REPRODUCTIVE PERFORMANCE OF HOLSTEIN-FRIESIAN COWS GENETICALLY SELECTED FOR HEAVY OR LIGHT MATURE BODYWEIGHT

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ABSTRACT

Over recent years, concern has developed over the declining fertility and survival of New Zealand dairy cows in parallel with the increasing proportion of US Holstein-Friesian genetics in the national herds. A long-term trial at Massey University has shown that in a spring-calving pastoral systems cows selected for high mature bodyweight (H), which have a high proportion of US Holstein genetics, had lower first service conception rate (FSCR) than cows selected for low mature bodyweight (L), which have a low proportion of US Holstein genetics. The experiments in this thesis were conducted to examine whether differences in the activity of the central and peripheral reproductive endocrine axes during the postpartum periods of H and L cows might underlie the different FSCR between strains. Analysis of herd records over 3 years showed that L cows had higher ($P<0.05$) FSCR (60%) than H cows (47%), but there was much variation in FSCR in H between years. No differences were found between strains in the intervals from calving to first ovulation and calving to first behavioural oestrus.

Experiment 1 examined the endogenous LH and FSH secretion patterns in H and L cows (n=7/group) on Days 14, 21, 28 and 35 after calving, in order to evaluate the time-course of postpartum restoration of the hypothalamo-pituitary axis. The overall mean amplitudes of LH episodes were greater ($P<0.01$) in H (0.33 ± 0.02 ng/ml) than L cows (0.27 ± 0.02 ng/ml). In anoestrous cows, LH concentrations and episode amplitudes were greater in H than L cows. However, patterns of LH secretion were identical between strains during the mid luteal phase.

Changes in LH responses to a GnRH agonist (buserelin) were studied in mixed age and 2 year-old cows after calving (Experiment 2). LH responses to buserelin (10μg/iv) on Days 21, 28, 35 and 42 after calving were greater ($P<0.001$) in L than H cows, but that there was no change in responses with time (Experiment 2a). Responses to buserelin were therefore studied on Days 7, 14, 21 and 28 after calving using mixed-age H and L cows (Experiment 2b, n=7/group) and first calved 2 year-old L and H cows (Experiment 2c; H: n=6 and L: n=7). LH responses increased significantly ($P<0.01$) as time postpartum increased, but there were no differences between LH responses in L and H cows in either Experiments 2b or 2c.

The positive feedback effect of oestradiol benzoate (1 mg, i/m) upon LH was examined in groups of 12 L and H cows on Days 7 and 21, or on Days 14 and 28 after calving (Experiment 3). LH responses to oestradiol increased ($P<0.05$) and FSH responses ($P=0.07$) tended to decrease as time postpartum advanced, but there were no significant differences between strains in the responses. The results from Experiments 1-3 showed that the intrinsic activity of the hypothalamo-pituitary axis is similar between strains during the postpartum period.

Patterns of ovarian activity were examined by determining progesterone concentrations in thrice weekly milk samples collected between calving and the end of the mating period (Experiment 4). The percentage of cows that started to cycle within 21 days after calving was significantly ($P<0.05$) higher in Year 2 (78%) than in Year 1 (28%), but there were no differences between strains. No were there any differences between strains in the incidence of atypical ovarian patterns. Between Days 1 and 7 after insemination, progesterone concentrations were identical in H and L cows that conceived (P) or failed to conceive (NP). Progesterone concentrations in non-pregnant, H (HNP) cows declined earlier than in L cows during the late luteal phase. Progesterone concentrations were significantly ($P<0.05$) lower in HNP cows on Day 16 than in all other animals and lower in HNP than HP cows on Day 14 ($P<0.05$). This decline may be the result of either premature luteolysis or luteal inadequacy.

Studies of follicular and luteal activity between Day 5 postpartum and the second behavioural oestrus or the planned start of mating (i.e. during the pre-mating period: Experiment 5) showed that large follicles emerge earlier in H than in L cows, corresponding to the earlier resumption of oestrous cycles found in Experiment 4. H cows tended to have more cycles in which the interval from heat to ovulation was $\geq$48 h ($P=0.06$) and fewer cycles with three follicular waves ($P=0.12$) than L cows. CL size increased significantly with time after ovulation ($P<0.001$), and tended ($P=0.08$) to be smaller in the mid luteal phase of H than L cows. Progesterone concentrations and luteal size of H cows reached maxima earlier (Day 11.4) than in L cows (Day 12.6); as also occurred in Experiment 4. Progesterone concentrations declined 1.3 days earlier in H cows than L cows, and more H cows had progesterone concentrations of <2 ng/ml three or four days before the next ovulation than did L cows. These observations suggest that there are differences in the timing of the onset of luteolysis between strains, which may significantly affect the fertility in H cows.

In conclusion, the results from this thesis suggest that differences in fertility between strains probably do not lie in the areas of the hypothalamo-pituitary function, although the finding that follicular activity commences earlier in H cows may be related to the higher levels of endogenous LH secretion in anoestrous H than L cows. There were, however, differences in ovarian and luteal function between H and L cows which could explain the observed differences of fertility. The mechanisms by which these differences of ovarian and luteal function contribute to the differences in fertility between the strains and the way such mechanisms relate to the genetic differences between the strains, requires further investigation.

DEDICATION

I dedicate this thesis to my mother, the late Sew Kim Thiengtham, who had gone through lots of difficulties just to provide me with a sound fundamental education. She did not hold any university degree, but totally believed in the value of education for a better future of her children.

Her persistence and determination in all what she did always inspire me in pursuit of the excellence. Her unconditional love, example, attitude and good memories about her have been pushing me and providing strength for me to press on.

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LIST OF ABBREVIATIONS

AI	artificial insemination
AP	the anterior pituitary
AUC	area under the curve
BCS	body condition score
bTP-1	bovine trophoblastic protein
CL	corpus luteum
d	day
DF	dominant follicle
E_2	Oestradiol-17β
FSCR	first service conception rate
FSH	Follicle stimulating hormone
GnRH	Gonadotrophin releasing hormone
H	Heavy strain
h	hour
hCG	human chorionic gonadotrophin
IFN-τ	Interferon-tau
IGF-I	Insulin-like growth factor I
L	Light strain
LH	Luteinising hormone
LLC	large luteal cells
LW	liveweight
mg	milligram
min	minute
ml	millilitre
mm	millimetre
NEB	negative energy balance
ng	nanogram
NZ	New Zealand
OHF	overseas Holstein Friesian
OT	oxytocin
OTR	oxytocin receptor
P_4	Progesterone
PG	Prostaglandins
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PGI_2	Prostaglandin I_2
SLC	small luteal cells
wk	week
μl	microlitre

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1 Chapter 1: Literature review

1.1 Introduction

Reproductive performance of dairy cows is a key determinant of production efficiency and profitability in seasonal and non-seasonal dairy production systems, because it affects milk yield, number of replacement heifers, genetic progress and involuntary culling rate (Gröhn and Rajala-Schultz, 2000; Xu and Burton, 2000). The economic losses related to poor fertility of the herd have been very well documented (Esslemont and Peeler, 1993).

The majority of New Zealand (NZ) dairy farms operate under a low cost system, in which the herds have a single concentrated seasonal calving pattern (Holmes *et al.*, 2002a). The calving period is planned to start between late winter and early spring and lasts for only 10-12 weeks each year (Macmillan *et al.*, 1984a; 1990; Holmes, 2001). This allows the herd to match feed demand with feed supply, mainly from pasture (Macmillan *et al.*, 1984a; Holmes, 2001). In this type of herd management system, it is critical that the cows are fertile, because cows need to achieve a high pregnancy rate in a very short mating period (Holmes, 2001) and a herd's calving pattern will reflect the submission rate and conception pattern during the previous season's mating programme (Macmillan *et al.*, 1984b, 1985b; Xu and Burton, 1996). Thus, in order to survive in the herd, a cow has to be able to cope with the stress of producing considerable amount of milk from a grass diet and still conceive in time to calve during the same month next year. Likewise, the replacement heifers have to reach puberty and be ready to be mated by the age of 15 months and calve by two years of age (Holmes *et al.*, 2002a).

There is evidence that selection primarily for higher milk yield generally brings about lower reproductive performance in dairy cows. Moreover, high milk yield is associated with high risk factors for several reproductive disorders, lower first service conception rate, delayed insemination and conception and increased risk of involuntary culling in dairy cows (Faust *et al.*, 1988; Nebel and McGilliard, 1993; Larson *et al.*, 1997; Gröhn, and Rajala-Schultz, 2000).

Fertility of NZ dairy cows is relatively higher than that of cows overseas (Grosshans *et al.*, 1996; Mee, 2000). However, with considerable amounts of overseas genetics recently introduced into the national breeding programme (in order to increase genetic potential for yield of milk and protein in particular) there is concern over the declining survival rate and

reproductive performance, (as measured by conception rate to first service) of NZ dairy cows in association with genetic improvement in yield (Burton *et al.*, 1999; Harris and Kolver, 2000; Verkerk *et al.*, 2000; Harris *et al.*, 2001). Similar trends have been reported elsewhere (for example in the Netherlands: Hoekstra *et al.*, 1994, UK: Lamming *et al.*, 1998; Royal *et al.*, 1999, Ireland: O'Farrell *et al.*, 1997; Dillon *et al.*, 2001, Langhill, Scotland: Dillon *et al.*, 2001; Australia: Fulkerson *et al.*, 2001). These problems stem from the fact that the Holstein-Friesian cows derived from overseas sires have been selected for production performance in all-year-round calving and concentrate-based, high input dairy systems. In such production systems, reproductive performance is less important than in seasonal pasture-based systems. Thus, there is concern over the suitability of these cows for seasonal pasture-based feeding systems (Harris and Winkelman, 2000).

Increased proportions of North American Holstein genotypes in NZ dairy cows have not resulted only in increased milk yield but also in increased body weight. Recent studies at Massey University on reproductive performance of Holstein Friesian cows genetically selected for heavy (H) and light (L) mature bodyweight have shown that H cows had a lower conception rate to the first service, but a shorter interval from calving to ovulation than L animals (García-Muñiz, 1998; Laborde *et al.*, 1998b). The reasons for these differences are unknown.

However, it is of importance to note that the H cows in the Massey study have higher proportion of overseas genetics than the L counterparts. Detailed information on the breeding strategy for these two groups of animals has been reported elsewhere (Garcia-Muniz, 1998). Further detailed investigations into this area may provide insight to better understanding of the reasons behind the declining of fertility in cows with a high proportion of overseas genetics under seasonal pasture-based systems. In addition, understanding of the neuroendocrine and endocrine factors that control reproductive events in these animals may facilitate the construction of strategies aimed at improving their fertility under the NZ dairy production system.

1.2 Reproductive performance of dairy cows in NZ dairy system

Seasonal calving, pasture-based, dairy herd management systems require the herds to operate at high reproductive rates and to achieve compact calving patterns (Holmes *et al.*, 2002a). This can be partly achieved by minimising the proportion of cows that fail to conceive during the mating period (Macmillan, 1985b), since the cows that calve later

during the planned calving period are likely to produce less milk, conceive later during the mating period, and so to calve late next year (Macmillan, 1979, Holmes, 1986) with a higher chance of being involuntarily culled (Harris, 1989). Consequently, it has been shown recently that late calving and anoestrus are main contributors to poor in-calf rate in NZ dairy herds (Xu and Burton, 2000). Thus, low fertility rates or low in-calf rates will have carry-over effects on fertility, the herd's calving pattern and productivity in subsequent years (Macmillan *et al.*, 1984b; 1985b; 1990). Interestingly, fertility traits are used as an indirect selection tool at farm level, so as the cows fail to conceive during mating period, they will be culled to maintain a 365-d calving interval (Harris and Kolver, 2001).

Common measures designed to maximise reproductive performance in seasonal dairy herds have been described in detail by Macmillan (1985b); Williamson (1998) and Holmes (2001). The calving pattern reflects the submission rate and conception pattern during the previous season's mating (Macmillan *et al.*, 1984b; Macmillan, 1985b; Crosse *et al.*, 1994), highlighting the importance of these two parameters in determining fertility in the herd (Xu and Burton, 1996). Submission rate, defined as the percentage of the herd that has been mated during a specific period of time after mating starts (Holmes *et al.*, 2002a), is related to the interval between the actual calving dates and the start of the mating period, the postpartum anoestrous period (calving to first ovulation) and oestrus detection rate (Ryan and Mee, 1994). To achieve a high submission rate, all cows should resume their oestrous activity before the start of the mating period and be mated at the right time (Holmes *et al.*, 2002a). The success of mating can be determined by the percentage of mated cows that conceived and did not return to oestrus. In other words, the pregnancy rate or in-calf rate is defined as the number of cows which successfully become pregnant by specific dates (e.g. the percent diagnosed pregnant 3 to 7 weeks after the start of mating; Williamson, 1998; Holmes *et al.*, 2002a). First service conception rate is a function of nutrition, management, AI technique and heat detection rate and accuracy (Macmillan, 1985b; Ryan and Mee, 1994). It is also positively correlated to the number of days from calving and the number of ovarian cycles preceding the service (Macmillan and Clayton, 1980; Visser *et al.*, 1988; Butler and Smith, 1989). Even through culling cows which conceive late in the mating season can improve the calving pattern (Williamson, 1998), it is vital that compact calving is not achieved by high culling rates for infertility, as this proves to be costly (Crosse *et al.*, 1994).

Current selection for milk yield in the NZ dairy system, related to the use of overseas Holstein Friesian genetics (OHF; Harris and Winkelman, 2000), continues to change the genetic composition of the national herd (Holmes *et al.*, 2002b). The consequences of this practice include increases in milk production, feed intake and body size; and decreases in body condition score (BCS), fertility and longevity (Harris and Winkelman, 2000; Kolver *et al.*, 2000; Verkerk *et al.*, 2000; Harris and Kolver, 2001). The latter problems may be related to the ability of these high OHF genetic cows to undergo a high degree of nutrient partitioning in favour of lactation and body tissue reserve mobilisation during early lactation (Bauman and Currie, 1980; Veerkamp *et al.*, 1995; Fulkerson *et al.*, 2001). In addition, under pasture-fed conditions, quantity and quality of pasture together with the ability of these high OHF genetic cows to consume sufficient feed required to meet their genetic potential to produce milk and to maintain body condition (i.e. their grazing ability: Holmes *et al.*, 2002b) may worsen the problem. Negative energy balance, losing BCS and metabolic disorders during early lactation are major causes of subfertility in high producing cows at pasture (Buckley *et al.*, 2000).

Moreover, breeding values estimated in one production system may not be good predictors of merit in the other (Cromie *et al.*, 1998). Consequently, there is concern about the effect of the interaction between genotype and environment on milk production and functional traits in NZ dairy systems (Holmes *et al.*, 2002b). Indeed, genotype x feeding systems (grazing and total mixed ration) interactions seem to exist with regard to milk production and survival (Kolver *et al.*, 2000; Westwood *et al.*, 2002) and also with the incidence of mastitis (Lacy-Hulbert *et al.*, 2002). A significant interaction between genetic merit and level of feeding on pregnancy rate of dairy cows was reported recently (Fulkerson *et al.*, 2001). The effects of genotype (strain) and feeding system interactions on health, fertility and survival largely remain to be defined for the NZ system. Such information may help to make sensible decisions in the selection of suitable cows for NZ seasonal grazing systems.

1.3 Reproductive performance of heavy- and light-strain cows

Recent studies at Massey University on the impact of genetic selection for mature body weight in dairy cows in seasonal calving, pasture-based management system (García-Muñiz, 1998; Laborde, 1998; Caicedo-Caldas, 2000) have revealed similar trends to those in studies of all-year round calving systems in the US (Hansen *et al.*, 1998; 1999). Selection for production traits increases skeletal size (Mason *et al.*, 1957), but at the same

time there is a substantial genetic antagonism between milk yield and body weight and services per conception (Badinga *et al.*, 1985). Even so, milk yields were similar in large and small lines of cows in the US trial of Hansen *et al.* (1998), despite a marked difference in weight between the strains. By contrast, García-Muñiz (1998) and Lopez-Villalobos *et al.* (2001) found that the H cows, which had a higher proportion of North American genetics, were heavier and produced more milk than the L cows. The differences between the two studies may have been due to differences in selection objectives between them.

There have been conflicting results for feed intake capacity of H and L cows. It was found that both strains had similar feed intake capacity and lactation curves under grazing conditions (Caicedo-Caldas *et al.*, 2001; Lopez-Villalobos *et al.*, 2001). Surprisingly, Laborde (1998) found that H cows had similar energy balance status and body condition during the early postpartum period, but H cows achieved higher pasture intakes and had different grazing behaviour from L cows (Laborde *et al.*, 1998a, Caicedo-Caldas *et al.*, 2001). The reason of this discrepancy is unknown.

Puberty age and weight

There is limited evidence about differences in weight and age at puberty of H and L heifer calves. García-Muñiz (1998) found that H heifers were heavier and older at puberty than L heifers. The percentage of H heifers that reached puberty at 12 months (83%) was significantly lower than that of L heifers (94%; García-Muñiz, 1998). Working with OHF and NZ high (H) and low (L) genetic merit heifers, McNaughton *et al.* (2002) also found that OHF heifers were heavier and older than NZL and NZH heifers at puberty and that more NZH than OHF heifers reached puberty by planned start mating. However, no comparative endocrine studies have been undertaken in H and L heifer calves around the onset of puberty.

Reproductive outcomes after calving

Oestrous cycles commence sooner after calving in H cows than L cows (Laborde *et al.*, 1998b). García-Muñiz (1998) reported shorter intervals from first service and the start of mating to conception in the L cows than in the H cows. In addition, Verkerk *et al.* (2000) also reported a shorter interval from calving to first postpartum ovulation in OHF cows than in NZ cows. It is evident that the introduction of overseas genetics into the herd is not associated with an extended duration of postpartum anovulatory anoestrus, yet a problem of subfertility in modern cows due to the increased prevalence of a prolonged anoestrous

period after calving is also evident (Macmillan *et al.*, 1996; Opsomer *et al.*, 1998). Therefore, the relationship between postpartum cyclic activity and reproductive performance is debatable and requires further investigation.

More importantly, the main finding from the studies with H and L cows is the higher conception rate to first insemination in L than H cows (García-Muñiz, 1998; Laborde *et al.*, 1998b), resulting in extended conception and calving patterns in the H cows. This finding confirms the contention from the US strain trial that smaller cows required fewer services to conception (Hansen *et al.* 1998). This may subsequently impact upon submission rates, calving pattern, days in milk, milk yield, culling rate and longevity of the mature cows.

Reproductive endocrine differences between H and L cows

There is no information about whether there are differences in reproductive endocrine activity between H and L cows, particularly during early lactation until the resumption of cyclicity. It is envisaged that future studies in this aspect may lead to a better understanding of the problems related to declining in fertility in high genetic merit dairy cows that are evident around the world. It should also be noted that the implications of the findings available from the studies conducted in all year round calving and concentrate feeding systems may not be applicable in the NZ context.

In a comparative study between US and NZ lactating cows, Bilby *et al.* (1998) found that circulating progesterone concentrations after administration of Controlled Internal Drug Release (CIDR) devices were inversely related to bodyweight and milk yield. This suggests the possibility of the differences in the metabolic clearance rate of progesterone in these groups of cows. However, there is no information regarding the pattern of P4 during the early postpartum period in H and L cows.

Pancarci (1999) found the oestrous cycle characteristics, follicular wave patterns and follicular populations in genetically high-producing cows were virtually identical to those in their low-producing counterparts. Bilby *et al.* (1998) also found that US cows had greater follicular growth but slower follicular turnover and tended to have lighter Corpora lutea (CLs) than NZ cows. However, Verkerk *et al.* (2000) could not find genetic effect on follicular characteristics when compared between OHF and NZ cows. Laborde (1998) reported that ovulatory follicles were larger in synchronised H cows than those in the L counterparts. Hence, there could be genetic differences in ovarian follicular function

and/or in the interactions between luteal function and conception success in H and L cows. However, further studies in the area of ovarian follicular dynamics, luteal function and P4 profiles in H and L cows after calving are required before such a conclusion can be drawn with confidence.

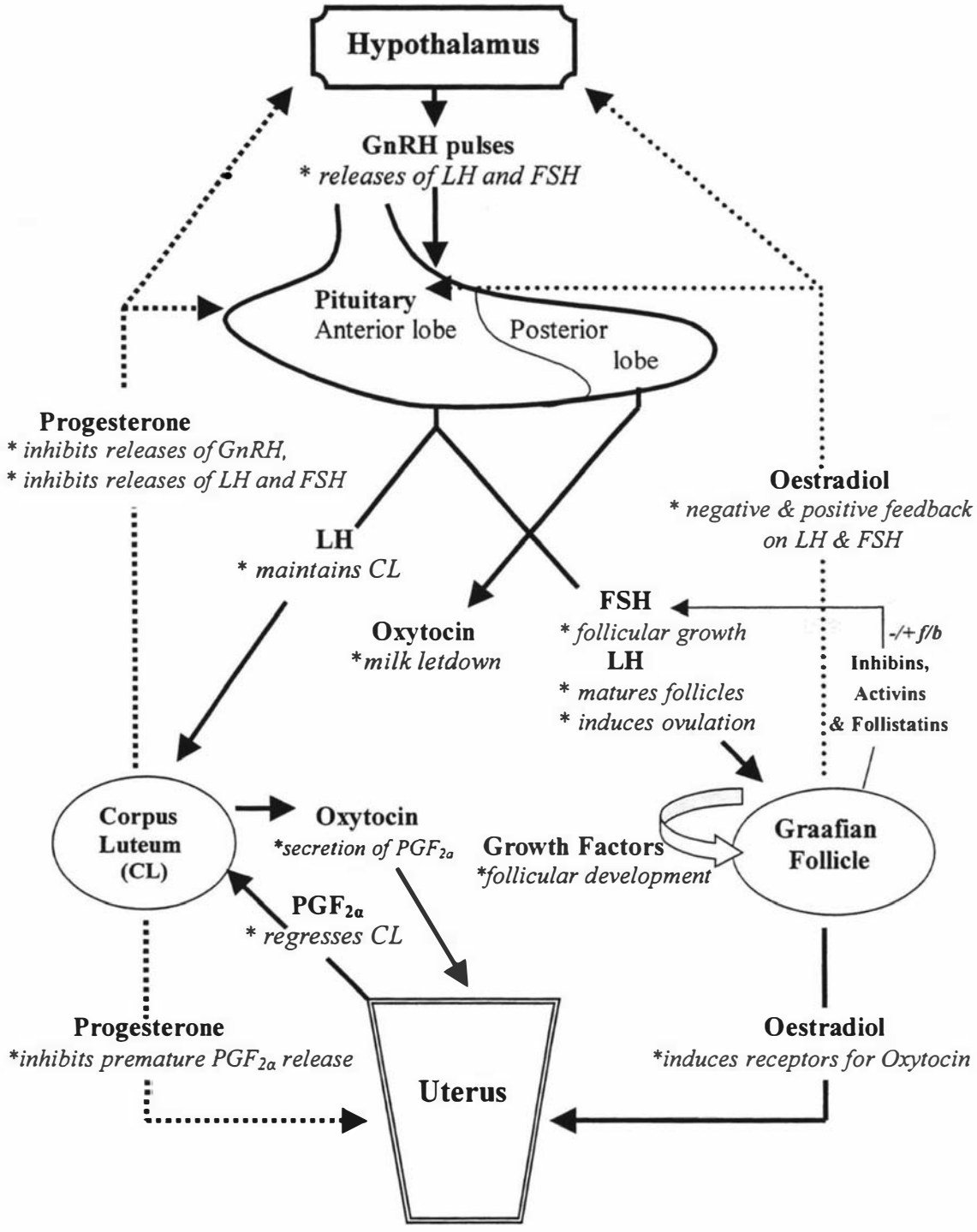
Taken together, these results emphasise the need for further studies of the reproductive endocrine system to clarify the underlying mechanisms that are involved in these differences between H and L dairy cows. There is very limited evidence available concerning the reproductive endocrine patterns of dairy cows under NZ dairy system. Therefore, it needs to be identified whether there are any differences in the reproductive endocrine system between these two lines of cows, and whether these differences are due to genotype *per se*, management factors or genetic x environment interactions.

1.4 Neuroendocrine regulation of gonadal function in the cow

During the past three decades, a large body of evidence has accumulated regarding bovine reproductive physiology and endocrinology. The complex systems regulating bovine reproductive function and activity will be described in this chapter. Reproductive activity in female mammals is governed by neuroendocrine mechanisms involving two main components viz. the central nervous system (CNS) and the peripheral endocrine system (Karshch, 1984; Hafez *et al.*, 2000). The CNS component perceives and integrates information from external and internal environments, such as light, temperature, stress, food supply, suckling stimuli, social cues and endocrine signals, and transforms it into chemical massages or hormones to regulate gonadal functions (Edqvist and Stabenfeldt, 1993a; Hafez *et al.*, 2000). The endocrine system operates via blood-borne hormones and feedback loop mechanisms (Hafez *et al.*, 2000). In addition, a complex of internal autocrine and paracrine mechanisms influencing the anterior pituitary and gonadal functions has been one of very rapid development in reproductive biology of farm animals, but will not be covered in detail in this review.

The main interactions between the hypothalamo-pituitary axis and the ovary are illustrated in **Figure** 1-1.

Figure 1-1: Schematic summary of the hormonal interactions of the hypothalamo-pituitary-gonadal axis controlling reproductive activity in cows



1.1.1 Hypotahlamic secretion of gonadotrophin–releasing hormone (GnRH)

In response to neural signals, hypothalamic nerve cells release a decapeptide neurohormone, gonadotrophin–releasing hormone (GnRH; also previously known as luteinising hormone releasing hormone; LHRH), in a pulsatile manner into the hypothalamohypophyseal portal vessels, for transport to the secretory cells of the anterior pituitary (AP) lobe (Hartigan, 1992; Edqvist and Stabenfeldt, 1993a). This neurohormone is synthesised by neurones whose cell bodies lie within the arcuate nucleus of the hypothalamus. It is then stored in the axons in the medial basal hypothalamus (Hartigan, 1992, Leshin *et al.,* 1992). It exerts stimulatory actions (Braun *et al.*, 1985) by binding to specific receptors on anterior pituitary gonadotroph cells, controlling release of the gonadotrophins (luteinising hormone (LH) and follicle stimulating hormone; FSH), into the circulation (Hartigan, 1992; Turzillo and Nett, 1999).

The synthesis and episodic release of GnRH from GnRH neurons is controlled by a complex integrated system located in the CNS called the *oscillator* or *pulse generator*, which involves various neurotransmitters, opiate peptides, hormonal and neural feedback mechanisms and endogenous hypothalamic and brain rhythms (Karsch, 1984; Dyer, 1988). The nature of the GnRH pulse generator activity within the hypothalamus has been the focus of neurophysiology research for decades. Major findings have been made in primate, rodent and non-bovine ruminant species. In goats, Mori *et al.* (1997) described an electrophysiological approach to record the characteristic increases in neuronal activity related to pulsatile LH secretion using a multiple unit activity (MUA) recording technique adapted from the methods used in monkeys, rats and sheep. These MUA volleys were associated with GnRH pulse generator activity and LH secretion (Tanaka *et al.*, 1992).

There seem to be at least two subpopulations of neurons regulating GnRH secretion (Mori *et al.*, 1997). The first group resides in the median basal hypothalamus (MBH), which may regulate pulsatile LH and FSH secretion from the pituitary by influencing either/or the amplitude and the pulse frequency of these two hormones (Edqvist and Stabenfeldt, 1993b). The second group resides in the preoptic area, which is responsible for the preovulatory GnRH surge (Tanaka *et al.*, 1992). Interestingly enough, Kaneko *et al.* (2002) recently found that the preovulatory LH surge, but not pulsatile LH secretion, was suppressed in cows immunised against oestradiol. As this also induced follicular cysts, this finding indirectly suggests differential functional actions of a GnRH surge generator and a GnRH pulse generator in the bovine hypothalamus.

The pattern of GnRH secretion in the cow has not been very well documented. However, using surgical cannulation of the third ventricle for frequent collection of cerebrospinal fluid (CSF) from mature ovariectomised cows, Gazal *et al.* (1998) demonstrated that the pulsatile secretion pattern of GnRH in the third ventricle CSF is highly correlated with pulses of LH and with the preovulatory LH surge. This observation concurs with those made in ewes (Skinner *et al.*, 1997). Yoshioka *et al.* (2001), using a technique similar to that described by Gazal *et al.* (1998) in intact heifers, found that about 80% of the GnRH pulses identified in CSF coincided with a pulse of LH during pro-estrous and the early luteal and mid-luteal phases. These results provide clear evidence that, during the reproductive cycle of cattle, changes in LH secretion patterns in serum are solely the consequence of pulse patterns of GnRH released into the CSF. Therefore, LH secretion can be used as an indirect index of GnRH release (Clarke and Cummins, 1982; Edqvist and Stabenfeldt, 1993b).

Self-priming effect of GnRH

GnRH is capable of priming the anterior pituitary (AP) to augment GnRH stimulation. Foster (1978) found that by giving two synthetic GnRH injections to the cows 1.5 h apart, the total LH release after the second injection (as indicated by the areas under the peak) was greater than that after the first injection. In prepubertal heifers receiving hourly GnRH injections, MacDonald and Page (1986) found that the magnitude of LH release after the third GnRH injection was greater than that of the second injection. Padmanabhan *et al.* (1981) reported similar results *in vitro*. This self-priming effect, via a direct effect on pituitary cells, may play a vital role in preparing the AP to release gonadotrophin at the pubertal oestrus and at the first postpartum oestrus (Padmanabhan *et al.*, 1981). Increased pulsatile stimulation of GnRH may also play a role in up-regulation of the AP GnRH receptors during the preovulatory period and, consequently, increases LH release in response to further GnRH stimulation leading to the generation of the LH surge (Turzillo and Nett, 1999).

1.1.2 Pituitary gland and hypothalamo-pituiatry axis

The pituitary gland is divided into three parts, the anterior, intermediate and posterior lobes (Karsch, 1984; Hafez *et al.*, 2000). The AP gland connects to the median eminence within the hypothalamus by a venous portal system, in which neuronal messages from hypothalamus, including GnRH, change into blood-borne humoral messages (Karsch,

1984; Edqvist and Stabenfeldt, 1993b). In the anterior lobe, five cell types are responsible for secreting six different hormones (Hafez *et al.*, 2000). The basophilic cells or gonadotrophs contain granules full of gonadotrophins, so are responsible for producing and secreting both LH and FSH.

Hormones released from the posterior pituitary gland (i.e. oxytocin (OT) and vasopressin) are not produced within the gland but produced via direct neural links with the hypothalamus (Hartigan, 1992; Edqvist and Stabenfeldt, 1993b). OT, which is synthesised in the paraventricular and supraoptic nuclei of the hypothalamus, is transported to and stored in the posterior pituitary, and acts (i) upon myoepithelial cells in the mammary gland in response to sucking stimuli, to eject milk and (ii) to control uterine contraction in response to tactile stimulation of the reproductive tract during parturition (Edqvist and Stabenfeldt, 1993a, b).

The regulation of the pituitary gonadotrophin secretion in the female animals involves complex neurotransmitter and neuromodulator systems in the hypothalamus. As mentioned above, GnRH pulsatility is of importance for maintaining different aspects of gonadotrophin secretory function, including pituitary GnRH receptor up-regulation, subunit gene expression, and LH and FSH storage and secretion (Molter-Gérard *et al.*, 1999; Vizcarra *et al.*, 1999). In sheep, different GnRH pulse frequencies have been shown to selectively modulate patterns of gonadotrophin storage and secretion in gonadotroph cells (Molter-Gérard *et al.*, 1999). Low GnRH pulse frequency boosts FSH storage, although the mechanism involved in this phenomenon has not been fully elucidated. In ovariectomised ewes with a hypothalamo-pituitary disconnection, LH was undetectable in plasma, but when given exogenous GnRH, LH pulse amplitudes were directly related to the amount of releasable LH in the AP (Clarke and Cummins, 1985). The size of this LH pool and the amplitudes of LH pulse are dependent upon the frequency of GnRH pulses. Hence, when the frequency of GnRH pulses is decreased the amplitude of LH responses increases in proportion to the size of the releasable LH pool in the AP (Clarke and Cummins, 1985). As in sheep, LH and FSH secretion and the ratio of LH to FSH in cows are differentially regulated by the duration, frequency and dose of exogenous GnRH pulses (Vizcarra *et al.*, 1999).

Because the FSH secretion pattern is less obviously pulsatile than that of LH (Webb *et al.*, 1980), the presence of separate releasing factors for LH and FSH has been postulated from time to time. In heifers treated with an LHRH antagonist, LH secretion is suppressed to a

greater extent than FSH secretion (Fike *et al.*, 1997; Ulker *et al.*, 2001), suggesting that there is divergent regulation of LH and FSH secretion by LHRH. However, the ability of LHRH antagonists to suppress LH secretion is due to direct blocking of secretion from the AP, whereas altered FSH secretion in these animals may be determined by a change in ovarian follicular dynamics leading to subsequent changes in the timing of changes in FSH secretion (Fike *et al.*, 1997). In cattle, LH and FSH are released differentially in response to GnRH challenges (Schams *et al.*, 1974), further suggesting the presence of different releasing mechanisms for these two hormones. It is, therefore, clear that FSH synthesis is fully under GnRH control, but the extent to which its secretion depends upon GnRH pulses requires further investigation. In addition, at specific stages of the reproductive cycle, the involvement of other factors may account for some of the differential responses of LH and FSH to GnRH. Inhibins, activins and follistatins are examples of such factors (Findlay, 1993; Kaneko *et al.*, 1993; Padmanabhan *et al.*, 2002). The different half-lives of LH and FSH may also account for divergent secretion pattern of these hormones (Clarke, 1989).

Cellular hormonal signal transfer

The endocrine signal perceived by the target tissue relies mainly on the characteristics of the signals, as determined by their amplitude, duration, frequency and form. Also, only cells that possess specific receptors to bind a particular hormone can detect its signal. The activated membrane receptors then initiate signal transduction cascades that lead to changes in enzyme activities, gene expression and cellular function (Hartigan, 1992). The polypeptide hormones (e.g. GnRH) and glycoprotein hormones (e.g. LH, FSH) bind to receptors located on the cell membrane and activate a second messenger (e.g. cyclic adenosine monophosphate; cAMP or phosphoinositide) that either triggers the release of stored hormone from cells or promotes the synthesis of new proteins (McLeod and Phillips, 1998). Conversely, the steroid hormones (e.g. oestrogen (E2), progesterone, androgens) diffuse passively into the target cells and interact with receptors which are located within the nucleus or cytoplasm of the cell. In the latter case, the hormone-receptor complex then crosses the membrane into the nucleus, where it interacts with chromosomes to activate synthesis of the new proteins that elicit the characteristic response to that hormone (Hartigan, 1992; McLeod and Phillips, 1998). The biological responses of a target cell to a particular hormone are controlled by the concentration of circulating hormone, the concentration of receptors and the affinity of the hormone-receptor

interaction (Ojeda, 2000). The sensitivity of a target cell to a hormone depends on the number of specific receptors available for binding to that hormone (Hartigan, 1992). The hormone concentrations in circulation at particular time, therefore, reflect the functional capacity of the gland in which they are synthesised and secreted, since the metabolic clearance rate is relatively stable (Edqvist and Stabenfeldt, 1993a).

Endocrine signals pulse and surge

In ruminants, two functionally distinct modes of LH secretion occur. Firstly, *episodic/tonic* LH secretion occurs during the oestrous cycle and is vital for ovarian steroidogenesis and corpus luteum (CL) maintenance (Rahe *et al.*, 1980; Hartigan, 1992). This type of LH secretion is evident as a sudden release into circulation followed by decaying concentrations as the hormone is eliminating from the circulation (Rahe *et al.*, 1980; McLeod and Phillips, 1998). Secondly, *the preovulatory LH surge*, which is defined as a sustained and massive outflow of LH from the AP (Rahe *et al.*, 1980; Hartigan, 1992), occurs during oestrus and is involved in the final maturation of the oocyte, ovulation, oestrous behaviour and CL formation (Karsch *et al.*, 1992). This sustained and massive release of LH (surge) is due to a continuous discharge of GnRH into the portal circulation (Moenter *et al.*, 1991; Karsch *et al.*, 1992). The LH surge terminates when the pituitary is depleted of releasable stores of the hormone.

1.1.3 Gonadotrophin regulation of the ovary

GnRH-mediated LH and FSH release from the AP regulates follicular and oocyte growth, antrum formation, steroidogenesis, ovulation and maintenance of the CL (Vizcarra *et al.*, 1997). Evidence supporting this can be found in many recent studies. For example, it is clear that when pulsatile gonadotrophin secretion is suppressed by prolonged GnRH agonist treatment (Gong *et al.*, 1995; 1996; Vizcarra *et al.*, 1997; Garverick *et al.*, 2002; Lindsey *et al.*, 2002), follicular growth is arrested at less than 4.5 mm in diameter (Garverick *et al.*, 2002). There is also blockage of the development of dominant follicles (DF) beyond 9 mm in diameter and prevention of the preovulatory LH surge and ovulation (Gong *et al.*, 1995). Exogenous FSH supplements in heifers which increase FSH concentration enhance follicular growth and differentiation (Crowe *et al.*, 2001; Garverick *et al.*, 2002). Therefore, both LH and FSH are vital for ovarian follicle growth, steroidogenesis and ovulation.

1.1.3.1 Gonadotrophin requirements for follicular growth and development

The major functions of female gonads are the development and release of the mature oocyte for fertilisation and the production of ovarian steroid hormones (mainly E2 and P4) and non-steroidal substances with hormonal actions (such as prostaglandins (PG), OT, inhibin and relaxin). The growth and development of a follicle from the primordial stage to the preovulatory follicle is continuous and is controlled by complex interactions between the gonadal hormones, growth factors, extraovarian factors and the hypothalamo-pituitary-ovarian system (Campbell *et al.*, 1995; Monget and Monniaux, 1995; Webb *et al.*, 1999). The mechanisms controlling the transition from the primordial to primary stage of the follicles are not clear.

A number of antral follicles within the ovary are selected from the pool of growing follicles to continue to grow rapidly. This process is called *follicle recruitment* (Webb *et al.*, 1999). The development of antral follicles, which are larger than 4 mm, to form preovulatory follicle is dependent on both LH and FSH (Campbell *et al.*, 1995). FSH promotes the growth and differentiation of ovarian follicles and is associated with the formation of a fluid-filled antrum or Graafian follicle (Crowe *et al.*, 2001). The repeated transitory rises in FSH concentrations during oestrus, the anovulatory postpartum period and early in life before puberty are related to follicular wave emergence (Adams *et al.*, 1992; Evans *et al.*, 1994b; Beam and Butler, 1997). The growth of small follicles (up to 5 mm diameter) can occur with limited gonadotrophin stimulation, whereas growth of follicles 6 to 9 mm (Gong *et al.*, 1996) or beyond 3-4 mm (Lussier *et al.*, 1994) requires FSH stimulation, and growth beyond 9 mm needs LH pulses (Gong *et al.*, 1996).

The follicular dominance process appears to be controlled by a number of mechanisms working in concert (Armstrong and Webb, 1997). The roles of LH in the maintenance, maturation and ovulation of the DF) are well established (Ireland, 1987; Savio *et al.*, 1993), and as proved earlier by the study using LH antiserum by Snook *et al.* (1969). Following recruitment, medium size follicles become oestrogenically active, but only one follicle from this group will be selected and continues to grow (Webb *et al.*, 1999). This follicle acquires LH responsiveness whilst declining FSH concentrations caused by its own E2 and inhibin secretions during the growth phase suppresses the growth of other follicles (Beg *et al.*, 2001). Low FSH concentrations are required for continued growth of the DF in cattle (Bergfelt *et al.*, 2000). The fate of other subordinate follicles, so called E2 inactive follicles (which have more P4 and androgens than E2 in their follicular fluid) is to

undergo atresia (Ireland and Roche, 1982a). A negative relationship between the growth of the DF and the total number of smaller follicles is evident as one of the features of follicular dominance (Ginther *et al.*, 1996; 2000). Recent studies have also demonstrated the actions of the intra-ovarian hormones through paracrine mechanisms that enable the selected follicle to continue to grow and form a DF in the presence of low concentrations of FSH, while other follicles cannot (Webb *et al.*, 1999). In addition, decreased IGFBP-2 and -4 in follicular fluid and increased theca cell binding sites for LH/hCG may be involved in follicular development and the process of dominance in cattle (Stewart *et al.*, 1996; Armstrong *et al.*, 1998). This process will not be described in detail in this review.

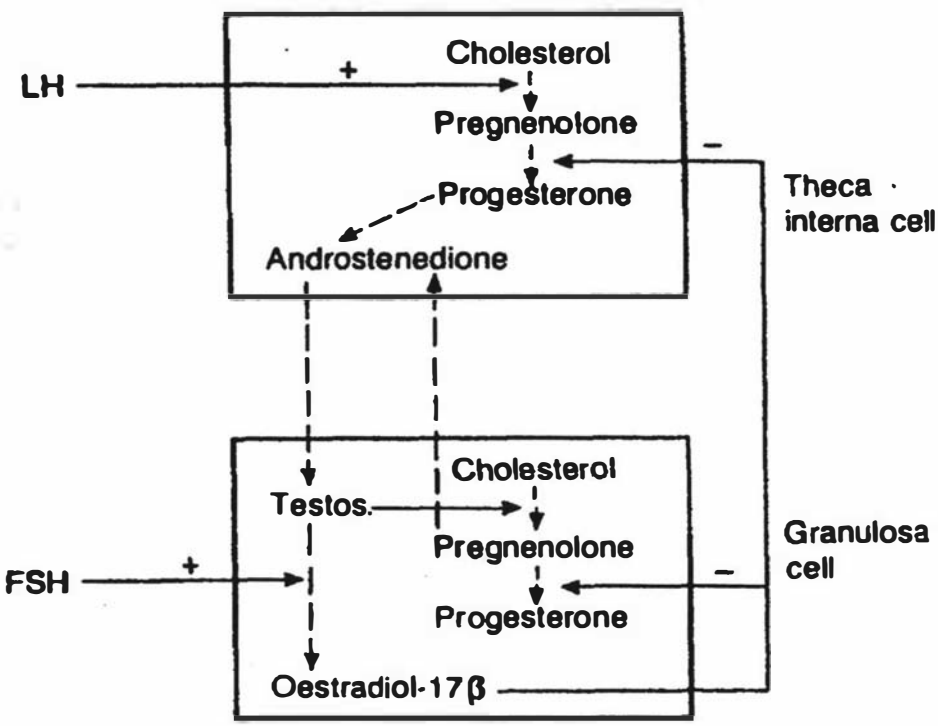
1.1.3.2 Gonadotrophin, ovarian steroidogenesis and ovulation

The growing follicle contains two types of steroid-secreting cells: theca interna cells and granulosa cells (Hartigan, 1992). The response of the follicle to the gonadotrophin signals depends on the presence of specific receptors on the surface of these ovarian cell types (Mariana *et al.*, 1991). LH binds to its receptors on theca cells to promote synthesis of androgens and small amounts of E2 from low-density lipoprotein. While LH receptors on the granulosa cell membrane only appear during the preovulatory phase under the influence of FSH stimulation, FSH also stimulates the aromatisation of androgens of thecal origin in granulosa cells to form oestrogen, mainly in the potent form of oestradiol-17β (E2: Edqvist and Stabenfeldt, 1993a). This is known as the "*two-cell*" theory (Fortune, 1986; Fortune and Quirk, 1988).

The DF continues to produce E2, while escaping atresia, thereby developing the potential to ovulate. Its follicular fluid contains more E2 than P4 and androgens (Ireland and Roche, 1982a). During the transition from DF to pre-ovulatory follicle (i.e. after the initiation of luteolysis and before the LH surge) an important mechanism that leads to increased E2 production is an increase in the gene expression of steroidogenic enzymes in theca cells (Tian *et al.*, 1995). Pituitary FSH, plus E2 secreted by the granulosa cells of this follicle, are responsible for up-regulating gene expression for aromatase (which synthesises E2 from androgen) and LH receptors (Kaneko *et al.*, 1993). Development of LH receptors is vital for the development of LH responsiveness of these cells (Hansel and Convey, 1983). Furthermore, granulosa cells produce inhibin, which regulates FSH concentrations by inhibiting its secretion from the AP (Kaneko *et al.*, 1993). If LH or FSH support is removed (such as in the case of Norgestomet implantation: Savio *et al.*, 1993) the largest

follicle will regress. In fact, low LH pulse frequency leads to atresia of DF, whereas one pulse per h of LH leads to ovulation of the DF (Evans *et al.*, 1997).

Figure 1-2: Schematic illustration for regulation of steroidogenesis by theca interna and granulosa cells of bovine preovulatory follicles, so called the "Two-cell theory" (after Fortune, 1986).



The frequency of LH pulses increases during the follicular phase of the cycle (Rahe *et al.*, 1980) and each individual pulse of LH is associated with a marked increase in the secretion of androgens and E2 by the follicle (Baird, 1984). During the luteal phase, there are minor rises of E2 on Day 4 and Days 10-13 (Shemesh *et al.*, 1972) and Days 5-8 (Mason *et al.*, 1972; Dieleman *et al.*, 1986). These mid-cycle rises are coincident with two or three waves of follicular growth that occur during the cycle (Savio *et al.*, 1988; Knopf *et al.*, 1989; Ko *et al.*, 1991). Rising concentrations of E2 initially have a negative feedback effect on LH and FSH secretion. Thereafter, the rising E2 concentrations trigger the preovulatory surge of GnRH, leading to the gonadotrophin surge (Wise and Maurer, 1994), which is required for ovulation (Stumpf *et al.*, 1991; Turzillo and Nett, 1999). Thus, the DF signals its stage of development to the hypothalamus and the AP, by sustained E2 production (Taya *et al.*, 1996).

LH induces ovulation and initiates luteinsation only in those follicles which have become fully developed and which have acquired a full complement of granulosa LH receptors (Wise and Maurer, 1994). Antral follicles that are immature or are in early stages of atresia do not ovulate in response to this surge but undergo atresia, and other follicles from the gonadotrophic-responsive pool will be recruited to replace them (Campbell *et al.*, 1995). Elimination of the LH surge results in abnormal follicular secretion patterns of steroids and glycosaminoglycans (which are associated with follicular atresia) and altered ovulation, leading to disturbed oocyte maturation and decreased oocyte quality (Wise and Maurer, 1994).

1.1.3.3 Follicular dynamics in cattle

In cattle, it is evident that, as suggested earlier by Rajakoski (1960), ovarian follicular growth and regression occurs in waves throughout the oestrous cycle (Savio *et al.*, 1988; Sirois and Fortune, 1988; Roche and Boland, 1991), with most animals showing either two (Ginther *et al* 1989a) or three waves of follicular growth per cycle (Savio *et al.*, 1988; Sirois and Fortune, 1988). Follicular activity, which is characterised by regular, periodic surges of FSH and emergence of follicular waves, is also present during pregnancy (Ginther *et al.*, 1989c; Taylor and Rajamahendran, 1991a), puberty (Hopper *et al.*, 1993; Adams *et al.*, 1994; Evans *et al.*, 1994a; 1994b) and the postpartum anoestrous period (Savio *et al.*, 1990a). In each follicular wave, a group of small follicles, 4 to 5 mm in diameter, is recruited from primordial follicles to become active and to continue to grow (Savio *et al* 1988; Knopf *et al* 1989; Adams *et al.*, 1992).

Considering follicular wave development, the secondary FSH surge (Day 1) is vital to initiate of the first follicular wave of the oestrous cycle (Walters and Schallenberger, 1984; Turzillo and Fortune, 1990; Adams *et al.*, 1992). Suppression of FSH surges with bovine follicular fluid inhibits further growth of the DF and blocks the emergence of new waves (Turzillo and Fortune, 1990; 1993), confirming the role of FSH in regulating the follicular wave (Bergfelt *et al.*, 1994). This accords with studies indicated that the granulosa cells of follicles of all sizes start to have FSH receptors from the preantral stage (McNatty *et al.*, 1999).

By the second day of the wave, only one follicle is selected, based on diameter and E2 secretion (Evans and Fortune, 1997) to become dominant over the others (as indicated by divergence in diameter; Ginther *et al.*, 1997), while the others cease to grow and undergo

regression via atresia (Ginther *et al.*, 1989b). In the last wave of the oestrous cycle, the DF becomes the pre-ovulatory follicle which will eventually ovulate after the LH surge (Savio *et al.,* 1990a). Follicles which become dominant during the luteal phase, do not ovulate if luteal regression does not occur, but undergo atresia (Dieleman *et al.*, 1986; Ireland and Roche, 1987) and, following DF regression, a new wave emerges (Ginther *et al.*, 1989b). If the CL regresses, this follicle will ovulate in response to the LH surge (Ireland and Roche, 1987).

