A Comparison of Reproductive Performance and Physiology of Three Genotypes of Holstein Friesian Dairy Cattle

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CANDIDATE'S DECLARATION

This is to certify that the research carried out for my Doctoral thesis entitled "A comparison of reproductive performance and physiology of three genotypes of Holstein Friesian dairy cattle " in the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Turitea Campus, New Zealand is my own work and that the thesis material has not been used in part or in whole for any other qualification.

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SUPERVISOR'S DECLARATION

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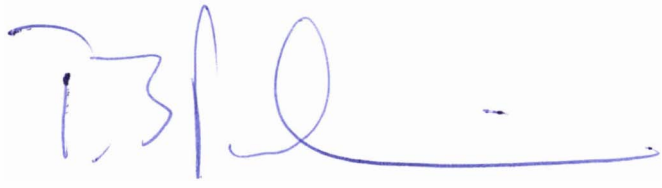

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Abstract

It is important to achieve a consistently high reproductive performance in the seasonal, pastoral-based dairy production systems found in New Zealand. A decline in dairy cattle reproductive performance has been reported in many countries and this decline has been suggested to be due to the incorporation of Holstein genetics into the Friesian populations. The use of Holstein genetics (referred to as overseas genetics) has increased rapidly in New Zealand in the past 10 years. This thesis investigates the reproductive performance and physiology of animals in the Dexcel Holstein Friesian Strain Trial, from the onset of puberty, through to the end of the second lactation in a pasture-based dairy production system (total 272 animals). Two strains of New Zealand genetic origin, of either high (NZH) or low (NZL) genetic merit were compared to high genetic merit Holstein-Friesian animals of overseas genetic origin (predominantly North American and Dutch origin, OS).

Differences in live weight at puberty were identified between NZ and overseas strains. Nulliparous OS heifers were found to have longer oestrous cycles and luteal phases than NZ heifers, but pregnancy rates between the strains were not different. Body condition score at calving was found to be an important predictor of the length of the postpartum anovulatory interval. Postpartum anovulatory intervals were significantly shorter ($p<0.05$) in OS than NZH animals. Final pregnancy rates were not different between the strains. The timing of luteal regression, following an unsuccessful first insemination was found to be more variable in OS than NZH cows, with some OS cows initiating luteal regression prior to the timing of maternal recognition of pregnancy.

The results from this thesis showed that there are differences in reproductive performance between the strains and that some OS animals are able to perform well in pasture-based dairy production systems. In conclusion, provided OS genetics are proven in New Zealand before they are widely used, there is no reason to recommend against the use of OS Holstein Friesian genetics.

Further investigations should focus on the area of body condition score mobilisation and the control of the timing of luteolysis, which are areas where differences between the strains were identified.

Acknowledgments

I would firstly like to thank Colin Holmes who worked hard to get permission for my involvement with the Dexcel Strain Trial. It would not have been possible to base myself at Dexcel without a supervisor, and although she was already busy with numerous other projects Gwyn Verkerk took on the challenge. I would like to thank Gwyn for her advice and feedback during my time at Dexcel and for always being the first to put her hand up to volunteer to help with the 2am blood sampling. The technicians from the fertility group, particularly Peter Gore went out of their way to help me settle in, to make my trials run smoothly and to show me the tricks of Excel. Many hours were spent chatting while bloods were spinning and being processed. I really enjoyed the friendly atmosphere of the fertility group, and the assistance from Susanne Meier and Lucia Chagas in collecting samples, even when it was freezing cold and pouring with rain and they had papers to write or trials to plan.

The farm managers and farm staff at Dexcel were always available to lend a hand, even when we wanted to completely disrupt farm routines to collect our samples. I would particularly like to thank John Milburn and the staff of #2 Dairy for putting up with my twice and three-times weekly milk sampling, which inevitably occurred during the busiest period on the farm.

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Thanks also to Trish O'Donnell, Angela Sheahan and Eleanor Smith, the lab staff at the University of Western Australia, particularly Margaret Blackberry, and the animal health group at LIC for the RIA and ELISA analysis.

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Finally, I would like to thank my parents for their encouragement and support of my educational endeavors, and for believing that one day I would get a job!!

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1 General Introduction

The intimate linkage of reproduction and lactation makes reproduction an essential component of dairy production systems. Successful female reproduction requires the integration of endocrine, paracrine and autocrine signals to cause the timely onset of puberty, development of follicles, regular oestrous cycles, ovulation and behavioural oestrus. With a suitable uterine environment to nourish the embryo pregnancy is maintained, eventually leading to birth, and the initiation of lactation. Physiological variation at each of these points has the potential to affect the success of reproductive outcomes.

The New Zealand dairy production system is quite distinct from that found in the Northern Hemisphere. This brings unique challenges in terms of reproduction. The narrow window between calving and mating in a seasonal production system (a maximum of 83 days) requires cows that begin cycling soon after calving, display appropriate oestrus behaviours and conceive readily. Dairy animals selected in the Northern Hemisphere, in dairy systems that are far removed from extensive, pastoral-based systems, have not been exposed to the same selection pressure for reproductive performance, and have been selected to produce very high milk yields from a diet that is heavily supplemented by concentrate feeds. It might therefore be expected that animals selected in New Zealand will perform differently in pastoral dairying systems, and may have different physiological adaptations, than animals selected in overseas dairying systems. The substantial introduction of North American and European Holstein-Friesian genetics into New Zealand provides an opportunity to compare the two genotypes.

This thesis focuses on investigating and explaining differences (if any) in reproductive physiology and performance between three different strains of Holstein-Friesian dairy cattle, from approximately 9 months of age to the end of their 2nd lactation, in the Dexcel Holstein-Friesian Strain Trial. The Dexcel Strain Trial was designed to test the productive and reproductive performance of New Zealand and OS origin Holstein-Friesian cattle and to determine suitable management systems for OS origin animals in a

pasture-based, seasonal dairy production system. Three genotypes were included in the trial, New Zealand origin high breeding worth (NZH), New Zealand origin low breeding worth (NZL), daughters of NZ 1970s bulls and bred from low genetic merit dams and overseas origin high breeding worth (OS).

The objectives of the work presented in this thesis were to:

1. Describe the New Zealand dairy production system and the importance of reproductive performance to this system (Chapter 2).
2. Review the physiological control of reproduction in dairy cattle, from birth to maturity (Chapter 2).
3. To determine whether differences exist in age and live weight at puberty, and heifer reproductive performance between the strains in the Dexcel Strain Trial (Chapter 4).
4. To determine whether differences exist between the strains in the reproductive endocrinology of the oestrous cycle in nulliparous heifers in the Dexcel Strain Trial (Chapter 5).
5. To investigate postpartum reproductive function, and endocrinology in lactating animals in the Dexcel Strain Trial (Chapter 6).
6. To identify whether differences exist in post-insemination progesterone secretion between New Zealand high genetic merit and overseas high genetic merit Holstein Friesian dairy cattle (Chapter 7).
7. To investigate postpartum hypothalamo-pituitary axis responsiveness in New Zealand and overseas Holstein Friesian dairy cattle (Chapter 8).
8. Discuss the implications of the findings of this thesis for the use of overseas Holstein Friesian genetics by New Zealand dairy farmers (Chapter 9).

2 *Literature Review*

This literature review begins with a brief outline of the New Zealand dairy industry and the importance of reproductive performance in a seasonal system of dairy production. The onset of puberty is then considered, followed by a section on the oestrous cycle and associated hormones. The initiation of reproductive activity in the postpartum cow will then be considered, leading to the relationship between genetics and fertility and the scope of this thesis.

2.1 The New Zealand Dairy Industry

There are around 3.5 million dairy cows in New Zealand, which are managed in 13,649 herds, with an average herd size of 271 cows (Livestock Improvement Corporation, 2002). Dairying is expanding rapidly in the South Island with an average herd size of 394 cows, compared to 246 in the North Island. The proportion of herds milking 300 or more cows has increased from 1.5% of herds in the 1980/81 season to 30 % of herds in the 2001/2002 season. The New Zealand dairy production system is seasonally-based, with the majority of cows calving during the late winter and early spring, very little milk is produced during the months of June and July (Livestock Improvement Corporation, 2002). Calving dates vary, depending upon climatic conditions. Average calving dates are earlier in Northland and Auckland (early to mid July) than in the South Island (early August). Milder winters, faster winter pasture growth rates and earlier onset of spring pasture growth explains much of the difference in average calving date between Northern and Southern New Zealand. In Northland in recent years there has been a swig towards split calving. A portion of the herd is calved in autumn, allowing winter growth to be utilized for milk production, and a portion of the herd to be dried off over the summer months, when grass growth is often slower than during the winter.

The Jersey was the predominant dairy breed up until the late 1960s (Livestock Improvement Corporation, 2002). Since then, the Holstein-Friesian has increased in popularity and now makes up 54% of the national dairy herd. Jersey cows make up 15% of the national herd, outnumbered by the Holstein-Friesian x Jersey cow, which constitute 23% of the national herd. The Ayrshire cow is a minority breed, comprising just 1% of the national herd. Other minority dairy breeds such as the Brown Swiss,

Milking Shorthorn, Guernsey and their crosses account for the remaining 7% of cows (Livestock Improvement Corporation, 2002).

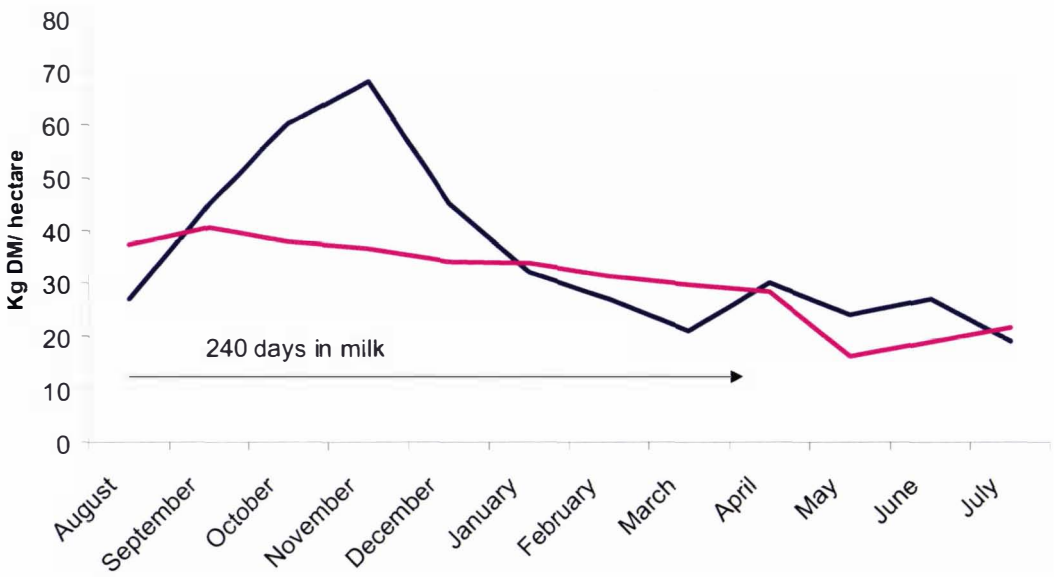
For economic and physical reasons, pasture is the main feed source (Holmes *et al.*, 1987). Farmers are paid per kg of milksolids produced. The price that the farmer receives for milk supplied to the dairy company is relatively low and highly variable. In the 1999/2000 season farmers received $3.78/kg milksolids and $5.35/kg milksolids in the 2001/2002 season, the highest inflation adjusted payout since the 1974/1975 season (Livestock Improvement Corporation, 2002). The inflation-adjusted price of milk has been declining since the 1950's. The New Zealand farmer received around 30 cents ($NZ) per litre of milk in 1993, compared to around 60 cents per litre in Canada and the EC. In addition to the basic milk price, Canadian farmers received an estimated extra 40 cents per litre in the form of government support, whilst the New Zealand farmer received around 2 cents per litre (Howse, 1997). For this reason, the cost of milk production must be low in order for the industry to be profitable. New Zealand possesses only a small-scale grain growing industry, therefore concentrate feeds are expensive to buy, and require storage and special feeding out facilities. Conversely, cows harvest the pasture themselves, directly from the paddock eliminating feeding out and storage costs. New Zealand's temperate climate allows cows to live outside year round with adequate pasture growth to meet cow demands for most of the year, eliminating housing costs. Calving and drying off dates are determined by the pattern of pasture growth over the year. Figure 2-1 illustrates the pattern of grass growth over the year and how this relates to the feed demand of the cow. Note that there is a surplus of feed during the spring period, which can be harvested in the form of silage or hay and fed to the cows during periods of feed deficit during the summer and winter periods (Holmes *et al.*, 1987).

All animals within a herd are dried off over a short period of time. As a consequence, those cows that calve earlier have a longer lactation length. Lactation length is a key determinant of productivity and is determined by the average dates of calving and of drying off of the herd (Macmillan *et al.*, 1984). In theory, a cow should lactate for 305 days, with a dry period of 60 days before calving (Capuco *et al.*, 1997) to maintain a 365-day calving interval. The dry period is important for mammogenesis in preparation for the next lactation (Capuco *et al.*, 1997). In practice, however, 305-day lactations are

rare in New Zealand as calving is spread over weeks, and as feed deficits and cow condition loss during the autumn often necessitate drying off.

The average lactation length for a New Zealand cow is around 268 days, an average which is worked out from tanker pick up information, adjusted for calving spread (Livestock Improvement Corporation, 2002). Drying off dates may vary substantially from season to season, and from region to region due to the influence of weather conditions and cow condition score (Holmes *et al.*, 1987). A compact calving period ensures that the average lactation length is maximised, by making the most of the available pasture in spring (Macmillan *et al.*, 1984).

Figure 2-1: Daily pasture growth rate vs. daily pasture requirements for a Friesian x Jersey herd stocked at 2.7 cows per hectare, producing 289 kg MS/cow/year and calving on 1 August. No young stock is grazed on the farm. From: Holmes *et al.* (1987). __pasture growth, __pasture requirements



2.2 The importance of reproductive performance on New Zealand dairy farms

Reproductive performance is a critical component of dairy farming, particularly in a seasonal system. Xu and Burton, (1996) stated that 'under the New Zealand seasonal dairy production system, the reproductive performance of dairy cows is undoubtedly one of the most important determinants of production efficiency and profitability'. A high reproductive performance is essential to maximise lactation length and dairy farm profitability (Macmillan *et al.*, 1984).

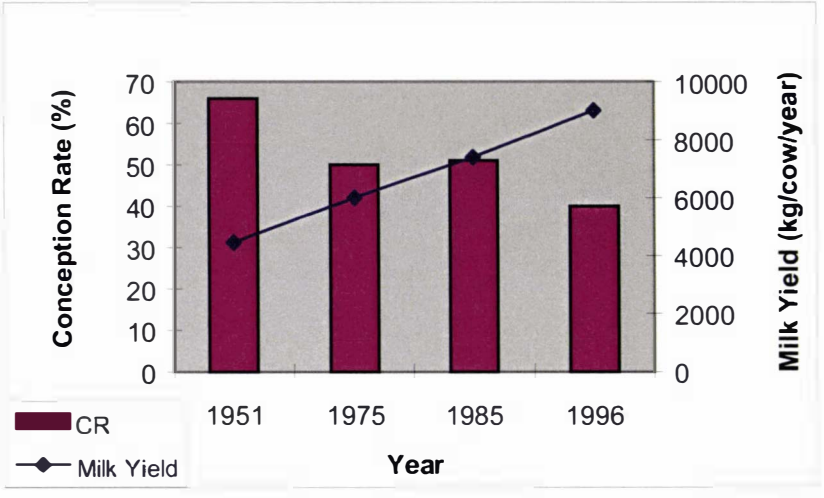
Cows that do not achieve a 365-day inter-calving interval calve progressively later in each successive calving period. Their lactation length is shorter than a cow that calved early, providing that all animals are dried off at the same time. Further, cows that fail to conceive at all are culled from the herd and must be replaced, or are carried over. Other than debt servicing the cost of producing or purchasing replacement heifers is the single largest cost of milk production (Penno, 1994).

Relative to many other countries, New Zealand cows achieve a high reproductive performance. In cows identified as cycling on the basis of pre-mating heats or rectal palpation prior to the start of mating, a 69% non-return rate was achieved in the first round of artificial insemination (Xu and Burton, 1996). This is above the target conception rate of 60% for lactating New Zealand dairy cows (Xu and Burton, 1996). However, there has been a growing concern in recent years that certain groups of New Zealand dairy cows are failing to meet reproductive targets.

The average conception rate to first service in 19 large herds in the USA in 1995 was around 40% (Butler *et al.*, 1995). Figure 2-2 illustrates the change in conception rate and milk production in New York dairy herds. A fertility study of 91 herds in the UK during the 1988-1989 calving season revealed an all service conception rate of 49% for cows with a yield average of 5800 litres (Esslemont, 1992). A comparison of reproductive performance of British Friesian cows between 1975 and 1982 and Holstein-Friesian cows between 1995 and 1998 reported a significant decline in fertility. In this study pregnancy rates to first service declined from 56% to 40%; an average decline in pregnancy rate approaching 1% per year (Royal *et al.*, 2000). There has been a substantial introduction of North American Holstein genetics into the UK herd over this time. The proportion of Holstein genetics has increased from close to 0% in 1975 to approximately 80% in 1997, whilst average milk yield per annum has increased from around 4817kg in 1975 to 6638kg in 1996 (Royal *et al.*, 2000). Lamming *et al.* (1998) estimated that first service conception rates in the UK were declining by 1% every three years; however the large data set of Royal *et al.* (2000) indicates a more precipitous decline. The pregnancy rate to first service reported by Royal *et al.* (2000) is in close agreement with the conception rate to any particular service of 40% reported by

Darwash *et al.* (1999) in a sample of more than 6000 inseminations in Holstein-Friesian cows in the UK between 1994 and 1997.

Figure 2-2: The change in milk yield and conception rate in New York dairy herds between 1951 and 1996. Adapted from Butler (1998).



Using conception rate to first artificial insemination as a measure of fertility in New Zealand dairy cattle (Jersey and Holstein-Friesian) Burton *et al.* (1999) failed to find evidence of a significant decline in cow fertility in the early to mid 1990s, although variations in conception rates between herds and between seasons were identified. The proportion of Holstein-Friesian genetics in animals born from 1991 onwards has increased rapidly so the population (herds in 1993-1996) sampled by Burton *et al.* (1999) would have had a significantly lower proportion of Holstein genetics than is currently present. Anecdotal evidence from farmers indicates that high producing Holstein-Friesian cows are proving difficult to get back in-calf. A small-scale trial at Dexcel (formerly Dairying Research Corporation) Hamilton, New Zealand compared New Zealand and overseas (OS) origin Holstein-Friesian dairy cattle and reported greater empty rates in pure OS origin cows managed under New Zealand pastoral conditions in each of the 3 years (see Table 2-1). All animals were heifers in the first season of the trial.

A Massey University study of Holstein-Friesian cows selected to for light or heavy mature live weight found a significantly lower first service conception rate in the heavy line animals (65 vs. 54% respectively). Pedigree analysis revealed that the heavy line

animals had a significantly greater proportion of OS origin (in particular North American) genetics, an average of 28% in the heavy line and 9% in the light line (Laborde, 1998). It was suggested that the increase in OS genetics, rather than the increase in live weight could be contributing to the reduced reproductive performance. Genetic material from North American Holsteins has been used within New Zealand since the 1920s and the proportion of OS origin Holstein-Friesian genetics within the New Zealand Holstein-Friesian population is increasing. Initially, OS Holstein Friesian genetics were confined to a small population of herds producing milk for liquid consumption (town milk), but these animals became the foundation herd of the New Zealand Holstein Friesian (Harris and Kolver, 2001). From the 1960s, Jersey animals were crossed with NZ HF animals and imported North American Holstein Friesian cattle (Harris and Kolver, 2001). However, the widespread use of North American origin genetics did not begin to have a significant impact on New Zealand dairy cattle for a number of years. The average New Zealand Holstein-Friesian cow born in 1978 and 1998 had 2 and 38% overseas Holstein genetics, respectively and 95% of NZHF animals born in 1998 had some overseas Holstein genetics, compared to just 6% in 1978 (Harris and Winkelman, 2000). The average proportion of OS genetics within the Jersey population has increased from 1% to 9% over the same period. The national database of cow records was examined to determine whether reproductive performance had changed over this time. Between 1973 and 1996 submission rates in the first 21 days of mating fell by 11% in Holstein-Friesian cows, but only by 5% for Jersey cows. There was no evidence of a change in conception rate to first artificial insemination in either breed (Burton *et al.*, 1999). However, a decrease in the number of cows mated during the early mating period will lead to a less compact calving pattern the following season, and later average calving date, with subsequent effects on days in milk and fertility. Cows with a high proportion of OS genetics had fewer calves born from AI and a longer interval from the start of the mating period to the first mating, in line with these decreases in submission rate; these animals with a high proportion of OS genetics also weighed more and produced more milk (Harris *et al.*, 1999).

Table 2-1: The percentage (and number) of empty cows of either New Zealand (NZ) or overseas (OS) Holstein-Friesian genetic origin, fed on a generous pasture diet, from (Kolver, 2001).

	NZ	OS
1998/1999 season (all 2- year-olds)	0	22 (2)
1999/2000 season	7 (1)	38 (5)
2000/2001 season	7 (1)	62 (8)

In contrast to the situation in New Zealand, much of the northern hemisphere dairy industry is not strictly seasonal, with cows calving year-round. Although a 365-day calving interval has been identified as economically optimum for Friesian type dairy cattle (Schmidt, 1989; Peters, 1996) a regular feed supply allows extended lactations. In the USA the use of bST has been associated with the trend towards calving intervals of greater than 365-days (Washburn *et al.*, 2002). In North America the average inter-calving interval ranges from 393-405 days (Nieuwhof *et al.*, 1989) but has more recently been reported to be greater than 435 days (Lucy, 2001b). Only 40% of British dairy cows were found to have conceived within 85 days of calving (Lamming *et al.*, 1998). Bulls originating in northern hemisphere countries will not have been exposed to the same intensity of selection for reproductive performance as bulls of New Zealand origin (Garrick and Lopez-Villalobos, 1998). It is speculated that the reproductive performance of New Zealand cows in the future may be inferior relative to the cows of today if genetic material continues to be sourced from foreign breeding programmes that are based on very different productive systems from our own (Garrick and Lopez-Villalobos, 1998). In support of this notion are the findings of Harris & Winkleman, (2000) who reported only 33% of NZ HF with a high proportion of OS genetics survived to 5th lactation in Sire Proving herds, compared to 60% of HF with a low proportion of OS genetics. The Irish system of dairy production is broadly similar to that of New Zealand, being largely seasonally based, with pasture the main feed source. The introduction of high producing Holstein-Friesian genetics into the Irish Friesian population has been associated with a decline in reproductive performance. High genetic merit animals of largely US Holstein origin had an average empty rate of 22% over two seasons, compared to 6% for local medium genetic merit animals. First service conception rates were 52.1 and 41.5% in medium genetic merit and high genetic merit animals respectively and differences between the two groups were significant, or approached significance for both measures of fertility (Snijders *et al.*, 2001).

Reproductive failure was the primary reason given for culling cows in surveys of dairy herds in USA (Bascom and Young, 1998) and in the UK (Esslemont and Kossaibati, 1997). More than 20% of cows culled were culled for reproductive failure. In Australian Holstein-Friesian cows run under pastoral conditions (similar to those in New Zealand), reproductive failure was again the most common reason for culling cows. Reproductive failure was the primary reason given for culling in 32% of cases, with over half of these cows failing to conceive (Stevenson and Lean, 1998). Of New Zealand dairy cattle that are culled each year 43% are culled for reproductive failure (Verkerk *et al.*, 2000). Involuntary culling, such as culling for reproductive failure, represents a cost to the system. The costs to the industry are two-fold, firstly the monetary cost of having to raise or purchase a replacement animal, and secondly the loss in genetic gain for milk production through culling of reproductively unfit animals instead of low producers which would otherwise have been removed from the herd. Clearly reproductive failure is a costly worldwide problem in dairy herds today.

2.3 Puberty

Puberty in females is the age when mature gametes are produced and reproductive activity is initiated (Foster, 1999). In this section the onset of puberty will be examined, focusing on *Bos taurus* cattle, the factors that control the onset of puberty and the practical implications of these facts on farm.

2.3.1 Reproductive tract growth

Puberty is not only associated with the development of mature endocrine responses, but the physical development necessary for pregnancy. Rapid puberal growth of the reproductive tract commences during the 7th month of age, and is mostly completed by 10 months of age in Holstein heifers (Desjardins and Hafs, 1969). Uterine weight increased dramatically (by approximately 400%) at the pubertal oestrus in ewes, compared to the corresponding weight in prepubertal herdmates of the same age and weight (Lewis and Beradenelli, 2001). Uterine weight had increased further after 2 oestrous cycles, and was heaviest in pluriparous mature ewes. Uterine diameter was increased in pubertal ewes compared to prepubertal ewes, whilst oviductal weight and length did not differ between ewes at the pubertal, first or third oestrus, but was greater in mature ewes (Lewis and Beradenelli, 2001).

Ovarian development in terms of folliculogenesis will be covered in section 3.1. Briefly, follicular activity begins long before puberty. The wave-like pattern of follicular growth seen in mature animals (that is characterised by the growth and regression of dominant follicles) has been reported from as early as 2 weeks old in heifers (Evans *et al.*, 1994) and is well established by 36 weeks (Adams *et al.*, 1994).

2.3.2 Reproductive Endocrinology of Puberty

In Hereford x Friesian heifers mean plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations decreased between 3 and 15 weeks of age and then increased from 15 to 35 weeks of age (Dodson, 1988). Schams *et al.* (1981) reported a similar pattern of gonadotropin secretion and low progesterone concentrations (<0.1ng/ml) from 1 to 9 months of age. Elevated progesterone concentrations were recorded 8-12 days before first oestrus (elevations of 0.9 ± 0.1 ng/ml).

The development of a pulsatile pattern of LH secretion in the first month of life has been demonstrated in heifers (Schams *et al.*, 1981; Dodson, 1988), bulls (Amann and Walker, 1983) and lambs (Foster *et al.*, 1975). Luteinizing hormone pulse frequency was lowest at 3 weeks of age. By 7 weeks of age all heifers had established an LH pulse frequency of at least 1 pulse/ 24 hours. The amplitude of LH pulses increased linearly between 3 and 35 weeks of age, with an LH pulse frequency of ≈5pulses/24 hours at 35 weeks (Dodson, 1988), although in the short period before puberty LH episode frequency was far lower than reported in the follicular phase of the adult (Dodson, 1988).

A short period of elevated progesterone concentrations has been reported in heifers (Berardinelli *et al.*, 1979) and in lambs before first oestrus (Berardinelli *et al.*, 1980). In cattle, short cycles often occur during the postpartum period (Savio, Boland and Roche, 1990). The transient progesterone rise in postpartum cows is believed to be due to ovulation of a follicle that has not properly responded to the preovulatory gonadotropin surge (Hunter *et al.*, 1986). It is likely that a similar occurrence explains the observed rise in progesterone preceding puberty in the heifer. Normal cyclic activity followed the

short 'luteal phase' (period of elevated progesterone concentrations) in all heifers in the study of Dodson (1988). It was proposed that the progesterone produced synchronized follicular development, ensuring that the second preovulatory surge occurred at the correct time, relative to follicular development.

The prepubertal phase of the heifer is marked by a gradual increase in LH pulse frequency, and in mean FSH concentrations and the absence of progesterone secretion until just prior to first oestrus.

2.3.3 Endocrine control of puberty onset

Two main theories exist to explain the hormonal control of the onset of puberty, the gonadostat theory, and the central drive theory. The gonadostat theory Ramirez & McCann (1963) suggests that the onset of puberty is dependent upon a decrease in the hypothalamic sensitivity to the negative feedback of oestrogen on LH secretion. In the prepubertal period it is believed that oestradiol, produced by the ovaries, feeds back to the hypothalamus and pituitary to inhibit LH secretion. A decrease in the negative feedback of oestradiol allows LH secretion to increase, promoting follicular development (and oestradiol secretion), and eventually ovulation.

Evidence suggests that the immature lamb has a relatively mature hypothalamic-hypophysial-ovarian system, but the LH pulse frequency limits ovarian follicular development. In the immature lamb LH pulse frequency is low, one LH pulse every 2-3 hours, whereas in the post pubertal lamb (Foster *et al.*, 1975) and the mature ewe 1 pulse per hour, or more are reported (Karsch *et al.*, 1983). Ovariectomy of lambs at 2 weeks of age results in an increase in LH pulse frequency to 1 pulse/ hour by 7 – 9 weeks of age Foster, (1975), a frequency which is not usually attained until around 30 weeks of age (at puberty) in the intact lamb. A rapid increase in LH secretion following prepubertal ovariectomy has been reported in heifers (Day *et al.*, 1984), lambs (Foster, 1975)b and rats (Andrews and Ojeda, 1977). This suggests that a factor secreted by the ovary is influences LH pulse frequency in intact immature animals. Beef heifers, ovariectomized approximately 100 days prior to expected puberty and given an oestradiol implant following ovariectomy had a significantly lower LH pulse frequency than heifers that did not receive oestradiol. LH pulse frequency increased with time in both groups of ovariectomized heifers, but was lower in oestradiol treated animals (Day,

1986). The increase in LH pulse frequency over time probably mimics the natural increase in LH pulse frequency in intact heifers as puberty approaches, and the postulated decrease in oestradiol negative feedback. In ovariectomized lambs treated with oestradiol, LH pulse frequency was suppressed until the onset of puberty in intact controls, suggesting a decrease in oestradiol inhibition of LH pulse frequency around this time (Foster and Ryan, 1979). Oestradiol seems to have a negative effect on LH pulse frequency in ovariectomized, prepubertal heifers and lambs, which decreases as animals approach the normal time of puberty.

Ovulatory potential is established well before puberty. The administration of hourly doses of LH to lambs at 18-20 weeks of age causes an endocrine response identical to that of the follicular phase, including an increase in oestradiol concentration and a preovulatory LH surge, ultimately resulting in ovulation (Foster *et al.*, 1984). Increasing oestradiol concentrations in response to LH pulse frequency ultimately cause the preovulatory LH surge. Administration of oestradiol to the prepubertal lamb or heifer results in a large increase (or surge) in LH secretion (Foster and Karsch, 1975; Swanson and McCarthy, 1978). Therefore, whilst low concentrations of oestradiol seem to inhibit LH pulse frequency in the prepubertal lamb, greater concentrations are stimulatory, resulting in an LH surge.

A potential mechanism for the prepubertal decline in negative feedback of oestradiol on LH secretion was proposed by Day (1987). The number of oestradiol receptors at the hypothalamus and pituitary declines prior to puberty. It has been estimated that during the 50 days before puberty oestradiol receptors at hypothalamic sites decline by 35%. Pituitary oestradiol receptor numbers began to decline about 50 days before puberty and continued to decline up to the time of puberty. Therefore, it is possible that the increase in LH pulse frequency that is seen as heifers approach puberty is due to a decrease in the ability of oestradiol to inhibit GnRH secretion, through a reduction in receptor numbers. Alternatively, the decline in receptor numbers at the pituitary could enhance the response to GnRH, by reducing a source of inhibition (Day, 1987). It seems most likely that if the decline in oestradiol receptor numbers reported is important then the decrease at both the hypothalamus and pituitary plays a role in reducing oestradiol inhibition of LH release.

There is evidence to suggest that the onset of puberty in primates (and the rat) does not fit the 'gonadostat hypothesis', (for review see Plant (1994). Whilst a decrease in hypothalamic-pituitary sensitivity to steroid negative feedback is a component of the puberty process, it does not appear to be the initiating factor (Ojeda, 1991). The post-ovariectomy gonadotropin response of the immature primate female is smaller than in heifers and gilts, not consistent with the expected rise in gonadotropin levels if a gonadostat is the major determinant in the timing of puberty onset (Plant, 1994). In humans, in agonadal patients, or patients with gonadal dysgenesis, the pattern of gonadotropin secretion from infancy to adulthood is similar to normal, but gonadotropin values are dramatically higher than normal (Winter and Faiman, 1972; Conte, 1975; Levine, 1983). Gonadal function, particularly the release of steroids, is not the main determinant of the pattern of gonadotropin secretion, but affects gonadotropin values. These data suggest that the increase in gonadotropin secretion around puberty is not a direct result of changes in steroid feedback sensitivity in humans and primates, but the result of an increase in the drive of the hypothalamic-pituitary unit to produce gonadotropin. In the rat the resetting of the gonadostat follows the first preovulatory surge (Andrews *et al.*, 1981).

Whether the resetting of the gonadostat is a driving factor, or consequence of the onset of puberty in the heifer is not clear. Dodson, (1988) postulated that puberty in the heifer awaits the final maturation of some central component regulating gonadotropin secretion as none of the events preceding, or immediately following first ovulation initiate the change in sensitivity to ovarian steroids. Supporting the gonadostat theory is evidence that there is a rapid gonadotropin response to ovariectomy, which can be reversed by oestradiol implants (Day, 1986). The strength of oestradiol inhibition of LH secretion seems to decrease with time, fitting with the proposed mechanism of action of decreasing numbers of oestradiol receptors at the hypothalamus and pituitary gland as puberty approaches (Day, 1987).

2.3.4 Precocious puberty

Precocious puberty refers to the attainment of oestrous cycles before the normal age and weight at puberty in that particular species. Precocious puberty is of particular concern for beef producers, where heifers may be exposed to fertile bulls whilst still suckling their dams. In an investigation of precocious puberty in beef heifers the frequency of

precocious puberty varied over the two seasons observed. The average age of animals which exhibited precocious puberty was 194 ± 12.4 days and cyclic luteal function lasted for an average of 65 ± 10.5 days (Wehrman, 1996). The potential control of precocious puberty remains to be determined, however, it has been suggested that a failure to develop hypersensitivity to oestrogen and the ability of the ovaries to secrete 17β-oestradiol may be responsible. In the absence of a suppressive action of oestradiol on LH secretion, oestradiol secretion by the ovary promotes LH secretion, which in turn promotes follicular growth and oestradiol secretion. Eventually the concentration of oestradiol secreted by the growing follicles is sufficient to trigger the preovulatory LH surge. Ovulation and luteinization follow. Oestrous cycles would cease when the inhibitory effects of oestradiol on LH secretion develops and the heifers return to an anoestrous state until puberty (Wehrman, 1996). The hypothesis used to explain the phenomenon of precocious puberty fits well with the gonadostat hypothesis, however, no evidence to support this explanation is provided.

2.3.5 Other factors that may determine the onset of puberty

Live weight/nutrition

Live weight is considered to be a key determinant of the onset of puberty (Foster, 1999; Hafez and Hafez, 2000). Manipulation of growth rate has resulted in a change in the age, but not the live weight, at puberty (Barash *et al.*, 1994; Lammers, 1999). However, others have reported that heifers fed a high-energy diet were younger and heavier at puberty than heifers that were fed a lower energy diet (Bergfeld, 1994). The animals that were the heaviest at 3 and 6 months reached puberty earlier than lighter animals (Hawk *et al.*, 1954), whilst heifers which grew faster before weaning tended to be younger and heavier at puberty (Arije and Wiltbank, 1971). Inadequate nutrition delays the onset of puberty, whilst a high plane of nutrition results in rapid growth and earlier puberty (Barash *et al.*, 1994). Indeed an inverse relationship between level of intake and age at puberty in heifers has been demonstrated (Short and Bellows, 1971; Yelich *et al.*, 1996). These observations can be explained by the onset of puberty being determined more by live weight and growth rate, than by age. However, it has been suggested that rather than a specific body weight, that a certain carcass composition must be obtained to trigger the onset of puberty. The 'fatness hypothesis' suggests that a critical amount of fat must be accumulated, relative to lean body mass before

ovulation can occur in the young female (Frisch, 1990). The scientific community has spent much time debating the relative merits of this hypothesis.

Much of the evidence for the 'fatness hypothesis' comes from studies on human subjects. The fatness hypothesis was first suggested when it was found that there was a relationship between weight and the prepubertal growth spurt (Frisch and Revelle, 1971). When body composition was calculated, using changes in total body water as a measure of fat percentage, there was an improvement in the correlation (Frisch *et al.*, 1973). During the prepubertal growth spurt there was a greater increase in fat deposition than lean tissue deposition, with the ratio of lean mass to fat declining from 5:1 to 3:1 at menarche. Menarche has been attained at progressively younger ages over the past century in America, perhaps explained by the abundant caloric supply of most children and the resultant increase in growth rates. Children are therefore reaching 'pubertal weight' at an earlier age (Frisch, 1990).

Severely anorexic women are characterized by ammenorhea (the absence of menstrual cycles); likewise athletes whom train heavily are prone to irregular or entirely absent menstrual cycles (Frisch and McArthur, 1974; Frisch *et al.*, 1981). The body fatness hypothesis has been used to explain the absence of menstrual cycles in such women. However, the relationship between heavily training athletes and menstrual disorders should be viewed with caution. Only sometimes are menstrual abnormalities found to correlate with body weight and fat reserves (Bronson, 1991). Schwartz *et al.* (1981) studied amenorrheic long-distance runners, and compared this group with runners who were regularly menstruating, and a control group of nonrunning subjects. On average, body fat percentage was lower in the amenorrheic group than in the menstruating groups. However, some women reported to be regularly menstruating were as lean as the leanest of the amenorrheic group. Thus, a low percentage of body fat did not guarantee amenorrhoea.

Onset of puberty in young female rats fed either high fat or low fat chow occurred at different ages and body weights, but at a similar body fat composition (Frisch *et al.*, 1977). Carcass fat content of poultry was not significantly different between chickens that differed in body weight and age at first egg production (Lewis and Perry, 1989). However, other similar studies in rodents have found no relationship between fatness

and age at either first ovulation or vaginal opening (Glass *et al.*, 1979; Perrigo and Bronson, 1983). Treatment with growth hormone reduced back fat thickness in heifers, but had no effect on the onset of puberty (McShane *et al.*, 1989). A certain body fat composition, and therefore degree of maturity, may be one of many thresholds to the onset of puberty, combined with level of nutrition and other factors such as photoperiod.

In a review of the energetic regulation of ovulation Bronson (1991) argued that the fatness hypothesis is too simple and is based on correlations, which do not necessarily represent cause and effect. They also argue that there is no known linkage between the fat stores and the GnRH pulse generator, which ultimately determines puberty and ovulatory cycles. However, since this paper was written the hormone leptin has been discovered. Leptin is a 167 amino acid protein, which is coded for by the ob gene and mainly produced by white adipose tissue (Rohner-Jeanrenaud, 1996). Whole body leptin production correlates directly with body mass index (BMI) and percentage body fat mass in humans (Klein *et al.*, 1996) and body fatness in sheep (Delavaud *et al.*, 2000). In rodents, leptin acts to reduce the secretion of neuropeptide-Y (NPY) (a potent stimulator of food intake), thereby reducing food intake; whilst a reduction in leptin concentrations (as occurs during weight loss) acts upon the arcuate nucleus to increase production of NPY and stimulate food intake (Schwartz and Seely, 1997). A decrease in leptin concentrations also reduces sympathetic nervous system activity and thermogenesis in brown adipose tissue, via NPY, to conserve energy during weight loss (Billington *et al.*, 1994). Mice of the ob/ob genotype are hyperphagic and obese, due to mutation in the ob gene which prevents normal leptin production (Campfield *et al.*, 1995). Leptin receptor mutations, which prevent leptin binding in the Zucker fatty rat and the diabetes-prone mouse, also produce obesity (Auwerx and Staels, 1998).

There is evidence to suggest that leptin is involved in the control of reproductive function. The ob/ob mouse is grossly obese and is also infertile. Administration of leptin reverses obesity and restores reproductive function in the ob/ob mouse (Chehab *et al.*, 1996). Administration of leptin to acutely fasted mice maintained normal oestrous cycles compared to control animals, where dioestrus was prolonged and vaginal oestrus was delayed (Ahima, 1996). Leptin receptors are present on the ovary; however, to determine whether leptin acts at a higher level in the reproductive axis Nagatani *et al.* (1998) administered leptin to ovariectomized rats. Leptin administered to fasted,

ovariectomized and oestrogen treated rats restored fasting state LH concentrations to fed state concentrations, demonstrating that leptin is acting centrally. In mice, leptin administration accelerates the onset of puberty in normal females, relative to untreated controls, as measured by age at vaginal opening, age at vaginal oestrus and age at vaginal cycling (Ahima *et al.*, 1997). Likewise, Chehab *et al.* (1997) reported leptin-treated mice grew more slowly, but were younger and lighter when copulatory plugs were detected and hence were younger at parturition. LH concentrations were significantly lower in leptin-treated mice than in controls, whilst oestradiol-17β concentrations tended to be lower, although the difference was not significant (Chehab *et al.*, 1997). It is notable that the onset of puberty has not been advanced in rats treated with leptin (Cheung *et al.*, 1997), and that other laboratories have not been able to repeat the results reported above in mice (Cunningham *et al.*, 1999).

The level of nutrition affects plasma leptin concentrations in sheep (Blache *et al.*, 2000). In prepubertal heifers, food restriction decreased leptin mRNA in adipose tissue and circulating leptin concentrations. The frequency of LH pulses was lower in fasted than in control animals (Amstalden, 2000). Leptin concentrations in heifers were affected by two different feeding levels, with restricted heifers having lower leptin concentrations and reaching puberty later, but at the same live weight as control animals (Luna-Pinto and Cronje, 2000). Leptin concentrations increased during the prepubertal period (Luna-Pinto and Cronje, 2000). When leptin concentrations were aligned retrospectively by week of puberty onset, a peak in leptin concentrations was observed around 2 weeks before puberty onset (Diaz-Torga *et al.*, 2001). Leptin concentrations increased around 2 weeks after an increase in LH concentrations, indicating that changes in leptin concentrations had not affected LH release (Diaz-Torga *et al.*, 2001). Leptin concentrations declined during the postpartum period (Kadokawa *et al.*, 2000; Block *et al.*, 2001). However, in ruminants it is not clear whether leptin has a direct effect on reproduction, particularly whether it affects LH secretion.

It is clear that nutrition has an effect on the onset of puberty, but the magnitude of this effect, and the underlying endocrine mechanisms remain to be determined. In terms of estimating when animals will reach puberty, live weight is a better predictor than age, is easily measurable on farm, and the pattern of live weight gain/loss provides an estimate of the nutritional status of the animal.

