. The persistence of uterine infections could therefore be due to the maintenance of relatively high concentrations of PGE_2 (Slama, et al., 1991). Thus,

changes in the $PGF_{2\alpha}/PGE_2$, ratio may be responsible for a sub-chronic puerperal immunosuppression that subsequently permits the development (and the severity) of uterine infection from the normal postpartum contamination. Once ovulations have occurred, such changes may exacerbate those induced by progesterone; given that $PGF_{2\alpha}$ is luteolytic, whereas PGE_2 is generally regarded as being luteotrophic (Weems et al., 1983).

Whether and, if so, how postpartum uterine infection limits ovarian activity is not yet clearly understood. It has generally been held that restoration of ovarian activity, being driven by the hypothalamo-pituitary axis, is largely unrelated to the process of involution and/or the presence of uterine infection. However, in a study by Peter and Bosu (1988), the patterns of bacterial recovery and changes in follicle growth would suggest a causal relationship.

Schams et al. (1978) observed that the postpartum preovulatory LH surge was delayed in cows with an abnormal puerperium. An understanding of the mechanism by which follicle growth is inhibited by uterine infection may also provide valuable clues toward elucidating the process of cyst formation in postpartum dairy cows (Bosu et al., 1987).

Any inhibition of folliculogenesis that is induced by the presence of uterine infection is probably mediated through endotoxin produced by Gram negative bacteria in the uterus. There is evidence that endotoxin alters the function of hypothalamo-pituitary-ovarian axis leading to delayed follicular growth in infected cows. Intrauterine infusion of *E. coli* endotoxin has resulted in suppression of follicular growth and preovulatory LH surges (Peter et al., 1989), which might suggest that postpartum uterine infections or products of infection suppress synthesis and/or secretion of gonadotrophin, resulting in delayed onset of folliculogenesis (Peter and Bosu, 1988).

The data from the present study confirm previous findings that the postpartum uterus is infected with a broad spectrum of bacteria, for, although bacteria were not identified to species level, there was a wide range of colony types and growth of both aerobic and anaerobic bacteria. Previous studies have indicated that the uteri of postpartum cows contain large numbers of bacteria. Species most frequently isolated are *A. pyogenes*, together with *Bacteriods* species and *F. necrophorum*, with *E. coli* and *Streptococcus* species (Kash, 1999).

However, given the wide range of bacterial species that are present, the main focus of the present study was to establish the total bacterial load of bacterial infection (including whether it was aerobic, or anaerobic) in H and L cows over the postpartum period and examine the relationship between this and the process of uterine involution.

Data from the present study showed that the majority of animals had aerobic and anaerobic bacteria present in first 2 weeks postpartum; and it was noted that H cows always had higher levels of contamination compared to L cows. Specifically, on Days 4, 7 and 10 post partum, the anaerobic bacterial load was significantly ($P<0.05$) greater in H than L cows, whilst on Days 7 and 10, the total bacterial load (aerobic plus anaerobic) was also greater ($P<0.05$) in H than L cows. It is feasible that the higher anaerobic bacterial count in the heavy strain predisposed those cows to a greater level of aerobic contamination, since the presence of the anaerobic organism *F. necrophorum* has been associated with a facilitation of aerobic bacterial growth (Price and McCallum, 1986, Markusfeld, 1993). Thereafter, the presence of the aerobic bacteria might have caused delayed conception (Fivaz and Swanpoel, 1978).

The present results also provide some evidence that cows managed under New Zealand conditions eliminated post-partum bacterial contamination more quickly than

occurred in studies of housed cows in Northern Hemisphere conditions (Bekana et al. 1996, Pugh et al., 1994) since, by the end of second week only 30% of the of either strain had any bacterial contamination in the current study. Similar results have been provided by Hussein and Daniel (1992) from a study of dairy cows managed under pastoral conditions in Australia. On the other hand, Bekana et al. (1996) showed that the rate of elimination of post partum contamination was longer in housed cows; reporting that maximal bacterial contamination occurred during the second week post-partum and declined sharply and disappeared towards the end of the third week. Similarly, Griffin et al. (1974) reported that after Day 28, a progressive decrease in bacterial contamination continued, and only 33% of cultured samples yielded positive results. In another study (Pugh et al., 1994), the incidence of uterine infection in dairy cows, which calved in maternity stalls or sod lots, was greater than the incidence in beef cows, which calved on pasture.