It is more common for cows to have two waves than three waves (Rajakoski, 1960). Three waves usually occur in longer cycles (Savio *et al.,* 1990a). In fact, Savio *et al.* (1990a) found a positive relationship between the duration of the oestrous cycle and the number of follicular waves. In a three-wave cycle, the non-ovulatory follicle emerges at around Day 9 of the cycle (Adams *et al* 1993a). It starts to decrease in size or fails to grow when the ovulatory follicle emerges (Quirk *et al.*, 1986). In most cases, the selection process of the follicle destined to ovulate occurs between Day 16 and Day 17 in the three-wave cycle (Pierson and Ginther, 1988) or around Day 12 in the two-wave cycle (Quirk *et al.*, 1986; Sirois and Fortune, 1988).

1.1.3.4 Ovarian steroids and feedback control

The effects of ovarian steroids are to regulate female reproductive functions by acting on both peripheral tissues and the HPA. In particular, the variation in gonadotrophin response to GnRH stimulation under different physiological conditions is the result of differences in gonadal steroid hormone environment (Convey, 1973; Noakes, 2001). The steroid hormones E2 and P4 and some gonadal peptide hormones such as inhibin, activin and follistatin regulate the hypothalamo-pituitary system via *negative* and *positive feedback mechanisms* (Edqvist and Stabenfeldt, 1993b; McLeod and Phillips, 1998). A negative feedback loop displays a reciprocal hormonal effects as a decrease in the rate of secretion of itself or of other hormones. On the other hand, a positive feedback involves a stimulatory effect of hormone to increase the rate of other hormone secretions (McLeod and Phillips, 1998). The purpose of these feedback mechanisms is to maintain hormone balance and to synchronise physiological events and reproductive behaviour (Allrich, 1994).

Mean LH and FSH concentrations, LH pulse frequency and amplitude increase in cattle after ovariectomy, as negative feedback effect of E2 on gonadotrophin secretion is

removed (Hobson and Hansel, 1972; Schallenberger and Peterson; 1982). High plasma P4 concentrations suppress gonadotrophin pulse secretion by a negative feedback inhibition of GnRH pulse frequency at the hypothalamic level (Ireland and Roche, 1982b; Bolt *et al.*, 1990; Kawate *et al.*, 1993; Bergfeld *et al.*, 1995) and in the AP (via altered GnRH receptor gene expression; Turzillo and Nett, 1999). Furthermore, there is evidence that decreasing plasma P4 concentrations allow the LH pulse frequency to increase (Ireland and Roche, 1982b; Kawate *et al.*, 1993), but do not affect FSH secretion (Roche and Ireland, 1982b). In fact, immunisation against steroids (E2 and P4) had no effect on FSH secretion but increased follicular development as a result of enhanced LH secretion by decreases in steroid negative feedback (Chang *et al.*, 1987).

There is consistent evidence that E2 and P4 together modulate the effects of GnRH secretion on pituitary gonadotrophin release (Azzazi *et al.*, 1983). High P4 concentrations enhance the chronic negative feedback effect of low amounts of E2, whilst low P4 concentrations facilitate the positive feedback of E2 in provoking the pre-ovulatory LH and FSH surge (Price and Webb, 1988; Stumpf *et al.*, 1991; O'Rourke *et al.*, 2000). Moreover, the combined effect of P4 and E2 was more effective than either hormone alone in suppressing basal LH secretion after ovariectomy (Price and Webb, 1988; Stumpf *et al.*, 1991). Oestrogen appears mainly to inhibit FSH secretion (Price and Webb, 1988) and is thought to affect gonadotrophin secretion through an effect on both the hypothalamus and the AP (Stabenfeldt and Davidson, 2002a).

Two peptide hormones are synthesised and secreted by the granulosa cells of the bovine ovary that selectively regulate FSH secretion from the AP. Activins enhance FSH secretion and inhibins suppress FSH secretion (Findlay, 1993; Kaneko *et al.*, 1993). Activins may also increase LH secretion, but inhibin does not affect LH. Inhibin and E2 are thought to play a suppressive role in FSH secretion in the cow (Martin *et al.*, 1988). Martin *et al.* (1991) and Mihm and Austin (2002) suggested the role of inhibins in growth and atresia of DFs during the oestrous cycle in cattle. There is a possibility that activin may be involved in the establishment and maintenance of the DF (Knight and Glister, 2001; Mihm and Austin, 2002; Mihm *et al.*, 2002), as it can suppress P4 and OT production by granulosa cells from preovulatory bovine follicles *in vitro*. Follistatins also inhibit FSH by binding and neutralising activins (Findlay, 1993; Mihm and Austin, 2002), thereby reducing their bioavailability (Knight and Glister, 2001).

1.5 Therapeutic use of exogenous GnRH treatments in the cow

1.1.4 Therapeutic use of a single exogenous GnRH injection

The response to exogenous GnRH depends on dose, route of treatment, potency of the GnRH analogue used, frequency and duration of treatment, and physiological status of the animal (Schams *et al.*, 1974; Chenault *et al.*, 1990; Vizcarra *et al.*, 1999). In addition, in many earlier studies reported that the magnitude of the response depends on route of administration and time since the previous treatment (Schams *et al.*, 1974; Braun *et al.*, 1985; Chenault *et al.*, 1990). GnRH agonist treatment does not interfere with the activity of hypothalamic GnRH secreting neurones (D'Occhico and Aspden, 1999), so its impact depends upon its direct effects upon secretion of LH and FSH from the AP (Stevenson *et al.*, 1993).

LH and FSH responses (in terms of area under the curve, time to peak and peak concentration of LH and FSH) have been used to evaluate the responsiveness of AP to GnRH stimulation (Fernandes *et al.*, 1978; Chenault *et al.*, 1990). A single injection of relatively high doses of GnRH or its agonist, either intramuscularly or intravenously, induces LH and FSH release in cattle during the early postpartum period (Fernandes *et al.*, 1978; Foster *et al.*, 1980; Nash *et al.*, 1980; McDougall *et al.*, 1995c), prepubertal period (Barnes *et al.*, 1980) and oestrous cycle (Foster *et al.*, 1980; Alam and Dobson, 1987).

During the postpartum period, the gonadotrophin response to GnRH depends upon the number of days after calving (Fernandes *et al.*, 1978; Foster *et al.*, 1980; Alam and Dobson, 1987), pre-treatment P4 concentrations (Williams *et al.*, 1982b; Azzazi *et al.*, 1983), pre-treatment plasma E2 concentrations (Fernandes *et al.*, 1978; Zaied *et al.*, 1980; Kesner *et al.*, 1981; Williams *et al.*, 1982a; Azzazi *et al.*, 1983), pre-treatment LH concentrations (Zaied *et al.*, 1980), uterine infection (Janowski *et al.*, 1998), nutritional status and body condition (Beal *et al.*, 1978; Rutter and Randel, 1984; Kelly *et al.*, 1988).

1.1.5 The efficacy of a single GnRH injection during the postpartum period

Numerous studies have examined the physiological and endocrine effects of GnRH treatment during the early postpartum period and its efficacy as a treatment to improve pregnancy rates and fertility in cattle (Thatcher *et al.*, 1993; Heuwieser *et al.*, 1994). These studies involved GnRH treatment in cows during the early postpartum period (Britt *et al.*, 1974; Nash *et al.*, 1980; Zaied *et al.*, 1980; Williams *et al.*, 1982a; 1982b; Cavestany and

Foote, 1985a; Heuwieser *et al.*, 1994; McDougall *et al.*, 1995c), at time of first breeding after calving (Chenault, 1990; Heuwieser *et al.*, 1994), at the time of AI (Schels and Mostafawi, 1978; Chenault, 1990; Drew and Peters, 1994; Valks, 1996) and during the luteal phase after AI (Lee *et al.*, 1985; Macmillan *et al.*, 1986; Chenault *et al.*, 1990; Macmillan and Thatcher, 1991; Stevenson *et al.*, 1993; Drew and Peters, 1994). In general, the outcomes of the treatment were affected by differences in management practices, study design, dose of GnRH, use of PG to induce oestrus, physiological state at the time of treatment, reproductive disorders or disease, parity, body condition and environment of the herds involved in these studies (Thatcher *et al.*, 1993; Heuwieser *et al.*, 1994; Beckett and Lean, 1997).

The ability of GnRH to induce LH release during the early postpartum period (< 40 days after calving) has been used to induce a preovulatory LH surge, hasten the onset of ovulation and shorten the interval between calving and conception (Britt *et al.*, 1974; Nash *et al.*, 1980; Pratt *et al.*, 1982; Cavestany and Foote, 1985a). However, in order for a single GnRH injection to induce ovulation, a large follicle (10 to 15 mm) must be present at the time of GnRH treatment (Garverick *et al.*, 1980; McDougall *et al.*, 1995c). In fact, elevated pre-treatment plasma LH and E2 concentrations and follicular growth are of importance for GnRH to induce ovulations during Days 12 to 14 postpartum (Zaied *et al.*, 1980).

Even though GnRH treatment can induce ovulation in postpartum anoestrous cows, the subsequent luteal phases are usually subnormal, i.e. the life span of the induced CL is less than 14 days and ovarian cyclicity may not be re-established (Garverick *et al.*, 1980; Pratt *et al.*, 1982; McDougall *et al.*, 1995c; Taponen *et al.*, 1999). In ewes, the inadequate luteal function after repeated injection of GnRH may be due to a poor response to the LH surge, indicative of a deficiency in the final stages of the follicle maturation (Hunter *et al.*, 1986). However, priming with P4 enhanced luteal P4 secretion on Days 12 and 16 of the induced cycle (Williams *et al.*, 1982b). This pre-treatment with P4 resulted in reduced incidence of abnormal luteal phases in hCG and GnRH-induced cycles (Pratt *et al.*, 1982; Sheffel *et al.*, 1982; Hunter *et al.*, 1986; Garverick *et al.*, 1992).

GnRH treatment at the first or second mating after calving shows inconsistent results upon pregnancy rate (Chenault, 1990; Stevenson *et al.*, 1990; Drew and Peters, 1994; Valks, 1996). Meta-analysis showed that blanket treatment of cows after calving may shorten the duration of postpartum anoestrus period, but subsequent fertility is not improved (Lean *et*

al., 2003). However, the effects of such GnRH treatment may be due to induction of a secondary LH surge (Ryan *et al.*, 1994) and luteinisation of mature follicles (Valks, 1996).

GnRH treatment of repeat-breeding dairy cows at oestrus increased the ratio of large to small luteal cells in the CL and increased serum P4 concentrations earlier after ovulation and up to 40 days after treatment (Mee *et al.*, 1993). However, serum P4 concentrations after oestrus in GnRH-treated cows were lower than in control cows during Days 3-5 (Ryan *et al.*, 1994) or the first 7 days of the oestrous cycle (Lucy and Stevenson, 1986). The latter authors also found delayed or slowly rising P4 concentrations after ovulation, which may result in reduced early embryonic death. In repeat breeder cows, GnRH treatment at AI increases pregnancy rate (Morgan and Lean, 1993).

GnRH treatment during the mid-luteal phase (Days 11 to 14 after AI) may induce an LH surge, an accessory CL, a short term increase in plasma P4 concentrations or extension of the luteal phase and may increase the chance of successful maternal recognition of pregnancy (Thatcher *et al.*, 1993; Lean *et al.*, 2003). Some studies have shown altered follicular development, increased cycle length (by prolonging the life-span of the CL) and altered E2 and P4 concentrations during late dioestrus, although the responses in pregnancy rates have been inconclusive (Macmillan *et al.*, 1986; Sheldon and Dobson, 1993; Stevenson *et al.*, 1993). Interestingly, plasma E2 concentrations between Days 12 and 16 were reduced when GnRH was injected in the mid-luteal phase (Days 11 and 13 of the oestrous cycle; Mann and Lamming, 1995b), possibly associated with altered follicular dynamics (Stevenson *et al.*, 1993). Such reduced E2 concentrations may be related to a weakening luteolytic signal, which increases the chances of an embryo preventing luteolysis (Mann and Lamming, 1995b), by allowing the embryo to have more time to grow and produce the signal for maternal recognition of pregnancy (Stevenson *et al.*, 1993). This may explain, partly, the improved conception rate after treatment in these cows in other studies mentioned above.

1.1.6 Factors affecting pituitary responsiveness to GnRH stimulation

The ability of the AP to respond to GnRH stimulation is associated with the number of GnRH receptors on the membranes of gonadotroph cells (Schoenemann *et al.*, 1985a; Turzillo and Nett, 1999). There is evidence that steroid hormone secretion from the ovary is one of the factors that regulates the number of GnRH receptors (Schoenemann *et al.*, 1985b). The results from *in vitro* (Padmanabhan *et al.*, 1982; Hashizume *et al.*, 2002) and

in vivo (Kesner *et al.*, 1981) studies have shown that exposure of bovine pituitary cells to E2, or high circulating E2 concentrations, increased both pituitary sensitivity to LHRH and basal LH release and synthesis; an effect that may be mediated through an increase in the number of GnRH receptors on the gonadotroph cells (Gregg *et al.*, 1990). Thus, changes in the steroid hormones environment during different physiological stages and ovarian development modify the responsiveness of the AP to GnRH.

The responsiveness of the AP to GnRH is higher at the time just preceeding the preovulatory gonadotrophin surge than at other periods of the cycle (Schams *et al.*, 1974); an effect which is coincident with the progressive increase in number of GnRH receptors in the AP between the mid luteal phase and the onset of oestrus (Schoenemann *et al.*, 1985a, Kawate *et al.*, 1991). In sheep, LH release in response to synthetic GnRH injection was significantly greater at the onset of oestrus than at 20 and 48 h after oestrus and on Day 10 of the oestrous cycle (Forster, 1978).

In suckling beef cows, the number of GnRH receptors increased between Day 1 and Day 15 after calving (Nett *et al.*, 1988). Moreover, changes in GnRH-induced LH release between Day 5 and Day 10 after calving (Fernandes *et al.*, 1978; Foster *et al.*, 1980) also indicate that the responsiveness to GnRH of the AP is related to physiological status of the animal and is enhanced by 7-10 days after calving. Priming the AP with exogenous E2 over the first 16 days postpartum first stimulated, and then inhibited, GnRH-induced LH release (Azzazi *et al.*, 1983). Thereafter, the rate of increase in pituitary responsiveness to GnRH challenge was higher in the E2-treated cows than in those with no steroid treatment. The authors suggested that high E2 concentrations might increase GnRH receptor numbers during the first 16 days after calving and subsequently enhance the AP responsiveness to the priming effect of GnRH. The high oestrogen concentrations in plasma during this period could increase the LH synthesis and decrease the amount of LH released (Azzazi *et al.*, 1983). Recently, the *in vitro* study by Hashizume *et al.* (2002) has shown that IGF-1 enhances GnRH-induced LH release without modifying the number of GnRH receptors in the AP, and that IGF-I interacts with E2 to increase the responsiveness of the AP to GnRH stimulation.

Although there is limited evidence to show whether the response of the AP to a single GnRH injection is related to the genetics of the cow, Royal *et al.* (2000a) found that LH concentration 30 min after GnRH challenge in pre-pubertal Holstein-Friesian heifers showed genetic correlations with high reproductive efficiency in subsequent lactations.

Moreover, this response has been utilised as a physiological marker to link with the reproductive potential of the replacement stock. On the other hand, Fajersson *et al.* (1999), by classifying the AP responses of beef cows to GnRH injection between Days 5 and 8 postpartum into early (10 to 30 min) or late (60 to 120 min) LH peaks, found that the timing of the responses was related to the duration of the interval from calving to the first ovulation (i.e. which was 8 days shorter in the late group than in the early group). This physiological response may also have potential as an indirect measure of reproductive merit in cattle (Williams and Stanko, 1996).

1.1.7 The effect of continuous or prolonged GnRH treatment

On the other hand, the responses of the AP to chronic and continuous treatments of GnRH agonist display two distinctive phases (D'Occhico and Aspden, 1999). In the acute response phase, the AP is stimulated, resulting in increased LH and FSH secretion. This may last for few days and then return to basal concentrations (Gong *et al.*, 1995). Then follows a long period of suppressed gonadotrophin pulsatile (but not basal) secretion until the treatment is withdrawn (Gong *et al.*, 1995; Mann and Lamming, 2000a).

The effects of prolonged periods of administration of GnRH and its agonists have been studied by giving: 1) repeated injections during postpartum anoestrus (Schams *et al.*, 1974; Peters *et al.*, 1986) or to cycling heifers (Gong *et al.*, 1995), 2) pulsatile infusion (Vizcarra *et al.*, 1997; 1999); 3) continuous infusion via osmotic minipump (Gong *et al.*, 1996; Mann and Lamming, 2000a; Garverick *et al.*, 2002) and 4) sustained-release implants (Britt *et al.*, 1974; Thatcher *et al.*, 2002). The outcomes of these treatments are variable, depending on the duration of the treatment. In general, all these treatments can result in down-regulation of the GnRH receptors in the AP, depression of basal LH secretion, elimination of episodic LH secretion, and regression of the gonad (Peters *et al.*, 1986; Chenault *et al.*, 1990; Gong *et al.*, 1995).

1.6 Exogenous oestradiol administration in cattle

In ovariectomised cows, administration of exogenous E2 decreases LH secretion within 3 h then induces an LH surge (Hobson and Hansel, 1972; Beck and Convey, 1977; Short *et al.*, 1979; Kesner *et al.*, 1981; Stumpf *et al.*, 1991). Similar responses occur during the follicular phases of cycling cows (Alam and Dobson, 1987; Nanda *et al.*, 1988) and during the postpartum period (Zaied *et al.*, 1981; Stevenson *et al.*, 1983; Peters, 1984a; Schallenberger and Prokopp, 1985; Alam and Dobson, 1987). Oestradiol-17β increases

numbers of GnRH receptors (Schoenemann *et al.*, 1985b), which in turn leads to increased pituitary sensitivity to GnRH (Beck and Convey, 1977; Turzillo and Nett, 1999). However, if endogenous P4 concentrations are also high (i.e. >0.50 ng/ml: Short *et al.*, 1979; Stevenson *et al.*, 1983; Schallenberger and Prokopp, 1985) or the cow is pre-treated with P4 (Schoenemann *et al.*, 1985b; Nanda *et al.*, 1988) or is given E2 during the luteal phase (Hobson and Hansel, 1972; Short *et al.*, 1979; Alam and Dobson, 1987; Nanda *et al.*, 1988), this phenomenon does not occur.

The interval between the administration of E2 and the peak LH response declines, and magnitude of LH peak increases, from Week 1 to Week 4 postpartum in both dairy (Stevenson *et al.*, 1983) and beef (Peters, 1984a) cows, suggesting a progressive recovery of the hypothalamo-pituitary responsiveness to the positive feedback effects of E2 leading to the first ovulation after calving.

1.7 Endocrine activity during the peri-pubertal period in cattle

Puberty in the female can be defined as the age at the first behavioural oestrus and ovulation, which is followed by a normal luteal phase (Moran *et al.*, 1989). For puberty to be reached requires that the reproductive neuroendocrine system has matured to the point at which it can sustain regular oestrous cycles. When hypophysial stalk transection was performed in 5 months old beef calves, there was no evidence of episodic LH secretion and circulating LH concentrations were low after the surgery (Anderson *et al.*, 1981). At 16 months of age, these calves showed neither signs of oestrous behaviour nor of ovarian follicular activity, indicating the pivotal role of hypothalamic stimulation by GnRH in sustaining LH and FSH release during peripubertal development.

The AP of calves has the ability to respond to exogenous GnRH at a very young age (Barnes *et al.*, 1980; Schams *et al.*, 1981; MacDonald and Page, 1986; Grasselli *et al.*, 1993), although ovulation was not induced (Grasselli *et al.* 1993). In addition, exogenous E2 can induce a LH surge in prepubertal heifers by 3 months of age (Nakada *et al.*, 2001). Although the surge system is capable of functioning from a very early age, it remains quiescent until the onset of puberty (Moran *et al.*, 1989). These data suggest that key components of the hypothalamic-pituitary-gonadal system and feedback mechanisms are functional long before the onset of puberty.

The ovaries of prepubertal heifers are functional inasmuch as they contain growing follicles (Day *et al.*, 1987; Evans *et al.*, 1994a; 1994b). Follicular waves are also present in

animals as young as 2 weeks of age (Evans *et al.*, 1994a), although follicles undergo atresia without reaching pre-ovulatory size (Schillo *et al.*, 1992; Hopper *et al.*, 1993; Evans *et al.*, 1994b). There are no changes in the maximum diameter of the dominant or the second largest follicles or in DF growth and regression rates in the 12 weeks before the onset of puberty or in the postpubertal ovulatory cycles (Evans *et al.*, 1994a). As puberty approaches, the number of large follicles (>12 mm in diameter: Day *et al.*, 1987) LH pulse frequency (Page *et al.*, 1987; Evans *et al.*, 1994a) and FSH concentrations increase (Melvin *et al.*, 1999).

Schams *et al.* (1981) reported two periods of increase in LH and FSH concentrations in prepubertal calves. The first occurs between 3 to 5 months of age after which concentrations decline. The subsequent rise coincides with the first ovulation at 9-13 months of age (Schams *et al.*, 1981; Day *et al.*, 1984; Dodson *et al.*, 1988) at puberty. It has been postulated that, in prepubertal animals, gonadotrophin secretion is inhibited by a hypersensitivity of *a hypothalamic gonadostat* to the inhibitory effect of very low circulating concentrations of E2, resulting in the low circulating LH concentrations present in very young calves (Day *et al.*, 1984; 1987; Moran *et al.*, 1989; Day and Anderson, 1998). As the animals approach puberty, the sensitivity to steroids declines and LH secretion subsequently develops its characteristic pulsatile pattern (Day *et al.*, 1984; Moran *et al.*, 1989; Melvin *et al.*, 1999). This decrease in negative feedback of E2 on release of LH pulses is consistent with the finding that the number of receptors for E2 in the medial basal hypothalamus and/or the AP decreases as puberty approaches (Day *et al.*, 1987). LH pulse frequency increases during peripubertal ovarian follicular development, the follicles reach about 13 mm in size and produce enough E2 to induce the preovulatory gonadotrophin surge and the behavioural oestrus at puberty and first ovulation occurs (Evans *et al.*, 1994a; Melvin *et al.*, 1999). Changes in FSH concentrations around the time of puberty are less dramatic (Evans *et al.*, 1994a).

On the other hand, Day and Anderson (1998) highlighted an alternative model for maturation of the reproductive endocrine axis in cattle, based on the fact that the oestradiol negative feedback is present early in life and does not decline until about 1 yr of age. During this period, low LH concentrations merely reflect minimal GnRH secretion. As the peripubertal period approaches, hypothalamic maturity is evident and the AP begins to respond to GnRH stimulation, leading to increased LH and FSH secretion (Dyer *et al.*, 1990).

1.8 Endocrine activity during the oestrous cycle

The normal oestrus cycle of the cow consists of 4 periods, i.e. oestrus (Day 0), metoestrus (Days 1-3), dioestrus (Days 4-18) and proestrus (Days 19 to the onset of oestrus); a total of 17 to 25 days (Bearden and Fuquay, 2000). The cycle can also be described according to the activity of the gonads: the follicular phase, which includes oestrus and proestrus, and the luteal phase, which includes metoestrus and dioestrus (Stabenfeldt and Davidson, 2002b). The patterns of gonadotrophin, E2 and P4 secretions during the bovine oestrous cycle have been described by Mason *et al.* (1972); Shemesh *et al.* (1972); Lemon *et al.* (1975); Rahe *et al.* (1980); Schallenberger *et al.* (1984); Walters and Schallenberger (1984) and Walters *et al.* (1984).

1.1.8 Follicular phase

This phase begins with luteolysis in the non-pregnant animal, when plasma P4 concentrations fall from luteal phase values to below 0.05 ng/ml (Lemon *et al.*, 1975). This removes the negative feedback of P4 upon the pulsatile release of LH (Rahe *et al.*, 1980; Ireland and Roche, 1982b). Consequently, rising LH concentrations allow rapid growth of the DF, which, in turn brings about progressive increases in E2 production by the DF and rising circulating concentrations of E2 during the three days preceding oestrus (Bodensteiner *et al.*, 1996).

1.1.9 Preovulatory phase

During this phase, an increase of GnRH receptor numbers precedes the enhancement of the responsiveness of the AP to GnRH that is required for the occurrence of the preovulatory gonadotrophin surge (Kawate *et al.*, 1991). The pre-ovulatory surge of E2 precedes, and stimulates, the gonadotrophin surge. LH concentrations are maximal between 15-31 h before ovulation (Shemesh *et al.*, 1972; Rajamahendran *et al.*, 1989). The E2 surge also initiates oestrous behaviour (Lemon *et al* 1975; Dieleman *et al.*, 1986). Ovulation occurs 24-30 h after the onset of oestrus (Rajamahendran *et al.*, 1989). It is of interest to note that, in the cow, there is a concomitant pre-ovulatory FSH surge (Walters and Schallenberger, 1984). Even though its significance is not clear, Noakes (2001) suggests that it may be involved in the ovulation-inducing hormone complex. It is evident also that FSH basal concentrations and pulse amplitude increase 4-12 h after LH surge, resulting in a second FSH surge (Dodson, 1978a; Walters and Schallenberger, 1984). This

secondary FSH surge stimulates the growth of small antral follicles to grow beyond 4 mm in diameter (Adams *et al.*, 1992; Sunderland *et al.*, 1994).

1.1.10 Ovulation and luteinisation

The LH surge induces changes in both biochemistry and structure of the preovulatory follicle, which result in oocyte maturation and ovulation (Komar *et al.,* 2001). LH stimulates increased PGF synthesis and the release of steroids, PGE_2 and cytokines from pre-surge follicles, which, in turn, lead to increased steroidogenesis and ovulation (Shemesh and Hansel, 1975b; Algire *et al.*, 1992; Acosta *et al.*, 1998). The preovulatory gonadotrophin surge triggers a shift of steroidogenesis from E2 to P4 secretion by up-regulating the P450 side-chain cleavage enzyme gene expression, which results in P4 synthesis and secretion (Voss and Fortune, 1993; Komar *et al.,* 2001). The LH surge also up-regulates prostaglandin synthetase gene expression, which increases PG secretion required for ovulation and P4 receptors necessary for luteinisation of follicular cells (Algire *et al.,* 1992).

In cattle, oxytocin (OT) is locally synthesised in, and secreted by, follicles and the CL (Schams *et al.*, 1985; 1987; Flint *et al.*, 1990). The main source of ovarian OT is the large luteal cells of the CL (Schams, 1987). Bovine granulosa cells isolated after gonadotrophin surge are able to synthesise and secrete OT *in vitro* (Voss and Fortune, 1992), suggesting an autocrine role of OT during the transition from the late follicular to early luteal phase (i.e. during luteinisation of granulosa cells) which involves a loss of aromatising capacity and an increase in progesterone (P4) and OT production (Berndtson *et al.*, 1996). In addition, both OT and P4 act in an autocrine manner in bovine granulosa cells to activate receptors that are needed to form fully functional luteal cells during *in vitro* luteinisation (Lioutas *et al.*, 1997). Nevertheless, the endocrine mechanisms involved in the sudden shift of pre-ovulatory follicular cells to form functional luteal cells are not fully understood. This subject has been reviewed by Smith *et al.* (1994).

1.1.11 Luteal phase

The luteal phase begins following ovulation and ends with luteolysis about 18 days later. During the luteal phase, follicles grow and regress (Matton *et al.*, 1981) and, although they will not ovulate, they still produce enough E2 to enhance the P4 negative feedback effect on LH secretion. The pattern of LH secretion during Days 3 to 8 of the oestrous cycle has been characterised as a three-fold decrease in the frequency of LH pulses and a two-fold

increase in the amplitude, although FSH secretion does not change with time after oestrus (Parfet *et al.,* 1989). Although LH concentrations are low, there is sufficient to maintain CL function (Rahe *et al.,* 1980; Schallenberger *et al.,* 1984; Parfet *et al.,* 1989).

1.1.12 Corpus luteum formation, development and function

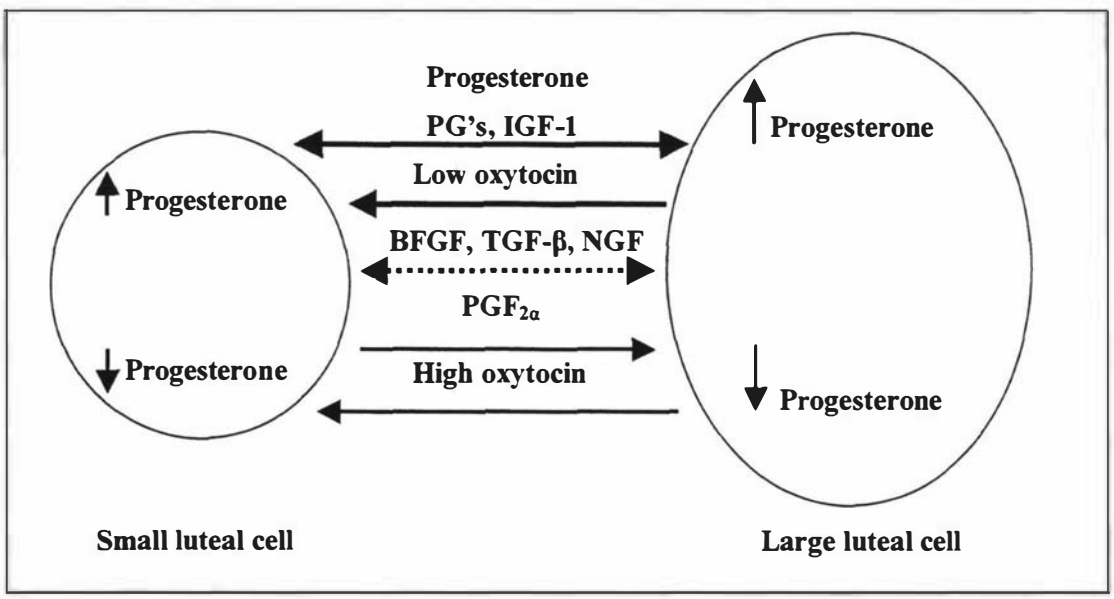
After ovulation, the wall of the follicle collapses and the granulosa and theca layers become deeply infolded and undergo hyperplasia to form the CL (Donaldson and Hansel, 1965a; Lobel and Levy, 1968). Both granulosa and theca cells of the follicle undergo functional luteinsation, as defined by a decrease in androgen aromatising capacity, an increase in P4 production and by changes of enzymatic activities. These changes are accompanied by tissue remodelling (Lobel and Levy, 1968; Henderson and Moon, 1979; Smith *et al.*, 1994). With prominent mitotic activity during the first seven days after ovulation (Lobel and Levy, 1968), granulosa cells differentiate into large luteal (LLC) cells and the theca cells into small luteal (SLC) cells (Donaldson and Hansel, 1965a; Alila and Hansel, 1984) and, by mid-cycle (Day 12), LLC occupy a larger volume of the CL than do SLC (O'Shea *et al.*, 1989). The SLC can transform into LLC as the CL matures (Donaldson and Hansel, 1965a; O'Shea, 1987). In the early CL, from Days 2 to 5, blood flow increases in line with the increase in CL size, volume and plasma P4 concentration (Acosta *et al.*, 2003). The formation and maintenance of blood supply network in the CL during its functional lifespan is under the effect of LH stimulation (luteoangiotrophic effect: Redmer *et al.*, 1988). Blood vessels are important as P4 production by LLC and SLC is primarily dependent on lipoproteins delivered by the luteal blood supply (Wiltbank, 1994).

Earlier *in vivo* and *in vitro* works in cows (Ursely and Leymarie, 1979) and pigs (Lemon and Mauleon, 1982) suggested that LLC and SLC interact to produce P4. This was later confirmed by del Vecchio *et al.* (1995). Findlay and Risbridger (1987) suggested that the control of P4 production by the CL might be partly associated with an interaction between these two luteal cell types, particularly during luteolysis. The SLC have high numbers of LH receptors, a low number of PGF receptors, so have the ability to respond to LH (and $PGF_{2\alpha}$ during the early luteal phase) by increasing P4 production (Ursely and Leymarie, 1979;). Whereas LLC, with many receptors for PGF, but few for LH cannot respond to LH but have baseline production of P4 (Rao *et al.*, 1979; Duby *et al.*, 1985; O'Shea *et al.*, 1989). Therefore, the LLC seem to be the targets of the luteolytic effects of $PGF_{2\alpha}$, while

the SLC are responsible for the luteotrophic effects of LH, and possibly for the luteotrophic effect of $PGF_{2\alpha}$ and prostaglandin E_2 (PGE_2: Alila *et al.*, 1988; Braun *et al.*, 1988).

The LLC also have the ability to synthesise and secrete other proteins (e.g. relaxin and OT); the latter in response to both luteal and uterine $PGF_{2\alpha}$ (Abdelgadir *et al.*, 1987; Pate, 1996). Low OT concentrations stimulate P4 production in dispersed bovine luteal cells, but high concentrations are inhibitory (Tan *et al.*, 1982). Taken together, Pate (1996) simplified and illustrated the model showing the important roles of intercellular communication and interactions between LLC and SLC in the regulation of the bovine CL function involving the luteal paracrine factors (**Figure 1-3**).

Figure 1-3: Schematic illustration of interactions of autocrine and paracrine factors within the LLC and SLC of the bovine CL (after Pate, 1996).



The function and lifespan of the CL are controlled by many hormones, including LH, which can prolong its lifespan (Donaldson and Hansel, 1965b), by $PGF_{2\alpha}$, which is luteolytic (Inskeep, 1973) and by E2, which also exerts a luteolytic effect (Hansel, 1975).

1.1.13 Luteotrophic effect of LH

In cattle, LH is the primary luteotrophin, and is responsible for the formation and maintenance of the bovine CL (Hoffmann *et al.*, 1974; Milvae *et al.*, 1996). In other species, mainly rodents, prolactin is also luteotrophic, but there is no evidence for such a

role in cattle (Hoffmann *et al.*, 1974). Administration of LH or hCG in the early or mid luteal phase of the cow can extend the functional lifespan of the CL, sustain P4 secretion and increase the number of CLs (Diskin and Sreenan, 1986; Santos *et al.*, 2001). Snook *et al.* (1971) found a correlation between circulating P4 concentrations and LH concentrations between Day 3 and Day 15 of the oestrous cycle, suggesting the luteotrophic action of LH during the mid-luteal phase. However, initial luteinisation and growth and development of the CL are independent of the action of LH, but P4 production by the mid-cycle CL is relatively dependent of acute LH stimulation (Wiltbank, 1994). In addition, Okuda *et al.* (1999) also suggested that the increase in luteal P4 secretion from the early to mid-luteal phase is not correlated with either the basal concentrations or pulse frequency of LH, but during this period, as during pregnancy, increasing expression of LH receptors in the CL are coincident with increasing luteal P4 synthesis (Garverick *et al.*, 1985: Okuda *et al.*, 1999).

The significance of the LH surge quality on the quality of oocyte and functional luteal cells is well recognised. The LH pulses during the 48 h before the preovulatory LH surge are required for maturation of the follicle to an extent that will allow for formation of a CL with typical size and capacity to secrete P4 (Quintal-Franco *et al.*, 1999).

1.1.14 Other luteotrophic factors

Rothchild (1981) suggested that the ability of the mammalian CL to secrete P4 was not dependent on the pituitary support (i.e. that there is autonomy of P4 secretion). Reviewing studies in many species, he suggested that the CL can grow and secrete P4 for almost the full duration of a normal lifespan in the absence of the pituitary. This hypothesis is in line with evidence available from earlier studies in the cow involving hypophysectomy, granulosa cell culture, LH and hCG administration and LH antiserum treatment (Schomberg *et al.*, 1967; Snook *et al.*, 1969; Henderson and McNatty, 1977). Moreover, the P4 receptor gene expression is present in the bovine CL (Rueda *et al.*, 2000), suggesting a possible local autocrine role. Kotwica *et al.* (1998) also showed the effect of P4 on its own synthesis in bovine CL *in vitro* mainly during the early stage of development. The mechanism by which P4 exerts its effect on CL function is not known, but there is some evidence that P4 can increase LH receptor numbers in the developing CL (although not in the mature CL) which may affect luteal sensitivity to LH and luteal functional maturation during the early luteal phase (Jones *et al.*, 1992; Okuda *et al.*, 1999).

In common with other species, it appears therefore that P4 is a significant luteotrophin in cattle (Rothchild, 1981).

However, some current findings seem to contradict the above contention. For example, in LHRH antagonist-treated heifers, Peters *et al.* (1994) and Ulker *et al.* (2001) demonstrated that pulsatile LH secretion is more important for CL development and function than is basal LH secretion, particularly until Day 12 after ovulation. Peters *et al.* (1994) also found that when LH pulses were absent after LHRH antagonist treatment during the early luteal phase, the development of SLC into the LLC was affected, resulting in reduced LLC numbers and, thereby, reduced P4 production in the late luteal phase (Peters *et al.*, 1994; Ulker *et al.*, 2001). It is possible that the LHRH antagonist treatment may wipe out other factor(s) that may be involved in the process of CL formation, development and function, or may modify the *in vivo* endocrine environment after treatment. More probably, it is likely that low basal LH concentrations after the treatment may be insufficient to maintain the function of mature CL (Wiltbank, 1994).

Of some interest in this regard is the suggestion that luteotrophic factors other than LH may be involved in stimulating P4 production in LLC (Wiltbank, 1994). These involve a number of growth hormones and growth factors, including growth hormone, insulin, insulin-like growth factor-I (IGF-I), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β) and nerve growth factor (NGF: McArdle and Holtorf, 1989; Miyamoto *et al.*, 1992; Sauerwein *et al.*, 1992; Liebermann *et al.*, 1996). In addition, Hansel and Dowd (1986), Weems *et al.* (1998) and Alila *et al.* (1988) also showed that PGE_2 and prostacyclin (PGI_2) have a luteotrophic effect on the bovine CL both *in vivo* and *in vitro*. In the SLC, PGE_2, $PGF_{2\alpha}$ and PGI_2 exert a luteotropic action *in vitro*, but in the LLC, only PGE_2 and PGI_2 are luteotropic, while $PGF_{2\alpha}$ exerts an antiluteotrophic action (Alila *et al.*, 1988). The mechanisms by which these substances regulate the bovine CL function have been reviewed in detail by Davis *et al.* (1996). Wiltbank (1994) concluded that the mid-cycle CL function may rely on both luteotrophic support and a relative autonomy of the CL from trophic stimulation. He, therefore, suggested the need to search for a better understanding of the interactions between luteal cells and better definition of the way in which *in vivo* mechanisms can be inferred from *in vitro* findings.

1.1.15 Progesterone secretion during the luteal phase

During the period between Days 1 and 4, granulosa-derived cells (LLC) develop into functioning luteal cells which are capable of secreting P4. By Days 3 to 6 after ovulation, the CL begins to produce and secrete considerable amounts of P4 (Robertson, 1972). By Day 7, SLC cells undergo mitosis, leading to a threefold increase in CL size and increased P4 secretion (Donaldson and Hansel, 1965a). This is reflected in peripheral P4 concentrations which increase rapidly from Day 3 to Day 8 (Stabenfeldt *et al.*, 1969). Interestingly, Mann *et al.* (2001) found a positive correlation between CL weight and plasma P4 concentrations on Day 5, but not on Day 16 after oestrus. Garverick *et al.* (1985) also reported that CL weight, luteal P4 and plasma P4 concentrations increased from Days 4 to Day 7 or 10 and declined by Day 19.

The CL is fully mature by 8-12 days after ovulation, when P4 secretion is maximal (Robertson, 1972; Mihm and Roche, 1997; Parkinson *et al.*, 1994). This contention is in agreement with the finding that the CL reaches its maximum diameter around Day 9 and increases its P4 secretion capacity from Days 3 to 4 to produce peak circulating concentrations on Day 13 to Day 15 (Robertson and Sarda, 1971; Taylor and Rajamahendran, 1991b). The increase in size is related to luteal growth, resulting mainly from cell proliferation rather than cell hyperplasia (Zheng *et al.*, 1994).

However, when the mitotic phase has passed, the fully formed luteal cells are able to respond to exogenous luteotrophin stimulation by increasing P4 production (Lobel and Levy, 1968). Nevertheless the rate of P4 secretion from Day 8 to Day 17 is slower than during the first 8 days. In the late luteal phase, P4 synthesis declines rapidly as the onset of luteolysis occurs, with concentration declining to baseline over a period of 2-3 days (Stabenfeldt *et al.*, 1969; Robertson and Sarda, 1971).

1.1.16 Luteolysis

Normal luteolysis has been defined as lysis or structural demise of the CL, in association with loss of the capacity to synthesise and secrete P4 and loss of the cells that comprise the CL (Niswender *et al.*, 2000). Knickerbocker *et al.* (1988) summarised the main mechanisms by which uterine $PGF_{2\alpha}$ causes regression of the CL. These included an abrupt reduction in luteal blood flow (Nett and Niswender, 1981), alteration of structure and function of luteal cells, induction of chemoattractants for eosinophils, blockage of LH-stimulated steroidogenic pathways, effects on calcium mobilisation (Wiltbank *et al.*, 1991)

and loss of activity of enzymes involved in P4 synthesis and secretion (McCracken *et al.*, 1999).

Role of the uterus in CL regression

The control of the CL's life span by the uterus has been extensively investigated in ruminant species. In hysterectomised heifers and ewes, the functional life span of the CL is prolonged well beyond the normal time of regression (Wiltbank and Casida, 1956; Moor and Rowson, 1966a), suggesting a direct luteolytic action of the uterus on the CL (Moor and Rowson, 1966a; Niswender *et al.*, 2000). When conception does not occur, luteolysis occurs in response to the pulsatile release of $PGF_{2\alpha}$ by the uterus (Pate and Condon, 1989; Juengal *et al.*, 1993; 1999; Pate, 1994). Recent findings have shown that the uterus responds to luteal OT to promote the release of the endometrial $PGF_{2\alpha}$ pulses that are required for luteal regression (McCracken *et al.*, 1999). In addition, the results from studies that involved hysterectomy and surgical anastomosis in cows have indicated the involvement of a venoarterial pathway in the local uteroovarian luteolytic effect (Ginther and Campo, 1974; Mapletoft *et al.*, 1976).

$PGF_{2\alpha}$ as luteolysin in cattle

In ruminants, $PGF_{2\alpha}$ produced by the endometrium under the influence of OT and, perhaps, ovarian E2, is the luteolytic factor that induces luteal regression (Hansel, 1975). Injection of $PGF_{2\alpha}$ or its analogue can bring about and accelerate the onset of the CL regression (Heath *et al.*, 1983; Parfet *et al.*, 1989). Likewise, heifers injected with arachidonic acid, a precursor of PGF, also exhibited a decline in circulating plasma progesterone concentration (Hansel, 1975). It is evident that, in the cow, the release of $PGF_{2\alpha}$ from the uterus occurs during the 2-3 days prior to and during luteolysis as a series of rapid pulses with a duration of 1-5 h (Kindahl *et al.*, 1976b). Schramm *et al.* (1983) also demonstrated that five pulses of $PGF_{2\alpha}$ infusion over a period of 25 h induced luteal regression in sheep, but continuous infusion did not. This relatively short period of pulse frequency of $PGF_{2\alpha}$ coincided with a period of decreasing P4 concentrations. However, no such pulsatile secretion of $PGF_{2\alpha}$ occurs in pregnant heifers (Kindahl *et al.*, 1976a).

Hormone control of uterine $PGF_{2\alpha}$ release

It is now widely recognised that complex endocrine and local mechanisms, involving E2 from the dominant follicle, OT from the CL and P4 from the CL, regulate $PGF_{2\alpha}$ synthesis and secretion from the uterus. Early studies by Lobel and Levy (1968) and Peterson *et al.*

(1975) suggested that, in the normal oestrous cycle, increasing E2 production by the pre-ovulatory follicle contributes to the initiation of luteolysis. In fact, Fogwell *et al.* (1985) showed that the non-ovulatory follicles which are present during late dioestrus are necessary to trigger luteal regression. In ewes, Hansel (1975) showed that luteolysis did not occur in response to PGF treatment unless E2 was also detectable in jugular blood samples. Moreover, E2 is also produced in the bovine CL and stimulates $PGF_{2\alpha}$ secretion in mid-luteal cell cultures *in vitro* (Okuda *et al.*, 2001). Thus, both $PGF_{2\alpha}$ and E2 are both necessary for a full luteolytic effect.

Luteal function was extended after destruction of ovarian follicles 9-15 days after oestrus, suggesting that secretions of ovarian follicles are required for spontaneous luteal regression (Villa-Godoy *et al.*, 1985). Administration of E2 (Salfen *et al.*, 1999) early in the luteal phase shortened the luteal phase. Luteolysis was delayed when follicular growth was suppressed (Salfen *et al.*, 1999). Thus, E2 seems to be a luteolytic factor in cattle with an inhibitory action on the CL, and it suppresses P4 production by blocking the LH stimulatory effect at a step after cAMP (Williams and Marsh, 1978).

The development of oxytocin receptors (OTR) in the endometrium is the key event which initiates luteolysis (Wathes and Lamming, 1995). During normal luteolysis, the presence of the endometrial OTR permits luteal OT to generate $PGF_{2\alpha}$ episodes (Soloff and Fields, 1989; Mann *et al.*, 1999) by activating of prostaglandin synthase activity (Tsai *et al.*, 2001). This in turn results in more OT being released from the CL (Flint *et al.*, 1986). Consequently, $PGF_{2\alpha}$ binds to its receptors on LLC and induces luteolysis and also enhances the positive feedback mechanism between CL and endometrium resulting in additional release of $PGF_{2\alpha}$ (Flint *et al.*, 1986; Silvia and Taylor, 1989). It has been found that the endometrial OTR concentrations increased sharply during the onset of luteolysis in the cow (Jenner *et al.*, 1991) and were significantly higher in non-pregnant than pregnant heifers (Parkinson *et al.*, 1990). The ability of OT to stimulate uterine $PGF_{2\alpha}$ secretion increases during the end of the oestrous cycle (Lafrance and Goff, 1985). The regulatory mechanisms of luteolysis in domestic animals have been reviewed recently (Pate, 1994; McCracken *et al.*, 1999; Niswender *et al.*, 2000).

It appears that P4 and E2 also play a major role in the control of the development of OTR and $PGF_{2\alpha}$ pulses leading to CL regression (Vallet *et al.*, 1990; Silvia *et al.*, 1991; McCracken *et al.*, 1999). Mann (2001) found a stimulatory effect of E2 on endometrial $PGF_{2\alpha}$ production and an inhibitory action of P4 on bovine endometrial OTR

concentrations *in vitro*. Moreover, both elevated E2 concentrations between Days 14 and 17 of the oestrous cycle (Pritchard *et al.*, 1994) and raised E2:P4 ratios have been associated with failure to conceive in the cows (Randel *et al.*, 1971; Erb *et al.*, 1976; Maurer and Echternkamp, 1982).

The effects of $PGF_{2\alpha}$ on the CL

$PGF_{2\alpha}$ is the primary luteolytic hormone in most mammals. It appears to exert its antisteroidogenic actions (inhibition of P4 synthesis and secretion by the luteal cells) via activation of the protein kinase C system. Its cytotoxic effects appear to be mediated via a marked increase in the intracellular concentrations of free calcium (Niswender *et al.*, 1994). A number of other luteolytic actions of $PGF_{2\alpha}$ have been recognised including (a) vasoconstrictive effects (reduced blood flow to CL, thereby reducing circulating P4 concentrations: Wiltbank, 1994), (b) cytotoxic effects (Niswender *et al.*, 1994) and (c) uncoupling LH receptors from adenylate cyclase (cAMP: Marsh, 1971). The luteolytic action of $PGF_{2\alpha}$ in the bovine species could therefore involve both an inhibition of the LH induced synthesis of cAMP and an inhibition of the downstream actions of cAMP (Benhaim *et al.*, 1987). It has been suggested that the decrease in gonadotropin receptors following $PGF_{2\alpha}$ injection is a reflection of a general decline in the structural, functional and metabolic integrity of luteal cells (Rao *et al.*, 1984).

Endothelin-1 (ET-1) secretion from both steroidogenic and endothelial cells of the bovine CL is a further potential paracrine control mediating $PGF_{2\alpha}$-induced luteolysis (Girsh *et al.* 1996a; 1996b). There is evidence that, in heifers, exogenous $PGF_{2\alpha}$ induces increased luteal output of ET-1 and probably results in decreases in luteal blood flow (Milvae, 2000). This subject still requires further research.

Functional luteolysis and antisteroidogenesis

Although gene transcription, as indicated by the concentrations of mRNA encoding steroidogenic enzymes, may decline in response to $PGF_{2\alpha}$, this does not result in a sufficiently rapid reduction in the concentrations of the enzymes to cause the precipitous decline in plasma progesterone concentrations that occurs during luteolysis (Rodgers *et al.*, 1995). The initial decrease in plasma P4 concentrations during luteolysis, therefore, does not appear to be due to loss of steroidogenic luteal cells, since the numbers of these cells do not decline until well after plasma P4 concentrations have decreased (Braden *et al.*, 1988). Nevertheless, Niswender *et al.* (2000) suggested that the decrease in plasma P4

concentrations is most likely to be due to the decreased luteal blood flow and decreased steroidogenic capacity of each luteal cell. Thus, the exact mechanism for the initial decline in plasma P4 concentrations during luteolysis is still not fully understood.