Seasonal effects on puberty

The sheep is recognized as a seasonal breeder. Shortening days after the summer solstice signal the onset of the breeding season and animals begin cycling after a period of anoestrum (about 210 days) since lambing in spring (for mature ewes). Season has an impact on pubertal development in sheep. Autumn born lambs attain the appropriate size for puberty during the anoestrus, non-breeding season (spring). However, reproductive cycles do not begin until the normal start of the breeding season in late summer/autumn (Foster, 1981). By reversing the normal photoperiod that autumn born lambs are exposed to puberty occurs at close to the normal age (Foster, 1981).

Cattle are not usually considered to be seasonal breeders, however, season does have an impact on cow reproduction and puberty onset. In the UK animals calving in the spring have a longer post-partum anoestrus interval than autumn calving cows, an effect which remained when differences in body weight at calving were included in the analysis (Lamming *et al.*, 1981). Melatonin implants were administered to spring-born calves for 5 weeks, at around 105 days of age and the effect on puberty onset examined during the following winter and spring. Significantly more melatonin-treated heifers had reached puberty than control heifers at each occasion that animals were examined to evaluate puberty onset (Tortonese and Inskeep, 1992). Age at puberty was reduced in heifers born in spring and early summer if they were exposed to photoperiods of 18 h of light: 6 h of dark during autumn and winter months (Hansen *et al.*, 1983). Autumn born heifers reach puberty at a younger age than their spring born contemporaries (Schillo *et al.*, 1982; Schillo *et al.*, 1983), and late-spring born heifers reach puberty at a younger age than early born heifers (Arije and Wiltbank, 1971). Others have reported the opposite, with spring born heifers reaching puberty at younger ages than those born in the autumn (Hawk *et al.*, 1954; Roy *et al.*, 1980). When comparing age at puberty, care should be taken to ensure that nutrition and live weight gain was equal between the treatments as live weight and nutrition are key determinants of puberty onset. The effect of photoperiod on puberty onset is not clear, but evidence with changes in photoperiod and the use of melatonin implants suggest that exposure to a lengthening photoperiod, after exposure to a short day photoperiod is able to hasten puberty onset (Tortonese and Inskeep, 1992).

2.3.6 Puberty in a seasonal system of dairy production

The common practice on New Zealand dairy farms is to mate heifers at 13-16 months, so that they calve at around 24 months. Heifers usually begin calving a few days before the main milking herd because they are mated slightly earlier, to allow for the longer postpartum anovulatory period in 2-year-old animals (McDougall, 1994).

In order to conceive at 13-16 months heifers must already have reached puberty and be cycling regularly.

There is some evidence to suggest that conception rates are lower at pubertal than at subsequent oestrus events. Litter size in gilts was greater at second oestrus than in at the pubertal oestrus (Robertson, 1951). In ewe lambs, pregnancy rates associated with breeding at puberal oestrus were lower than pregnancy rates of ewe lambs bred at a subsequent oestrus (Hare and Bryant, 1985). Byerley, (1987) found that heifers bred to puberal oestrus had significantly lower conception rates than those bred to the third oestrus following puberty. The occurrence of non-puberal oestrus (NPO) may be responsible for part of the difference in conception rates. Non-puberal oestrus is characterized by behavioural oestrus without luteal function. A total of 25% of heifers in the study of (Byerley, 1987) exhibited NPO. It is preferable for heifers to have already reached puberty prior to the start of the mating period.

It is relatively easy to measure live weight on the farm, and it is a practical way to monitor whether animals are likely to reach puberty at the required age/time. To help farmers achieve high pregnancy rates in heifers, growth targets have been set, to allow comparison between the heifers' actual weights with the targeted weights. Meeting growth targets on time is important in a seasonal production system, to ensure that animals reach puberty well before the start of mating, and continue to grow through their first pregnancy and lactation. Live weight and condition score have an effect on milk production and reproductive performance of cows after their first calving (Chagas, 2003). A well-grown heifer will produce more milk and is more likely to begin cycling, conceive and maintain a 365-day calving interval and remain in the herd. Therefore, well-grown and conditioned heifers are an investment in the future production of the herd.

2.4 Endocrinology of the Oestrous cycle

The oestrous cycle of the cow (*Bos taurus*) is typically around 21 days in length with 60-70% of animals exhibiting oestrus at intervals of between 17 and 25 days (Peters and Ball, 1987). Oestrous cycles are initiated at puberty and occur regularly throughout the lifetime of the female cow. Each oestrous cycle involves follicular development, leading to the ovulation of a follicle. Assuming adequate nutrition and health, oestrous cycles only cease during pregnancy and for a short period following parturition. In seasonal breeding species oestrous cycles only occur during the breeding season. The cow is not considered to be a seasonal breeder. The cycle can be divided into 4 phases for descriptive purposes, see Table 2-2 (Bearden and Fuquay, 1980; Peters and Ball, 1987).

Table 2-2: Summary of the main events of the oestrous cycle, assuming a 21-day cycle length (adapted from (Bearden and Fuquay, 1980)).

Stage of oestrous cycle	Day of Cycle	Main events
Oestrus	Day 1	Receptive to the bull
Metoestrus	Days 2-4	Ovulation and corpus luteum formation
Dioestrus	Days 5-16	Corpus luteum function
Proestrus	Days 17-21	Rapid follicle growth

Oestrus is characterized by certain behaviours, particularly standing to be mounted by other herdmates (or the bull if present), and mounting other oestral animals (Hurnik and King, 1987; Coe and Allrich, 1989). The duration of oestrus behaviour is highly variable, reported as ranging from 2.4 to 27.5 hours (Coe and Allrich, 1989) from 4 to 20 hours (Darwash *et al.*, 2001) and from 1 to 18 hours (Hurnik and King, 1987). Ovulation occurred approximately 25 hours after oestrus began (Chenault *et al.*, 1975) or 12 hours after the termination of oestrus (Allrich, 1994).

The corpus luteum forms after ovulation, from the remaining cells of the ovarian follicle, and begins producing progesterone (Niswender and Nett, 1994). Follicular development continues during what can be termed the luteal phase of the oestrous cycle, but under the influence of progesterone a follicle cannot ovulate (Nanda *et al.*, 1988). If conception has not occurred prostaglandin$F_{2\alpha}$ ($PGF_{2\alpha}$) produced by the uterus causes luteolysis of the corpus luteum (Goding, 1974), ending progesterone production and

leading to the growth of an ovulatory follicle. Increasing oestradiol production as the follicle grows leads to oestrus behaviour, the LH surge and ovulation.

2.4.1 Folliculogenesis

The oestrous cycle cannot be considered without an examination of folliculogenesis, the early stages of which begin in the foetal ovary. During foetal life the number of germ cells in the bovine ovary reaches approximately 2 million. At birth 90-99% of these germ cells have degenerated. Of the viable oocytes left at birth, less than 1% will ever end in ovulation (van den Hurk *et al.*, 1997). The oocytes present at birth are termed primordial follicles; an oocyte surrounded by a single layer of squamous pregranulosal cells (van den Hurk *et al.*, 1997).

Primordial follicles are arranged in non-growing or static pools (Roche and Boland, 1991). Groups of follicles gradually and successively leave the resting pool and begin to grow. Each day a group of around 6 follicles enter the rapid growth phase (Scaramuzzi *et al.*, 1980). Follicle growth can occur from early infancy through to the end of the reproductive period (Gougen, 1993).

Cell division in the granulosa cells surrounding the primary oocyte forms a multilayered granulosa, and the oocyte becomes known as a secondary follicle (van den Hurk *et al.*, 1997). Up to 6 layers of granulosa cells may form; granulosa cells are the major site of hormone production. The thecal cell layer forms in the early stages of secondary follicle development, followed by the formation of the zona pellucida, which separates the growing oocyte and innermost granulosa cell layer (van den Hurk *et al.*, 1997). The development of further cell layers and an antrum follows, after which the follicle becomes known as a tertiary follicle (Bearden and Fuquay, 1980). In cattle, antrum formation begins at a diameter of 200-400 μm. The growth rate of follicles increases after antrum formation (Roche *et al.*, 1998).

During the oestrous cycle, follicle development occurs in a wave like pattern, with two or three waves per cycle (Savio, Boland and Roche, 1990; Fortune *et al.*, 1991). Each follicular wave is characterized by the emergence of approximately 5-7 growing follicles of greater than 5mm diameter (Webb *et al.*, 1994; Gordon, 1996). A single

follicle within each wave is selected as the dominant follicle. The dominant follicle continues to develop, and prevents the formation of a new follicular wave, whilst the subordinate follicles become atretic and regress (Fortune *et al.*, 2001). The dominant follicle can only ovulate when the endocrine environment is suitable, otherwise it too regresses and a new follicular wave begins (D'Occhio and Kinder, 1995). A new follicular wave also develops following ovulation.

2.4.2 Control of the oestrous cycle

The hypothalamic-pituitary-ovarian axis is the platform that controls oestrous cycles and fertility (Nett *et al.*, 1987). The hypothalamus is the ultimate endocrine control centre, releasing gonadotropin releasing hormone (GnRH), a 10 amino acid decapeptide (Ling *et al.*, 1973) into the hypothalamo-hypophysial portal system. Output from the hypothalamus is modified by feedback loops from the pituitary and ovary to provide co-ordinated control of the bovine oestrous cycle. GnRH acts upon the pituitary gland to cause the release of the gonadotropins, LH and FSH into the blood stream (Mol and Rijnberk, 1997). In turn the gonadotropins bind to ovarian receptors to cause follicular development (Mol and Rijnberk, 1997). The actions of both LH and FSH will be discussed in greater detail in the next section.

Secretion of GnRH is episodic throughout the majority of the oestrous cycle, and occurs through the co-ordinated, rhythmic depolarization of GnRH neurons located within the hypothalamus (Karsch, 1987), particularly in the medial preoptic area in sheep (Lehman *et al.*, 1986). The episodic pattern of release may be the result of rapidly changing sensitivity of GnRH receptors. GnRH receptor numbers initially increased in response to continuous GnRH infusion (2.5 μg/h), but then decreased as infusion continued to 12 and 24 hours (Nett *et al.*, 1981). Despite the episodic nature of the secretion of GnRH throughout most of the oestrous cycle, a surge of GnRH occurs to initiate the LH surge and ovulation (Moenter *et al.*, 1990; Moenter *et al.*, 1991).

Oestradiol acts upon the hypothalamus causing the surge of GnRH release (Kesner *et al.*, 1981; Moenter *et al.*, 1990), and upon the pituitary, increasing gonadotropin release in response to GnRH (Reeves *et al.*, 1971), in rats (Aiyer *et al.*, 1974), in monkeys (Knobil, 1974) and in cattle (Kesner *et al.*, 1981). GnRH release during the surge is believed to switch from strictly pulsatile, to an undisrupted pattern of discharge

(Moenter, Brand and Karsch, 1992; Moenter, Brand, Midgley *et al.*, 1992; Caraty *et al.*, 1995). During the GnRH surge LH concentrations increase, peak, then decline prior to the decline in GnRH concentrations (Kesner *et al.*, 1981; Moenter *et al.*, 1991). The LH surge does not end due to a lack of GnRH stimulation (Moenter *et al.*, 1991), but due to the depletion of releasable LH in the pituitary.

During the luteal phase of the oestrous cycle, progesterone acts in concert with oestradiol, suppressing the release of GnRH from the hypothalamus, resulting in a relatively low pulse frequency. Progesterone blockade of oestradiol-induced GnRH secretion was elegantly demonstrated by Kasa Vabu *et al.* (1992). Progesterone concentrations equivalent to those in the luteal phase, induced by implants, blocked the GnRH surge response to oestradiol implants, but exogenous GnRH administration was able to induce an LH surge, despite luteal phase progesterone concentrations. The pituitary is capable of responding to GnRH during the luteal phase, but progesterone acts upon the hypothalamus to inhibit GnRH secretion.

Luteolysis of the corpus luteum releases the secretion of GnRH from progesterone suppression, resulting in an increase in GnRH pulse frequency. In intact ewes average GnRH pulse frequency increased from 0.66 ± 0.14 during the luteal phase to 1.26 ± 0.14 / hour in the follicular phase (Clarke *et al.*, 1987). Oestradiol secretion increases at this time, due to the presence of a growing follicle/s (the follicular phase). Increasing oestradiol triggers behavioural oestrus (Blache *et al.*, 1991) and the GnRH surge (Moenter *et al.*, 1990; Moenter *et al.*, 1991), which in turn triggers the LH surge and ovulation (Kesner *et al.*, 1981; Kesner and Convey, 1982).

2.4.3 The Gonadotropins

Follicle stimulating hormone (FSH)

Follicle stimulating hormone (FSH) is a glycoprotein hormone, released from the gonadotrope cells of the anterior pituitary (Mol and Rijnberk, 1997).

Unlike LH, which is released from gonadotrope cells in acute pulses, FSH is released constitutively and is not stored after synthesis, which implies that rates of FSH secretion are controlled through alterations in rates of synthesis (Farnworth, 1995). Gonadotropin

releasing hormone (GnRH) is a major stimulator of FSH release (Kesner and Convey, 1982), as demonstrated by the cessation of FSH secretion after active immunization against GnRH (McNeilly *et al.*, 1986). Activin has also been found to stimulate FSH secretion (Rivier and Vale, 1991). Oestradiol (Price and Webb, 1988; Kaneko *et al.*, 1995) and inhibin released by the dominant follicle feedback to the pituitary to reduce FSH secretion (Quirk and Fortune, 1986; Beard *et al.*, 1990; Turzillo and Fortune, 1990; Rivier and Vale, 1991; Glencross *et al.*, 1992; Glencross *et al.*, 1994). Oestradiol can also exert positive feedback on FSH secretion, triggering the preovulatory FSH/LH surge (Kesner *et al.*, 1982).

In vitro studies indicate that in cattle FSH is involved in the proliferation and differentiation of preantral granulosa cells (Hulshof *et al.*, 1995). Granulosa cells of preantral follicles possess FSH receptors (Richards *et al.*, 1976). Follicle stimulating hormone is able to induce its own receptors on granulosa cells. Binding of FSH to its receptor stimulates oestradiol production from androgen precursors (Price and Silva, 1999) and oestradiol acts in synergy with FSH to promote LH receptor formation (Richards *et al.*, 1976).

The initial FSH/LH surge at oestrus is followed approximately 24 hours later by a smaller secondary surge of FSH alone (Bleach *et al.*, 2001). Each wave of follicular growth is preceded by a rise in the serum concentrations of FSH (Adams *et al.*, 1992; Sunderland *et al.*, 1994). FSH concentrations increase around 2 days before emergence of the dominant follicle, with peak FSH concentrations occurring around 0.7 days before the emergence of the dominant follicle (Adams *et al.*, 1992). During the growth phase of the dominant follicle, FSH concentrations are low and do not increase again until the regression or ovulation of the dominant follicle.

Follicle stimulating hormone is believed to play a role in the selection of the dominant follicle (Bergfelt *et al.*, 2000; Ginther *et al.*, 2000); for review see (Ginther, Beg *et al.*, 2001). Gibbons *et al.* (1997) showed that any 5mm follicle at the emergence of the wave is capable of becoming the dominant follicle. The future dominant follicle emerges before the future largest subordinate follicle, whilst FSH concentrations are high (Ginther *et al.*, 1997; Kulick *et al.*, 1999) and is almost always the first follicle to reach 8mm diameter (Ginther *et al.*, 1997). The dominant follicle is able to survive the

decline in FSH concentrations, whilst smaller follicles regress (Bergfelt *et al.* 2000), possibly through the acquisition of LH receptors (Ginther, Beg *et al.*, 2001) which appear on the follicle just prior to deviation (Beg *et al.*, 2001).

Therefore, FSH is important in follicular growth, stimulating the emergence of each follicular wave and oestradiol production, there also appears to be a role for FSH in the selection/deviation of the dominant follicle.

Luteinizing Hormone (LH)

The second gonadotropin is luteinizing hormone (LH), also a glycoprotein hormone that is secreted from the gonadotrope cells of the anterior pituitary (Mol and Rijnberk, 1997). The pattern of LH secretion varies during the oestrous cycle (Rahe *et al.*, 1980). LH secretion is normally described by pulse frequency and amplitude. During the early luteal phase low amplitude, high frequency pulses are reported, reducing in frequency and increasing in amplitude in the midluteal phase of the cycle. A high frequency, low amplitude pattern of LH release is resumed prior to the preovulatory LH surge (Rahe *et al.*, 1980).

The secretion of LH is stimulated by GnRH (Mol and Rijnberk, 1997) and modified by ovarian steroids (Kesner and Convey, 1982; Karsch *et al.*, 1983) and factors such as nutrition (Foster and Olster, 1985) and season (Day, Imakawa, Pennel *et al.*, 1986). Ginther *et al.* (2001) reported that progesterone had a negative effect on LH pulse frequency.

Oestradiol has both stimulatory and inhibitory effects on LH secretion. In ovariectomized cows oestradiol administration enhanced LH secretion, so that LH concentrations increased linearly with increases in oestradiol concentration (Kinder *et al.*, 1991). Oestradiol suppression of LH pulse frequency and amplitude has been reported in ovariectomized ewes (Tamanini *et al.*, 1986) and cattle (Price and Webb, 1988). Hobson & Hansel, (1972) found that oestradiol benzoate administration (2 x 400μg injections given 12 hours apart) induced an LH surge in ovariectomized animals. Beck & Convey, (1977) reported that LH concentrations initially decreased, then increased dramatically in response to 17β-oestradiol. In intact cows during the

postpartum period a single 1mg injection of oestradiol benzoate resulted in an LH surge (Nanda *et al.*, 1988).

It is accepted that an increase in oestradiol concentrations is required to induce the preovulatory LH surge (Kesner *et al.*, 1981; Moenter *et al.*, 1990; Moenter, Brand and Karsch, 1992). Whether oestradiol is inhibitory or stimulatory to LH secretion appears to depend on the concentration of oestradiol present. In the oestrous cycle oestradiol does not act alone to inhibit LH secretion. Plasma progesterone concentrations seem to have an inhibitory effect on the oestradiol induced LH surge. During the follicular phase exogenous oestradiol benzoate elicits a normal LH surge in healthy animals. However, animals treated with oestradiol benzoate during the luteal phase of the cycle, or when plasma progesterone concentrations were >0.7ng/ml during exogenous progesterone treatment, failed to respond with an LH surge (Nanda *et al.*, 1988). The absence of progesterone is necessary to allow oestradiol to stimulate an LH surge. Progesterone also reduces LH pulse frequency, probably through actions at the hypothalamus (Tamanini *et al.*, 1986). An increasing LH pulse frequency is necessary for final follicular maturation and oestradiol production (McNatty, 1984). There is a transient rise in LH concentrations around follicle deviation (the selection of the dominant follicle) (Ginther *et al.*, 1999), which coincides with the appearance of LH receptors on the dominant follicle (Beg *et al.*, 2001). After follicle deviation the dominant follicle is dependent upon LH pulses to continue to grow (Ginther, Bergfelt *et al.*, 2001).

2.4.4 Reproductive steroids

Oestradiol

The control of oestradiol production in the ovarian follicle has been comprehensively reviewed, (see (Leung and Armstrong, 1980; Hsueh *et al.*, 1984; Erickson *et al.*, 1985). Production of oestradiol occurs in growing ovarian follicles (Leung and Armstrong, 1980). Both the theca and granulosa cells are involved in oestradiol production. Theca cells are equipped to convert cholesterol to pregnenolone and then to androgen (androstenedione and/or testosterone). The androgen then crosses the basal lamina into granulosa cells and is converted to oestradiol by cytochrome P450 aromatase (Hansel and Convey, 1983; Price and Silva, 1999; Silva, 2000). Thecal cells seem to lack aromatase activity, whilst granulosa cells do not contain the 17α-hydroxylase and

$C_{17,20}$-lyase enzymes that are required to produce androgens from C_{21} precursors, therefore they are reliant on receiving a supply of androgens from the theca cells (Leung and Armstrong, 1980; Hsueh *et al.*, 1984; Erickson *et al.*, 1985). Thus, both cell types are essential for oestradiol production, the two-cell model of steroid production. Luteinizing hormone stimulates theca-cell androgen production (Armstrong and Dorrington, 1977), whilst the production of oestradiol by granulosa cells is stimulated by FSH and insulin (Price and Silva, 1999; Silva, 2000). Oestradiol increases LH and FSH receptor formation on rat granulosa cells (Richards *et al.*, 1976). Oestradiol concentrations in the dominant follicle increase just before deviation (Beg *et al.*, 2002), perhaps promoting the formation of LH receptors to enable the dominant follicle to survive the decline in FSH concentrations around deviation.

Oestradiol is involved in the regulation of gonadotropin secretion, and in the expression of oestrus behaviour (Peters and Ball, 1987). Oestrus behaviour is induced by the actions of oestradiol upon the hypothalamus, after prior exposure to progesterone (Blache *et al.*, 1991). A threshold value of oestradiol induces oestrus; but concentrations of oestradiol above the threshold do not stimulate more intense oestrus behaviour (Coe and Allrich, 1989; Allrich, 1994). In the presence of raised progesterone concentrations, threshold concentrations of oestradiol fail to induce oestrus (Davidge *et al.*, 1987; Fabre-Nys and Martin, 1991).

Late follicular phase concentrations of oestradiol associated with oestrus also induce the preovulatory LH surge, through the actions of oestradiol on the hypothalamus to cause GnRH release (Moenter *et al.*, 1990) and at the pituitary, enhancing GnRH induced LH secretion (Kesner *et al.*, 1981). Oestradiol has stimulatory effects on the expression of the GnRH receptor gene in ewes. These effects are blocked during the luteal phase, possibly by an as yet unidentified factor released by the corpus luteum (Turzillo *et al.*, 1998). The interval from the onset of oestrus to the LH surge has been reported as averaging 3 hours in a frequent sampling regime (Chenault *et al.*, 1975), variation between 6 and 18 hours has also been reported (Martin *et al.*, 1978), but samples were collected less frequently in the latter study.

Progesterone

Progesterone is released from the corpus luteum that forms after the ovulation of a follicle (Niswender and Nett, 1994). Progesterone has inhibitory effects on LH pulse frequency, thought a direct action on the pituitary gland to affect its responsiveness to GnRH (Nett *et al.*, 2002). Progesterone seems to be able to block the positive effects of oestradiol on GnRH receptor gene expression (Turzillo *et al.*, 1998). Progesterone has important roles in the preparation of the uterus for pregnancy, and in the early nourishment of the conceptus (Niswender and Nett, 1994). Continued progesterone secretion is essential in the maintenance of pregnancy (Al-Gubory, 1999). The corpus luteum is the main source of progesterone during gestation in cattle (Estergreen *et al.*, 1967), although the placenta also synthesizes progesterone (Chew *et al.*, 1979). Ovariectomy during gestation results in abortion, generally within days. If ovariectomy is followed by progesterone supplementation the pregnancy can be maintained (Estergreen *et al.*, 1967). In sheep the corpus luteum is only essential until around day 60 of gestation as after this time the placenta produces sufficient progesterone to maintain gestation (Al-Gubory, 1999).

Luteal regression is a key event timing the duration of the oestrous cycle (Lamming and Mann, 1995). Luteolysis is caused by episodic release of $PGF_2\alpha$, in response to the binding of ovarian (luteal) oxytocin to endometrial oxytocin receptors (McCracken *et al.*, 1984). The $PGF_2\alpha$ is released into the uterine-ovarian vein, where, through counter current exchange it is transferred to the ovarian artery, avoiding dilution in the general circulation (Land *et al.*, 1976). The $PGF_2\alpha$ then travels to the corpus luteum (Land *et al.*, 1976). Pulsatile release of $PGF_2\alpha$ begins around day 17/18 of the oestrous cycle in cattle (Kindahl *et al.*, 1976). Oxytocin receptors are elevated around oestrus, decline during the early part of the cycle and then begin to rise again around day 15-17 (Jenner *et al.*, 1991).

The process by which $PGF_2\alpha$ acts to cause luteal regression is beginning to be understood. Injection of $PGF_2\alpha$ is followed by an initial increase, then decrease in luteal blood flow, the initial increase in luteal blood flow may be the trigger of the luteolytic cascade (Acosta *et al.*, 2002). Upon reaching the corpus luteum $PGF_2\alpha$ has been found to up-regulate production of endothelin-1 (Girsh *et al.*, 1996; Ohtani *et al.*, 1998) and angiotensin II (Hayashi and Miyamoto, 1999; Schams *et al.*, 2003). These peptides are

potent vasoconstrictors and are the probable cause of the observed decrease in luteal blood flow (Schams *et al.*, 2003). In cell cultures endothelin-1 has been found to inhibit basal and LH stimulated progesterone secretion by luteal cells (Girsh *et al.*, 1996). Thus, functional luteolysis, that is the decline in progesterone concentrations, is likely due, in part, to a decline in luteal blood flow and direct effects of the peptides endothelin-1 and angiotensin II on progesterone production.

2.4.5 Inhibin, Activin and Follistatin

Inhibin

Inhibin, a dimeric protein hormone, consisting of two dissimilar subunits α and β, is secreted by the granulosa cells of the ovarian follicles (Wrathall and Knight, 1995). Ovarian follicles are the main source of inhibin, but the adrenal glands may also be a production site (Webb *et al.*, 1994). Austin *et al.* (2001) has identified eight molecular weight forms of bovine inhibin in follicular fluid, >160, 110, 77, 58, 49, 48, 34 and 29 kDa. These represent the 6 precursor inhibin forms and the free α subunit (the 29 kDa form). Fully processed inhibin has been reported as a 32kDa (Bleach *et al.*, 2001) or 34kDa molecule (Austin *et al.*, 2001).

A key action of inhibin is the regulation of FSH release by the pituitary gland (Bleach *et al.*, 2001). Steroid-free bovine follicular fluid (bFF) is a major source of inhibin. Administration of bFF causes decreases in serum FSH concentrations during treatment in intact (Bleach *et al.*, 2001) and ovariectomized cattle (Beard *et al.*, 1989; Beard *et al.*, 1990). The repeated injection of bFF suppresses the FSH surge that follows the combined FSH/LH ovulatory surge, and delays the first wave of follicular development relative to saline treated controls (Fortune *et al.*, 1991). The second follicular wave was also delayed in bFF treated animals. The growth of follicles beyond 6mm diameter was prevented during bFF treatment (Bleach *et al.*, 2001). Treatment of ovariectomized prepubertal heifers with purified inhibin also resulted in a decrease in serum FSH concentrations during treatment, but with no effect on LH secretion (Beard *et al.*, 1989). Beard *et al.* (1990) reported that bFF inhibits the expression of the gene encoding the β-subunit of FSH in heifers. Likewise, Mercer *et al.* (1987) reported pituitary levels of FSH-β mRNA were reduced significantly by inhibin in hypothalamo-pituitary disconnected sheep. The combined evidence suggest that inhibin, either purified or in

bFF suppresses FSH secretion, through inhibition of the expression of the β-subunit of FSH.

Inhibin production increases as follicles grow and develop. Plasma inhibin A concentrations increase in association with the growth of the dominant follicle (Bleach *et al.*, 2001). The increasing inhibin concentrations feed back to the pituitary, reducing FSH synthesis and release, and preventing the emergence of the next follicular wave. An inverse relationship between circulating inhibin and FSH concentrations has been reported during the oestrous cycle of a number of species, including cattle, sheep, goats, horses, humans and pigs (Taya *et al.*, 1991; Taya *et al.*, 1996). However, during the preovulatory LH/FSH surge, high inhibin concentrations are associated with high FSH concentrations, as the rapid release of GnRH overrides inhibin inhibition.

Plasma inhibin A concentrations decline precipitously following the LH/FSH surge at ovulation (Bleach *et al.*, 2001). The release of inhibin-suppression of FSH synthesis and secretion results in a second peak of FSH, which occurs shortly after the combined FSH/LH surge. This FSH surge starts of another wave of follicular development.

Inhibin has been found to exert a stimulatory effect on basal and LH-induced androstenedione production in bovine and rat thecal cells, *in vitro* (Hsueh *et al.*, 1987; Wrathall and Knight, 1995). Inhibin-induced increases in androgen production increase the amount of substrate available for follicular oestradiol production. Increasing oestradiol and inhibin production decreases the secretion of FSH, causing atresia of non-dominant antral follicles. The dominant follicle is equipped to survive the decrease in FSH levels though the local action of factors such as IGF-1 (Findlay, 1993).

In cattle, immunization against inhibin and/or oestradiol resulted in significant increases in plasma FSH concentrations throughout the study period, whereas oestradiol immunization alone had no effect on FSH concentrations (Kaneko *et al.*, 1995). Antral follicle numbers increased simultaneously with the increase in FSH concentrations. Immunization against inhibin alone resulted in an increase in FSH and oestradiol plasma concentrations. Inhibin-antiserum administration stimulated the growth of a large number of small, medium and large follicles, probably as a result of the increase in FSH concentrations (Kaneko *et al.*, 1995). Active immunization against inhibin results in an

increase in the ovulation rate of sheep (Wrathall *et al.*, 1992), cattle (Glencross *et al.*, 1992; Glencross *et al.*, 1994), pigs (Brown *et al.*, 1990) and horses (McCue *et al.*, 1992). Although ovulation rates were increased in the study of Wrathall *et al.* (1992), there was no difference in the number of viable lambs produced between immunized and control ewes, due to ova/embryo wastage and a high proportion of small, stillborn lambs. Thus, the potential usefulness of inhibin vaccines to increase fecundity on farm must be questioned, although, there may be a place for inhibin vaccines in IVF programmes to increase the yield of oocytes (Taya *et al.*, 1996).

Inhibin, therefore, seems to have a powerful role in the regulation of FSH secretion, and thecal androgen production. Inhibin is one factor that influences ovulation rate, through inhibition of FSH secretion.

Activin and Follistatin

Activin is another dimeric protein hormone existing as a homodimer (βAβA or βBβB) or heterodimer (βAβB) (Findlay, 1994). Activin is formed in pituitary gonadotropes (Mol and Rijnberk, 1997) and granulosa cells of ovarian follicles (Wrathall and Knight, 1995). Activin stimulates the secretion of FSH from cultured rat pituitary cells (Ling *et al.*, 1986; Vale *et al.*, 1986; Mol and Rijnberk, 1997), (i.e. has the opposing action to inhibin). Incubation of ovine pituitary cells in the presence of activin A caused a dose dependent increase in basal and GnRH-induced FSH release (Muttukrishna and Knight, 1991). Where inhibin has been reported to have a stimulatory effect on GnRH-induced LH release, activin A has an inhibitory effect (Hsueh *et al.*, 1987; Muttukrishna and Knight, 1991). (Shukovski *et al.*, 1991) showed that activin can enhance aromatase activity and oestradiol production in bovine granulosa cells. Within the follicle activin may help to delay atresia and luteinization (Knight and Glister, 2001). Activin has a variety of regulatory actions in other cells (Mather *et al.*, 1992). Both inhibin and activin are part of a large superfamily, including transforming growth factor and mullerian inhibiting substance amongst others (Mather *et al.*, 1992).

Follistatin is a glycoslyated single-chain peptide with FSH-suppressing activity (Robertson *et al.*, 1987). At least 6 forms are known, ranging from 31-39 kDa molecular mass. In bovines the ovarian granulosa cells are the major site of follistatin production (Singh and Adams, 1998). The mRNA levels of follistatin change with

folliculogenesis and atresia (Findlay, 1993). The expression of follistatin mRNA increases as the follicles increase in size (Shukovski *et al.*, 1992). Follistatin expression is greater in dominant than subordinate follicles at all growth phases (Singh and Adams, 1998). Follistatin production is regulated by FSH and activin and its expression is maximal during the mid-growing phase, with levels decreasing progressively through the static and regressing phases (Singh and Adams, 1998).

It is postulated that follistatin alters granulosa cell function in an autocrine fashion, promoting luteinization or atresia. *In vitro* follistatin promoted oxytocin and progesterone production, which are markers of luteinization, in LH stimulated immature bovine granulosa cells (Shukovski *et al.*, 1991). Activin had a dose-dependent inhibitory effect on markers of luteinization in LH stimulated immature bovine granulosa cells (Shukovski *et al.*, 1991). It has been proposed that follistatin acts through neutralization of the effects of activin which possess two binding sites for follistatin (Findlay, 1993). Indeed follistatin binding to activin inactivates the bioactivity of activin *in vitro*, with one follistatin molecule binding to one activin molecule (Kogawa *et al.*, 1991). The addition of follistatin to granulosa cell cultures previously treated with activin reversed the inhibitory effect of activin on oxytocin and progesterone production (Shukovski *et al.*, 1991). Increasing follistatin concentrations as the follicle grows would cancel out the actions of activin on the growing follicle and promote luteinization. Follistatin is also able to bind inhibin, however, the bioactivity of the bound inhibin molecule has not been studied (Mather *et al.*, 1992).

The full interactions between inhibin and its related peptides remain to be elucidated.

2.4.6 Growth Hormone (GH) and the Insulin-like growth factors

Growth Hormone

Large numbers of dairy animals are now being treated with recombinant bovine growth hormone (GH) in the United States of America (Deaver, 1999). Growth hormone receptors are present on some reproductive tissues, including the ovary, although receptor numbers are low (Lucy, Collier *et al.*, 1993), therefore, it is important to evaluate potential effects on the reproductive performance of dairy cattle. As well as direct tissue actions GH binds to the liver to cause the release of IGF-1 (Cohick and Clemmons, 1993; Carter-Su *et al.*, 1996). Often GH and IGF-1 concentrations are

correlated as GH causes IGF-1 release (Chase *et al.*, 1998). Insulin like growth factor-1 is the primary negative feedback regulator of GH, a decrease in IGF-1 concentrations promotes an increase in GH concentrations (Berelowtiz, M.M. 1981). This relationship is disrupted by undernutrition where IGF-1 concentrations fall and the response to GH is attenuated (Clemmons and Underwood, 1991). A similar phenomenon is observed during the postpartum period, with elevated GH concentrations, but low IGF-1 concentrations, probably through a reduction in GH receptor 1A on the liver (Kobayashi *et al.*, 1999). Insulin-like growth factor-1 is also produced locally within the ovary (Cohick and Clemmons, 1993).

Growth hormone administration has been reported to increase the rate of twinning in cattle (Butterwick *et al.*, 1988; Wilkinson and Tarrant, 1991). An increase in the rate of twinning was found only in cows treated intramuscularly (Cole *et al.*, 1991), there was no difference in the twinning rate between cows treated sub-cutaneously and control animals. Others have failed to report an increase in ovulation rate in cattle receiving GH (Gong *et al.*, 1991). Ovulation rate is not increased by GH treatment in non-lactating sheep (Davis *et al.*, 1990), and the response to equine chorionic gonadotropin (eCG) was not different between GH treated and untreated lactating goats during the non-breeding season (Driancourt and Disenhaus, 1997). High milk production is a risk factor for increased twinning in dairy cattle (Kinsel, 1998), thus the increase in twinning noted in some studies may have been associated with an increase in milk production, rather than a direct effect of GH on follicle selection.

Ultrasonography of the ovaries of GH treated cattle has shown an increase in the recruitment of small follicles in non-lactating beef heifers (Gong *et al.*, 1991). This finding was later confirmed (Gong *et al.*, 1997), but only in heifers where GH administration significantly increased IGF-1 and insulin concentrations. In lactating dairy cattle the number of medium sized, rather than small follicles was increased (De la Sota, 1993). During undernutrition the IGF-1 response to GH is attenuated (Clemmons, 1991), thus the follicular response to GH may depend on the lactational or nutritional state of the animals being treated.

Whether GH has an effect on plasma progesterone is not clear. Bovine luteal tissue expresses the GH receptor mRNA and protein (Lucy, de la Sota *et al.*, 1993). Daily

measurement of IGFs difficult, with less than 1% of the total IGF pool believed to be free from IGFBPs (Hossner *et al.*, 1997).

IGF-1 binding sites are present on ovarian follicles (Spicer, Alpizar and Vernon, 1994 b). The number of IGF-1 binding sites on granulosa cells increased with follicle size (Spicer, Alpizar and Vernon, 1994 b). A proliferative action of IGF-1 on small granulosa cells has been demonstrated in cattle (Spicer *et al.*, 1993). FSH-induced progesterone production is enhanced by IGF-1 in granulosa cells (Spicer *et al.*, 1993; Gong *et al.*, 1994). IGF-1 has been found to increase thecal cell numbers in culture, and to synergise with LH to enhance progesterone and androstenedione production (Stewart *et al.*, 1995). In addition, IGF-1 increased LH/hCG receptors in cultured bovine thecal cells (Stewart *et al.*, 1995). IGFBP binding activity is lower in large preovulatory follicles than in large oestrogen inactive and small and medium follicles (Echternkamp, 1994), increasing the amount of bioactive IGF-1 available to bind to its receptor. Concentrations of IGF-1 are higher in the dominant follicle than the second largest follicle (Beg *et al.*, 2001). In particular, IGFBP-2 was almost non-detectable in dominant follicles (Yuan *et al.*, 1998).

It is postulated that increasing the amount of bioactive IGF-1 within the follicle leads to an increase in the number of LH/hCG receptors. IGF-1 increased the number of LH binding sites in bovine thecal cells (Stewart *et al.*, 1995). A negative correlation existed between the amount of IGFBP-2 and IGFBPs identified as possibly IGFBP-4 and IGFBP-5 and the number of thecal LH/hCG binding sites (Stewart, 1996). An increase in the number of LH receptors may allow greater thecal androgen production and, subsequently, greater oestradiol production by the granulosa cells. In support of this notion the oestradiol concentration of follicular fluid in bovine follicles was negatively correlated with the activity of IGFBP-2 and with two IGFBPs identified as possibly IGFBP-4 and IGFBP-5 (Stewart, 1996). Oestradiol and FSH increased the number of IGF-1 binding sites in small, but not large granulosa cells (Spicer, Alpizar and Vernon, 1994).

It is postulated that low serum IGF-1 concentrations reduce the number of LH receptors on follicles in GH receptor deficiency cattle. This may explain the lack of follicular growth during the mid-luteal phase in GH receptor deficiency cattle. LH pulse

frequency is reduced during the luteal phase, when LH receptors numbers are reduced the stimulatory effect of LH on follicular growth is reduced, and follicular growth ceases until LH pulse frequency increases at luteolysis (Chase *et al.*, 1998).

During the decline in serum FSH concentrations, early in the oestrous cycle, an increase in the growth rate and oestradiol secretion of the first dominant follicle has been associated with increased intrafollicular concentrations of IGF-I and markedly reduced concentrations of IGFBP-2 and –3, and total IGFBP (Mihm *et al.*, 1997). Increasing IGF-I and decreasing IGFBP concentrations are likely to be important in the survival of the dominant follicle. This hypothesis is supported by positive correlations between IGF-I concentrations and follicle size and intrafollicular oestradiol activity (Spicer *et al.*, 1988). Also, increased oestrogen activity of follicles was accompanied by a decrease in total IGFBP (Echternkamp, 1994). The increase in IGF-I and the decrease in IGFBPs are likely to be key biochemical events enabling the DF to continue to grow and develop whilst the subordinate follicles regress (Mihm *et al.*, 1997).

It seems clear that IGF-I and the various IGFBPs play a role in the regulation of follicular growth. One key role for IGF-I appears to be the stimulation of LH receptors in thecal cells, an essential developmental step for ovulation to occur.
Future research will enhance understanding of the mechanisms through which the IGFs and IGFBPs modify follicular growth, and may allow a definitive conclusion on the role of growth hormone vs. IGF to be drawn.

2.4.7 Insulin

In vitro culture of bovine granulosa cells has revealed a greater sensitivity to insulin than other species (Spicer, Alpizar and Echternkamp, 1994). Insulin has been identified as mitogenic, stimulating granulosa cells (Spicer *et al.*, 1993) and thecal cell proliferation (Stewart *et al.*, 1995). A stimulatory effect on thecal cell steroidogenesis has also been reported in the presence of LH (Stewart *et al.*, 1995). Insulin also stimulates oestradiol production by granulosa cells (Spicer *et al.*, 1993; Gong *et al.*, 1994). These effects may serve to enhance the ovulatory potential of the dominant follicle, as first wave dominant follicles that ovulate are characterized by their greater steroidogenic output than first wave dominant follicles that failed to ovulate (Beam and Butler, 1997). Gong *et al.* (2002) found that by feeding a diet that increased plasma

insulin concentrations, the interval from calving to ovulation was decreased, but numbers were insufficient to determine whether overall reproductive performance was enhanced

2.4.8 Summary of the major endocrine effects on folliculogenesis

A surge of FSH follows 24 hours after the preovulatory LH/FSH surge (Bleach *et al.*, 2001), and is followed by the emergence of the first follicular wave of the oestrous cycle. Inhibin and oestradiol, released from growing follicles, suppress FSH release, and FSH concentrations decline. An enhanced sensitivity or response to gonadotropins is believed to be a key component in the selection of the dominant follicle (Fortune *et al.*, 2001; Ginther, Beg *et al.*, 2001). Both oestradiol and IGF-1 increase gonadotropin responsiveness and stimulate steroid production of the future dominant follicle (Richards *et al.*, 1976; Spicer *et al.*, 1993; Stewart *et al.*, 1995). The dominant follicle is then able to survive the decline in FSH concentrations, and switch to a dependence on LH (Ginther, Beg *et al.*, 2001). LH receptors appear on the future dominant follicle just prior to deviation (Beg *et al.*, 2001). The appearance of these receptors is coincident with a transient increase in LH concentrations (Ginther *et al.*, 1999). After deviation LH is crucial to continued growth and oestradiol production of the dominant follicle (Ginther, Bergfelt *et al.*, 2001).

Activin and follistatin are two inhibin-related peptides that are involved in follicle development. Within the follicle, activin has a role in delaying the onset of atresia and luteinization (Knight, 1996), whilst follistatin promotes luteinization and atresia (Shukovski *et al.*, 1991), possibly through inactivating the effects of activin. Follistatin concentrations increase as follicles grow (Shukovski *et al.*, 1992) with follistatin binding to and inactivating activin (Kogawa *et al.*, 1991), resulting in the promotion of luteinization or atresia of the dominant follicle. Whether the dominant follicle ovulates (and luteinizes) or undergoes atresia is determined by plasma progesterone concentrations that in turn determine LH pulse frequency.

The first wave dominant follicle develops in the presence of rising progesterone concentrations from the corpus luteum. Progesterone reduces LH pulse frequency, probably through actions at the hypothalamus (Tamanini *et al.*, 1986) and blocks the

oestradiol induced LH surge (Nanda *et al.*, 1988). Thus, the first-wave dominant follicle does not ovulate, and undergoes atresia.