It is interesting to speculate about reasons for the more rapid elimination of infection from H and L cows that have been reported from overseas studies. The most likely explanation is that, by calving outdoors, in an environment in which there is little opportunity for build-up of pathogenic organisms, the uteri of parturient cows are exposed to fewer, and less pathogenic, organisms than are their housed counterparts. Difference in nutrition may have played a role, although the lower energy status in the diet of pasture-fed cows would generally be held to be predisposing, rather than mitigating, factors to the development of uterine infection (Ruder et al., 1981). Also, the level of production is lower in pastoral than intensive housed systems, which may have further affected the probability of contamination, since increased milk production per cow increased the incidence of silent heat and post partum metritis (Erb et al., 1985, Butler and Smith, 1989). Moreover, in the tudy of Kash (1999), it

was observed that a higher frequency of milking affected the incidence of uterine infection. It was suggested that higher milk production, with consequent changes to cows' metabolism, might have influence their resistance to infection (Gisi et al., 1986). Whether differences in the level of protein in the diet affected the speed of elimination of bacterial contamination is unclear. On one hand, higher protein concentrations have been associated with beneficial effects on involution (Miettinen 1990, Ruder et al., 1981). On the other hand, high levels of NPN in the diet, especially where this results in raised blood (and, hence, intrauterine) urea concentrations (Westwood et al., 1998), such as occurs in the spring in pasture-fed cows, is generally held to be deleterious to the uterine environment and immune function.

The conclusion from the study of bacterial contamination is that heavy-strain cows have significantly higher contamination levels in the first 2 weeks postpartum than light-strain cows. It was noted that the levels of contamination were associated with visible signs of endometritis, nor was there an extended duration of infection, yet the levels of contamination that were present appear to have been negatively related to the ability of cows to conceive after the start of mating. Whether this was due to alterations in immunity due to changes in the metabolism associated with higher capacity to produce milk, to higher PGE_2 production, lower $PGF_{2\alpha}$ secretion (see below) or simply due to different anatomical characteristics between the strains remains to be resolved.

4.4 Prostaglandin $F_{2\alpha}$

$PGF_{2\alpha}$ is derived from PGH_2, which in turn is synthesised from arachidonic acid, through the cyclooxygenase pathway. Prostaglandin F metabolite (PGFM: 13,14-dihydro, 15-keto prostaglandin $F_{2\alpha}$) is derived from $PGF_{2\alpha}$ through the consecutive

actions of 15-hydroxy prostaglandin dehydrogenase and prostaglandin Δ^3-reductase in the lungs. It is not possible to measure $PGF_{2\alpha}$ in the peripheral circulation because of this rapid metabolism; hence quantification of PGFM in peripheral blood is generally used as an index for $PGF_{2\alpha}$ synthesis in tissue. Uterine tissues are able to synthesize different types of prostaglandins, including both PGE_2 and $PGF_{2\alpha}$: the former, as previously discussed, possibly being related to the development of uterine infections.

Prostaglandin $F_{2\alpha}$ is considered by some authors as being a key regulatory hormone, primarily being that of a luteolysin, during the bovine peripartum period (Kindahl et al., 1992). Peter and Bosu (1987) suggested that increases in uterine $PGF_{2\alpha}$ production might cause the premature regression of an existing corpus luteum, which will result in lowered progesterone concentrations. In a study by Rodriguez-Martinez et al. (1987) it was found that progesterone exerts an immunosuppressive effect upon the uterus. Therefore, by secreting $PGF_{2\alpha}$, the uterus may be acting to completely terminate corpus luteum function (and thereby lower progesterone concentrations to baseline values) in an attempt to rid itself of the infectious organisms.

Higher peripheral postpartum PGFM concentrations have been associated with a more rapid decrease in the volume of ovaries ipsilateral to the previously gravid uterine horn and a smaller diameter for the regressing CL of pregnancy (Lewis et al., 1984). The regressing CL of pregnancy dampens follicular development during the postpartum period and prolongs the time interval for follicles to develop to half mature size (Dufour and Roy, 1985). Thus, the higher release of PGFM by the postpartum uterus may cause more rapid degeneration of the CL of pregnancy, which would promote follicular growth and contribute to higher ovarian activity in ovaries ipsilateral to the previously gravid uterine horn (Guilbault et al., 1987).