Lipoproteins can enhance luteal P4 production and suppress de-novo cholesterol synthesis *in vitro*. Only the former effect of lipoproteins is inhibited by $PGF_{2\alpha}$ (Pate and Condon, 1989), so it has been suggested that $PGF_{2\alpha}$ allows entry of cholesterol (a substrate for steroid hormone synthesis) into the cell, but prevents its utilisation for steroidogenesis. In addition, $PGF_{2\alpha}$ suppresses cholesterol synthesis, as well as decreasing the rate of conversion of cholesterol to P4 (Pate and Condon, 1989). In fact, structural changes in the bovine CL in early luteal regression are associated with increased in cellular content of esterified sterols (Hafs and Armstrong, 1968), lipid content (Lobel and Levy, 1968) and lysosome content (Parry *et al.*, 1980).

Both interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) may also be involved in antesteroidogenic and cytotoxic effects during luteolysis by inducing PG production and inhibiting LH-stimulated P4 production, and bring about the decline in steroidogenesis and luteal cell viability (Fairchild and Pate, 1991; Benyo and Pate, 1992).

It is likely that CL function ceases (as indicated by reduced P4 secretion) before structural luteolysis (as seen by cell apoptosis) occurs (Juengel *et al.*, 1993), suggesting the possible autocrine role of P4 in controlling its own function. Recent findings suggested that P4 could play an active role in the inhibition of luteal regression by a direct effect on the CL (Rueda *et al.*, 2000).

Blood flow

The flow of blood through the CL is essential not only for removal of P4 from CL into the circulation but also for enhanced delivery of hormones, nutrients and substrates to the CL (Wiltbank, 1994). By Day 18, both morphological and cytochemical signs of degeneration are prominent, especially the collapse of the vascular system and declines in enzyme activities and P4 secretion, whereas the vascular network of the CL of pregnancy remains intact (Lobel and Levy, 1968). It could be argued that the functional life of the CL begins with vascularisation of the granulosa and ends abruptly with the collapse of the vascular system within the luteal tissue (Lobel and Levy, 1968; Acosta *et al.*, 2003).

Structural luteolysis

Although definitive luteolysis starts on Days 17-18 of the cycle, early degenerative changes are present by Day 14-16 in the CL of the cycle (Hopkins, 1989; Parkinson and Lamming, 1990). Luteolysis leads to the onset of luteal cell death, followed by a decline in P4 secretion with structural involution of the CL (Juengel *et al.*, 1993; Pate, 1994) and formation of the inactive corpus albicans, which is composed of connective tissue and collagen (McCracken *et al.*, 1999). The structural changes initially occur at the submicroscopic level in cellular membranes of the CL, which appear to be related to the loss in cellular function coinciding with a decline in P4 secretion ability of the CL (Carlson *et al.*, 1982).

Luteal regression from Day 17 to Day 21 was primarily linked with cell deletion and decreased cell size (as shown by a decrease in luteal DNA content and the occurrence of cell apoptosis and decreased protein synthesis capacity; Zheng *et al.*, 1994). Signs of luteal cells degenerations involve nuclear changes, increased peripheral vacuolation and condensation of cytoplasm (Donaldson and Hansel, 1965; Hafs and Armstrong, 1968) and degeneration of mitochondria and other cell organelles (Parry *et al.*, 1980). In addition, luteolysis is related to marked increases in vacuoles in the luteal cells and in degenerated endothelial cells as shown by pyknotic and disintegrated nuclei (Lobel and Levy, 1968). Parkinson *et al.* (1994) also found lower proportions of luteal cell cytoplasm-containing vacuoles in pregnant cows than in cyclic cows from Day 12 after oestrus. Some changes in lysosomal enzyme activity have also been suggested to be associated with the degenerative changes in the structural involution of the CL during luteolysis (McCracken *et al.*, 1999).

Immune system

It is evident that immune cells participate in the structural luteolysis (McCracken *et al.*, 1999). In fact, the first signs of luteal degeneration in cyclic CL begin on Day 11-17 with an infiltration with leucocytes, well before the onset of luteolysis and increase steadily until the end of the cycle (Lobel and Levy, 1968; Bauer *et al.*, 2001). The immune cells, primarily macrophages and T lymphocytes are present in the CL and play important roles in luteolysis (Bauer *et al.*, 2001; Pate and Keyes, 2001). Interleukin-1β (IL-1β) could act as a regulator of luteal PGF production during periods when progesterone concentrations are low, such as during luteal development and regression (Nothnick and Pate, 1990).

Raising P4 concentrations reduced basal as well as IL-1β-stimulated production of 6-keto-$PGF_{1\alpha}$ (inactive form of PGI_2), PGE_2 and $PGF_{2\alpha}$ *in vitro* (Nothnick and Pate, 1990). The ability of IL-1β to stimulate luteal $PGF_{2\alpha}$ production via up-regulating the enzyme involved in $PGF_{2\alpha}$ synthesis while inhibiting luteal P4 production suggests that IL-1β may facilitate regression of the CL (Townson and Pate, 1996).

1.1.17 CL of pregnancy and pregnancy recognition

The successful outcome of a pregnancy depends upon complex interactions between the uterus (i.e. endometrium), the CL and the conceptus. Much research has been undertaken to gain a better understanding of this important phenomenon. The mechanisms involved in the maternal recognition of pregnancy vary widely among species and may involve direct luteotrophic stimulation of the CL, reduced uterine secretion of $PGF_{2\alpha}$, and/or inhibition of actions of $PGF_{2\alpha}$ at the level of the CL (Wolfenson *et al.*, 1985; Niswender *et al.*, 1994).

The high amplitude luteolytic pulses of $PGF_{2\alpha}$ that come from the uterus at the onset of luteolysis do not occur in pregnant cows (Kindahl *et al.*, 1976a; Wolfenson *et al.*, 1985; Parkinson and Lamming, 1990). Moreover, the responsiveness to LH of luteal tissues is higher in terms of P4 release *in vitro* from pregnant cows than from cyclic animals (Lukaszewska and Hansel, 1980). There is also evidence that the CL of early pregnancy in ewes is resistant to the luteolytic effect of $PGF_{2\alpha}$ (Silvia and Niswender, 1984). All of these lead to CL maintenance and sustained P4 secretion.

In addition, both endometrium and embryo secrete a considerable amount of PGE_2 during early pregnancy, which may have either a luteotrophic action on the CL or suppress the luteolytic action of E2 and $PGF_{2\alpha}$ (Gimenez and Henricks, 1983; Weems *et al.*, 1998). However, Parkinson *et al.* (1991) found no luteotrophic effect of PGE_2 when administered directly into the aorta of mid-luteal phase heifers, suggesting that its action may involve other components of the utero-ovarian system.

During pregnancy, both luteal and circulating concentrations of OT are low (Schams *et al.*, 1983). The $PGF_{2\alpha}$ secretion in response to OT challenge is attenuated in pregnant cows on Day 18 after oestrus (Lafrance and Goff, 1985; Parkinson *et al.*, 1990) and endometrial OTR concentrations are lower in pregnant than cyclic cows (Parkinson *et al.*, 1990). Circulating E2 concentrations are lower around the time of the onset of luteolysis in pregnant than cyclic cows (Henricks *et al.*, 1972; Schallenberger *et al.*, 1989) which, given the association between uterine OTR concentrations and plasma E2 concentrations

(Parkinson *et al.*, 1990), suggest that altered E2 dynamics may also assist in the early pregnancy maintenance.

The critical period of maternal recognition of pregnancy in the cow occurs on Day 16 postoestrus (Northey and French, 1980; Humblot and Dalla Porta, 1984). The presence of an embryo in the uterus at this time, the injection of homogeneates from 17-18-day-old bovine embryo into the uterus or the uterine transfer of trophoblastic vesicles all delay normal luteolysis (Moor and Rowson, 1966b; Northey and French, 1980; Heyman *et al.*, 1984). These findings show that the early signals that inhibit luteolysis are of trophoblastic origin. The developing conceptus produces an antiluteolytic protein between Days 15-17 of gestation which prolongs the life span of the CL (Knickerbocker *et al.*, 1986; Godkin *et al.*, 1988b; Plante *et al.*, 1988). This protein was initially called bovine trophoblast protein (bTP-1) but was later identified as an interferon and named bovine interferon-τ (bIFN-τ; Stewart *et al.*, 1992). It is produced by the trophectoderm of the peri-implantation conceptus (Godkin *et al.*, 1988a). Infusion of bIFN-τ into the uterine lumen of cyclic cows extends luteal function (Meyer *et al.*, 1995).

The mechanism of IFN-τ action in prolonging the life span of CL is through a local action upon the uterine epithelium, which abrogates the OT-induced increase in pulsatile secretion of $PGF_{2\alpha}$ (Robert *et al.*, 1992; 1996; Meyer *et al.*, 1995; Mann and Lamming, 2001). IFN-τ interacts with type 1 IFN receptors in the uterine epithelium to block E2 receptors and OTR gene expression, inhibiting the ability of E2 and OT to stimulate luteolytic pulses of $PGF_{2\alpha}$ (Mann *et al.*, 1999; Robinson *et al.*, 1999; Thatcher *et al.*, 2001). The positive feedback loop between luteal OT and uterine $PGF_{2\alpha}$ is thereby interrupted, allowing maintenance of the CL and sustaining the circulating P4 concentrations thereafter (Thatcher *et al.*, 2001).

In addition to the role of IFN-τ upon OTR, other antiluteolytic actions of this protein involve stabilising or increasing P4 receptors in the endometrium, inducing the synthesis of an inhibitor of enzymes required for $PGF_{2\alpha}$ synthesis in the endometrium, inhibiting endometrial E2 receptors and/or initiating post receptor mechanisms (Bazer *et al.*, 1991; 1994; Binelli *et al.*, 2000). In a recent study, Wang *et al.* (2003) also speculated that abnormal IFN-τ and/or P4 secretions could result in pregnancy failure for reasons other than failure to maintain the CL function.

1.1.18 Luteal dysfunction

Subnormal function of the CL has been associated with subfertility in cattle (Folman *et al.*, 1973). Garverick and Smith (1986) classified subnormal CLs into two types, (i) a short lifespan (Odde *et al.*, 1980) and (ii) a normal lifespan with decreased P4 secretion (Pratt *et al.*, 1982). The first CL after calving, like that of the first pubertal oestrus, often has a shorter lifespan (7-10 days) than normal and produces lower P4 concentration than CL of subsequent cycles (Odde *et al.*, 1980; Manns *et al.*, 1983; Eger *et al.*, 1988; Eldon, 1991; Perry *et al.*, 1991).

It has been suggested that the cause of this short-lived CL may be insufficient LH support leading to altered follicular development and/or premature or early increased $PGF_{2\alpha}$ release from the involuting uterus or altered responsiveness to $PGF_{2\alpha}$ (Copelin *et al.*, 1988; Braden *et al.*, 1989; Roberson *et al.*, 1989). In fact, lack of gonadotrophin support is unlikely to be a primary cause of premature luteal regression of short-life CL (Duby *et al.*, 1985; Garverick *et al.*, 1988) but, rather, the cause seems to be the premature induction of luteolysis, associated with the presence of the endometrial OTR (Zollers *et al.*, 1989; Hunter, 1991). Copelin *et al.* (1988) showed that putative short-lived CLs were no more responsive to $PGF_{2\alpha}$ than ones expected to have a normal lifespan. However, preferential biosynthesis of $PGF_{2\alpha}$ in the subnormal CL may further alter the ratio of luteotrophin:luteolysin facilitating premature luteolysis (Hu *et al.*, 1990). In addition, P4 receptors on Day 5 were higher in cows having a normal luteal phase (Zollers *et al.*, 1993), suggesting that the uterus of cows with short-lived CLs may lose P4 dominance at an earlier time in the cycle and allow the premature synthesis of OTR. Mann and Lamming (2000b) showed that low preovulatory E2 concentrations in the first postpartum ovulation were associated with high levels of OTR in the following luteal phase.

The possible causes of inadequate P4 production were suggested to involve inadequate preovulatory follicular development, abnormal luteal maturation or lack of CL maintenance (Farin and Estill, 1993). Robinson *et al.* (1989) showed that low P4 concentrations during the luteal phase may involve altered LH secretion and, thus, ovarian folliculogenesis during the subsequent cycle. The increased duration of the luteal phase and luteal P4 concentrations from the first to the third postpartum ovarian cycle reflects progressive increase in the CL activity during this period (Odde *et al.*, 1980; Eldon, 1991).

On the other hand, persistent CLs are described as having sustained function over the appropriate time (beyond Day 17 or 18 of the oestrous cycle: Farin and Estill, 1993), so that the cow fails to return to oestrus (Foley, 1996). The loss of an embryo or a foetus, or inflammatory conditions of the uterus or horns can disrupt the normal luteolytic mechanism, causing persistence of the CL (Lynn *et al.*, 1966; Gilbert *et al.*, 1990; Noakes *et al.*, 1990). Lamming and Bulman (1976) found that two percent of cows exhibited spontaneous persistent CL with high milk P4 for at least 30 days after ovulation, suggesting that luteolysis did not occur at the normal time (i.e. 17 days after ovulation).

1.9 Progesterone concentrations and pregnancy outcome

1.1.19 P4 concentrations after mating, embryo survival and conception

The P4 secreted from the CL is responsible for the maintenance of pregnancy (Farin and Estill, 1993). Establishment of pregnancy in the cow is associated with a prolongation of luteal function beyond the 18 days duration of the luteal phase in the non-pregnant animal (Gordon, 1996). The extent to which P4 concentrations determine, or are determined by, pregnancy outcome remains a greatly controversial subject. On one hand, there are a number of studies which suggest mechanistic reasons for a beneficial effect of high P4 concentrations upon pregnancy outcomes. For example, decreased P4 concentrations in the uterus, coupled with increased E2 concentrations can contribute to early embryo loss (Mann and Lamming, 1995c; Mann *et al.*, 2002). The role of P4 in the maternal regulation of the growth and development of bovine conceptus, which is positively related to uterine bIFN-τ, protein and growth factor secretion during early pregnancy, has also been well established (Garrett *et al.*, 1988; Kerbler *et al.*, 1997; Mann *et al.*, 1998; Green *et al.*, 2001). In addition, luteal P4 has been identified as an anti-apoptotic agent that can repress the onset of apoptosis in the CL via its own receptor-dependent mechanism (Rueda *et al.*, 2000). Interestingly, P4 has been reported to be capable of suppressing the ability of OT to induce endometrial secretion of $PGF_{2\alpha}$ *in vitro* (Skarzynski *et al.*, 1999; Bogacki *et al.*, 2002).

On the other hand, it is unclear whether differences in plasma or milk progesterone profile, which are detected during the early and mid luteal phase of pregnant compared to non-pregnant cows cause or are caused by pregnancy success. As embryo losses in cows are followed by luteolysis and resumption of the new cycle (Sreenan and Diskin, 1983), it remains questionable whether luteolysis is the potential cause or effect of these losses. The

divergence of plasma or milk P4 concentrations between pregnant and cycling cows occurs between Day 6 (Erb *et al.*, 1976), Days 10-16 (Henricks *et al.*, 1972; Lamming *et al.*, 1989;) and the onset of luteolysis (Day 18-19; Shemesh *et al.*, 1968; Lukaszewska and Hansel, 1980; Parkinson and Lamming, 1990). Such divergences during mid-luteal phase has been suggested to reflect either i) an antiluteolytic effect of the embryo, or ii) luteal dysfunction and resultant embryo death (Sreenan and Diskin, 1983).

a. Early luteal phase P4 concentrations (Day 1 to Day 7)

Many attempts have been made to correlate P4 concentrations after insemination with the chances of conception or subfertility in dairy cows. Green *et al.* (2001) reported that CL weight and P4 content were related to the degree of embryo development on Day 5 post-oestrus. A delayed rise in P4 concentrations (indicating subnormal luteal function) during the first 5 days after insemination was related to a shorter subsequent luteal phase and reduced pregnancy rate (Shelton *et al.*, 1990; Lamming and Darwash, 1995; Starbuck *et al.*, 2001). In fact, Henricks *et al.* (1972) and Lamming *et al.* (1989) found similar patterns of P4 production up to Day 7 after oestrus in non-inseminated pregnant and inseminated non-pregnant cows. In addition, Verkerk and Macmillan (1998) found that milk P4 concentrations on Day 4 or 8 after insemination could not predict the probability of either return to service or pregnancy status. Conversely, Thompson *et al.* (1980) reported that P4 concentrations were higher from Days 5 to 16, with a faster rate of P4 increase between Days 5 and 10 in the pregnant than non-pregnant heifers. Higher P4 concentrations 6 days after ovulation in cows that conceived than in those that failed to conceive have also been reported (Erb *et al.*, 1976).

A delayed rise in P4 concentrations during the early luteal phase has also been associated with smaller embryos that produced less bIFN-τ and were less capable of inhibiting luteolysis (Mann *et al.*, 1999). In addition, low P4 concentrations during the early luteal phase may enhance later responsiveness to OT on Days 15 and 16 of the cycle (Mann and Lamming, 1995c; Shaham-Albalancy *et al.*, 2001), leading to stronger luteolytic signals. Unfortunately, advancing the P4 rise by exogenous administration at the start of luteal phase advances the development of the luteolytic mechanism, but delaying the rise does not delay its development (Mann *et al.*, 1994).

b. Mid-luteal phase to early luteolytic phase P4 concentrations (Day 10 to 21)

Many studies have demonstrated that P4 concentrations were lower during the second half of the luteal phase (i.e. around Days 10-18 of the oestrous cycle) in cows that undergo luteolysis than in cows which are pregnant (Henricks and Dickey, 1970; Henricks *et al.*, 1971; Lukesewska and Hansel, 1980; Hansel, 1981; Lamming *et al.*, 1989; Parkinson and Lamming, 1990; Pritchard *et al.*, 1994; Mann *et al.*, 1995; Larson *et al.*, 1997). It has been argued that such divergences suggest the presence of embryo-derived antiluteolytic or luteotrophic signals at least by Day 12 after oestrus (Lamming *et al.*, 1989; Thatcher *et al.*, 1989).

On the other hand, many other reports revealed no difference in plasma P4 concentrations between pregnant and cyclic cows between Days 10 and 17 (Pope *et al.*, 1969; Edgerton and Hafs, 1973; Folman *et al.*, 1973; Geisert *et al.*, 1988). Furthermore, Taylor and Rajamahendran (1991a) found no differences between pregnant and non-pregnant cows in the maximum size and growth rate of CL, peak circulating P4 concentrations during mid-luteal phase and profile of P4 during 24 days after AI. The reason behind this discrepancy is unknown, except that there must clearly be other factor(s) involving in the chance of conception rather than P4 concentration *per se*.

Moreover, high plasma P4 concentrations during mid-luteal phase of at least one oestrous cycle before insemination has been positively correlated to the chance of conception (Folman *et al.*, 1973; Holness *et al.*, 1981; Rosenberg *et al.*, 1990). Interestingly, P4 concentrations at the time of first insemination were related to a lower chance of conception (Waldman *et al.*, 2001), whereas repeat-breeder cows tended to have high P4 concentrations before ovulation and a late postovulatory rise in plasma P4 (Båge *et al.*, 2002). Thus it also seems that the pattern of P4 profile during the luteal phase before and after insemination is one of the factors indicating and affecting the chance of conception in the cow.

1.1.20 Progesterone supplementation and pregnancy success

The results of exogenous P4 supplementation in order to improve conception outcomes are very variable. In fact, meta-analysis showed that P4 supplementation after insemination does not improve pregnancy rates (Lean *et al.*, 2003). Administration of P4 advanced the conceptus growth and development associated with early synthesis and release of polypeptides from endometrial explant cultures on Day 5 (Garrett *et al.*, 1988). Pregnancy

was maintained beyond Day 40 in P4 treated cows. Improved pregnancy rates have been reported in trials with a variety of methods of P4 supplementation (Diskin and Sreenan, 1986; Robinson *et al.*, 1989), but not in others (Van Cleeff *et al.*, 1991; Rhodes *et al.*, 2001). Similar variable results have also been found when hCG or GnRH have been administered in order to increase P4 concentrations (Helmer and Britt, 1986; Sianangama and Rajamahendran, 1992; Tefera *et al.*, 2001). Thatcher *et al.* (1994) concluded that supplemental P4 during the luteal phase is unlikely to exert a major stimulation upon embryo survival.

1.10 Endocrine changes during the postpartum period

The function of the hypothalamo-pituitary-ovarian-axis has a pivotal role for the postpartum cow to return to normal oestrous cycles. Several reviews of endocrine functions during the postpartum period in the cow have been published (Wettemann, 1980; Lamming *et al.*, 1981; Garcia, 1982; Peters and Lamming, 1984a; 1984b; 1986; 1990; McNatty, 1988; Hussain and Daniel, 1991; Jolly, 1993; Roche and Diskin, 1995; Opsomer *et al.*, 1996), highlighting a number of endocrine factors and mechanisms which are important for the resumption of normal reproductive function after calving. During late gestation, high steroid concentrations suppress LH synthesis and secretion in the AP, thus limiting LH release after calving (Nett, 1987: Rahe *et al.*, 1988). After parturition, mean E2 and P4 concentrations decline and remain low during the early postpartum period (Azzazi *et al.*, 1983; Kaker *et al.*, 1984).

1.1.21 Hypothalamo-pituitary axis

Nett *et al.* (1988) suggested that the decreased LH secretion during the early postpartum period is probably due to high steroid (E2 and P4) concentrations in circulation during late gestation, resulting in low LH stores in the AP (Nett *et al.*, 1988; Rahe *et al.*, 1988). Thus, on Day 275 of pregnancy and Day 2 postpartum, the pituitary content of LH is low compared to that present on Day 3 of the oestrous cycle (Rahe *et al.*, 1988). Therefore, low circulating concentrations of LH are evident during the early postpartum period which is probably the main factor limiting the initiation of ovarian activity after calving (Nett *et al.*, 1988). This is vital because follicle development and maturation is dependent on gonadotrophin support (Vishwanath *et al.*, 1996). The amount of the available GnRH in the hypothalamus does not seem to be a limiting factor (Carruthers *et al.*, 1980; Nett *et al.*,

1988) since it is evident that there are sufficient stores of GnRH to initiate cyclic activity. Therefore, it is the release of GnRH that is suppressed (Nett et al., 1988).

The AP is less sensitive to GnRH during the early postpartum period, but its responsiveness starts to be restored by as early as 7 to 10 days after calving (Fernandes *et al.*, 1978; Azzazi *et al.*, 1983). In addition, Rahe *et al.* (1988) reported low number of GnRH receptors in the AP during the first two days after calving. This further indicates that the GnRH receptors and releasable pool of LH at pituitary level are probably sufficient. The LH stores in the AP was low between Days 1 and 15 postpartum (Nett *et al.*, 1988). However, about a third of the cows still do not regain normal pituitary responsiveness to exogenous GnRH and ovarian function by three weeks postpartum (Torres *et al.*, 1997). There is a progressive increase in the LH response to GnRH stimulation between Days 5 and 19 (Kesler *et al.*, 1977). Whether there is any difference in the AP responses after calving between dairy cows with different genotype or strain is unknown.

After a rise in the early postpartum period, plasma FSH concentrations thereafter appear to be less variable (Dobson, 1978; Schams *et al.*, 1978: Webb *et al.*, 1980; Lamming *et al.*, 1981). This early secretion of FSH initiates follicle growth and development. It is therefore believed that FSH secretion is not the limiting factor controlling the initiation of ovarian activity postpartum. In the other words, the pituitary content of FSH seems to be sufficient to drive follicular growth and development from very soon after calving (Nett *et al.*, 1988; Rahe *et al.*, 1988).

The key pre-requisites for resumption of ovarian activity and oestrous cycling in the postpartum cow are, therefore, increasing LH concentrations and the restoration of episodic LH secretion pattern (Edgerton and Hafs, 1973; Goodale *et al.*, 1978; Lamming *et al.*, 1981; Peters *et al.*, 1981; Peters and Riley, 1982; Azzazi *et al.*, 1983; McDougall *et al.*, 1995a). The resumption of episodic LH secretion pattern probably reflects the resumption of episodic release of GnRH from the hypothalamus, although GnRH-induced LH release also increases with time postpartum (Fernandes *et al.*, 1978; Foster *et al.*, 1980; Azzazi *et al.*, 1983; McDougall *et al.*, 1995b). However, changes in LH pulse secretion pattern are closely related to the increased pituitary content of LH (Nett *et al.*, 1988), and may reflect increased responsiveness of the AP to GnRH rather than just an increased magnitude of GnRH pulses. Recently, Stagg *et al.* (1998) confirmed that both mean LH concentrations and LH pulse frequency significantly increased during the six

follicle waves that precede the first ovulatory wave in beef cows, but FSH concentrations remain relatively unchanged. The interval between each pulse also decreased as first ovulation approached (Stagg *et al.*, 1998). Schallenberger and Hutterer, (1982) suggested that a certain threshold frequency of LH secretion is needed before E2 can elicit a positive feedback and initiate regular oestrous cycles after calving.

1.1.22 Ovarian follicular development after calving

Follicular development resumes very quickly after calving, with antral follicles being present 7-10 days after calving (Morrow *et al.*, 1966). Follicular growth and development, as characterised by growth of small (< 4 mm) and medium (5-9 mm) follicles, resumes within two weeks after calving (Murphy *et al.*, 1990; McDougall *et al.*, 1995a; Kamimura *et al.*, 1994). Later on, DFs develop. Most of the first DFs eventually ovulate (Savio *et al.*, 1990b). It is now clear that the prolonged anoestrous period after calving is primarily due to the failure of the AP to generate the pre-ovulatory LH surge in response to rising circulating E2 concentrations (Alam and Dobson, 1987; Gyawu and Pope, 1990) rather than an intrinsic inability of DF to release pre-ovulatory concentrations of E2 (Murphy *et al.*, 1990; Savio *et al.*, 1990a; Kamimura *et al.*, 1993; 1994; Stagg *et al.*, 1995).

1.1.23 Uterine involution and $PGF_{2\alpha}$

After parturition, four major changes are required before successful pregnancy can occur: uterine involution, repair of the endometrium, elimination of bacterial contamination which is acquired during calving by uterine defence mechanisms and the resumption of ovarian activity (Sheldon, 1999). The uterine involution process involves uterine contraction, resulting in a reduction of uterine size, loss of tissue whereby the caruncles undergo necrosis and are expelled and tissue repair to replace the endometrial lining (Gier and Marion, 1968; van Camp, 1991).

The reduction in size of the uterus follows a logarithmic pattern during the first few days postpartum (Schirar and Martinet, 1982). The time required to complete involution varies, ranging from 25 to 50 days (Hussain and Daniel, 1991). Two to three days after calving, the uterine lochia, which consists of the sloughed uterine caruncles, remains of foetal fluids, membranes and blood from the ruptured umbilical and endometrial vessels, starts to be discharged. Discharge of lochia lasts for a period of 14 to 18 days (Gier and Marion, 1968; Hussain and Daniel, 1991).

The early postpartum period is also characterised by the large amounts of $PGF_{2\alpha}$ that are secreted by the involuting uterus (Kaker *et al.*, 1984). This high-level secretion has to decline below a certain threshold value before the reestablishment of ovarian cyclicity and ovulation can occur (Mann *et al.*, 1983; Kindahl *et al.*, 1992).

The relationship between uterine involution and ovarian cyclicity after calving is not yet fully established, however, it is evident that greater release of $PGF_{2\alpha}$ from the endometrium during the early postpartum period is associated with an enhanced rate of uterine involution (Lindell *et al.*, 1982; Kindahl *et al.*, 1992). The duration of the period of increased uterine $PGF_{2\alpha}$ secretion is also negatively correlated with the number of days to complete uterine involution and with the interval between calving and the resumption of normal ovarian activity (Madej *et al.*, 1984). In NZ dairy cows, Nation *et al.* (1999) found a bias in the distribution of the first DF postpartum and ovulatory follicle towards the contralateral ovary of pregnancy. In addition, Sheldon *et al.* (2003) found that the previously-gravid and non-gravid uterine horns are smaller in cows that ovulated the first DF than in cows in which the follicle regressed. The appearance of a DF in the ipsilateral ovary to that bearing the CL may be a reflection of a utero-ovarian signal associated with more rapid uterine involution relative to the side of pregnancy (Nation *et al.*, 1999; Sheldon *et al.*, 2003).

1.1.24 CL function during postpartum period

P4 determinations in sequential samples of plasma and milk (whole, skim or milk fat) collected, for example thrice per week, have been used under field conditions to investigate the incidence and nature of atypical P4 profiles and their effect on fertility (Kimura *et al.*, 1987; Lamming and Darwash, 1998; Royal *et al.*, 2000b; Lamming and Royal, 2001). The P4 concentrations during the postpartum anoestrus period in the cow are low (Donaldson *et al.*, 1970) and normally display a short rise (i.e. a short luteal phase) before the resumption of the pattern of a normal cycle (Stevenson and Britt, 1979; Lamming *et al.*, 1981). Atypical plasma P4 concentrations were found in some inseminated cows, suggesting abnormal pregnancy or conceptus losses (Bulman and Lamming, 1978).

1.11 Prerequisites for resumption of cyclicity in the postpartum cow

Once ovarian follicles have developed and the positive feedback mechanism of the AP to circulating E2 is re-established, then the first ovulation follows. This occurs at a minimum

of around 10 days postpartum (Peters, 1984b). Oestrous behaviour is almost always absent (silent heat) from the first postpartum ovulation, since a P4 priming effect is required both for behavioural oestrus and normal ovulation (Williams *et al.*, 1982b; Dimmick *et al.*, 1991; Taylor and Rajamahendran, 1994).

There is strong evidence that the main regulators of LH pulse frequency preceding the first ovulation after calving in dairy cows are negative energy balance after calving, body weight and body condition score (BCS) loss early after calving (Henricksen and Jensen, 1985; Canfield and Butler, 1990; Butler, 2001). In a seasonal pasture-based system, McDougall *et al.* (1993) confirmed that the anoestrous cows were in lower energy balance and lower BCS than cycling herd-mates. The results from the Australian InCalf study have also shown that balanced nutrition and sufficient energy intake from pasture (as reflected in higher percentages of milk protein) are vital to obtain high 3-week submission rates, whilst low BCS before calving and a high rate of BCS loss in early lactation are associated with negative effects on 3-week submission rate (Morton, 2000).

Many studies have shown that an early resumption of ovarian cyclicity and luteal function after calving is beneficial to fertility (Thatcher and Wilcox, 1973; Macmillan and Clayton, 1980; Holt *et al.*, 1989; Darwash *et al.*, 1997), although this has not been confirmed in all studies (Smith and Wallace, 1998; Opsomer *et al*, 2000). Conception rates increase with increased number of oestrous cycles (up to 4) in the first 60 days postpartum (Thatcher and Wilcox, 1973; Stevenson and Call, 1983), whilst cows that fail to exhibit oestrus in the first 30 days postpartum are likely to have more services per conception than those that do (Gordon, 1996). In a seasonal calving system, it is even more critical for cows to resume their cyclicity as early as possible after calving, since the mating period is so short (Macmillan, 1985a; Holmes *et al*, 2002a). The cows that calve late may have to be mated while they are in their first or second postpartum heat, so such cows are more likely to be empty at the end of the mating period than those which calved early.

1.12 Progesterone analysis as a tool for pregnancy diagnosis

Since the mating period is short in the seasonal calving system, it is very important that the non-pregnant animals are identified soon after mating by a method that is accurate and cost effective. Then the result can be readily used on-farm for reproductive management, culling and feeding budget decision-making purposes. Apart from observing oestrous behaviour to identify non-returning to cycle, confirmation of pregnancy status in cattle can

be done by rectal palpation, the use of plasma or milk P4 assay or ultrasonography (Hickey, 1990; Gordon, 1996).

Rectal palpation of the reproductive tract is the most practical and common method, but can possibly have negative effects on the animal. Strategic milk P4 analysis (at 21, 24, 28 or 42 days after AI), cow-side type devices for P4 determination and ultrasonography (at more than 26 days after AI) provide non-invasive approaches and are reliable means of pregnancy diagnosis (Heap *et al.*, 1976; Nebel, 1988; Hickey, 1990; Pieterse *et al.*, 1990; Bajema *et al.*, 1994). Measuring milk P4 23 to 25 days after insemination has been reported to be highly accurate in predicting non-pregnancy (Heap *et al.*, 1976; Cavestany and Foote, 1985b). It is less accurate for positive pregnancy diagnosis, but Shemesh *et al.* (1978) reported that two milk P4 determinations, at 24 and either 40 or 44 days after insemination ensure maximum reliability for pregnancy detection.

Progesterone profiles during early pregnancy can be studied by examining milk P4 concentrations, since these reflect the circulating pattern of P4 in blood. The P4 concentrations in blood and milk act as an indicator of luteal function and may be used to distinguish between pregnant and non-pregnant cows (Foulkes, 1991). Milk P4 determination can be used as a practical aid to monitor postpartum ovarian and CL functions, confirm oestrus, identify time of AI and detect early pregnancy in dairy cattle (Fagan and Roche, 1986; Kimura *et al.*, 1987; Nebel, 1988; Ruiz *et al*, 1989; Foulkes, 1991; Kang and Kim, 1998; Opsommer *et al*, 1998).

Recently, many new tests have been developed, such as plasma or milk concentrations of oestrone sulfate, bovine pregnancy-associated glycoprotein 1 (bPAG 1), bovine pregnancy-specific protein B (bPSPB) and early pregnancy factor (EPF) to provide a wide range of indicators of pregnancy status in dairy cows (Holdsworth *et al.*, 1982: Sasser *et al.*, 1989; Henderson *et al.*, 1994; Mueller *et al.*, 1998; Szenci *et al.*, 1998). Some of these tests gave good agreement with conventional pregnancy-testing methods, e.g. palpation *per rectum* and plasma progesterone assays (O'Connor, 1994). However, limitations to each method, including cost or economic advantages, time to perform, accuracy and sensitivity, method involved and practical application of the method, will determine its application on-farm in the future.

1.13 Conclusion

In summary, there is concern about a problem of subfertility in high producing cows which have been selected mainly for milk yield. Current evidence gives inconclusive answers to the cause of such decline of fertility. For example, in the seasonal pasture-based systems, cows with higher proportions of overseas genetics showed lower fertility compared to home-bred animals, but it is not clear whether this difference is dependent upon genetic or environmental effects, or the interactions of both (G x E).

Reproductive performance in the cow is controlled by the interactions of the hypothalamo-pituitary-ovarian axis. Follicular growth, development, steroid production, oocyte maturation and ovulation are dependent on gonadotrophin support. Ovarian steroids, in turn, regulate the release of GnRH and gonadotrophin by positive and negative feedback mechanisms. FSH plays vital role in the process of follicular dominance and steroidogenesis, whilst LH pulsatility is also very important in regulation of steroidogenesis in the pre-ovulatory period. The gonadotrophin surge triggers ovulation and shifts in ovarian hormone production in the pre-ovulatory follicles leading to the formation of the CL. Formation and maintenance of the CL are thereafter regulated by LH. Luteal hormones, including P4, E2, OT, PG and other autocrine and paracrine factors, such as growth factors, play major roles in regulating the ovarian activity during the oestrous cycle. The maintenance of luteal function, in order to keep high concentrations of P4 which are required for the establishment and maintenance of pregnancy is regulated by the presence of the conceptus. Embryonic secretion of the protein-IFN-τ ensures the survival of the CL by preventing luteolytic effect of $PGF_{2\alpha}$. Adequate luteal P4 is then secreted uninterruptedly to allow maintenance of pregnancy until additional sources of progesterone (e.g. placenta, adrenal or foetus) start to be produced after the end of the first trimester of gestation. The essential components of the key endocrine factors determining the establishment of ovarian activity during the onset of puberty or after calving are the establishment or the resumption of the LH pulsatility and the positive feedback mechanism by E2 which is associated with the expression of oestrous behaviour, gonadotrophin surge and ovulation.

The impact of selection for different mature bodyweight upon the activity of the reproductive endocrine system is questionable. Differences in reproductive outcomes in cows which are selected for different mature bodyweight may be related to either the direct or indirect effects or both on the function of the reproductive endocrine axis. The

direct effects may lie in the intrinsic differences in the function of the hypothalamo-pituitary axis after calving, reflecting in the ability to resume ovarian cyclicity and, subsequently, a chance to conceive. Alternatively, the intrinsic differences in the ovarian and luteal function may affect follicular characteristics (oocyte fitness and quality) and steroidogenesis (P4 secretion) in a way that the ability to maintain pregnancy declines. On the other hand, the indirect effects may be exerted by an altered metabolic endocrine axis as a consequence of changes in metabolic demand and nutritional balance either at the hypothalamo-pituitary level (i.e. GnRH and gonadotrophin secretions) or at ovary level (i.e. follicular development and the CL function). Thus, it is logical to focus on the differences in the reproductive endocrine system after calving in these cows. In fact, the survey into the reproductive performance of the Heavy (H) and the Light (L) Holstein Friesian cows in seasonal pasture-based system has led to the needs for further studies involving the reproductive endocrine system in order to clarify the underlying mechanisms that may be involved in their differences in fertility.

Therefore, the main aim of this thesis is to use these two groups of animals which genetically differ in bodyweight, H and L, as a model to explore the differences between the reproductive endocrine axes governing the reproductive function in groups of cows with different overseas genetic backgrounds and to compare the follicular dynamics and reproductive performance after calving in these groups.

Hypothesis:

The studies in this thesis were based on the hypothesis that the differences in the following parameters of postpartum endocrine activity may underline the difference in fertility between H and L cows:

- Endogenous gonadotrophin release after calving.
- The anterior pituitary responsiveness to GnRH after calving.
- The re-establishment of the positive feedback control mechanism after calving.
- The ovarian follicular growth and development during the early postpartum period and luteal function after ovulation.
- Patterns of follicular dynamics during the early postpartum period.

Objectives of this study:

The objectives of this study were to determine the differences between H and L cows in the following:

1. Pulsatile secretion patterns of gonadotrophin postpartum.
2. The AP responsiveness to GnRH challenge during the early postpartum period.
3. The establishment of the positive feedback mechanism by E2 postpartum.
4. The reproductive performance after calving using milk P4 profile analysis.
5. Luteal function during early postpartum and pregnancy.
6. Follicular dynamics postpartum.

2 Chapter 2: General materials and methods

2.1 Animal and general management

Animals

The activity of the reproductive endocrine axis and reproductive performance of two strains of Holstein-Friesian cattle, which had been genetically selected for heavy (H) or light (L) mature body weights at the Dairy Cattle Research Unit (DCRU), Massey University (García-Muñiz *et al.*, 1998; Laborde *et al.*, 1998b), was examined during the postpartum and pre-pubertal periods. Previous studies using these animals showed that the H cows were 10% (or 46 kg) heavier at maturity than L cows (García-Muñiz *et al.*, 1998). Estimates of the genetic background of the cows in this herd during 1999-2001, based upon pedigree information, showed that on average the H strain animals were 40 % North American Holstein, while the L strain were 18 % North American Holstein. The percentages of North American Holstein in each group of H and L cows used in each experiment are also estimated and presented. Within each experiment, animals were balanced by calving date and, where mixed-age cows were used, by age. All of the animals had had uncomplicated calvings and had established normal lactations. All procedures using experimental animals were approved by the Massey University Animal Ethics Committee.

Grazing and pasture management

The cows were managed in one herd and were rotationally grazed on mixed ryegrass (*Lulium 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).

General reproductive management

Each year, the cows calved during late winter and early spring (July to October). From mid September, oestrus detection was performed by the stockman twice daily during morning and afternoon milking times, with the help of tail painting (Macmillan *et al.*, 1988). All observed heats were recorded. During the first 4 weeks of the mating period, oestrous cows were bred by artificial insemination (AI). At the end of the AI mating

period, bulls were grazed with the cows for 4 weeks. The animals were then pregnancy tested by rectal palpation in February/March. The general herd management and details of the reproductive management during the study period (1999-2001) are summarised in **Table 2-1**.

Table 2-1. The general herd and reproductive management at the Dairy Cattle Research Unit (DCRU), Massey University during 1999-2001 (H=Heavy cows, L=Light cows).

Year	1999-2000	2000-2001	2001-2002
Planned start calving	28/07/99	29/07/00	1/08/01
Mean calving date	28/08/99	16/08/00	19/08/01
First day to mean calving date (days)	20	44	18
Calving duration (days)	65	117	60
Tail paint start (days before PSM)	40	40	40
Planned start mating (PSM)	25/10/99	24/10/00	24/10/01
Finished AB	28/11/99	25/11/00	27/11/01
Natural mating start	29/11/99	26/11/00	28/11/01
Finished mating	13/01/00	23/12/00	17/01/02
Pregnancy test (date)	1/2/00, 7/3/00	12/01/01	21/03/02
Empty cows at the end of mating	7L	5L	9L
	3H	5H	7H
Culling cows : Empty	6L	4L	7L
	1H	5H	6H
Culling cows : Other causes	2L	x	x
	1H	1H	x
Culling rate (%)	14L	12L	16L
	11H	15H	16H
Stocking rate (cows/ha)	2.6	2.6	2.2
Average pasture cover (KgDM/ha)			
August	2,550	2,199	2,075
September	1,954	2,091	2,141

2.2 *Production data of the herd*

Milk yield of each cow was recorded using in-line Metatron milk meters (Westfalia Separator). Milk composition data, including concentrations of protein and fat, were calculated from monthly herd test records (for the whole herd in 2000/2001 and for the cows used in Experiment 5 in 2001/2002). Because of the incomplete data regarding milk yield from the DCRU herd records during 1999/2000 calving season, only milk protein percentages were selected for use in comparing the metabolic loads in these cows during the first and second herd testings in Experiments 1 and 2a. As these data underpin the

following experimental chapters, the analysis and results of these data will be presented in this chapter. Analysis of production data in all three calving seasons (1999 to 2001) showed no statistical differences in production between strains (see Appendix 1).

Liveweight (LW) and body condition scores (BCS) of the cows were measured fortnightly. The analysis of these data revealed no significant difference between lines in LW or BCS changes during early lactation (first 4 to 6 wks after calving).

2.3 Hormone assay methods

Blood sampling

Blood samples were collected via coccygeal or jugular venipuncture into lithium heparinised vacutainers (Becton Dickinson, USA), except for blood samples from the serial sampling in Experiment 1. Within 1 hour of collection, blood samples were centrifuged at 4°C and 1000 g for 20 min to allow removal of the plasma. The plasma was separated and stored at –20 °C until assayed.

Gonadotrophin assays

Concentrations of LH and FSH were measured in plasma by standard double antibody radioimmunoassay methods. Heterologous assays were used for both hormones, using bovine LH and FSH (NIDDK bLH; AFP11743B and USDA bFSH-1-2; AFP5318C) for standards and as tracer (after iodination by the chloramine-T method), and anti-ovine gonadotrophin antisera (NIDDKanti-oLH-1; AFP192279 and NIDDK anti-oFSH-1; AFPC5288113). Samples were assayed in duplicate, using 50 µl aliquots of plasma. The limit of sensitivity (defined as twice the standard deviation of blank values) for both assays was 0.05 ng/ml. Other details of the assay procedures were as described by Parkinson and Follet (1994) for the assay of ovine gonadotrophins. The assays were validated for use in this laboratory by demonstrating parallelism between a series of standards made in assay buffer and (a) a sample of plasma which was serially diluted in assay buffer or (b) a pool of plasma to which increasing concentrations of standard had been added (Abdullah, 2000). Assay validation data are shown in Table 2.2.

Assay procedures

Plasma samples were thawed overnight in the fridge or rapidly in water. On the first day, 50 µl of standards and quality control samples (Low, Med, High) were added to

appropriate tubes and 50 µl assay buffer to non-specific binding (NSB) and total binding (Bo) tubes (triplicate). Standards were prepared by serial dilution of the highest standard (stored in 400 µl aliquots) in assay buffer (0.05M phosphate buffer; pH 7.05, containing 0.0375% EDTA (disodium salt), 0.0875% NaCl, 0.01% Na azide and 0.05% bovine serum albumin). To overcome potential "serum/plasma effects", 25 µl of serum/plasma containing negligible hormone concentration (as determined in previous assays) was added to NSB, Bo and standards tubes. Plasma samples were assayed in duplicate 50 µl/sample. In samples with high concentration, 50 or 100 µl/tube of assay buffer was added in order to bring values into the most accurate (middle) part of the standard curve (for the samples from Experiment 2 and 3). Twenty µl of normal rabbit serum (1:400 dilution in assay buffer) was added to NSB tubes. Thereafter, 20 µl of 1:125,000 NIDDK-rabbit-anti-oLH-1, batch # AFP-192279 or 1:25,000 NIDDK-rabbit-anti-oFSH-1, batch # AFPC5288113 diluted in assay buffer were added to all tubes except TC and NSB. All tubes were vortex mixed and left overnight at 4°C.

On the second day, 20 µl of ^{125}I-labelled hormone (diluted to 5000 cpm/20 µl assay buffer) was added to all tubes. Total counts (TC) tubes were covered. All tubes were vortex mixed and incubated for > 20 h at 4°C.

On the third day, 20 µl of 1:60 donkey anti-rabbit serum (DARS; IDS, Boldon, UK) in assay buffer was added to all tubes except TC. Tubes were again vortex mixed and incubated for > 20 h at 4°C. On the final day, 200 µl of 'diluent IV' (0.5% egg white in assay buffer) was added to all tubes (except TC tubes) which were then centrifuged at 3750 g at 4°C for 1 h. The supernatant was aspirated and the pellet counted for 2 min/tube. Radioactivity (c.p.m.) in the precipitate was counted in a gamma counter (MultiGamma LKB Wallac, Turku, Finland). An attached computer was used to calculate unknowns from the standard curve using RIACALC DM programme version 2.25 by WallacOy 1985-1987.

Progesterone (P4) assay

Concentrations of progesterone were measured using ELISA kits (Ridgeway Science Ltd., Cirencester, UK), as described by Sauer *et al.* (1986) and Groves *et al.* (1990). Duplicate 10 µl samples of plasma (Experiments 1-3 and 5) or milk (Experiment 4) were dispensed into 96-well microtitre plates that were coated with anti-progesterone antibody. Enzyme-linked label (progesterone-11α-glucuronide-alkaline phosphatase; 200 µl) was then added

and the plates were incubated for 2.5 h at room temperature. After washing, alkaline phosphatase substrate (p-nitrophenol phosphate liquid substrate system; Sigma Chemical Co., Poole, UK; 200 µl) was added and the plates were incubated for a further 45-60 min. until the colour difference between the blanks and the highest concentration standards was noticeable. Optical densities were measured at 550 nm and the concentrations of progesterone in unknown samples were estimated by making comparisons with standards (0.5 to 20 ng/ml) prepared in charcoal-stripped anoestrous cow plasma (for the plasma progesterone assay) or in progesterone-free milk from oestrous cows for milk progesterone assay (range from 1 to 50 ng/ml). The limit of sensitivity (defined as twice the standard deviation of the optical densities of wells containing progesterone-free anoestrous cow plasma/progesterone-free milk), inter- and intra-assay coefficients of variation are shown in **Table 2-2**.

Table 2-2: Assay validation details.

Assay	Sample	Sensitivity	Intra-assay CV	Inter-assay CV
LH	50 µl	0.05 ng/ml	9.07 %	13.57 %
FSH	50 µl	0.05 ng/ml	8.71 %	10.25 %
Plasma progesterone	10 µl	0.10 ng/ml	9.32 %	14.92 %
Milk progesterone 1999	10 µl	0.17 ng/ml	7.51 %	17.66 %
Milk progesterone 2000	10 µl	0.13 ng/ml	4.12 %	13.77 %

2.4 Statistical analyses

In most cases, the endocrine data were normalised by $\log_e$ transformation if they were not normally distributed and were subjected to analysis of variance (ANOVA) with respect to strain effect (i.e. H or L) and time after calving, in a repeated measures model in which individual animals were nested within strain. Where statistically significant effects were noted, least significant differences were calculated (Snedecor and Cochran, 1967) to determine the points at which the means differed. All analyses were made using GENSTAT 5 release 4.1. All categorical data were analysed using the Chi-square (χ^2) test

for the effect of treatment or G-test (log-linear goodness of fit test; Sokal and Rohlf, 1969) for effects of treatment and seasons.

Mean milk progesterone concentrations, strain and pregnancy status data in Experiment 4 were subjected to regression analysis, with respect to time, using linear and quadratic regression analysis. The standard least-square methods were used to test the difference of the regression equations. Specific details of further statistical analyses were described in each particular experiment.

3 Chapter 3- Experiment 1: Endogenous secretion of gonadotrophins in postpartum Holstein-Friesian dairy cows selected for heavy or light mature bodyweight

The material in this chapter has been accepted for publication by the *New Zealand Veterinary Journal*, as "Post-partum gonadotrophin secretion in Holstein-Friesian dairy cows differing genetically in liveweight" by Thiengtham, J., McNaughton, L.R., Holmes, C.W. and Parkinson, T.J.