The demise of the dominant follicle leads to a decline in oestradiol and inhibin concentrations and a surge of FSH, followed by the appearance of the next follicular wave. The pattern of development of the second follicular wave is identical to the first until the final stages of development. The endocrine environment whilst the second dominant follicle is actively growing and morphologically and functionally dominant determines whether the follicle will ovulate or not. If progesterone concentrations are declining, due to luteolysis, then the LH pulse frequency increases, stimulating oestradiol production by the growing follicle. If sufficient oestradiol is produced the preovulatory GnRH is triggered (Moenter *et al.*, 1990; Moenter *et al.*, 1991), leading to the LH surge and ovulation (Kesner *et al.*, 1981; Kesner *et al.*, 1982). However, if the corpus luteum is still actively secreting progesterone then LH pulse frequency is insufficient to maintain the growth and development of the dominant follicle, which then undergoes atresia.

Atresia of the second wave dominant follicle is followed by the development of a third follicular wave, the dominant follicle of which will ovulate providing progesterone concentrations are low enough.

2.5 Pregnancy

2.5.1 Maternal recognition of pregnancy

Interferon-τ (IFN-τ) is produced by the trophoblast cells of the preimplantation conceptus from around day 12 to day 25 in cattle and reaches a peak around days 17-19 (Farin *et al.*, 1990). IFN-τ extends luteal lifespan and inhibits pulsatile release of $PGF_2\alpha$, by preventing the end-of-cycle increase in oxytocin receptors in pregnant animals (Wathes and Lamming, 1995). The corpus luteum is therefore able to discharge oxytocin without triggering a luteolytic response in pregnant animals, whilst in nonpregnant animals oxytocin released from the corpus luteum triggers $PGF_2\alpha$ pulses.

Production of $PGF_2\alpha$ occurs in pregnant animals, but the pattern of production is different from that in cyclic animals (Zarco *et al.*, 1988). Concentrations of oxytocin receptors in pregnant animals are lower than in cyclic animals around the time of luteolysis (Sheldrick and Flint, 1985; Jenner *et al.*, 1991; Robinson *et al.*, 1999). The coupling between oxytocin and $PGF_2\alpha$ pulses is disrupted during pregnancy. Fewer pulses of $PGF_2\alpha$ were observed in pregnant ewes, despite similar frequencies of oxytocin pulses in pregnant and non-pregnant ewes (Hooper *et al.*, 1986).

2.5.2 Endocrine environment of pregnancy

Total oestrogen concentrations increase during gestation in the cow, whilst progesterone concentrations increase rapidly during early pregnancy and are relatively constant for most of gestation (Randel and Erb, 1971). Oestradiol 17α increased, whilst progesterone decreased between 34 and 3 days before parturition. Progesterone concentrations dropped rapidly in the two-three days preceding parturition. Total oestrogen concentrations dropped rapidly after parturition (Hunter *et al.*, 1971). Plasma IGF-1 concentrations decreased rapidly around parturition, then gradually increased during the postpartum period (Kobayashi *et al.*, 1999). Leptin concentrations decreased significantly from the pre- to post-partum period (Kadokawa *et al.*, 2000; Block *et al.*, 2001), reaching a nadir 10.1 ± 2.2 days after parturition (Kadokawa *et al.*, 2000).

2.5.3 Gestation length

The gestation of the cow is around 280-285 days (Peters and Ball, 1987). Gestation length is longer in cows carrying male calves (Hunter *et al.*, 1971), and is also affected by the genetics of the dam and sire (Peters and Ball, 1987). Livestock Improvement Corporation, one of the main breeding companies in New Zealand, has been selecting some bulls on gestation length and since 2000 has been offering semen from bulls whose daughters have shorter gestations (www.lic.co.nz).

2.6 The postpartum anoestrous period

Following parturition there is a period during which ovulation and oestrus are absent. The period from calving to the first oestrus is known as the postpartum anoestrus interval(Lamming *et al.*, 1981). During this period of anoestrus, uterine involution occurs and the endocrine patterns return to normal from the elevated progesterone and

oestrogen and low gonadotropin concentrations of late pregnancy. The length of the PPAI is highly variable, depending upon nutritional status, milk yield, milking &/or suckling frequency, environmental factors and health and reproductive disorders (Lamming *et al.*, 1981). Extended postpartum anoestrus intervals are the major cause of infertility in the New Zealand dairy industry, which requires that all cows calve at intervals of 365 days (Macmillan, 2002).

2.6.1 Postpartum anoestrus interval

The first ovulation is often not accompanied by behavioural oestrus; therefore, the postpartum anoestrus interval is often longer than the interval from calving to first ovulation. Savio *et al.* (1990) found 94% of cows showed no sign of oestrus at the first post-partum ovulation. Likewise the first postpartum ovulation of 13/23 cows occurred without behavioural oestrus (Schams *et al.*, 1978). Direct comparisons between postpartum anoestrus intervals and post-partum anovulatory intervals (PPAI) are therefore not appropriate.

In a study of New Zealand pasture-grazed dairy cattle McDougall (1994), reported that PPAI were longer in 2 year olds than cows >3years old (40.2 vs. 27.2 ± 6.2 days, respectively) and longer in Friesians than Jerseys (39.3 ± 3.1 vs. 27.9 ± 2.9 days). PPAI of 28 ± 3.2 days and 31 ± 2.5 days were reported for mixed-age, pasture-fed, New Zealand Holstein-Friesians that had been bred for heavy and light mature weight in New Zealand (Laborde, 1998). In Canadian Holstein Friesian cows the average PPAI was 24.1 ± 1.5 days (Zurek *et al.*, 1995). Using milk progesterone concentrations, measured thrice weekly, the average interval from calving to commencement of luteal activity was 27.9 ± 0.6 days in UK Holsteins (Royal *et al.*, 2000). In the 1970s the interval from calving to luteal activity was reported to be slightly shorter at 24.1 ± 0.63 days in British Friesians and Ayrshires (Bulman and Lamming, 1978).

Nutrition and body condition are two factors that influence the length of the postpartum period anoestrus period (McGowan, 1981; McDougall, 1994). In pasture fed cattle that had been calved for at least 45 days, anoestrus animals had significantly lower condition scores than their cycling counterparts (McDougall, 1993). A significant negative correlation between body condition score at calving and postpartum interval from

calving to the start of luteal activity has been reported (Royal, 1999). The interval to first ovulation was significantly correlated with body condition at calving and the change in body condition from 6 weeks prepartum to calving (Chagas *et al.*, 2002).

During early lactation, high producing cows are unable to consume sufficient energy to meet energy expenditure, since peak milk production is attained prior to maximum feed consumption (Bauman and Currie, 1980). As a consequence, these cows go into negative energy balance and mobilise body tissue reserves to meet the energy deficit. In pasture-based dairying systems the volume of pasture that must be consumed and slow rates of intake can limit energy intakes. Negative energy balance (NEB) is believed to be an important factor affecting the interval from calving to first ovulation. The majority of the variation in energy balance within a herd is due to variation in intake, rather than variation in milk production (Villa-Godoy *et al.*, 1988), and is also related to body condition at calving (Butler, 2000). Overconditioned cows develop more severe NEB and take longer to return to positive energy balance than normal herdmates, probably due to depressed feed intake (Rukkwamsuk, 1999).

A weak correlation between predicted energy balance and days to first ovulation was reported by Lucy (1991), leading to further investigations into this relationship. Number of days to first ovulation was significantly correlated with energy balance and 4% fat-corrected milk yield in the first 21 days post-partum (Beam and Butler, 1998). Cows in which the first post-partum dominant follicle did not ovulate were in deeper NEB than cows in which the dominant follicle did ovulate (Beam and Butler, 1998). The interval from calving to first ovulation was highly correlated with the interval from calving to NEB nadir (Canfield and Butler, 1991). Butler & Smith (1989) reported that maximum negative energy balance occurred around Week 1 to 2 of lactation and cows ovulated an average of 10 days after maximum negative energy balance. A lower nadir of energy balance, larger total energy deficit in early lactation and longer interval to return of positive energy balance were related to a longer interval from calving to start of luteal activity (de Vries, 2000).

Despite variations in energy balance status, cows establish follicular waves and dominant follicles during the early post-partum period (Savio, Boland, Hynes *et al.*, 1990; McDougall and Macmillan, 1993; Beam and Butler, 1997), indicating that

metabolic conditions do not limit the formation of follicular wave or dominant follicles but may affect the ovulatory ability of the dominant follicle (Butler, 1998). Negative energy balance appears to interfere with the hypothalamo-hypophysial axis, preventing the development of the appropriate LH pulse pattern for ovarian follicular development and ovulation (Butler and Smith, 1989). Animals, e.g. cows, and man in deep negative energy balance show an alteration in LH pulsatility, indicating that the release of GnRH is affected (Judd, 1998). Episodic LH peaks increase after the nadir of NEB (Canfield and Butler, 1990; Canfield and Butler, 1991). Luteinizing hormone pulse frequency during the first follicular wave postpartum is higher in cows that ovulate the first dominant follicle (Beam and Butler, 1994). It is postulated that the improvement of energy balance during early lactation may signal the initiation of ovarian activity by allowing the normal function of the hypothalamo-hypophysial axis (Butler and Smith, 1989).

Energy balance has been reported to affect the size distribution of follicles during the first 25 days post-partum (Lucy, 1991). As NEB improved towards zero there was a decrease in the number of small class I and II follicles (3-5 and 6-9 mm diameter respectively) and an increase in class III follicles (10-15mm diameter) (Lucy, 1991). Others have reported no change in the distribution of follicle sizes with energy balance (Beam and Butler, 1997).

IGF-1 and insulin have been proposed as potential endocrine links between metabolic state and the HPA axis (Beam and Butler, 1999). Concentrations of IGF-1 were greater in cows that were in positive than in negative energy balance (Spicer *et al.*, 1990). Cows that ovulated the first wave dominant follicle had greater plasma or serum IGF-1 concentrations than cows where a non-ovulatory follicle developed (Beam and Butler, 1997). Follicular fluid IGF-1 concentrations are 20-30% below plasma IGF-1 concentrations (Spicer, 1991). Concentrations of IGF in serum and follicular fluid are positively correlated in follicles greater than 4mm (Echternkamp *et al.*, 1990). Reduced IGF-I concentrations could lead to failure of final follicular maturation and the LH surge, due to inadequate oestradiol concentrations (Beam and Butler, 1998). IGF-1 declines around parturition (Taylor *et al.*, 2000) and increases gradually in the postpartum period (Spicer *et al.*, 1990). The link between ovarian IGF-I concentrations and energy balance status requires further research.

Insulin was positively correlated with energy balance and is a possible link between energy balance and reproductive function (Lucy, 1991). Insulin receptors are present both at the brain and on ovarian follicles.

As discussed in Section 2.42, leptin is another potential link between energy status and reproduction. Leptin administration to mice in negative energy balance can restore fertility (Barash *et al.*, 1996). Leptin may be one pathway through which energy balance status is conveyed from the peripheral to the central system. However, leptin concentrations did not differ significantly between cows differing in body condition score, body weight and energy balance, although cows that ovulated the first DF tended to have higher leptin concentrations than cows that did not ovulate the first DF (Frajblat *et al.*, 1998). Leptin concentrations decrease significantly from the pre- to post-partum period (Kadokawa *et al.*, 2000; Block *et al.*, 2001), reaching a nadir 10.1 ± 2.2 days after parturition (Kadokawa *et al.*, 2000). Lactating cows had lower leptin concentrations during the postpartum period than cows that calved and were not milked (Block *et al.*, 2001). Leptin concentrations remained relatively constant from parturition to 56 days postpartum when measurements ceased (Block *et al.*, 2001). However, Kadokawa *et al.* (2000) reported that leptin concentrations increased during the postpartum period, and that the interval from parturition to the nadir of leptin was correlated with the interval from parturition to ovulation. This may indicate that changes in leptin concentrations, rather than absolute leptin concentrations are the important signal.

There is evidence to suggest that leptin receptors and leptin receptor mRNA is present in the ovarian tissue of cattle, amongst other species. Bovine granulosa and thecal cells respond to leptin *in vitro* (Spicer and Franciso, 1998), with high affinity leptin binding to granulosa cells (Spicer and Francisco, 1997). Leptin inhibits insulin-induced oestradiol and progesterone production of granulosa cells from both small and large bovine follicles but has no effect on basal or insulin-induced granulosa cell numbers (Spicer and Francisco, 1997). At the lowest concentration (10ng/ml), leptin was effective in decreasing insulin-induced oestradiol production by 30% and progesterone by 58%. Leptin concentrations in prepartum cows of around 7ng/ml (Block *et al.*, 2001), or around 2ng/ml (Kadokawa *et al.*, 2000) have been reported. In pasture-fed

dairy cattle leptin concentrations of around 0.5 – 1.5 ng/ml have been reported during the pre- and immediate postpartum periods (Chagas *et al.*, 2002). Concentrations in postpartum animals were initially lower, but then increased towards prepartum levels. Whether this reflects a negative impact of leptin on reproductive performance, or a normal physiological role for leptin remains to be determined.

Future work may allow the mechanisms linking nutrition and reproductive performance to be more clearly defined, so that nutrition can be manipulated to maximize reproductive performance. From a practical point of view calving cows in good (but not obese) condition (between condition score 5 and 6 in NZ system), and feeding them generously after calving is a good start to achieving a high reproductive performance and milk production.

2.6.2 The first postpartum oestrous cycle

Often the first oestrous cycle after calving is not of normal duration (period between two ovulations). The first oestrous cycle was shorter than normal in 13/23 cows, extended in 3/23 animals and of normal length only in the remaining 7 animals (Schams *et al.*, 1978). Significant differences in the length of the period of progesterone elevation during the first oestrous cycle were recorded. Peak value progesterone concentrations were lower in abnormal than normal length cycles (Schams *et al.*, 1978) consistent with sub-optimal luteal function. Savio *et al.* (1990) found similar variability in the length of the first post-partum cycle. Almost half of all cows (44%) had a long first cycle, whilst 28% had normal and 28% short length cycles. Cycles tended to be short when the first ovulatory dominant follicle was detected later than 20 days post-partum. Similarly, half of all first cycles were long (>25 days) or short (7-17 days) in a survey of 685 UK Holstein-Friesians. The proportion of short cycles decreased significantly from the first to second cycle (Royal, 1999). The overall incidence of long inter-ovulatory intervals was greater for the first two cycles post-partum, resulting in a longer average inter-ovulatory interval during the first two post-partum oestrous cycles (Royal *et al.*, 2000). The first post-partum ovulatory event is often not accompanied by behavioural oestrus, and the subsequent oestrous cycle has around a 50% likelihood of being either longer or shorter than normal.

First service conception rates were 6-7% higher in Jersey and Jersey x Friesian cows that had had at least one pre-mating oestrus, compared to cows that were mated at their first detected oestrus. Extra pre-mating oestrus events had a further small positive effect (+2%) on conception rate (Macmillan and Clayton, 1980). Mating heifers at the pubertal oestrus results in a significantly lower conception rate than mating to the third oestrus after puberty (Byerley, 1987). Cows inseminated within 30 days of calving to their first detected oestrus had a conception rate of 32%, compared to 67% for cows that had been calved for more than 39 days and had more than 1 detected oestrus (Macmillan and Clayton, 1980). It is impossible to determine whether the improvement in conception rate is due to time postpartum, or a beneficial effect of subsequent oestrous cycles. It may, however, be due to effects upon the uterine environment. Uterine involution occurs during the early postpartum period, and, in healthy cows is generally completed around day 30 postpartum (Moller, 1970; Zain *et al.*, 1995). Thus, the uterus of some cows inseminated within 30 days of calving would still be undergoing involution, with ovulation occurring during the period of uterine involution. Work with ewes has shown normal fertilization rates in early post-partum ewes (achieved using laparoscopic intrauterine insemination), but very poor embryo survival after the return of embryos to their uterus of origin (McKelvey *et al.*, 1989). A higher incidence of inadequate luteal function has been observed in ewes induced to ovulate at day 21 vs. day 35 postpartum (Wallace *et al.*, 1989). The increased incidence of short and long cycles, and disrupted luteal phases recorded after first oestrus suggests that corpus luteum function may be subnormal early in the postpartum period. This would be expected to lead to a lower conception rate, probably due to lower embryo survival.

Early re-establishment of oestrus cycles after calving has been associated with improved reproductive performance, relative to animals with delayed resumption of oestrus cycles (Thatcher and Wilcox, 1973; Lucy *et al.*, 1992; Darwash *et al.*, 1997). However, Smith and Wallace (1998) reported that multiparous cows which ovulated before day 21 postpartum had significantly lower all service conception rates, and a reduced percentage of cows pregnant by 100 and 150 days postpartum, no animals were inseminated before Day 42 postpartum. Exposure to progesterone makes the early postpartum uterus of ewes susceptible to infection (Lewis, 2003), thus ovulation early in

the postpartum period could increase the likelihood of a uterine infection and the associated negative reproductive outcomes.

Cows that begin to cycle within a reasonable period after calving (42 days), and that exhibit oestrous cycles of normal length, combined with appropriate oestrous behaviour, have the best chances of conceiving at the appropriate time postpartum.

2.7 Genetics and Fertility

Traditionally, genetic selection has been based almost exclusively on milk production (Pryce and Veerkamp, 1999). The negative genetic correlation between yield and fertility indicates a moderate unfavourable association between these traits (Pryce and Veerkamp, 1999), such that continued selection for yield traits alone will result in a decline in reproductive performance. Fertility traits have relatively low heritabilities, but genetic estimates suggest that there is sufficient genetic variation to allow genetic progress to be made (Pryce and Veerkamp, 1999). Fertility has been included in the Swedish national breeding programme since 1975, and is also included in the breeding programmes of Denmark, Norway and Finland (Lindhe and Philipsson, 1999,), whilst France, the Netherlands, Germany and Israel are now producing genetic evaluations for fertility (Pryce and Veerkamp, 1999).

In New Zealand the system of genetic evaluation was changed to the Breeding Worth (BW) index in 1996 (Montgomerie, 2002). With the introduction of the breeding worth came the inclusion of longevity and animal live weight into the evaluation model, reflecting the fact that live weight influences feed requirements and hence feed conversion efficiency. Fertility was introduced into the BW in May 2002. As of May 2002 the BW comprised 6 traits, with the current effective weighting of each trait (%) in brackets: yield of milkfat (13.3), yield of protein (34), yield of milk (16.8), live weight (18.1), fertility (10.3) and longevity (7.5). The BW for individuals is calculated by multiplying the breeding value by the current economic value of each trait (Montgomerie, 2002). The average fertility BV is worse for Holstein-Friesians than for Jersey sires, but there is significant variability within breeds (Animal Evaluation Unit, 2002).

The inclusion of fertility in the breeding worth calculation will not have an overnight impact on reproductive performance. The evidence provided here would suggest that the increase in the proportion of overseas Holstein-Friesian genetics within the Friesian population might have a detrimental effect on reproductive performance in New Zealand.

2.8 Thesis scope

The substantial introduction of North American and European Holstein-Friesian genetics into New Zealand provides an opportunity to compare the two genotypes.

This thesis will focus on investigating and explaining differences (if any) in reproductive physiology and performance between three different strains of Holstein-Friesian dairy cattle, from approximately 9 months of age to the end of their 2nd lactation, that are being compared in the Dexcel Holstein-Friesian Strain Trial. Traits investigated include the onset of puberty, heifer oestrous cycle characteristics and reproductive performance and postpartum reproductive performance and physiology in lactating 2- and 3-year-old animals.

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3 General Materials and Methods

3.1 Experimental site

3.1.1 Location and climate

Experiments were performed at Number 1 and Number 2 Dairy and at the Grazing Unit, Dexcel, Hamilton, Waikato, New Zealand (latitude 37.783°S, longitude 175.317°E). The Waikato region is home to over 30% of the nation's dairy farmers and over 1,000,000 dairy cattle (Livestock Improvement Corporation, 2003). The Hamilton weather station is located on the Number 1 Dairy. Average rainfall is 1190mm per year (Figure 3-1) with 2009 hours of sunshine per year (National Institute of Water and Atmospheric Research, www.niwa.co.nz/edu/resources/climate/station/). The Waikato has a temperate climate, as illustrated in

Figure 3-2. The highest mean temperatures coincide with the lowest mean rainfall. Pasture growth rates are influenced by both temperature and rainfall. The average pasture growth rates at Number 2 Dairy are presented in Figure 3-3. Annual pasture production is around 17 t DM/ha.

Figure 3-1: The average distribution of rainfall in Hamilton over the past 23 years, by month (http://www.niwa.co.nz/edu/resources/climate/meanrain/rain.xls).

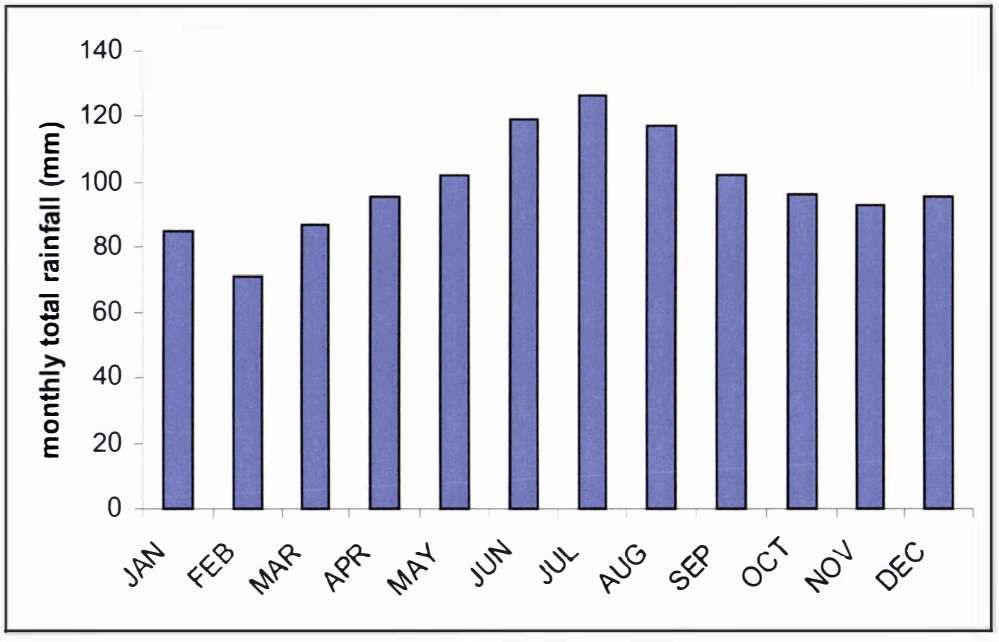
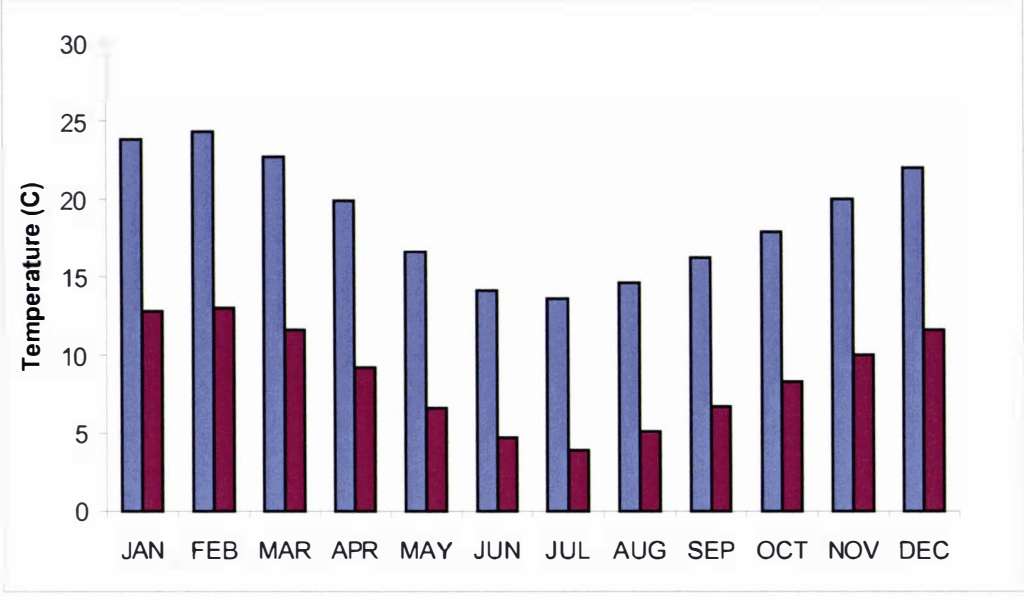


Figure 3-2: Maximum and minimum daily air temperatures for Hamilton over the past 23 years, by month, showing the seasonal variation in temperature, from the National Institute of Water and Atmospheric research, http://www.niwa.co.nz/edu/resources/climate/maxairtemp/. ■ maximum ■ minimum

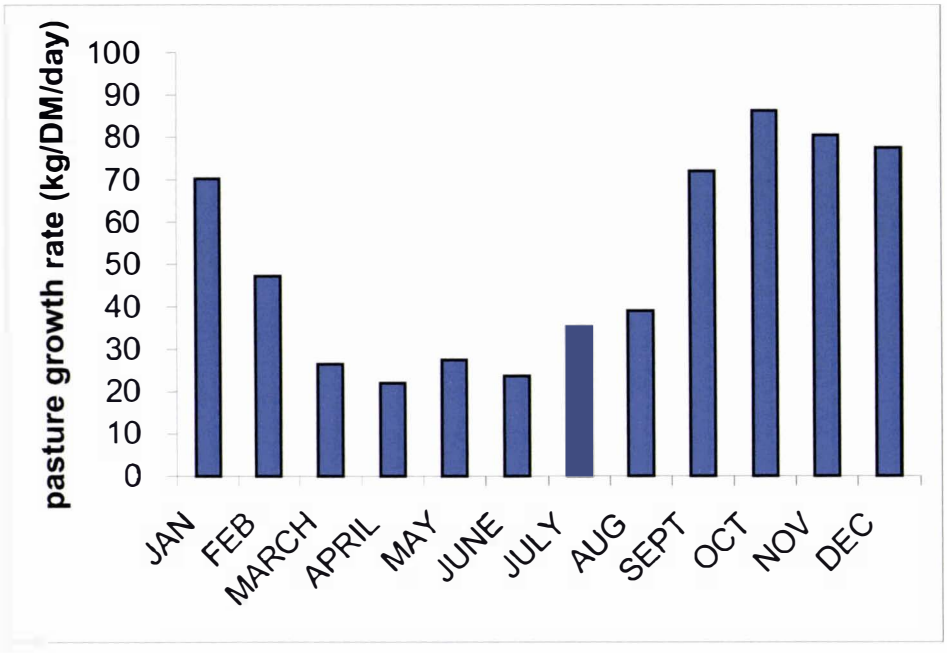


3.1.2 Soils and pastures

Three main soil types at Number 2 Dairy are a peat loam (Te Rapa silt loam), a poorly drained silt loam (Te Kowhai) and a free draining volcanic ash soil (Horotui) (Macdonald *et al.*, 2002). At the Grazing Unit Te Rapa soils predominate, whilst at Number 1 Dairy the dominant soils are Te Kowhai.

The predominant pasture species on all farms are perennial ryegrass (*Lolium perene*) and white clover (*Trifolium repens*).

Figure 3-3: The average daily pasture growth rates from the June 1998 to May 2001 at Number 2 Dairy, by month. Each farmlet had 200 kg nitrogen applied per hectare.



3.2 Experimental animals

Animals in the Dexcel Holstein-Friesian Strain Trial (see below) were used for the majority of experiments. Where strain trial animals were not used, animal details are given in the Materials and Methods section of the particular experiment.

3.2.1 Strain Trial Animals

General description

The aim of the project was to generate three strains of Holstein Friesian animals with different genetic backgrounds. Overseas (OS) animals were on average 90.1 % OS genetics, whilst NZH were an average of 75.4 % New Zealand genetics with a similar predicted breeding worth (BW) as the OS animals (Table 1). The NZL line were 93.3% New Zealand genetics and 90-100 BW points lower than either the NZH or OS lines, with production characteristics representative of the 1970s dairy cow. The first two intakes of strain trial animals (born in 1999 and 2000) were used for experiments in this thesis.

Dam selection

A search of the national database of cow genetic information was undertaken to identify animals suitable as being dams of each strain. Dams were selected firstly on breeding values, with emphasis on milk characteristics and then on pedigree information. Selected animals had to be free of bovine tuberculosis and enzootic bovine leucosis. Dams of OS and NZH had to have at least 3 generations of the appropriate sire breed (i.e. OS or NZ) and be at least 75% OS (or NZ) genetics. The selection criteria were slightly different for dams of NZL animals. These animals had to have a low BW; this tended to be animals that had a high milk fat to protein ratio and had to have less than 10% OS genetics. Breeding worth information for animals that were contract mated is contained in Table 3-1. Dams had to be in-calf when records were examined, and have been herd tested that year. Once suitable animals had been identified the owners were approached. Four animals were mated for every strain calf required.

Table 3-1: Breeding values (BV) and overall breeding worth (BW) of dams that were contract mated to produce the first crop of strain trial animals.

	OS	NZH	NZL
Number	288	250	285
BW	78	86	-10
BV - Live weight	76	49	53
BV- Milk	1049	952	457
BV- Fat	37	39	14
BV- Protein	44	36	7

Treatments: overseas (OS), New Zealand high (NZH), New Zealand low (NZL)

Sire selection

OS – 17 sires were represented, with the number of daughters ranging from 1 to 18; the sires used had a high BW (Table 3-2), and were of 100% OS origin.

NZH – 13 sires were represented, with the number of daughters ranging from 1 to 30 per sire. The sires used had a high BW and similar milk characteristic breeding values to the OS strain (Table 3-2) and a high proportion (usually at least 75% New Zealand genetics) of New Zealand genetics.

NZL – 13 sires were represented, these sires were bulls that were used commercially during the late 1960s and 1970s, and who had stocks of semen stored for experimental use. By today's standards the BW are low (Table 3-2; although these bulls were assessed in breeding index, which was chiefly related to fat production).

All mating options were assessed for co-efficient of inbreeding, to avoid a depression in reproduction/production traits through inbreeding. The sires that were used for the NZH line are in part the same as the ones that Moorepark in Ireland has used. The sires that were used were the same for the 1999 and 2000 born crop of heifers. All heifers were DNA tested to verify parentage and those that failed the parentage test were excluded from the trial.

Heifer rearing

Animals were collected and brought to Dexcel at between 4 and 15 days of age.

Calves were fed colostrum or whole milk and a calf starter ration (compound feed) to achieve growth rates of around 0.7kg/day for NZ and around 0.8 kg/day for OS strains. Barley straw was introduced from Day 7 onwards, to assist with rumen development. Calves were housed in groups of 20-40, until deemed sufficiently robust to be managed outdoors, usually by 2-3 weeks of age. Calves were weaned at between 8 and 10 weeks of age, when they were eating sufficient pasture and compound feed to maintain satisfactory growth rates. NZ strain animals weighed a minimum of 71 kg at weaning, and OS animals 79 kg. After weaning heifers grazed pasture with compound feed supplementation for at least 4 weeks. Silage and compound feed were reintroduced when insufficient pasture was available to meet target growth rates. Target live weights at 6, 12, 18 and 24 months are shown in Table 3-3.

The first intake of heifers (183 animals) was grazed in four herds, in adjacent paddocks: one herd of each strain, plus a preferentially fed herd of animals that were behind growth targets. The second intake of heifers was smaller (73 animals) and was grazed as one herd.

Table 3-2: a) Breeding values for traits incorporated into the breeding worth index in sires of the 1999 and 2000 strain crop animals, as at October 2002.

	Fat	Protein	Volume	Live weight	Survival	Longevity	Fertility
OS	33.34	44.32	1317.85	93.69	-26.63	-266.32	-2.35
NZL	6.2	5.22	387.46	40.99	7.35	73.54	0.74
NZH	33.74	35.39	953.01	50.44	-5.65	-56.53	-1.48

b) Breeding worth, and percentage North American Holstein genetics of sires of the 1999 and 2000 crop strain animals as at October 2002.

	BW ($)	% North American Holstein genetics
OS	109	100
NZL	-24	0
NZH	131	26.6

c) Predicted overall breeding worth (BW), and individual trait breeding values (BV) of strain trial animals when the contracts to produce the first intake of animals were issued.

	OS	NZH	NZL
BW	84	86	-10
BV – Live weight	84	48	49
BV- Milk	1268	999	440
BV- Fat	38	39	10
BV- Protein	47	37	7

Treatments: overseas (OS), New Zealand high (NZH), New Zealand low (NZL)

Table 3-3: Live weight targets for New Zealand (NZ) and overseas (OS) strain heifers, weights in brackets exclude live weight gain associated with pregnancy.

	NZ strains	OS strain
6 months	162 kg	179 kg
12 months	256 kg	276 kg
18 months	380 kg (375 kg)	425 kg (420 kg)
24 months	528 kg (458 kg)	578 kg (508 kg)

Lactating animals

On 1 June, pregnant heifers were moved from the Grazing Unit to Dexcel Number 2 Dairy, an established farmlet grazing facility. The three genotypes were run in 11 farmlets, 4 farmlets each of OS and NZH animals and 3 farmlets of NZL animals. Each farmlet was balanced for geographic location, soil type and pasture type. Each farmlet had 150kg of nitrogen fertiliser per hectare applied annually, with an expected annual pasture production of 17.0tDM/ha. The farmlet herds were balanced for genetic merit, calving date, live weight and age within strain. One herd was randomly allocated to each farmlet. The number of animals per farmlet was increased in Year 2 of the trial. Table 3-4 illustrates the age structure of the herd in Years 1 and 2. Where insufficient animals of a particular strain were available animals of a similar age were substituted into the herds; data from these animals were excluded from analysis.

Table 3-4: Age structure of the herds in Years 1 and 2 of the Dexcel Holstein-Friesian strain trial.

		OS	NZH	NZL
Year 1	All 2 year olds	17	17	12
Year 2	2 year olds	5	5	4
	3 year olds	15	15	11

Treatments: overseas (OS), New Zealand high (NZH), New Zealand low (NZL)

Each genotype was run under a range of feeding systems, to provide from moderate to generous annual feed allowances as shown in Table 3-5. Feed allowance was manipulated by altering stocking rate and supplementary feed inputs. Maize silage was the supplementary feed used, with copra meal added to maintain the crude protein concentration in the diet (pasture and supplement) above 14%. Maize silage was added to the 6, 6.5 and 7 t DM per cow systems at a rate of 500 kg, 1 tonne, and 1.5 tonnes per cow respectively in year one of the trial. In the second year of the trial, up to 500kg of maize grain was fed from calving in the highest fed herd (see Table 2-1) of each genotype and the amount of maize silage was reduced by 500kg. Farmlets were managed according to the decision rules of Macdonald and Penno (1998).

Table 3-5: Feed allowances of the herds expressed as a) tonnes of dry matter per cow, per year and the associated herd number, and b) as kg live weight per tonne of dry matter.

a)	Feed allowance (t DM/cow/year)					
	4.5	5	5.5	6	6.5	7
NZL	1		2	3		
NZH		4	5	6	7	
OS			8	9	10	11
b)	Kg live weight/ t DM					
NZL	111		91	84		
NZH		100	91	84	77	
OS			100	92	85	79

Treatment: overseas (OS), New Zealand high (NZH), New Zealand low (NZL)

3.3 Sample and data collection procedures

3.3.1 Blood sample collection

Blood samples were collected from each cow by coccygeal venipuncture into heparinised blood collection tubes (Vacutainers, Beckton, Dickson and Company, USA). After collection, samples were placed into ice water and centrifuged (12 minutes at 1831g) within 2 hours. Plasma was harvested and stored at -20°C until analysis. For determination of metabolite concentrations samples were collected without anticoagulant and were allowed to clot overnight at room temperature. Samples were then centrifuged for 12 minutes at 1831g, and the serum harvested and stored at -20° until analysis.

3.3.2 Milk samples

A representative sample of whole milk was collected on Tuesdays and Fridays for twice weekly sample collection, or on Mondays, Wednesdays and Fridays during thrice weekly sample collection. Samples were collected into milk meters in the dairy, then processed through a divider where a 30ml sub-sample was collected into 30ml plastic containers (Techno-plas, Australia), with the addition of a potassium dichromate tablet (Merck, Darmstadt, Germany). At the end of milking all samples were inverted to mix

the sample thoroughly. Samples were stored at between 2 and 4°C, in the dark, for up to 7 days before analysis.

3.3.3 Ultrasonography

Ovarian structures were examined daily following the morning milking using a linear array probe (7.5 mHz) with an Aloka DX210 scanner (Medtel, Auckland, New Zealand). Follicle sizes were estimated by averaging the horizontal and vertical diameters estimated using the measurement function of the ultrasound display. Follicle diameters measured by ultrasonography are 2-3mm smaller than measurements of dissected follicles, as ultrasonography measures only the antrum of the follicle and excludes the follicle wall (Sirois and Fortune, 1988).

3.3.4 Live weight and condition score data

Live weights were measured using load-cell scales (Trutest, NZ Ltd). Condition scores were assessed by experienced staff on a 10 point scale (1= emaciated, 10 = obese; Macdonald and Macmillan,(1993).

3.4 Hormone Assays

3.4.1 Oestradiol

Concentrations of oestradiol in plasma were determined using a modified method for the Serono Estradiol MAIA assay kit (Biodata S.p.A., Montecelio, Italy). Briefly, 200ul aliquots of plasma are extracted into diethyl ether by mixing for 15 min. After freezing in an ethanol/dry ice bath the solvent layer containing the oestradiol was decanted and the solvent evaporated off in a fume hood. Samples were then reconstituted in a phosphate buffer (0.1 M PBS, pH 7.0). Lyophilized (I^{125}) oestradiol was added to tubes, including standards and controls that were then incubated for 1 hour at room temperature. Iodinated oestradiol was added to each sample and incubated for a further 3 hours. A second antibody (covalently bound to magnetic particles) was then added to each tube, mixed and incubated for 20 minutes. Tubes were then placed in magnetic racks and the supernatant decanted off to separate the bound and free antibody. Samples were counted for 2 minutes on a gamma counter (Wallac 1470 Wizard, Perkin

Elmer Life Sciences). This method has been validated for use in cattle (Prendiville *et al.*, 1995).

3.4.2 Luteinizing Hormone (LH)

Concentrations of LH in plasma were determined using a double antibody radioimmunoassay as described by (McDougall, Williamson *et al.*, 1995). LH (ovine LH, batch number AFP 8614B) for iodination and the standards were obtained from the National Hormone and Peptide Program, California, USA (courtesy of Dr Parlow). LH was iodinated using the Chloramine T method (see Appendix A for a description of the method).

The primary antibody, raised in a rabbit against highly purified ovine LH (NIAMDD-oLH-24), was used at an initial dilution of 1:60 000 (and around 12,000 counts per minute) and obtained from Tim Manley at Agresearch Mosgiel. The antiserum exhibited low (<1%) cross reactivity with ovine and cervine growth hormone and bovine thyroid stimulating hormone and little reactivity (<0.1%) with ovine FSH or ovine prolactin. The secondary antibody (sheep anti-rabbit) was raised in-house and used at a dilution of 1:80. Briefly, on day 1 standards and controls (100µl standard or control, 100µl filler plasma and 100µl protein buffer), samples (100µl sample and 200µl protein buffer) have primary antibody (100µl, at a 1:60,000 dilution) added and were incubated for 24 hours at room temperature. Iodinated LH was added the next day (100µl) and incubated for a further 24 hours at room temperature. The second antibody was added on day 3 (100µl) and tubes were incubated for at least 18 hours at 4°C. Finally, 1ml of wash buffer was added, tubes are centrifuged at 4°C, the supernatant decanted and tubes were counted for 1 min on a gamma counter (Wallac 1470 Wizard, Perkin Elmer Life Sciences).

3.4.3 IGF-1

IGF-1 concentrations in extracted bovine plasma were determined using a double antibody radioimmunoassay at the University of Western Australia, Perth.

Recombinant human IGF-1 hormone for iodination was obtained from Bachem, California, USA, batch number ZO 206. The primary antibody, human anti-IGF-1 was obtained from Dr Parlow at the National Hormone and Peptide Program, California,

USA, batch number AFP 4892898. The second antibody was Donkey anti-Rabbit sera (DARS), with normal rabbit sera (lot number 42371, IDS, UK). The primary antibody was iodinated using the Chloramine T method.

The first day of the assay involved the extraction of plasma IGF-I using the acid-ethanol cryoprecipitation method (Breier *et al.*, 1991). All overnight incubations were at 4°C. On the second day 100µl of standard, controls and samples and 100µl of primary antibody (1:130,000 dilution, between 10,000 and 15,000 counts per minute) was added to the tubes and incubated overnight. On Day 3 100µl of iodinated IGF-I tracer was added and the tubes incubated overnight. On day 4 the second antibody, 100µl of DARS (1:10 dilution) was added, then 1ml of 5% polyethylene glycol was added to separate the free IGF-I antigen from the bound and samples incubated overnight. On day 5 samples were centrifuged and the supernatant decanted off, leaving variable amounts of tracer antigen and sample antigen attached to the primary antibody. Tubes were counted for 1 min on a gamma counter

3.4.4 Growth Hormone

Concentrations of GH in plasma were determined using a double antibody radioimmunoassay that has been validated for bovine use by Alpha Scientific Ltd, Hamilton, New Zealand. GH (bovine GH, batch number AFP 9884 C) for iodination and the standards, and also the primary antibody of rabbit, anti-Ovine GH-3 (AFP 0802210 Rb) was obtained the National Hormone and Peptide Program, California, USA courtesy of Dr Parlow. The GH was iodinated using the Iodogen method (see Appendix A for a description of the method).

The secondary antibody (sheep anti-rabbit) was raised in-house. Briefly, on day 1 standards and controls (100µl standard or control, 100µl filler plasma and 100µl protein buffer), samples (100µl sample and 200µl protein buffer) had primary antibody (100µl, at a 1:130,000 dilution, 10,000 to 15,000 counts per minute) added and were incubated for 24 hours at room temperature. Iodinated GH was added the next day (100µl) and incubated for a further 24 hours at room temperature. The second antibody was added on day 3 (100µl, 1:80,000 dilution) and tubes were incubated for at least 18 hours at 4°C. Finally, 1ml of wash buffer was added, tubes were centrifuged at 4°C; the

supernatant tipped off and counts per minute determined on a gamma counter (Packard Cobra-II, Auto Gamma).