Normal uterine involution has been associated with a massive synthesis of $PGF_{2\alpha}$ lasting from 7 to 15 days after calving (Edqvist et al., 1978, Eley et al., 1981, Lindell et al., 1982); whilst abnormal uterine involution has been associated with a prolonged duration of high level of $PGF_{2\alpha}$ (Lindell et al., 1982). In cows with a normal puerperium (as in the present study) the amplitude and duration of this release is negatively correlated with the time required for completion of uterine involution (reviewed by Kindahl et al., 1992). Uninfected cows with the longest duration of elevated $PGF_{2\alpha}$ concentrations also have the most rapid involution process (see Kindahl et al., 1992). If the duration of $PGF_{2\alpha}$, release is too short, the involution process is delayed, and if the duration of its secretion is longer the involution process is accelerated. On the other hand, in cows with an abnormal puerperium this correlation is positive, inasmuch as increased prostaglandin release is associated with prolonged involution process (Kindahl et al., 1992, Del Vecchio et al., 1992, 1994).

Cows with bacterial infection have high PGFM concentrations immediately after parturition, but these concentrations fall rapidly within 2 weeks post-partum (early PGFM elevation). Thereafter, concentrations increase again (although peak values are less than in the immediate postpartum period) and remain elevated during the period of uterine infection (late PGFM elevation). Bacteriological contamination directly contributes to these high concentrations of PGFM during the early postpartum period (Konigsson et al., 2002). Late PGFM elevation has been negatively correlated to the duration of uterine infection and the rate cervical involution (Konigsson et al., 2002). This suggests a link between uterine endocrinology, bacteriology and involution in cows with post partum infection with aerobic and anaerobic bacteria (Konigsson et al., 2002). Bekana et al. (1996) also showed that pronounced and sustained periods

of pulsatile release of $PGF_{2\alpha}$ occurred in the presence of increasing degrees of bacterial contamination. These additional periods of $PGF_{2\alpha}$ release were positively correlated with the time required for uterine involution (as determine by rectal palpation).

From the aforegoing results, it can be concluded that intrauterine infection does not prolong the duration of the immediate post-partum release of $PGF_{2\alpha}$. However, a second period of release is associated with the increased frequency of uterine infections, indicating that $PGF_{2\alpha}$ may play a role for the early elimination of the infections (Bekana et al., 1996). Interestingly, a correlation has also been found between the duration of elevated PGFM concentrations, the time required for completion of uterine involution and interval from parturition to the first ovulation (Madej et al., 1984).

In the present study, there were temporal changes in plasma PGFM concentrations, which were significantly different between strains over time.

The relationship between PGFM concentrations and fertility in the present study warrants further consideration. As well as differences between H and L strains on (Days 4 to 10) and over the period from Day 17 to Day 35, in terms of mean PGFM concentrations, there were substantial differences in both PGFM profiles and fertility outcomes between individual animals within strains. In this context it is of interest to note the observation by Guilbault et al. (1987) that partial suppression of prostaglandin synthesis early postpartum reduced ovarian activity of both ovaries during the first 60 days postpartum, whereas that replacement with only $PGF_{2\alpha}$ enhanced ovarian activity specifically on the ovary ipsilateral to the previously gravid uterine horn. In the present study, H cows had significantly lower fertility than L

cows, taking the interval between calving and first observed oestrus ($p<0.05$) and the intervals between calving and first insemination ($p<0.01$) as markers of fertility analysis. In H cows (although not in L cows), PGFM concentrations during the early postpartum period were significantly ($P<0.01$) related with the interval between calving and first insemination.

The extent to which postpartum uterine involution affects the resumption of oestrous cycles is a complex issue, which in general, has been under-researched. However, one key observation on the subject was that of Guilbault et al. (1987), who found that the residuum of the regressing CL of pregnancy impaired follicular development during the postpartum period and prolonged the time interval for follicles to develop to half mature size. Similarly, Lewis et al. (1984) found higher peripheral postpartum PGFM concentrations were associated with a more rapid decrease in ovarian volume of the ovary ipsilateral to the previously gravid uterine horn, due to a more rapid diminution of the diameter for the regressing CL of pregnancy. Thus, a higher rate of release of $PGF_{2\alpha}$ by the postpartum uterus may cause a faster regression of the CL of pregnancy which, according to the hypothesis of Guilbault et al. (1987), would promote follicular growth and contribute to higher ovarian activity in ovaries ipsilateral to the previously gravid uterine horn.