3.1 Introduction

For cows to achieve a 365-day calving interval, they need to conceive by 80-85 days postpartum (Webb *et al.*, 1980; Peters and Lamming, 1986; Fagan and Roche, 1988; Opsomer *et al.*, 1996) and, in a seasonal pastoral system, this must be achieved as synchronously as possible (Macmillan, 1979; Morton, 2000). An early resumption of ovarian activity after calving is required to achieve this target (Savio *et al.*, 1990b), and a prolonged postpartum anovulatory period delays the first service after calving, resulting in a delay to re-conception, an increase in calving interval and a less compact calving pattern (McDougall *et al.*, 1995a).

In late gestation, both gonadotrophin secretion and follicular development are profoundly suppressed by the exposure of the hypothalamo-pituitary axis to the prolonged period of elevated concentrations of progesterone and oestrogen that is characteristic of pregnancy. Hence, during the postpartum period, restoration of the activity of the central reproductive endocrine axis is essential for the restoration of ovarian follicular activity. Reappearance of FSH and episodic LH secretion from the anterior pituitary are therefore well recognised as the major regulators of ovarian activity through the postpartum period (Stevenson and Britt, 1979; Peters *et al.*, 1981; Peters and Lamming, 1983).

Several studies have demonstrated a negative relationship between body size and reproductive performance in both dairy and beef cows (Badinga *et al.*, 1985; Markusfeld and Ezra, 1993; Hansen *et al.*, 1998). Previous studies in Holstein Friesian cows from the heavy (H) and light (L) mature live weight selection-strains at Massey University have shown that H cows had a lower conception rate to the first service, but a shorter interval from calving to ovulation than L animals (García-Muñiz, 1998; Laborde *et al.*, 1998b). The reasons for these differences are not clear, but may be related to the activity of the central reproductive endocrine axis during the postpartum period.

The objective of this experiment was to examine the endogenous patterns of secretion of LH and FSH in these cows during the first six weeks after calving, in order to compare the duration of postpartum functional restoration of the hypothalamo-pituitary axis between the two strains. The study was based on the hypothesis that differences in (a) conception rate and (b) the interval from calving to first ovulation between these two strains of animals are related to differences in gonadotrophin secretion profiles during the early postpartum period.

3.2 ***Materials and methods***

Animals and experimental design

The patterns of LH, FSH and progesterone were studied during the postpartum period in spring-calving (August), mature Holstein-Friesian dairy cows, in two strains of animals that had been genetically selected (García-Muñiz, 1998) for high (H) or low (L) mature liveweight (n=7 per group).

On the 14th, 21st, 28th and 35th days after calving, serial blood samples (5 ml, sodium heparin anticoagulant) were taken at 15-min intervals for 8 h, through jugular venous cannulae that had been inserted, under local anaesthetic, immediately prior to the start of sampling. During blood collection periods, the cows were housed in a stall barn and fed pasture silage *ad libitum* plus 1 kg of compound feed (12.5 MJME/kg DM; 16% crude protein). At other times, the cows were grazed on a generous allowance of clover/ryegrass pasture. They always had *ad libitum* access to water. Within 1 hour of collection, blood samples were centrifuged at 4°C and 1000 g for 20 min to allow removal of the plasma. The plasma was stored at –20 °C for later assay.

Hormone assays

Concentrations of LH and FSH were measured in plasma by standard double antibody radioimmunoassay as described in Chapter 2. Concentrations of progesterone were measured using ELISA kits (Ridgeway Science Ltd., Cirencester, UK) as described in Chapter 2. Concentrations of LH were measured in all samples. Concentrations of FSH and progesterone were measured once per day in plasma that was pooled over the entire sampling period. In addition, milk samples were collected three times per week from each of the cows in the experiment from the time of calving, for determination of milk progesterone concentrations (see Chapter 6). These samples were collected to define more

accurately the resumption of oestrous cyclicity in the postpartum period, to define the reproductive status of the cows on the days of blood sampling as well as to indicate the times at which ovulations occurred.

Data and statistical analyses

The time of ovulation was inferred from milk progesterone data with the assistance (where such data existed) of stockmen's observations of oestrus. Concentrations of <2 ng/ml were considered to represent basal values (i.e. these were indicative of no luteal activity: Stevenson and Britt, 1980), but the presence of concentrations >2.0 ng/ml for at least 2 consecutive samplings were taken to indicate the presence of active luteal tissue. Since the first rise in progesterone concentrations occurs 4.5 ± 1.1 days after oestrus (Bulman and Lamming, 1978), the timing of ovulation was taken as 5 days before the time of the first rise in progesterone concentrations (Darwash *et al.*, 1997).

The secretion pattern of LH was determined for each individual cow on each collection day. An episode of LH secretion was considered to have occurred when (a) the peak concentration was least 2 standard deviations greater than the mean concentration present in each cow on each collection day (b) peak concentrations occurred within 2 samples of the previous nadir and (c) the rate of decay was within the limits of the known half life of LH (Geschwind and Dewey, 1968; Lamming and McLeod, 1988). Episode frequency and amplitude were calculated from this information.

All endocrine data were subjected to analysis of variance with respect to strain (i.e. H or L) and time after calving, in a repeated measures model in which individual animals were nested within strain. Where statistically significant effects were noted, least significant differences were calculated (Snedecor and Cochran, 1967) to determine the points at which the means differed. LH episode data (frequency and amplitude) were subjected to generalised linear regression analysis with respect to strain and time. Progesterone concentrations were used as a covariate in all of these analyses. Frequency data were analysed using Poisson distribution model.

After Day 14, data were affected by the reproductive status of the animals, inasmuch as some animals had ovulated whereas others had not. In order to make valid comparisons of this information, LH data were separated into those which had been obtained from cows that were anoestrous, those which had been obtained from cows in the follicular or early luteal phase (Days 0 to 8 of the oestrous cycle; where Day 0 is the day of oestrus) and

those which had been obtained from cows in the mid luteal phase (Days 8 to 18). Data from the follicular/early luteal phase were discarded, as there were insufficient observations on each day to allow meaningful interpretation of LH patterns. Data from anoestrous cows were subjected to one-way analysis of variance with respect to strain (H or L), in a repeated-measures analysis, in which time postpartum was used as a covariate. Individual animals were nested within strain. Data from cows in the mid luteal phase were similarly, but separately, analysed. In the latter analysis, only data from the first luteal phase (including animals with a short first luteal phase) were used.

3.3 Results

There was a significant ($p<0.001$) interaction between strain and days postpartum in overall mean LH concentrations (Figure 3-1 and Table 3-1). Concentrations were significantly ($P<0.05$) higher in H than L cows on Days 14, 21 and 35 postpartum and tended ($0.10>P>0.05$) to be higher on Day 28. Mean LH concentrations over all periods of sampling averaged 0.30±0.04 ng/ml in H and 0.21±0.03 ng/ml in L cows. The frequency of LH episodes (Table 3-2) did not differ significantly between either strains or days postpartum. However, there was a significant ($P<0.01$) main effect of strain upon the amplitude of LH episodes (Table3-2), such that the overall mean amplitude was greater in H cows (0.33±0.02 ng/ml) than in L cows (0.27±0.02 ng/ml). Some individual 8-h profiles of LH concentrations in representative cows from H and L strains are presented in Appendix 3.

After Day 14, data were affected by the reproductive status of the animals, since some cows had resumed oestrous cyclicity (and were at different stages of the cycle at the time of blood sampling) and some remained anoestrous. No cows had resumed ovarian cycles before Day 14 (as determined by progesterone concentrations of <1.0 ng/ml on and before Day 14). On Day 21, one H cow was in the mid-luteal phase (milk progesterone concentration: 12.3 ng/ml) and four others had ovulated one or two days previously. Three L cows were in the early luteal phase and two L cows had ovulated one or two days previously. On Day 28, six H cows and three L cows were in the mid-luteal phase. One further L cow displayed a short first luteal phase (Days 20–33), so had small elevations of progesterone in milk (≈3.0 ng/ml), but not in plasma, on Day 28. Five H cows ovulated on Days 37 or 38, so, on Day 35, these were undergoing luteal regression and had low

Figure 3-1: Overall mean values of plasma LH concentrations of light- and heavy-strain cows during the study period. The values between strains were significantly different on Days 14, 21 and 35 postpartum.

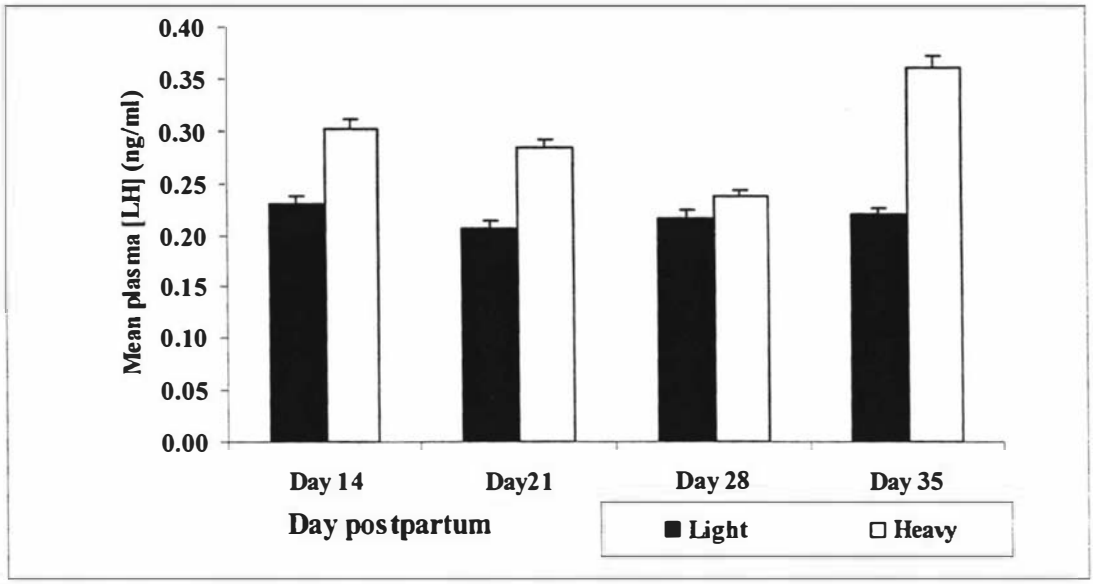


Figure 3-2: Overall mean values of plasma LH concentrations of light- and heavy-strain cows during the study period, after separation of data into that derived from anoestrous and luteal-phase cows. The value in H cows was higher than in L cows ($P<0.01$) during the anoestrous period, but there was no difference during the mid-luteal period.

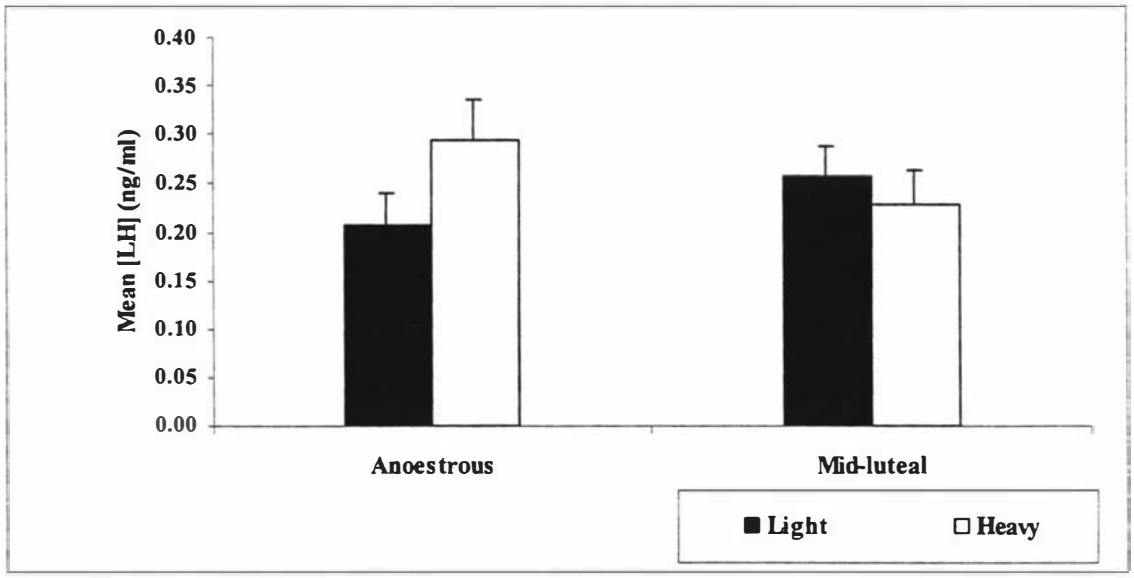


Table 3-1: Mean (± s.e.m.) plasma LH and FSH concentrations in light- and heavy-strain Holstein-Friesian cows during the postpartum period.

Days postpartum	LH concentration (ng/ml)	
	Light strain	**Heavy strain**
14	0.23 ± 0.03 *	0.30 ± 0.42
21	0.21 ± 0.04 *	0.28 ± 0.04
28	0.22 ± 0.04	0.24 ± 0.03
35	0.22 ± 0.03 *	0.36 ± 0.06
Days postpartum	**FSH concentration (ng/ml)**	
	Light strain	**Heavy strain**
14	0.19 ± 0.02	0.27 ± 0.06
21	0.33 ± 0.06	0.30 ± 0.07
28	0.32 ± 0.06	0.25 ± 0.03
35	0.24 ± 0.04	0.17 ± 0.02

Mean LH concentrations differ between strains where marked (*: $P<0.05$). For FSH concentrations, neither differences between strains nor between days postpartum were statistically significant ($P\approx0.50$).

Table 3-2: Mean frequency and mean (± s.e.m.) amplitude of LH episodes in light- and heavy-strain Holstein-Friesian cows during the postpartum period.

Day postpartum	Frequency (episodes/8 h)		Amplitude (ng/ml)	
	Heavy	Light	Heavy	Light
Day 14	2.6	2.3	0.33 ± 0.03	0.28 ± 0.02
Day 21	2.3	1.9	0.31 ± 0.04	0.29 ± 0.04
Day 28	1.4	2.3	0.29 ± 0.01	0.30 ± 0.03
Day 35	3.1	1.4	0.33 ±0.04	0.23 ± 0.03

Episode frequency did not differ between strains or days postpartum. Overall mean episode amplitude was greater in heavy than light cows ($P<0.05$), but differences between strains on individual days postpartum were not significant.

Table 3-3: Interval between calving and first ovulation, and progesterone status of cows on Days 14, 21, 28 and 35 post partum.

Strain	Interval (days)		Cows with milk progesterone <1.0 ng/ml			
	Mean (± s.e.m.)	Range	Day 14	Day 21	Day 28	Day 35
Heavy	22 ± 3	17-24	7/7	5/7	1/7	3/7
Light	28 ± 7	15-57	7/7	4/7	3/7	4/7

Table 3-4: Analysis of variance for mean LH endogenous concentrations in light and heavy-strain cows during the postpartum period, after separation of data into that derived from anoestrous and luteal-phase cows.

Anoestrous cows					
Strain	**Mean LH concentration (ng/ml)**	**s.e.m.**	**s.e.d.**	**F-ratio**	**Significance**
Light	**0.205**	0.094	0.022	14.21	P=0.004
Heavy	**0.297**	0.038			
+ Covariate (days postpartum)	r=-0.0071			2.37	P=0.124
Luteal phase cows					
Treatment	**Mean LH concentration (ng/ml)**	**s.e.m.**	**s.e.d.**	**F-ratio**	**Significance**
Light	0.232	0.036	0.038	0.28	P=0.614
Heavy	0.252	0.039			
Covariate (days postpartum)	r=0.015			0.68	0.437

Table 3-5: Mean frequency and mean (± s.e.m.) amplitude of LH episodes during the anoestrous periods and during the first postpartum luteal phase in light- and heavy-strain Holstein-Friesian cows.

	Anoestrous		**Mid luteal phase**	
LH episodes	**Heavy strain (n=11)**	**Light strain (n=11)**	**Heavy strain (n=8)**	**Light strain (n=7)**
Amplitude	0.36 ± 0.06 [a]	0.25 ± 0.06 [b]	0.32 ± 0.07	0.29 ± 0.07
Frequency	2.4	1.8	2.0	2.4

Means with different superscripts on the same line differ significantly (P<0.05) from each other. (n) indicates the number of blood sampling periods that contributed to the analysis.

progesterone concentrations. Four L cows ovulated between Days 31 and 36 and two L cows were still anoestrous by Day 35 (see Table 3-3).

There were significant ($P<0.01$) differences in mean LH concentrations between anoestrous L (0.21±0.01 ng/ml) and H (0.30±0.03 ng/ml) cows (Figure 3-2 and Table 3-4) whilst the amplitude of LH episodes was also greater in anoestrous H (0.33±0.06 ng/ml) than L (0.25±0.06 ng/ml) cows (Table 3-5). Episode frequency did not differ between strains (2.0±0.3 episodes/8 h in both strains). Neither mean LH concentrations nor the amplitude or frequency of LH episodes differed between strains in the mid-luteal phase (Figure 3-2, Table 3-5).

There were no significant effects of strain or time postpartum (both $p\approx0.50$) upon plasma FSH concentrations (Table 3-1).

3.4 Discussion

The pulsatile secretion patterns of LH and FSH of the cows in this study generally followed the patterns that have previously been described after calving (Webb *et al.*, 1980; Peters *et al.*, 1981; Savio *et al.*, 1990b). Pulsatile LH secretion was detected from 14 days postpartum yet, although overall mean concentrations increased between Days 14 and 35, there were no changes in the amplitude or frequency of LH episodes over the duration of the experiment. This was in contrast to the results of other studies which have demonstrated changes in LH pulse frequency during the first few weeks after calving (Stevenson and Britt, 1979; Schallenberger and Hutterer, 1982; Schallenberger, 1985), although a similar lack of change in LH episodes was reported by Watson and Williams (1987). Furthermore, Peters and Lamming (1984a), reported that plasma LH concentrations and pituitary sensitivity to GnRH reach a maximum at 12 to 15 days postpartum.

However, given the effects of progesterone upon endogenous secretion of LH (Ireland and Roche, 1982b; Nett, 1987; Bergfeld, *et al.*, 1996), it was to be expected that the timing of postpartum resumption of oestrous cycles would affect overall results, especially as H cows tended to show earlier luteal function after calving than L cows. It was for this reason that the effect of reproductive status was taken into account in the re-analysis of the data. When these data were analysed with respect to reproductive status, it was evident

that, for cows in their first luteal phase, patterns of LH secretion were indistinguishable between H and L cows. Parkinson and Lamming (1990) showed that LH episode amplitude and frequency are remarkably constant throughout the luteal phase of the bovine oestrous cycle, so this result is largely as expected. On the other hand, the observation that mean concentrations and the amplitude of LH episodes in anoestrous cows were greater in H strain cows than in L strain cows was not expected. Since some of the aforementioned studies have shown progressive increases in LH secretion during the postpartum period, it was important to note that parameters of LH secretion in anoestrous cows in the present experiment were unrelated to the covariate of time after calving.

It has been well documented that the re-initiation of cyclicity after calving is due to a resumption of secretion of gonadotrophin-releasing hormone (GnRH), which, in turn, leads to progressive restoration of the gonadotrophic activity of the pituitary. The resumption of cyclic ovarian activity after calving requires increasing plasma LH concentrations, accompanied by the development of a pulsatile pattern of LH secretion (Stevenson and Britt, 1979; Lamming *et al.*, 1981; Peters *et al.*, 1981; Schallenberger and Hutterer, 1982). Thus, the LH pulse frequency between Days 10 and 17 postpartum is negatively correlated with the time to the onset of ovarian activity (Stevenson and Britt, 1979; Peters *et al.*, 1981). In addition, it is likely that the increased LH secretory activity of H compared with L cows that was observed in the present experiment is related to the earlier resumption of oestrous cycles in the H cows as reported by García-Muñiz (1998) and Laborde *et al.* (1998b).

Further, it has recently been reported that cows whose first ovulation occurred within three weeks of calving, and which were inseminated from the sixth week postpartum, had a longer calving-to-conception interval, lower conception rate and greater number of services per conception than those that ovulated after three weeks postpartum (Smith and Wallace, 1998). The lower pregnancy rate in these early-ovulating cows may reflect a proportion of such cows becoming infertile and, therefore, being unlikely to conceive for reasons other than those of postpartum ovarian (i.e. endocrine) activity. Such results may explain the previous observations of García-Muñiz (1998) and Laborde *et al.*, (1998b), in which H cows had a shorter interval from calving to first ovulation, but lower first-service conception rate and overall pregnancy rate than did the L animals. Given that LH concentrations and episode amplitude were greater in H than L cows, and that H cows

resumed oestrous cyclicity more quickly than L cows, it seems unlikely that their infertility was due to deficiencies in endogenous endocrine activity.

Conversely, the absence of any strain differences in plasma FSH concentrations between strains or between times postpartum, suggests that selection for mature live weight is unlikely to have affected this aspect of pituitary function in these cows during the postpartum period. Indeed, the FSH data in this study confirm that its secretion is not a limiting factor to the onset of ovarian cycles postpartum, at least between the 14th and 35th days after calving. This is, perhaps, to be expected from the work of Lamming *et al.* (1981) and Peters and Lamming (1984a), who demonstrated that a threshold level of FSH may be required for follicular growth and development early in the postpartum period, and from the more recent studies (Beam and Butler, 1997; Crowe *et al.*, 1998) which have revealed that recurrent 2- to 4-day cyclical elevations of FSH concentrations resumed within 5 days postpartum, each of which was associated with emergence of a follicular wave.

3.5 Conclusion

Overall concentrations of LH were higher in H cows than in L cows throughout the postpartum period. Parameters of LH secretion were similar in H and L cows during their first luteal phase, but the mean concentrations and episode amplitude were higher in anoestrous H cows than in L animals. There were no differences in FSH concentrations between strains. Hence, it is unlikely that inferior first service conception rates of H cows are primarily due to differences in LH secretion between H and L cows during the postpartum period. The results do, however, explain the earlier resumption of ovarian activity after calving in H than L cows as reported earlier. Further study is required to determine the factors involved in the reduced fertility of H compared to L cows under pasture-fed condition.

4 Chapter 4- Experiment 2: LH responses to buserelin administration in postpartum dairy cows selected for heavy or light mature bodyweight

The material in this chapter has been accepted for publication by the *New Zealand Veterinary Journal*, as "Post-partum gonadotrophin secretion in Holstein-Friesian dairy cows differing genetically in liveweight" by Thiengtham, J., McNaughton, L.R., Holmes, C.W. and Parkinson, T.J.

4.1 Introduction

Previous studies have shown that heavy (H) cows have a lower conception rate to the first service, but a shorter interval from calving to ovulation than light (L) animals (García-Muñiz, 1998; Laborde *et al.*, 1998b). Results from Experiment 1 showed that there were some differences in LH secretion characteristics between H and L cows during the postpartum period. LH concentrations and episode amplitude were greater in H than L cows whilst they were anoestrous, but not after ovarian cyclicity had been re-established. Consequently, it seems unlikely that the lower first service conception rates of these H strain cows were due to deficiencies in endogenous endocrine activity.

Apart from the development of the episodic secretion pattern of LH that is required for the resumption of oestrous cycles, the restoration of the ability of the anterior pituitary and the LH surge mechanism in response to hypothalamic signals is a further crucial step in the transition from the postpartum anoestrous period into regular oestrous cycles (Schallenberger *et al.*, 1982; Peters, 1984b).

In dairy cows, the anterior pituitary starts to regain responsiveness to GnRH as soon as 7 to 10 days postpartum (Kesler *et al.*, 1977; Fernandes *et al.*, 1978), although the magnitude of the LH response to GnRH subsequently increases as time postpartum progresses (Azzazi *et al.*, 1983; Alam and Dobson, 1987). Such an increase in anterior pituitary responsiveness to GnRH is responsible for the increase in endogenous LH release that permits the postpartum resumption of ovarian cyclicity.

As might be expected, there is much cow-to-cow variation in the time-course of these changes, and Osawa *et al.* (1996) have shown differences between individual cows in their responses to GnRH on Day 7 postpartum. In beef cows, Fajersson *et al.* (1999) found that the timing of the GnRH responses was related to the duration of the interval from calving to the first ovulation.

However, there is no information available about the timing and magnitude of anterior pituitary response to exogenous GnRH challenge in H and L cows. The purpose of this study was to investigate whether the differences in responsiveness of the anterior pituitary to GnRH during the early postpartum period underlie differences in the patterns of LH secretion and the time to resume ovarian activity after calving between H and L strain cows. As the period of postpartum anoestrus in first-calved heifers is typically longer than in mature cows (Holmes *et al.*, 2002a), this present study was also set up to examine differences in responsiveness of the anterior pituitary to GnRH in first-calved heifers that might explain such differences in the period of postpartum anoestrus between first-calved heifers and mature cows. This was achieved by examining, during the postpartum period, the pituitary function in the release and time to peak of LH in response to exogenous GnRH challenge in mixed aged cows and 2-year old heifers from the H and L strains.

4.2 Materials and methods

Animals and experimental design

The ability of the pituitary to secrete LH in response to exogenous GnRH (buserelin) during the early postpartum period was examined in mixed age H and L strain cows and first calved heifers. These animals respectively contained 37.5% and 10.5% North American Holstein genetics. Mixed age cows were balanced between strains for parity, calving date and BCS in Experiments 2a and 2b. The 2-year old heifers used in Experiment 2c were balanced for calving date and, as far as possible, BCS.

Experiment 2a

Fourteen mixed-age H and L strain cows (n=7 per group) received an exogenous GnRH challenge as an intravenous bolus injection of 10μg buserelin (Receptal: Intervet, Auckland, NZ) on Days 21, 28, 35 and 42 postpartum. During the challenge period, cows were housed in a barn and fed pasture silage *ad libitum*. At other times they were managed at pasture. Blood samples (3 ml, using sodium heparin as anticoagulant) were collected through indwelling jugular venous cannulae that had been placed under local anaesthesia immediately before the start of the experiments. Samples were collected at 10 min intervals from 30 min before buserelin administration until 90 min afterwards, with two further samples collected at 30 min intervals (120 and 150 min).

Experiment 2b

Fourteen mixed age cows (n=7 per group) were given 10 μg buserelin (as described for Experiment 2a) on Days 7, 14, 21 and 28 postpartum. Blood samples (3ml) were collected by jugular or caudal venepuncture (lithium heparin anticoagulant) immediately before and 60, 120, 150, 180, 210, and 240 min after intravenous injection of buserelin.

In this experiment, cows were restrained in a crush during the challenge and blood sampling periods. At other times, the cows grazed on mixed ryegrass/white clover pasture.

Experiment 2c

The same procedure as that had been used in Experiment 2b was applied to groups of first-calved (2 year-old) heifers (6H and 7L) on Days 7, 14, 21 and 28 postpartum.

Hormone assays

All blood samples were centrifuged at 1000g for 20 min at 4°C. Plasma was separated and stored at -20°C until assayed for LH, FSH and progesterone concentrations.

LH, FSH and progesterone concentrations were measured in plasma by the methods as described in Chapter 2. Concentrations of LH were measured in all samples, whilst those of FSH were measured only in the samples collected prior to and at 120 min after the injection of buserelin in Experiment 2a and in those collected prior to, at 210 min and at 240 min after the injection of buserelin in Experiments 2b and 2c. Concentrations of progesterone were measured in a pooled sample of plasma from each cow at each sampling period.

Data and statistical analyses

LH data were normalised by $\log_e$ transformation. The area under the curve (AUC) of LH response was determined by trapezoidal summation of all LH values during the 240-min sampling period after GnRH challenge (Experiments 2b and 2c). Both transformed LH data, AUC and FSH data were subjected to analysis of variance with respect to the factors of strain and time postpartum, in a repeated-measures model in which individual animals were nested within strains. Plasma progesterone concentration was used as a covariate to determine whether responses were affected by reproductive status. Where statistically significant effects were present, *post hoc* comparison of means was achieved by determination of least significant differences (Snedecor and Cochran, 1967).

4.3 Results

Experiment 2a:

All cows in this experiment exhibited significant increases in LH concentrations in response to buserelin, with peak values occurring at 120 or 150 min after buserelin administration (Figure 4-1). Peak LH concentrations in L cows (11.5±0.6 ng/ml) were significantly ($P<0.001$) greater than in H cows (8.3±0.8 ng/ml), when data from all days were amalgamated, although neither differences between days postpartum nor between strains of cows at different days postpartum were statistically significant. The LH response to the challenge was not related to the covariate, progesterone concentrations. There were no significant differences in FSH concentrations either between strains of cow or days postpartum. LH and FSH responses to buserelin challenge are presented in Table 4-1.

Experiment 2b and 2c

It is possible that the lack of a difference between strains in Experiment 2a might have been due to the experiment having started at a relatively late time after calving (Day 21). In addition, peak values of LH response in Experiment 2a were attained at either 120 min or 150 min after the challenge. It is possible that some of these peak values would have been missed since the period of blood samplings was terminated at 150 min after the challenge. It was postulated that an earlier start to the experiment (Day 7) might increase the chances of detecting differences between the strains. Therefore, the time and blood sampling protocol were altered in Experiments 2b and 2c to re-examine the LH response starting on Day 7 after calving and collecting blood samples beyond 150 min after the challenge. Under the constraints of seasonal calving in this herd, these three experiments were undertaken in two different calving seasons, i.e. Experiment 2a was done in 1999 and Experiment 2b and 2c were done in 2000.

As in Experiment 2a, all animals in Experiments 2b and 2c also displayed significantly ($P<0.01$) increases in LH concentrations in response to buserelin. In both Experiment 2b (Figure 4-2) and 2c (Figure 4-3) peak LH concentrations were attained at between 150 and 180 min after buserelin administration. As expected, LH response to buserelin administration, as determined by peak LH concentrations and the area under the LH response curve, increased markedly (both $P<0.001$) with time after calving. However, there were no significant differences (Experiment 2b; $P=0.77$, Experiment 2c; $P=0.19$) between the LH responses of both the two strains at any time during the study periods

Table 4-1: LH and FSH responses in light- and heavy-strain cows to buserelin challenge; Experiment 2a.

Maximum LH concentration (ng/ml) after receiving 10 µg buserelin				
	Light	**s.e.m.**	**Heavy**	**s.e.m.**
Day 21	11.6	1.32	8.6	1.86
Day 28	10.9	1.36	9.3	1.86
Day 35	11.1	0.55	6.3	1.27
Day 42	12.3	1.30	9.2	1.37

Analysis of variance for LH data		
Source of variation	**Variance ratio**	**F Probability**
Strain	3.08	0.113
Time (i.e. relative to giving buserelin)	319.70	<.001
Day (post partum)	0.88	0.458
Strain.time	9.34	0.003
Strain.day	0.25	0.864
Day.time	0.65	0.587
Strain.day.time	0.40	0.753
Covariate (progesterone concentration)	2.663	0.529

Mean FSH concentration (ng/ml) after receiving 10 µg buserelin				
	Light	**s.e.m.**	**Heavy**	**s.e.m.**
Day 21	0.85	0.03	1.11	0.03
Day 28	1.48	0.03	1.12	0.05
Day 35	1.12	0.03	0.97	0.03
Day 42	0.77	0.05	0.88	0.03

Figure 4-1: Mean LH response to buserelin injection between 21 and 42 days postpartum in mixed age light- and heavy-strain cows: Experiment 2a.

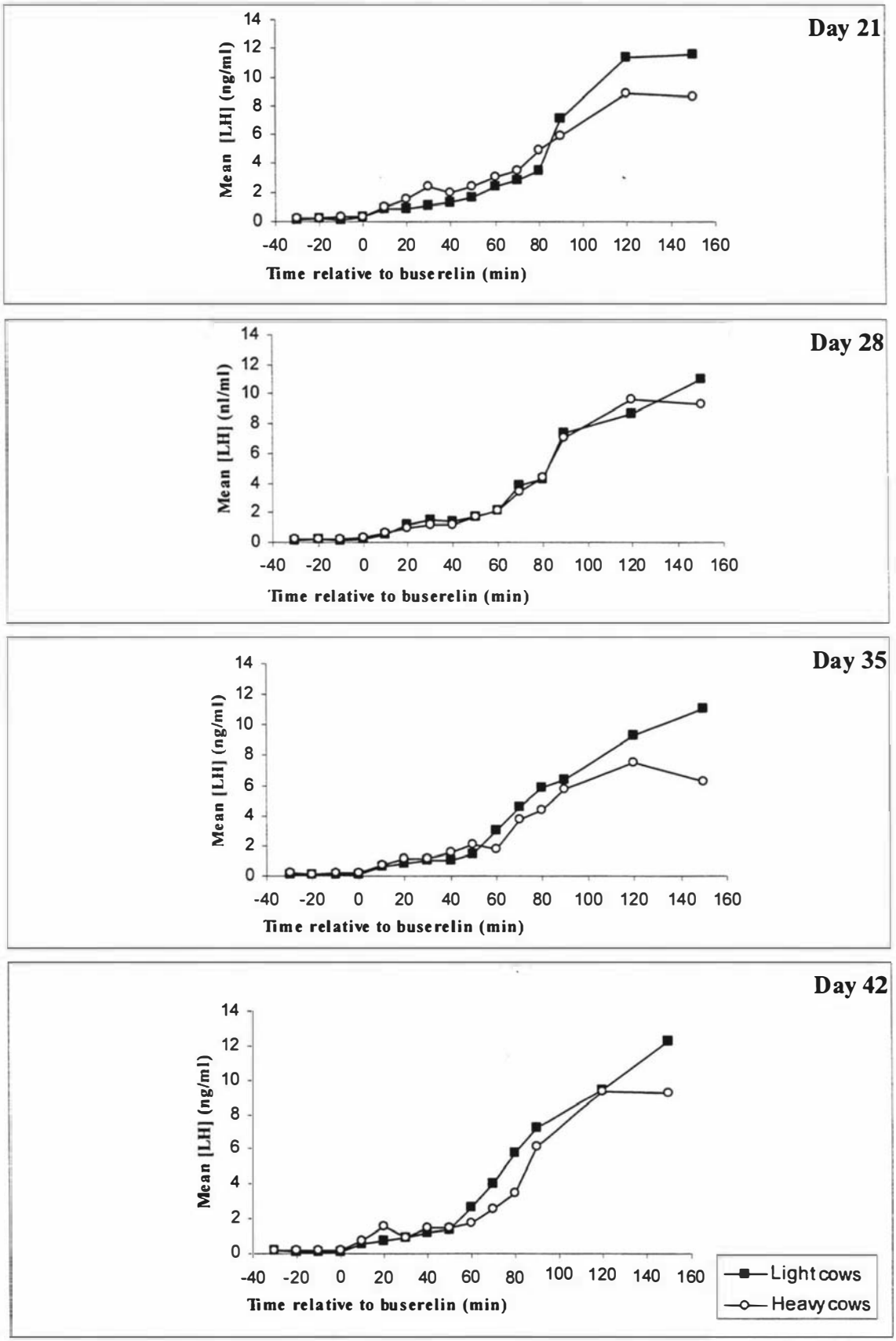


Figure 4-2: Mean LH response to buserelin injection on Days 7, 14, 21 and 28 postpartum in mixed age light- and heavy-strain cows: Experiment 2b.

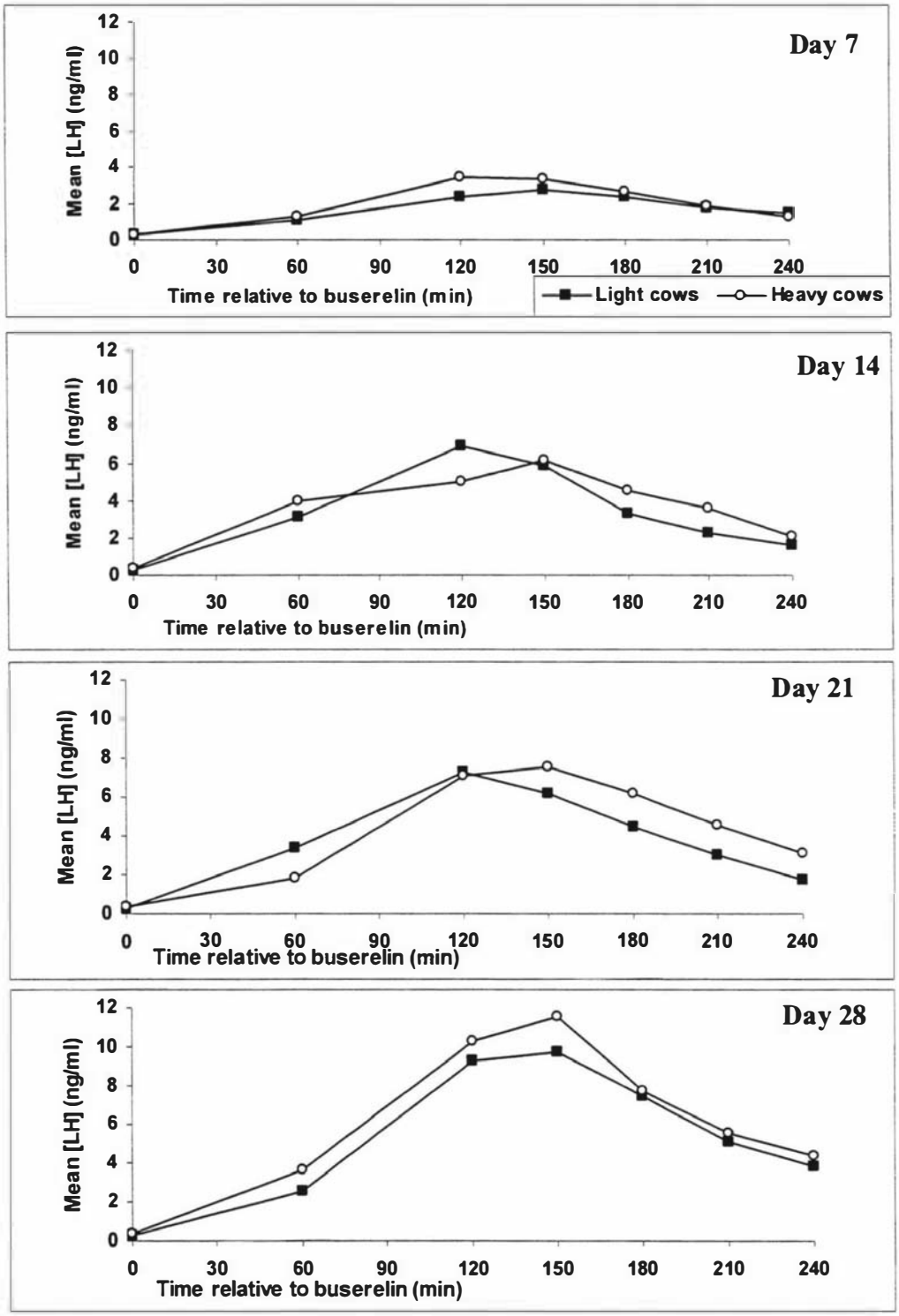
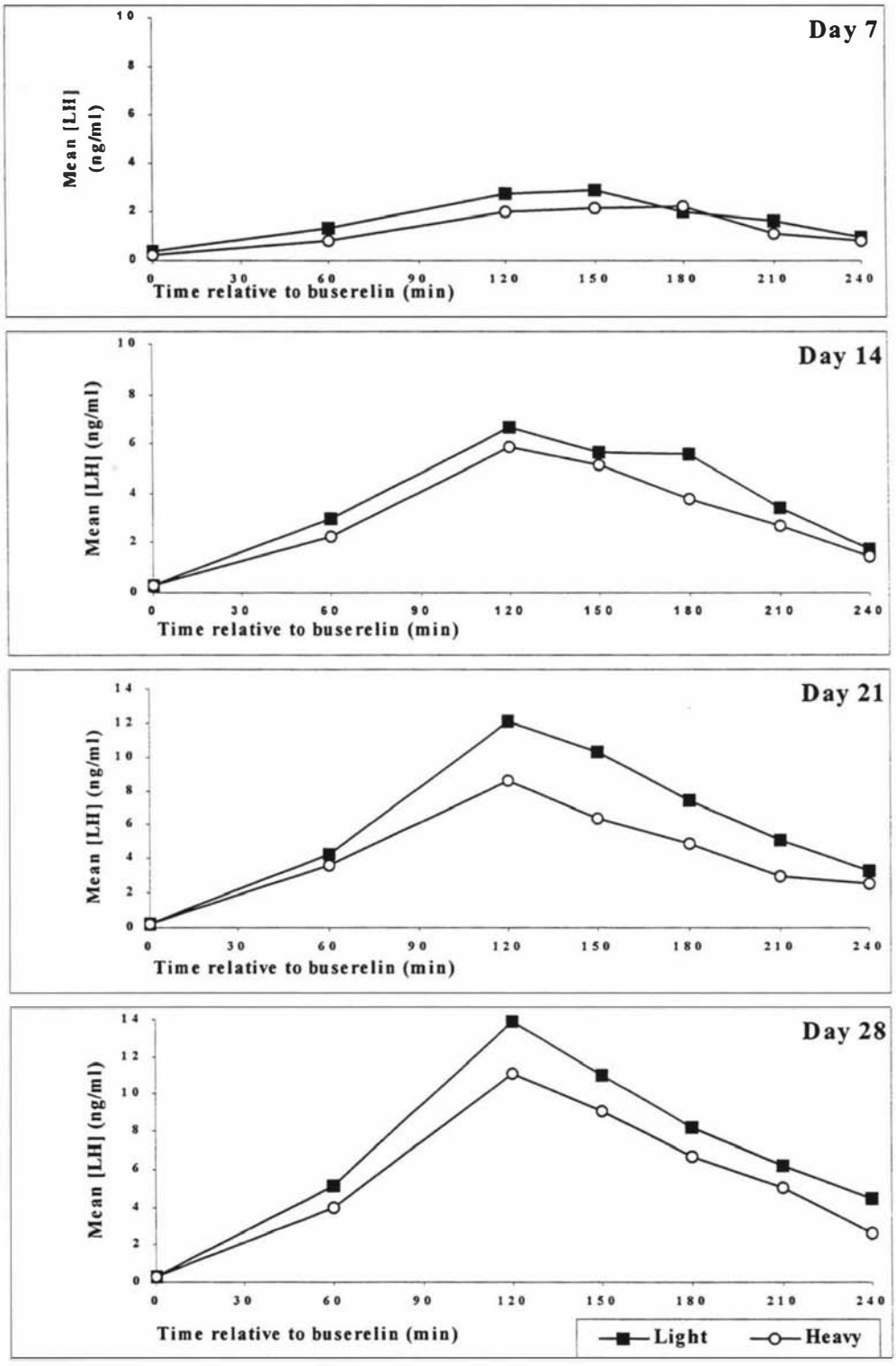


Figure 4-3: Mean LH response to buserelin injection between 7 and 28 days postpartum in first calved light- and heavy-strain heifers: Experiment 2c.



(Figures 4-4 and 4-5). In both experiments, LH responses to buserelin administration were unrelated to plasma progesterone concentrations. There were no significant differences ($P>0.05$) between mature cows (Experiment 2b) and first-calved heifers (Experiment 2c) in the LH responses to GnRH at any time. The amplitude of LH response in first-calved heifers was similar ($P>0.05$) to that of cows on any given day.

Across strains, all cows also exhibited significant increases in FSH concentrations in response to buserelin. However, there were no differences in FSH concentrations either between strains of cow or days postpartum.

4.4 Discussion

The results of these experiments indicate that pituitary function, in terms of the release and time to peak of LH in response to exogenous GnRH challenge, is generally similar in the two strains of cows between the 7th and 42nd days after calving, whether assessed in mixed age cows or in first-calved heifers. The endocrine challenge protocols that were employed in these studies have been widely used for the investigation of the activity of the reproductive neuroendocrine axis in postpartum cattle, and the results that were obtained are consistent with earlier reports in the literature. For example, administration of GnRH, at an equivalent dose to that of buserelin used in the present experiments, produced LH responses of both a similar amplitude and interval to maximal values to those previously reported (Britt *et al.*, 1974; Lamming *et al.*, 1981; Chenault *et al.*, 1990).

The resumption of the anterior pituitary function is a key factor to determine the resumption of the first cyclic ovarian activity after calving (Lamming *et al.*, 1981). Surprisingly, the findings in the present study do not support the observations by Laborde *et al.* (1998b) that indicated that the interval from calving to the first ovulation was shorter in H than in L cows.

Kanchev and Baruah (1994) found that pituitary responsiveness to GnRH had been regained within 7 days postpartum in dairy cows. In the present study, even though the LH responses were relatively lower on Day 7 than later days, all cows did exhibit a significant increase in LH concentrations in response to GnRH on Day 7. By contrast, fourteen percent (3/22) of cows in the study of Osawa *et al.* (1996) failed to show any response to GnRH challenge on Day 7. In fact, it was of interest to note that, in the present experiments, a significant LH response to buserelin was observed on Day 7 postpartum, whereas both Schams *et al.* (1974) and Fernandes *et al.* (1978) reported negligible LH

Figure 4-4: Mean maximal LH response of light- and heavy-strain mixed age cows to 10 mg buserelin between 7 and 28 days postpartum. Differences between strains were not statistically significant (P=0.77).

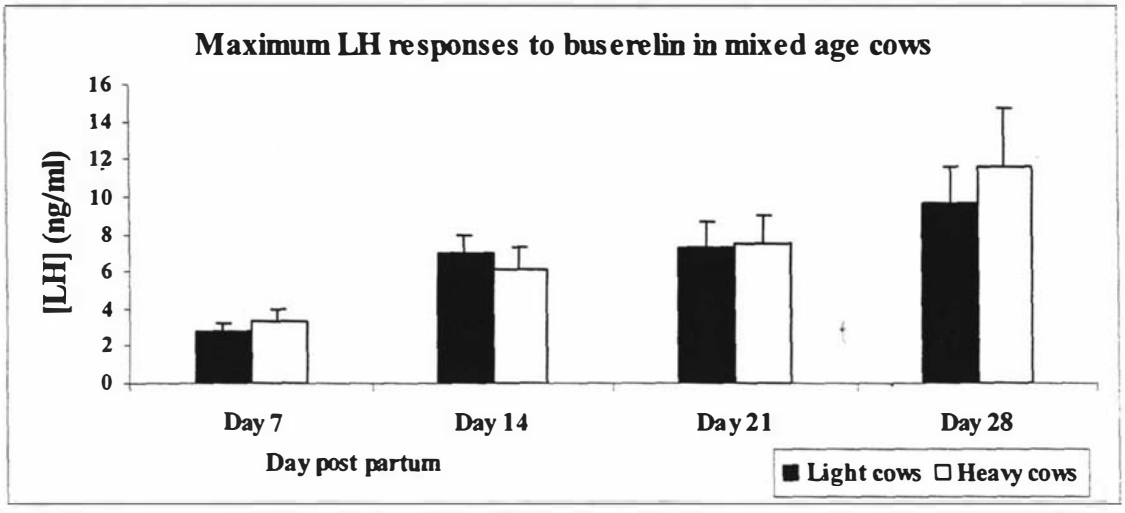
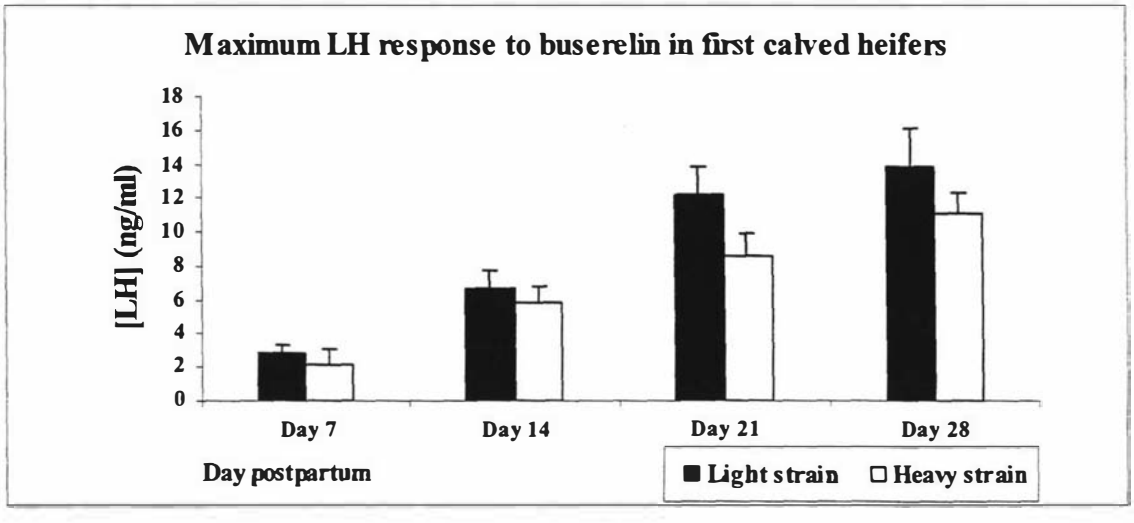


Figure 4-5: Mean maximal LH response of light- and heavy-strain first calved heifers to 10 mg buserelin between 7 and 28 days postpartum. Differences between strains were not statistically significant (P=0.19).



responses before Day 10. It is possible that differences in genetics, nutritional status and BCS of the cows, and in the route of GnRH administration may contribute to these differences in the pituitary function.