3.4.5 Insulin

Insulin concentrations in plasma were measured in a double-antibody radioimmunoassay as per the method of Tindal *et al.* 1978 in the Animal Science Laboratory, University of Western Australia. Highly purified crystalline bovine insulin obtained from Sigma, Australia and then dissolved in dilute HCl, pH 2.5 to 3 was used. The primary antibody was donated by Dr. Peter Wynn (CSIRO Division of Animal Production, NSW, Australia) was raised against bovine insulin (BI 4499, Ely Lilly Pty Lt, Australia) in guinea pigs. The secondary antibody was donkey anti-guinea pig serum.

On Day 1, duplicate 100 µl of samples and standards were incubated with 300µl of buffer that included 0.25% BSA, 50µl of primary antibody (1:40,000 dilution, 10,000 to 15,000 counts per minute) was then added. Tubes were incubated at 4°C overnight. The next day 50µl of tracer was added, tubes were vortexed and incubated for 48 hours at 4°C. On Day 5, 100µl of secondary antibody (1:4 dilution) and 100µl of normal guinea pig serum was added and the tubes vortexed and incubated overnight at 4°C. Finally 1ml of 2% polyethylene glycol 6000 (Sigma, St Louis, MO, USA) was added to all tubes (except TCs) and tubes were centrifuged at 1500 x g for 25 minutes at 4°C (Beckman, J-6M/E, USA). The supernatant was then decanted and pellets left to dry overnight before the activity of the precipitate was determined on a gamma counter (Packard Cobra-II, Auto Gamma).

3.4.6 Leptin

Leptin concentrations in plasma were measured in a double-antibody radioimmunoassay, in the Animal Science Laboratory, University of Western Australia according to the method of Blache *et al.* (2000). Bovine recombinant leptin was obtained from Dr. Ross L. Tellam (CISRO Tropical Agriculture, Indooroopilly, Queensland, Australia). The primary antibody was raised against the bovine recombinant leptin described above, in a male emu (*Dromaius novaehollandiae*) in the Animal Science Laboratory, Faculty of Agriculture, University of Western Australia by

Dr. Dominique Blache and Mrs. Margret Blackberry. The secondary antibody was sheep anti-emu and was also raised at the University of Western Australia.

On Day 1, duplicate 100 µl of samples and standards were incubated with 50µl of primary antibody (1:5000 dilution) and 50µl of normal emu serum (1:500 dilution). Tubes were incubated at 4°C overnight. The next day 50µl of tracer (^{125}I-b/o-leptin, 10,000 counts per minute) was added and incubated for 48 hours at 4°C. On Day 5, 100µl of secondary antibody (1:12 dilution) was added to precipitate the antibody-antigen complex and the tubes incubated for 48 hours at 4°C. On Day 7 1.0ml of 3% polyethylene glycol 6000 (Sigma, St Louis, MO, USA) in buffer was added to all tubes, except the TCS, before centrifugation at 2000 x g for 30 min at 4°C (Beckman, J-6M/E, USA). The supernatant was then decanted and pellets left to dry overnight before the activity of the precipitate was determined on a gamma counter (Packard Cobra-II, Auto Gamma).

3.4.7 Plasma and milk progesterone

Radioimmunoassay

Concentrations of progesterone in plasma were determined using a kit (Coat-a-Count, DPC, California, USA). The assay tubes came coated with rabbit antibodies to progesterone and 100µl samples were added to the tubes. Iodinated progesterone (tracer, 1ml) was also added and tubes were incubated at room temperature for 3 hours. Supernatant was then tipped off and counts per minute determined on a gamma counter. This method has been validated for use in cattle (Dieleman and Bevers, 1987).

Enzyme-linked immunosorbant assay

Whole milk samples were analysed for progesterone concentrations using a microtitre plate enzyme-linked immunosorbant assay (ELISA) kit (Ridgeway Science Ltd, Alvington, Gloucestershire), validated by Sauer *et al.* (1986).

Samples were removed from the refrigerator at least 2 hours before analysis and warmed to 20°C. Samples were thoroughly mixed and a 200µl subsample pipetted into a dummy plate. The plates are also brought to room temperature, the foil stripped from

the wells and the wells emptied and tapped dry. To each well 10µl of sample or standard was added (0.5 to 15ng/ml), followed by 200µl of progesterone-enzyme label (progesterone-11-α glucuronide-alkaline phosphatase). The plate was then vortexed and left for 1- 1.5 hours at room temperature.

After incubation the plate was washed three times with cold water and tapped dry each time. Finally 200µl of substrate in substrate buffer was added, and the plates vortexed again and left for the colour to develop. The samples were read on a 570nm plate reader, the time of reading depending on the appearance of the standard curve and the value of the blank, which needed to be around 1.5 (absorbance at 470 nm), this could take as long as an hour.

Quality control samples could not be stored for the entire 2-year period of the trial. When quality control samples began to deteriorate new quality control samples were selected from previously analysed samples. Therefore, the concentration of quality control samples varied from batch to batch.

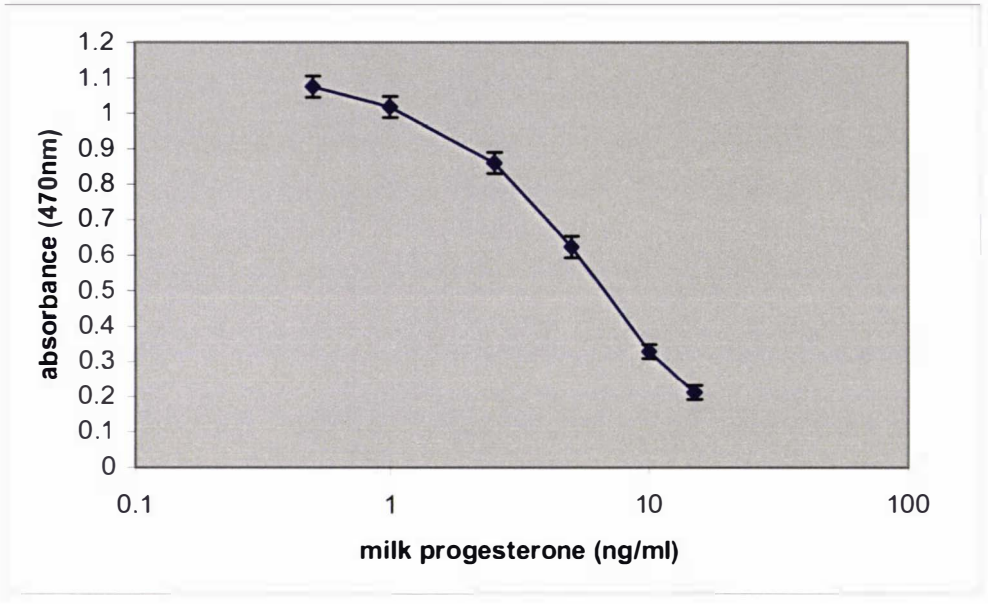
A composite standard curve (Figure 3-4) was constructed from standard curve data from one plate from each week of the analysis period over the two years of the trial. The limit of sensitivity of this assay (defined as twice the standard deviation of the optical densities of the blank wells) was 0.23 ng/ml. To detect the onset of luteal activity such precision is not required, with 2-3ng/ml the concentration of interest. Therefore the lowest standard used on the standard curve was 0.5 ng/ml and the highest standard 15 ng/ml. The intra-assay coefficients of variation for quality control standards were 12% for an average concentration of 1.24ng/ml, 12.5% for an average concentration of 2.5 ng/ml and 8.5% for an average concentration of 8.7ng/ml. Examples of three individual cow progesterone profiles are found in Appendix B.

3.4.8 Validation of the Ridgeway Milk Progesterone ELISA

Progesterone concentrations can be determined by radioimmunoassay (RIA), or enzyme immunoassay (ELISA). The aim of this trial was to validate the Ridgeway Sciences ELISA for use in the Dexcel Laboratory, and to compare the results from this test with those of a previously validated RIA (Coat-a-Count, DPC, California).

Ethical approval for this trial was granted by the Ruakura Animal Ethics Committee, (approval number 4210). Milk with a low endogenous progesterone concentration (as determined by analysis with RIA) was used to make up standards for use in the remainder of the experimental protocol for the ELISA assay. Standard values of 0.5, 1, 2.5, 5, 10 and 15ng/ml were used. Standards were analysed at a variety of positions on the ELISA plate to test for the presence of edge effects.

Figure 3-4: Composite standard curve. Each point is the average of 40 plates (one plate per week of the analysis period over the two years of the trial), plus or minus the standard error.



Milk samples were collected from 6 pregnant animals and 22 postpartum Holstein-Friesian dairy cattle at a morning milking. The sample collected was a representative sample of the morning's milk production. Two subsamples of each sample were collected into 35 ml containers, one containing a potassium dichromate tablet (Merck, Damstadt, Germany), the other without preservative.

The samples were analysed in duplicate by RIA to determine concentrations of progesterone. The same samples were also analysed by ELISA, in triplicate. Five and seven days later all preserved samples were again assayed by ELISA, in triplicate.

Progesterone was added to milk with a low progesterone concentration to provide a range of 2, 3, 4, 8 and 12 ng/ml. Samples were analysed by RIA, in duplicate, and by ELISA in triplicate.

Milk samples (n=9) with a progesterone concentration of greater than 6 ng/ml were selected (based on RIA results) and diluted serially with milk of very low progesterone concentration. Concentrations of progesterone were determined by ELISA in triplicate, and by RIA, in duplicate. Twelve ELISA plates were used during the trial.

Results

No edge effects were apparent in samples analysed using the ELISA.

Figure 3-5 illustrates the relationship of results from preserved samples analysed by RIA and ELISA. Concentrations of progesterone determined by ELISA were higher than concentrations determined by RIA, particularly at higher progesterone concentrations.

At concentrations of less than 1.5 ng/ml as determined by RIA, the ELISA reads only slightly higher, on average 0.153 ng/ml. At RIA values of between 1.5 and 2 ng/ml the average over-estimate is 0.618 ng/ml, which increases to 0.803 ng/ml for RIA values between 2 and 6 ng/ml. At RIA values over 6 ng/ml the ELISA reports concentrations an average of 3.465 ng/ml higher than the RIA.

Figure 3-5: The relationship between preserved samples analysed by either radioimmunoassay (RIA) enzyme-linked immunosorbant assay (ELISA).

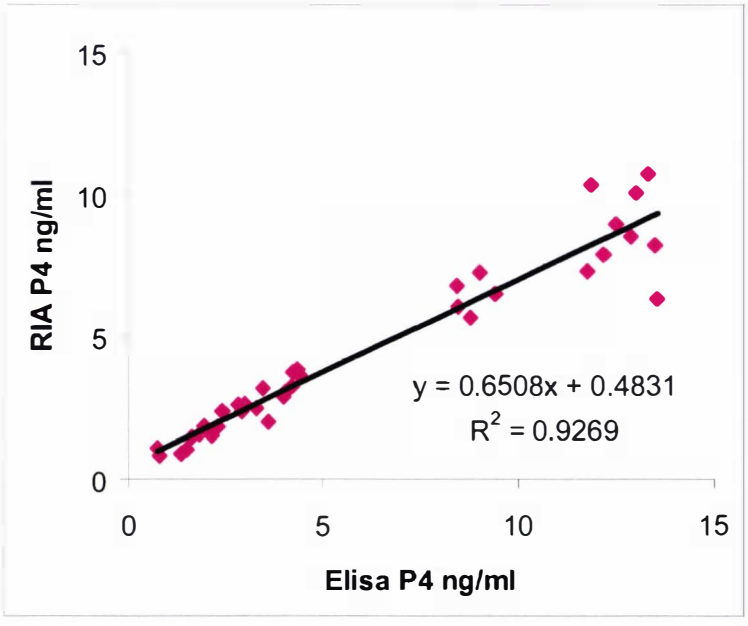


Figure 3-6 illustrates the relationship of preserved and unpreserved samples analysed by RIA. For concentrations of less than 2 ng/ml the average difference between preserved and unpreserved samples was almost zero. There were only two values recorded between 2 and 6 ng/ml therefore an estimation of the difference between preserved and unpreserved was not made. For samples that read above 6 ng/ml, preserved samples read an average of 0.79 ng/ml below unpreserved samples.

Figure 3-6: The relationship between milk progesterone concentrations determined by radioimmunoassay (RIA) in preserved or unpreserved whole milk samples.

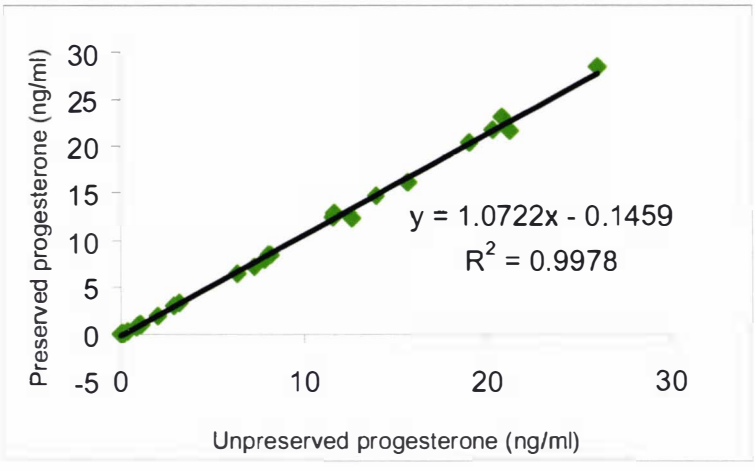
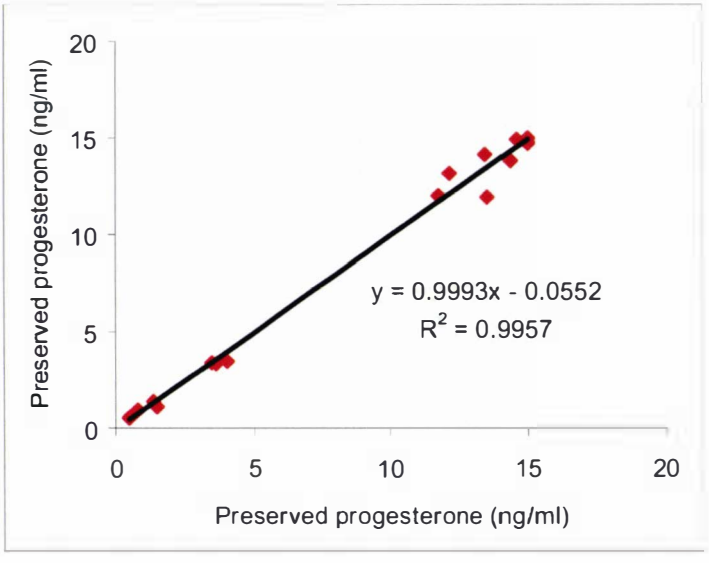


Figure 3-7 illustrates the relationship of preserved and unpreserved samples analysed by ELISA. The working range of the assay is around 0.5 to 15ng/ml, samples that fall outside this range are reported as <0.5000 or >15.00 ng/ml respectively. Samples that recorded the above concentrations (at the limit of detection of the assay) were excluded from the following analysis. The difference between preserved and unpreserved samples was small and inconsistent. Overall, unpreserved samples read slightly higher (approaching 0.1 ng/ml).

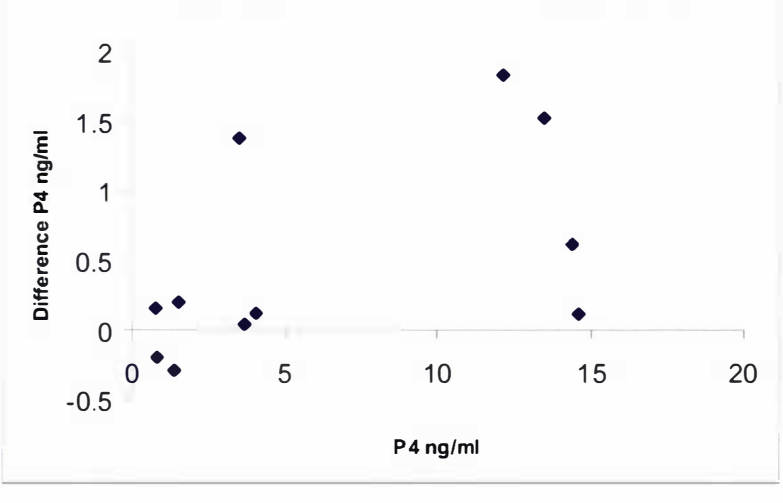
Figure 3-7: The relationship between milk progesterone concentrations determined by enzyme-linked immunosorbant assay (ELISA) in preserved or unpreserved whole milk samples.



Sample age effects

Figure 3-8 demonstrates the difference in recorded concentrations between samples analysed on the 13th and 20th June. There was little difference in progesterone concentration with sample age for samples with a concentration of less than 5ng/ml. At the upper end of the scale there was a tendency for older samples to have higher progesterone concentrations, but this may be related to the variability of the assay at this level. For concentrations around the cut-off value for luteal activity (currently 3ng/ml) there did not appear to be a consistent difference in progesterone concentrations between samples analysed either the day after collection, or a week after initial analysis.

Figure 3-8: The difference in progesterone concentrations between samples analysed on the 13th of June, vs. the same samples analysed again on the 20th June.



Parallelism

Data were $\log_e$ transformed and analysed in Genstat. Serial dilution resulted in significantly non-parallel lines in samples analysed by ELISA. When three cows with starting values that were above the sensitivity of the assay were excluded parallelism was demonstrated. There was no evidence to suggest that the lines were not parallel. In the same samples analysed by RIA there was no evidence to suggest that the lines were non-parallel.

Spikes

Figure 3-6 shows the mean recovery of spikes of 12, 8, 4, 3, and 2 ng/ml as determined by RIA and ELISA. The recovery of the RIA was relatively constant at all levels. The ELISA had greater recovery than the RIA, which may be related to the ELISA tending to read higher than the RIA.

Table 3-6: Mean recovery of spikes of progesterone added to whole milk samples after analysis by radioimmunoassay (RIA) or enzyme-linked immunosorbant assay (ELISA).

	RIA	ELISA
Expected concentration (ng/ml)	Recovery % (sed = 3.467)	Recovery % (sed = 3.467)
12	80.98	105.92
8	80.42	110.12
4	87.27	106.66
3	82.81	96.16
2	82.36	98.69

3.4.9 Conclusions

It is reasonable to use the ELISA to determine the onset of luteal activity, and to monitor luteal activity during the postpartum period. However, using ELISA values in analysis carries risk, as it is more variable at the top end of the assay, for values above 6 ng/ml with the current standard curve.

It was concluded that the ELISA was best suited to determine the onset of luteal activity, but that for accurate measurement of higher concentrations the RIA was preferable.

Therefore, the use of an ELISA is recommended when values are used to determine luteal activity, but not to monitor differences in progesterone concentrations because the accuracy is not high enough.

3.5 Metabolites

Metabolite concentrations (triglycerides, urea, β-hydroxybutyrate and cholesterol) were determined by Alpha Scientific Ltd (Hamilton, New Zealand), using enzyme immunoassay, read on a spectrophotometeric auto-analyser (Hitachi 717, Hitachi Ltd, Tokyo, Japan).

3.5.1 Triglyceride

Triglyceride concentrations were determined using a kit supplied by Roche Diagnostics Ltd, based on the method of Wahlefeld. The coefficient of variation at a concentration of 1 mmol/litre was less than 5%. The minimum detectable concentration of this assay is 0.05 mmol/litre

3.5.2 β-hydroxybutyrate

Concentrations of β-hydroxybutyrate were determined using an enzymatic kit supplied by Sigma Diagnostics kit (catalogue number 310-A), with reagents prepared by Alpha Scientific. The coefficient of variation for samples at concentration of 2mmol/litre was less than 5%.

3.5.3 Cholesterol

Cholesterol concentrations were determined in a single assay using a kit supplied by Roche Diagnostics Corporation. The coefficient of variation for samples of 3.5 mmol/litre was less than 2%. The minimum detectable concentration of the assay is 0.08 mmol/litre.

3.5.4 Urea

Urea concentrations were determined in a single assay, using a kit supplied by Roche Diagnostics. At a concentration of 6mmol/litre the coefficient of variation was less than 5%. The minimum detectable concentration of this assay is 0.83 mmol/litre.

3.6 References

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4 Puberty and heifer growth

4.1 Abstract

Live weight and age at puberty were determined in three strains of Holstein-Friesian dairy cattle in each of two consecutive years. The strains were overseas-origin high-breeding-worth genetics (OS), New Zealand-origin high-breeding worth genetics and New Zealand-origin low-breeding worth genetics (NZL), similar to the New Zealand dairy cows of the 1970s. Onset of puberty was monitored by weekly measurement of progesterone in a total of 271 heifers. Animals were considered to have attained puberty when plasma progesterone concentrations were greater than 2ng/ml in two out of three consecutive samples. Live weights were measured fortnightly and interpolated to estimate live weight at puberty. Animals that were not pubertal before the planned start of mating were treated to induce oestrus. Reproductive performance was compared between the three strains. Live weight at puberty differed between the strains, (OS > NZH > NZL, $P<0.05$) but not between years. Age at puberty was affected by strain in Year 1 (OS > NZH > NZL, $P<0.01$), but not in Year 2. The relationship between live weight breeding values and live weight at puberty was investigated. The reproductive performance of heifers was found to not differ between the three strains. The greater live weights required for heifers to attain puberty in the OS genotype animals must be recognised in target live weights for rearing of heifers. Live weight breeding values can be used to set appropriate target live weights for Holstein-Friesian heifers.

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4.2 Introduction

Puberty in females can be defined as the age when mature gametes are produced and reproductive activity is initiated (Foster, 1999). In the field, puberty is usually measured as either first ovulation (indirect measurement through monitoring plasma progesterone concentrations) or first behavioural oestrus, using bulls and field observation to detect oestrus. There is evidence to suggest that early onset of puberty is correlated with a shorter postpartum anoestrus period. Charolais heifers that were genetically younger at puberty had a genetic aptitude for shorter postpartum anovulatory periods (Mialon *et al.*, 2000).

In the New Zealand system of dairy production, heifers normally enter the herd as 2 year olds, having conceived at around 15 months of age. Heifers born late in the calving period are younger at conception and at calving than their early born counterparts. Early onset of puberty is therefore advantageous, to ensure that all heifers are pubertal and cycling regularly before the start of mating, which begins at a calendar date, regardless of the age of the heifer.

There is no one factor that determines when an animal will reach puberty. Level of nutrition, live weight, age, degree of body fatness and photoperiod have been identified as some of the traits that influence puberty onset (Frisch *et al.*, 1977; Foster *et al.*, 1989; Foster, 1994). In seasonal breeders photoperiod is important, as when all other criteria have been met, it is ultimately photoperiod that determines whether ovulation will occur. Cattle, particularly *Bos taurus*, are generally not considered to be seasonal breeders, but some papers have reported an effect of season on onset of puberty, but the effect is not consistent between papers (Menge *et al.*, 1960; Roy *et al.*, 1980; Schillo *et al.*, 1983). However, a significant negative correlation between photoperiod one month prior to parturition and the postpartum anoestrous period was apparent in the study of Peters and Riley (1982), suggesting an effect of photoperiod in addition to nutrition.

Many studies have reported that puberty is more dependent upon body weight than upon age (Bryant and McRobbie, 1991; Yelich *et al.*, 1996; Hafez and Hafez, 2000). Heifers fed a control diet were significantly younger at puberty than heifers fed a relatively restricted diet, followed by a high energy diet to promote compensatory growth, yet

body weight at puberty was not different between the two groups (Barash *et al.*, 1994). Likewise, heifers on an accelerated growth regime reached puberty at a younger age, but similar bodyweight to heifers on a standard growth treatment (Lammers, 1999). Poorly nourished animals may be prepubertal at the start of the mating period, increasing the likelihood of late conception or being non-pregnant at the end of the breeding season. Animals with a larger mature weight would have to grow faster to reach puberty at the same age as animals with a smaller mature weight (Garcia-Muniz, 1998). The Holstein-Friesian (HF) dairy cow of North America and Europe attains a greater live weight than the New Zealand Holstein-Friesian (NZHF) under NZ conditions (Harris and Kolver, 2001). In recent years there has been a significant increase in the proportion of OS genetics within the NZHF population (Harris and Winkelman, 2000). One consequence of this increase in OS HF genetics is likely to be an increase in size and weight at puberty, requiring faster growth rates to reach puberty at the same age as NZ genetic origin animals (Garcia-Muniz, 1998). NZHF dairy cattle selected for heavy mature live weight, and with a greater proportion of OS HF genetics, were older and heavier at puberty than cattle selected for light mature live weight (Garcia-Muniz, 1998).

In NZ young stock are fed almost exclusively on pasture, the quality and quantity of which varies according to climatic vagaries. On such a variable diet it may prove difficult for heifers that are genetically larger to grow fast enough to reach puberty and conceive by 15 months of age. Ideally, heifers will be cycling regularly prior to the start of mating as this has a beneficial effect on conception rates. Lower conception rates have been reported in heifers mated at pubertal, compared to third oestrus (Byerley, 1987), pregnancy rates in ewe lambs bred at oestrus were lower than in lambs bred at subsequent oestrus (Hare and Bryant, 1985) and in gilts litter size increased between pubertal and subsequent oestrus (Robertson, 1951). Blanket CIDR synchronization/oestrous induction of heifers improves reproductive performance relative to untreated controls and animals that underwent a double PG synchrony, probably due to a proportion of untreated heifers being anoestrous at the start of the mating period (Macmillan *et al.*, 1990).

The purpose of this experiment was to monitor the onset of puberty in HF animals of three genotypes, raised under NZ conditions, in order to determine age and live weight

at puberty and to determine reproductive performance at heifer mating. An additional objective was to develop a model to predict live weight at puberty from the information collected. It was postulated that OS animals would be older and heavier at puberty than NZ genotype animals, and that there would be no difference in age and live weight at puberty between the two NZ genotypes. A difference in reproductive performance was not expected.

4.3 Materials and methods

4.3.1 Animals, samples and assays

This trial was approved by the Ruakura Animal Ethics Committee, approval numbers RAEC # 3445 and 3781.

The onset of puberty in heifer calves of three genetic strains was monitored. Details of the three strains are given below, with further details in Chapter 3, section 2.1, including breeding value information.

- 1998 - Overseas Holstein-Friesian, High Genetic Merit (OS) n=111.
- 1998 - New Zealand Holstein-Friesian, High Genetic Merit (NZH), n=97.
- 1970 - New Zealand Holstein-Friesian, Low Genetic Merit (NZL), n=63.

Animals were grazed at #4 grazing unit Dexcel, Hamilton, New Zealand. In Year 1 (2000; n=190), animals were grazed in 4 herds, one of each strain, plus a preferential group of the lightest animals from each strain. Pasture formed the bulk of the diet, with grass silage and concentrates (Harvey Farms Animal Feeds) fed during feed shortages. The preferential group received pellets at a higher rate and for a longer period, to try and increase weight gain in this group. The four groups of animals were grazed at generous feeding levels in adjacent paddocks on pasture of similar quality. In Year 2 all animals were run as one herd (2001; n=81). Grass silage and concentrates were fed during the winter of Year 2 after a severe autumn drought.

Blood samples were taken weekly from each heifer that had reached 195 kg live weight (NZ strains) or 215 kg (OS strain). All prepubertal heifers were sampled for the last four weeks of the sampling period, regardless of their live weight. Sampling ceased 14 days prior to the start of mating to allow induction of oestrus in heifers that were

prepubertal. Progesterone concentrations of plasma were measured by radioimmunoassay (Coat-a-Count™, DPC, USA). Inter-assay co-efficients of variation for plasma pools of 4.43, 3.04 and 0.40 ng/ml were 7.3, 5.4 and 12.4% respectively. Intra-assay co-efficients of variation for the same plasma pools were 6.8, 5.7 and 13.6% over 52 assays. The minimum detectable concentration of the assay was 0.061ng/ml. Further details of assay methodology are given in 3.4.7. Puberty was considered to have occurred when plasma progesterone concentrations of 2ng/ml were present in two out of three consecutive samples. The date of puberty was recorded as the time of the first of these samples. Live weights were measured fortnightly using load-cell scales (Trutest, NZ Ltd) and live weight at puberty was estimated by interpolation.

The plasma progesterone records of the heifers were examined at the end of September, at which time any animals that had progesterone concentrations of >1ng/ml were considered to be either cycling or close to cycling, despite not having reached the definition of puberty. The remaining animals (Year 1 n=13; Year 2 n=12) were treated with an 8-day CIDR (Interag, Hamilton, New Zealand), followed by prostaglandin $F_{2\alpha}$ (Lutalyse, 5mls, Pharmacia) at the time of CIDR removal. Heifers that were in oestrus 24 hours after CIDR removal were inseminated. All other animals were given 0.75mg oestradiol benzoate (ODB, CIDROL, Bomac Laboratories Ltd, New Zealand), and then fixed-time inseminated to Jersey bulls on the following day. Heifers were then returned to the main herd. Pubertal animals were naturally mated to yearling Jersey bulls, with the bulls introduced to the herds on 10th October and being removed 20th December in Year 1. The corresponding dates were 4th October and 6th December in Year 2. The mating period was earlier in Year 2 as the planned start of calving for the trial had been moved forward by a week to better match cow demand to pasture growth.

4.3.2 Analysis of data

Proportional data were analysed using the chi-squared test (SAS version 8.1). Continuous data were analysed using the mixed procedure of SAS, with a model that included the fixed effect of strain and a random effect of sire, nested within strain.

A further analysis was conducted in S-Plus 2000 using frailty models to estimate age and weight at puberty for each strain if all animals had had the opportunity to reach puberty.

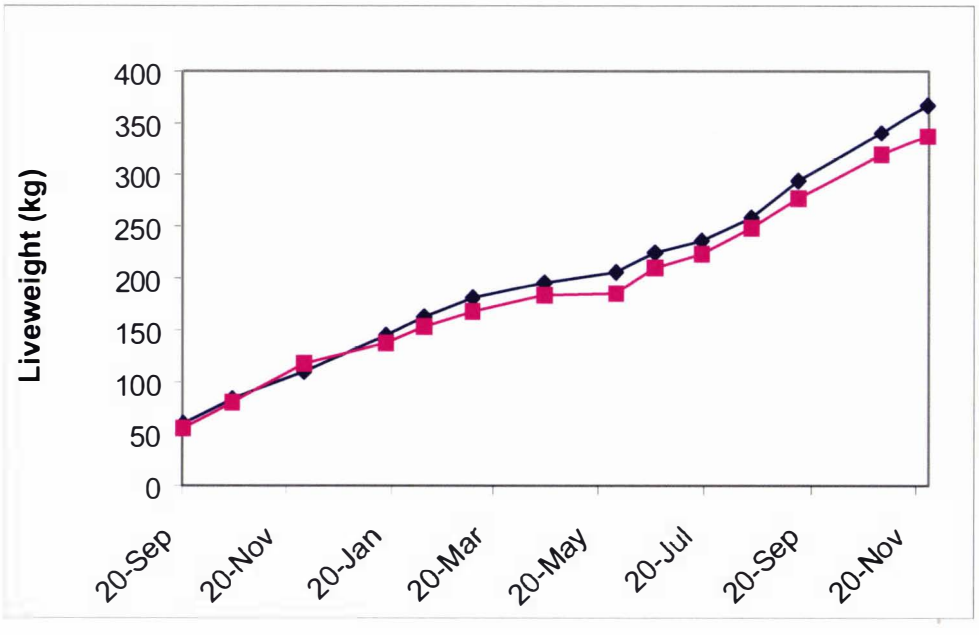
A forward regression, run in regression procedure of SAS, (SAS version 8.1) was conducted to develop a model to predict live weight at puberty. The variables offered were the heifers breeding value for live weight, daily gain from October to October (approximately 3 to 15 months of age), strain, fortnight of birth over a 14-week period, and year.

4.4 Results

4.5 Heifer growth

Average live weight, by month of the rearing period are presented in Figure 4-1. Average heifer live weights in May (approximately 10 months of age) were 20 kg heavier in Year 1, than Year 2 of the trial.

Figure 4-1: Average live weight of all heifers in the strain trial from approximately 2 months of age to 16 months of age, by year. Year 1 (—◆—), Year 2 (—■—). Error bars have been emitted for clarity.



4.6 Proportion pubertal and distribution of puberty onset

Over the two years when sampling was undertaken a total of 56/271 animals failed to reach puberty before the start of mating. Strain had a significant effect on the proportion of pubertal animals at the end of the sampling period (Table 1). A greater proportion of animals reached puberty in 2000 compared to 2001 ($P<0.05$). To remove the effect of birth date (see Tables 3 and 4), only those animals that were at least 400

days of age at the end of the sampling period were included in a further analysis (Table 2). This age was chose because on a dairy farm with a start of calving date of 20th July, heifers kept during the first 6 weeks of the calving period would be a minimum of 400 days of age 1 week before the planned start of mating.

Table 4-1 Proportion of animals, by strain, that reached puberty before the start of mating, in Year 1 (2000) and Year 2 (2001) and for both years combined.

	Year 1	Year 2	Total
OS	56/76 (74%)[a]	20/35 (57%)[a]	76/111 (68%)[a]
NZH	70/73 (96%)[b]	20/24 (83%)[b]	90/97 (93%)[b]
NZL	36/41 (88%)[ab]	13/22 (73%)[a†]	49/63 (78%)[a]
Total	162/190 (85%)	53/81 (65%)	215/271 (79%)

Treatments: New Zealand high (NZH), New Zealand low (NZL) and overseas (OS)

[abc] Proportions with different superscripts within a column differ significantly (P<0.05)

[†] Difference between NZL and NZH approaches significance p=0.068

Table 4-2: Proportion of animals that were at least 400 days of age at the end of the sampling period and that had reached puberty.

	OS	NZH	NZL
Year 1	79%[a]	97%[b]	89%[ab]
Year 2	68%	83%	61%

Treatments: New Zealand high (NZH), New Zealand low (NZL) and overseas (OS)

[abc] Proportions with different superscripts within a column differ significantly (P<0.05)

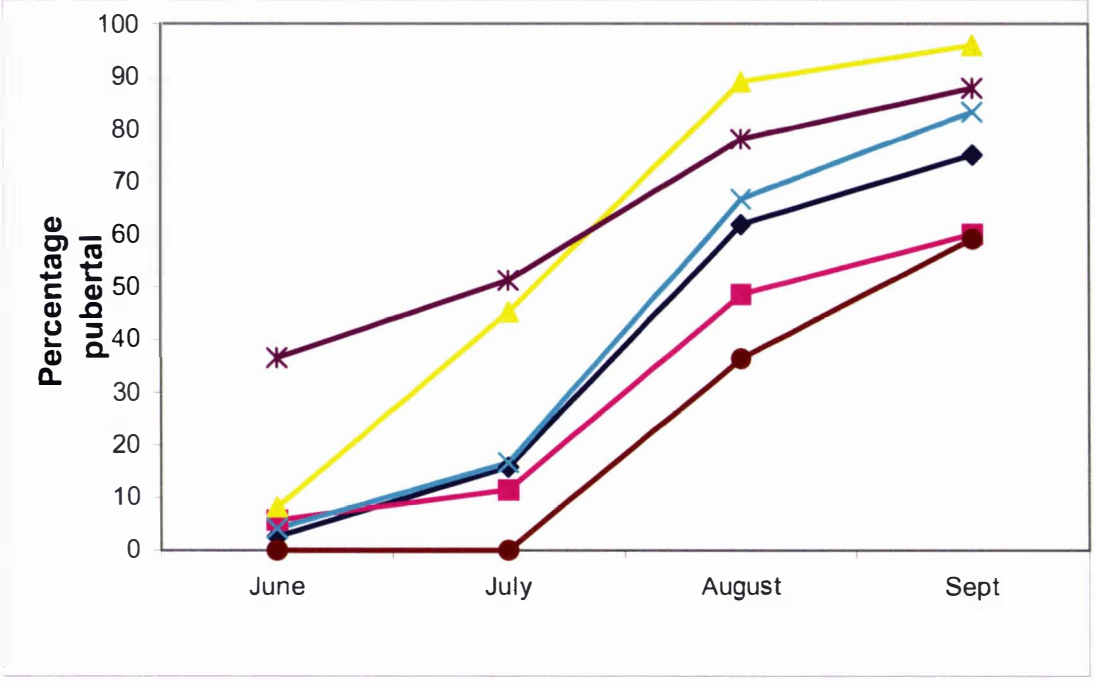
The distribution of puberty onset differed between Year 1 and Year 2. By the end of July 35% of animals in year 1 had reached puberty, compared to 9% of animals in year 2 (p<0.0001). This had increased to 76% and 50% respectively, by the end of August (P<0.0001). In both years 41% of animals reached puberty during August. Figure 4-2 illustrates the distribution of puberty onset between strains and between years.

4.7 Year 1

Results for age at puberty, live weight at puberty, average daily live weight gain and predicted mature live weight, by strain for Year 1 are shown in Table 4-3. Significant strain differences were evident for both age and live weight at puberty. Across the three

strains, age at puberty was different between Year 1 (353 days) and Year 2 (377 days) ($p<0.0001$).

Figure 4-2: Cumulative distribution of puberty onset by strain in year 1 and year 2. Overseas Year 1; —◆—, Overseas Year 2; —■— New Zealand high Year 1; , New Zealand high Year 2; —×—, New Zealand low, Year 1; —*—, New Zealand low Year 2; —●—.



4.8 Year 2

Results for age at puberty, weight at puberty, mean daily live weight gain and predicted mature live weight, by strain for Year 2 are given in Table 4-4. Age at puberty was not different between strains, whilst live weight at puberty was significantly ($P<0.05$) different.

4.9 Censored data

Data were adjusted to take into account the possible effect of the animals that failed to reach puberty by the end of the sampling period on the age and live weight at puberty. Table 4-5 gives the estimated live weight and age at puberty for each strain, within each year. Within each strain there was no difference in live weight at puberty between the

two years. Both New Zealand strains were older at puberty in Year 2 than Year 1 ($P<0.05$). There was no difference in age at puberty between years for OS animals.

Table 4-3: Mean date of birth, age and weight at puberty, mean daily live weight gain and predicted mature live weight for heifers in Year 1 (2000). Predicted mature live weights were supplied by Livestock Improvement Corporation.

	OS n = 76	NZH n = 73	NZL n = 41
Mean birth date	10th August	7th August	13th August
Mean (± sem) age at puberty (d)	373 ± 6.0[a]	356 ± 6.9[at]	329 ± 6.7[b]
Mean (± sem) live weight at puberty (kg)	274 ± 4.4[a]	253 ± 4.9[b]	230 ± 4.9[c]
Mean (± sem) daily live weight gain in the 12 months prior to mating (kg/day)	0.73 ± 0.01[a]	0.68 ± 0.01[b]	0.67 ± 0.01[b]
Predicted mature live weight (kg)	640	540	540

Treatments: Overseas (OS), New Zealand high (NZH) and New Zealand low (NZL)

[abc] Values within rows, with different superscripts, differ significantly (P<0.05)

† Difference between NZH and OS approaches significance (P=0.07)

Table 4-4: Mean date of birth, age and weight at puberty, mean daily live weight gain and predicted mature live weight for heifers in Year 2 (2001). Predicted mature live weights were supplied by Livestock Improvement Corporation.

	OS n = 35	NZH n = 24	NZL n =22
Mean birth date	3rd August	27th July	10th August
Mean (± sem) age at puberty (d)	374 ± 6.5	380 ± 6.5	381 ± 8.1
Mean (± sem) live weight at puberty (kg)	271 ± 6.0[a]	258 ± 5.9[a]	237 ± 7.3[b]
Mean (± sem) daily live weight gain in the 12 months prior to mating (kg/day)	0.67 ± 0.01[a]	0.66 ± 0.01[a]	0.59 ± 0.01[b]
Predicted mature live weight (kg)	640	540	540

Treatments: Overseas (OS), New Zealand high (NZH) and New Zealand low (NZL)

[abc] Values within rows, with different superscripts, differ significantly (P<0.05)

Table 4-5: Censored means for live weight and age at puberty for Holstein-Friesian heifers of different genetic strains.

		OS	NZH	NZL
Live weight at puberty (kg)	Year 1	282.4 ± 5.3^{a}	256.9 ± 6.4^{b}	235.7 ± 6.11^{c}
	Year 2	288.5 ± 6.9^{a}	266.7 ± 7.8^{ab}	251.6 ± 7.8^{b}
Age at puberty (days)	Year 1	384.6 ± 6.0^{a}	360.3 ± 6.9^{b}	339.0 ± 7.0^{c}
	Year 2	403.2 ± 8.1	388.4 ± 9.1	396.2 ± 9.4

Treatments: Overseas (OS), New Zealand high (NZH) and New Zealand low (NZL)

^{abc} Different superscripts within rows denote significant differences.

4.9.1 Model

All variables offered to the model (breeding value (BV) for live weight, daily gain, fortnight of birth, strain and year) were significant ($P<0.05$). Live weight BV accounted for 42% of the variation in live weight at puberty, but no other variable was able to account for more than 6% of the variation in live weight at puberty. When all variables were included in the model 55% of the total variation was explained ($p<0.05$). The equation for a model including only live weight breeding value would be

Weight at puberty = 204.14 + 0.86 live weight BV

4.10 Heifer reproductive performance

The reproductive performance of heifers was similar between strains and between years. The percentage of non-pregnant animals was less than 5% in both years, and did not differ between strains. Figure 4-3 and Figure 4-4 show the cumulative percentage of pregnant animals during the mating period.