Thiengtham (2004), in a parallel study on the herds of H and L cows, showed an earlier, rather than later resumption of follicular activity and oestrous cycles in H compared to L cows. Whether this earlier resumption of oestrous cycles in H cows is beneficial, however, is much less clear. Opsomer et al. (2000), for example, conducted an epidemiological study of risk factors for postpartum ovarian disturbances and concluded that an early resumption of post partum ovarian activity, characterized by an early rise of progesterone, was also clearly a risk factor for

developing prolonged luteal phases and consequent functional anoestrus. Cows showing their first progesterone rise within 24 days after calving were significantly more at risk of having prolonged luteal activity than those with later first ovulations. Likewise, Etherington et al. (1984) induced ovulation early in the postpartum period by injecting cows with GnRH 10 days after calving, resulting in an increased number of uterine infections. This in turn led to a higher number of cases of pyometra and anoestrus due to luteal retention; and generally had detrimental effects on reproductive performance of such cows. These studies give weight to the notion that when ovulation occurs before uterine involution is completed, cows are at greater risk of developing reproductive problems (Opsomer et al., 1996).

Such observations are therefore of some importance in understanding the results of the present study in the context of the parallel results of Thiengtham (2004). In his study, ovarian follicular activity was resumed earlier in the post-partum period in H than in L cows, inasmuch as H cows exhibited significantly earlier emergence of the first two (and non significantly earlier emergence of the third and fourth) follicular waves, an earlier appearance of large follicles and a trend towards earlier ovulations. Conversely, and despite this earlier onset of follicular activity, several parameters of luteal activity were poorer (mid-cycle progesterone concentrations, interval between luteolysis and ovulation) in H than L cows. Moreover, in the present study, individual H strain cows still had positive microbiological cultures on Days 24 and 35 postpartum. These might be the cows which could, as a result of an early onset of oestrous cycles before uterine contamination had been eliminated, have been at a risk of developing uterine infections once luteal progesterone secretion commenced.

From these measurements of PGFM in the postpartum period, it can be concluded that H cows had less $PGF_{2\alpha}$ secretion from Day 11 until Day 35 postpartum than did L

cows. It is suggested that such a reduction of $PGF_{2\alpha}$ secretion in H cows might have been associated with a failure to eliminate bacterial contamination as expeditiously as occurred in L cows. Perhaps higher $PGF_{2\alpha}$ or its continued presence in the early postpartum period provided an additional stimulus in L cows that favours uterine contraction thereby lowering total contamination. With the earlier onset of ovarian activity in H than L cows, the former were at risk of developing infections once a luteal phase was established. Collectively, results from the present study and other studies (primarily Guilbault et al., 1987), suggest that the involuting uterus may affect ovarian function via release of $PGF_{2\alpha}$ into the ovarian circulation as well as the bacterial load in the uterine lumen.

4.5 Collagen breakdown products

Early investigations of uterine involution were based on palpation *per rectum* and on *in vivo* biopsy or post mortem anatomo-histologic observations (Morrow et al., 1966, Tennant et al., 1967, Gier and Marion 1968, Studer and Morrow, 1978, Studer 1983). Later, the development of ultrasonography allowed a non-invasive characterization of anatomical uterine involution (Okano and Tomizuka, 1987). The present study has added to the data provided by such studies by describing the process of uterine remodeling through quantification of the collagen remodeling that characterises involution, as determined by urinary concentrations of pyridinium crosslinks. No other studies of excretion of pyridinium cross-links during the process of involution of the bovine uterus were found in the literature, other than the preliminary studies of Kaidi et al. (1991). As far as could be ascertained, it is also the first time that such a method has been used to evaluate differences of the process of involution between breeds/strains within a species.

Tissues of the postpartum uterus must undergo the degenerative and regenerative process of involution to return to the non-gravid state and prepare a suitable environment to establish the next pregnancy. Large amounts of collagen are added to the extracellular matrix of the gravid reproductive tract of the cow, particularly during late gestation, to support the increasing foetal load (Kaidi et al., 1995). Rapid and extensive degradation of this collagen after birth causes the decrease in uterine size and weight that occurs during postpartum involution (Kaidi et al., 1995). Urinary excretion of the pyridinium cross-links, a biochemical marker of collagen degradation, has been reported to increase during the period of postpartum uterine involution in dairy cows (Kaidi et al., 1991, Liesegang et al., 2000). Therefore, a marker of collagen catabolism may provide an useful means to monitor postpartum uterine involution in a study of the process in different strains of animals.

Pyridinium cross-links are small molecules that are found in mature, fibril-forming collagens and link two of the three non-helical, amino-terminal or carboxy-terminal ends of one collagen molecule to a site on the helical portion of an adjacent collagen molecule (Eyre, 1996). When collagen molecules are cleaved, these cross-links are released while still connected to peptide fragments, which may be further be cleaved in the liver or kidneys. The cross-links themselves, however, are not metabolized and are excreted unchanged (Eyre, 1996). Consequently, the measurement of urinary cross-links excretion has been developed and extensively studied as a clinical tool to investigate collagen breakdown (e.g. during tumor formation) in humans.