Moreover, as expected, there was a progressive increase in the LH response to GnRH with time postpartum in Experiments 2b and 2c, which were undertaken between Days 7 and 28 postpartum, but not in Experiment 2a (Days 21 to 42 postpartum). Peters and Lamming (1984a) reported that plasma LH concentrations and pituitary sensitivity to GnRH become maximal between 12 and 15 days postpartum. Thus the results of the present experiments are largely consistent with those in the literature, indicating that the LH response mechanism is fully restored by about the 20th day after calving, a figure that is similar to that reported by Peters and Lamming (1984a: Day 15).

The results in the present study also showed that the LH responses to GnRH in first-calved heifers were identical to those in mature cows, indicating that the LH response mechanism may be restored at a similar rate in these two groups of animals and the pools of releasable LH also undergo similar changes. It is, therefore, unlikely that the differences in the postpartum anoestrous period between these two groups of cows can be explained only by the responsiveness of the pituitaries to GnRH during the postpartum period.

It is curious why there was a difference in the results between Experiment 2a as compared with Experiments 2b and 2c. Perhaps it may stem from interactions with season of calving, nutritional status, BCS, sampling protocol and/or after unknown factors. Nevertheless, the biological significance of these differences in the LH responses to buserelin administration between strains found in Experiment 2a is unclear and difficult to explain. It is not clear whether the lower LH responses to buserelin administration in H cows than in L cows during Days 21 to 42 postpartum can contribute to altered follicular and/or luteal function resulting in the lower first service conception rates in H than L cows (as observed earlier by Laborde *et al.*, 1998b). However, it appears that the pools of releasable LH undergo similar changes in the two strains up to Day 21 to 28, although there may be a slight divergence later during Days 21 to 42 postpartum.

It was also of interest to note that the response to GnRH was unaffected when progesterone was used as a covariate, since endogenous secretion of LH is undoubtedly affected by progesterone concentration (Goodman and Karsch, 1980). However, data from the sheep indicates that the effects of progesterone upon LH secretion are primarily

modulated by alterations of the rate and amplitude of endogenous GnRH secretion rather than by direct effects upon the pituitary (Clarke and Cummins, 1984). Hence, administration of exogenous GnRH by-passes this mechanism, allowing the pituitary to display an unimpeded response to trophic stimulation. In the absence of measurements of GnRH in bovine hypophyseal portal blood, it has been assumed that similar mechanisms pertain in the cow (Nett, 1987).

In consequence, it is clear that the effects of GnRH administration in the present experiments were compatible with those previously reported in the literature. Hence, it can be concluded that there is no difference in the responsiveness of the pituitaries of cows in the L and H strains to GnRH during the postpartum period; a conclusion that is underlined by the range of animals and stages of the postpartum periods from which such observations were made.

4.5 Conclusion

There are no significant differences between strains of Holstein-Friesian cows in terms of their responsiveness to GnRH (i.e. buserelin) in the early postpartum period (up to Day 21). Later on, there is some evidence of a divergence of response between strains, inasmuch as there were higher overall mean concentrations in Experiment 2a in L compared with H cows between Days 21 and 42 postpartum. In other words, the pools of releasable gonadotrophins undergo similar changes in the two strains during the early postpartum period, although there may be a slight divergence later. Consequently, these data suggest that the mechanism responsible for earlier postpartum ovulatory activity may operate through differences in ovarian responsiveness to gonadotrophins rather than through the activity of the hypothalamo-pituitary axis itself.

5 Chapter 5- Experiment 3: The effect of oestradiol treatment on LH secretion in postpartum dairy cows selected for heavy or light mature bodyweight

The material in this chapter has been accepted for publication by the *New Zealand Veterinary Journal*, as "Post-partum gonadotrophin secretion in Holstein-Friesian dairy cows differing genetically in liveweight" by Thiengtham, J., McNaughton, L.R., Holmes, C.W. and Parkinson, T.J.

5.1 Introduction

Within the seasonal, pasture-based dairying systems in New Zealand, reproductive performance of the cows is subject to two major constraints: the duration of postpartum anoestrus and conception rate (Macmillan, 1985a). These appear to be of greater significance in Holstein cows than in Friesian cows. However, results from Experiment 1 revealed that heavy strain (H) cows had higher LH pulse frequency and mean concentrations during the postpartum anoestrous period than light strain (L) cows, whilst Experiment 2 showed that the responsiveness of the anterior pituitary to exogenous GnRH stimulation during early lactation of mature cows and 2-year old heifers was similar in both strains. Thus, these results do not support the notion that either of these constraints mentioned above are dependent upon, or mediated by, intrinsic differences in the resumption of LH secretions in the postpartum period between strains.

However, pituitary function after calving is not only dependent upon the stimulatory signals from the hypothalamus but also on feedback from ovarian steroids. For example, it has been suggested that the prolonged anoestrous period after calving involves an increase in hypothalamic sensitivity to oestradiol negative feedback leading to suppression of GnRH secretion and, thus, LH release (McDougall *et al.*, 1998). A major component of the endocrine activity of the postpartum period is the restoration of the positive feedback interaction between oestradiol and LH secretion (Schallenberger and Prokopp, 1985). The length of time required for this restoration is likely to be related to the duration of postpartum anoestrus (Alam and Dobson, 1987). Re-establishment of the positive feedback mechanism of oestradiol itself further requires not only the development of GnRH receptors within the pituitary (Schoenemann *et al.*, 1985b), but also of oestrogen receptors within the hypothalamus (Nett *et al.*, 1988). In other words, the hypothalamo-pituitary axis must be able to respond to high concentrations of oestradiol in circulation

produced by preovulatory follicles in order to release the preovulatory gonadotrophin surge leading to the ovulation of large follicles.

In dairy cows, administration of exogenous oestradiol stimulates a release of LH which is similar both in magnitude and duration to the preovulatory LH surge (Beck and Convey, 1977). The positive feedback effect of oestrogens upon the hypothalamo-hypophyseal axis inducing the pre-ovulatory LH and FSH surge (Price and Webb, 1988) is a major component of postpartum re-establishment of ovarian cyclicity (Stevenson *et al.*, 1983). Moreover, a delay in the re-establishment of the positive feedback mechanisms to ovarian steroid-induced gonadotrophin surge might be expected to impair fertility and delay the resumption of ovarian activity and subsequently conception after calving. In normal cows, the hypothalamo-pituitary responsiveness to exogenous oestradiol returns to full competency somewhere between 5 and 30 days postpartum (Zaied *et al.*, 1981; Peters, 1984a; Schallenberger and Prokopp, 1985; Alam and Dobson, 1987).

This component of the hypothalamo-pituitary-ovarian axis may play a role in the difference in fertility between cows from the H and L strains. The working hypothesis for this study was that differences between the strains in the responsiveness of the anterior pituitary to oestradiol during the postpartum period underlie differences in the time to resume ovarian activity after calving. In addition, the results from Experiment 2a showed that the differences in LH response to GnRH between H and L cows might have indicated a different response during the LH surge. If this proves to be the case, it might explain differences in the first service conception rate between these two strains of animals. The objective of this experiment was to determine the responsiveness of the hypothalamo-pituitary axis to oestradiol injection in H and L dairy cows on Days 7, 14, 21 and 28 postpartum.

5.2 Materials and methods

Animals and experimental design

The re-establishment of positive feedback secretion of LH in response to oestradiol was studied using 24 mixed age cows (12 of each strain). The cows were assigned into four groups (two groups each of 6H and 6L cows) and were given 1 mg oestradiol benzoate (Cidirol; Bomac Laboratories Ltd, Manakau, NZ) on Days 7 and 21, or on Days 14 and 28 after calving. Forty-eight hours before the administration of oestradiol, each cow was given a luteolytic dose (500 μg) of cloprostenol (Estrumate; Schering-Plough Animal

Health, Upper Hutt, NZ), to ensure the removal of any endogenous source of progesterone.

Blood sampling and hormone assays

Blood samples (5 ml, using lithium heparin as the anticoagulant) were collected by caudal venepuncture at 0, 12, 15, 18, 21, 24, 27, 30, 33, 36, and 42 h relative to the time of oestradiol administration, for measurement of LH and FSH concentrations. Blood samples were centrifuged at 1000g for 20 min at 4 °C and plasma was then separated and stored at -20°C until assayed.

Concentrations of LH and FSH were measured in all samples. Progesterone concentrations were measured in the first (0), second (12 h) and last (42 h) samples of each challenge. Concentrations of LH, FSH and progesterone were measured in plasma by the methods as described in Chapter 2.

Data and statistical analyses

An LH surge was deemed to have occurred when peak LH concentrations exceeded the mean of all values from an individual animal (or animal/day sampling period) by two standard deviations of the mean. The peak value was then examined and, if it was not above 2ng/ml, then a surge was not considered to have occurred, even if the first criterion had been satisfied. LH data from each series of samples were thereafter synchronised around the time of the maximal LH concentrations. The area under the curve (AUC) of LH response was determined by trapezoidal summation of all LH values during the 42-h sampling period after oestradiol injection. Then, LH and FSH data were subjected to analysis of variance after having been synchronised around peak values. Progesterone concentration was used as a covariate. The AUC of the LH response and intervals between oestradiol administration and peak LH concentrations were also subjected to analysis of variance with respect to the effects of strain and day postpartum.

5.3 Results

All cows exhibited a significant increase in LH concentrations after the administration of oestradiol benzoate, except for two cows which failed to display a significant increases in LH concentrations after the challenge on Days 21 (one H cow) and 28 (one L cow). These animals had high plasma progesterone concentrations ($\geq$2 ng/ml) at the time of challenges.

Table 5-1: Analysis of variance for the area under the curve of the LH response to oestradiol administration.

Source of variation	Variance ratio	F Probability
Strain	1.24	0.269
Day postpartum	9.97	<0.001
Strain.day	0.01	0.911
Covariate (progesterone concentration)	2.00	0.928

Table 5-2: Mean values for the interval between oestradiol benzoate administration and attainment of peak LH concentrations.

Time postpartum	Interval to peak LH concentrations (h)
Day 7	33.8 ± 1.7^{a}
Day 14	28.2 ± 2.0^{b}
Day 21	25.7 ± 2.2^{b}
Day 28	26.7 ± 2.2^{b}

Means that are labelled with a different letter are significantly different from each other ($P<0.01$).

Figure 5-1: Mean (± s.e.m.) areas under the curve of (a) LH responses to oestradiol benzoate administration to light- (stippled bars) and heavy-strain (open bars) cows between Days 7 and 28 postpartum. LH responses were significantly ($P<0.05$) different between days where indicated by bars labelled with different letters, but differences between strains were not significant.

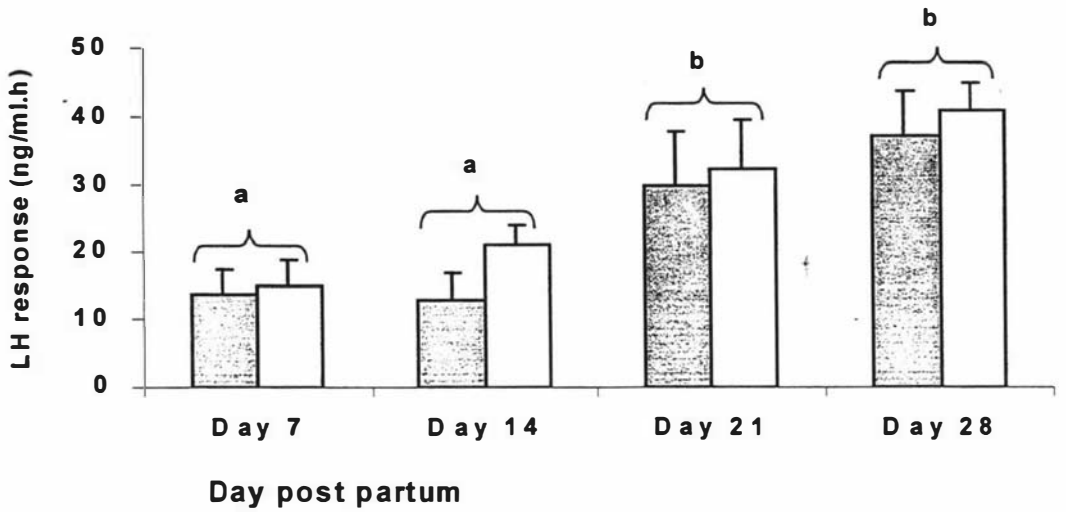
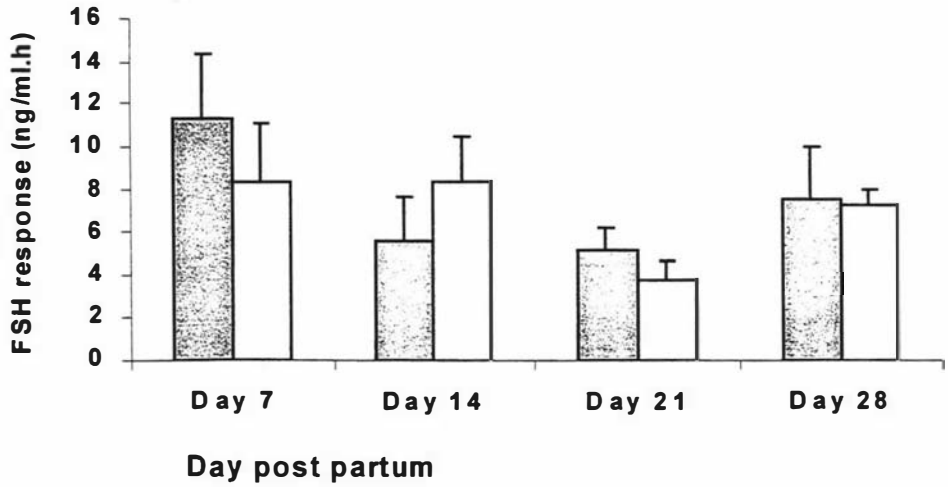


Figure 5-2: Mean (± s.e.m.) areas under the curve of FSH responses to oestradiol benzoate administration to light- (stippled bars) and heavy-strain (open bars) cows between Days 7 and 28 postpartum. Differences between FSH responses on different days post partum approached, but did not reach statistical significance ($P=0.078$). Differences between strains were not statistically significant.



Therefore, the data from these cows on that particular day were not used for further analysis.

Mean amplitude and area under the curve of the LH response increased with time postpartum, but responses were similar in the two strains at each time postpartum (Figure 5-1). The response was unrelated to the covariate, progesterone concentration (Table 5-1). The interval between oestradiol administration and the attainment of peak LH concentrations also varied with time postpartum (Table 5-2), such that intervals significantly ($P<0.001$) decreased as time postpartum increased.

FSH responses to oestradiol decreased with time postpartum (Figure 5-2), although these differences just failed to reach statistical significance ($P=0.078$). FSH concentrations were maximal on Day 7 and reached a nadir on Day 21.

5.4 Discussion

The results in the present experiment indicated that there was no significant effect of strain upon the ability of cows to secrete LH in response to the exogenous oestradiol injection over the period between 7 and 28 days postpartum. Since the recovery of the ability of oestradiol-17β to stimulate a preovulatory LH surge is an essential component of the duration of the postpartum anoestrous period (Schallenberger *et al.*, 1982; Peters, 1984a; Schallengerger and Prokopp, 1985), these results confirm the observations of Experiments 1 and 2 that there are no substantial differences between strains in the activity of the hypothalamo-pituitary axis during the postpartum period.

In previous studies, there was no LH surge in response to exogenous oestradiol injection on either Day 1 (Alam and Dobson, 1987) or Day 5 postpartum (Schallengerger and Prokopp, 1985; Alam and Dobson, 1987). In the literature, as in the present experiments, a progressive increase in the amplitude of the LH response to oestradiol occurs as the postpartum periods progresses (Peters, 1984a). In addition, Alam and Dobson (1987) also found a significant negative correlation between the time to peak LH concentration and day postpartum. Thus the data from the present experiment showed the expected progressive increase in LH responsiveness over the postpartum period, yet did not reveal any differences between strains at any time. A similar trial in which overseas Holstein cows and NZ Holstein-Friesian cows were given oestradiol challenges on Day 16 and 17 (i.e. when the first postpartum ovulation is likely to occur; Peters and Lamming, 1986) confirmed that there was no difference of the LH responsiveness, even between such

6 Chapter 6- Experiment 4: Reproductive performance and milk progesterone patterns of postpartum dairy cows selected for heavy or light mature bodyweight

Part of the material contained in this chapter has been published as "Thiengtham, J., Parkinson, T.J. and Holmes, C.W. 2002. Relationships between milk progesterone profiles and conception in strains of Holstein-Friesian cattle genetically selected for high and low-mature bodyweight. *The 6th International Symposium on Reproduction in Domestic Ruminants*. Crieff, UK. Abstract A82"

6.1 Introduction

Low fertility in the modern dairy cow has been a major concern worldwide, especially in NZ under seasonally managed, pasture-fed conditions. The reasons for this infertility are unclear, but data from Experiments 1, 2 and 3 indicate that differences in fertility between heavy-strain (H) and light-strain (L) cows after calving are not due to the differences in the function of the hypothalamo-pituitary axis. Identical hormonal environments during the resumption of the ovarian activity, and similar responses of the anterior pituitary to endocrine challenges in these cows show that it is unlikely that the differences between the first service conception of H and L cows could be explained in such terms. However, they might be explicable in terms of differences in ovarian and/or luteal function. The establishment of pregnancy is dependent upon the presence of progesterone (P4), in order to develop an uterine environment which is suitable for embryonic development (Mann and Lamming, 1999). The role of progesterone, however, goes beyond a simple permissive state, since several studies have shown that high progesterone concentrations are associated with better embryonic survival (Sreenan and Diskin, 1983; Diskin and Sreenan, 1986), and because of the complex interactions that take place between embryo, endometrium and corpus luteum around the time of maternal recognition of pregnancy (Peters, 1996; Mann and Lamming, 1999). It was therefore postulated that differences in luteal activity might be responsible for the divergence of conception rates between H and L cows.

Reproductive activity of cows after calving can be monitored by several methods, such as examination of the herd records, examination of the reproductive tract (by means of rectal palpation or ultrasonography) or determination of progesterone concentrations in plasma or milk (Fagan and Roche, 1986; Rajamahendran and Taylor, 1990; Opsomer *et al.*, 1998). Milk progesterone profiles based on twice on thrice-weekly samples after calving can be

used as a tool to monitor ovarian and luteal activity and to identify cows showing abnormal patterns of reproductive activity during the early postpartum period (Lamming and Bulman, 1976; Lamming and Darwash, 1998; Opsomer *et al.*, 1998). Several studies have investigated the factors affecting fertility by monitoring the reproductive patterns after calving using the pattern of progesterone profiles (e.g. Bulman and Lamming, 1978; Lamming and Darwash, 1998).

Nutritional status during the dry period through to early lactation is one of the most important factors influencing postpartum reproductive performance of cows which have been selected for high milk yield (Butler and Smith, 1989). It has been suggested that, as genetic potential for milk production of the cow increases, so does body reserve mobilisation. Such changes can be measured by body condition score (BCS) changes during early lactation in both concentrate-based (Butler *et al.*, 1981) and pasture-based systems (Buckley *et al.*, 2000; Snijders *et al.*, 2001). Low BCS and loss of BCS during the first four weeks of lactation are associated with a reduced chance of conception to first service (Domecq *et al.*, 1997; Beam and Butler, 1998). Likewise, cows with more severe negative energy balance lost more BCS during the first four weeks postpartum and showed longer periods from calving to first ovulation than cows that had small BCS losses (Butler *et al.*, 1981; Butler, 2001).

In the present study, data from the analysis both of herd records and of milk progesterone profile characteristics during the early postpartum period (from calving until the end of mating period) were used 1) to monitor and compare the reproductive outcomes, onset of ovarian cyclicity and patterns of luteal activity between H and L cows; and 2) to determine the relationships between milk progesterone concentrations and pregnancy outcomes in these cows.

6.2 Materials and methods

Animals and experimental design

One hundred and fifty nine lactations from spring calving, Holstein-Friesian cows, that had been genetically selected for heavy (H) or light (L) mature body weight (Year 1: H=37; L=41; Year 2 H=41; L=40) were used in this study. The H and L cows contained an average of 37.5% and 10.5% North American Holstein genetics, respectively. The cows were managed in one herd and were milked twice a day. They were fed a generous allowance of mixed ryegrass and white clover pasture, with supplementary maize silage

when required. LW and BCS of the cows during the first four weeks after calving were recorded. Other details of herd reproductive management are summarised in Chapter 2.

Milk sampling and hormone assays

Milk samples (15 ml) were collected during morning milking thrice weekly (Monday, Wednesday and Friday) from 5-7 days after calving until the end of December, over two lactations 1999 (Year 1) and 2000 (Year 2). Milk samples were stored at -20°C until assayed, and no preservative was used. Concentrations of milk progesterone (P4) were determined using ELISA kits as described in Chapter 2. The limit of sensitivity, inter- and intra-assay coefficients of variation are presented in Table 2-2 (Chapter 2).

Analysis of data

In order to characterise ovarian activity and luteal function after calving, milk progesterone concentrations were plotted against days postpartum for each animal. These profiles were used to determine the reproductive activity after calving. Milk progesterone concentrations ≥3 ng/ml were considered to indicate the presence of an active CL (Lamming and Darwash, 1998). A short luteal cycle was defined as one with the luteal phase interval of ≤ 10 days in duration (Lamming *et al.*, 1981).

The incidence of atypical ovarian activity during the early postpartum period was identified using progesterone profiles as described in Lamming and Darwash (1998), including:

1. Delayed ovulation type I (DOI; P4 concentrations <3 ng/ml for ≥45 days postpartum).
2. Delayed ovulation type II (DOII; P4 concentrations <3 ng/ml for ≥12 days between two luteal phases).
3. Persistent CL type I (PCLI; P4 concentrations ≥3 ng/ml for ≥19 days during the first postpartum oestrous cycle).
4. Persistent CL type II (PCLII; P4 concentrations ≥3 ng/ml for ≥19 days during subsequent postpartum cycles).
5. Late embryo mortality (LEM; P4 concentrations ≥3 ng/ml for ≥19 days days after insemination, followed by declined P4 concentrations).

Reproductive performance

The herd's reproductive records, including calving date, observations of oestrous behaviour, artificial insemination (AI) records and pregnancy outcomes, together with milk progesterone profiles of all cows were reviewed and used to calculate the following parameters:

1. Interval from calving to first observed oestrus postpartum (C-to-FH; d)
2. Interval from calving to first progesterone concentration >3 ng/ml (C-to-P_4 rise)
3. Interval from calving to ovulation (C-to-Ov)
4. Interval from calving to first AI (C-to-AI)
5. Interval from first progesterone >3 ng/ml to conception (P_4 rise-to-Con)
6. Interval from calving to conception (C-to-Con)
7. Services per conception (Ser/Con) and
8. First service conception rate (FSCR).

Ovulation was defined to have occurred 5 d before a progesterone rise to >3ng/ml, so long as elevated progesterone concentrations were maintained for at least two consecutive samples (McDougall, 1994). Pregnancy was confirmed by high progesterone concentrations at the end of the study period (end of December) and calving date. The milk progesterone data derived from progesterone profiles were correlated with the herdsman's observations of oestrus and AI records. The percentages of cows calved within 21 days of the planned start of calving, submission rate during the first three weeks of mating period, cows with $\geq$1 natural mating, empty rate at the end of mating period and culling rate were also calculated from the herd records. The definitions of these parameters are given in Table 6-1.

Analysis of milk progesterone profiles

Milk progesterone concentrations were plotted against days of the oestrous cycle for each animal in order to characterise patterns of progesterone secretion during the cycles of each strain. Since there were differences in oestrous cycle length, progesterone data were retrospectively normalised by using the day of oestrus to be Day 0. The data set was thereafter subjected to regression analysis with respect to strain and day. Milk

progesterone concentrations, taken from individual progesterone profiles, were used to calculate mean progesterone values for each day of the cycle.

Luteal activity and pregnancy outcomes

Luteal activity was characterised by collecting progesterone concentration data from individual cows during the first 16 days after first insemination. These data were subjected to linear and quadratic regression analysis, with respect to time post-insemination, strain and pregnancy outcome. Although only data from cows with normal cycles were used (i.e. for cows that did not conceive to first AI, and for which the inter-service interval was between 18-24 days; Wood, 1976), it was not possible to examine their luteal activity during the late luteal phase by the above method, since there was a range of lengths of luteal phases. Data were therefore re-synchronised around the day on which they returned to oestrus (i.e. backwards from the end of the cycle). The significant differences of regression lines between groups of cows related to strains (H and L) and pregnancy status, conceived (P) or failed to conceive (NP), were tested using analysis of variance.

Statistical analyses

Reproductive data and milk progesterone concentrations were subjected to analysis of variance to test the effect of strain and days. Categorical data, including the incidence of atypical ovarian patterns and short luteal cycle together with the FSCR, 3-wk calving rate, 3-wk submission rate, cows with $\geq$1 natural mating, empty rate and culling rate, were subjected to Chi-square (χ^2) analyses with respect to strain and G-test analysis (Sokal and Rohlf, 1969) with respect to strain and year.

6.3 ***Results***

There were no significant differences in yield of milksolids, LW or BCS during early lactation between the strains (Tables 6-2, 6-3 and 6-4). Data from both years showed that there were no significant ($P>0.05$) differences between strains in the proportion of cows in each strain that lost either LW or BCS during the first 4 weeks of lactation. However, data for Year 2 showed that the proportion of cows that lost more than 30 kg of LW (12/30) was significantly ($P<0.05$) higher for H cows than for L cows (5/34).

Reproductive performance

Results of the reproductive performance of H and L cows are shown in Table 6-5. There was a significant ($P<0.05$) difference in FSCR between H (42 %) and L (65 %) cows in

Table 6-1: Definitions of reproductive parameters derived from herd's records and milk progesterone profiles.

Parameter	Definitions
Interval from calving to first observed oestrus postpartum (C-to-FH)	Duration from calving date to first observed behavioural oestrus detected by tail painting
Interval from calving to first ovulation postpartum (C-to-1st Ov)	Duration from calving date to 5 days before the first milk progesterone>3 ng/ml.
% Silent heat before 1st ovulation postpartum	Percentage of cows showed no sign of behavioural oestrus before 1st ovulation postpartum
Interval from 1st P_4>3ng/ml to conception (P_4 rise-to-Con)	Duration from the date of first milk progesterone >3 ng/ml to the date of conception confirmed by date of subsequent calving
Interval from calving to conception (C-to-Con)	Duration from calving date to date of conception
3-wk calving rate	Percentage of cows calved within 21 days of the planned start date of calving
3 wk submission rate	Percentage of cows inseminated during the first 3 weeks of mating period
Service per conception (Ser/Con)	Number of services to achieve a conception
First service conception rate (FSCR)	Percentage of cows that conceived to the first service confirmed by subsequent calving date
Empty rate	Percentage of cows that failed to conceive by the end of the mating period
Culling rate	Percentage of cows removed from the herd before next calving

Table 6-2: Mean values for daily yields of milksolids for light- and heavy-strain cows during early lactation (first 4 weeks) Year 1 (1999: H: n=36; L: n=40) and Year 2 (2000: H: n=40; L: n=40) calving seasons.

Strain of cow	**Milksolid yields (mean±SE)during early lactation (kg/cow daily)**	
	Year 1 (1999)	**Year 2 (2000)**
Light	2.23 ± 0.05	2.18 ± 0.08
Heavy	2.30 ± 0.06	2.22 ± 0.06

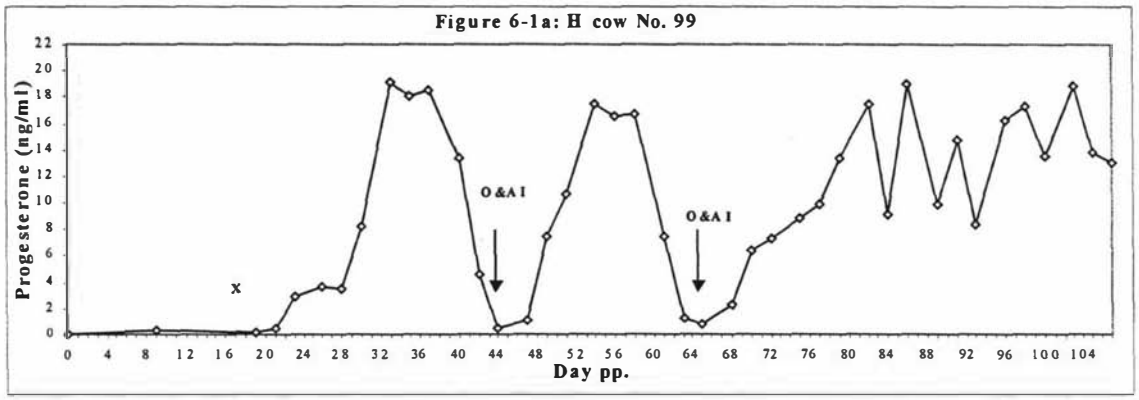
Table 6-3: Mean values for changes in lightweight during the first four weeks of lactation in light- and heavy-strain cows in Years 1 and 2.

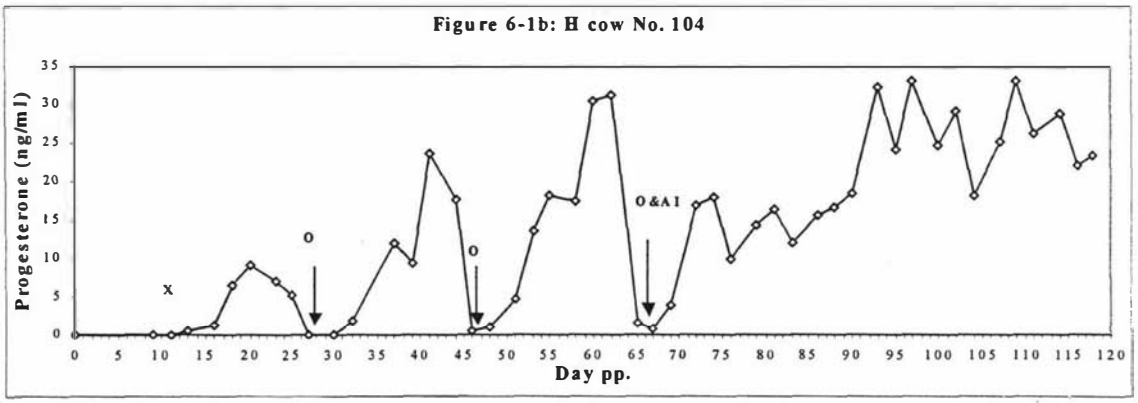
Strain of cow	**Liveweight changes (mean±SE) during early lactation (kg/cow/4 wks)**	
	Year 1 (1999)	**Year 2 (2000)**
Light	-8.81±4.47	-12.44±2.93
Heavy	-13.17±2.11	-18.13±3.33

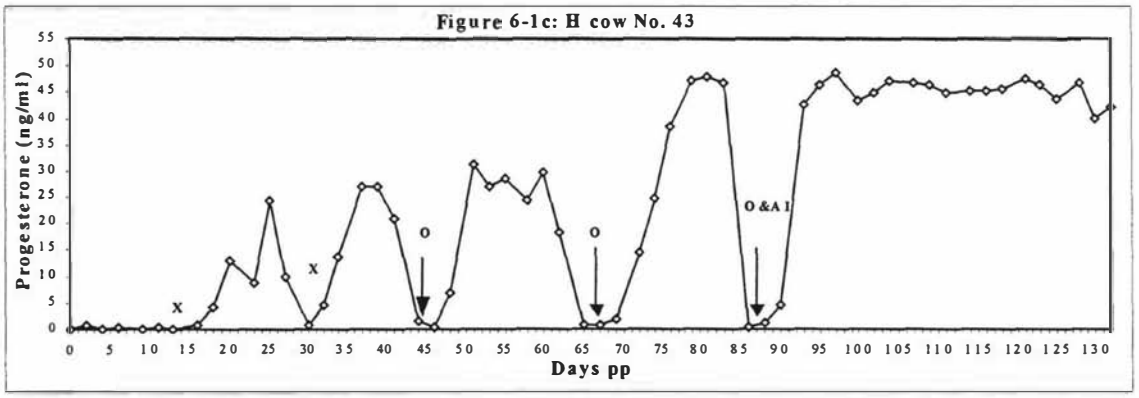
Table 6-4: Mean values for changes in body condition score during the first four weeks of lactation in light- and heavy-strain cows during Year 1 and 2.

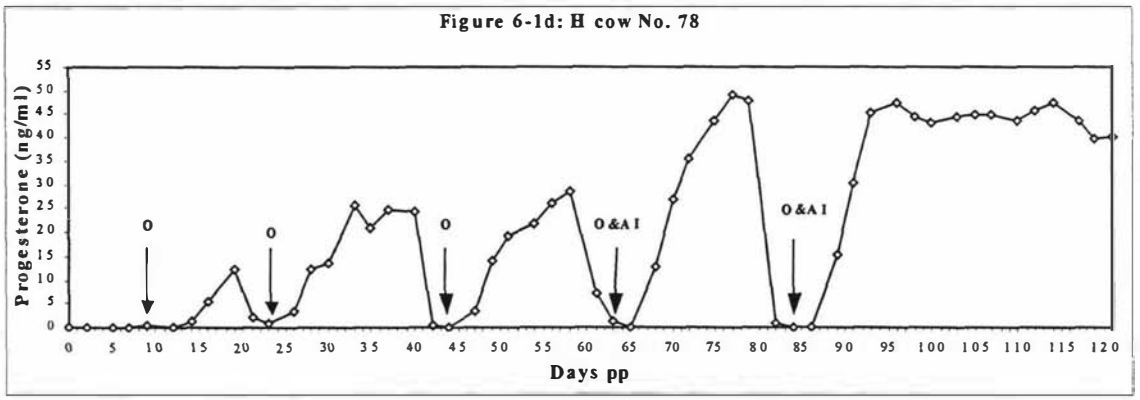
Strain of cows	**Body condition score changes (mean±SE) (unit per 4 wks)**	
	Year 1 (1999)	**Year 2 (2000)**
Light	-0.10±0.03	-0.10±0.05
Heavy	-0.15±0.04	-0.19±0.05

Figures 6-1a to **6-1d:** Representative milk progesterone profiles of H cows during the study period. x=silent heat, O=behavioural oestrus and O&AI=behavioural oestrus followed by artificial insemination.

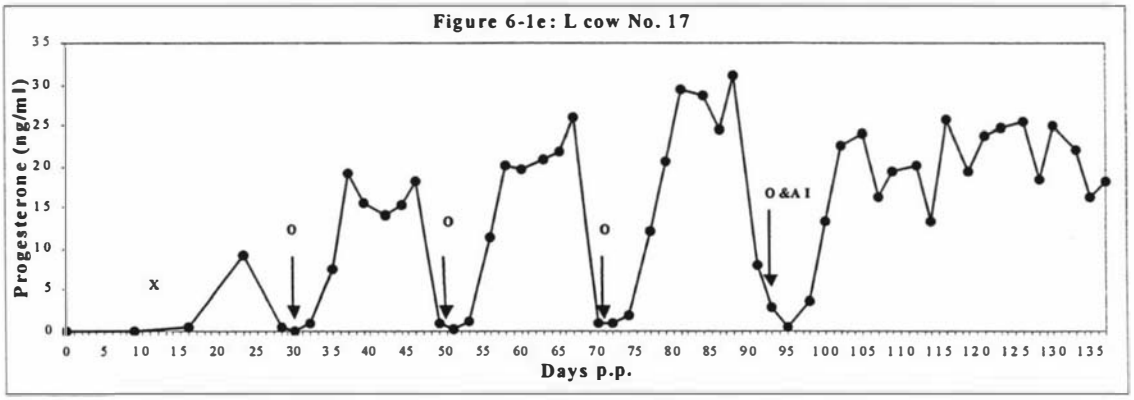


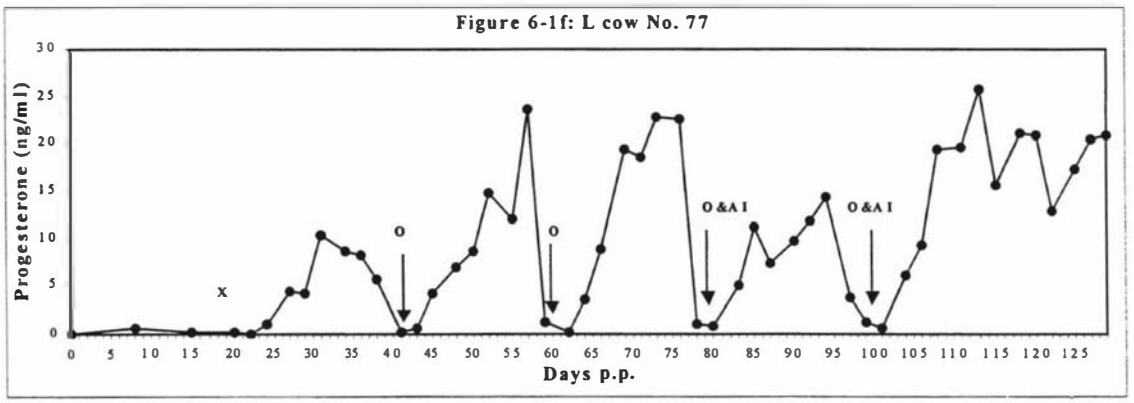


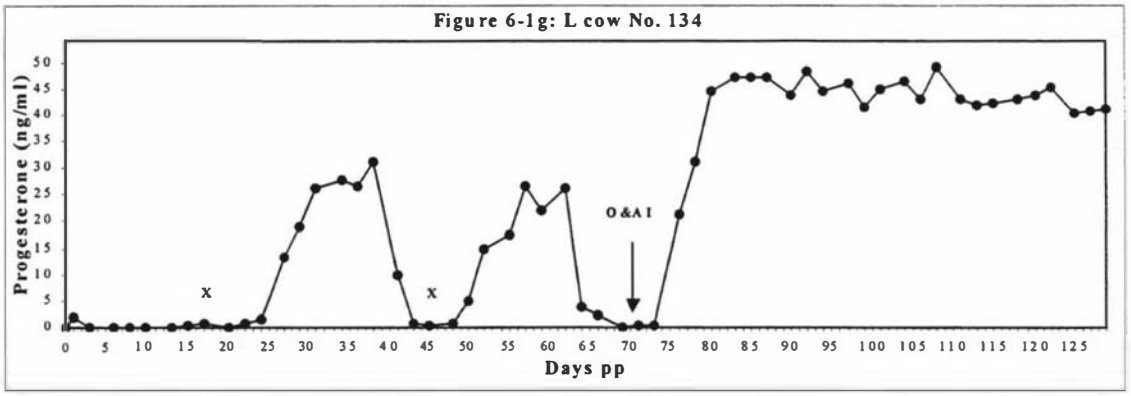




Figures 6-1e to **6-1h:** Representative milk progesterone profiles of L cows during the study period. x=silent heat, O=behavioural oestrus and O&AI=behavioural oestrus followed by artificial insemination.







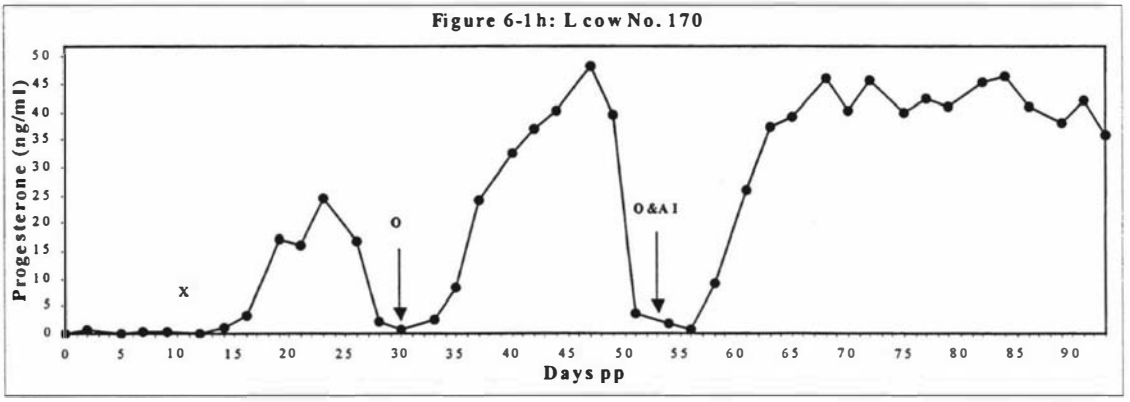


Table 6-5: Reproductive performance of light- (L) and heavy-strain (H) cows during a 2-year study period. Data are presented as mean ± s.e.m.

Reproductive parameters	Year 1		Year 2	
	H (n=37)	L (n=41)	H (n=41)	L (n=40)
3-wk calving rate (%)	54	56	75	70
3-wk submission rate (%)	75	76	95	95
C-to-FH (d)	44.1 ± 2.9	46.1 ± 2.9	44.6 ± 2.9	41.7 ± 3.0
C-to-1st Ov (d)	25.6 ± 2.7	25.1 ± 2.4	13.7 ± 0.7	14.9 ± 0.9
C-to-P_4 rise>3ng/ml (d)	30.6 ± 2.9	30.1 ± 2.2	18.7 ± 0.8	19.9 ± 1.0
% Silent heat before 1st ovulation postpartum	81 (30/37)	80 (33/41)	85 (35/41)	78 (31/40)
C-to-1st AI (d)	71.6 ± 2.1	71.6 ± 2.5	79.8 ± 3.3	80.6 ± 2.9
C-to-Con (d)	78.5 ± 3.2	80.4 ± 4.4	93.9 ± 3.1	85.6 ± 3.2
1st P_4 rise-to-Con (d)	51.4 ± 3.1	52.0 ± 4.5	77.9 ± 3.2	65.0 ± 3.3
Ser/con	1.42	1.43	1.74	1.37
FSCR (%)	57.9	62.2	**42.5[a]**	**65.0[b]**
Cows conceived by natural mating (%)	16	12	22	15
Empty rate (%)	3	14	15	8
Culling rate (%)	10.8	14.6	14.6	12.5

[a, b] Means in the same line with different superscripts are different ($P < 0.05$).

Year 2 but not in Year 1 (H=58 %, L=62 %; P>0.05). Combined FSCR data from both years were not significantly difference between strains (H= 51%, L= 64 %; P>0.05). However, combined data for the three years (1999-2001) showed a significant difference between strains in FSCR (H=47 % versus L=60 %; P<0.05, see also Experiment 5). The results from G-test using the data from the three years also showed that FSCR was dependent upon strains but not upon year and FSCR is significantly (P<0.05) lower in H cows than in cows. There were no significant differences in any other reproductive data between strains, either for data from each year, or for combined data from both years.

Progesterone profile analysis after calving

Representative patterns of milk progesterone concentrations of H and L cows during the study period are shown in Figures 6-1a to 6-1h. The first rise of progesterone to >3 ng/ml indicated the initiation of the first cycle after calving regardless of its luteal phase interval. The distribution of these data was similar between strains but not between years (Figure 6-1). In Year 1, more than 80 % of cows resumed luteal activity within 35 days after calving. However in Year 2, all cows (100%) had resumed luteal activity by the same time after calving. Seventeen percent of cows started to cycle within 14 days after calving in Year 2, whilst none did so in Year 1. The results from G-test analysis showed that the proportions of cows that started to cycle within 21 days after calving were significantly (P<0.05) different between years, but not between strains. The percentage of cows that started to cycle within 21 days after calving was significantly (P<0.05) higher in Year 2 (78%) than in Year 1 (28%). Whilst there were no significant differences between strains in the incidence of short luteal phases after calving, short luteal cycles occurred in both the first and the second cycles after calving (in 55 % and 7 % of all cows in Year 1 and 2 respectively).

The incidence of atypical ovarian patterns in H and L cows for Year 1, Year 2 and both years combined are presented in Table 6-6. There was no difference between strains in the incidence of the abnormal ovarian patterns. However, across strains, combined data showed that the incidence of DOI was significantly higher in Year 1 (13%) than in Year 2 (1%: P<0.05). The results from G-test analysis also confirmed that the incidence of DOI was dependent upon year, but not upon strain. The incidence of PCLII in data combined from both years tended to be higher in H cows (10%) than in L (4%: P=0.12) cows. Total incidences of all types of atypical patterns of ovarian activity in both Year 1 and 2 were similar (33% versus 36%; P≈0.50).

Figure 6-1: Cumulative proportions (%) of heavy- (open circles = Year 1, open squares= Year 2) and light- strain (solid circles= Year 1, solid squares= Year 2) cows that resumed luteal activity (as indicated by milk progesterone concentration >3 ng/ml) at different times postpartum (dpp) during the 2-year study period.

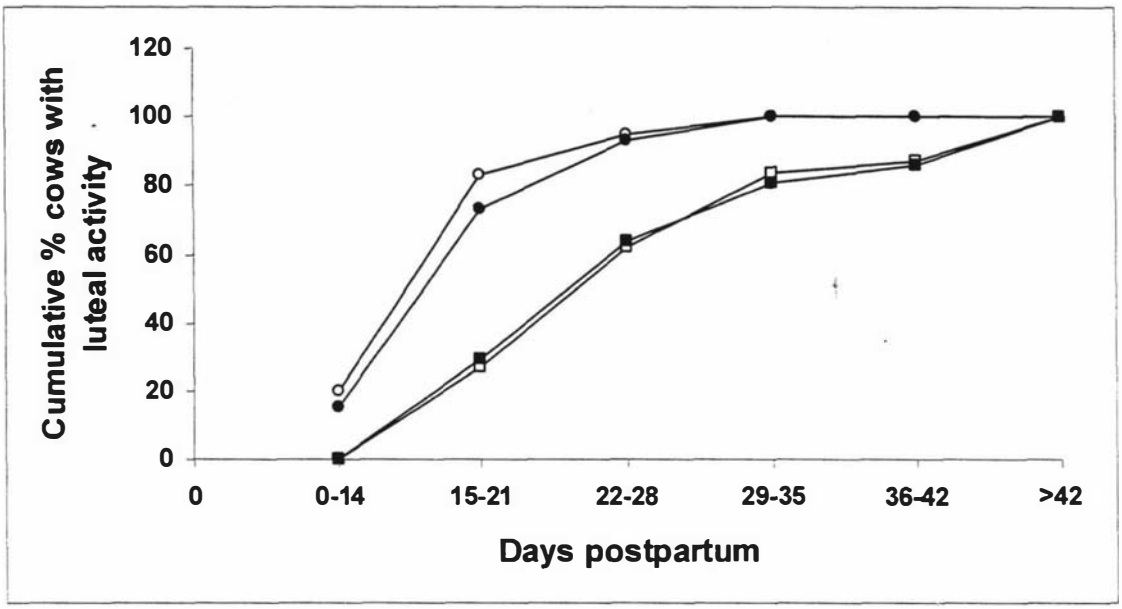


Figure 6-2: Milk progesterone concentrations before behavioural oestrus (Day 0) in light- and heavy-strain cows: Year 2 (2000).

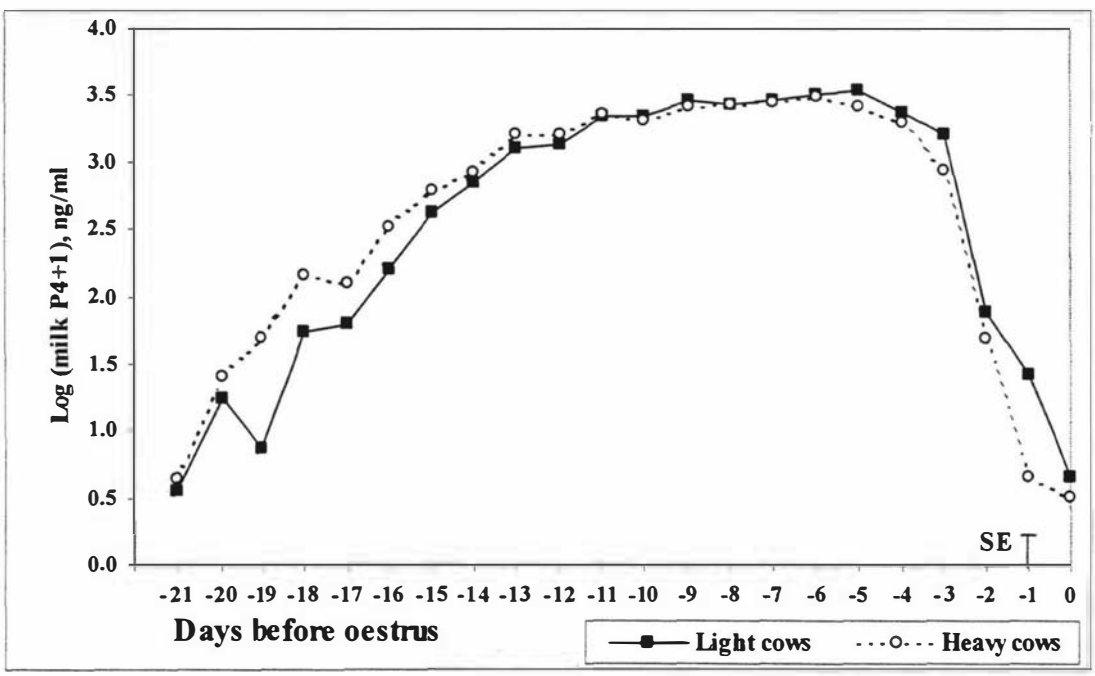


Table 6-6: The incidence of atypical ovarian patterns (%) in light- and heavy-strain cows during two years of study.