Figure 4-3: Cumulative percentage pregnant by strain and by week of the mating period in Year 1. New Zealand High ■ New Zealand Low ■ overseas ■

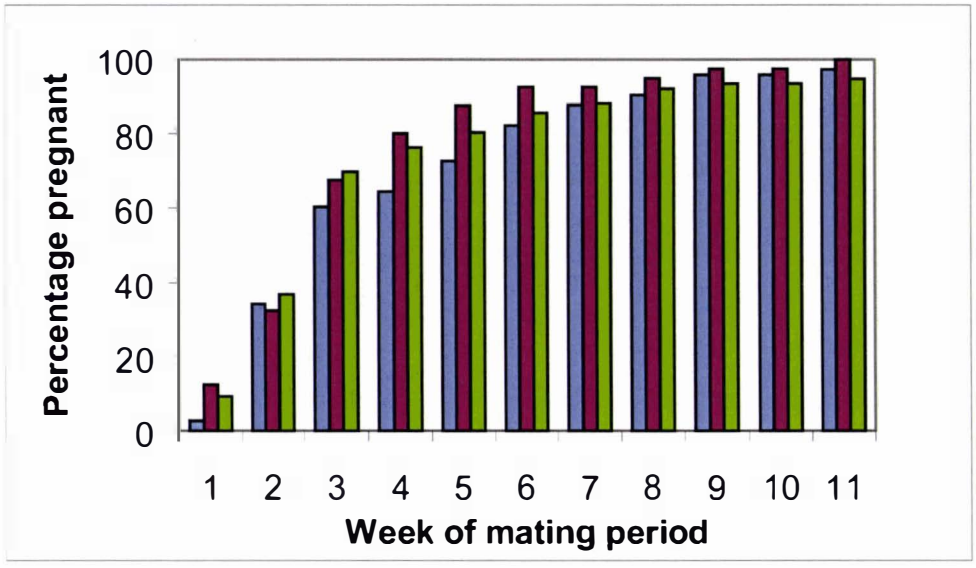
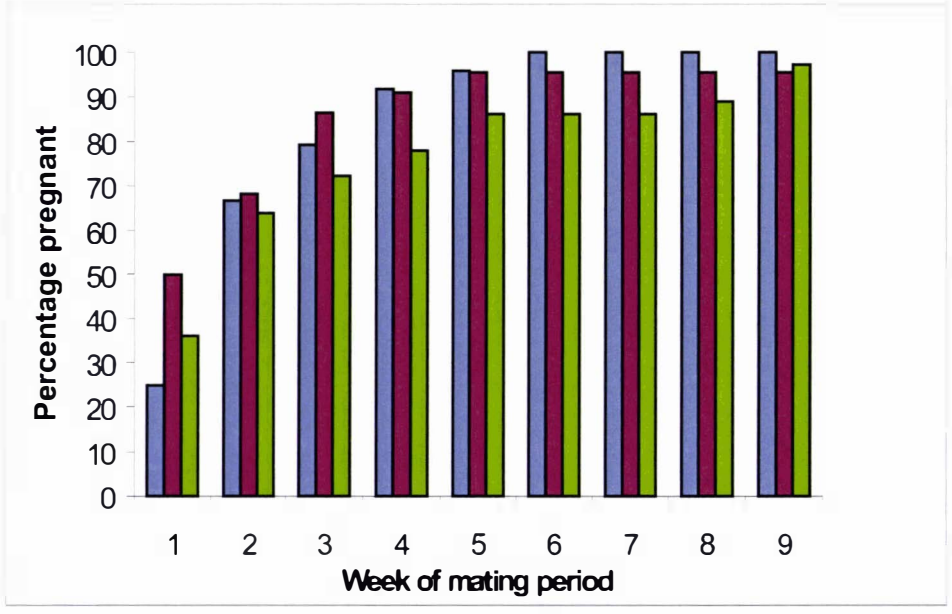


Figure 4-4: Cumulative percentage pregnant, by strain and by week of the mating period in Year 2. New Zealand High ■ New Zealand Low ■ overseas ■



4.11 Discussion

4.11.1 Puberty

These results indicate that genetic strain of Holstein-Friesian influences age at puberty, largely through the influence of strain on live weight. Strain had a significant effect on the proportion of animals that reached puberty only in Year 1 when overall growth rates were acceptable. In Year 1 more NZH than OS heifers reached puberty by the planned start of mating. Likewise, differences in age at puberty between strains were recorded only in Year 1 of the trial. Overseas animals were heavier at puberty than animals of either NZ strain, and NZL animals were lighter at puberty than both NZH and OS heifers. Due to inadequate feeding during Year 2 (associated with a drought period), the effect of strain on age at puberty was not evident. Adjustment of the data for the proportion of each strain that reached puberty at the end of the sampling period resulted in little change from the results seen with analysis of the raw data.

In a related trial in Ireland, more NZ Holstein-Friesians (imported as embryos, with the same sires used to produced the NZH in the present trial) reached puberty by the start of mating than Irish high merit Holstein-Friesians (cows that have a significant proportion of North American and European genetics; McGrath *et al.* (2001). Live weights at puberty recorded in the present trial are similar to those recorded in other trials with heifers of similar genetic background (Table 6). The differences in live weight between the NZ lines at puberty, as reported in the present trial, are similar to that between two NZ strains selected for heavy and light mature live weight (Garcia-Muniz, 1998). In the present study, live weight at puberty was similar to that recorded in other trials (Barash *et al.*, 1994; McGrath *et al.*, 2001); Table 6), but age at puberty was greater than in these trials. This would suggest that growth rates in the present trial were lower than in these other reports, which is likely associated with rearing on pasture rather than a concentrate-based diet.

Age at puberty is responsive to live weight and level of nutrition. The conditions of Year 2 influenced growth rates, such that animals were 20 kg lighter at the end of May in Year 2. Thus, the proportion of animals that were pubertal in each of the strains was lower in Year 2 than in Year 1, yet live weights at puberty were similar to those

recorded in Year 1 for each strain. After the drought period, concentrates (June to September) and silage (June to December) were fed to increase nutrient intakes and live weight. The effects of this accelerated growth, which appeared to have assisted the onset of puberty, possibly occurred as a consequence of increased pulsatile secretion of luteinizing hormone (Day, Imakawa, Zalesky *et al.*, 1986; Kurz *et al.*, 1990). It may also have caused the loose synchronization of the onset of puberty, as described by (Gonzalez-Padilla *et al.*, 1975), to give the more compact pattern in Year 2, with no differences in age at puberty between the strains. The introduction of supplementary feed occurred at a time that allowed the OS animals to reach pubertal live weight at the same age as in Year 1, but would have been too late to allow the NZ strains to reach pubertal live weight at the same age as the previous year. Thus age at puberty was affected by strain in Year 1, but not in Year 2.

These results support the contention that live weight is more important than age in determining the timing of the onset of puberty (Hafez and Hafez, 2000). OS animals were heavier at puberty than NZ genotype animals. This difference was predicted by the greater live weight BV of OS compared to NZ genetic origin animals. The difference in live weight at puberty between the NZ strains was unexpected, given that they had similar predicted live weight BVs. This suggests that either NZL animals reach puberty at a lower proportion of their mature weight than NZH animals, or that the live weight BV for NZL cattle was overestimated. NZL animals in this study were significantly lighter than NZH animals from six months of age, despite similar condition scores and live weight BVs.

Selection over the past 20-30 years seems to have resulted in NZHF that need to reach heavier live weights to reach puberty, suggesting that mature live weights may also differ between the NZ strains. Genetic correlations between body size and fat, protein and milk yields have been reported as 0.34, 0.37 and 0.39 respectively in NZ HF (Ahlborn and Dempfle, 1992). At this level of correspondence selection for yield traits would be expected to result in larger cows (Ahlborn and Dempfle, 1992). Thus, selection on breeding index (BI), which was largely based on fat production, probably has contributed to the apparent increase in live weight over the past 20- 30 years. However, the breeding worth index (BW) replaced the BI index in 1996. Live weight is

included in the calculation of BW, with a negative economic value. The inclusion of live weight may be expected to slow the increase in live weight that has apparently occurred over the past 20-30 years. However, the continued widespread use of Holstein-Friesian bulls with a high proportion of OS genetics, and high live weight breeding values is likely to result in the continued slow increase in the live weight of the New Zealand dairy cattle population.

Attainment of a proportion of mature live weight has been proposed as one trigger for puberty onset (Hafez and Hafez, 2000). Estimates of the proportion of mature weight that NZH, NZL and OS had attained at puberty (47, 43 and 43% respectively), based on predicted mature live weights are similar to those calculated by (Garcia-Muniz, 1998) for heifers selected for light or heavy mature live weight (both 47%), and differing in proportion of OS genetics (Table 4-6). Nonetheless, even if OS heifers reach puberty at a lower proportion of mature live weight than NZH animals they still need to attain a heavier absolute live weight, requiring a faster growth rate, and higher feed consumption to reach puberty at the same age as a NZ-origin heifer. Target live weights, therefore, need to be increased for OS-genetic-origin animals. Penno *et al.* (1995) suggested that HF heifers should weigh 300 kg at mating, based on results with 1992 born HF heifers with an expected average of 17% OS genetics (Harris and Kolver, 2001). For animals with a high proportion of OS genetics, a target of 340 kg would be more appropriate, whilst 300 kg is a minimum live weight target for NZH animals. Feeding levels in this trial were inadequate for most OS animals to reach this target live weight at mating. Setting growth targets that are related to mature live weight (live weight BV), and feeding heifers to ensure targets are met are crucial to ensure animals are cycling before the start of the mating period.

Live weight breeding value explained considerably more variation than all other factors offered in the regression. The live weight breeding value is an estimation of mature live weight for an individual animal (B. Montgomerie, personal communication). The relatively high explanatory value of the live weight breeding value indicates a relationship between puberty onset and genetically determined mature live weight. For the purpose of developing a model for use by farmers, a model including genetically determined mature live weight seems most appropriate, as this information is readily

available to farmers, the relevant equation is given in the results section. However, in practice an equation to estimate target live weights at various ages would be more useful. A larger data set, including other breeds, would be required to develop such a model with a reasonable degree of accuracy. A model has now been developed where live weight breeding value is used to estimate mature live weight, and subsequently target live weights required at specific ages during development (Bryant, 2003).

Table 4-6: Mean age, live weight and percentage of mature live weight at puberty for various strains of Holstein Friesian (HF) dairy cattle.

Location and heifer type	Age (days)	Live weight (kg)	Reference
Ireland, NZ high BW (NZIre)	300	241	(McGrath *et al.*, 2001)
Ireland, Medium merit Irish (MM)	352	291	
Ireland, high merit Irish (HM)	369	352	
NZ, HF selected for heavy mature weight, 46% OS genetics (NZheavy)	345	241	(Garcia-Muniz, 1998)
NZ, HF selected for light mature weight, 12% OS genetics (NZlight)	300	221	
NZ, NZHF 1970 (NZ 1970)	348	218	(Pleasants *et al.*, 1975)
Israel, OS high genetic merit (OSIsrael)	318	271	(Barash *et al.*, 1994)

The genetic origin of replacement heifers and their BV for live weight must be considered when setting live weight targets for heifer growth. Setting appropriate targets for the type of animals being farmed, and meeting these are crucial to produce well-grown heifers that are cycling and ready to conceive at the start of the mating period. The present results indicate that target live weights should be highest for the OS and lowest for the NZL strains. Live weight breeding values can be used as an aid in setting target weights for heifers.

4.11.2 Reproductive Performance

The pattern of conception, and overall pregnancy rate after the heifers first mating period was similar between strains and between years. However, during Year 1, NZH animals had a lower than expected number of conceptions in the early breeding period.

This was related to a problem with one of the bulls, which was rectified early in the breeding period. The result of this was a slightly later mean calving date for the NZH animals than for the other two strains in Year 1 of the trial. Conception rates in heifers have remained constant, despite a decline in the reproductive performance of lactating animals (Butler and Smith, 1989). This data confirms that under New Zealand conditions the reproductive performance of OS heifers is not different from those of New Zealand origin, in heifers that were pubertal, or treated to induce oestrus at the planned start of mating.

The effect of the proportion of pubertal animals on conception patterns could not be quantified in this trial, due to the induction of oestrus in all prepubertal animals before mating started. However, it is logical to expect that animals that were not cycling at the start of the breeding period would be more likely to conceive late, or not at all, as is the case for cows that are anoestrous at the start of the mating period.

4.12 Conclusion

The results from the current trial demonstrate that live weight is a more important determinant of the timing of the onset of puberty than age, and demonstrates that seasonal effects can have a large impact on the timing of puberty onset, through effects on live weight. Differences in the average live weight required to attain puberty were reported for NZ and OS strains, related chiefly to differences in mature live weight. The best way to predict live weight at puberty onset is by the live weight BV, which is specific to the individual animal and reduces within strain variation.

Farmers are unlikely to know whether heifers are cycling or not at the planned start of mating. It is therefore important that farmers are aware of the potential mature live weight of the animals that they farm, and suitable target live weights for these animals. Live weight breeding values provide an indication of mature live weight. By achieving target live weights a high level of reproductive performance can be obtained from all strains of heifer. If there is doubt about whether heifers have reached puberty a CIDR oestrus induction/synchrony scheme could be utilized to achieve high reproductive performance.

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5 *Comparison of oestrous cycle characteristics between NZH, OS and NZL heifers.*

5.1 Abstract

Oestrous cycle characteristics were compared in nulliparous New Zealand high (NZH), New Zealand low (NZL) and overseas (OS) origin Holstein Friesian heifers. Heifers were blood sampled daily, from one oestrus event, to the subsequent oestrus event during a synchronised oestrous cycle. Concentrations of progesterone, IGF-1, insulin and leptin were measured in all samples and concentrations of oestradiol measured in samples from Days 3 to 8. Tailpaint scores, in conjunction with progesterone concentrations were used to determine oestrous cycle, and luteal phase lengths, which were found to differ between the three strains. Oestrous cycle, and luteal phase lengths were greater in OS than NZH heifers ($p<0.05$). However, concentrations of progesterone were greater in the NZL strain than the NZH or OS strain ($p<0.05$). Although body condition scores were not different between the three strains, concentrations of leptin and IGF-1 were higher in the NZH strain ($p<0.05$). Concentrations of leptin and insulin were significantly correlated for all strains ($p<0.05$). Previous studies of heifers of high and low genetic merit have failed to find differences in reproductive endocrinology between the strains, but differences in progesterone secretion and IGF-1 concentrations have been reported in lactating dairy cows. The differences in metabolic hormones observed in the current trial may be an indication of physiological differences in the regulation of energy metabolism between the strains that should be investigated in lactating animals.

5.2 Introduction

New Zealand and Overseas Holstein Friesians have been selected in vastly different dairy production environments. The seasonal nature of the New Zealand system has lead to indirect selection on reproductive performance. Whilst New Zealand Holstein Friesians are generally considered to have a relatively high reproductive performance, as measured by conception rate (Xu and Burton, 1996; Burton *et al.*, 1999), the New Zealand dairy industry has a problem with extended postpartum anoestrous intervals (Macmillan, 2002). OS Holstein Friesians are considered to have relatively poor reproductive performance, with low first service conception rates in lactating animals (Butler *et al.*, 1995; Royal *et al.*, 2000). However, first service conception rates in heifers remain high (Pursley *et al.*, 1997).

In a large-scale on-farm trial Royal *et al.* (2000) reported that the reproductive performance of British Friesians had declined between the late 1970s and mid 1990s, possibly in part due to the increase in OS Holstein genetics within the Friesian population. Over this period, the interovulatory interval increased from 20.2 to 22.3 days and luteal phase length increased from 12.93 to 14.8 days. In the USA, cows selected for increased production had lower progesterone concentrations and shorter luteal phases than control cows. In addition, postpartum plasma IGF-1 concentrations were lower in selected than control cows after 21 days postpartum, despite similar energy balance between the two lines (Lucy and Crooker, 1999). Although differences were not consistent between the two studies, it appears that oestrous cycle physiology may be altered by selection for milk production. In order to more fully investigate the reproductive physiology of the oestrous cycle of Strain Trial animals a group of pubertal heifers was monitored, to determine whether any differences between strains exist, and for possible later comparison with oestrous cycle characteristics in lactating animals. We postulated that oestrous cycle length would differ between NZ and OS strains of Holstein Friesian, in line with the findings of Royal *et al.* (2000).

5.3 Materials and Methods

5.3.1 Animals

This project was approved by the Ruakura Animal Ethics Committee (approval number; 3659). Three genotypes of pubertal, yearling Holstein Friesian cattle were available (for details on strain generation see 3.2.1). Fifteen animals from each of three genotypes (NZL, NZH, OS) of Holstein Friesian cattle were selected, based on the date of onset of puberty, so as to ensure animals that would have had at least 2 oestrous cycles prior to the start of the experiment (pubertal cycle, and a further cycle).

5.3.2 Management

Selected animals were separated from their original groups and run as one mob. Animals were grazed at the Grazing Unit, Dexcel, Hamilton, New Zealand, on pasture sward of predominantly perennial ryegrass (*Lolium perene*) and white clover (*Trifolium repens*). Food intake was *ad libitum*, with animals shifted to new grazing during the morning and sampled each day around 1pm.

Heifers were given two injections of 250mg cloprostenol (Estrumate, Schering-Plough Animal Health Ltd, Upper Hutt, New Zealand), administered 11 days apart to synchronise oestrus. Heifers were tail painted (Macmillan and Curnow, 1977) and observed daily for oestrus behaviour. Blood samples were collected by coccygeal venipuncture (heparin anticoagulant) daily from 24 hours after the second cloprostenol injection; samples were handled according the protocol described in 3.3.1. All animals that exhibited oestrus within 6 days of the second cloprostenol injection were included in the trial group (OS and NZL n=12, NZH n=13). Details of the animals selected to participate in the trial are given in Table 5-1. Sampling continued from synchronized oestrus to the next observed oestrus, as indicated by tail paint scores (Macmillan and Curnow, 1977) and behaviour, and confirmed by progesterone analysis, if there was any doubt.

Table 5-1:Mean age and weight at puberty, date of onset of puberty, and weight and condition score in animals who were included in the trial.

	Age at puberty	Weight at puberty	Date of puberty onset	Weight at trial start	Condition score at trial start
OS n=12	343 days	259 kg	15-Jul-00	299 kg	4.9
NZH n=13	342 days	242 kg	16-Jul-00	277 kg	4.9
NZL n=12	334 days	231 kg	13-Jul-00	269 kg	4.9

Treatments: Overseas (OS), New Zealand high (NZH) and New Zealand low (NZL).

5.3.3 Hormone Assays

Progesterone Assay

Samples were analysed for progesterone concentrations using a radioimmunoassay kit (Count-a-Count, DPC, California), (see 3.4.7 for further assay methodology). Over two assays intra-assay co-efficients of variation for plasma pools of 4.87, 3.37 and 0.41 ng/ml were 11.5 7.93 and 14.3% respectively. Inter-assay co-efficients of variation for plasma pools of 4.87, 3.37 and 0.41 ng/ml were 2.31 5.33 and 7.37% respectively. The minimum detectable concentration was 0.023 ng/ml.

Oestradiol Assay

Concentrations of oestradiol were determined in plasma on days 3 to 9 of the cycle using the Serono Estradiol MAIA assay kit (Biodata S.p.A., Montecelio, Italy), a previously reported method (Prendiville *et al.*, 1995). Further details of the assay method are given in 3.4.1.

Over two assays intra-assay co-efficients of variation for plasma pools of 15.12, 4.5 and 0.78 pg/ml were 11.2, 11.9 and 35.7% respectively. Inter-assay co-efficients of variation for plasma pools of 15.121, 4.54 and 0.775 pg/ml were 0, 0 and 28.2% respectively. The minimum detectable concentration was 0.069 pg/ml.

Insulin-like growth factor-1 Assay

Concentrations of IGF-1 were determined in a double antibody radioimmunoassay at the University of Western Australia, Perth (see 3.4.3 for further assay methodology).

Intra-assay co-efficients of variation for plasma pools of 21.3 ng/ml and 47.4 ng/ml and 98.5 ng/ml were 10.0, 6.1 and 4.9% respectively. The minimum detectable concentration was 7.1ng/ml.

Leptin Assay

Samples were analysed at the University of Western Australia, Perth, in a double antibody radioimmunoassay (See 3.4.6 for further assay methodology). Intra-assay co-efficients of variation for plasma pools of 0.85 ng/ml and 1.49 ng/ml and 2.51 ng/ml were 8.4, 5.1 and 2.5% respectively. The minimum detectable concentration was 0.1 ng/ml.

Insulin Assay

Samples were analysed at the University of Western Australia, Perth, in a double antibody radioimmunoassay (see 3.4.5 for further assay methodology).

Intra-assay co-efficients of variation for plasma pools of 1.38 μU/ml and 2.97 μU/ml and 10.93 μU/ml were 7.3, 6.3 and 4.8% respectively. The minimum detectable concentration was 1.193 μU/ml.

5.3.4 Statistical Analysis

Continuous data (cycle length and luteal phase length and non-luteal phase length) were analysed using a simple mixed model (Proc Mixed, SAS) where cycle length = strain, sire was included as a random effect, nested within strain. For the cycle length calculation sire was found to have a no effect, so it was left out of the model for this variable. Likewise, maximum progesterone concentration and day of maximum progesterone concentration were determined in proc mixed, with the effect of sire left out for the same reason.

Progesterone, oestradiol, insulin, IGF-1 and leptin data were also analysed using a simple model in proc mixed in SAS. This model was run on separately on each day of the cycle. The model included the fixed effect of strain and the random effect of sire, nested in strain where the sire effect was found to be greater than 0; otherwise sire was excluded from the model. A repeated measures analysis was also conducted for hormones whose pattern of secretion did not change over the oestrous cycle (leptin, IGF-1 and insulin) in a mixed model (Proc Mixed, SAS). A simple model was

constructed where leptin = strain, with a repeated effect of day and a random effect of sire nested within strain. The effect of condition score on leptin concentrations was also tested.

5.3.5 Definitions

Determination of cycle length

Cycle lengths were determined on the basis of tailpaint scores and plasma progesterone concentrations. Tailpaint scores were recorded daily (range 0-5;(Macmillan *et al.*, 1988)). Where tailpaint scores were the same on two consecutive days the first day of that score was considered to be the oestrus event. Where tailpaint scores did not change over the anticipated period of oestrus diagnosis was made solely on the basis of plasma progesterone concentrations, the day of oestrus was recorded as two days after progesterone concentrations declined to below 1ng/ml. Oestrus occurs 2-3 days after progesterone levels begin to decline (Peterson *et al.*, 1975), and in the present trial, heifers were in oestrus two days after progesterone concentrations declined to below 1ng/ml, therefore this was the basis for oestrus detection in the isolated case where tailpaint scores were not indicative of oestrus. Cycle length was calculated from day of oestrus (day 1) to the day prior to the next oestrus event.

Luteal phase length

The luteal phase was defined as beginning when plasma progesterone concentrations reached 1ng/ml or more in the first of at least 2 consecutive samples and ending at the day previous to progesterone concentrations declining below 1ng/ml.

5.4 Results

5.4.1 Oestrous cycle length

There was a significant difference in the overall oestrous cycle length between the genotypes ($P<0.05$) as illustrated in Figure 5-1. Luteal phase length also differed between strains ($P<0.05$) as illustrated in Figure 5-2. There was no difference in follicular phase length between the strains as illustrated in Figure 5-3. Luteal phase length explains a significant ($P<0.0001$) amount of the variation in cycle length recorded ($r^2 = 0.74$). The relationship was linear (as illustrated in Figure 5-4). At a given oestrous cycle length, luteal phase length can be estimated by the equation luteal phase length = 0.8755 (oestrous cycle length) – 2.8591.

Figure 5-1: Oestrous cycle length in three genotypes of yearling Holstein-Friesian heifers. Significant (P<0.05) differences between bars are denoted by different letters (a and b).

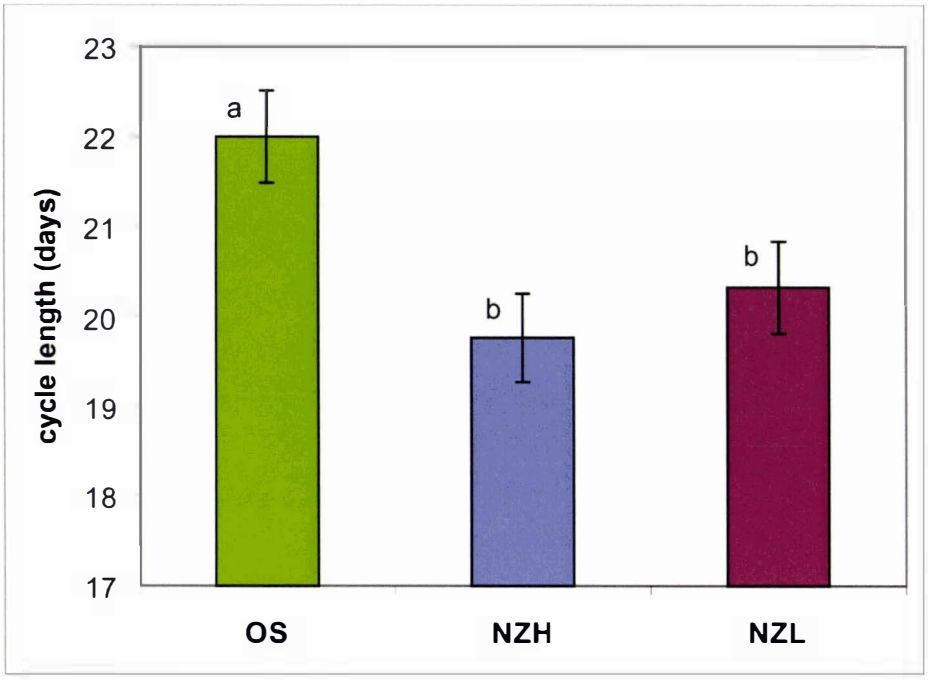


Figure 5-2: Luteal phase length (P4 > 1ng/ml) in three genotypes of yearling Holstein-Friesian heifers. Different letters (a, b) indicate a significant (P<0.05) difference between bars, * indicates the difference between New Zealand low and overseas animal approaches significance (P<0.1)

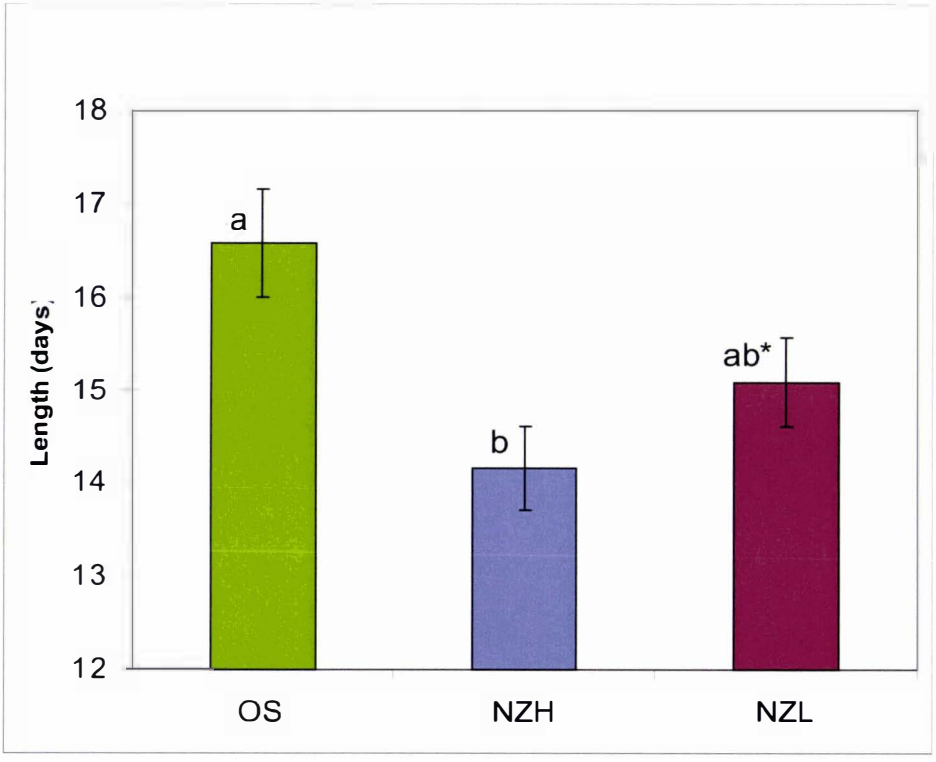


Figure 5-3: Non-luteal phase length (P4 < 1ng/ml, follicular phase) in three genotypes of yearling Holstein-Friesian heifers. Overseas (OS), New Zealand high (NZH) and New Zealand low (NZL).

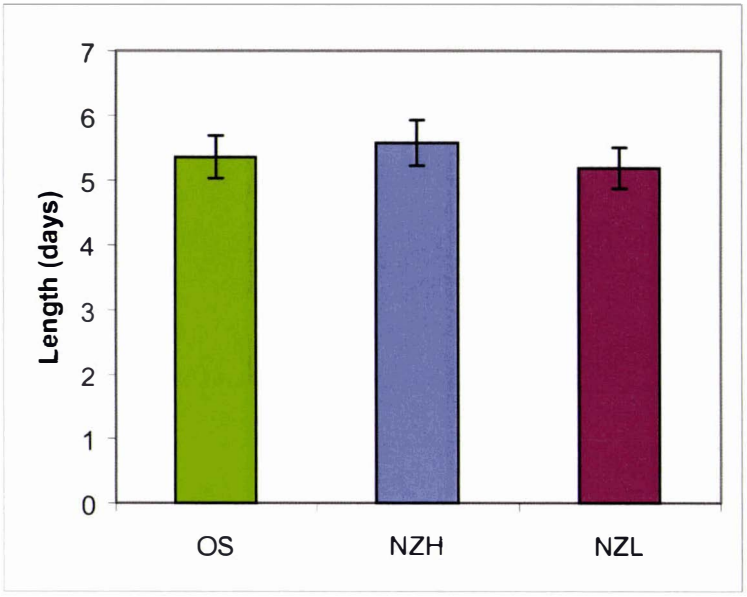
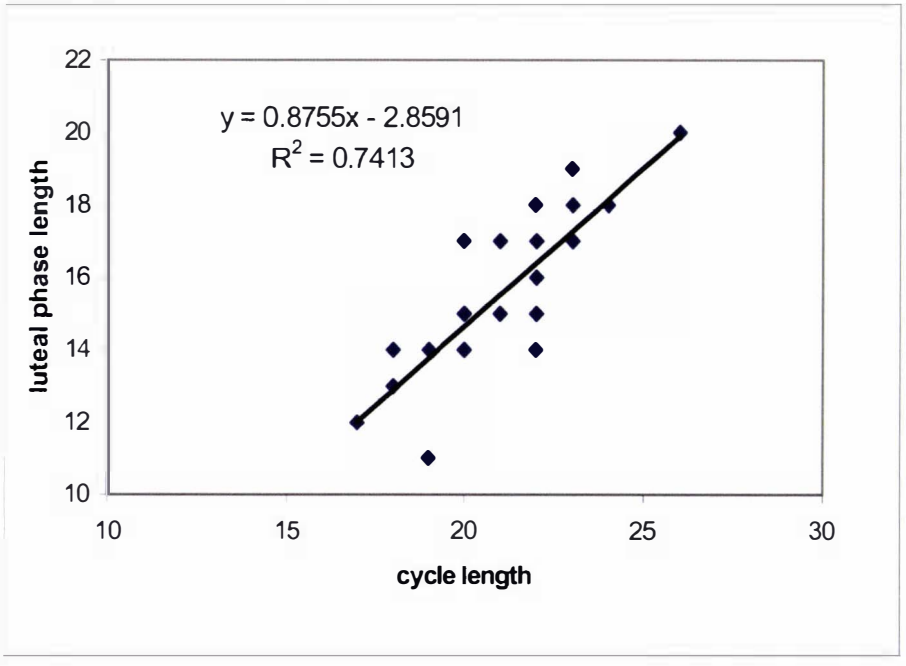


Figure 5-4: The relationship between luteal phase length (days, P4 >1ng/ml) and oestrous cycle length (days) in three genotypes of Holstein-Friesian heifers (n=37). Some heifers had identical co-ordinates, therefore les s than 37 data points appear on the graph.



5.4.2 Hormone concentrations over the oestrous cycle

The day on which maximal oestradiol concentrations occurred differed between NZL and OS animals (Day 5 and Day 6 respectively; $P<0.01$), but was not different between NZL and NZH, or NZH and OS (Table 5-2, Figure 5-5).

Figure 5-5: Mean plasma oestradiol concentrations from day 3 to day 9 of the oestrous cycle in three genotypes of Holstein-Friesian heifers. New Zealand high (—■—), New Zealand low (—▲—) and overseas (—◆—).

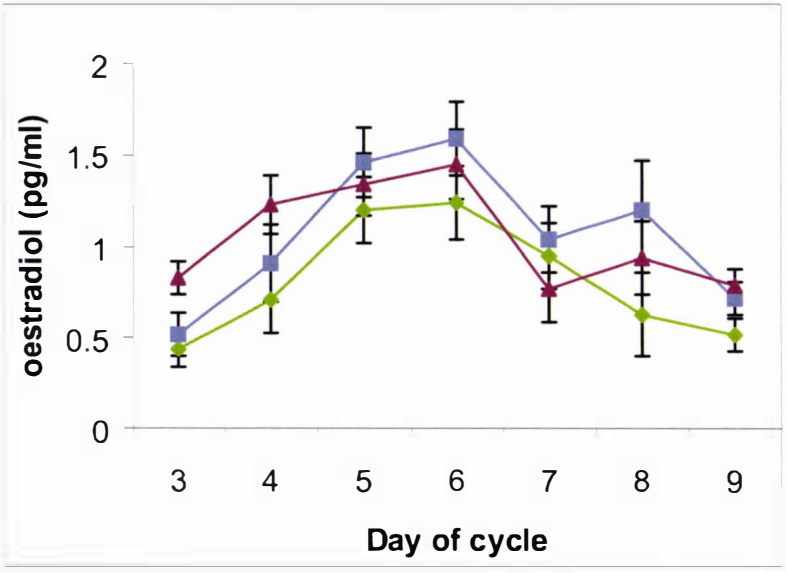


Table 5-2: Plasma oestradiol concentrations (LSMean, pg/ml ± sem), by strain and by day over days 3 to 8 of the oestrous cycle.

	OS	NZH	NZL
Day 3	0.44 ± 0.10^{a}	$0.52 \pm 0.12^{ab\dagger}$	$0.83 \pm 0.09^{b\dagger}$
Day 4	0.71 ± 0.18^{a}	0.91 ± 0.21^{ab}	1.23 ± 0.16^{b}
Day 5	1.20 ± 0.18	1.46 ± 0.19	1.34 ± 0.17
Day 6	1.24 ± 0.20	1.59 ± 0.20	1.45 ± 0.19
Day 7	0.95 ± 0.18	1.04 ± 0.18	0.77 ± 0.18
Day 8	0.63 ± 0.23	1.20 ± 0.27	0.94 ± 0.20
Day 9	$0.52 \pm 0.09^{\dagger}$	0.72 ± 0.09	$0.79 \pm 0.09^{\dagger}$

Treatments: Overseas high (OS), New Zealand high (NZH) and New Zealand low (NZL).

^{abc} different superscripts within a row indicate a significant (P<0.05) difference, †, indicates the difference approaches significance (P<0.1).

Strain had a significant (P<0.05) effect on progesterone concentrations on individual days of the oestrous cycle. NZL animals generally had higher progesterone concentrations than NZH and OS animals between Days 3 and 13 of the cycle. At days 19 and 20 the trend was reversed and OS animals tended to have greater progesterone

concentrations, see Figure 5-6 and Appendix C. One NZH animal had a luteal phase of normal duration, but low progesterone concentrations (not exceeding 4ng/ml). The inclusion, or exclusion of this animal did not affect the results.

Table 5-3: Maximum progesterone (P4) concentrations, and day of maximum P4 concentrations, by strain.

	OS	NZH	NZL
Maximum P4 concentration (ng/ml)	12.32 ± 1.03	11.78 ± 0.99	12.52 ± 1.03
Day of maximum P4 concentration	16.25 ± 0.69^{a†}	15.15 ± 0.66^{a}	14.33 ± 0.69^{a†}

Treatments: Overseas (OS), New Zealand high (NZH) and New Zealand low (NZL).

^{abc} Different superscripts within a row indicate a significant difference (P<0.05), †, indicates difference between OS and NZL approaches significance (P<0.1).

Maximum P4 concentration recorded during the oestrous cycle did not differ between genotypes (Table 5-3). The day on which maximum progesterone concentrations occurred differed between NZL and OS animals (P=0.057). The day of maximum progesterone concentration was significantly related to overall cycle length (P<0.01), when cycle length was tested as a covariate.

IGF-1 concentrations were significantly (P<0.05) higher in NZH than OS and NZL animals on a number of days over the oestrous cycle (see Figure 5-7) and mean IGF-I concentrations in NZH animals were significantly (P<0.01) higher than in OS or NZL animals (167.35 ± 9.88 vs. 128.0 ± 10.29 and 126.74 ± 10.29 ng/ml, respectively). Results were similar when the repeated effect of day was included in the analysis.

Mean leptin concentrations were significantly (P<0.05) greater in NZH than OS animals (P<0.05), but were not different between NZH and NZL, or NZL and OS animals (NZH 2.81 ± 0.14; OS 2.29 ± 0.15 and NZL 2.52 ± 0.15 ng/ml leptin). The same results were obtained whether the analysis was conducted with or without repeated measures. Figure 5-8 shows the pattern of leptin concentrations over the oestrous cycle, indicating days where leptin concentrations differed significantly between strains.

Mean concentrations of insulin were not different between strains, but were significantly (P<0.05) higher in OS compared to NZH and/or NZL, as indicated by * in Figure 5-9.

Figure 5-6: LSMean progesterone concentrations over the oestrous cycle (day 1= oestrus) in three genotypes of yearling Holstein-Friesian heifers. Overseas (—◆—), New Zealand high (—■—), New Zealand low (—▲—) and * denotes a significant (P<0.05) difference between NZL and NZH or OS.

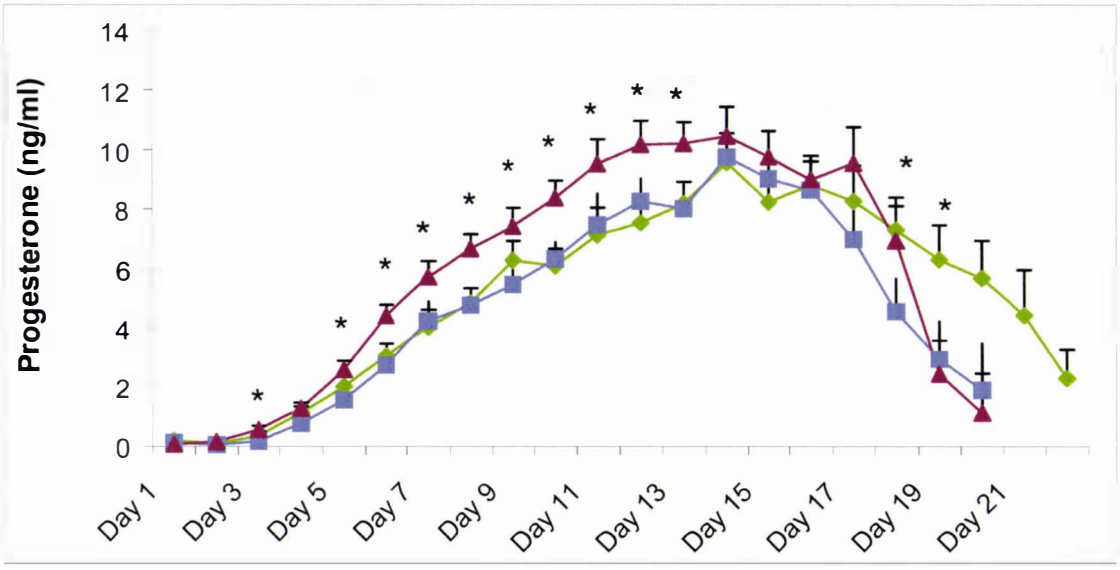


Figure 5-7: LSMean IGF-1 concentrations over the oestrous cycle in three genotypes of Holstein Friesian yearling heifers. New Zealand high (—■—), New Zealand low (—▲—), and overseas (—◆—), * denotes a significant (P<0.05) difference between NZH and either NZL or OS.

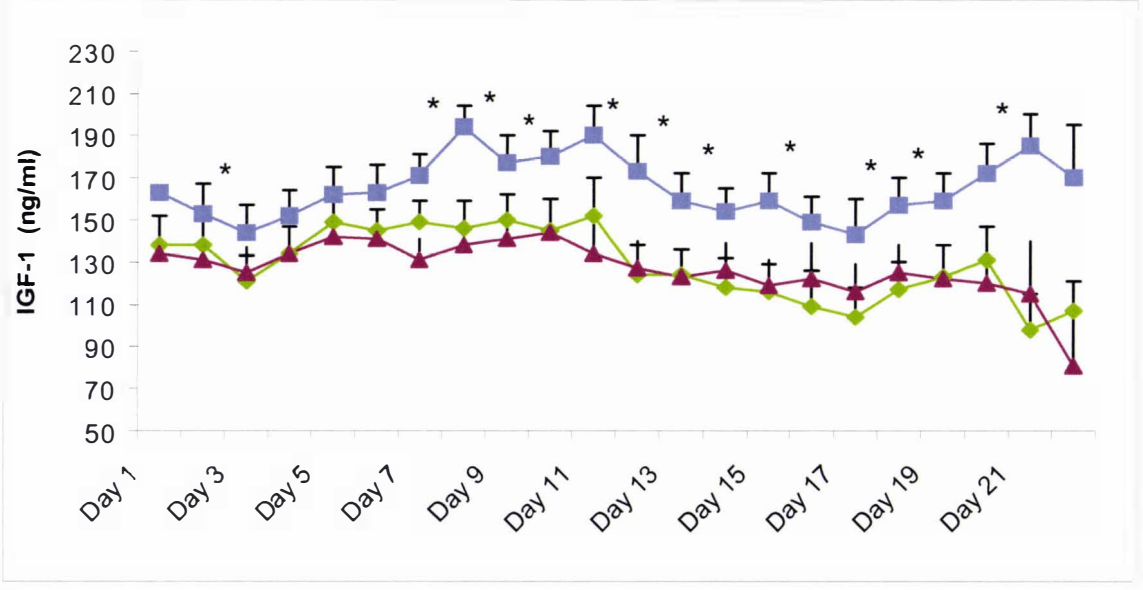


Figure 5-8: LSMean leptin concentrations over an oestrous cycle in three genotypes of Holstein Friesian yearling heifers. New Zealand high (—■—, NZH), New Zealand low (—▲—, NZL) and overseas (—◆— , OS), * denotes a significant (P<0.05) difference between NZH and either NZL or OS.