The technique of HPLC has been regarded as the 'gold standard' for measuring pyridinium cross-links. In one study on uterine involution in sows, urine samples were collected and a similar ELISA Kit was used in this study and validated using HPLC as a cross-reference. The HPLC estimates of the concentration of total (free and peptide-

bound) collagen cross-links hydroxylysyl pyridinoline, and lysyl pyridinoline in the urine samples were highly correlated (P<0.001) to that of the ELISA estimates of the concentrations in the urine samples (Belstraa et al., 2005). Similarly, human studies have found comparable performance between immunoassays and the HPLC technique (Gomez et al., 1996).

In many instances, the subtle changes in the uterus in terms of changes in size are difficult to recognize by rectal palpation alone. The ELISA used in the present experiment represents a reliable means to measure collagen cross-links in cow urine based on the parallelism of serial dilutions of cow urine to the manufacturers' standard curves. Such a novel method constitutes a simple, yet highly accurate, repeatable, and non-invasive means to monitor uterine involution in the cow. Therefore, a powerful new useful research tool that would provide researchers with a novel, easier yet accurate tool to study the different factors that interplay in the uterine involution and its effect on fertility. Such a tool could have potential management applications in the future.

The present experiment therefore provides novel data on urinary excretion of pyridinium cross-links by postpartum cows that are consistent with increased catabolism of uterine collagen over the postpartum period. The results generally agreed with literature from other species and with the results of the cervical measurements experiment data.

Data on the excretion of pyridinium cross-links were correlated with the PGFM results and cervical measurements. Cervical measurements did not reveal any significant difference in the rate of involution over the entire period between the two strains. Although there were no statistically significant differences between strains in the data for excretion of pyridinium cross-links, there was a trend (P=0.07) for values

in L cows to be higher than in H cows on Day 11 past partum. A study of larger numbers of cows might allow significant differences to be observed. Nonetheless, it was of note that PGFM concentrations also tended (P=0.10) to differ on Day 11 as well. This result agrees with literature in which higher PGFM concentrations are correlated with normal uterine involution, especially in the first two weeks postpartum (Edqvist et al., 1978, Eley et al., 1981, Lindell et al., 1982).

4.6 General conclusion

Despite the significantly higher bacterial contamination (aerobic on Days 7 and 10; anaerobic on Days 4 to 10) in H than L cows, PGFM concentrations decreased earlier (Day 11) and reached basal values earlier (Day 17) in H than L cows. On the other hand, on Days 17, 28 and 35, L cows had significantly higher PGFM concentrations than H cows. Moreover, H cows have a significantly greater bacterial contamination than L cows. Perhaps such data might suggest that the immune system of H cows was less able to efficiently eliminate the postpartum contamination than was that of L cows.

On the other hand, there were no significant differences (P>0.5) in terms of physical involution (as determined by cervical diameter and urinary collagen crosslink excretion). Whether it is possible to explain the differences in ovarian activity between the strains that were observed by Thiengtham (2004) remains open to conjecture. At a more basic level, however, it seems likely from previous studies of the contribution of chronic/subclinical uterine damage/infection to the repeat breeder syndrome, that the increased levels of bacterial contamination in H than L cows may well have contributed to the lower fertility of the former animals. The lower PGF

concentrations of H than L cows are a further part of the evidence from the present study that this may indeed be the case. Whether such greater levels of bacterial contamination resulted from interrelationships between PGF, physical and/or tissue-level involution and the resumption of ovarian activity remains unresolved; however the present study contains at least circumstantial evidence that this may be so. Further investigation of the genesis of uterine PGF secretion in the two strains would be of great interest in this context.

The novel use of the collagen breakdown products, pyridinum cross-links, opens a new dimension of rather easier and more accurate method of investigating uterine involution in cattle. The availability of an ELISA for these substances will make further research in this area much easier yet accurate than was the case when using HPLC. Many of the questions raised by the present study could be investigated further using this method.

In summary, it can be concluded that whilst H cows exhibited similar physical involution characteristics to those of L cows, they had higher levels of post-calving bacterial contamination and lower levels of PGF secretion during involution than did L cows. Both of these characteristics are likely to contribute to poorer involution and/or a greater risk of damage to the endometrium during the postpartum period. As such, it is probable that they would contribute to the lower fertility in the animals with the higher proportion of overseas genetics.

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