Ovarian pattern	Year 1		Year 2		Combined	
	H (n=37)	L (n=41)	H (n=41)	L (n=40)	H (n=78)	L (n=81)
Normal (%)	68	66	63	65	65	64
DOI (%)	14	13	0	2	6	7
Total DOI (%)	13 (10/78) [a]		1 (1/81) [b]		x	
DOII (%)	0	2	5	8	3	5
PCLI (%)	3	7	12	10	8	9
PCLII (%)	8	2	12	5	10	4
LEM (%)	8	10	7	10	8	10
Total (%)	33 (26/78)		36 (29/81)		35	36

[a, b] Means in the same row with different superscripts are different ($P<0.05$).

DOI =Delayed ovulation type I occurs after calving.

DOII =Delayed ovulation type II occurs after resumption of ovarian cyclicity.

PCLI = Persistent CL type I occurs after calving.

PCLII = Persistent CL type II occurs after resumption of ovarian cyclicity.

LEM=Late embryo mortality.

Figure 6-3: Milk progesterone profiles during the first 7 days after insemination.

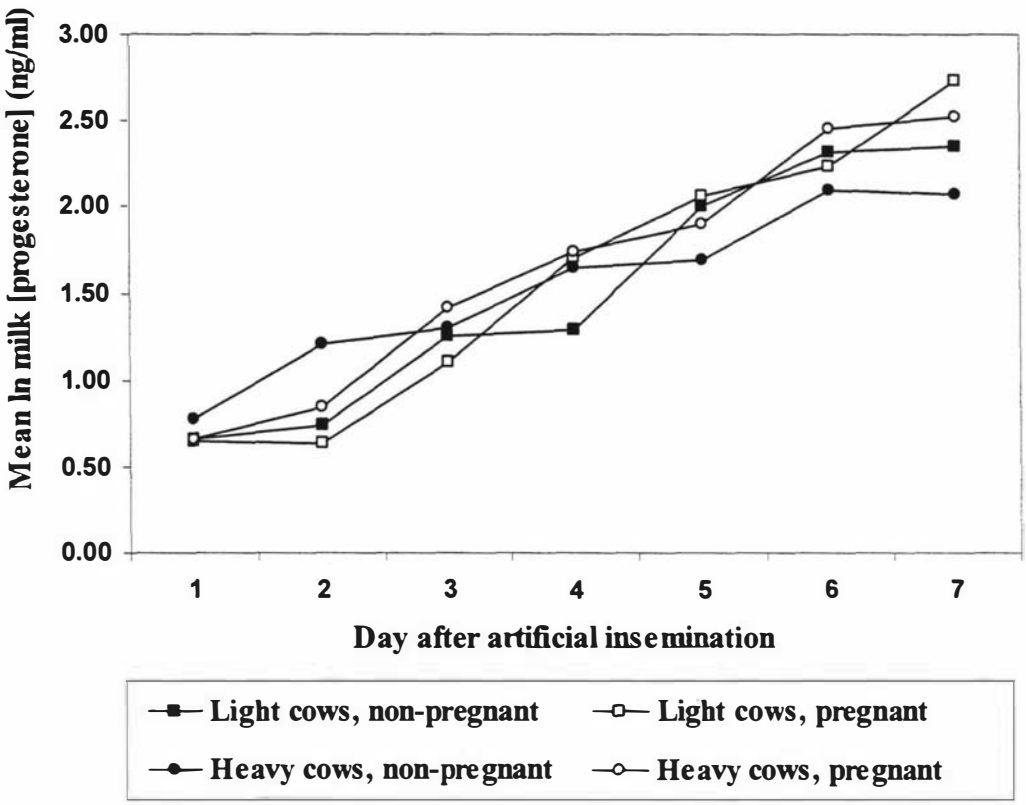


Figure 6-4: Milk progesterone profiles between Days 8 and 16 after first insemination.

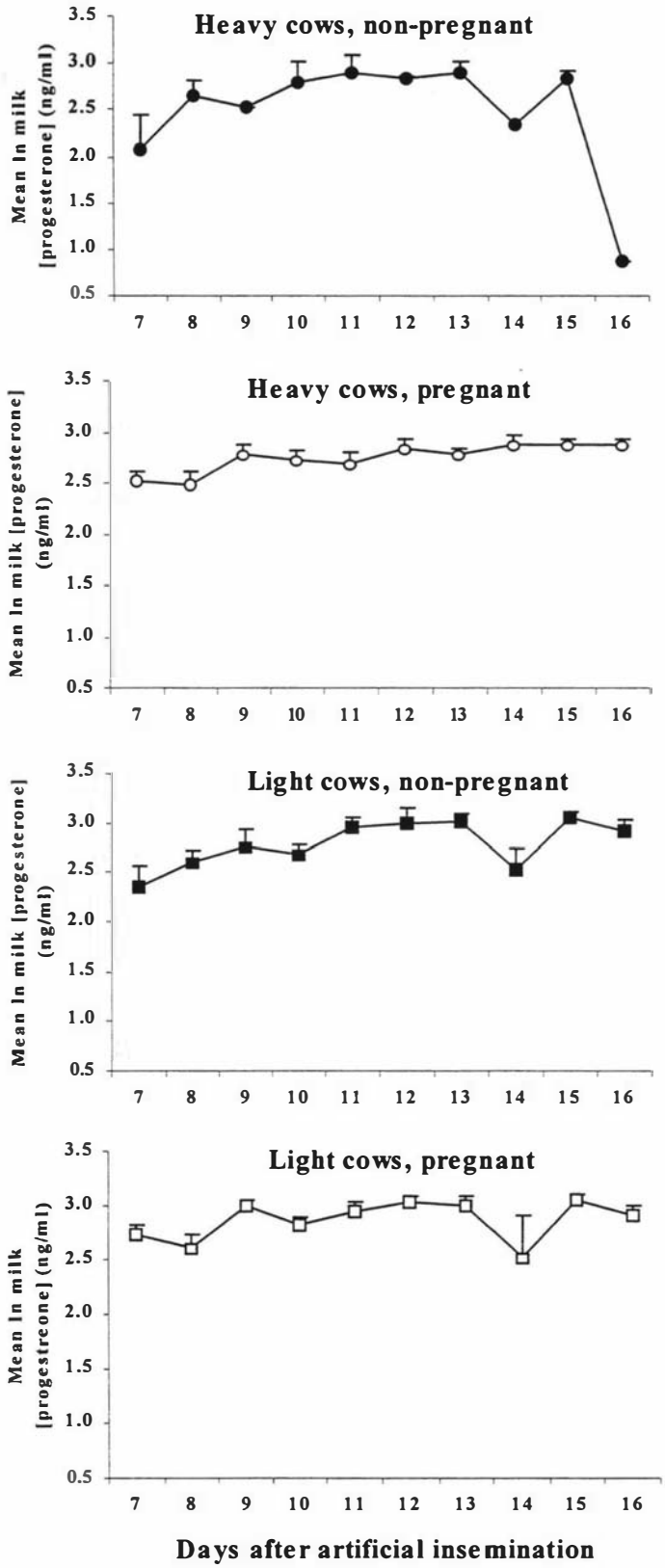
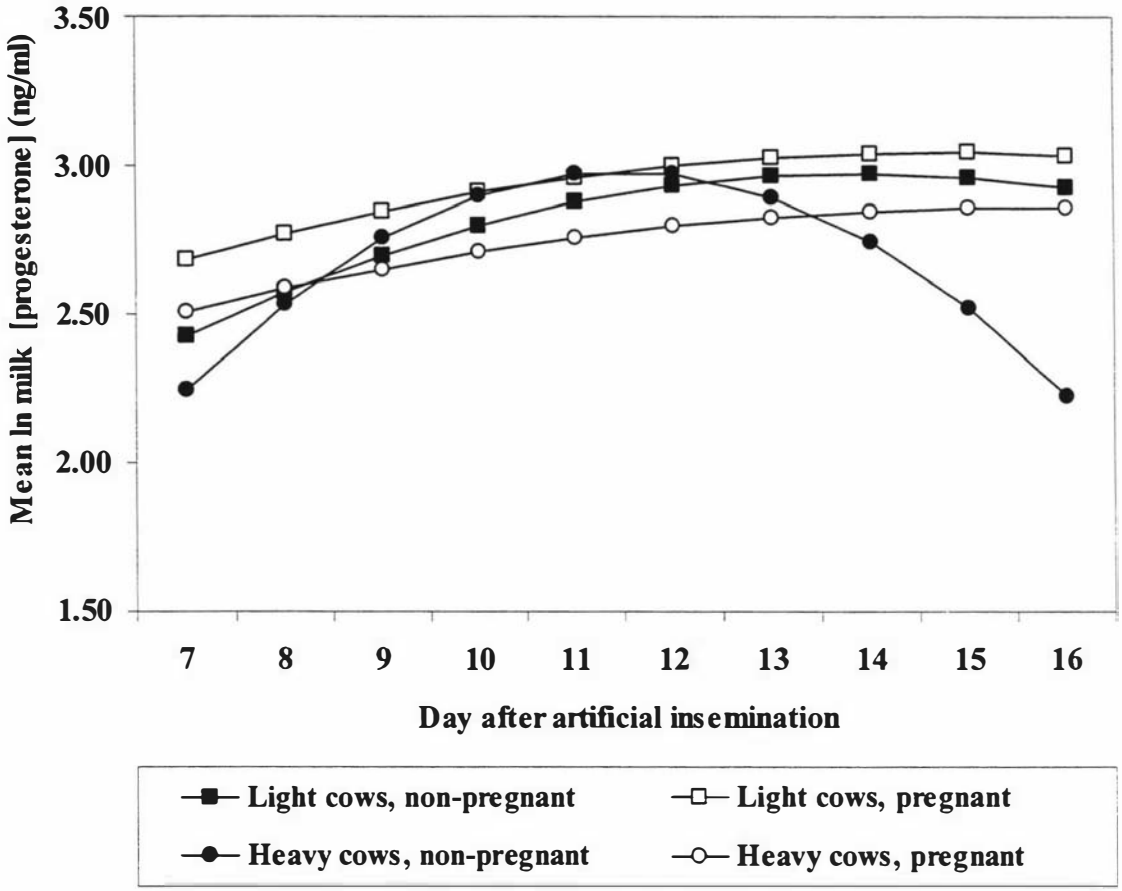


Figure 6-5: Fitted regression lines (quadratic) for milk progesterone data between Days 7 and 16 after first insemination.



Luteal activity and pregnancy outcomes

Progesterone data from normal oestrous cycles were identical between years, so these data were combined for both years for further analysis. Results from the analysis of milk progesterone concentration pattern during the oestrous cycle of H and L cows showed that there was no difference between strains in both years. The pattern of progesterone concentration in $\log_e$ form during the oestrous cycle of H and L cows in Year 2 is presented in Figure 6-2.

Milk progesterone concentrations were identical in all cows between Days 1 (day of AI=Day 0) and 7 (Figure 6-3) whether or not they conceived. Thereafter, concentrations in non-pregnant H (HNP) were significantly ($P<0.05$) lower than pregnant H (HP) cows on Day 14 and were also lower in HNP cows than in all other animals on Day 16 ($P<0.05$) (Figures 6-4a to 6-4d). Regression analysis (Figure 6-5) showed that concentrations in HP, pregnant L (LP) and non-pregnant L (LNP) cows were equally well described by linear and quadratic regressions:

Linear regressions:

HP cows: $\log_e$ milk progesterone (ng/ml+1) = $2.214+0.0525t$

LP cows: $\log_e$ milk progesterone (ng/ml+1) = $2.517+0.0338t$

LNP cows: $\log_e$ milk progesterone (ng/ml+1) = $2.288+0.0400t$

where t=time in days after insemination

By contrast, in HNP cows, the quadratic expression was a significantly ($P<0.001$) better description of the data than the linear model.

Quadratic regression:

$$\log_e \text{ milk progesterone (ng/ml+1)} = -1.89+0.849t-0.0371t^2$$

Differentiation of this fitted quadratic equation showed that the point of inflection occurred on Day 11 (11.4).

For comparison, the linear regression for HNP cows was:

$$\log_e \text{ milk progesterone (ng/ml+1)} = 2.621+0.0061t$$

It was found that the linear model substantially underestimated both the maximum and minimum values that were actually measured.

6.4 Discussion

Reproductive performance

The results from this study, in which both herd records and milk progesterone profiles were used to monitor reproductive status of the cows in the herd showed that many aspects of the reproductive performance of H and L cows are similar to each other. The exception to this generality was FSCR, which was consistently lower in H than L cows. Other parameters of reproductive performance were within the range of values reported from the NZ national database (Hayes, 1996 and Xu and Burton, 2000), from the InCalf study (a large-scale Australian survey; Morton, 2000) and a UK trial (Darwash *et al.*, 1997).

It was interesting to note that the difference in FSCR between H and L cows was significant in Year 2 (42.5 versus 65.0 %, for H and L cows respectively), but not in Year 1. This reflects the overall variation between FSCRs that have been achieved over the years in H and L cows, both in the present study and in the work of Laborde *et al.* (1998b). Over the eight years for which the H and L strains have been studied, FSCR has been relatively constant in the L strain, at about 60%, whereas, in the H strain, it has varied from minima of about 40% (as in Year 2 of the present study), through to figures that are indistinguishable from the L strain cows (55-60%).

Laborde *et al* (1998b) and the present trial have provided at least some evidence that these year-to-year variations in performance in the H strain are closely linked to the availability of feed in different seasons. Indeed, the extent to which differences in FSCR between strains within years represent differences in nutritional status between years is of some importance, even though it was not the main purpose of this thesis to study nutritional-reproductive interactions. Firstly, the fact that, in Year 1, the changes in liveweight (LW) and BCS of the cows were small, and were indistinguishable between strains, implies that the strains were in similar nutritional status, which is compatible with the similar reproductive performance of the two strains in that year. However, in Year 2, there were differences in patterns of LW change, for, whilst similar numbers of cows in each strain lost LW, the H cows did lose more LW than L cows. In other words, H cows were, overall, in more severe negative energy balance (NEB) than L cows.

The level of NEB experienced by high producing cows is itself controlled by many factors, including BCS at calving, dry matter intake (including feed quality and feed availability) and the genetic potential of the cow for both milk production and for tissue

mobilisation to meet the demands of production (Butler, 2000). Within the pastoral dairying systems of Australia and New Zealand, cows generally calve in relatively lower BCS [4.5- 5.0 (0-10 scale)] and produce less milk than cows in North America, so lose less LW during early lactation (Abe *et al.*, 1994; Mackel *et al.*, 1996; Morton, 2000). Therefore, the depth and duration of NEB period may be less severe in these animals (Westwood *et al.*, 2002).

NEB, both in terms of depth and duration, is of some importance to reproductive outcomes, as shown in many studies (Butler *et al.*, 1981; Butler and Smith, 1989; Senatore *et al.*, 1996). One study of particular interest in that of Fullkerson *et al.* (2001) with pasture-fed cows, who showed that there is a positive relationship between LW changes over the four weeks preceding the start of the mating period and pregnancy rate. Cows that became pregnant had gained, on average, 0.95 kg per cow, whilst non-pregnant cows had lost 4 kg per cow. In other studies, it has been shown that it is unlikely that the first ovulation after calving will occur until NEB reaches its nadir and starts to return to a positive value. For example, in the study of Butler *et al.* (1981), the mean EB during the first 20 days of lactation was inversely related to the interval from calving to first ovulation.

Nonetheless, there is conflicting evidence about the effect of selection for differences in mature LW or milk production on C-to-Ov and C-to-FH intervals. In terms of the studies upon H and L strain cows, Laborde *et al.* (1998b) found that H cows had significantly shorter C-to-Ov interval than L cows, although no such difference was found in the present study. However, McNaughton *et al.* (2003) also found longer intervals between calving and the resumption of ovarian cyclicity in NZ than OS cows. Conversely, neither the results of the present study, nor those of Laborde *et al.* (1998b), nor those of McNaughton *et al.* (2003), support the contention of Harrison *et al.* (1989), who suggested that a delay in resumption of oestrous cyclicity is responsible for the delay in the time from calving to conception. Nor does it support the contention that cows with higher proportions of overseas genetics have an impaired ability to initiate ovarian and oestrus cycles before the planned start of mating (as reported by Harris *et al.*, 2001).

In terms of the present study, these studies provide strong evidence that, in seasons in which H cows lost significant amounts of LW during early lactation, they were at risk of failure to conceive. This would probably have contributed to the lower FSCR in H than L cows in Year 2. However, since such lowered FSCR occurred in the absence of a

significant difference in either the time of resumption of oestrous cycles or in the incidence of atypical ovarian activity (see below), it appears that any effect of NEB upon conception rate in H cows is independent upon the patterns of ovarian activity before the start of mating.

Progesterone profile analysis

Analysis of milk progesterone profiles provided information on many aspects of reproductive activity in the cows in this trial, including the timing of resumption of oestrous cycles, the relation between ovulation and oestrus behaviour, incidences of abnormal ovarian cycles and changes of progesterone concentrations during the luteal phase.

As expected, the first ovulation was not accompanied by an observable behavioural oestrus in the majority of cows. Thus, only in about 15-20% of first ovulations was a behavioural oestrus recorded. This was reflected in longer intervals from calving to first observed oestrus than from calving to first ovulation, and from calving to milk progesterone rise to >3ng/ml intervals in both years of the study. Curiously, however, in the study of Laborde *et* al. (1989b), a higher proportion of cows displayed oestrus at their first ovulation (H=30%, L=40%) than in the present study. The reason for these differences is unclear, but, examination of records later in the breeding season, in which a high level of correlation between the herd manager's observations of oestrus and actual ovulations (as determined by nadirs of progesterone concentrations), means that it is unlikely that it was to be due to the herd manager's ability to detect oestrus efficiently.

The proportions of short cycles and the incidence of atypical ovarian cycles found in this study were generally in line with those previously reported. For example, Pope *et al.* (1969), Savio *et al.* (1990a) and Eldon (1991) have shown that the first cycle after calving is generally of short duration, but the second and subsequent cycles are of normal length. Likewise, Lamming and Darwash (1998) and Royal *et al.* (2000b) reported incidences of atypical patterns of ovarian activity that were very similar to those observed after calving in H and L cows in the present study The total incidence of abnormal patterns of ovarian activity was similar both between strains and years, although, in Year 1, but not in Year 2, a higher proportion of cows had Type I delayed ovulations (DOI). This higher incidence in Year 1 may, in turn, be related to the later resumption of oestrous cycles (as reflected by the longer interval from calving to first progesterone >3 ng/ml) in Year 1 than in Year 2,

for Lamming and Darwash (1998) and Lamming and Royal (2001) observed that cows with DOI showed longer intervals to the re-establishment of oestrous cycles than did normal cows.

Whether the incidence of atypical ovarian cycles in the present study contributes to the lower FSCR in H than L cows is, however, debateable. On one hand, Lamming and Darwash (1998) demonstrated that atypical ovarian activity after calving is detrimental to reproductive parameters such as the intervals from calving to first AI and to conception, submission rate, FSCR, total conception rate and numbers of service per conception. However, the data set from the present study is not big enough to make a meaningful analysis of relationships between atypical ovarian activity and reproductive outcomes; nor, in terms of the main interest of this thesis, does the lack of significant differences in incidences of such activity between strains, provide any evidence that it contributes to the low FSCR of H cows. On the other hand, delayed luteolysis in the period before the planned start of mating (PCLII) might contribute to lower conception rates (Lamming and Royal, 2001), but, again, in the context of the present study, a lack of difference in the incidences of this abnormality between strains makes it unlikely to have contributed to lower FSCR in H than L cows.

Progesterone concentration and pregnancy outcomes

Whether the pattern of progesterone concentrations during the luteal phase before and after insemination affects the chance of conception in the cow has been one of the more contentious aspects of the endocrinology of bovine pregnancy. In the present study, milk progesterone concentrations were indistinguishable in H and L cows for the first 7 days after oestrus and, although there was a divergence of concentrations in the mid-luteal phase, significant differences of concentration did not occur until the peri-luteolytic period. In the literature, several studies have reported higher progesterone concentrations in pregnant than non-pregnant cows from around Day 10 after insemination (Garverick *et al.*, 1971; Erb *et al.*, 1976; Lukaszewska and Hansel, 1980; Thompson *et al.*, 1980; Shelton *et al.*, 1990; Larson *et al.*, 1997; Starbuck *et al.*, 2001). Other studies have found that the divergence occurs later, between Days 10 and 16 (Pope *et al.*, 1969; Henricks *et al.*, 1972; Edgerton and Hafs, 1973; Bulman and Lamming, 1978; Bloomfield *et al.*, 1986; Diskin and Sreenan, 1986; Lamming *et al.*, 1989; Parkinson and Lamming, 1990). Yet other studies (e.g. Folman *et al.*, 1973; Roche *et al.*, 1981; Sreenan and Diskin, 1983; Geisert *et al.*, 1988) found that divergence of progesterone concentrations does not occur

until late in the cycle, somewhere between Day 16 and the definitive onset of luteolysis on Days 18 or 19. The reason behind the discrepancy between these many trials is not clear, except that there must be a wide range of factors involved in maintenance of pregnancy in addition to the progesterone concentration after insemination *per se*. Nonetheless, the latter studies are in closest agreement with the present investigation, although, even in the present trial there was evidence for a pre-luteolytic limitation of progesterone secretion from the corpus luteum of some non-pregnant animals. Evidence for such a contention comes from the quadratic regression analysis of progesterone profiles of pregnant and non-pregnant cows, in which it was found that inflection point of the progesterone profile in H non pregnant (HNP) cows occurred on Day 11. If so, it would indicate that the actual divergence of progesterone concentration in HNP cows occurred earlier than that reported by Lamming *et al.* (1989) and Parkinson and Lamming (1990), even though concentrations were not significantly different from other animals until much later in the luteal phase.

The significance of different timings of divergence of progesterone concentrations is of some importance to understanding its significance in terms of the probability of animals becoming pregnant. For example, it has been suggested that the rate of increase in CL size and progesterone secretion (Donaldson and Hansel, 1965a; Mann *et al.*, 2001) in the first third of the luteal phase may be determined by differences and/or inadequacies in the pre-ovulatory follicle that gave rise to them (Maurer and Echternkamp, 1982; Wilmut *et al.*, 1986). Since progesterone has major effects upon the activity of the uterus during the early luteal phase (including the suppression of motility, development of uterine glands, secretion of histotroph and, possibly, localised immunosuppression; Garrett *et al.* 1988; Mann and Lamming, 1999), rates of change of progesterone concentrations during the first 5 days of pregnancy are associated with rates of conceptus growth and development (Garrett *et al.* 1988; Larson *et al.*, 1997). In other words, high progesterone concentrations during very early pregnancy permit the development of a histotrophic uterine environment which is suitable for optimal embryonic development (Thatcher *et al.* 1989). It is therefore of interest to note that Vasconcelos *et al.* (2001) found differences in serum progesterone concentrations (and a tendency for a difference in luteal volume) between Days 7 and 14 in cows with small ($\leq$14 mm) versus those with larger ($\geq$15 mm) pre-ovulatory follicles. In the present study, the similarity of progesterone concentrations in all groups of cows between Days 1 and 7 post ovulation does not give any real support to the notion of

follicle-driven differences in luteal activity. Nonetheless, further investigation into the follicular function is required before meaningful conclusions can be drawn from the early luteal phase progesterone data.

The causes of mid-luteal phase divergences of progesterone concentration is similarly contentious, although there are studies, such as that of Albihn *et al.* (1991) in repeat breeder heifers, who found a positive correlation between the size of the embryo and progesterone concentrations between Days 6 and 11 after oestrus, which suggests the existence of causal relationships between progesterone concentrations and embryonic survival. One school of thought is that embryo-derived luteotrophins are responsible for the divergence of concentrations. LH is, of course, the primary luteotrophic factor in cattle (Milvae *et al.*, 1996), but other factors, such as oestrogens, prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2), which are produced by the conceptus and/or the gravid uterus (Milvae and Hansel, 1983; Shelton *et al.*, 1990; Wilson *et al.*, 1992; Weems *et al.*, 1998), may also affect luteal function. Injection of PGI_2 directly into the bovine corpus luteum significantly increased the peripheral plasma progesterone concentration (Milvae and Hansel, 1980). The combination between E2 and PGE_2 is even able to prevent luteolysis in cows (Reynolds *et al.*, 1983). Indirect effects of PGE_2 on the corpus luteum may be related to increased ovarian blood flow, with a subsequent increase in progesterone secretion (Ford and Chenault, 1981; Reynolds *et al.*, 1983; Parkinson *et al.*, 1991; Wilson *et al.*, 1992). The extent to which such factors affected luteal function in the animals in the present trial is unclear, yet the data from the quadratic regression analysis indicates that the corpora lutea of HNP cows were significantly different in their function from other animals from as early as the mid luteal phase. Whether these differences relate to differences of embryonic luteotrophic action, or to some other factors, is beyond the scope of the present investigation.

Perhaps the most interesting observation of the present study is that, in HNP cows, progesterone concentrations started to decline before those of LNP cows. Both when measured forwards from the time of ovulation and when assessed retrospectively from the time of the second ovulation (i.e. the one at the end of the first insemination cycle), luteolysis in HNP cows started on Day 15 or 16, 1-2 days earlier than in LNP cows. This is in addition to the previously mentioned observation that regression analysis of progesterone concentrations shows that maximal concentrations occur in HNP cows on Day 16.

The reasons for these differences in the behaviour of the corpora lutea of non-pregnant H and L cows in the peri-luteolytic period are of some importance to assessing their significance in terms of the different FSCRs between the strains. Firstly, Hansel (1981) suggested that the death of the blastocyst in the very earliest stage of pregnancy may cause early luteal regression. Whether this might apply in HNP cows is unclear, however, since the present study did not collect direct data on early embryonic failure. The only data on embryonic mortality gathered in the present study was inferential data on late embryonic mortality, based upon patterns of progesterone concentrations. Such data showed that LEM in this study did not differ between strains and, moreover, was lower than those reported in other studies (Butterfield and Lishman, 1990; Darwash *et al.*, 1997). Since studies of repeat breeders have shown that they are subject to both early and late embryonic mortality (Ayalon, 1978; Sreenan and Diskin, 1986), it seems unlikely that one strain would have a higher level of early embryonic mortality than the other, since their LEM rates are indistinguishable.

Secondly, the decline in milk progesterone concentrations on Days 15-16 could be considered to represent a failure of the antiluteolytic signal of the maternal recognition of pregnancy. This suggestion also raises a further difficult question; namely, does failure of the embryo lead to failure of the antilutoelytic signal, or does failure of the corpus luteum cause the loss of the embryo.

Evidence certainly exists in the literature to support the notion that inadequate progesterone concentrations can lead to embryonic failure. Albihn *et al.* (1991) showed that low plasma progesterone concentrations just before the critical time of embryo development and pregnancy recognition may result in pregnancy failure, whilst Shelton *et al.* (1990) suggested that lowered progesterone secretions and/or a change in ovarian steroid hormone ratios during this critical time could modify changes in the uterine environment, so that the embryo survival may be compromised. Likewise, Lamming *et al.* (1989) suggested that cows that lost an embryo early had significantly lower milk progesterone concentrations from Days 7 to 16 than in pregnant and cyclic animals. Hence, it is possible that early embryo mortality in HNP cows could have occurred before the time of maternal recognition of pregnancy, although, again, the present study has not directly investigated the time of embryo loss.

However, it is also possible that the early decline in progesterone concentrations represents a failure of maternal recognition of pregnancy. Maternal recognition of

pregnancy, as defined by the critical period when the presence of an embryo is necessary to prevent luteal regression occurs on Day 16 (Northey and French, 1980; Humblot and Dalla Porta, 1984). At this time, the protein, interferon-tau (IFN-τ), is secreted from the embryonic trophoblast, which inhibits luteolytic $PGF_{2\alpha}$ release from the uterus by inhibiting the development of oxytocin receptors on the endometrium (Mann *et al.,* 1999; Robinson *et al.,* 1999). This results in luteal maintenance and sustains progesterone concentrations thereafter (Thatcher *et al.*, 2001). Normal establishment of pregnancy is, therefore, dependent upon the ability of the embryo to develop normally (especially in terms of the time and rate of embryonic expansion into a filamentous blastocyst) and secrete sufficient amounts of IFN-τ to prevent luteolysis

It could therefore be argued that the early decline of progesterone concentrations in the HNP cows in the present study represents a failure of maternal recognition of pregnancy. However, the timing of the first decline of progesterone concentrations, on Day 15, argues against this suggestion, since this is actually before the time of the antiluteolytic signal. Consequently, it is more likely that it actually represents a premature initiation of luteolysis before the normal embryonic interferon message can be delivered. Certainly, there is good evidence that the first luteal phase is of short duration and is terminated at around Day 12 by premature luteolysis (Mann *et al.*, 1983; Inskeep, 1995). Even in 'normal' corpora lutea of non-pregnant cows, there is evidence that early luteal degeneration can begin as early as Day 13-14, with changes in luteal cell morphology and population, perivascular lymphocytic infiltration and fibroblast proliferation (Hafs and Armstrong, 1968; Lobel and Levy, 1968; Parkinson *et al.*, 1994). In pregnant cows, by contrast, such changes do not occur or are very much less pronounced (Parkinson *et al.*, 1994). Such pre-luteolytic degenerative changes of the corpus luteum would be compatible with the finding of the present study that maximum progesterone concentrations (as defined by quadratic regression analysis) occurred well before the definitive onset of luteolysis. More significantly, no such premature limitation of luteal function occurred in the LNP cows. Thus, in all groups of cows (HP, LNP and LP) other than the HNP animals, there was no decline in luteal function during the first 16 days after insemination. In other words, the decline in progesterone concentrations in LNP cows did not occur before Day 16 after insemination, indicating that no such decline occurs until the end of the classical luteolysis process and, more importantly, indicating a fundamental difference in luteal activity between the two strains.

If this is the case, it is likely that the lower progesterone concentrations of HNP than other cows are probably due to the inhibitory effect of $PGF_{2\alpha}$ on their corpora lutea. The main evidence to support this contention from the present study is the lack of any difference in the progesterone concentrations of LNP, LP and HP cows before the onset of definitive luteolysis. That is, if differences between the corpora lutea of cyclic and pregnant cows were due to a luteotrophic effect of pregnancy, HP and LP cows would have higher progesterone concentrations than LNP cows during the middle part of the luteal phase (perhaps between Day 11 or 12 and Day 15 or 16). This was certainly the conclusion of Parkinson and Lamming (1990) and could be explained in terms of the observations of Hu *et al.* (1990), who showed that the subnormal CL shows preferential production of $PGF_{2\alpha}$ resulting in a shift in the ratios of luteotrophin:luteolysin that affect it in the mid luteal phase. Moreover, during the postpartum period, the short life of corpora lutea may not be due to the absence of luteotropic support, but to the action of a luteolytic mechanism (Duby *et al.*, 1985).

Therefore, declining progesterone concentrations after mating may be due to: 1) failure in maternal pregnancy recognition or 2) premature luteolysis (CL demise). However, in the present study low progesterone concentrations in HNP cows on Day 15 is, therefore, too early to be the result of classic luteolysis. This indicates that premature luteolysis has probably occurred in these cows. The cause of premature luteolysis in the short luteal phases is related to the premature release of $PGF_{2\alpha}$ from the uterus accompanied by earlier elevated level of oxytocin receptors (OTR) or increased oxytocin-induced release of $PGF_{2\alpha}$ (Zollers *et al.*, 1989; 1993; Garverick *et al.*, 1992; Mann *et al.*, 1998). Perhaps the same situation occurred in the H cows in the present trial

Higher bacterial counts were found in the uteri of H (3.4×10^6) compared with L (1.2×10^6) cows (F. Daoud, personal communication). It might be argued, by analogy with the mare (in which mild uterine infection is associated with premature release of $PGF_{2\alpha}$, premature luteolysis, a rapid decline in progesterone and an early return to oestrus; Peter and Bosu, 1987; Pycock, 2001), that a similar process might take place in cows with mild, or residual endometrial infections, contributing to their premature luteolysis. On the other hand, uterine infection in the cow is more commonly associated with luteal retention, so perhaps the effect of infection has to be considered in terms of its well-recognised contribution to the repeat breeder phenomenon. Infection by anaerobic, tissue-damaging organisms impairs the function of the endometrium (Gonzalez, 1984) and results in

inadequate growth and development of the embryo during the critical period leading up to the maternal recognition of pregnancy. Therefore, an inadequate embryonic signal is delivered to the mother and, subsequently, premature luteolysis ensues. It is, therefore, plausible that the relatively high level of bacterial contamination during the postpartum period in H cows might be a potential factor affecting their conception rates.

Finally, it should also be noted that the time at which oestrous cycles recommence after calving may also affect conception rates. Traditionally, it has been considered that an early resumption of oestrous cycles is beneficial (Thatcher and Wilcox, 1973; Macmillan and Clayton, 1980; Holt *et al.*, 1989; Darwash *et al.*, 1997), but Smith *et al.* (1998) showed a detrimental impact of early resumption of luteal activity after calving on pregnancy rates in multiparous cows. Likewise, in the work of Laborde *et al.* (1998b) it was evident that the H cows which resumed a luteal activity earlier after calving showed lower first service conception rates than animals which started cycling at a longer interval after calving. The observation reported by Laborde *et al.* (1998b) appears to be in consistent with above contention made by Smith *et al.* (1998).

6.5 Conclusion

Heavy cows had lower values for FSCR in all three years, but the differences were significant only for Year 2 and for all three years combined. These data and others data showed that variations in FSCR between years were larger in H cows than in L cows, with some evidence that this may be linked to the H cows being more sensitive to feed restrictions. There was no difference between strains in the interval from calving to first ovulation and calving to first behavioural oestrus. The higher incidence of PCLII in H cows may contribute to their lower fertility compared with L cows. H cows which fail to conceive show significant decreases in milk progesterone concentrations well before the expected time of luteolysis. Such a decline in concentrations could be due to luteal inadequacy or a premature induction of luteolysis. It remains to be clarified whether this early decline in luteal activity in H cows stems from the follicular characteristics while entering the ovulatory stage, or from the alterations in CL function and/or responsiveness to the uterine luteolytic signal after ovulation as a result of genetic selection for liveweight.

Nevertheless, it is clear that the luteal function of HNP cows is remarkably different from that of all other groups. The finding that in HNP cows progesterone concentrations start to

decline on Day 15, nearly 3 days before this occurs in LNP cows is clearly significant in terms of the animals' ability to sustain a pregnancy. Whilst it is questionable as to whether the cause of this early decline in progesterone is an inadequate follicle as its precursor, a premature luteolytic event, the effects of chronic uterine infection or some failure of embryo-maternal interaction, the significance of this observation, in terms of observed reproductive outcomes, is substantial.

7 Chapter 7- Experiment 5: Follicular dynamics in dairy cows selected for heavy or light mature bodyweight during the postpartum anoestrous period and the first oestrous cycle

7.1 Introduction

Analysis of reproductive performance data over a 2-year period in Experiment 4 (Chapter 6) showed that heavy strain (H) cows have a lower first service conception rate than light strain (L) cows. These results confirm earlier findings of García-Muñiz (1998) and Laborde *et al.* (1998b) who showed similar trends in reproductive performance between the strains over an 8-year period (see Appendix 2-1). The difference in first service conception rate is unlikely to be due to different intervals from calving to conception (Experiment 4), or to differences in the function of the hypothalamo-pituitary axis between strains (Experiments 1-3). However, the results of Experiment 4 suggest that differences in luteal activity between the two strains might explain the difference in conception rate. Since it has been postulated that both the activity of the CL and conception rates are associated with the quality of follicle from which it originated (Maurer and Echternkamp, 1985: Burke *et al*, 1996). Such results may be of relevance to the present study, since Laborde (1998b) found differences in size of the pre-ovulatory follicle and CL between H and L cows after oestrus synchronisation. Thus, it is possible that genetic selection for mature bodyweight may have modified the dynamics of ovarian follicular activity during the postpartum period.

Postpartum ovarian activity and follicular dynamics of dairy cows can be monitored daily with a noninvasive ultrasound imaging technique (Rajamahendran and Taylor, 1990; McDougall *et al.*, 1995a). The emergence of the first postpartum follicular wave occurs after FSH concentrations have been elevated for 5-14 days (Beam and Butler, 1997) and the first single, dominant follicle (DF) emerges between Days 10 and 30 after calving (Rajamaendran and Taylor, 1990; Savio *et al.*, 1990b). Under grazing conditions, McDougall *et al.* (1995a) found that the first ovulation occurs between 13 to 93 days after calving, after one to nine follicular waves. The process of ovarian follicular development after calving is under the control of both gonadotrophins and various autocrine and paracrine factors (Darwash *et al.*, 1999).

Limited information exists on ovarian follicular dynamics of pasture-fed cows during the postpartum period. No data exist on the associations between systemic FSH concentrations and ovarian follicular dynamics in H and L cows during the period from calving to the first ovulation.

Therefore, the purpose of this study was to characterise ovarian follicular growth and development, and their association with FSH concentrations in H and L cows between calving and first ovulation. The hypothesis of the study is that differences in these parameters between strains may contribute to the differences in ovarian and luteal function (Experiment 4) and, hence, in conception rate, between these strains.

7.2 Materials and methods

Animals and experimental design

Twenty-four, freshly calved Holstein Friesian dairy cows (12 each from the H and L lines), were selected from the DCRU herd. The H and L cows in this study contained 45% and 21% North American Holstein genetics, respectively, and were 3 to 8 years old. Animals were balanced between strains for parity, calving date and, as far as possible, calving condition score. Cows that had had assisted calvings or any abnormalities of the puerperium were excluded. Animals were managed in one group on mixed ryegrass and white clover pasture. Supplementary maize silage was also given when pasture quality or quantity was inadequate. BCS changes of the cows were assessed every week from calving until the end of the study. Animals were observed twice daily for oestrous behaviour (using the tail painting technique) for 40 days before the planned start of mating.

Ovarian ultrasonography

Ovaries were scanned daily from Day 7 after calving. Cows were examined daily until they had shown their first full oestrous cycle after calving (as defined by the disappearance of the largest follicle, i.e. the dominant follicle; DF) and the presence of the corpus luteum (CL) 3 days after its disappearance: Pierson and Ginther, 1984). In cows with long anoestrous periods, the examinations were terminated when either hormonal intervention (CIDR) was instigated or artificial insemination (AI) began. Cows were drafted after morning milking (5.30-8.30 a.m.) and held in a pen whilst awaiting ultrasound examination. They were put back on to pasture during the waiting period if it was likely that they would have to wait for longer than 1 h for examination. Scanning was

performed while the animals were restrained in a crush, using transrectal B-mode ultrasound (100 Falco Vet, Pie Medical Equipment BV, Netherlands) with a 7.5 MHz linear array transducer. The ultrasound examination was performed according to the protocol described by Sirois and Fortune (1988) and Lucy *et al.* (1991).

Ultrasound images were recorded on videotape for subsequent detailed examination and measurement. Videotaped images were compared with sketch maps of each ovary that were drawn at the time of scanning. The follicle identity method (Ginther, 1993) was used to indicate the position of each individual follicle more that 3 mm in diameter on the ovaries of each cow on each day of examination. All follicles that were larger than 3 mm in diameter were recorded and followed until the ovulation of the largest follicle occurred, which was firstly confirmed by the presence of a CL and, later, by plasma progesterone concentrations >1 ng/ml. The diameter of the CL was also recorded.

Follicular dynamics

For each cow, diameters of all follicles that were present on each day of examination (i.e. all follicles with a diameter of >3 mm) were plotted in relation to days after calving. Follicular data were used to classify the DF into four classes according to Lucy *et al.* (1991) and Beam and Butler (1997), Class 1 (3-5 mm), Class 2 (6-9 mm), Class 3 (10-15 mm) and Class 4 (>15mm). The synchronous growth and development of a group of follicles 3-4 mm in diameter was considered to be a follicular wave (Knopf *et al.*, 1989). The DF was defined as the largest follicle present in the ovary at each particular moment in time. Ovulation was defined as the disappearance of a large follicle followed by the presence of a CL in the same position that had previously been occupied by the DF (Savio *et al.*, 1988).

Other parameters that were examined in relation to follicular dynamics included whether there was an association between the rise in FSH concentration and the emergence of follicular waves, the interval from calving to the first follicular wave, the number of follicular waves before the first ovulation and the percentage of first DFs that were ovulated. Each of these parameters was compared between H and L cows.

Blood sampling and hormone assays

Blood samples (5 ml) were collected via coccygeal venipuncture into heparinised vacutainers prior to each ultrasound examination. Plasma was separated by centrifugation at 1000g for 20 min at 4°C and was stored at -20°C. Concentrations of FSH and

progesterone were measured in these samples, as described in Chapter 2. In this study, a significant elevation of FSH concentrations was defined by peak FSH concentrations which exceeded the mean of all values from an individual animal by two standard deviations.

Reproductive performance

The records of oestrus observations, AI and pregnancy outcomes, together with plasma progesterone concentration data of all cows were reviewed and used for calculating days from calving to first observed oestrus (C-to-FH), calving to first ovulation (C-to-Ov), calving to first plasma progesterone > 1 ng/ml (C-to-P4rise), observed oestrus to ovulation (H-to-Ov), service per conception (Ser/Con) and first service conception rate (FSCR), as defined in Chapter 7 (Table 7-1).

Plasma progesterone profiles

Plasma progesterone concentrations were plotted against days of the oestrous cycle for each animal, in order to characterise the progesterone secretion patterns. Since there were differences in the length of the oestrous cycle between individual cows, the analysis of plasma progesterone concentration data during the full oestrous cycle was undertaken using the method described in Experiment 4. Progesterone data were retrospectively normalised by using the day on which the DF ovulated (Day 0=day of ovulation) either forwards from the ovulation at the start of the luteal phase, or backwards from the ovulation at its end. Only data from cows with normal cycles (18-24 days, Wood, 1976) were used. Progesterone data were subjected to regression analysis with respect to day of the cycle within strains after logarithmic transformation ($\log_e$+1) to normalise the data. Significant differences of regression lines between strains (H and L) were tested using analysis of variance.

Luteal regression was analysed by dividing the period of decline in progesterone concentrations into two phases. The start of the initial decline was defined as the day before that on which progesterone concentrations had decreased from maximum values during that cows' luteal phase by twice the standard deviation (2SD) of all progesterone data for that cow. The start of the second phase was defined as the day before the first date on which progesterone concentrations were less than 2 ng/ml. The end of both phases was the first day on which progesterone concentrations were below 1.0 ng/ml. These data were used to calculate the rate of decline of progesterone concentrations, the day on which the

phases started and the time interval from the start of the phase until the first day in which progesterone concentrations were below 1.0 ng/ml. All of these data were statistically analysed using the Student's t-test.

Short and normal luteal phase data

Examination of the incidence of short luteal phases could not be undertaken within each strain, because there was a limited number of cycles to compare between strains. Hence, data from both strains were pooled. Short luteal phases were defined as being ≤ 10 days (Odde *et al.*, 1980). The effects of the interval from emergence (>5mm in diameter) of the DF until ovulation, maximum diameter of the DF, maximum CL diameter and ratios of maximum progesterone concentration to maximum CL diameter during the cycle upon the incidence of short luteal phase were determined. Student's t-test was used to test whether the distributions of means of these parameters were different in relation to the duration of luteal cycles.

Statistical analyses of follicular data

Data for maximum DF size, maximum CL size, progesterone and FSH were subjected to analysis of variance, with respect to strain (H or L) and time after calving, in a repeated measures model in which individual animals were nested within strain. Cycle number was used as a covariate. The effect of strain upon days to first ovulation, days to observed oestrus, successive follicular wave number between calving and first ovulation, the maximum size of the pre-ovulatory DF, FSH concentrations and numbers of follicles in each Class (Class 1, 2, 3 and 4) were also subjected to analysis of variance.

The relationships between progesterone concentration and day postpartum, CL size and progesterone concentration, FSH elevation and successive DF number associated with each follicular wave number after calving and between DF and day postpartum were tested by linear regression analysis, and tested for differences between strains. Correlation analysis was made to determine whether milk protein yield, milk protein percentage, days postpartum or FSH concentrations were related to maximum DF size or successive DF number in each strain of cows.

The Chi-square (χ^2) test was used to compare the proportions of cows with plasma progesterone concentrations less than 2 ng/ml on the different days before the ovulation of the next oestrous cycle (Days –5 to –1; where Day 0=day of second ovulation), the proportions of cows ovulating the first DF after calving, the proportions of cows with 2- or

3-waves before ovulation, and the proportions of cows having an interval from the observed oestrous activity (as indicated by tail paint removed) to ovulation ≥ 2 days, within each strain of cows

7.3 Results

Two cows in L strain (17 %) developed follicular cysts from the first DF after calving until the end of the study period. Data from these cows were excluded from analysis. Five H and four L cows had already started to resume follicular wave activity by the time the examination started, as indicating by the presence of a few follicles ≥ 5 mm on the first day of examination (Day 7 postpartum). It was difficult to locate the ovaries in the first few scans due mainly to the size of the involuting uterus and its depth within the abdomen.

The proportion of H cows that increased BCS after calving (3/12) was significantly lower than that of L cows (9/12; $P<0.05$). On average, L cows improved BCS but H cows lost BCS during the study period. However, mean differences were small (Table 7-1) with less than 1/10 of a BCS point difference between strains. BSC changes of the cows in each strain during the study period are presented in Table 7-1. Data for milk production by these two groups of cows are presented in Appendix 1-5, 1-6 and 1-7.

Reproductive outcomes

The reproductive performance and follicular data are presented in Table 7-2. There were no differences in service per conception or first service conception rate (FSCR) between the H and L cows that were used in this study. However, when data from the whole herd (2001) were analysed, the results showed that H cows tended ($P=0.07$) to have poorer first service conception rates than L cows (H=39% versus L=55%). The H cows also trended to have a higher percentage of cycles associated with silent heats before the first postpartum ovulation ($P=0.10$) and higher percentages of cycles for which the intervals from heat to ovulation were longer than 48 h ($P=0.06$) than in the L cows. Even though the percentage of cycles in the L cows that had three follicular waves before ovulation (38%, 3/8) was more than twice that in H cows (17%, 3/18), the difference failed to reach statistical significance ($P=0.12$). There were no significant differences in any other reproductive or follicular data between strains.

Follicular dynamics

Representative profiles of follicular development, FSH concentrations and progesterone concentrations are shown in Figures 7-1 (a and b). Overall, H cows had significantly ($P<0.05$) shorter intervals from calving to first DF emergence that L cows. Regression analysis confirmed this finding and also showed than H cows had significantly shorter intervals from calving to the emergence of the successive DF number 2, 4 and 5 (i.e. the second, fourth and fifth DFs after calving, but not the third or sixth). The maximum sizes of DFs in H and L cows was identical ($P=0.83$). Of the first DFs which emerged after calving, 50 percent ovulated in both strains. In H cows, the remaining DFs became atretic whereas in L cows 17% (2/12) of the first DFs became cystic before undergoing atresia. The relationship between strain and the days of emergence of DF numbers 1 to 6 after calving is shown in Table 7-3.

FSH elevation concentrations and DF development

Overall, analysis of the association between the elevation of FSH concentrations and emergence of DF showed that 80 % of FSH elevations was followed by the development of the follicular wave within 1-2 days. Mean FSH concentrations during these elevations were significantly ($P=0.02$) higher in H cows (1.08 ng/ml) than in L cows (0.85 ng/ml). Regardless of strain, FSH elevation concentrations were not related to either successive DF number or time postpartum. Regression analysis results showed that H cows had higher mean FSH elevation concentrations associated with the emergence of the successive DFs in follicular wave numbers 2, 3 and 4 after calving than L cows ($P<0.05$). FSH elevation concentrations associated with the emergence of each successive DF of the follicular numbers (1-6) after calving in H and L cows are presented in Table 7-4.

Follicle classes

No significant differences in Class 1, 2 or 3 follicles were found between H and L cows. The effect of time postpartum was significant for all classes ($P<0.01$) but neither strain nor the interaction between strain and day had significant effect on the Class 1, 2 and 3 follicles. There was a significant effect of the strain x day interaction on Class 4 follicles ($P=0.002$). The effect of strain on the possibility of developing Class 4 follicles is presented in Figure 7-2, which shows that H cows had a higher chance of having Class 4 than L cows between Days 14 and 23 after calving.