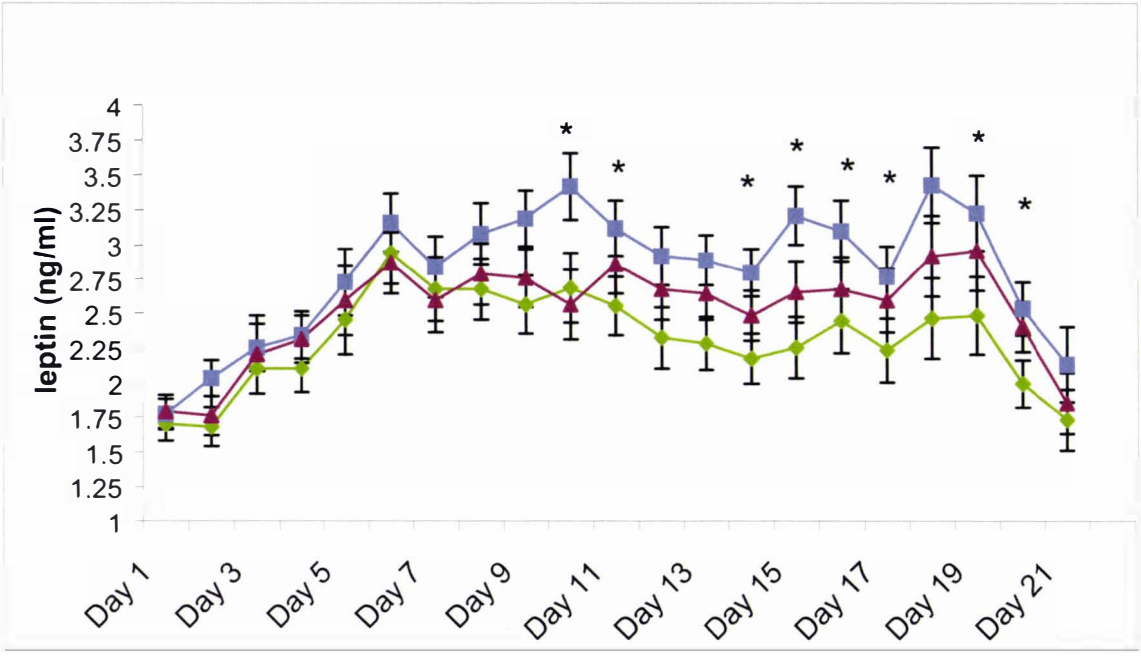
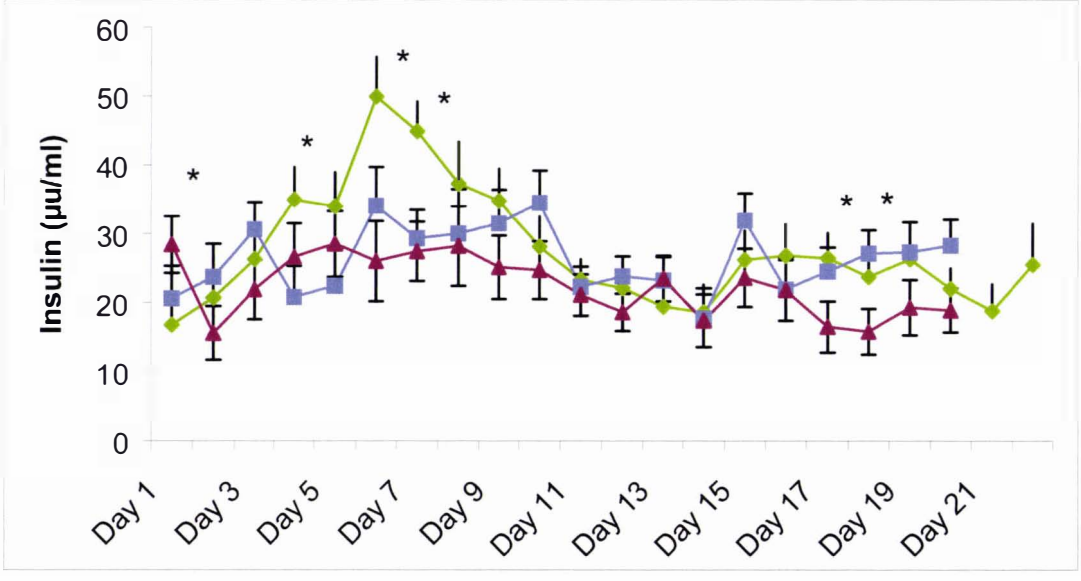


Figure 5-9: LSMean (+ sem) insulin concentrations during an oestrous cycle in yearling New Zealand high (—■—), New Zealand low (—▲—) and overseas (—◆—) Holstein Friesian heifers, * denotes a significant (P<0.05) difference between overseas and either New Zealand low or New Zealand high.



Although the OS heifers were heavier at the start of the trial, there was no difference in the proportion of estimated mature live weight attained by heifers in each of the strains. Growth rates in the month prior to the trial were not different between the strains, and were significantly (P<0.01) lower than growth rates achieved during the trial (Table 5-4). There was no correlation between live weight at the start of the trial and leptin concentrations. Significant correlations between metabolic and reproductive hormones are reported in Table 5-5.

Table 5-4: Live weight gain in the month preceding the start of the trial, and during the trial period, by strain.

	Pre-trial live weight gain (kg/day)	During trial live weight gain (kg/day)
NZH	0.82	1.26[a]
NZL	0.84	1.33[ab]
OS	0.95	1.51[b]

Treatments: New Zealand high (NZH), New Zealand low (NZL) and overseas (OS)

[abc] Different superscripts within a column indicate a significant difference (p<0.05).

Table 5-5: Correlations of metabolic and reproductive hormones, by strain.

		Insulin	Insulin-like growth factor-1	Progesterone
Leptin	NZH	0.38	0.39	0.17
		P<0.0001	P<0.0001	P=0.004
	NZL	0.15	0.13	0.24
		P=0.01	P=0.04	P=0.0001
	OS	0.57	0.26	0.16
		P<0.0001	P<0.0001	P=0.007
Insulin	NZH		0.25	NS
			P<0.0001	
	NZL		0.22	NS
			P=0.0006	
	OS		0.32	NS
			P<0.0001	

Treatments: New Zealand high (NZH), New Zealand low (NZL) and overseas (OS)

5.5 Discussion

Results from the current study suggest that there are some interesting differences in oestrous cycle physiology and endocrinology between the three strains.

5.5.1 Oestrous cycle lengths

The finding that overseas Holstein-Friesians had a longer oestrous cycle than New Zealand Holstein-Friesians is in agreement with reports from lactating dairy cattle as Bilby (1998) and Fahey *et al.* (2003) both reported that New Zealand cows had shorter oestrous cycles than OS cows. Luteal phase length was also greater in OS than NZ cows (Bilby, 1998) and had increased in British Friesians over a period of 20 years, coincident with a significant increase in the proportion of Holstein genetics (Royal *et al.*, 2000). Oestrous cycle length in heifers from the control (analogous to the 1960s dairy cattle) and select (current day) University of Minnesota dairy herd did not differ (M. Lucy, personal communication), but oestrous cycle length was similar to that recorded for the OS strain in the current study. These findings would suggest that there is a strain effect on oestrous cycle length.

The different cycle lengths recorded in this experiment are similar to those recorded by Burke et al. (1995), for differences in length between 2 and 3 wave cycles (19.2 vs. 22.4 days respectively). Without having used ovarian ultrasonography, or frequent FSH samples we cannot say whether a difference in the number of 2 and 3-wave cycles per strain might explain the difference in cycle lengths that were observed. However, previous studies suggest that the number of follicle waves per oestrous cycle is similar between NZ and OS strains. Fahey *et al.* (2003) found that all cows exhibited two follicular waves per cycle, likewise, Bilby (1998) found that the majority of cows had two waves of follicular growth.

Although Fahey *et al.* (2003) found that oestrous cycle length differed between NZ and OS strains, significantly, the duration from the emergence of the dominant follicle to ovulation was not different between strains, so the difference in cycle length is unlikely to affect fertility. Reproductive performance of heifers in the Strain Trial was not different between the strains (see Chapter 4). It would seem that the difference in cycle length between the strains is not having an impact on heifer reproductive performance. This result concurs with the reported high reproductive performance of nulliparous

heifers in the USA (Pursley *et al.*, 1997), despite apparently longer oestrous cycles. The continued reporting of high reproductive performance in heifers strengthens the hypothesis that the decline in reproductive performance is related to factors associated with calving and the onset of milk production (Lucy, 2001a). Whether cows require assistance at calving and the incidence of periparturient diseases, including retained foetal membranes, have been found to have a significant negative effect on reproductive performance in New Zealand herds (McDougall, 2001). There is also likely to be a small effect directly attributable to milk production, as genetic correlations indicate that milk production is negatively associated with fertility (Pryce and Veerkamp, 1999). These negative effects are absent when nulliparous heifers are mated and likely account for a significant proportion of the differences in conception rates between heifers and lactating dairy cattle.

5.5.2 Endocrinology of the heifer oestrous cycle

Progesterone

The secretion of progesterone from the corpus luteum is necessary to maintain a suitable uterine environment for the growth and development of the conceptus and embryo (Kotwica *et al.*, 2002). Progesterone concentrations during the early luteal phase play an important role in controlling the development of the embryo, particularly its ability to secrete interferon-tau (Mann *et al.*, 1998). Corpora lutea that have a normal lifespan, but reduced progesterone secretion may be seen after an induced ovulation (Hunter, 1991). Despite the oestrous cycle that was studied in the current trial being the result of an induced ovulation there was only one animal that had sub-luteal progesterone secretion, but a normal length luteal phase. Progesterone concentrations over an oestrous cycle were not different between Minnesota Selection line control heifers (analogous to 1960s dairy cattle) and heifers selected for high milk production (Weber *et al.*, 2003), but in the current study progesterone concentrations in the control line (NZL) were higher than in the two strains that had been selected for increased milk production. Pregnancy rates were not improved in heifers when progesterone was supplemented on days 7-13 (van Cleef *et al.*, 1991), coincident the time when progesterone concentrations were higher in NZL heifers. A recent meta-analysis (Lean *et al.*, 2003) also reported that post-insemination supplementation with progesterone failed to increase pregnancy rates. However, in lactating cows low progesterone

concentrations (<3ng/ml) 5 days after insemination were associated with severely reduced conception rates compared to cows with high progesterone concentrations at this time (Mann *et al.*, 2003). Although progesterone concentrations were lower in the NZH and OS strains than the NZL strain at a similar stage of the oestrous cycle, the differences in progesterone concentrations were much smaller than the difference between the high and low progesterone groups reported by Mann *et al.*(2003), and may not have been large enough to cause a difference in reproductive performance. The higher progesterone concentrations in the NZL strain did not translate into an improved reproductive performance during the heifer-mating period (see Chapter 4), therefore the physiological significance of this finding must be questioned.

Oestradiol

The pattern of oestradiol release in the current trial was similar to that observed by Bleach *et al.* (2001), who also worked with heifers with synchronized oestrous cycles. The increase in oestradiol concentrations in the current trial coincided with the expected time of emergence and rapid growth of the first dominant follicle (Bleach *et al.*, 2001) and are therefore taken to indicate the growth of the first dominant follicle. Preovulatory oestradiol concentrations were not different between cows selected for increased milk yield and control animals (Lucy and Crooker, 1999) and nor were oestradiol concentrations associated with the expected emergence and development of first-wave dominant follicle different between the strains in the current trial. The small differences in oestradiol concentrations at isolated time points in the current trial were probably not physiologically significant.

5.5.3 Metabolic hormones

Insulin-like growth factor-1 (IGF-1) is a metabolic hormone that declines during periods of undernutrition (Clemmons and Underwood, 1991). Receptors for IGF-1 are found on granulosa cells (Spicer, Alpizar and Vernon, 1994) and IGF-1 has been found to have a stimulatory effect on granulosa cell proliferation and oestradiol production (Spicer *et al.*, 1993). Concentrations of IGF-1 in follicular fluid are in part determined by peripheral IGF-1 concentrations. Follicular and peripheral IGF-1 concentrations were significantly correlated with in intermediate and large, but not small follicles from sheep (Leeuwenberg *et al.*, 1996). Intrafollicular production of IGF-1 is believed to contribute

to the total follicular IGF-I pool in cattle as IGF-I mRNA has been detected in granulosa cell cultures (Spicer *et al.*, 1993). However, Armstrong *et al.* (2000) failed to detect mRNA encoding IGF-I in granulosa cells and expression was very low in thecal cells from bovine follicles. The increase in free IGF-I that has been reported to occur before diameter deviation in heifers (Ginther *et al.*, 2002), may be associated with intraovarian changes in insulin-like growth factor binding proteins (IGFBPs) (Armstrong *et al.*, 1998). The expression of insulin-like binding protein-2 is lower in large follicles than small or medium sized follicles (Armstrong *et al.*, 1998). Concentrations of IGFBP-4 are reduced in dominant follicles, but the expression of IGFBP-4 does not change with follicular size (Armstrong *et al.*, 1998), indicating that specific proteases that inactivate IGFBP-4 are acting. These proteases have been identified in ovine follicular fluid (Besnard *et al.*, 1996). The concentrations of IGF-I within the follicle are therefore determined partially by peripheral IGF-I concentrations, but modulated by intraovarian factors, so peripheral IGF-I concentrations may not be a useful indicator of follicular IGF-I availability.

Concentrations of IGF-I are affected by nutrition. Heifers or lactating dairy cattle offered high energy diets have higher IGF-I than cattle offered low energy diets (Armstrong *et al.*, 2001; Obese *et al.*, 2003). The animals in the current trial were well fed by New Zealand standards (*ad libitum* spring pasture), and were achieving reasonable growth rates, and the IGF-I concentrations reflect this.

Concentrations of IGF-I were significantly higher in the NZH strain, despite all animals being grazed together during the trial. Analysis of live weight data revealed that growth rates increased significantly during the trial, probably due to increasing pasture availability in the spring period, and the animals being grazed in a smaller herd, decreasing competition for feed. During the month prior to the trial growth rates were not different between the three strains, but during the trial period growth rates were higher in the OS strain than the NZH strain. These differences in growth rates are unlikely to explain the differences in IGF-I concentrations between the three strains. There was no difference in IGF-I concentrations between OS and NZ Holstein Friesian strains of equivalent genetic merit, in early lactation, fed either pasture or a total mixed ration (TMR), but IGF-I concentrations were greater in TMR fed cows, (Chapter 8). However, later in the postpartum period, IGF-I concentrations in cyclic animals were

highest in the NZ strain fed TMR, similar in the NZ strain fed pasture and OS fed TMR and lowest in the OS animals fed pasture, suggesting a genotype effect (Fahey *et al.*, 2003).

MacKenzie *et al.* (1988) found that baseline concentrations of IGF-1 were not different between Friesian bulls of high or low genetic merit. However, in the current study, the high genetic merit NZH strain had significantly higher concentrations of IGF-1 than the low genetic merit NZL strain. The IGF-1 results from lactating strain trial cows presented in Chapter 6 would suggest that the differences observed in the heifers in the current study do not continue through to lactating animals in early lactation, at a stage when energy balance is still likely to be negative.

The finding that leptin concentrations were higher in NZH than the other two strains was surprising, given all animals had similar condition scores and were well fed at the time of the trial. Condition scores were not different between the strains and there was insufficient between animal variation to correlate leptin and body condition scores. Whilst live weights differed between the strains, leptin concentrations and live weight were not correlated. Frajblat *et al.* (1998) found no difference in leptin concentrations in cows at a range of body condition scores during the postpartum period, yet in sheep Delavaud *et al.* (2000) reported a significant positive correlation between body fatness or body condition score and plasma leptin concentrations and Liefers *et al.* (2003) found that heavier cows had higher leptin concentrations than lighter cows. The body condition scoring system may not accurately reflect fat stores in animals that are growing rapidly, and is rarely used in growing cattle as part of normal farm practice in New Zealand.

Concentrations of leptin increase during the prepubertal period, and peak 2 weeks before puberty onset (Diaz-Torga *et al.*, 2001), but seem to plateau after puberty onset (Luna-Pinto and Cronje, 2000). Insulin is a positive regulator of leptin in humans (Utriainen *et al.*, 1996) and in cattle (Block *et al.*, 2003). Also in humans, Koutkia *et al.* (2003) found that changes in insulin preceded changes in leptin by 275 minutes, during an overnight fast. It was therefore not surprising to find that leptin and insulin concentrations were significantly correlated in the current study. However, it remains unclear why leptin concentrations were higher in the NZH strain.

Concentrations of IGF-1 were also significantly correlated with leptin in the current trial. In humans it has been suggested that although correlations between leptin and the IGF-1 system exist leptin does not regulate the IGF-1 system and vice versa (Gomez *et al.*, 2003). This correlation may have arisen through the stimulatory effects of insulin on both IGF-1 (Butler *et al.*, 2003) and leptin concentrations. In cattle, the factors other than fat mass that influence serum leptin concentrations are yet to be determined.

The significant correlation between progesterone and leptin was surprising, although the trend observed in Figure 5-8 would indicate that there is some cyclic variation in leptin concentrations. However, when the pattern of leptin secretion was compared in individual cows over the oestrous cycle (data not shown), no consistent pattern could be determined.

As well as a central role in the regulation of glucose metabolism insulin has been implicated as having important roles in reproduction. Insulin receptors are found on ovarian cells and insulin has been implicated as having a role in stimulating ovarian steroidogenesis (Spicer and Echternkamp, 1995). Previous studies have indicated that basal and glucose-stimulated insulin concentrations are higher in heifers and bulls of high compared to low genetic merit (MacKenzie *et al.*, 1988; Xing *et al.*, 1993). Despite the large difference in genetic merit between the NZL and NZH and OS strains in the current study there was no difference in basal insulin concentrations. Insulin concentrations were correlated with IGF-1 concentrations within strains in the current study. Insulin may have a role in controlling IGF-1 concentrations. Insulin has been found to stimulate IGF-1 production under hyperinsulinemic-euglycemic clamps (McGuire, Dwyer *et al.*, 1995; Butler and Butler, 2001; Back, 2002). Also, diabetic rats have low concentrations of IGF-1, which are increased by insulin treatment (Pao *et al.*, 1992). Recent research has demonstrated that insulin stimulates growth hormone receptor – 1A (GHR-1A) expression on the liver, and directly stimulates hepatic IGF- 1 gene expression (Butler *et al.*, 2003), probably through effects on the transcription factor Sp1 and the insulin-responsive binding protein (IRBP; (Kaytor *et al.*, 2001b; Kaytor *et al.*, 2001a; Pan *et al.*, 2001).

5.6 Conclusions

Differences in oestrous cycle length, while of interest, do not appear to be physiologically significant. Likewise, although progesterone concentrations were higher in the NZL strain, this was not found to have a beneficial effect on pregnancy rates, suggesting that progesterone secretion in the NZH and OS strains was adequate to support pregnancy.

The differences in metabolic hormones observed in the current trial may be an indication of physiological differences in the regulation of energy metabolism between the strains that will become more apparent in lactating animals. Thus, whilst interesting differences between the three strains were observed in oestrous cycle characteristics and reproductive and metabolic hormones, there was no difference in reproductive performance between the strains.

5.7 References

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6 *Postpartum anovulatory intervals and reproductive performance of three genotypes of Holstein-Friesian dairy cattle managed in seasonal pasture-based dairy systems.*

6.1 Abstract

Declining fertility of dairy cows in New Zealand (NZ) has been a cause of anxiety to the industry. This decline has occurred in parallel with the introduction of overseas (OS) Holstein-Friesian cows into NZ, so many within the industry believe that the decline in fertility has been caused by the introduction of OS genetics. This view has been fuelled by the many international reports of declining fertility in OS cows. In NZ, the main cause of sub-fertility has been an extended postpartum anovulatory interval (PPAI), although recent evidence suggests that conception rates may also be declining. This trial determined whether there are differences in PPAI, BCS and parameters of reproductive performance between NZ and OS Holstein-Friesians. One hundred and forty six records from 88 NZH animals of high genetic merit (NZH), 81 records from 48 NZ animals of low genetic merit (NZL), and 137 records from 88 OS high genetic merit animals that calved in Years 1 (all two-year-olds) and 2 (two- and three-year-olds) were included. Over both years, PPAI in two-year-olds were significantly shorter ($P<0.05$) in OS (20 days shorter) and NZL (12 days shorter) than in NZH animals. Body condition score at calving was not different between strains. In Year 1 of the trial, significantly ($P=0.001$) more NZH than NZL or OS two-year-olds were treated for anoestrus prior to the start of mating. Other measures of reproductive performance did not differ significantly between strains. Metabolic hormones (insulin, IGF-1 and GH) were investigated in the postpartum period, and on the whole, did not differ between the three strains. The physiological reasons for the differences in PPAI require further investigation.

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6.2 Introduction

In seasonal pasture-based dairying systems it is important to maintain a 365-day calving interval. This necessitates that ovulatory activity resumes early in the postpartum period and that conception rates during the short breeding season are high, so that the calving period is compact. The major form of infertility in New Zealand (NZ) dairy cows is an extended postpartum anovulatory interval (PPAI; (Macmillan, 2002)), a problem that can be exacerbated by low body condition score (BCS) at calving (McGowan, 1981; McDougall, Burke *et al.*, 1995). Untreated extended PPAI can affect the intervals from the planned start of mating to first insemination and conception, indicators of the compactness of the following calving period. Holstein cows, originating from North American genetic strains (Overseas; OS), have been widely used in NZ in recent years (Harris and Winkelman, 2000). This has coincided with worldwide concern over an apparent decline in the fertility of Holstein-Friesian dairy cattle, as evidenced by declines in conception and calving rates to first service (Butler *et al.*, 1995; Royal *et al.*, 2000). The 2002 Livestock Improvement Corporation Friesian Premier Sires Team had an average of 56.3% OS genetics, with a range from 6.25% to 100% OS genetics (J. Pryce, personal communication, 2002). The percentage of OS genetics is the percentage of the ancestors of an animal that were Holsteins born in the USA. In New Zealand cows with a high proportion of OS genetics have longer intervals to first service and are less likely to produce an artificial insemination sired calf than NZ strains, suggesting a possible delay in the initiation of postpartum cyclicity (Harris and Winkelman, 2000). The purpose of the work reported here was to examine and compare reproductive performance, and in particular length of PPAI, in cows of New Zealand genetic origin of high (NZH), or low (NZL) genetic merit and high genetic merit cows of OS origin (OS).

6.3 Materials and methods

6.3.1 Reproductive data

Ethical approval for the experiments detailed in this chapter was granted by the Ruakura Animal Ethics Committee, approval numbers 3879, 3962 and 4250. Details of animals that had reproductive data recorded are summarised in Table 6-1

For analyses of PPAI and BCS, only animals that calved within the first 6 weeks of the calving period were included. Body condition scores were assessed weekly from calving until the planned start of mating by experienced staff on a 10-point scale ((1= emaciated, 10 = obese; (Macdonald and Macmillan, 1993). Condition score at calving was assessed on the day of calving, when cows and calves were brought to the dairy to be separated. Further details are given in section 3.2.1 on the genetic origin of the animals and details of farm management practices.

Table 6-1: Summary of animals who had reproductive data recorded.

	Animals	No. of records	Breeding worth ($)[1]	Percentage of OS genetics
NZH	88	146	130	24.5
NZL	48	81	-20	6.9
OS	88	137	102	90.5

Treatments: New Zealand high (NZH), New Zealand low (NZL) and overseas (OS)

[1]Breeding worth as at October 2002.

6.3.2 Metabolic hormones

Blood samples were collected weekly from cows in the Dexcel Strain Trial for up to the first 8 weeks after calving. Sample collection ceased at the planned start of mating, so some animals had incomplete sets of samples. In Year 1, concentrations of insulin, IGF-1 and GH were determined in samples from five cows in Herds 1 and 3 (NZL), 4 and 6 (NZH) and 8 and 11 (OS) (details of the feeding levels of the herds are given in 3.2.1). In Year 2, samples from eight cows in each herd (4 x 2-year-olds and 4 x 3-year-olds) were analysed for the same hormones. Only cows that had a full set of eight samples available were used, so the data set was restricted to early calving animals.

6.3.3 Reproductive management

A management decision was made to bring the planned start of calving forward by one week between Years 1 and 2. In both years, treatment for prolonged PPAI was proactive. From one week before the planned start of mating (PSM, 2-October 2001 and 2002) animals were treated for anoestrus if they had been calved for at least 28 days and their milk progesterone concentrations remained below 2ng/ml. Oestrus was induced by the insertion of a controlled internal drug releasing device (CIDR; Pharmacia, Auckland) for 6-days, followed by 1mg of oestradiol benzoate (Cidirol, Bomac Laboratories Ltd, Auckland) 24-hours after CIDR removal. Transrectal ultrasonographic scanning was used to detect pregnancy at approximately 60 days after the planned start of mating and again 30-40 days after the bulls were removed. Pregnancies were aged at scanning to confirm dates of conception, relative to recorded dates of insemination and service. This information was used in the calculation of reproductive data, including percentage pregnant at 42 days after the PSM (P42), the interval from PSM to conception and conception rates to first service. A final pregnancy test was carried out in late lactation (late April/May). The 12-week in calf rates (percentage pregnant in 12-weeks) were based on these test results. The mating period finished after 12-weeks.

6.3.4 Hormone Assays

Insulin Assay

Samples were analysed at the University of Western Australia, Perth, in a double antibody radioimmunoassay (see 3.4.5 for further assay details).

The limit of detection of the assay was 0.78 μU/ml. For samples with a mean concentration of 5.2 μU/ml, the coefficient of variation (CV) was 9.7%, for samples with a mean concentration of 10.6 μU/ml the CV was 7.5% and for samples with a mean concentration of 32.6 μU/ml the CV was 3.7%.

Growth Hormone Assay

Samples were analysed at Dexcel, in a double antibody radioimmunoassay that has been validated for bovine use by Alpha Scientific Ltd (see 3.4.4 for further assay details).

The limit of detection of the assay was 0.24 ng/ml. Intra-assay coefficients of variation for samples with mean concentrations of 2.8, 6.3 and 11.1 ng/ml were 12.5, 7.9 and 10.3% respectively. Intra-assay coefficients of variation for samples with mean concentrations of 2.8, 6.3 and 11.1 ng/ml were 6.5, 4.2 and 4.8% respectively.

Insulin-like Growth Factor 1 Assay

Samples were analysed in a double antibody radioimmunoassay at the University of Western Australia, Perth (see 3.4.3 for further assay details).

Intra-assay co-efficients of variation for plasma pools of 3.8 ng/ml and 12.0 ng/ml and 33.4 ng/ml were 9.4, 6.3 and 5.4% respectively. The minimum detectable concentration was 0.95 ng/ml.

Progesterone Assay

Whole milk samples were collected twice weekly from all animals for determination of progesterone concentrations, starting within 7 days of calving. Progesterone concentrations were determined using an enzyme-linked immunosorbant assay kit (Ridgeway Sciences, Gloucestershire, UK), validated for use in cattle (Sauer *et al.*, 1986). Interassay coefficients of variation for samples with a mean concentration of 2.57 ng/ml and 9.95 ng/ml were 20.5% and 12.5% respectively. Further details of assay methodology can be found in 3.4.7

Definitions for luteal activity, and luteal phase length were adapted from Royal *et al.* (2000) and Fahey *et al.* (2004) to account for differing sampling frequency.
Luteal activity was defined as progesterone concentrations of >2ng/ml followed by >4ng/ml in two consecutive samples, with an adjustment for errors of timing associated with twice weekly sampling of –1.8 days.

A luteal phase was defined as the period of time during which progesterone concentrations were above 2ng/ml, then above 4ng/ml then above 2ng/ml in consecutive samples. The length of the luteal phase was measured from the first elevation of progesterone of ≥2ng/ml that was followed by a sample with a progesterone concentration of ≥4ng/ml, to the final consecutive milk sample of ≥2ng/ml. This twice-

weekly sampling regime underestimates the length of the luteal phase by an average of 3.6 days (Fahey *et al.*, 2004).

6.3.5 Statistical Analysis

Data were combined according to strains for analysis as feeding level (see 3.2.1 for a description of feeding levels) was not found to significantly affect any of the measures studied. This also increased the statistical power of the analysis. Postpartum anovulatory intervals were analysed using parametric frailty models with normal distribution, in which feeding level, age at calving, year where applicable and strain were included as fixed effects, and sire as a random effect, using the suvReg function in S-Plus 6.1 (S-Plus, Version 6.1). This procedure estimates PPAI for animals that failed to ovulate. Analysis of variance was used to examine the relationship of PPAI, time to conception and calving BCS and BCS loss, in Genstat, after the censor procedure had been used to estimate PPAI intervals for animals that failed to ovulate (GenStat, Version 6.1). Other continuous data were analysed by analysis of variance using mixed models (mixed procedure of SAS) with a model that included the fixed effect of strain, week and year and a random effect of sire, nested within strain. Correlations were determined in SAS. Categorical data were analysed using Chi-squared (SAS, Version 8.1).

6.4 Results

6.4.1 Calving dates

Mean calving dates were not different between strains, or between years, despite an earlier planned start of calving in Year 2 (12 July 2002) than in Year 1 (20 July 2001; Table 1). In Year 2 the mean calving date was significantly earlier in the first lactation heifers than in the 2nd lactation animals (18-Jul ± 3.1 days vs. 10-Aug ± 1.9 days; $P<0.0001$). Details of calving spread can be found in Table 6-2 and the cumulative percentage calved, by week of the calving period, is illustrated in Figure 6-1 and Figure 6-2.

6.4.2 Postpartum anovulatory intervals and body condition scores

A total of 60% (197/331) of animals that calved within 6-weeks of the PSC in both years had ovulated by one week before the PSM; see Table 6-2 for a breakdown by

strain. Over both years of the trial, mean PPAI in two-year-olds was significantly longer in NZH animals than OS and NZL ($P<0.05$) by 20 and 12 days, respectively. In Year 2 of the trial, PPAI were again longer in NZH than OS and NZL ($P<0.01$) animals by 15 and 14 days, respectively. There was no difference in PPAI between OS and NZL strains. The PPAI was 12 days longer in 3-year-olds than in 2-year-olds in Year 2 ($P<0.0001$). Body condition scores, and loss of body condition score from calving to Week 4 of lactation, by strain, are given in Table 6-2. The pattern of body condition score loss over lactation is illustrated in Figure 6-3 and Figure 6-4. In Figure 6-4 only cows that calved within two weeks of the planned start calving over both years of the trial are included, to remove the distorting effect of different calving dates from the pattern of condition score change.

Calving BCS in 2-year-olds was lower in Year 1 than in Year 2 (4.8 ± 0.04 vs. 5.6 ± 0.05 units; $P<0.0001$). In Year 2 BCS at calving was higher in 2-year-olds than 3-year-olds (5.6 ± 0.05 vs. 4.6 ± 0.04 units; $P<0.0001$). Body condition score loss from calving to week 4 of lactation was greater in Year 1 than Year 2 (0.6 ± 0.04 vs. 0.3 ± 0.04 units; $P<0.0001$). However, body condition score loss continued for longer in Year 2, so that by 12 weeks of lactation, body condition score loss from calving was greater in Year 2 than Year 1 (0.5 ± 0.05 vs. 0.7 ± 0.05; $p<0.01$). In Year 2 of the trial condition score loss from calving to week 4 of lactation tended to be greater in 2-year-olds than 3-year-olds (0.4 ± 0.05 vs. 0.3 ± 0.03 units; $P=0.08$), whilst condition score loss from calving to 8 and 12 weeks of lactation was significantly greater in 2-year-olds (0.9 ± 0.06 vs. 0.4 ± 0.04; $p<0.0001$ and 1.1 ± 0.07 vs. 0.5 ± 0.05; $p<0.0001$ respectively). A significant negative relationship between BCS at calving and PPAI was found in 2-year-olds (coefficient of -11.6 days $\pm$ 2.64; $P<0.001$) and in Year 2 of the trial (2- and 3-year-olds combined, coefficient of -15.8 ± 2.96 days; $P<0.001$). There was no relationship between BCS loss to 4-weeks postpartum and PPAI ($p>0.1$), nor was BCS loss to 12 weeks postpartum related to time from the start of the mating period to conception ($p>0.1$).

Figure 6-1: Cumulative percentage calved, by strain and by week of the calving period in Year 1. New Zealand high —, New Zealand low —, overseas —.

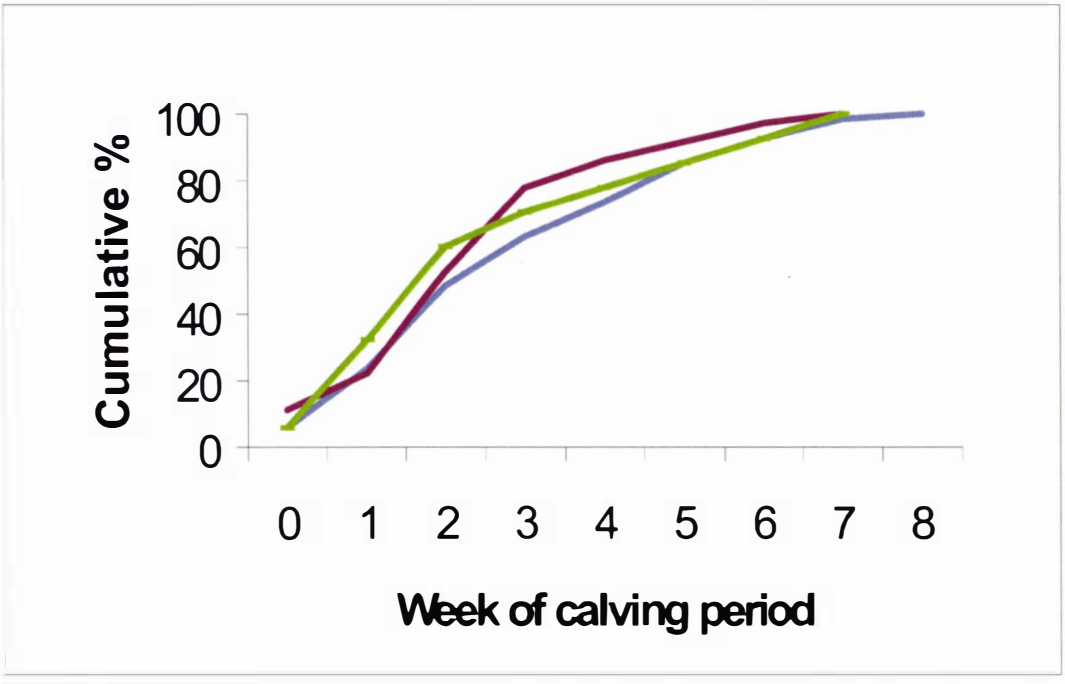


Figure 6-2: Cumulative percentage calved by strain and by week of the calving period in Year 2. New Zealand high —, New Zealand low —, overseas —.

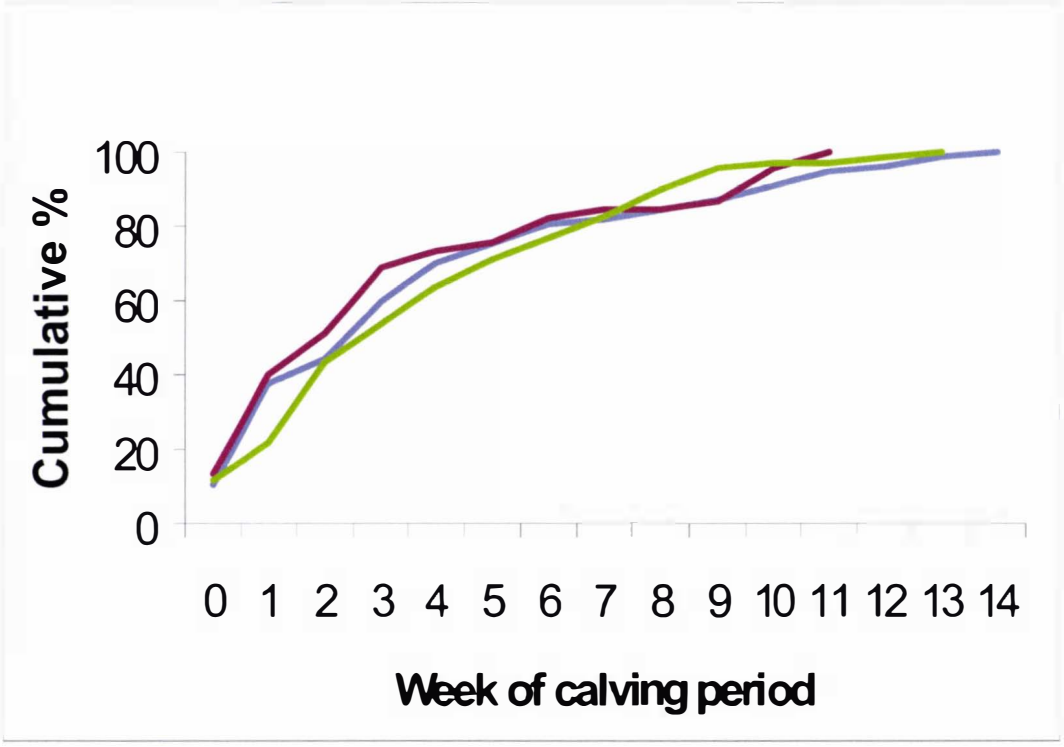


Figure 6-3: Mean body condition scores (1= emaciated, 10 = obese), in each strain from June 2001 to May 2003 (Year 1 and Year 2) with mean calving date and planned start of mating (PSM – 2-October) indicated. New Zealand high (—◆—), New Zealand low (—■—) and overseas (—▲—).

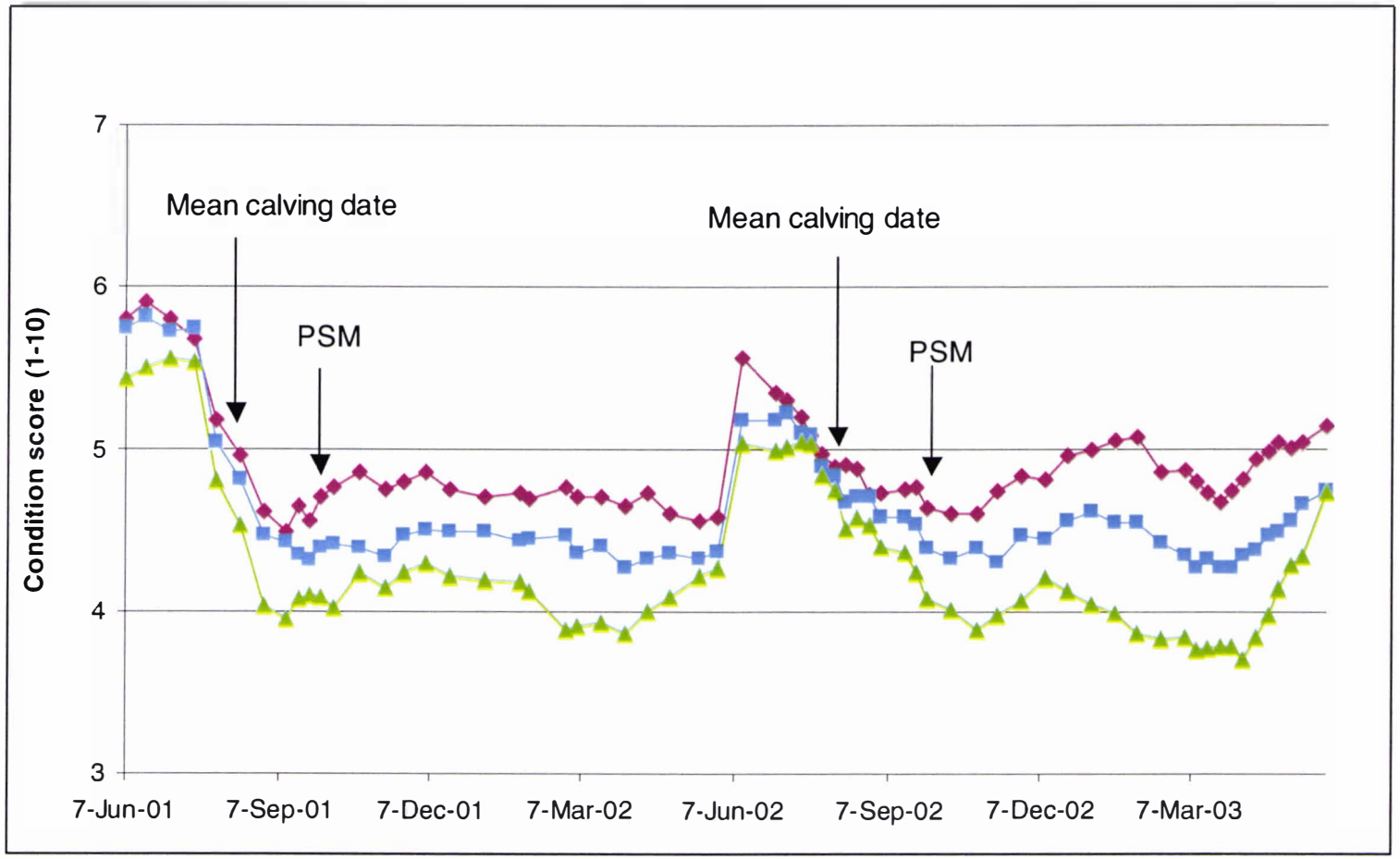


Figure 6-4: Mean body condition score (1=emaciated, 10=obese) over lactation 1 and 2 in a group of cows that calved within 2 weeks of the planned start of calving in both Year 1 (as 2-year-olds) and Year 2 (as 3-year-olds). Planned start of calving for this group of animals, and planned start of mating (PSM – 2-October) are indicated on the graph. New Zealand high, n=10 (—◆—), New Zealand low, n=7 (—■—) and overseas, n=7 (—▲—).

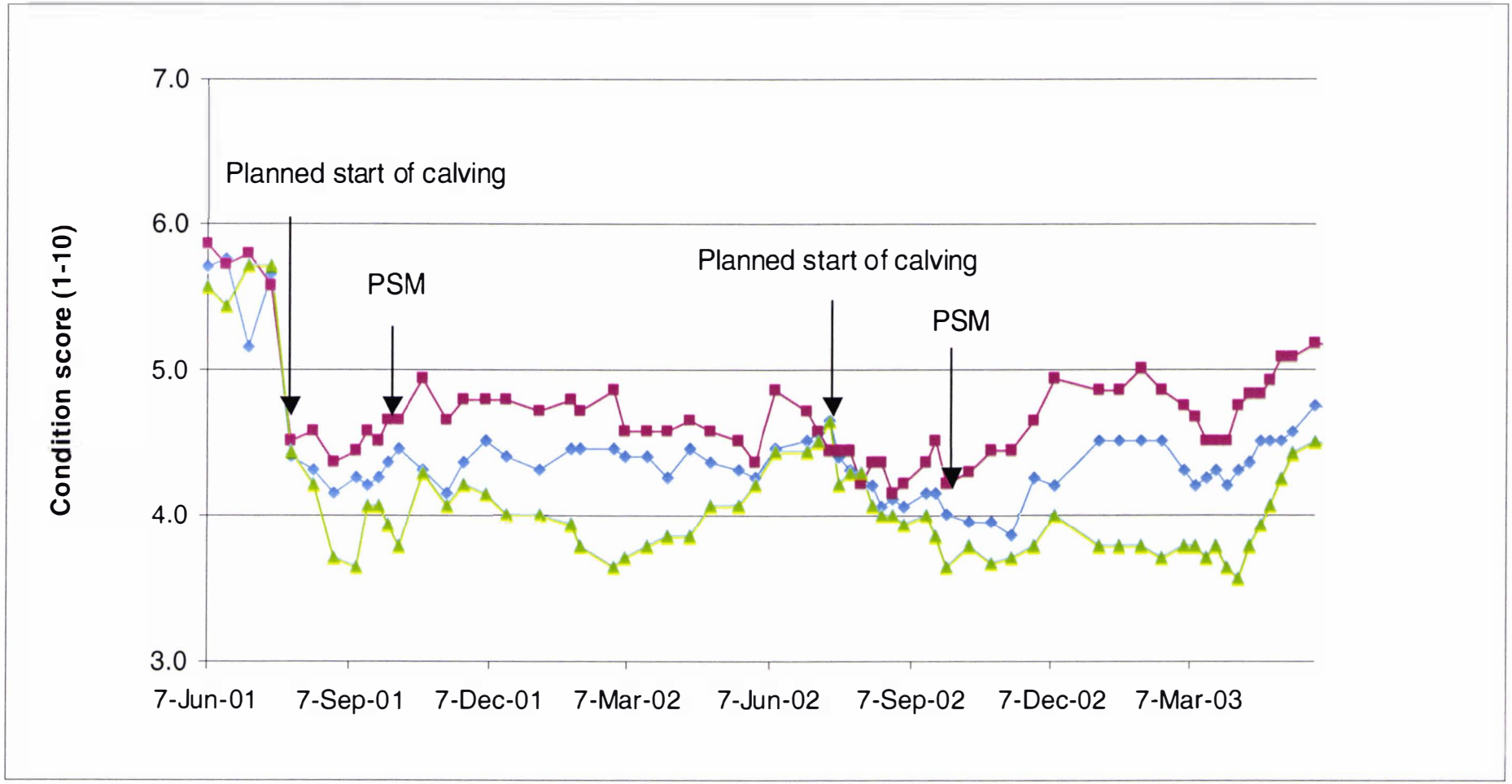


Table 6-2: Reproductive outcomes of the animals in Years 1 and 2. Planned start of calving (PSC), planned start of mating (PSM).

	Year	OS	NZH	NZL
Mean calving date	1	3-Aug ± 2.2	7-Aug ± 1.8	2-Aug ± 2.1
(Days ± sem)	2	4-Aug ± 2.6	4-Aug ± 3.0	31-Jul ± 3.6
Intervals from PSC to 50%	1	11 (21)	14 (28)	11 (16)
(and 75%) of animals calved	2	19 (39)	16 (35)	13 (34)
(in days)				
Treated for anoestrum (%)	1	34^{a}	75^{b}	53^{c}
	2	28^{ab}	36^{a}	18^{b}
21-day submission rate	1	88	91	92
	2	87	77	87
Interval from PSM to first	1	10.1 ± 1.29	6.1 ± 1.25	7.6 ± 1.77
service (days ± sem)	2	10.4 ± 1.22	11.0 ± 1.25	10.0 ± 1.19

Treatments: overseas (OS), New Zealand high (NZH), New Zealand low (NZL).

^{abc}Differing superscripts within a row indicate significance (P<0.05)

Table 6-3: Percentage of cows that ovulated during the postpartum period, and body condition score (BSC) and decrease in BCS, by strain, in animals that calved during the six weeks after the planned start of calving.

	Year	OS	NZH	NZL
Ovulated by one week before PSM (%)	1 + 2	70^{a}	42^{b}	70^{a}
Ovulated before day 21 pp (%)	1 + 2	18^{a}	4^{b}	17^{a}
Ovulated after day 43 pp (%)	1 + 2	14^{a}	24^{b}	24^{ab}
Body condition score (BCS)	1	4.8 ± 0.05	4.8 ± 0.06	4.9 ± 0.08
post-calving (± sem)	2	5.0 ± 0.09	5.0 ± 0.08	5.1 ± 0.11
Decrease in BCS from calving to	1	0.8^{a} ± 0.06	0.5^{b*} ± 0.06	0.3^{b*} ± 0.07
4-weeks postpartum (± sem)	2	0.4 ± 0.05	0.3 ± 0.05	0.2 ± 0.06

^{abc}Differing superscripts within a row indicate significance (P<0.05), *Difference between NZH and NZL approaches significance (P=0.06).

6.4.3 Reproductive performance

The length of the first cycle (interovulatory interval) could be recorded in only those animals for which the whole first cycle, and the subsequent rise in progesterone associated with the next cycle had been recorded. Milk sampling continued for longer in Year 1 (i.e. until the middle of December), therefore, almost all animals that started cycling had the length of the first cycle recorded. However, in Year 2 of the trial, twice-weekly sampling stopped at the planned start of mating, so only those animals that had started cycling earlier in the mating period had sufficient time to have a subsequent cycle recorded.

A total of 195 observations on the length of the first ovulatory cycle (interovulatory interval) were made over Years 1 and 2 of the trial. The length of the first postpartum ovulatory cycle was between 10 and 17 days in length for 38% of animals and greater than 25 days in length for 7% of animals. The incidence of short cycles tended to be greater in Year 2 of the trial ($p=0.057$), but the incidence of longer than average cycles did not differ between Years. Overall, the incidence of abnormal length first ovulatory cycles was 38% in Year 1 and 49% in Year 2. Table 6-4 shows the proportion of interovulatory intervals that were either of short, normal or long duration, by strain in both years of the trial.

Table 6-4: Interovulatory intervals of the first postpartum cycle, by strain, in Year 1 and 2, percentages are shown in brackets.

Duration of interovulatory interval	OS	NZH	NZL
17 days or less – Year 1	10 / 40 (25)	7 / 21 (33)	5 / 17 (29)
17 days or less – Year 2	12 / 45 (27)[a]	18 / 32 (56)[b]	20 / 38 (53)[b]
18 – 24 days – Year 1	24/ 40 (60)	14 / 21 (67)	10 / 17 (59)
18 – 24 days – Year 2	28 / 45 (62)[a]	13 / 32 (41)[ab†]	15 / 38 (39)[b]
25 or more days – Year 1	6 / 40 (15)	0 / 21 (3)	2 / 17 (12)
25 or more days – Year 2	5 / 45 (11)	1 / 32 (3)	3 / 38 (8)

Treatments: overseas (OS), New Zealand high (NZH) and New Zealand low (NZL).

[abc]Different superscipts within a row indicate a significant difference, † indicates the difference between NZH and OS approaches significance.

The length of the luteal phase increased between the first and second postpartum ovulatory cycles (13.4 vs. 15.1 days respectively; $p<0.005$). Luteal phase length was

more variable in the first postpartum cycle compared to the subsequent cycle, with a range of 47 days for the first luteal phase vs. 37 days for the second luteal phase.

Indicators of reproductive performance are summarised in Table 6-2, Table 6-3 and Table 6-4. A greater proportion of NZH and NZL animals were treated for anoestrus in Year 1 of the trial than in Year 2 ($P<0.001$). In Year 2 the proportion of 2-year-olds treated was significantly lower than for the 3-year-olds (10% vs. 35% respectively; $P<0.001$). See Table 6-5 for a breakdown of the number of animals treated for anoestrus in Year 2, by strain and by age.

Within a strain, and for cows which had calved within 6-weeks of the start of the calving period, yields of milksolids from Weeks 2 to 6 of lactation were not different ($p>0.1$) between cows that were treated for anoestrous, and cows that had ovulated spontaneously prior to the planned start of mating (NZH 60.7 vs. 61.9, NZL 51.5 vs. 50.9, OS 63.3 vs. 60.8 kg milksolids).

Table 6-5: Animals treated for anoestrus in Year 2 of the trial, by strain and by age.

	2 year olds		3-year-olds	
OS	1/20	5%	18/49	37%
NZH	4/20	20%	24/58	41%
NZL	0/12	0%	8/33	24%
Overall	5/52	10%	50/140	36%

Treatments: New Zealand high (NZH), New Zealand low (NZL), overseas (OS)

First service conception rates tended to be higher in Year 2 of the trial ($P=0.068$), but were not different between cycling and anoestrous treated animals (44 vs. 36%, respectively). When both years' data were combined P42 rates were higher in NZH than OS animals ($P<0.05$). The interval from PSM to first service was shorter in Year 1 than in Year 2, whilst the interval from PSM to conception was shorter in Year 2 than Year 1 ($P<0.05$).

Date of calving, relative to the planned start of calving had a significant effect on 6-week pregnancy rates. Cows that calved on or before the date 35 days after the planned start of calving had a 6-week incalf rate of 64%, compared to 38% for cows that calved

in the 6-weeks or later after the planned start of calving (chi-square; $p<0.0001$). Cows of NZH genetic origin that calved during Weeks 4 to 7 after the planned start of mating had significantly higher 6-week incalf rates than OS animals that calved during the same period (70% vs. 45% in NZH and OS respectively; $p<0.05$). Table 6-7 and Table 6-8 illustrate the relationship between calving week and 6-week in-calf rate.

Of animals that calved within 6 weeks of the PSC there was a trend ($p<0.1$) for 6-week-in-calf rates to increase as PPAI increased (see Table 6-9). Animals that ovulated before Day 21 postpartum had significantly lower 6-week-in-calf rates than animals that ovulated after Day 21 postpartum (48% vs. 69%; $p<0.05$). The same effect was observed when the data set included all animals that calved over both years of the trial (44% vs. 66%; $p<0.01$). Six-week-in-calf rates were not different between animals that ovulated before the planned started of mating compared to anoestrus treated animals for animals that calved within 6-weeks of the start of calving. However, when the data set was expanded to include all animals, animals treated for anoestrus tended to have lower 6-week-in-calf rates than animals that spontaneously ovulated during the postpartum period (53% vs. 63%; $p=0.062$). Within each strain, and within age groups, milksolids yield between Weeks 2 and 12 of lactation was not correlated with conception date ($p>0.1$).

6.4.4 IGF-1, Insulin and Growth Hormone

Postpartum anoestrus and IGF-1 concentrations

Concentrations of IGF-1 were higher in Year 1 than Year 2 (15.9 ± 0.5 vs. 8.3 ± 0.19; $p<0.001$). This may have been partially an age effect. In Year 2 of the trial concentrations of IGF-1 were higher in 2- vs. 3-year-olds (9.6 ± 0.3 vs. 7.1 ± 0.2; $p<0.0001$).

Concentrations of IGF-1, by strain and by week are illustrated in Figure 6-5 and Figure 6-6. In both years, concentrations of IGF-1 increased during the postpartum period and were significantly higher in Week 8 compared to Week 1 ($p<0.05$). Concentrations of IGF-1 were not different between the three strains during the first 8 weeks postpartum in Year 1 of the trial. In Year 2, concentrations of IGF-1 in NZL tended to be greater

than those in the NZH strain during the 8th week postpartum and were significantly greater than IGF-1 concentrations in the OS strain for Weeks 6, 7 and 8 ($p<0.05$).

In Year 2, when samples from all herds were analysed there was no difference in IGF-1 concentrations between herds within a strain, despite intended differences in feed allowances. The exception was the NZH strain, where IGF-1 concentrations in herd 4 were significantly lower than in herds 5 and 7. There was no difference in IGF-1 concentrations between herds 5, 6 and 7.

Table 6-6: Reproductive performance of Strain Trial animals, presented by strain. Conception rates for CIDR-treated and spontaneously ovulating cows are given separately. In-calf rate was after a 12-week mating period.

	Year	OS	NZH	NZL
First service conception	1- CIDR	22%	33%	47%
rate (%)	1- Cycling	40%	29%	47%
	2- CIDR	37%	33%	50%
	2- Cycling	44%	58%	43%
Interval from PSM	1	37.4 ± 3.83	33.0 ± 3.55	28.4 ± 4.58
to conception (days ± sem)	2	30.4 ± 3.35	26.1 ± 2.53	26.5 ± 3.57
Pregnant in first 6 weeks	1	51[a]	68[ab]	75[b]
of mating period, P42 (%)	2	61	71	64
In-calf rate (%)	1	78[a]	91[b]	97[b]
	2	83	87	87

Treatments: New Zealand high (NZH), New Zealand low (NZL) and overseas (OS)

[abc]Differing superscripts within a row indicate $P<0.05$.

Table 6-7: Effect of week in which cows calved, relative to the planned start of calving (PSC), on the proportion (and %) of animals pregnant at 6-weeks after the planned start of mating for all cows.

Cows calved in weeks:	Pregnant at 6-weeks	
	N	%
-2 – 1	72/108	67
2 – 3	79/125	63
4 – 5	36/57	63
6 – 7	17/39	44
8 – 9	5/15	33
10+	6/19	32

Table 6-8: Effect of calving week, relative to the planned start of calving, on the proportion (and %) of animals pregnant at 6-weeks after the planned start of mating, by strain.

Cows calved in weeks:	OS	NZH	NZL
-2 – 1	24/37 (65)	31/45 (69)	17/26 (65)
2 – 3	27/48 (56)	25/44 (57)	27/32 (84)
4 – 5	12/22 (55)	20/27 (74)	4/8 (50)
6 – 7	6/18 (33)	9/14 (64)	2/7 (29)
8+	3/12 (25)	5/15 (33)	3/7 (43)

Treatments: Overseas (OS), New Zealand high (NZH), New Zealand low (NZL).

Table 6-9: The effect of the length of the postpartum anovulatory interval (PPAI), or anoestrus treatment (CIDR) on the 6-week in-calf rate in Year 1 and Year 2 of the strain trial for animals that had calved within 6-weeks of the planned start of calving.

	Year 1		Year 2	
PPAI of less than 21 days	6/12	50%	8/17	47%
PPAI of 21 to 42 days, inclusive	17/31	55%	47/65	72%
PPAI of greater than 43 days	17/27	63%	35/45	77%
PPAI overall	40/70	57%	90/127	71%
CIDR treated	52/88	59%	28/44	64%

Figure 6-5: Concentrations of IGF-1 during the first 8- weeks postpartum in Year 1 of the strain trial. Each point represents the mean (± sem) for 10 animals per strain. New Zealand High (—◆—), New Zealand Low (—■—) and overseas (—▲—).

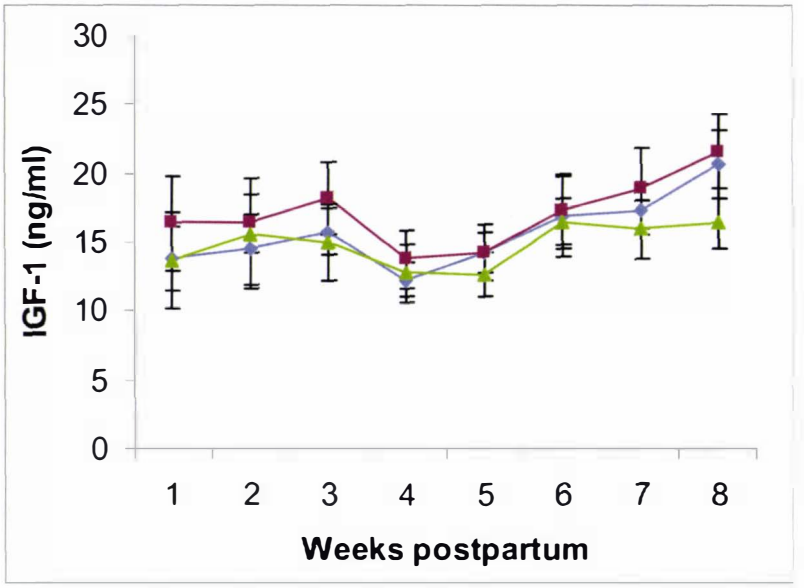
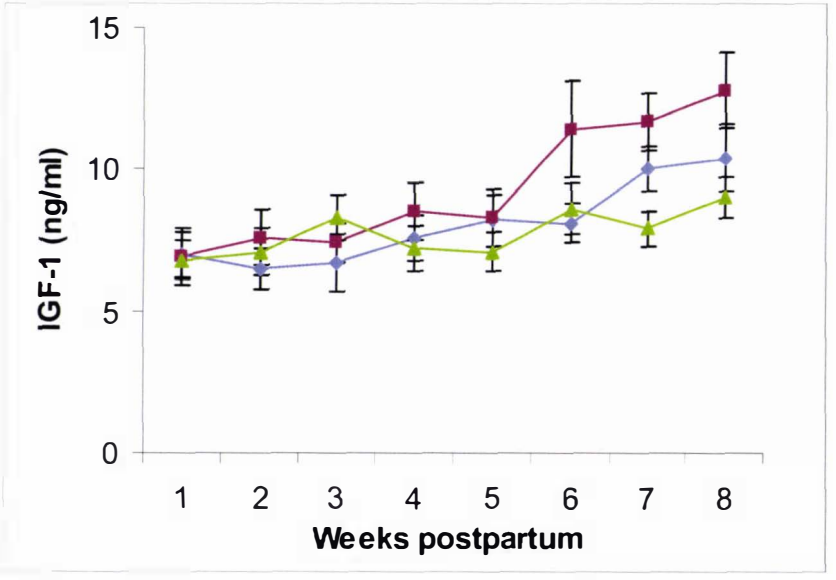


Figure 6-6: Concentrations of IGF-1 during the first 8-weeks postpartum in Year 2 of the strain trial. New Zealand high (—◆—), New Zealand low (—■—) and overseas (—▲—). Each point represents the mean (± sem) for 24 animals for the New Zealand low strain and 32 animals for the New Zealand high and overseas strains.



Concentrations of IGF-1 were significantly higher in Year 1 than Year 2. Therefore, correlations were determined within each year. The low numbers of samples analysed

in Year 1 (10 animals per strain) were considered insufficient to allow meaningful correlations to be determined. Therefore, correlation analysis was only performed on data from Year 2 of the trial.

IGF-1 concentrations in NZH in Week 1 of the postpartum period were significantly correlated with the length of the postpartum anoestrus interval ($r = -0.51$; $p=0.02$), with calving condition score ($r = 0.44$; $p=0.01$) and age at calving ($r = -0.65$; $p<0.0001$). Similarly, concentrations of IGF-1 in Week 2 of the postpartum period were significantly correlated with PPAI ($r = -0.53$; $p=0.02$), with calving condition score ($r = 0.50$; $p=0.004$) and age at calving ($r = 0.37$; $p=0.04$). In Weeks 3-8, there were no other significant correlations between IGF-1 concentrations and PPAI. Correlations between IGF-1 concentrations and age at calving and calving condition score were significant in all weeks, except Week 3.

For the NZL strain, the correlation between IGF-1 concentrations and calving condition score approached significance in Weeks 1, 2, 3, 6, and 8 ($r = 0.35$ to 0.40; $p<0.1$), and was significant in Weeks 4 and 7 ($r = 0.42$ to 0.52; $p<0.05$). The correlation was not significant in Week 5. The correlation between IGF-1 concentrations and age was only significant in Week 7 ($r = -0.49$; $p=0.01$). There was a tendency for time of calving, relative to the start of the calving period to be correlated with IGF-1 concentrations in Weeks 2 and 3, ($r= 0.39$ to 0.40; $p<-0.06$).

The correlation between IGF-1 concentrations and calving condition score was significant for OS animals in week 2 ($r = 0.51$; $p=0.02$) and approached significance in Weeks 6 and 7 ($r = 0.31$- 0.34; $p<0.1$). The correlation between IGF-1 concentrations and age at calving was significant only during Week 2 of the postpartum period ($r = -0.41$; $p=0.02$). There appeared to be a correlation between time of calving, relative to the start of the calving period and IGF-1 concentrations, the correlation was significant in Week 1 ($r= -0.38$; $p=0.03$) and approached significance in Week 2, the correlation was again significant in Week 3 ($r = 43$; $p=0.01$), but was a positive, rather than negative correlation.

The correlation between PPAI and IGF-1 concentrations, and PPAI and calving condition score are illustrated in Figure 6-7 and Figure 6-8.

When data from all strains was combined a significant correlation between IGF-1 concentrations and calving condition score was apparent for all except Week 3 ($r = 0.34$ –to 0.46; $p<0.01$). Age at calving was also significantly correlated with IGF-1 concentrations for all except Week 3 ($r = -0.41$ to –0.21; $p<0.05$). Concentrations of IGF-1 were significantly correlated with PPAI for Weeks 1, 2, 3 and 4, but not for any other weeks ($r = -0.33$ to –0.26; $p<0.05$). Whilst there was a significant correlation between IGF-1 concentrations and time of calving, relative to the start of the calving period only in Week 3 ($r = 0.35$; $p=0.0009$).

The correlation between PPAI and factors was examined, using IGF-1 concentrations from Week 1 postpartum. There were significant ($p<0.05$) negative correlations between PPAI and IGF-1 ($r = -0.28$), calving condition score ($r = -0.37$) and strain ($r = -0.34$), the correlation between time of calving, relative to the start of calving period approached significance ($r = -0.24$; $p<0.06$). The correlation between age and PPAI was positive, and approached significance ($r = 0.25$; $p<0.06$).

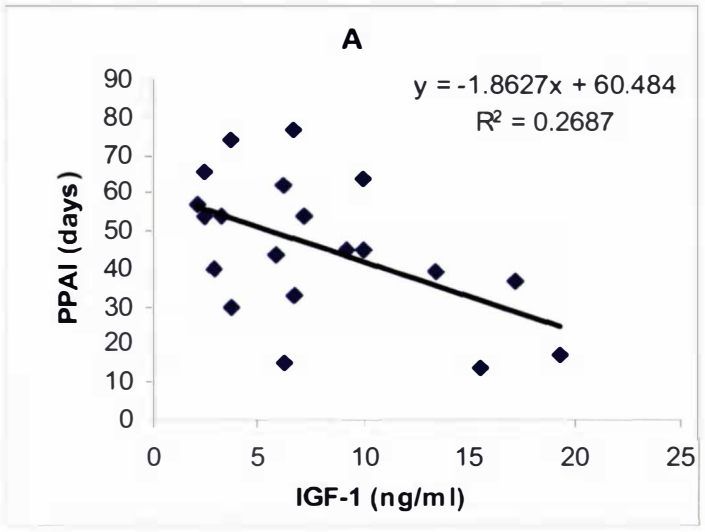
Correlations between IGF-1, insulin and GH were determined, by strain and by week during the first 8 weeks of the postpartum period. The majority of correlations were not significant, with the few significant correlations showing no consistent trend across weeks or within strains.

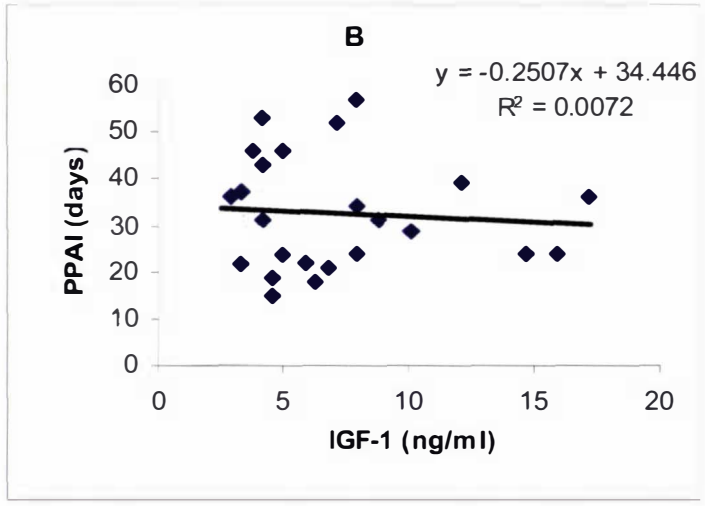
Concentrations of insulin did not differ between the strains, or between Years 1 and 2, as illustrated in Figure 6-9 and Figure 6-10. Insulin concentrations in the first week postpartum tended ($r= 0.23$; $p=0.06$) to be correlated with PPAI in Year 2 of the trial. Also, in Year 2, insulin concentrations in the first week postpartum tended ($p=0.06$) to be higher in animals that ovulated within 40 days of calving, compared to animals that took longer than 40 days after calving to ovulate (7.3 vs. 4.9 μu/ml). There were insufficient animals available to perform these same analyses on data from Year 1.

Concentrations of growth hormone (GH) were significantly higher in the OS strain than the NZH or NZL strains in both years ($p<0.05$). A significant effect of week, and a strain by week interaction were also detected ($p<0.05$). Concentrations of GH were significantly ($p<0.01$) lower in Year 1 than Year 2 (1.33 vs. 2.11, respectively). In Year

2, overall GH concentrations were lower in 2-year-olds than 3-year-olds ($p<0.05$). However, by Weeks 7 and 8 GH concentrations were not different between the two age groups ($p>0.05$). Within strains the age effect was significant ($p<0.05$) in the NZH and NZL strains, but not in the OS strain. See Figure 6-11 and Figure 6-12.

Figure 6-7: The relationship between length of the postpartum anovulatory interval (PPAI) and IGF-1 concentrations in week 1 of the postpartum period in A: New Zealand high cows, B: overseas cows and C: New Zealand low cows.





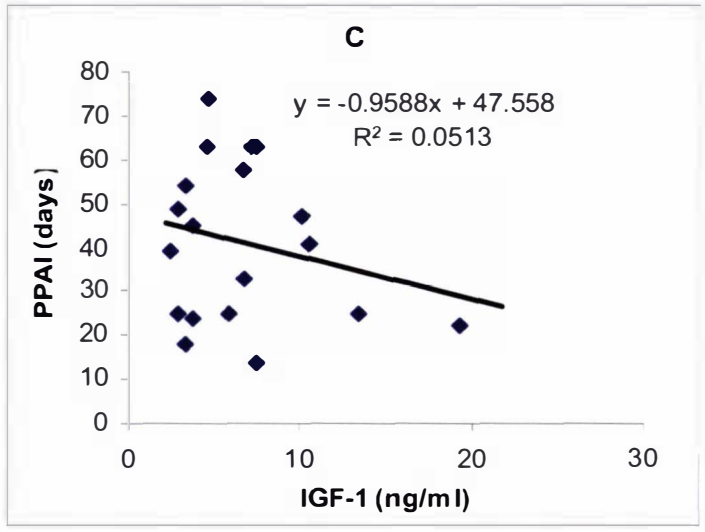


Figure 6-8: The relationship between calving condition score and insulin-like growth factor-1 (IGF-1) concentrations in the first week postpartum in New Zealand high, graph A, overseas, graph B and New Zealand low, graph C.

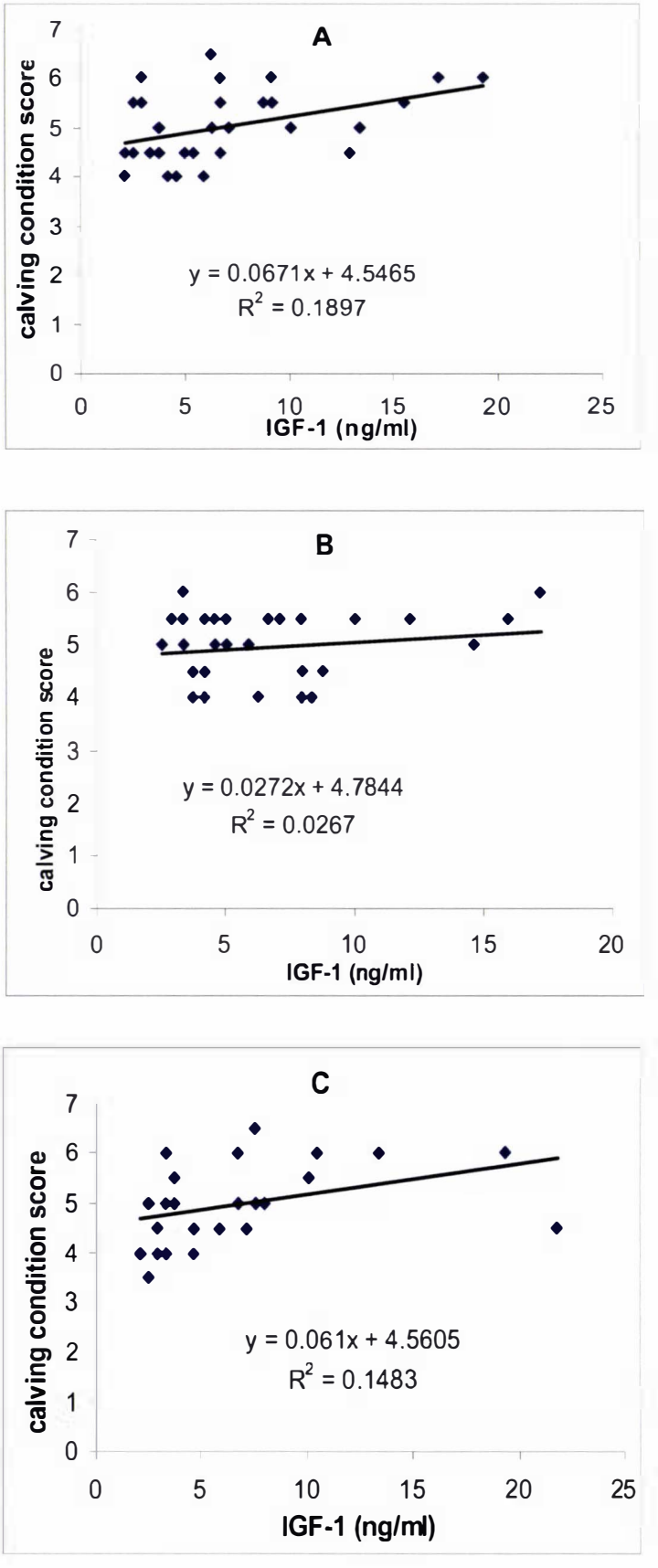


Figure 6-9: Concentrations of insulin during the first 8 weeks postpartum in Year 1. Each point represents the mean (± sem) for 10 animals per strain. New Zealand High (—◆—), New Zealand Low (—■—) and overseas (—▲—).. No significant differences on individual days.

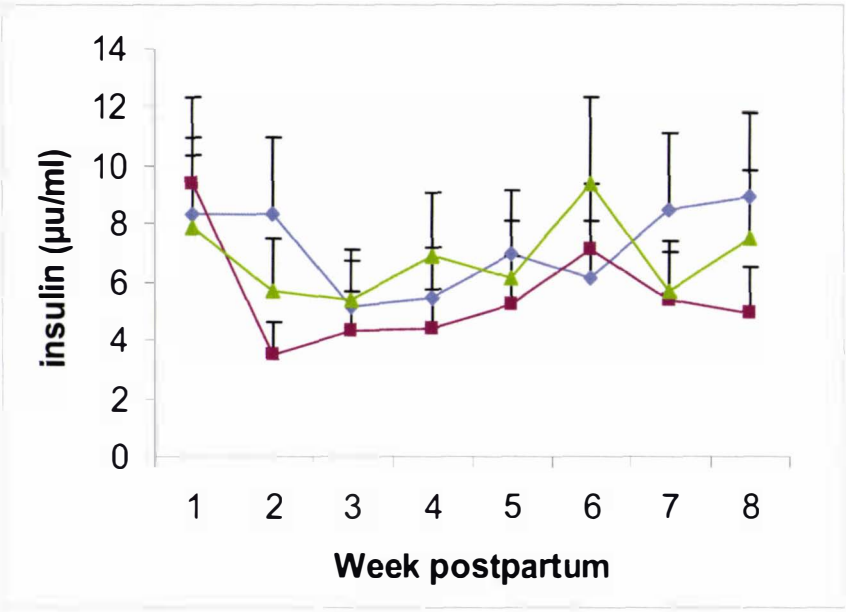


Figure 6-10: Concentrations of insulin during the first 8 weeks postpartum in Year 2. New Zealand high (—●—), New Zealand low (—■—) and overseas (—▲—). Each point represents the mean (± sem) for 24 animals for the New Zealand low strain and 32 animals for the New Zealand high and overseas strains. * significant difference between overseas and New Zealand low ($p<0.05$).

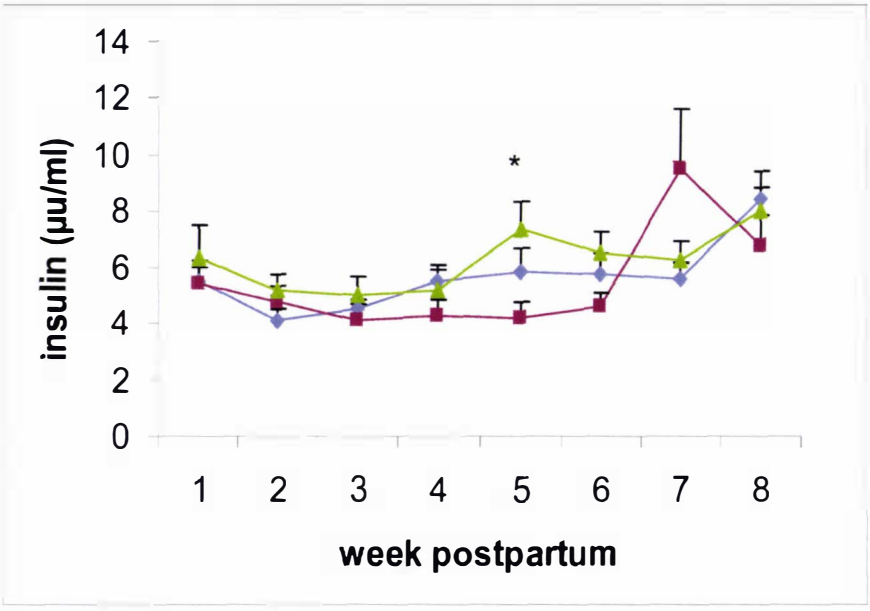


Figure 6-11: Concentrations of growth hormone (GH) during the first 8 weeks postpartum in Year 1. Each point represents the mean (± sem) for 10 animals per strain. New Zealand High (—◆—), New Zealand Low (—■—) and overseas (—▲—).* indicates a significant difference between overseas and/or New Zealand high or New Zealand low ($p<0.05$).

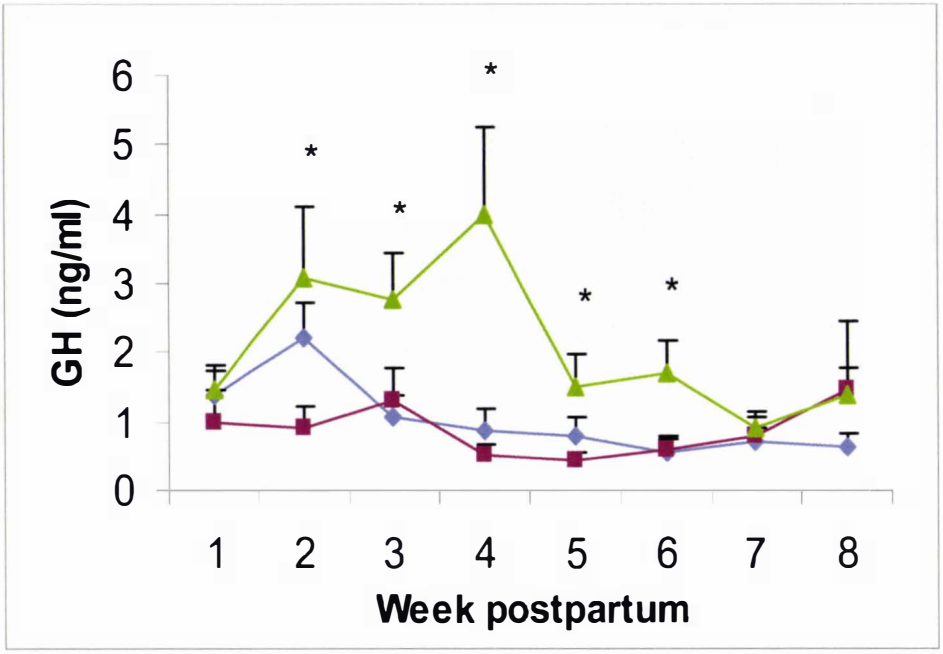
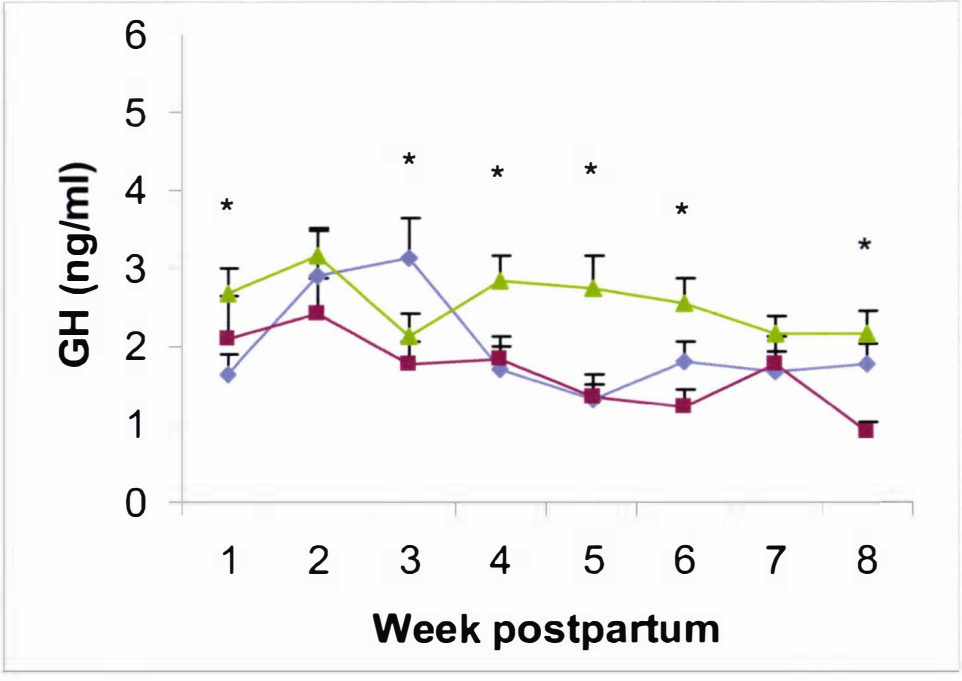


Figure 6-12: Concentrations of growth hormone (GH) during the first 8 weeks of the postpartum period in Year 2. New Zealand high (—◆—), New Zealand low (—■—) and overseas (—▲—). Each point represents the mean (± sem) for 24 animals for the New Zealand low strain and 32 animals for the New Zealand high and overseas strains. * indicates a significant difference between overseas and/or New Zealand high or New Zealand low ($p<0.05$).



6.5 Discussion

Postpartum anovulatory intervals were longer in NZH than OS and NZL animals. Meaning that the proportion of animals that had to be treated for anoestrus was higher in the NZH than in the OS and NZL strains. Despite the high proportion of treatment NZH animals had similar reproductive performance as OS and NZL strains. Thirty years of selective breeding, as represented by the difference in breeding worth, between the two NZ strains has resulted in an increase in duration of the PPAI, but no effect on pregnancy rates when anoestrus was treated before the start of mating. We could not determine what effect the greater PPAI in the NZH strain may have had on conception patterns due to the aggressive treatment of anoestrus.

6.5.1 Calving pattern

The calving pattern was more spread in Year 2 of the trial, but there were no differences between strains in either year. The difference in calving pattern between years can be explained by the change in the age structure of the herds, and the decision to bring the PSM forward to an earlier date between Years 1 and 2. All animals in Year 1 of the trial were 2-year-old heifers. Heifers would be expected to have a compact calving period, because as 15 month heifers they should have had high first-service conception rates (Lucy, 2001a), and any non-pubertal animals had been induced to ovulate prior to the heifer mating period (Chapter 4). Cows calving in Year 1 had just 67 days from the PSC to selection for treatment for anoestrus, because of the decision to change the PSM date. As a result, the incidence of CIDR treatment in Year 1 was high but, particularly in the NZH animals. The longer PPAI in NZH relative to the OS strain meant that proportionately more NZH animals were treated for anoestrous. In Year 2 of the trial the incidence of anoestrous was lower in the NZ strains than in Year 1, but was similar for the OS strains.

The extended period of condition score loss in heifers in Year 2 of the trial may have been related to the calving pattern. Mean calving dates for the heifers were earlier than for 3-year-olds, so that in early lactation heifers dominated the herds and there were smaller numbers of animals, leading to less competition for feed and with less opportunity for bullying. However, as calving progressed more 3-year-olds entered the herd and competition for feed increased. At this time it was likely to be the heifers

whose feed-intake was most restricted, leading to the mobilisation of body condition to support lactation and growth. This supposition is supported by the fact that the heifers lost more body condition than the 3-year-olds between calving and Weeks 4, 8 and 12 of lactation in Year 2. This suggestion is also supported by the increased rate of body condition score loss in heifers from weeks 4 to 12, when condition score loss had slowed in 3-year-old animals.

6.5.2 Postpartum anovulatory intervals

Age and condition score both affected the length of the PPAI, and therefore influenced the number of animals requiring treatment for anoestrus within the proactive treatment decision rules used in this trial. Older animals tend to have a shorter postpartum anoestrous period than 2-year-olds (McDougall, 1994). However, in Year 2, when the herds consisted of 2- and 3-year-olds, the PPAI was longer in 3-year-olds. McGowan (1981) reported that cows calving at a BCS of 6 had PPAI 12 days shorter than those calving at a BCS of 4. In the present trial, calving BCS had an even greater effect, with a reduction in PPAI of 12 days per unit of calving BCS in heifers, over both years. In Year 2 of the trial calving BCS was 1 unit higher in 2- than in 3-year-old animals, leading to a shorter PPAI in 2-year-olds. The difference in PPAI and the 23-day earlier mean calving date in the 2-year-olds explained much of the variation in the incidence of anoestrus treatment between 2- and 3-year-old animals. Calving heifers at an average body condition score of 5.6 was beneficial in minimising the need for anoestrus treatment in Year 2.

PPAI were longer in Year 1 than Year 2 across all strains. The lower calving BCS and greater loss of body condition from calving to Week 4 of lactation explained a significant proportion of the variation in PPAI between the two years.

Previous studies of Holstein-Friesian cows of different genotypes have reported a tendency for shorter PPAI periods in OS compared to NZ animals (Laborde, 1998; Verkerk *et al.*, 2000; Thiengtham *et al.*, 2003). The results from the current trial confirm that, even under pasture-based systems, OS genotype animals have the ability to resume oestrous cycles rapidly after calving. The interval from calving to luteal activity has been found to have a heritability of 0.16 in UK Holstein-Friesian cattle (Royal *et al.*, 2002). Selective breeding for higher milk yield over the past 20-30 years

appears to have been associated with an increase in PPAI in NZ cows, as judged by the difference between the high and low New Zealand strains. The widespread use of treatment for prolonged PPAI in NZ may be contributing to the development of longer PPAI in NZH animals, by enabling cows with a genetic tendency for a long PPAI to conceive during the artificial breeding period. It is likely that the six-week pregnancy rate in the NZH strain would have been lower had 75% of NZH cows not been treated with a CIDR device in Year 1. The true effect of extended PPAI on pregnancy rates in the current trial could not be determined due to the use of anoestrus treatments. Calving condition score was found to affect the PPAI in the present trial. However, there was no difference in calving condition score between strains, suggesting that genetic differences account for at least some of the differences in PPAI observed between strains.