Correlations among follicular activity and milk production traits

The results of correlation analyses within strain showed that DF number was negatively related to percentage of milk protein in L cows ($r=-0.69$; $P<0.05$) and percentage of milk protein was positively related to milk yield in H ($r=0.77$; $P<0.01$) but not in L cows. There were no correlations between DF number and maximum DF diameter, or between DF data and FSH elevation concentrations. When the data from both strains were combined, the results showed a negative correlation between percentage of milk protein and days postpartum ($r=-0.43$; $P<0.05$).

Plasma progesterone profiles

After ovulation (as detected by ultrasound examination), progesterone concentrations increased to >1 ng/ml by 4.4±1.2 days (range 1-7 d) post ovulation. The patterns of plasma progesterone concentrations in H and L cows in relation to the day of ovulation (Day 0) showed that values were identical in the two strains between Days 2 and 10 after ovulation, but thereafter values started to diverge (Figures 7-3 and 7-4). Moreover, progesterone concentration in H cows began to decline earlier towards the next ovulation than in L cows (Figure 7-4).

Analysis of variance showed that the rate of progesterone decline was dependent upon the day progesterone started to decline ($P=0.005$), but the difference between strains was not significant ($P=0.43$). Furthermore, during the first phase of progesterone decline, the rate, time of onset, time of completion and number of days for progesterone to decline to basal value were not significantly different between strains ($P=0.08$). However, during the second phase of decline, progesterone started to decline 1.5 d earlier ($P=0.01$) in H than in L cows. It also took 1.2 d longer in H cows for plasma progesterone to reach basal values ($P=0.05$) than in L cows. These findings were confirmed by results indicating that the proportions of H cows that had progesterone concentrations less than 2 ng/ml three and four days before the next ovulation was significantly higher than that of L cows ($P<0.001$).

Analysis of $\log_e$ progesterone concentrations after ovulation (Day 0) during the first two postpartum oestrous cycles showed a significant effect of day ($P<0.05$) but no strain x day effect ($P>0.05$). Analysis of plasma progesterone secretion patterns between Day 2 and Day 16 after ovulation showed that progesterone concentration in H cows started to decline earlier than in L cows (Figure 7-3). Regression analysis showed that plasma

Table 7-1: Mean values for changes in body condition score in heavy (n=12) and light (n=12) cows during the first 6 weeks of lactation.

Strains of cows	Body condition score changes (mean ± SE) (unit per 6 wks)
Heavy	-0.08±0.04
Light	0.02±0.02

Table 7-2: Reproductive performance and follicular data of light- and heavy-strain cows (mean ± SE).

Parameter		Heavy (n=12)	Light (n=12)	
Proportion of North American genetics (%)		**45**	**21**	
C-to-1st follicular wave emergence (d)		**10.0±0.9**	**12.7±0.7**	**P<0.05**
C-to-1st DF >10 mm (d)		**12.1±1.2**	**15.3±1.1**	
C-to-FH (d)		**24.0±2.0**	**32.6±4.2**	
C-to-Ov (d)		**21.2±1.5**	**26.2±4.1**	
C-to- 1st P4 rise to >1ng/ml (d)		**25.5±2.0**	**32.6±4.4**	
C-to-max. size of follicle that ovulated (d)		**21.2±1.5**	**26.2±4.1**	
Max. diameter of 1st ovulated follicle (mm)		**16.5±1.6**	**16.0±1.3**	
Max. diameter of DF1 (mm)		**13.5±1.3**	**14.5±0.9**	
Max. diameter of DF2 (mm)		**17.9±1.0**	**18.6±0.9**	
Fate of 1st DF (%)	**Ovulated**	**50 (6/12)**	**50 (6/12)**	
	Became atretic	**50 (6/12)**	**33 (4/12)**	
	Became cystic	**0**	**17 (2/12)**	
% of 1st postpartum Ov with silent heat		**58 (7/12)**	**25 (3/12)**	**P=0.10**
% of cycles with H-to-Ov interval >48 h		**50 (11/22)**	**20 (2/12)**	**P=0.06**
% of cycles with 3 follicular waves		**17 (3/18)**	**38 (3/8)**	**P>0.10**
Ser/Con		**1.83**	**1.58**	
FSCR (%)		**50**	**64**	
FSCR for the whole herd (%)		**39**	**55**	**P=0.07**

Figure 7-1a: Profiles of follicular dynamics, serum FSH and progesterone concentrations during the early postpartum period in a heavy cow (number 76) * denotes ovulation.

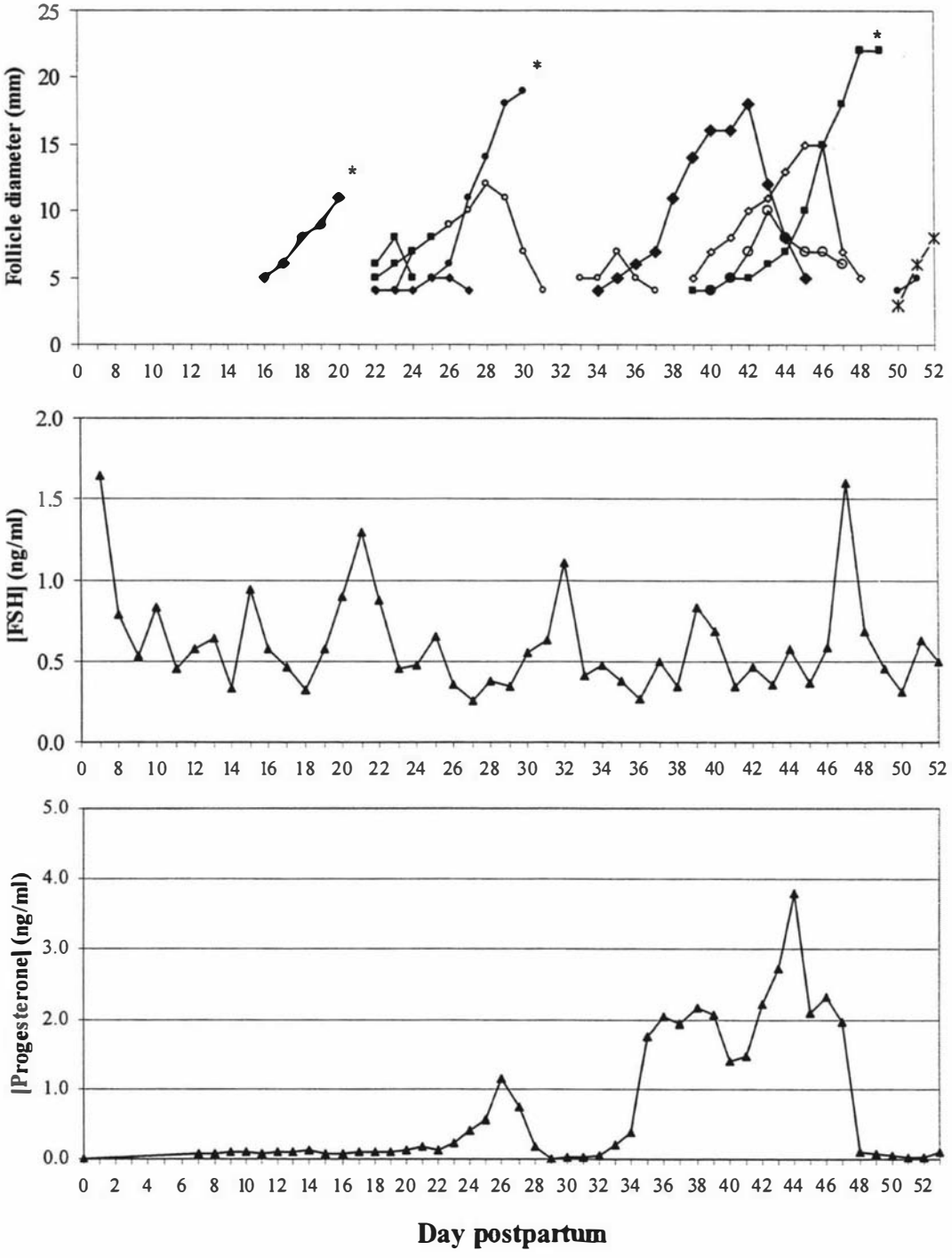


Figure 7-1b: Profiles of follicular dynamics, serum FSH and progesterone concentrations during the early postpartum period in a light cow (number 40) * denotes ovulation.

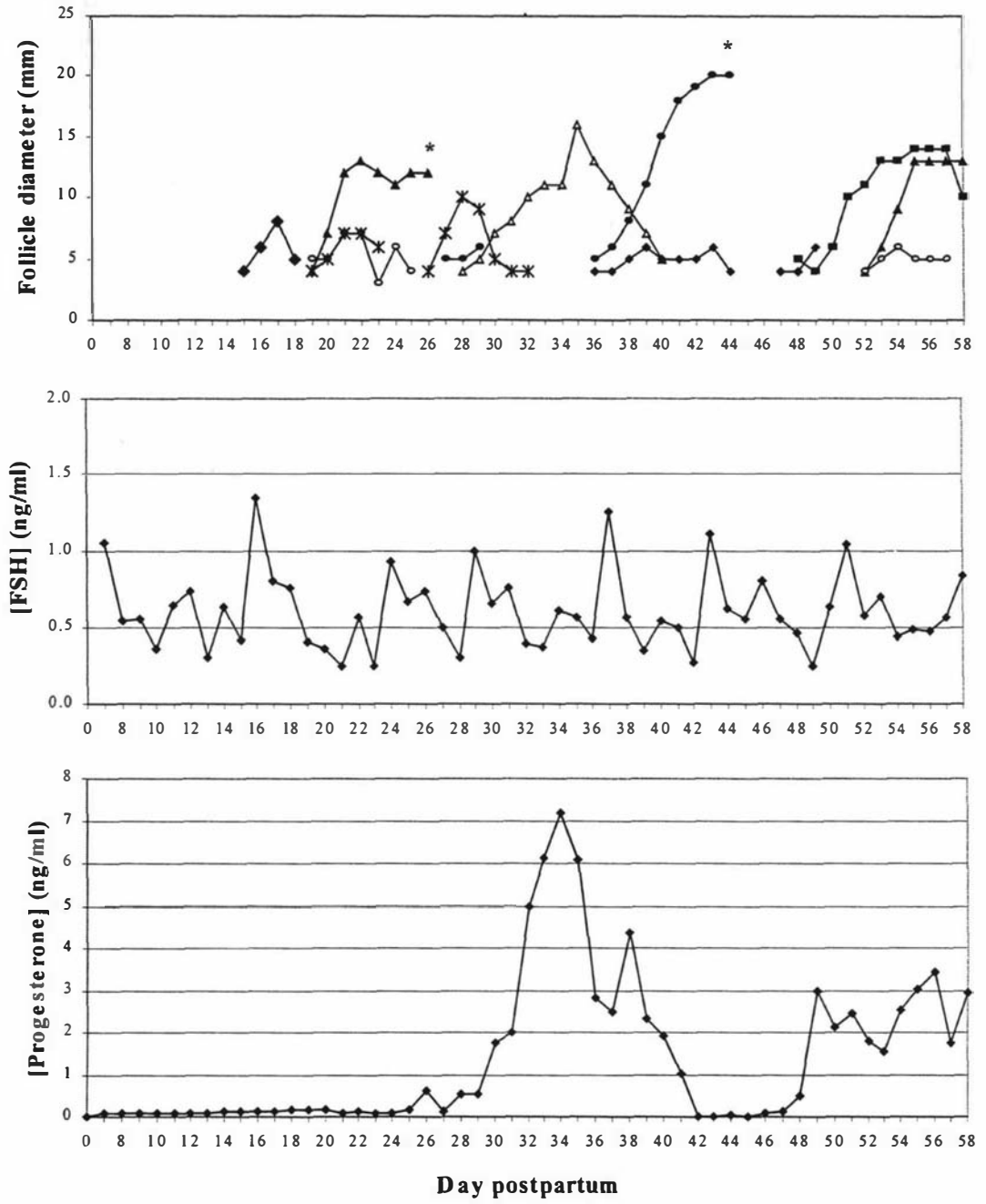


Table 7-3: Mean values for interval between the emergence of successive dominant follicle (DF) number following the follicular wave number 1 to 6 after calving in light- and heavy-strain cows (days).

Days to DF emergence DF number	Heavy (n=12)	Light (n=12)	difference	Significant level SED=1.33
1	10.0	12.7	2.7	*
2	18.3	19.8	1.5	*
3	27.8	28.8	1.1	NS
4	33.5	36.0	2.6	*
5	38.6	43.7	5.2	*
6	45.5	46.5	1.2	NS

* : P<0.05

Table 7-4: Mean values for FSH concentrations in light- and heavy-strain cows in relation to the emergence of each DF (1-6) after calving.

	FSH [ng/ml]			
DF Number	Heavy (n=12)	Light (n=12)	Difference (ng/ml)	Significance
1	0.93	0.88	0.05	NS
2	1.20	0.96	0.24	*
3	0.96	0.93	0.18	*
4	1.12	0.93	0.19	*
5	0.95	0.91	0.04	NS
6	0.90	0.88	0.02	NS

* : P<0.05

Figure 7-2: Probability of having a Class 4 follicle (diameter > 15 mm.) in heavy (n=12) and light (n=10) cows during Days 7 to 31 postpartum.

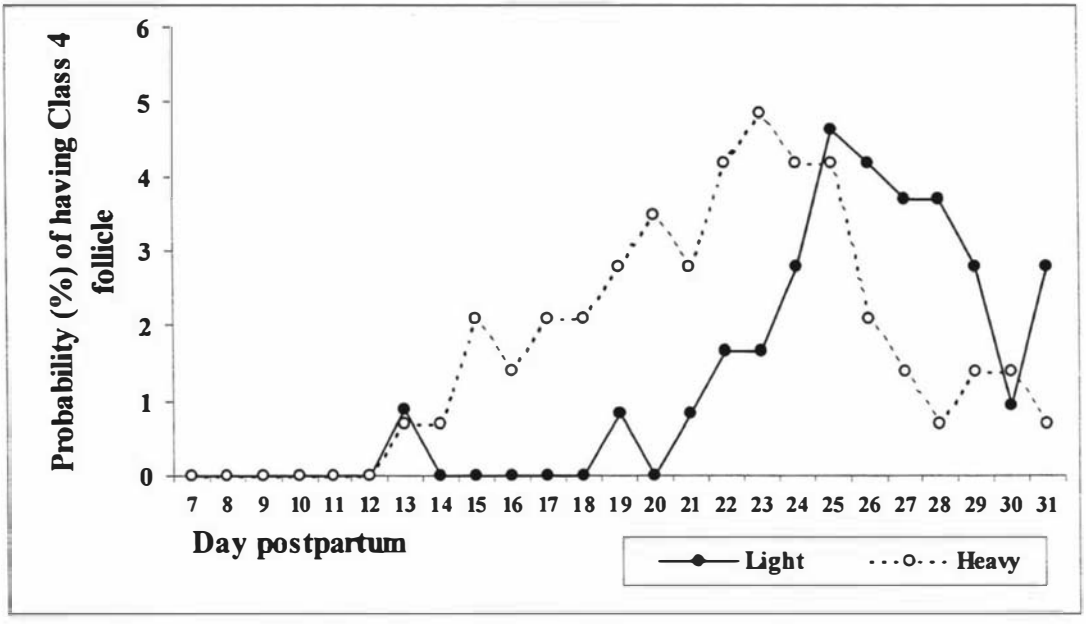
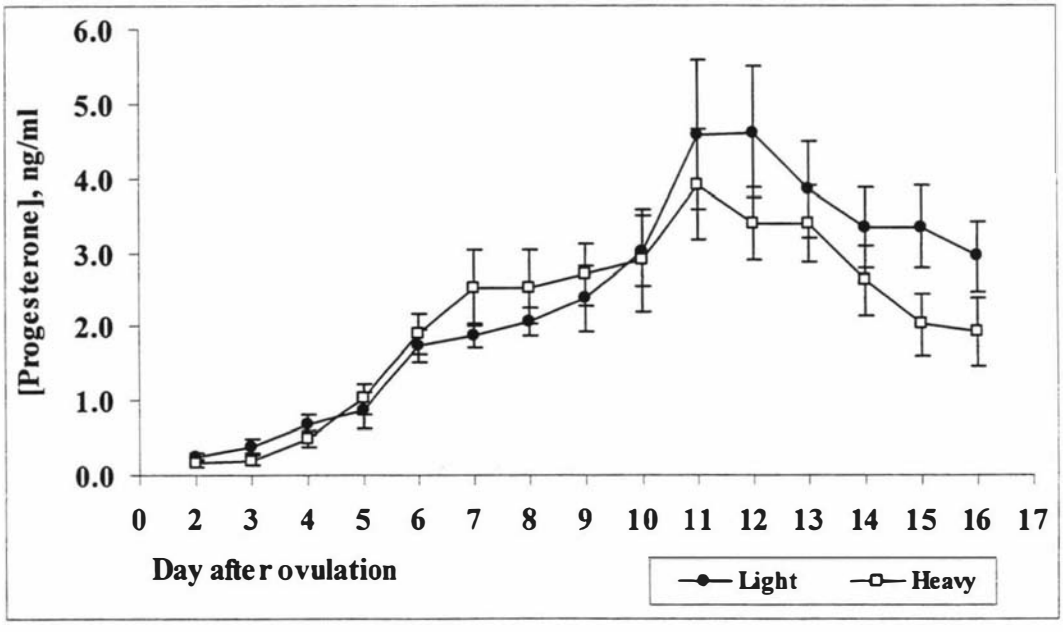


Figure 7-3: Plasma progesterone concentration during the full oestrous cycles in heavy (n=11) and light (n=7) cows between Days 2 and 16 after ovulation (Day of ovulation=Day 0).



progesterone concentrations from Days 9 to 16 after ovulation in cycling H and L cows were well described by the following quadratic regressions (Figure 7-5):

For L cows: $\log_e$ milk progesterone (ng/ml+1) = $-4.48+0.92t-0.0364t^2$

For H cows: $\log_e$ milk progesterone (ng/ml+1) = $-5.72+1.208t-0.053t^2$

where t=time in days after ovulation.

Analysis of variance of components fitted in the quadratic equations showed that these two equations have the same quadratic component but differ significantly in both the constant and linear components. In addition, differentiation of these fitted quadratic equations showed different points of inflection at which progesterone concentrations reached maximum values and started to decline thereafter. In L cows, the point of inflection occurred on Day 13 (12.6) as compared with Day 11 (11.4) in H cows. The rate of progesterone concentration changes on Day 16 in H cows was –0.41 ng/ml (–0.34 in log term). Light cows reached the same rate of progesterone decline on Day 17.3. For comparison, the rate of progesterone concentration change on Day 16 in L cows was –0.28 ng/ml (–0.25 in log term).

Progesterone concentrations and CL size

The effect of the interaction between strain x day on mean CL size approached significance (P=0.08). CL size significantly increased with days after ovulation (P<0.001). Changes of CL size after ovulation in H and L cows are presented in Figure 7-6.

Short and normal luteal phase data

Ninety percent (19/21) of the luteal phases that followed the first ovulation after calving were shorter than 11 d [H=92% (11/12); L=89% (8/9)]. The CLs with short life span originated from follicles that had a significantly longer period of development from emergence to ovulation than the ones that gave rise to CLs with normal life spans (9.6 d versus 7.3 d for short and normal cycles, respectively; P=0.04). Longer periods of development from emergence to ovulation had an impact on the cycle length, particularly during first two cycles after calving. Conversely, the maximum size of DFs destined to ovulate had no relationship with cycle length of the subsequent oestrous cycles. However, both maximum CL size (P=0.007) and the capacity of the CL to secrete progesterone (in terms of the ratios of maximum progesterone concentration and maximum CL diameter) were significantly (P=0.01) higher in normal cycles than in short cycles.

Figure 7-4: Plasma progesterone profiles (log_e+1) during the full oestrous cycle of heavy (n=11) and light (n=7) cows before the ovulation (Day 0=day of ovulation).

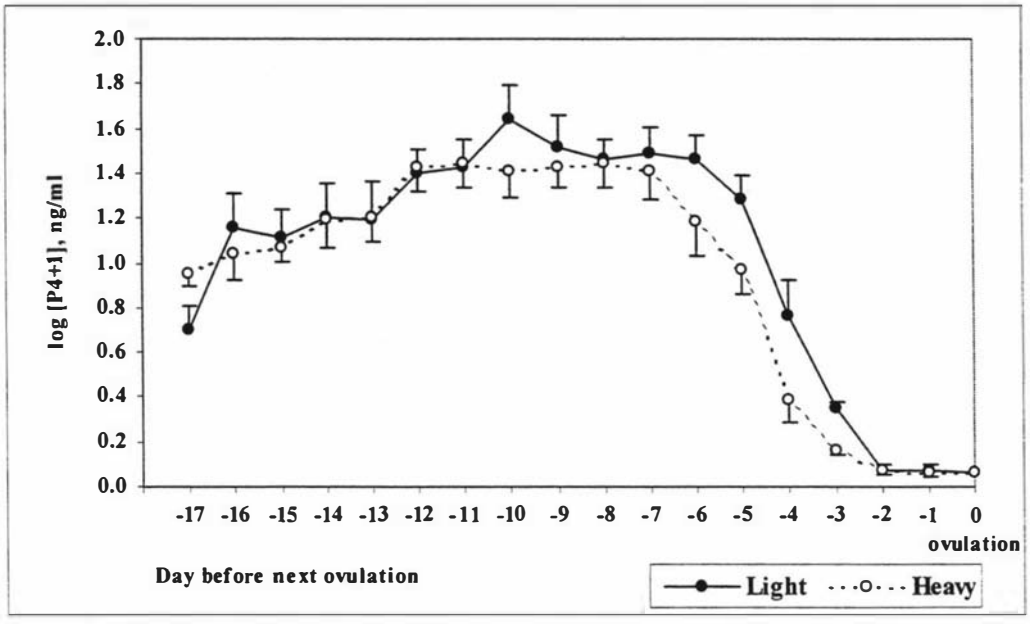


Figure 7-6: CL size (mm) during the full oestrous cycle in heavy (n=11) and light (n=7) cows between Days 2 to 14 after ovulation (Day 0).

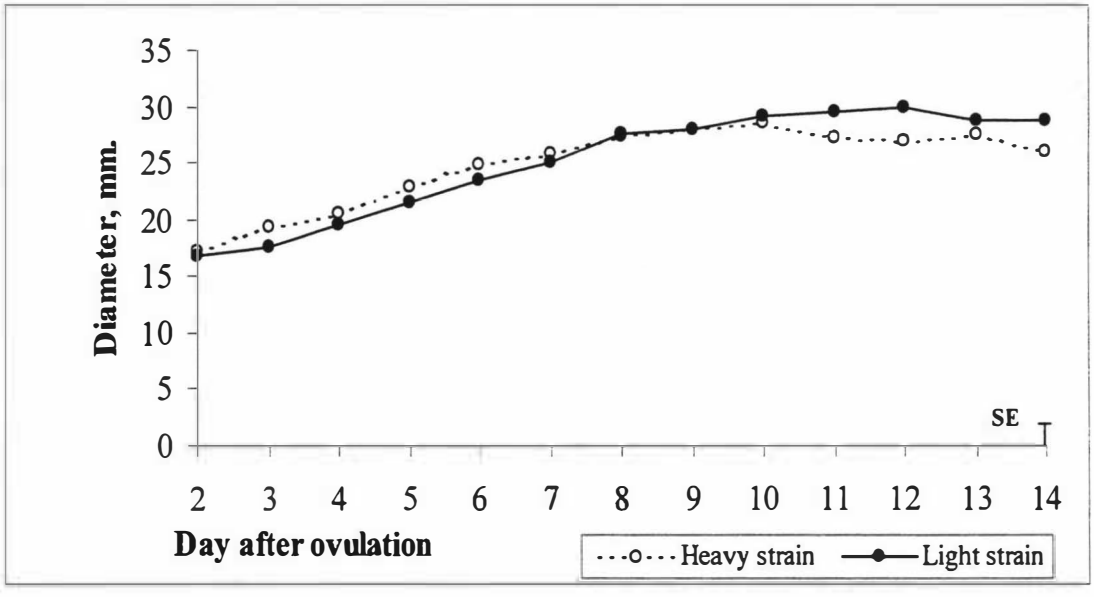
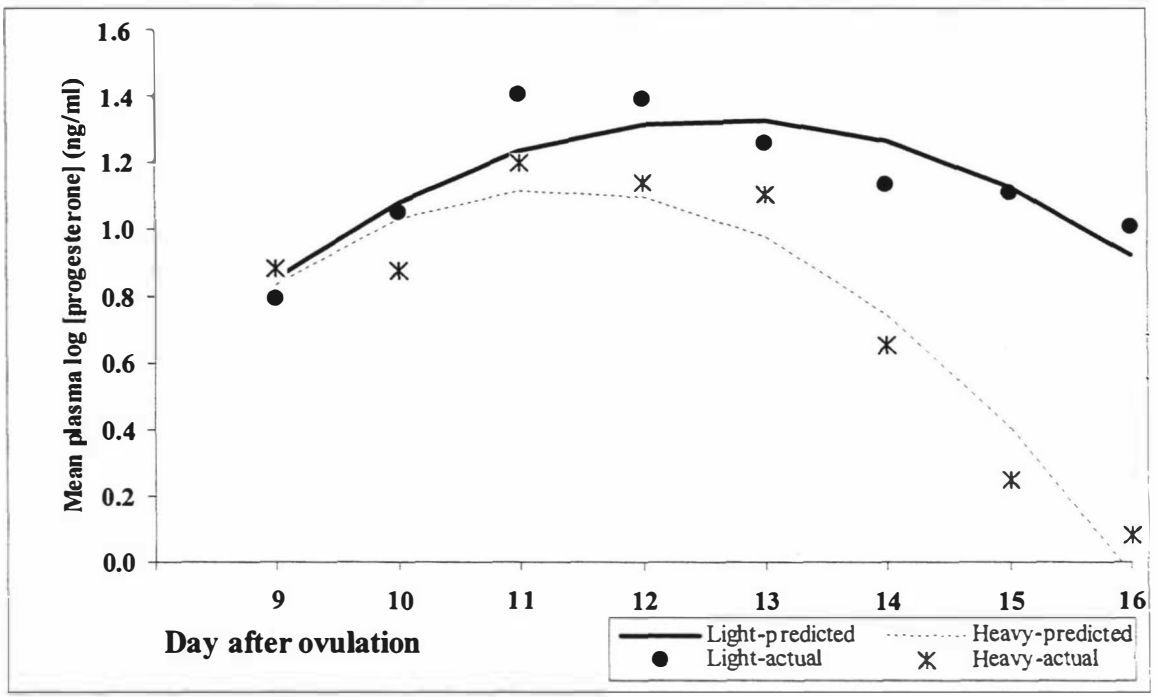


Figure 7-5: Fitted quadratic curves of estimate values and scatter plots of actual values for log mean plasma progesterone concentration of cyclic heavy (n=11) and light (n=7) cows between Day 9 and 16 after ovulation of the first three normal oestrous cycles postpartum.



For light cows: $\log_e$ milk progesterone (ng/ml+1) = -4.48 + 0.92t–0.0364t^2

For heavy cows: $\log_e$ milk progesterone (ng/ml+1) = -5.72 + 1.208t–0.053t^2

where t=time in days after ovulation.

Interval from observed behavioural oestrus to ovulation

There was no significant strain effect on the interval from observed behavioural oestrus to ovulation (P=0.29), but the effect of the covariate (cycle number) was significant (P=0.005). Overall mean interval from time from observed oestrus to ovulation was 1.5 d. The intervals increased significantly with the increasing cycle number after calving (cycle 1=1.0 d (n=11), cycle 2=1.2 d (n=18) and cycle 3=2.3 d (n=6); P<0.005).

7.4 Discussion

Reproductive outcomes

Patterns of reproductive performance observed in this experiment were generally similar to those reported previously, both by García-Muñiz, 1998 and Laborde *et al.* (1998b) and elsewhere in this thesis. The small numbers of animals meant that differences in reproductive outcome data between strains were not statistically significant (although differences of FSCR approached significance (P=0.07) even in this small group), but the trends of the data were identical to those previously reported; namely of lower conception rate and shorter interval from calving to first behavioural oestrus in H than L cows. Comparison of the present results with the long-term studies of the H and L cows is of some interest. Over the 8 years of the H and L trial, the overall FSCR was 60% (H=53 % and L=69%; P<0.001), but both Laborde *et al.* (1998b) and the present studies clearly showed that there has been considerable year to year variation in FSCR.

The intervals from calving to first ovulation and from calving to first behavioural oestrus were shorter than those reported by McDougall *et al.* (1995a) and Laborde *et al.* (1998b) for cows in seasonal herds, but they are within the range of values reported by Stevenson and Britt (1979) and Lamming and Darwash (1998) in all year round herds. Several NZ studies have shown that calving LW, BCS changes and nutritional status during the early postpartum period influence the intervals from calving to first ovulation and from calving to first behavioural oestrus in Friesian cows (Burke *et al.*, 1995; McDougall *et al.*, 1995b; Clark *et al.*, 2000). Mean BCS and milksolid production at four weeks after calving were negatively related to the intervals from calving to first ovulation (McDougall *et al.*, 1995b). In the present study, even though more H cows lost BCS than L cows, the intervals from calving to first ovulation and from calving to first behavioural oestrus were similar for both strains. In the earlier report from the same herd (Laborde *et al.*, 1998b) it was reported that H cows had a shorter interval from calving to first ovulation, but there

was no difference in the interval from calving to first behavioural oestrus. It therefore seems that the differences in nutritional (energy) status between H and L cows in the present experiment may differ from those in Experiment 4 and in the study of Laborde *et al.* (1998b). However, only a small number of cows were used in the present study, rather than the full herd examined by Laborde *et al* (1998b), so the conflict between the results may merely reflect the low power of the present experiment to detect differences in such intervals.

These results point to the unexpected conclusion that selection for high mature bodyweight does not appear to have brought about, or increased, anoestrus problems under pastoral management and feeding conditions. Again, the present evidence contrasts sharply with the contention of Harris *et al.* (2001), but agrees with the results from the recent findings by McNaughton *et al.* (2003), who reported shorter postpartum anovulatory intervals in cows that had high proportions of overseas genetics (90%) than in those with low proportions of overseas genetics (7% and 25%) under NZ pasture-fed conditions. By contrast, Harris *et al.* (2001) suggested that as the proportions of overseas genetics in the cow increase, its ability to resume ovarian and oestrus cyclicity before the planned start of mating is reduced. Likewise, Fulkerson *et al.*, (2001) reported that luteal activity and behavioural oestrus occurred later after calving in high genetic merit cows (61% North American genes) than in low genetic merit cows (22% North American genes). However, these effects do not appear to be direct consequences of selection for yield or bodyweight *per* se, for, on the other hand, within-breed selection for high milk solid yields in UK has not resulted in significant changes in the number of days to first observed oestrus in cows that were fed either low or high concentrate diets (McGowan *et al.*, 1996). Nonetheless, Taylor *et al.* (2001) reported a significantly longer time to first luteal activity in high genetic merit cows than in average genetic merit cows. In the US, Harrison *et al.* (1990) has shown that selection for high milk yield resulted in an increased number of days to first observed oestrus, but no change in the interval to first ovulation. The intervals to first service tended to be longer in animals that had been genetically selected for high milk yield (Nebel and McGilliard, 1993). Perhaps, since so many genetic and environmental factors may contribute to both the production and reproductive performance of cows, it has been difficult to isolate the contribution of the North American genetics to declining cow fertility, both in the international literature and in the present study. Given these difficulties, it may be that the differences in proportion of

overseas genetics and milk production in H and L cows in this study may not be sufficient to resolve such questions.

Although studying nutritional effects upon reproduction was not the main objective of this thesis, it seems clear from the results of Laborde *et al.* (1998b) and the present study, that in years in which feed was in short supply, the difference in the reproductive performance between H and L cows was increased, while when feed has been plentiful, the performance of H cows has been much more similar to that of L animals. Laborde *et al.* (1998b) also suggested that the long-term effect of selection for mature LW on fertility in pasture-fed cow and possibly the involvement of environmental factors such as nutrition and grazing management, might explain this discrepancy between the reproductive performance of H and L cows.

Follicular dynamics

It was remarkable to note that some cows had already started to show follicular wave activity from the beginning of the study period (on Day 7 postpartum). However, the time at which the first DF attained 10 mm in diameter was between Days 12 and 15 postpartum, which is consistent with previous reports (Savio *et al.*, 1990b, McDougall *et al.*, 1995a). Since ovarian follicular activity is mediated via the hypothalamic-pituitary axis (Webb *et al.*, 1992), the earlier follicular wave emergence in H cows than in L cows in the present study may be related to the observation reported in Chapter 3; namely that mean concentrations and episode amplitude of LH were higher in H cows than in L cows during the postpartum anoestrous period, although in association with an earlier resumption of oestrous cycles. The observation that there is higher chance for H cows to have Class 4 follicles (>15 mm) earlier in the postpartum period is consistent with this observation. With similar percentages of first DFs being ovulated in the two strains, H cows would have been expected to resume ovarian activity sooner than L cows. In fact, this was so, as H cows showed a trend of shorter intervals from calving to first ovulation (about 5 d) than L cows, even though the difference was not statistically significant (P=0.15).

Across strains, the positive correlations between DF number and days postpartum with maximum DF size lead to the contention that diameter of the DF tends to increase with the number of wave of DF and days postpartum (McDougall *et al.*, 1995a).

FSH elevations and DF development

Transient increases in FSH, which are associated with the emergence of the first DFs occur 7-14 d after calving (Savio *et al.*, 1990b; Crowe *et al.* 1998). Beam and Butler (1997) found even earlier rises of FSH concentrations (from Day 1 to 5 postpartum) followed by the emergence of the first DFs. In the present study, elevated FSH concentrations were associated with the emergence of follicular waves, although the amplitude of such elevations did not change as follicular wave number increased nor with time postpartum. This is in line with the observation of Stagg *et al.* (1998). However, the magnitude of the FSH elevations associated with the emergence of new follicular waves was higher in H cows than in L cows during the emergence of DFs number 2 to 4. The cause of this difference and its contribution to the differences in fertility between strains are unknown. However, it is consistent with the other observations of H cows, namely the higher LH concentrations in anoestrous H animals and the earlier emergence of DFs in these cows.

It has recently been suggested that the secretion pattern of FSH may result in the occurrence of 3-wave cycles rather than 2-wave oestrous cycles. Thus, lower FSH concentrations during the first and the second non-ovulatory follicular waves in cows with 3-wave cycles may be associated with reduced follicle development rate (Parker *et al.*, 2003). The results of the present study suggest that this may occur in the case of L cows, as FSH elevations associated with follicular wave emergence were lower than in H cows, whilst the proportion of 3-wave cycles was considerably higher in L cows (37%; n=8) than in H cows (17%; n=18). These figures can be compared with the 17% and 31% reported by Ginther *et al.* (1989a) and Townson *et al.* (2002) respectively. Further research to clarify the effect of these findings on the difference in FSCR between the strains seems warranted.

A lower proportion (50%) of the first DFs that developed after calving ovulated in the cows in the present study than was reported by Savio *et al.* (1990b; 74%), but the proportion was higher than that reported by McDougall *et al.* (1995a: 12%). Many factors may contribute to this difference, including genetic background of the cows, nutrition and farm management.

Follicular correlations

The observation of a relationship between DF number and percentage of milk protein in L cows but not in H cows, may indicate that interactions between milk production and

follicular activity may be different in the two strains. It could however just be a statistical artefact of small groups. Percentage of milk protein varies according to genetic factors, i.e. breed: Oldenbroek (1984), strain of cow: Pollott and Leaver (2002) and genetic merit for milk yield: Buckley *et al.* (2000); Aeberhard *et al.* (2001), diet composition (Petit *et al.*, 2001), stage of lactation (Aeberhard *et al.*, 2001), average body condition score (Pollott *et al.*, 2002) and feeding system (Fraser and Leaver, 1988; Fulkerson *et al.*, 2001). Aeberhard *et al.* (2001) suggested that differences in protein concentration in milk amongst farms were possibly related to the differences in intakes of energy and of protein as well as of feed protein/energy ratios.

Milk protein percentage or the ratio of milk protein:fat is often used as an indicator of energy deficit (Fulkerson *et al.*, 2001). Fulkerson *et al.* (2001) found that high producing cows fed low levels of concentrate had lower milk protein (2.9%) that were lower than high or low genetic merit cows fed either medium or high levels of concentrate (3.1%). The former group of cows were also expected to suffer the most severe and prolonged negative energy balance. The InCalf study provided evidence that milk protein percentage is positively related to FSCR and in-calf rate (Morton, 2000). Morton's interpretation of this relationship was not that high milk protein percentages result in better fertility, but rather that feeding strategies which brings about both increased percentage of milk protein and better fertility (Morton, 2000). However, the relationship between milk protein and fertility is not simple, with many genetic, environmental and management factors contributing to it (Fahey *et al.*, 2003). In the present study, milk protein percentage declined with time postpartum, probably reflected worsening negative energy balance (Holmes *et al.*, 2002a). However, it remains to be clarified whether any of the correlations between reproductive performance and milk production characteristics represent any causal relationship.

3-wave cycles

It has been noted previously that L cows had a higher incidence of 3-wave cycles than H cows. Whether this is related to FSH secretion patterns (as discussed earlier), or whether there is other cause, it may contribute to the higher pregnancy rates to first insemination in L cows. In the literature, there is evidence that the cows with 3 waves had higher pregnancy rates at 30-35 days after AI than those with 2 waves (81% versus 63%; Ahmad *et al.*, 1997; Townson *et al.*, 2002). In the present study, across the strains, there was no significant difference in first service conception rates between cows that were inseminated

at oestrus after 2 or 3 follicular waves (50% versus 69%). However, this finding should be interpreted taken with caution because of the small number of cycles used in the present analysis, especially for the L cows.

Plasma progesterone profile

In general, the plasma progesterone profiles of H and L cows in this study are similar to those previously described by Henricks *et al.* (1972) and Savio *et al.* (1990b).

The finding that overall progesterone concentrations were significantly lower in cyclic (i.e. non-inseminated) H than L cows between Days 2 and 16 after ovulation supports the assertion that luteal function of H cows differed from that of L cows. The cause of this difference is not known, but one suggestion is that higher pasture intake in H cows (Laborde *et al.*, 1998a) may be linked to a reduction in plasma progesterone concentration by an enhanced rate of progesterone excretion into faeces (Rabiee *et al.*, 2001). Be that as it may, it remains to be established whether this decreased progesterone concentration is due to luteal deficiency or altered progesterone metabolism in H cows. As the presence of high progesterone concentrations during the mid-luteal phase of at least one oestrous cycle before insemination has been positively correlated to the better chance of conception (Folman *et al.*, 1973; Holness *et al.*, 1981; Rosenberg *et al.*, 1990), this may, perhaps, contribute to the decreased FSCR in H cows.

That the changes of progesterone secretions with time during the normal oestrous cycle in these cows can be described by quadratic relationships has been previously reported in other studies (Lamming *et al.*, 1989; Parkinson and Lamming, 1990). Lamming *et al.* (1989) found that inseminated non-pregnant cows had lower progesterone concentrations from Days 7 to 16 than did cyclic non-inseminated cows. In the present study, regression analysis indicated that luteal function of H cows reached its maximum and started to decline earlier (Day 11.4) than in L cows (Day 12.6): a similar result to that of Experiment 4, in which luteal function of inseminated non-pregnant H cows also started to decline on Day 11.4. It appears that the functional processes of the CL of cycling H cows may be intrinsically different from those of L cows. As discussed in the previous chapter, the earlier decline in progesterone secretion in H cows may be due to either premature luteolysis or to subnormal CL function. Such a result is also consistent with the work of Meier *et al.* (2002) who found that progesterone concentrations declined earlier in US Holstein than NZ cows, also suggesting an earlier onset of luteal regression.

This early decline in plasma progesterone concentrations in H cows was also evident in Experiment 4. Taken together, these two experiments provide clear evidence of altered CL function in H cows. This conclusion is made on the basis of at least three lines of evidence. Firstly, H cows start to show signs of luteolysis (as indicated by the onset of the decline in plasma progesterone concentrations) earlier than L cows. Secondly, the maximum rate of progesterone decline occurred 1.3 days later in L cows than H cows (as estimated from the two strains of cows). Finally, in the H strain, more cows exhibited a decline in progesterone concentrations to <2 ng/ml three or four days before the next ovulation than in the L strain. Taken together, these observations suggest that there is a difference in the timing of the onset of luteolysis between strains. It is likely that such changes of luteal function may have a significant impact upon the fertility of H cows.

Progesterone concentrations and CL size

As expected, plasma progesterone concentrations increase as the CL increases in size between Day 2 and 14 after ovulation. This is in agreement with many earlier studies e.g. Kastelic *et al.* (1990); Assey *et al.* (1993). In cows, a significant correlation between the size of the developing CL and progesterone concentrations was found (Battocchio *et al.*, 1999). There were, however, no differences in the size of the CL between H and L cows from Day 2 to 14 after ovulation in the present study. Therefore, differences in progesterone concentrations during this time could be taken as further evidence of differences in luteal function (i.e. as the CL of the H cows appears to produce less progesterone per unit volume), so, perhaps there are differences of luteal cell function or ability to secrete progesterone rather than differences in CL size *per se.*

Short and normal luteal phase data

The incidence of short luteal phases that occurred in the present study is higher than that reported by Schams *et al.* (1978), Hinshelwood *et al.* (1982) and Eger *et al.* (1988). This may be due to differences in genetic background, management system, sampling protocol and the definition of short cycle. Daily blood sampling in this study may have allowed almost all short cycles to be detected, resulting in a relatively high incidence of such cycles compared with other studies.

The mechanisms associated with the development of short oestrous cycles are believed to involve inadequate pre-ovulatory follicular development, decreased LH support or premature release of $PGF_{2\alpha}$ from the uterus (Garverick and Smith, 1986; Lishman and

Inskeep, 1991; Garverick *et al.*, 1992: Bekana, 1997). In this study, the CLs of short luteal cycle phases were smaller than those of normal luteal phases, as in several previous studies (Odde *et al.*, 1980; Eger *et al.*, 1988; Bekana, 1997). The finding that the interval from first emergence of the dominant follicle to ovulation was related to development of short luteal phases is of interest, and is consistent with the study by Perry *et al.* (1991) in beef cows.

The present results also show that maximum size of the ovulatory follicle has no relationship with cycle length. However, Braden *et al.* (1989) found that the differences in number of gonadotrophin receptors and follicular oestradiol-17β of the preovulatory follicles may affect the life span of the subsequent CL. It is evident both in the present and in other works that an early resumption of ovarian activity after calving is frequently followed by short luteal phases (Hinshelwood *et al.*, 1982; Eger *et al.*, 1988). Hence, in the present study, short cycles may be due, in part, to the duration of the follicle development before ovulation and an early ovulation after calving. It is unlikely that strain of cows has any influence on these parameters.

Interval from observed oestrus to ovulation

Differences between H and L strains in reproductive performance could be due to their difference in the interval from onset of oestrus to ovulation. In the present study, more H cows had an interval longer than 48 h compared with L cows. Since bovine oocytes remain viable for only 8-10 h after ovulation, correct timing of insemination is essential for the establishment of a population of competent spermatozoa in the oviducts close to the site of fertilisation (Hunter, 1985). Cows ovulate about 21-30 h from the onset of standing oestrus (Rajamahendran *et al.*, 1989). Frozen-thawed semen is best inseminated 12-18 h before ovulation (Hunter, 1985). Hence for example, in the case of superovulated cows where the period of ovulation is protracted (up to 40 h or more) the functional sperm reservoirs would be depleted of viable sperm cells before ovulation was complete (Hunter, 1988). The risk of delayed AI is unlikely to occur in both strains as far as the duration from observed oestrus to ovulation is concerned. In fact, van Eerdenburg *et al.* (2002) found that pregnancy rate of cows that ovulated more than 48 h after AI was only 15 %. In addition, calving rate declined when AI was delayed for more than 24 h after first sign of oestrus (Watson *et al.*, 1987). Therefore, in a number of H cows that had an interval from observed oestrus to ovulation of >48 h, depletion of viable sperm cells before ovulation may occur and possibly contribute to the risk of low pregnancy rate.

7.5 Conclusion

The follicular dynamics during the postpartum period followed similar patterns in H and L cows. The first follicular wave emerged earlier in H than L cows. There was a higher probability of a Class 4 follicle (>15 mm in diameter) in H than L cows during this period. The patterns of elevations of plasma FSH concentrations preceding follicular waves were generally similar between strains, except that FSH elevations were higher prior to the emergence of DF in the follicular wave number 2, 3 and 4 in H than L cows. Proportions of first DF ovulated and intervals from C-to-FH and C-to-Ov were also similar between strains.

After ovulation, luteal function differed markedly between strains. In H cows, the overall mean progesterone concentrations were lower than in L animals between Days 2 and 16. Moreover, the proportion of cycles with intervals between standing oestrus and ovulation of >48 h tended to be higher in H cows than L cows. L cows had higher proportions of 3-wave than 2-wave cycles, which may have contributed to differences in FSCR between strains.

The results in this study support the contention that there are differences in ovarian and luteal function between H and L cows and these differences could be the causes for the observed differences between H and L cows in reproductive performance. To what extent this alteration of ovarian and luteal functions between these cows with different genetic makeups contributes to the differences in fertility between them during the early postpartum period under pasture-fed conditions, and the mechanism(s) involved, awaits further investigation.

8 Chapter 8: General discussion and conclusions

The decline of fertility in dairy cows has become a matter of global concern over the last four decades. For a long time, anecdotal evidence has existed to suggest that high yields are inimical to good reproductive performance, but, until recently, there was little evidence to suggest that there were negative genetic correlations between production and reproduction. This situation has changed markedly in recent years, with both negative genetic correlations and breed substitution effects being cited as significant genetic causes of impaired fertility. Dairy cattle fertility in New Zealand is a microcosm of these international trends. Traditionally, fertility in the NZ national herd has been high (Grosshans *et al.*, 1996), but the introduction of overseas Holstein genetics into the herd has been associated with declining fertility and survival of NZ dairy cows (Harris, 1989; Burton *et al.*, 1999).

A decline in fertility of the cows in the national dairy herd would be a serious issue for New Zealand's pastoral dairying system, since the ability of cows to calve, in a concentrated pattern, at a 365-day calving interval, is essential for the success of the system (Macmillan *et al.*, 1984; 1985a). High pregnancy rates have to be achieved within a short period of the planned start of mating, so cows have a relatively short period in which to resume oestrous cycles and complete uterine involution, if they are to be submitted for mating early in the breeding season and achieve high conception rates to those matings. Consequently, the factors affecting such a decline in fertility in the New Zealand production system may be different from those elsewhere, and an ideal type of cow for such a unique type of system may be different from that for the all-year-round systems (Macmillan *et al.*, 1996), needing at least as much emphasis upon reproductive performance as upon yield.

In the long-term, comparative, study of the efficiency of milk production of cows that has been undertaken at Massey University with cows that have been genetically selected for high (H strain) or low (L strain) mature bodyweights, parallel selection took place for the proportion of US Holstein genetics within the lines. Thus, H strain cows have a substantially greater proportion of Holstein genetics than do L strain cows (García-Muñiz, 1998). Therefore, use of these two strains not only allowed the investigation of the effects of body size *per se*, but also allowed making comparisons between the reproductive activity of cows with different genetic backgrounds.

Previous reports from the Massey trial (García-Muñiz, 1998; Laborde, 1998b) have shown that there is little difference in the efficiency of milk production from pasture between the two strains; but it is clear, from both the aforementioned studies and the results of this thesis, that the reproductive performance in H strain cows is not as good (in terms of age at puberty, per service conception rate and overall percentage of cows becoming pregnant) as in L strain cows. Thus, over the eight-years of the trial, first service conception rates (FSCRs) have been significantly ($P<0.001$) lower in H than L cows (H=53%, n=257; L=69%, n=271: see Appendix 2-1). Interestingly, throughout the trial, as in the present thesis, results have shown that the there is a considerable variation in the FSCR of H cows from year to year, whereas there was little variation in this parameter in L cows. Such observations indicate that the control of FSCR is not simply determined by genetic factors, but, more probably, given the year-to-year variability in FSCR of H cows implies that H animals are more susceptible to environmental effects (mainly nutritional; Kolver *et al.*, 2000; Verkerk *et al.*, 2000) than the L strain.

Gonadotrophin secretion

There are conflicting results considering the ability of the cows with different proportions of overseas genetics to cycle after calving. Laborde *et al.* (1998b) had reported a shorter interval from calving to first ovulation in H than L cows. In contrast, Harris *et al.* (2001) found that cows with higher proportions of overseas genetics had higher milk yields and liveweights and a reduced ability to initiate ovarian and oestrus cycles before the planned start of mating than did those with lower proportions of overseas genetics. However, working from first principles, it was postulated at the start of the present studies, that (a) H strain cows would have more severe NEB and display a longer postpartum anoestrous period than would L cows, (b) that this would be accompanied by impaired restoration of the hypothalamo-pituitary axis in H compared to L cows in the early stages of the postpartum period and (c) the impaired reproductive performance of H cows would be largely explicable in terms of extended periods of postpartum anoestrus. The role of the hypothalamo-pituitary axis in controlling reproductive activity during the postpartum period of dairy cows is well established (Lamming *et al.*, 1981; Britt, 1995), so the initial experiments in this thesis tested the hypothesis that differences in reproductive endocrinology or other aspects of reproductive physiology might be related to the differences in fertility between the strains.

However, the comparative studies of the endogenous secretion of LH and FSH (Experiment 1), the LH and FSH responses to buserelin administration (Experiment 2) and the positive feedback response of LH to oestradiol (Experiment 3), all indicate that the reproductive endocrinology of the H strain exhibits little evidence of impairment in comparison to that of L cows. Using endogenous secretion patterns of LH as an indicator of the GnRH pulse frequency (Clarke and Cummins, 1982: Yoshioka *et al.*, 2001), the similar LH pulse frequencies between strains suggest a similar pattern of GnRH secretion between strains. Likewise, the similar LH concentrations after administration of GnRH (buserelin) indicate that there is little difference in changes of pituitary responsiveness during the postpartum period between strains. Similar considerations apply to the similar responsiveness of the LH surge mechanism to oestradiol administration. In other words, the results from Experiments 2 and 3 indicate that the rate of re-establishment of a releasable pool of LH and/or the number GnRH receptors in the pituitary are unlikely to be contributing factors, give the similarity of responses between strains.

However, there were two results which stand out against this general trend. Firstly, anoestrous H cows had higher LH concentrations and LH episode frequency than anoestrous L cows. This might represent a fundamental difference in the physiology between the two strains, perhaps involving substances such as the endogenous opioid peptide, β-endorphin, which modulates LH secretion (Osawa *et al.*, 1998) or reduces GnRH pulse frequency (Gonong, 1991) in postpartum cows. On the other hand, it may be no more than an indication of the earlier onset of oestrous cycles in the H strain. Secondly, the higher LH responses to buserelin in L than H cows in the latter part of the postpartum period (Days 21-42; Experiment 2a) might indicate that, as the mating period approaches, there is better gonadotrophin support available for gonadal activity in the L cows; although the similar LH concentrations during the luteal phase of both strains argues against such a conclusion. Yet with these two caveats, the results from Experiments 1, 2 and 3 indicate that there are no gross differences in the rate of restoration in the hypothalamo-pituitary axis after calving of the two strains. Therefore, it appears that there has been negligible impact of selection for mature liveweight on hypothalamic-pituitary function during the oestrous cycle and/or postpartum period in these two strains. Consequently, the hypothesis that there are gross differences in the activity of the reproductive endocrine system after calving between H and L cows is not supported by the results of the present study.

Follicular activity

Instead, the evidence of the present study was that H cows resume ovarian activity earlier, rather than later, than L cows. Both the finding of increased endogenous LH secretory activity in anoestrous H cows when compared with L cows (Experiment 1) and the earlier development of dominant follicles (Experiment 5), may be related to the earlier onset of oestrous cycles in H animals that was reported by Laborde *et al.* (1998b) as a statistically significant difference, and which was observed in the present thesis (Experiment 4) as a non-significant trend between strains. In fact, these results contrast with that of Harris *et al.* (2001). Interestingly, McNaughton *et al.* (2003), in a parallel study of overseas Holstein and NZ Friesian cows, have also provided evidence that selection for higher milk yield or increasing the proportion of overseas genetics does not exacerbate the duration of postpartum anoestrus, but, rather (like the H cows in the present study) high merit cows have a shorter period of ovarian inactivity than low yielding/low genetic merit cows. This further corroborates the view that overseas Holstein animals have similar or more active hypothalamo-pituitary function after calving than do traditional New Zealand-bred cows.

Hence, the presence of follicles larger than 10 mm in diameter between Days 12 and 15 postpartum (Experiment 5) confirms the contention that absence of large follicles during the early postpartum period is not the limiting factor to the resumption of the reproductive activity in pasture-fed dairy cows (McDougall *et al.*, 1995a). More importantly, the observation that large follicles were present significantly earlier in H than L cows confirms that there is no impairment to the resumption of ovarian activity in H cows and, whilst the present study was unable to assess the 'fitness' of follicle or oocytes (at least at the animal-level) the evidence is of earlier resumption of ovarian activity in H than L cows, rather than the opposite situation as was originally envisioned. Interestingly, this earlier emergence of follicles occurred in the presence of minimal differences between gonadotrophin secretion patterns between strains, for others (e.g. Lamming *et al.*, 1981; Peters *et al.*, 1981; Azzazi *et al.*, 1983; Vishwanath *et al.*, 1996) have shown that the time-course of re-establishment of LH pulsatility (pulse frequency and pulse amplitude) is the key mechanism driving the resumption of ovarian activity after calving, whilst Nett *et al.* (1988) have also shown that the low circulating concentrations of LH during the early postpartum period may be a main factor limiting the initiation and maintenance of ovarian activity after calving in dairy cows.

On the other hand, there is evidence that differences in the patterns of FSH secretion associated with follicular wave emergence (Experiment 5) may affect reproductive performance, although Lucy and Crooker (2001) have shown that there is no evidence of any effect of selection for milk yield on patterns of FSH secretion during the postpartum period. Nevertheless, in the present study, it was interesting to note that lower concentrations of FSH at the time of follicular wave emergence were associated with a higher proportion of 3-wave cycles in L than H cows. Since 3-wave cycles have been associated with higher fertility than 2-wave cycles (Ahmad *et al.*, 1997; Townson *et al.*, 2002); and since alterations of FSH concentrations during the first and the second non-ovulatory follicular wave in cows with 3-wave cycles result in reduced follicle development rate (Parker *et al.,* 2003), the differences between strains in terms of FSH secretion patterns during the oestrous cycle may be related to the differences in fertility. Thus, whilst the present study did not confirm a statistically significant difference in conception rates between 2-wave and 3-wave cycles, this may be a reflection of the relatively small number of cycles that were available for study, rather than of a genuine lack of difference. Certainly the trend in the present study was compatible with the results that have been reported in the literature.

It therefore appears that whilst H cows showed earlier onset of oestrous cycles than L cows, they subsequently either failed to conceive or failed to maintain their pregnancy. Although Smith and Wallace (1998) have recently presented evidence that cows whose first ovulation occurs within three weeks of calving have a longer calving-to-conception interval, lower conception rate and greater number of services per conception than those that ovulated after three weeks postpartum, which supports the aforegoing conclusion, such a notion conflicts with many earlier reports. Most evidence in the literature (e.g. Thatcher and Wilcox, 1973; Macmillan and Clayton, 1980; Darwash *et al.*, 1997) shows that an early return to oestrous cycles has a beneficial, rather than detrimental effect upon fertility. On the other hand, most earlier studies have made comparisons within a breed (or have ignored the effect of breed), so it is not inconceivable that the effects of an early resumption of ovulation differs between breeds, depending, perhaps, upon other factors such as the rate and degree of uterine involution or the fitness of the oocyte that are ovulated.

It is also of interest to note that the finding of a trend towards a higher proportion of cycles with intervals of >48 h between the onset of oestrus and ovulation in H than L cows

(Experiment 5) raises the possibility of a potential risk of low pregnancy rate as a result of inappropriate timing of insemination in H cows (Hunter, 1985; Watson *et al.*, 1987; van Eerdenburg *et al.*, 2002). With insemination once daily, cows with delayed ovulation or a prolonged period between the onset of oestrus and ovulation may have reduced chances of conception as a result of too early insemination. Any such reduction in conception rate may also reflect the observation that prolongation of the period of dominance of the preovulatory follicles (persistent follicles) is related to a reduction of pregnancy rate due to a low quality of oocyte, disturbed early embryo development and early embryo death (Mihm *et al.*, 1994; Ahmad *et al.*, 1995; Revah and Butler, 1996).

Abnormalities of the oestrous cycle

It is possible that differences in the incidence of abnormal oestrous cycles between strains may also have affected fertility since, in Experiment 4, H cows had a higher incidence of persistent corpus luteum type II (PCLII) than did L cows, and since persistence of the corpus luteum has also been associated with lower conception rates (Lamming and Royal, 2001). It is interesting to speculate how, or whether, the higher incidence of PCLII in H cows is related to their earlier resumption of oestrous cycles, but the present study does not offer any clues upon this subject.

Likewise, the incidence of short luteal phases in the early postpartum oestrous cycles was relatively high, although it did not differ between stains (Experiments 4 and 5). This could be interpreted as evidence that the preovulatory follicles from which the short-lived corpora lutea were formed had similar characteristics between strains. It also suggests that the higher LH concentrations in anoestrous H than L cows do not result in qualitative differences between follicles, even though it may result in an earlier resumption of oestrous cycles. Thus, the incidence of short luteal phases does not appear to be dependent upon either genetic background (i.e. proportions of overseas genetics). Nor does it appear to be related to either the interval between calving and first ovulation, or to luteal function in subsequent cycles, so is probably of limited significance as a cause of impaired fertility.

Luteal function

Results of milk and plasma progesterone analyses from Experiments 4 and 5 provide clear evidence that luteal function differs between strains. Non-pregnant H cows, whether cycling or returning to oestrus after insemination, exhibited an earlier decline in progesterone secretion at the end of the luteal phase than did L cows. Very similar results

have recently been reported in New Zealand from other comparative studies of local and overseas animals (L. McNaughton, personal communication; Meier *et al.* 2002). Presumably, this premature decline of progesterone concentrations was either due to subnormal CL function or to premature luteolysis. The significance of these observations, in terms of potential effects upon pregnancy rates, is substantial, since many studies have indicated that the integrity of luteal function, in terms of the ability to produce and secrete progesterone, is clearly linked with pregnancy status (e.g. Mann and Lamming, 1999). Moreover, selection for higher milk yield has been linked with lower circulating progesterone concentrations (Lucy and Crooker, 2001), so it is not unreasonable to postulate that the H cows in the present trial did indeed have genetically impairment of luteal function.

It is of some interest to consider the causes of impaired luteal function in H strain cows. Firstly, evidence from Experiment 1 shows that there is identical luteotrophic support, in terms of LH patterns, during the luteal phase of both strains. Moreover, the sizes of the corpora lutea were identical between strains. Hence, it has to be concluded that lower progesterone concentration during the mid-luteal phase of H cows is due to either a lower progesterone production per unit mass of luteal tissue (Lucy and Crooker, 2001), more rapid metabolic clearance of progesterone from H than L cows, or that progesterone production from the corpus luteum is limited by external factors such as uterine $PGF_{2\alpha}$.

It is well established that metabolic conditions related to negative energy balance (NEB) of cows during early lactation may affect luteal function (as indicated by lower circulating progesterone concentrations: Villa-Godoy *et al.*, 1988; Ljøkjel *et al.,* 1995), possibly reflecting reduced luteal viability or impaired progesterone synthesis and secretion (Rabiee *et al.*, 2001; Westwood *et al.,* 2002). One way in which NEB may affect progesterone secretion is through the effects of the somatotrophic endocrine axis. Several studies have shown that selection for high yields has resulted in the cows which have high growth hormone and low IGF-I and insulin concentrations during the period of NEB during early lactation (Sharma *et al.*, 1994; Snijders *et al.*, 1998; Lucy and Crooker, 2001), because NEB is associated with reduced expression of IGF-I in the liver of high yielding cows (VandeHaar *et al.*, 1995). The dramatic increases in the nutrient requirement of the high yielding cows during early lactation can not be met from dietary intake alone, so during NEB, nutrient partitioning in favour of lactation occurs at the cost of body tissue reserves (Butler, 2000). Consequently, since IGF-1 has a significant regulatory role in

luteal function (McArdle and Holtorf, 1989), declining IGF-I concentrations may result in impaired luteal progesterone synthesis, resulting in lower circulating concentrations (Villa-Godoy *et al.*, 1990). Although nutritional effects were not studied directly in the present thesis, the marked year-to-year variation displayed by H cows in both FSCR and the degree of liveweight/BCS loss (Experiments 4 and 5), suggests that nutritional factors may also have played a role in determining pregnancy outcomes in those cows. If so, it is not beyond reason that IGF-1 mediated impairment of luteal function was a contributory factor to low FSCR in H cows.

On the other hand, it is possible that the lower progesterone concentrations in H cows could be due to higher metabolic clearance rates (Rabiee *et al.*, 2001), associated with higher feed intake (Laborde *et al.*, 1998a) in the larger animals. If so, it would imply that a difference in metabolic clearance rate is the factor which determines the difference in circulating concentrations. Moreover, subnormal progesterone concentrations can locally fail to prevent luteal apoptosis (Juengel *et al.*, 1993; Rueda *et al.*, 2000) leading to a further decease in progesterone secretion and, thus, a premature decline in circulating progesterone concentrations. If this were to be the case in the H cows, their premature drop in progesterone concentrations could be fully explained in terms of metabolic clearance rates. It therefore seems that detailed studies of metabolic conditions of these two strains in relation to luteal function during this critical time are warranted.

However, it seems more likely that the abrupt decline of progesterone concentrations in H cows could be explained in terms of premature initiation of luteolysis. If so, the early decline of progesterone concentrations in H cows could just be a further manifestation of classic short luteal phases that occur in postpartum cows. In this context, it is interesting to note that low progesterone concentrations during the previous oestrous cycle subsequently result in higher $PGF_{2\alpha}$ responses to oxytocin during the next cycle (Shaham-Albalancy *et al.*, 2001). Nonetheless, considering the possibility that the early decline in progesterone towards the end of cycle in HNP in Experiment 4 and in cycling H cows in Experiment 5 could be due to the effect of premature $PGF_{2\alpha}$ secretion from the uterus resulting in early onset of luteolysis, the mechanism that is involved is still unclear. Interestingly, the results from the present study also suggest that the early decline in progesterone towards the end of cycle in H cows seems to occur regardless of pregnancy status, although, in fact, very early embryonic loss could have contributed to this early decline (Lamming *et al.*, 1989). Conversely, it is very unlikely that the decline in progesterone represents a failure of the

antiluteolytic signal of the maternal recognition of pregnancy, since this event occurs on Day 16, by which time progesterone concentrations had already started to decline in HNP cows.

A further possible cause of premature luteolysis in the H strain cows derives from the greater degree of uterine infection (especially anaerobes) in H cows than in L cows during the early period of involution (F. Daoud, personal communication). Such infection, leading to local tissue damage within the endometrium, could cause premature release of $PGF_{2\alpha}$, resulting in the earlier decline in progesterone secretion in HNP cows. Moreover, giving that the function or structural integrity of luteal cells may be modulated by immune cells (Pate, 1995; Pate and Keyes, 2001), it is not impossible that changes in immune cells in the corpus luteum may occur in these animals. If so, this may further contribute to the early demise of the corpus luteum in H cows.

Consequently, whilst it is clear that the differences in luteal function between the H and L strains are substantial and, moreover, are likely to significantly contribute to the impaired reproductive performance of the H cows, it is unclear what causes these differences. Thus, further studies are required to determine whether this early decline of luteal activity in H cows stems from alterations to the uterine luteolytic signal or other controls of the luteolytic factors (e.g. oestradiol-17β, oxytocin, oxytocin receptors, cytokines and endothelin-1), or to changes in luteotrophic drives, (such as luteotrophic prostaglandins, angiogenic factors, growth factors), as a result of genetic selection for liveweight. Measurement of $PGF_{2\alpha}$ secretion pattern during early onset of luteolysis and $PGF_{2\alpha}$ secretion in response to the oxytocin challenge would be helpful to an understanding of the mechanism. Research into the detail of *in vitro* steroidogenesis and IGF-I secretion capacity of the luteal cells originating from H and L cows would also help towards a better understanding of the mechanisms underlying the differences in luteal function between the strains.

Follicle fitness and luteal function

It is well established that NEB during early lactation alters follicular function and reduces oocyte quality (Britt, 1992), as well as directly affecting luteal function. Maurer and Echternkamp (1985) also emphasised the possibility that follicular 'quality', as preovulatory follicles enter the final stages of maturation, is a major determinant of subsequent luteal integrity. However, in a more recent study, Snijders *et al.* (1998)

suggested that the decline of reproductive success in high genetic merit cows is less likely to be due to failure of follicular growth and ovulation than to being due to post-ovulation events. From the present study, it was clear that the differences between H and L cows in follicular activity were minor, so it is likely that the conclusion of Snijders *et al.* (1998) probably applies to them as well.

Consequences of impaired conception rates

As mentioned earlier, the introduction of North American genetics into the national breeding program occurred in parallel with a decline of fertility in the NZ dairy herd (Harris *et al.*, 2001). Indeed, the most significant consequences of poor fertility are likely to be the loss of animals of high genetic merit from the national herd, reflected in higher involuntary culling rates, and widening calving spreads. In addition, different FSCR between strains affects subsequent conception and calving patterns, so H cows may calve later during the next season. FSCRs tend to increase and number of services per conception decrease with increasing interval from calving to first service within 60 days postpartum (Williamson *et al.*, 1980). Consequently, late calving implies that H cows will be at an earlier stage postpartum at the start of the breeding season and, therefore, have fewer opportunities to conceive during the short period of mating. Thus, this "carry-over effect" has impacts upon calving pattern, culling rate, levels of production and survival rate (Macmillan, 1985a; Harris, 1989).

The fundamental cause of this problem is the antagonist effects of improved genetic potential for milk production upon reproductive performance (Nebel and McGilliard, 1993; Hoekstra *et al.*, 1994; Macmillan *et al.*, 1996). High yielding overseas cows have the ability to mobilise body reserve into milk during early lactation while feed intake is not sufficient. Consequently, the cows are well into negative energy balance during first few weeks after calving and have compromised reproductive performance. Nonetheless, it might be argued that the year-to year variations in FSCRs cows in the present study suggesting a predominant role of environment factors, such as pasture availability, since it is well-known that the reproductive performance of the herd can be influenced by season due to differences in herd nutrition and body condition scores (Hayes, 1998). However, the fact that the fluctuations in FSCR occurred in H rather than L strain cows must be interpreted as evidence that, even if the final cause is an environmental effect (e.g. nutrition), the H strain animals are more susceptible to adverse effects of that factor than are L cows. Yet perhaps it would be unwise to jump to conclusions about the genetic

versus environmental basis for the subfertility of H strain cows until long-term studies have been undertaken to definitively elucidate this question.

Conclusions

The present study shows that L strain cows have high FSCRs, which are relatively higher than those reported from many other parts of the world. H strain cows have lower FSCRs, which vary from year to year. This suggests the possibility that, within a pastoral dairying system, increased susceptibility to the effect of environmental factors such as level of feeding may contribute to the declining fertility that is seen in dairy cows (such as the H strain) containing a considerable proportion of overseas genetics.

Both strains seem to have identical HPA functions after calving. However, H cows showed higher LH pulsatility during the postpartum anoestrous period and higher magnitude of FSH elevations in association with the follicular wave emergence during the early postpartum period than L cows. As a result, ovarian activity resumed earlier in H than L cows after calving. Hence, contrary to expectations, in the seasonal pasture-based system, selection for higher mature LW with higher milk yield per cow by bringing in overseas genetic material has not resulted in a prolonged postpartum anoestrus period in such animals.

The most striking result from the present study is the remarkable differences in luteal function between H and L cows in terms of the timing of luteolysis in both inseminated and cycling animals. Hence, the decline of fertility in high yielding dairy cows is likely to be due to the ability of the animal to maintain luteal function after mating, rather than to impairment of ovarian follicular function. Whether this impairment of luteal function depends upon the quality of the preovulatory follicle, a premature onset of luteolysis or environmental effects remains to be determined. Whichever proves to be the case, the luteal function of H cows is impaired, leading to short luteal life span and subnormal progesterone concentration. There seems little doubt, however, that, whatever the causes of altered luteal function in H cows, it provides a credible explanation of their lowered conception rates.

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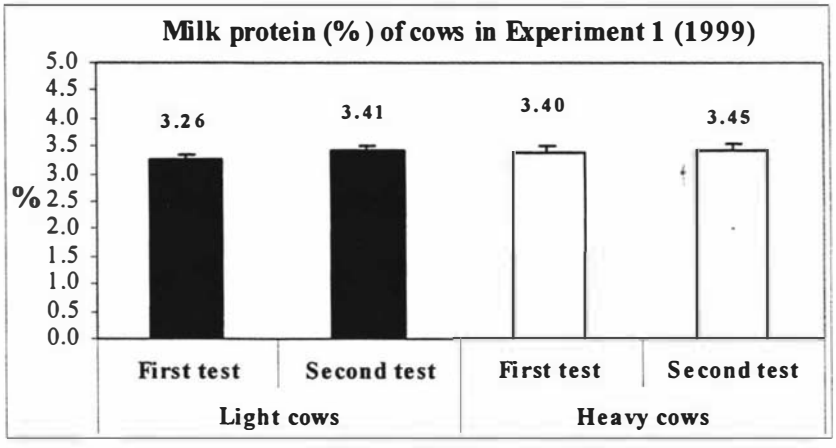
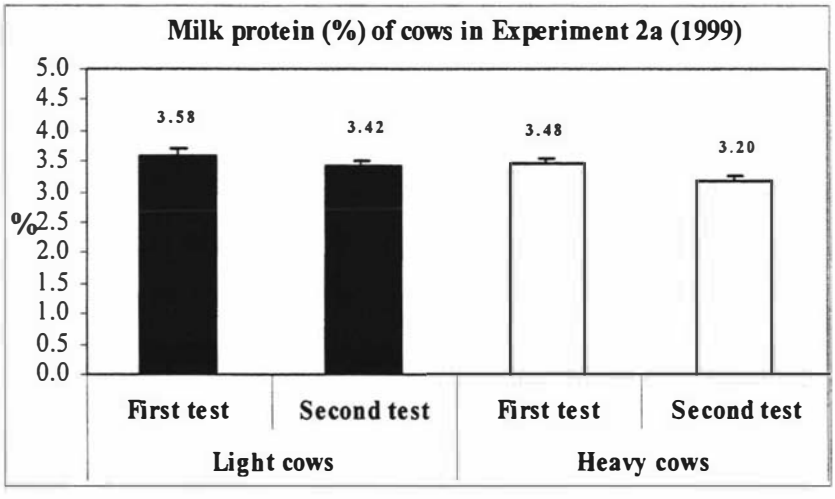
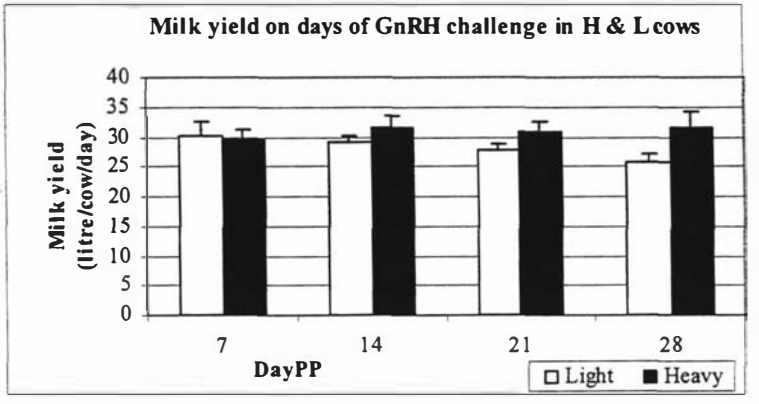
Appendix 1: Milk production and composition data

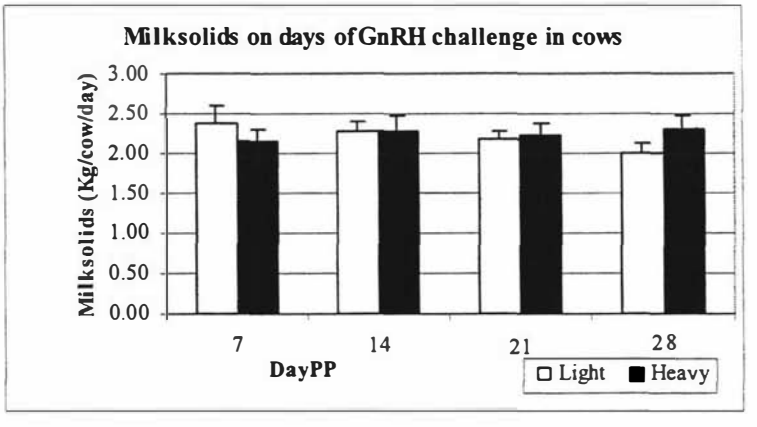
Appendix 1-1: Data for average milk protein percentage of H and L cows in Experiments 1 and 2a from the first and second herd test records (1999-2000 calving season).

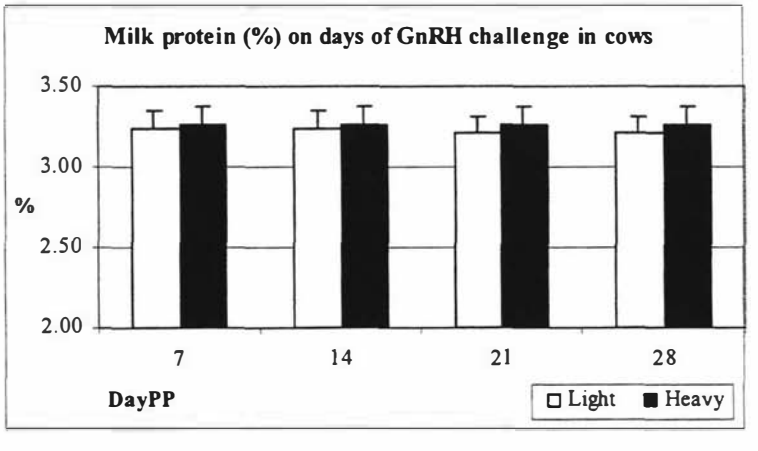




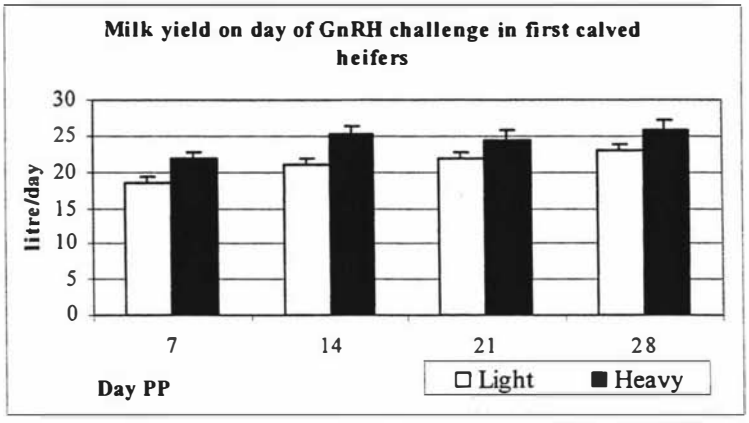
Appendix 1-2: Average milk yield (l/cow/day), yield of milksolid (kg/cow) and milk protein percentage of mixed age H and L cows in Experiment 2b (buserelin injection) during first 4-week postpartum in (2000-2001 calving season).

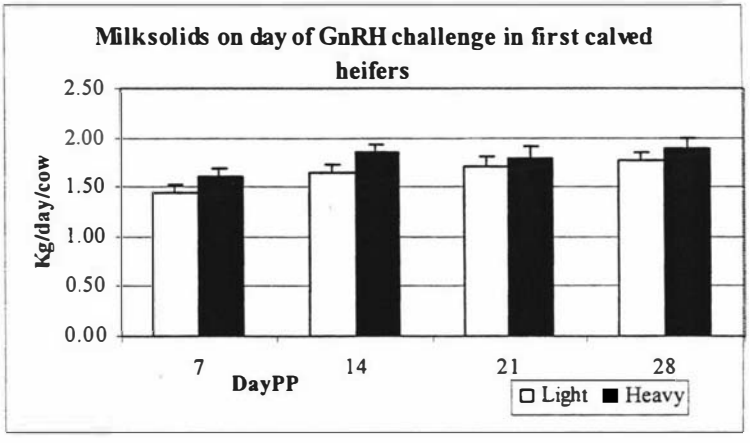


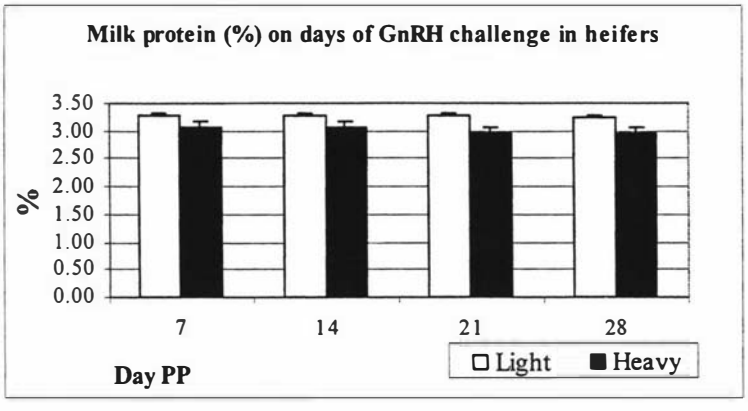




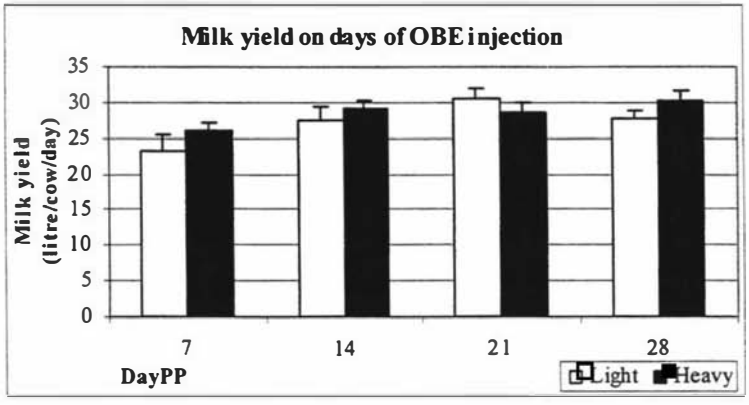
Appendix 1-3: Average milk yield (l/cow/day), yield of milksolid (kg/cow) and milk protein percentage of H and L first calved heifers in Experiment 2c (buserelin injection) during the first four weeks postpartum in 2000.

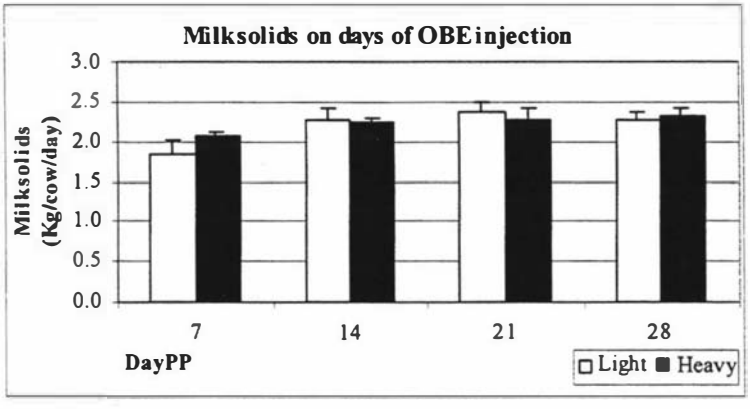


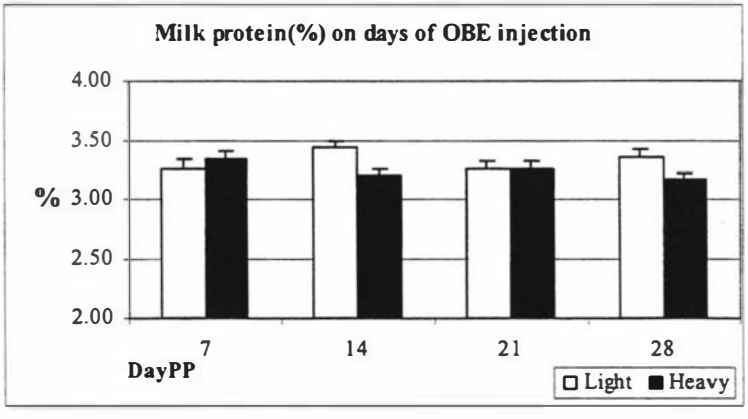




Appendix 1-4: Average milk yield (l/cow/day), yield of milksolid (kg/cow) and milk protein percentage of H and L cows in Experiment 3 (oestradiol benzoate injection) during the first four weeks postpartum in 2000.



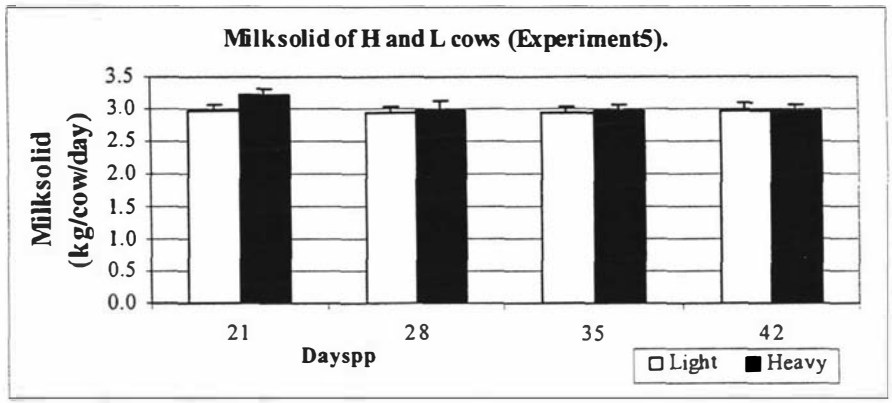




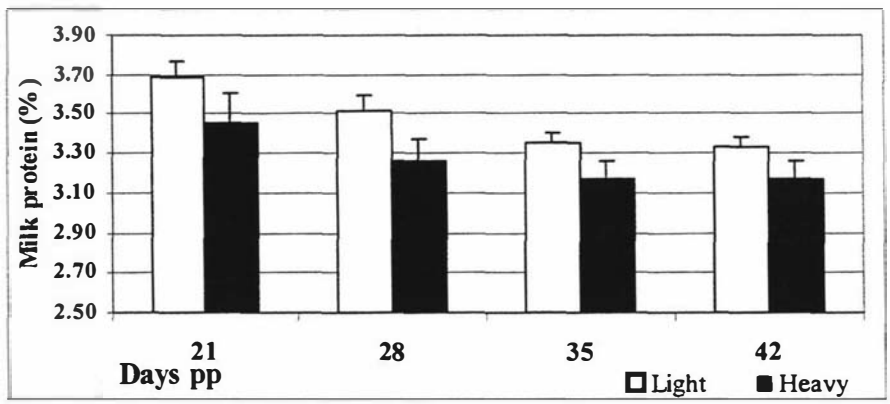
Appendix 1-5: Average milk yield (kg/cow/day) of H (n=12) and L (n=12) cows during the first six weeks in Experiment 5 (follicular study).

Time post partum	Heavy cows	Light cows
Day 21	36.3 ± 0.70	36.0 ± 0.93
Day 28	35.8 ± 0.84	35.9 ± 0.96
Day 35	36.8 ± 0.68	36.7 ± 0.89
Day 42	36.1 ± 0.76	35.9 ± 0.94

Appendix 1-6: Average yield of milksolid of H (n=12) and L (n=12) cows during the first six weeks in Experiment 5 (follicular study).

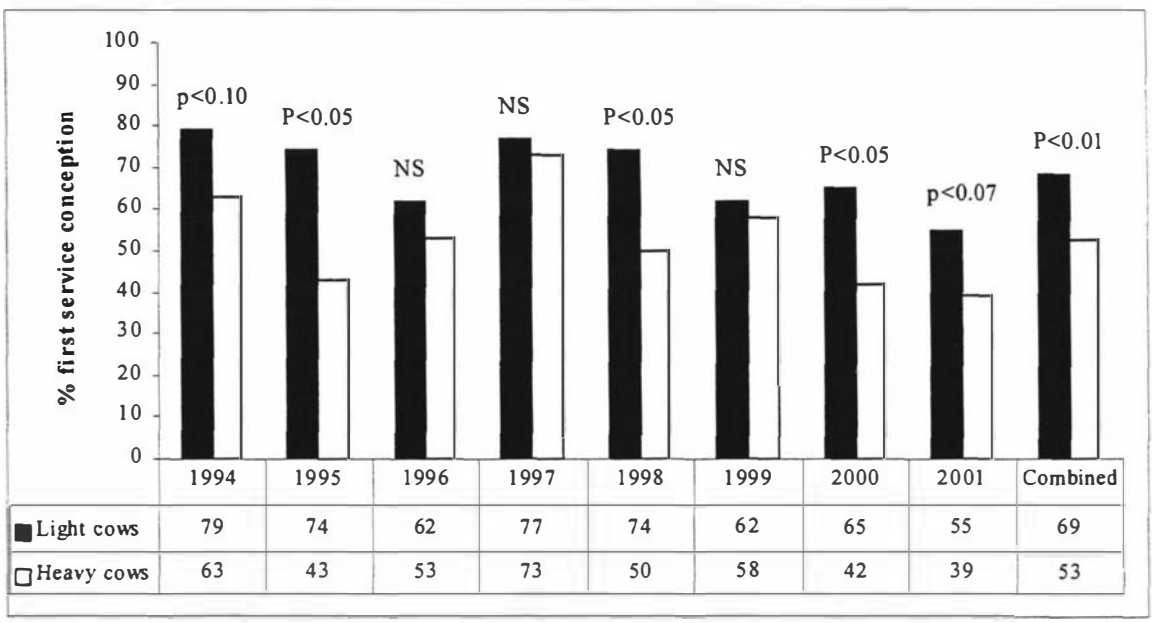


Appendix 1-7: Average milk protein percentage of H (n=12) and L (n=12) cows during the first six weeks in Experiment 5 (follicular study).



Appendix 2: Reproductive outcomes

Appendix 2-1: Conception rates for heavy (H) and light (L) strain cows during the 8-year study period. Differences between strains are presented not significant (NS) for 1994, 1996, 1997, 1999 and 2001, but are significant for 1995 (P<0.05), 1998 (P<0.05) and 200o (P<0.05). The overall mean conception rate for L cows over the eight years is significantly (P<0.01) greater than in H cows.



	1994	1995	1996	1997	1998	1999	2000	2001	Combined
■ Light cows	79	74	62	77	74	62	65	55	69
□ Heavy cows	63	43	53	73	50	58	42	39	53

Appendix 3: Representative LH profiles (Experiment 1)

Figure 1: Individual 8-h profiles of LH concentrations in a representative **Light** cow (No. 60) on Days 14, 21, 28 and 35 postpartum.

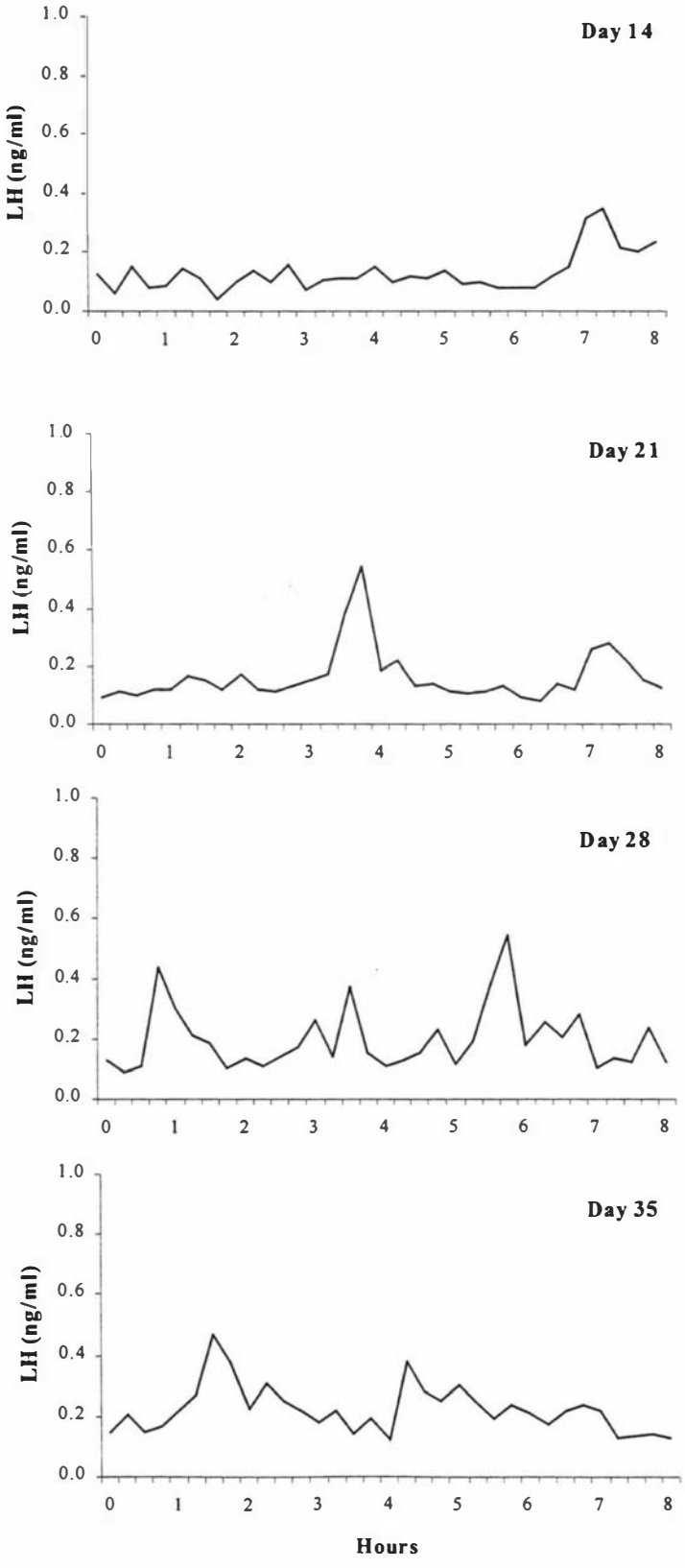


Figure 2: Individual 8-h profiles of LH concentrations in a representative **Light** cow (No. 22) on Days 14, 21, 28 and 35 postpartum.

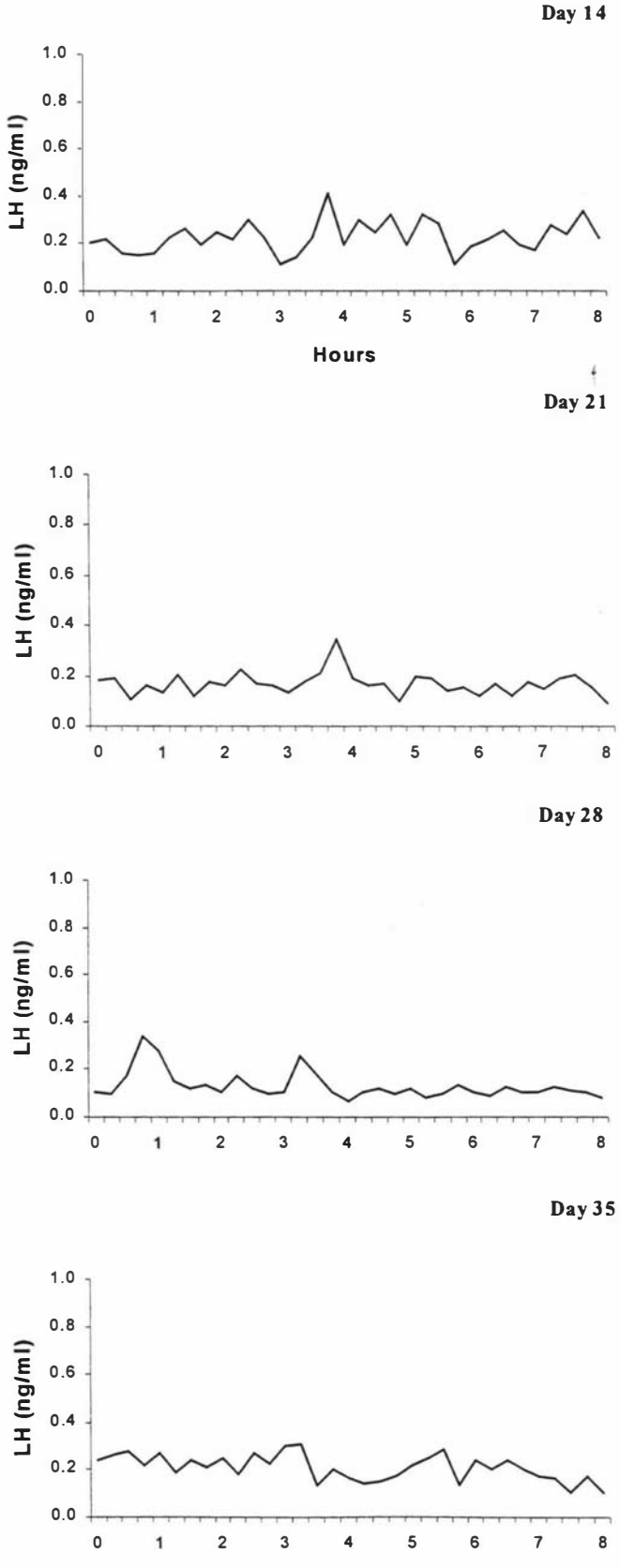


Figure 3: Individual 8-h profiles of LH concentrations in a representative **Heavy** cow (No. 69) on Days 14, 21, 28 and 35 postpartum.

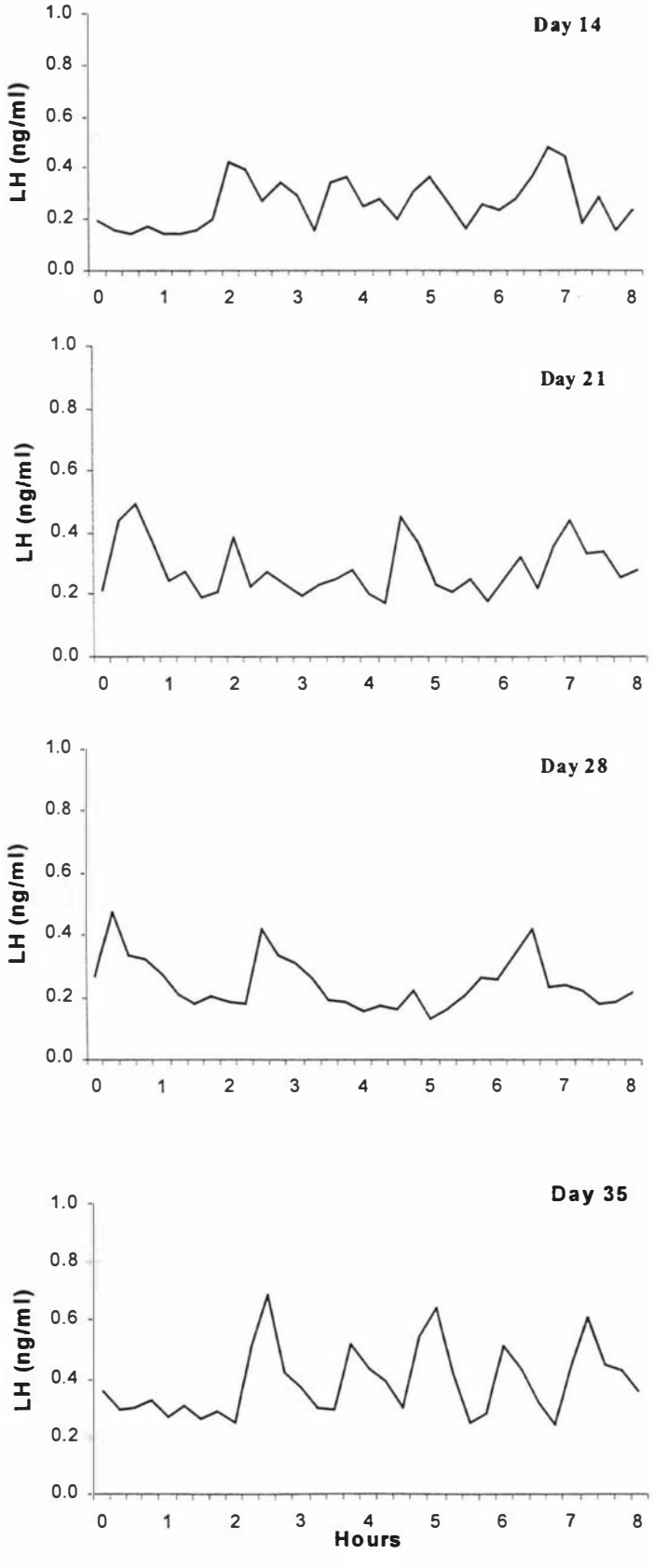


Figure 4: Individual 8-h profiles of LH concentrations in a representative **Heavy** cow (No. 153) on Days 14, 21, 28 and 35 postpartum.