Early re-establishment of oestrus cycles after calving has been associated with improved reproductive performance, relative to animals with delayed resumption of oestrus cycles (Thatcher and Wilcox, 1973; Lucy *et al.*, 1992; Darwash *et al.*, 1997). However, Smith and Wallace (1998) reported that multiparous cows which ovulated before day 21 postpartum had significantly lower all service conception rates, and a reduced percentage of cows pregnant by 100 and 150 days postpartum, no animals were inseminated before Day 42 postpartum. In the current trial 6-week in-calf rates were significantly lower in animals that ovulated before Day 21, compared to animals that ovulated after Day 21. Exposure to progesterone makes the early postpartum uterus of ewes susceptible to infection (Lewis, 2003), thus ovulation early in the postpartum period could increase the likelihood of a uterine infection and the associated negative reproductive outcomes.

6.5.3 First postpartum oestrous cycle

The proportion of short, normal and long first postpartum cycles in the current study was similar to previous reports in the literature. Schams *et al.* (1978) reported that the first oestrous cycle was shorter than normal in 13/23 cows, extended in 3/23 animals and of normal length in the remaining 7 animals. Savio *et al.* (1990) found similar variability in the length of the first post-partum cycle, but a higher proportion of long cycles than in the current trial. Almost half of all cows (44%) had a long first cycle,

whilst 28% had normal and 28% had short length cycles. Similarly, half of all first cycles were long or short in a survey of 685 UK Holstein-Friesians (Royal *et al.*, 2000). In Year 2 of the trial, when interovulatory data was analysed by strain, there appeared to be an effect of strain on the proportion of cycles that were either shorter than average or of normal length. However, this effect was not present in Year 1, when a smaller number of records were available for analysis. The incidence of short interovulatory intervals was lower in Year 1 than Year 2 of the current trial, due to the high proportion of short cycles in the NZH and NZL strains in Year 2 of the trial.

6.5.4 Reproductive performance

Anoestrus treatment

The proportionately higher use of anoestrous treatments in NZH and NZL strain in Year 1 contributed to short intervals from PSM to first service. Despite the interval from PSM to first service being shorter in Year 1 of the trial, the interval from PSM to conception was shorter in Year 2, due to higher first service conception rates. The use of progesterone treatments, such as CIDRs (Pharmacia, Auckland, New Zealand) to treat anoestrus has been associated with reduced first service conception rates (Xu and Burton, 1997). In the present trial (Table 6), animal numbers were insufficient to show a significant difference in conception rate between CIDR treated and the untreated cows. However, the first service conception rates in the CIDR treated animals in the present trial are similar to those reported in the literature for CIDR and other hormonal treatments (Xu and Burton, 1997; Xu and Burton, 2000; Xu *et al.*, 2003). Thus, the higher use of CIDR treatments in Year 1 may explain some of the difference in conception rate between the two years.

Milksolids yield from Weeks 2 to 6 of lactation was not different between CIDR treated, and cycling cows in the current study. Likewise, a survey of 8000 cows in commercial herds in the Waikato district of New Zealand found no relationship between milksolids production and the prevalence of anoestrous (Rhodes *et al.*, 1998) although in a within herd study, cows with higher yields were less likely to be anoestrous at the start of mating (Rhodes and Morgan, 1999). This may reflect the relationships between body condition score at calving, and milk production and PPAI (Chagas, 2003).

Conception rates

The overall first service conception rates (not non-return rates) in this trial are lower than the values of 60-65% reported for NZ by Macmillan & Day (1982) and Burton *et al.* (1999). However, they are similar to the 37.1% reported recently in Northern Ireland (Mayne *et al.*, 2002) and to first service calving rates in the UK of 40% (Royal *et al.*, 2000). The proportion of Holstein-Friesian genetics in animals born from 1991 onwards has increased rapidly (Harris and Kolver, 2001) so the herds sampled in 1993-1996 by Burton *et al.* (1999) would have had a significantly lower proportion of Holstein genetics than is currently present in the New Zealand Holstein-Friesian population. Recent data from Xu *et al.* (2003), from a large data set, reported a first service conception rate of 56.1% in New Zealand Holstein-Friesians, which whilst lower than the often reported 60-65%, remains significantly higher than in many other countries. A wide range in reproductive performance was found between herds, suggesting there is scope for improving reproductive performance through management (Xu *et al.*, 2003).

It must be noted that the present values for conception rates were derived from small numbers of cows, of one breed, and may not be directly comparable to industry averages. Further years' data is required before conception rates between the strains can be accurately quantified, as the statistical power to detect significant differences is limited by the small animal numbers.

Conception rates tended to be somewhat lower in Year 1 of the trial, probably due in part to the lower calving BCS and greater loss of body condition between calving and Week 4 of lactation, although body condition score loss out to Week 12 of lactation was actually greater in Year 2. The InCalf project in Australia has found that cows with a low pre-calving BCS, and cows with greater BCS loss in early lactation had lower first insemination conception rates (Morton, 2000a).

Calving pattern and final reproductive outcomes

A compact pattern of conception and therefore a compact subsequent calving period are important, where the aim is to maximise days in milk within seasonal systems in which all cows in a herd are dried off in a compact pattern. The InCalf report in Australia found that calving date, relative to the planned start of calving was an important determinant of reproductive performance, measured by 6 and 21-week in-calf rates in

seasonal systems. Cows that calved more that 9 weeks after the PSC had greatly reduced 6-week-in-calf rates, and a reduction in reproductive performance was evident even for cows that calved more than 3 weeks after the PSC (Morton, 2000a). Despite the aggressive anoestrus treatment regime used in the current trial, cows that calved more than 6 weeks after the planned start of calving had a reduced 6-week in-calf rates when compared to cows that calved within 5 weeks of the PSC. The small numbers of cows available would have made it difficult to demonstrate significant differences between strains, even if they had existed. However, the 6-week in-calf rates in cows that calved during Weeks 4-7 after the PSC were higher for the NZH than the OS strain. It is possible that the New Zealand production system has selected for cows that have some ability to cope with late calving and remain in the herd, as animals that fail to conceive are quickly culled from the system, and only a small proportion of non-pregnant animals are carried over for another lactation. Further data are required to investigate whether this is a real effect, or an artefact of small numbers.

Two-year-old Friesian heifers are at the greatest risk of extended postpartum anovulatory intervals under New Zealand conditions (McDougall, 1994; Macmillan, 1997). Friesian heifers that calved at high live weights had PPAI of 51 days, compared to PPAI of 77 days for those calving at low live weights (Macmillan, 1997). The use of CIDR treatments to induce ovulation in the current trial is likely to have altered the pattern of conception, particularly in Year 1, allowing animals to be inseminated and conceive at the start of the mating period that might otherwise not have been inseminated for several weeks. It is probable that six-week in-calf rates in Year 1 of the trial would have been reduced had CIDR treatments not been used, particularly in the NZH strain, where the greatest proportion of animals required treatment, compared to the other two strains. Ideally, animals would have not been treated with CIDR devices, to allow the natural reproductive performance of the three strains to be compared. The decision to treat animals with CIDRs was made to ensure sufficient production information was collected from these animals, which had been generated at great expense, and for the purpose of comparing milk production characteristics.

It has been suggested that the increase in milk yield per cow has contributed to the decline in reproductive performance that has been documented in the UK (Royal *et al.*, 2000) and USA (Butler *et al.*, 1995). Nebel and McGilliard (1993) reported increased

total lactation yields were associated with decreased conception rates, whilst Lean *et al.* (1989) found that peak milk yield was negatively related to fertility and Morton (2000b) found small decreases in fertility for cows with very low and very high milk fat yields. Genetic correlations between milk yield and fertility are small and negative (Lindhe and Philipsson, 1998; Pryce and Veerkamp, 1999; Roxstrom *et al.*, 2001), so that selection exclusively for milk yield is expected to lead to a decline in fertility (Philipsson and Lindhe, 2003), as evidenced by the decline in fertility in the UK and USA (Royal *et al.*, 2000). Although New Zealand has only recently introduced a fertility component into the genetic evaluation index (Montgomerie, 2002) the seasonal production system has likely helped to indirectly select for fertility. Animals that fail to conceive in the restricted breeding period are usually removed from the herd, as are some animals that conceive late, and artificial insemination is often only used for 6 weeks (followed by bulls, usually of a beef breed) so that only animals that conceive during the first few weeks of the mating period are able to contribute replacement heifers. In seasonal dairy production systems the effects of anoestrus treatment and calving date on reproductive performance must be removed, before the effect of milk yield can be considered. Animal numbers in the current trial were probably too small to detect whether associations between yield and fertility existed, and would have been confounded by differences in feeding levels between herds, with better-fed animals expected to produce more milk. Management factors (e.g. heat detection, animal health management and feeding allowances) also have a significant effect on the reproductive performance achieved between herds, so that higher producing herds achieve higher 6-week in-calf rates (Morton, 2000b), due to better animal husbandry.

Final (12-week) in-calf rates were different between the strains in Year 1 of the trial, but not in Year 2, whilst 6-week in calf rates were not different between strains, but were numerically greater in NZH than OS in both years. This is in contrast to previous findings at Dexcel where NZ and OS cows were fed a generous pasture diet and final in-calf rates were higher in the NZ herd in each of three seasons (Kolver, 2001). The difference in final in-calf rates increased each year, as the age structure of the herds changed. However, the animals used in that trial were imported as embryo's, whereas in the present trial all the original dams had conceived at least once in NZ prior to selection for this trial, and then had to conceive to artificial insemination to give birth to the cal used in the present trial. Therefore, there may have been some indirect selection

between dams for reproductive performance in NZ conditions. Of the OS animals that were not pregnant in Year 1, 7 out of 11 had the same sire, suggesting a sire genetic effect. Although estimates of heritabilities for fertility traits are low, there is sufficient genetic variability in the traits to allow genetic progress to be made by selection (Pryce and Veerkamp, 1999). In New Zealand the National Animal Evaluation Committee has now incorporated a fertility trait in the Breeding Worth index (Montgomerie, 2002). Fertility has been included in the Swedish national breeding programme since 1975, and is also included in the breeding programmes of Denmark, Norway and Finland (Lindhe and Philipsson, 1999,), whilst France, the Netherlands, Germany and Israel are now producing genetic evaluations for fertility (Pryce and Veerkamp, 1999) and the UK is currently developing a fertility index (Wall *et al.*, 2003). These measures are expected to help maintain fertility at the present level in the population.

6.5.5 IGF-1, GH and Insulin and interactions with reproductive performance

Growth hormone binds to the liver to cause the release of IGF-1 (Cohick and Clemmons, 1993; Carter-Su *et al.*, 1996), therefore GH and IGF-1 concentrations are often correlated (Chase *et al.*, 1998). Concentrations of GH are raised in the postpartum period, but the relationship between GH and IGF-1 is uncoupled, so that raised concentrations of GH do not lead to high concentrations of IGF-1 (Kobayashi *et al.*, 1999). The increase in GH concentrations may follow the postpartum decline in IGF-1 concentrations (Lucy, 2000), as IGF-1 is the primary negative feedback inhibitor of GH secretion (Berelowitz *et al.*, 1981). Concentrations of GH and IGF-1 were not correlated in the present study, suggesting that the expected uncoupling of the GH/IGF-1 relationship had indeed occurred. Hepatic GH receptors are down-regulated during the postpartum period (Kobayashi *et al.*, 1999), coincident with low concentrations of insulin (Gong, 2002), leading to speculation that low concentrations of insulin are responsible for the reduction in GH receptors (Butler, 2003), which in turn affects IGF-1 concentrations. The argument for a link between insulin and IGF-1 concentrations is strengthened by research where insulin has been found to stimulate IGF-1 production under hyperinsulinemic-euglycemic clamps (McGuire, Dwyer *et al.*, 1995; Butler and Butler, 2001). Also, concentrations of IGF-1 are reduced in diabetic rats, but restored by insulin treatment (Pao *et al.*, 1992). Recent research has demonstrated that insulin stimulates growth hormone receptor–1A (GHR-1A)

expression on the liver, and directly stimulates hepatic IGF- 1 gene expression (Butler *et al.*, 2003), probably through effects on the transcription factor Sp1 and the insulin-responsive binding protein (IRBP; (Kaytor *et al.*, 2001b; Kaytor *et al.*, 2001a; Pan *et al.*, 2001). However, insulin and IGF-1 concentrations were not correlated in the current study. During the postpartum period some tissues, or biochemical pathways in tissues, become "insulin resistant", in order to spare glucose for utilization by the mammary gland (Bauman, 2000). Insulin resistance is one possible explanation for the failure to observe a relationship between insulin and IGF-1 concentrations in the present trial.

Concentrations of GH were higher in the OS than NZH and NZL strains in the current trial and were lower in Year 1 than Year 2, but there was no difference in insulin concentrations, either between years or between strains. Insulin concentrations decline in the early postpartum period (Taylor *et al.*, 2000; Gong, 2002), but this decrease was seen only in Year 1 of the current trial. Concentrations of insulin were relatively constant from Weeks 1 to 4 postpartum in Year 2. Insulin concentrations are increased by feeding high vs. low energy diets (Armstrong *et al.*, 2001), and therefore, insulin could potentially act as a signal of metabolic status to the body, and in particular the ovary. *In vitro* studies have demonstrated that insulin enhances the development of bovine follicular cells by stimulating differentiation and steroidogenesis (Spicer and Echternkamp, 1995). These effects may serve to enhance the ovulatory potential of the dominant follicle, as first wave dominant follicles that ovulate are characterized by their greater steroidogenic output than first wave dominant follicles that failed to ovulate (Beam and Butler, 1997).

Gong *et al.* (2002) found that by feeding a diet that increased plasma insulin concentrations, the interval from calving to ovulation was decreased, although numbers of cows were insufficient to determine whether overall reproductive performance was enhanced. Under New Zealand conditions, twice-daily drenching with monopropylene glycol during the postpartum period resulted in a reduced PPAI, compared to undrenched controls (Chagas, 2003), possibly through an effect on insulin concentrations, as propylene glycol administration has been found to increase concentrations of insulin and glucose (Miyoshi *et al.*, 2001). The finding that concentrations of insulin in the first week postpartum were greater in cows with a PPAI of 40 days or less, compared to those with a PPAI of greater than 40 days, and the

relationship between insulin concentrations in the first week postpartum and PPAI was therefore unsurprising.

Concentrations of GH were related to age, but only in the NZ strains. In Friesian bulls GH concentrations declined as age increased (Tucker *et al.*, 1974). It was, therefore, surprising that mean concentrations of GH were higher in Year 2 than Year 1 of the trial, when 50% of animals sampled were 3-year-olds. IGF-1 is a feedback inhibitor of GH secretion (Berelowitz *et al.*, 1981), and concentrations of IGF-1 were significantly lower in Year 1 than in Year 2 of the trial, possibly causing less feedback inhibition of GH release, leading to higher GH concentrations.

However, nutritional differences between years may have been the underlying reason for the reduced IGF-1 concentrations and elevated GH concentrations in Year 2 of the trial. Growth hormone concentrations increase during under-nutrition (Houseknecht *et al.*, 1988). To investigate this possibility, condition score loss, as an indicator of level of nutrition, was compared between Years 1 and 2. It was found that condition score loss in the first four weeks postpartum was greater, and calving condition score lower in Year 1, suggesting poorer nutrition in the early postpartum period in that year. But when condition score loss past week 4 was compared (Figure 6-3 and Figure 6-4) it became apparent that condition score loss in the early postpartum period was faster in Year 1, and that the nadir in condition score occurred earlier, whereas in Year 2, condition score loss was more gradual, but continued over an extended period, so that by the twelfth week of lactation the difference between calving condition score and current condition score was greater for Year 2. Calving body condition scores were higher in Year 2. The extra loss of body condition appeared to maintain rather than increase milk production, as milk production in 2-year-old animals from Weeks 2 to 12 of lactation was not different between the two years.

The different pattern of condition score loss between the two years may explain the differences in GH concentrations, between years, between strains and between ages within a year. Condition score loss was particularly rapid in OS animals in the first 4-weeks of lactation in Year 1 of the trial, where GH concentrations increased significantly between Weeks 1 and 4. However, condition score loss was more gradual in Year 2 of the trial, and concentrations of GH were more static during the first 8

weeks after calving. Condition score loss in the two NZ strains was more gradual, and concentrations of GH were more static in the NZ strains. Although GH concentrations were lower in 2- than 3-year-olds overall in Year 2, by weeks 7 and 8 there was no difference in GH concentrations between the two age groups, possibly reflecting the continued condition score loss in heifers.

Concentrations of IGF-1 vary with feeding level (Yung *et al.*, 1996) and genetic merit: plasma concentrations of IGF-1 were lower in high compared to low genetic merit cows after Day 21 postpartum (Lucy and Crooker, 1999). In the present trial, although genetic merit did differ significantly between the NZL and the NZH and OS strains plasma IGF-1 concentrations were not different between the strains until after the 6th week postpartum, and only in Year 2 of the trial. Differences in IGF-1 concentrations were greatest between NZL and OS, although the difference in overall genetic merit (as measured by Breeding Worth) was greater between NZL and NZH. In nulliparous yearling heifers, concentrations of IGF-1 were significantly higher in the NZH strain over an oestrous cycle (Chapter 5). IGF-1 concentrations in OS and NZ cows have also been compared in the study of Fahey *et al.* (2003) and in the present thesis (Chapter 7). The former study found higher IGF-1 concentrations in NZ than in OS cows around Day 50 postpartum, although in the latter study, no differences were present at Day 17 or 18 postpartum. It would have been interesting to continue sampling until later in the postpartum period in the current study, to examine whether the differences between strains in IGF-1 concentrations that appeared to be emerging had actually continued. IGF-1 has important roles in follicular development, in particular, promoting the acquisition of LH/hCG receptors (Stewart *et al.*, 1995). Follicular concentrations of IGF-1 are positively correlated with IGF-1 concentrations in the circulation (Echternkamp *et al.*, 1990). If the differences in IGF-1 concentrations that appear to be emerging between the strains are real, it is possible that ovarian function could be affected.

Each strain was divided into herds and subjected to three (NZL) or four (NZH and OS) feed allowances, ranging from a restricted through to a relatively generous feeding level (Chapter 3). However, within a strain, concentrations of IGF-1 were only different between herds in the NZH strain, but the effect was not consistent, that is, the herd with the highest feed allowance did not have the highest concentration of IGF-1. This is not

consistent with published data. As mentioned previously concentrations of IGF-1 were greater in cows fed a total mixed ration (TMR) than in cows fed a pasture-based diet at Day 18 postpartum (Chapter 8). The differences in IGF-1 concentrations may reflect the greater dry matter intake (and hence greater energy intake) of early lactation animals fed the TMR (Kolver *et al.*, 2002). Previous research demonstrating significant effects of nutrition on IGF-1 concentrations utilized large differences in feed intakes between groups (McGuire, Bauman *et al.*, 1995). In the current trial, in a pasture-based system, it is unlikely that differences in feed allowances/intakes were large enough to have an impact on IGF-1 concentrations, at least during the spring period, when pasture was relatively plentiful.

The tendency for concentrations of IGF-1 to increase during the postpartum period has been reported elsewhere. In primiparous heifers, IGF-1 concentrations increased rapidly between calving and week 4 postpartum, and then increased very slowly to 20 weeks postpartum (Taylor *et al.*, 2003). Zulu *et al.* (2002), reported a steady increase in IGF-1 concentrations in mixed age cows from calving to 10 weeks postpartum. In contrast to such findings, Walters *et al.*(2002) reported that serum IGF-1 concentrations declined steadily in cows from around 32 to 100 days postpartum.

Concentrations of IGF-1 were significantly higher in Year 1 than Year 2 of the trial. Some of the difference between the years may have been due to an age effect. Previous research (Wathes *et al.*, 2001) found that primiparous cows had higher pre- and postpartum IGF-1 concentrations than multiparous cows: a finding that was replicated in Year 2 of the present trial. However, this explains only some of the effect, as the concentrations of IGF-1 in 2-year-old animals in Year 2 of the trial, were still significantly lower than in Year 1. There can be significant variability in pasture availability and climatic conditions between years that could affect energy balance, although this is difficult to assess in pastoral-based dairy production systems, due to difficulties in accurately estimating pasture intake. Intakes were assessed as part of another trial, but not until after the period when postpartum metabolites were measured for the current study. Condition score loss between calving and 4 weeks postpartum was greater in Year 1 of the trial, possibly indicating more negative energy balance in Year 1 of the trial. These differences between Year 1 and Year 2 may have been

expected to lead to higher concentrations of IGF-1 in Year 2 of the trial, which was not the case. We cannot explain why this occurred.

6.5.6 Correlations between PPAI, condition score and IGF-1

Beam and Butler (1998) reported a significant negative correlation between the time from calving to first ovulation and IGF-1 concentrations over the first 21 days postpartum. A similar relationship was observed in the present trial for the NZH strain, for IGF-1 concentrations in the first two weeks postpartum. However, a scatter plot of the data revealed the data were highly variable, particularly around low IGF-1 concentrations. So whilst a statistical relationship was found, it is difficult to envisage a biological effect of this relationship. No relationships between IGF-1 concentrations and PPAI were observed for the NZL and OS strains. IGF-1 concentrations were reported to be positively correlated with energy balance (Beam and Butler, 1998), perhaps suggesting that the relationship between PPAI and IGF-1 could be related to energy balance. A significant relationship between calving condition score and IGF-1 concentrations was found for all strains, but, although the correlation was statistically significant, visual analysis of the scatter plots and analysis of the r^2 values revealed the relationship was influenced by outliers, and that for each condition score there was a large range of IGF-1 concentrations. Thus, this relationship was not considered to be biologically significant.

In any attempt to explain why the OS strain ovulated earlier in the postpartum period than the NZH strain, attention must focus on the main difference between the two strains, which was the significantly higher concentrations of GH in the OS strain.

It has been suggested that there could be a relationship between the metabolic adaptations that lead to increased GH concentrations in the OS strain, and the release of luteinizing hormone (LH), which may explain the earlier onset of ovulatory activity during the postpartum period of the OS strain (M. Lucy, personal communication, 2003). This hypothesis relies on the existence of insulin resistance in the OS strain, which leads to increased GH concentrations in the OS strain, through the mechanism described below.

The increased concentrations of GH may be related to the interval from calving to first ovulation, through the actions of somatostatin at the pituitary. The release of GH from the pituitary is controlled by GH-releasing hormone and somatostatin (Butler *et al.*, 2003). In addition to the inhibition of GH secretion, there is also evidence that somatostatin inhibits luteinizing hormone (LH) secretion in male rats (Starcevic *et al.*, 2002) and in women (Saveanu *et al.*, 2001). It is suggested that GH concentrations increase due to a decrease in somatostatin concentrations, which also leads to increased release of LH from the pituitary, with stimulatory effects on follicular development. During the early postpartum period dominant follicles develop regardless of metabolic conditions (Savio, Boland and Roche, 1990; McDougall and Macmillan, 1993). However, unless the LH pulse frequency is sufficient to stimulate follicular maturation and oestradiol production (McNatty, 1984) the follicle fails to ovulate. The increase in GH may stimulate LH secretion sufficiently to elicit an ovulatory response in some animals.

A weakness in this hypothesis is the failure to find differences in IGF-1 concentrations between the strains, which would be expected if insulin resistance was causing reduced concentrations of IGF-1, and if the reduced concentrations of IGF-1 were causing an increase in GH secretion. Although PPAI were significantly shorter in the OS strain in both years of the trial, concentrations of GH were not greater in the OS than the NZH strain in the early postpartum period of Year 2. This hypothesis also contradicts previous research, where concentrations of IGF-1 were significantly greater in early postpartum cows where the dominant follicle ovulated compared to those where it failed to ovulate, although GH concentrations were not different between groups (Beam and Butler, 1998). Although this hypothesis may not adequately explain the differences in PPAI observed between the strains in the current trial, the area of energy partitioning and its relationship to reproduction is likely to be the area that holds the answers to explain the variation in PPAI in the current trial. OS animals seem to be able to ovulate early in the postpartum period, even in the face of sub-optimum calving body condition scores and rapid body condition score loss after calving in Year 1 of the trial. On the other hand the NZH strain, with similar calving body condition scores and body condition score loss to the OS strain, exhibited an extended PPAI, resulting in a high requirement for anoestrous treatments. These findings would suggest that there are strain differences in the area of energy partitioning to reproduction, but the current data

set does not provide sufficient information to explain the observations made in relation to PPAI.

The next step to investigate the earlier postpartum ovulation in the OS strain should be a series of LH pulse bleeds, to establish whether LH release differs between the strains early in the postpartum period. Such an experiment has already been conducted at Massey University, with a group of Holstein-Friesian dairy cattle that differ in live weight, and also in the proportion of overseas Holstein-Friesian genetics. Whilst LH pulse frequency was not different between the strains, mean LH concentrations and LH pulse amplitude were significantly higher in the heavy strain (with a greater proportion of OS genetics) on Days 14, 21, 28 and 35 postpartum. These differences in LH secretion in the postpartum period were accompanied by earlier postpartum ovulation in the heavy strain (Thiengtham *et al.*, 2003). For the purposes of the current trial LH pulse bleeds should begin early in the postpartum period (Day 7 onwards) as some animals in the current trial had begun ovulatory activity prior to Day 14. If differences in LH secretion between the strains are found then further investigations can continue from this starting point, to investigate factors which control the regulation of LH release in the different strains.

6.6 Conclusions

Data from the present study indicates that OS and NZL animals begin to cycle earlier postpartum than their NZH counterparts and the data reinforce the importance of BCS and BCS loss on the length of the PPAI. Earlier postpartum ovulation did not confer the expected reproductive advantage when anoestrous animals were treated prior to the planned start of mating in the present trial. In Year 1 when there was lower calving BSC and greater loss of body condition in the first four weeks of lactation there were a greater number of animals treated for anoestrus (particularly in the NZ strains). Further data is required to confirm whether the conception period is more compact in the NZH strain if CIDRs are not used, given the influence of anoestrus treatment on this variable. The present data did not show significant differences in reproductive performance between the three strains. Thirty years of selective breeding may have altered PPAI, but does not seem to have altered the reproductive performance of Holstein-Friesians of NZ

ancestry. The differences in PPAI between the NZH and NZL and OS merit further investigations.

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7 *Milk progesterone concentrations following first insemination in New Zealand High and Overseas Holstein Friesians*

7.1 Abstract

Milk progesterone concentrations were monitored in New Zealand high (NZH) and overseas (OS) Holstein Friesians, following first insemination to a spontaneous, natural oestrus. Milk samples were collected three times a week, and progesterone concentrations determined by radioimmunoassay. First insemination conception rates tended to be higher in NZH than OS animals where progesterone secretion was monitored following insemination ($p<0.1$), yet ovulatory activity had been established earlier in the postpartum period in the OS strain. Progesterone concentrations were not different between animals that conceived to first insemination, and those that failed to conceive, until 16 days after insemination when progesterone concentrations were significantly higher in pregnant cows ($p<0.05$). Milk progesterone profiles in animals that failed to conceive to first insemination did not differ between the strains until 16 days after insemination, when progesterone concentrations were significantly lower in the OS strain (13.6 ± 1.5 vs. 8.6 ± 1.0 ng/ml, in NZH and OS strains respectively, $P<0.01$). In some OS cows luteal regression was initiated prior to the maternal recognition of pregnancy, which would make conception impossible in these animals. Further, the rate of luteal regression seemed to be reduced in OS compared to NZH cows, with possible effects on the endocrine environment of the developing follicle. Mechanisms that control the onset of luteal regression should be investigated further, as this may a mechanism to explain the widely reported poor fertility in some high genetic merit Holstein Friesians.

7.2 Introduction

There have been many reports of a reduced reproductive efficiency in Holstein Friesians of predominantly North American genetic origin (OS). In Ireland, trial work suggests that cows with a high proportion (92%) of North American genetics cycle readily after calving, but require more inseminations to conceive, and have a higher non-pregnant rate and higher milk production than cows with a lower proportion (52%) of North American genetics (Dillon and Buckley, 1998; Buckley *et al.*, 2000). These data indicate fertilization failure, or early embryonic mortality, could be a factor in the reduced reproductive performance of OS Holstein Friesians.

In order to maintain pregnancy the corpus luteum must continue to secrete progesterone beyond the duration of a normal oestrous cycle (Thatcher *et al.*, 1984). Interferon-τ (IFN-τ) is produced by the trophoblast cells of the preimplantation conceptus from around Day 12 to Day 25 in cattle (Farin *et al.*, 1990). IFN-τ extends luteal lifespan and inhibits pulsatile release of $PGF_2\alpha$ at the end of the cycle, by preventing the increase in oxytocin receptors in pregnant animals (Wathes and Lamming, 1995). Progesterone concentrations rise steadily in pregnant animals (Lamming *et al.*, 1989), maintaining a suitable uterine environment for the developing embryo. Because of the importance of progesterone in determining pregnancy success there has been a significant body of research on the subject of progesterone concentrations and pregnancy outcomes. Incubations of luteal tissue from pregnant animals contain more progesterone than tissues from cyclic animals (Lukaszewska and Hansel, 1980). Monitoring milk progesterone concentrations during the postpartum period has become a standard method used to monitor reproductive activity (Bulman and Lamming, 1978; Bulman, 1980). Differences in progesterone concentrations between pregnant and non-pregnant animals have been detected from as early as 6 days post-insemination (Erb *et al.*, 1976; Larson *et al.*, 1997). Whilst others have reported higher progesterone concentrations from Day 10 post insemination in pregnant than non-pregnant cows (Lukaszewska and Hansel, 1980; Lamming *et al.*, 1989).

The aim of this trial was to compare milk progesterone profiles and pregnancy rates of spontaneously ovulating OS and NZH animals after their first postpartum insemination,

to determine whether there is a difference in milk progesterone profiles following insemination which could help to explain reproductive success or failure of the strains.

7.3 Materials and methods

7.3.1 Animals and samples

Ethical approval for this trial was granted by the Ruakura Animal Ethics Committee, approval number 4250.

Cows, during the second year of the Strain Trial from the NZH and OS strains were recruited into the study (n=107, 45 2-year-olds and 62 3-year-olds, 50 NZH and 57 OS). Cows had to have had at least one spontaneous ovulation prior to the planned start of mating and had to have been calved for at least 28 days at the planned start of mating. Animals that were treated with CIDR devices to induce oestrus were excluded. Twice weekly milk samples were collected from 7 days after calving until the planned start of mating. Thrice weekly milk sampling commenced after the first insemination. Samples (whole milk) were collected on Monday, Wednesday and Friday mornings (see 2.32). Samples were collected until cows were at least 26 days post insemination, or until cows returned to oestrus and were inseminated again, (provided that this insemination was accompanied by a fall in progesterone concentrations).

7.3.2 Progesterone Assay

Progesterone concentrations in milk samples that were collected prior to first insemination (collected twice weekly) were determined using an enzyme-linked immunosorbant assay kit (Ridgeway Science, Gloucestershire, UK). Interassay coefficients of variation for samples with a mean concentration of 2.57 ng/ml and 9.95 ng/ml were 20.45% and 12.5% respectively. Progesterone concentrations of whole milk from post-insemination thrice-weekly samples were measured by radioimmunoassay (Coat-a-Count™, DPC, USA) once a week. Inter-assay co-efficients of variation for milk pools of 4.37, 3.02 and 0.46 ng/ml were 4.2, 1.8 and 15.4% respectively. Intra-assay co-efficients of variation for the same milk pools were 6.8, 5.7 and 13.6% over 52 assays. The minimum detectable concentration of the assay was 0.061ng/ml. See 3.4.7 for further assay details.

7.3.3 Definitions

Cows were defined as pregnant when consecutive milk progesterone concentrations were above 3ng/ml until at least 26 days after insemination. Pregnancy diagnosis was later confirmed by ultrasound examination two months after the planned start of mating, and again 6 weeks later.

7.3.4 Analysis of data

Differences between days and strains were determined by ANOVA using the general linear model procedure in SAS, using a model where each day was analysed independently (Proc GLM) after an initial analysis was run in the mixed procedure (mixed model, ANOVA) of SAS to test the effect of sire (no effect was found). Analysing the data by day avoided the need to transform the data. Discrete data was analysed using a general linear model with a binomial error structure. Non-linear regression curves were fitted to the data in a stepwise non-linear regression in Genstat. The slope of the decline in progesterone concentrations in non-pregnant cows was determined from the first milk sample where milk progesterone concentrations were below 1ng/ml, to the two samples preceding this for individual cows. The difference in slope between strains was determined by ANOVA, using the general linear model procedure in SAS (Proc GLM).

7.4 Results

A total of 107 animals were sampled during the trial. Of these, seven were excluded from analysis as they were inseminated during the luteal phase of the cycle (three animals), or had inadequate luteal phase progesterone concentrations (progesterone concentrations of less than 3ng/ml during the luteal phase, two animals) or had missed heats (two animals). Data for the reproductive performance of this group of animals (n=100) are given first, follwed by data relating to milk progesterone concentrations.

7.4.1 Reproductive data

Of the 100 animals for which analysis was undertaken, 47 were of NZH genetic origin and 53 of OS genetic origin, 40 cows were 2-year-olds and 60 were 3-year-olds. Of the 47 NZH animals, 29 were pregnant by 26 days after insemination (based on milk progesterone profiles) compared to 23 out of 53 OS animals. The proportion of animals

that became pregnant at first service tended (p=0.067) to be greater in animals of NZH genetic origin than OS genetic origin. After ultrasound scanning one cow from the NZH strain and one cow from the OS strain were found to no longer be pregnant. Table 7-1 presents information on mean calving dates, PPAI (postpartum anovulatory interval) and the interval from 1st luteal activity to 1st service for the all animals, by strain and by pregnancy status. The length of the luteal phase preceding first service did not differ between cows that conceived or failed to conceive (p>0.1). Nor was there a difference in the proportion of short, or long luteal phases between groups.

The inter-service interval was calculated for animals that had a second service date recorded (NZH n = 17; OS n = 28). The inter-service interval was not different between NZH and OS strains (mean 20.9 days, standard deviation of 1.5 days and 2.7 days for NZH and OS respectively). However, the range of interovulatory intervals was greater in the OS strain (16 to 26 days), compared to the NZH strain (18 to 23 days). A quarter of OS animals had interovulatory intervals that were either shorter (2) or longer (5) than the 18-24 day interval that is considered normal, compared to none of the NZH strain (χ^2; p<0.05). Pregnancy rate to second service was 65% for the NZH strain and 46% for the OS strain, but the difference was not statistically different.

Table 7-1: Mean calving date (MCD), postpartum anovulatory interval (PPAI) and the interval from 1st luteal activity to 1st service (ILAS), in days, by strain and by pregnancy status. Within a strain there was no effect of pregnancy status on any of the measured variables.

	NZH		OS		P-value	
	NP	P	NP	P	Strain	Pregnancy
MCD	1-Aug ± 5	29-Jul ± 4	3-Aug ± 4	27-Jul ± 4	ns	ns
PPAI	39.9 ± 4.4	41.4 ± 3.0	28.1 ± 1.8	31.2 ± 2.7	P<0.001	ns
ILAS	28.5 ± 4.2	36.0 ± 3.2	40.0 ± 3.4	44.8 ± 4.9	P=0.02	ns

Treatments: New Zealand high (NZH), overseas (OS), non-pregnant (NP), pregnant (P).

7.4.2 Milk progesterone concentrations

Milk progesterone concentrations were not different between pregnant and non-pregnant animals until 16 days after first insemination, at which time milk progesterone concentrations were significantly (p<0.05) higher in pregnant than non-pregnant cows, (Figure 7-1).

Milk progesterone concentrations were then examined by strain and by pregnancy status. Milk progesterone concentrations were not different between non-pregnant cows of either NZH or OS genotype, as illustrated in Figure 7-2, except on Day 16 after insemination (13.6 ± 1.5 vs. 8.6 ± 1.0 ng/ml, in NZH and OS strains respectively, $P<0.01$).

Raw progesterone concentrations for individual cows are presented in Figure 7-3 (NZH) and Figure 7-4 (OS). These graphs illustrate that there was significantly more variation in the timing of progesterone decline in OS than NZH animals. The proportion of cows for which progesterone concentrations had been observed to decline below 1ng/ml was significantly ($p<0.01$) higher in the NZ than OS strain on Day 21 post-insemination.

The data were then reanalyzed looking at progesterone concentrations in non-pregnant animals in relation to time from second insemination, as illustrated in Figure 7-5. Milk progesterone concentrations tended to be higher in NZH animals 5 days prior to second insemination than OS animals (16.0 ± 2.1 vs. 11.2 ± 1.6 ng/ml in NZH and OS strain respectively, $P=0.088$).

Both linear and quadratic regression equations were investigated to model milk progesterone profiles for non-pregnant cows. Progesterone profiles for non-pregnant cows were best described (determined by comparison of r^2 values) by the following quadratic regression equations: -

$-2.457 + 0.8623\ day - 0.03537\ day^2$ for NZH non-pregnant animals and

$-0.668 + 0.5370\ day - 0.02287\ day^2$ for OS non-pregnant.

These equations are illustrated in Figure 7-6. The point of inflection occurred earlier in non-pregnant OS than NZ cows, 11.7 vs. 12.2 days respectively.

The slope between the first concentration of progesterone that was below 1ng/ml and the two preceding points was compared for NZ and OS non-pregnant cows. This analysis revealed that on average, the slope was steeper for the NZ strain, indicating a more rapid decline in progesterone concentrations (-3.3 ng/progesterone/day vs. –2.6 ng/progesterone/day; $p<0.01$).

Figure 7-1: Progesterone profiles after insemination in animals that conceived, or failed to conceive to first insemination (—♦— non-pregnant; —■— pregnant).

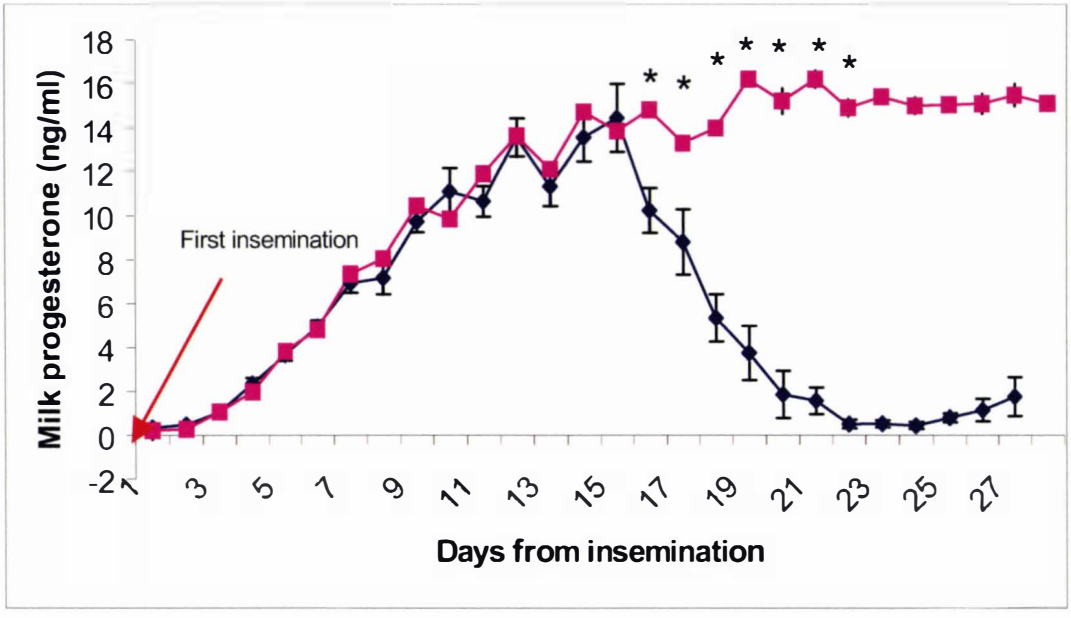


Figure 7-2: Progesterone profiles of non-pregnant cows following first insemination, by strain (—♦— New Zealand high non-pregnant; —■— overseas non-pregnant).

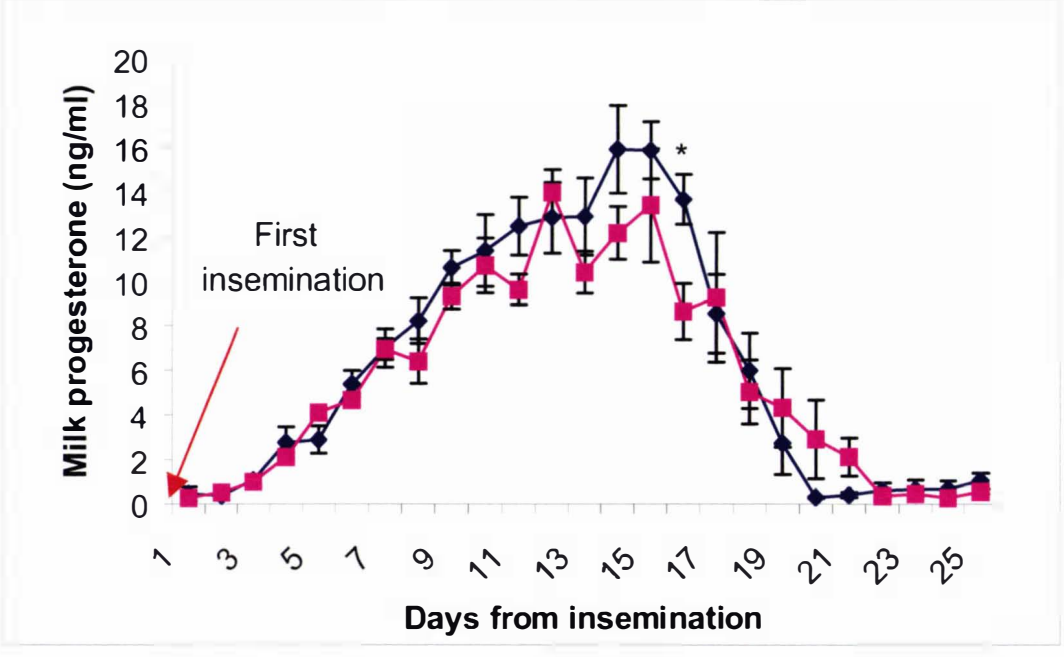


Figure 7-3: Progesterone concentrations from Day 10 post-insemination in New Zealand high animals that failed to conceive. Each line represents one animal.

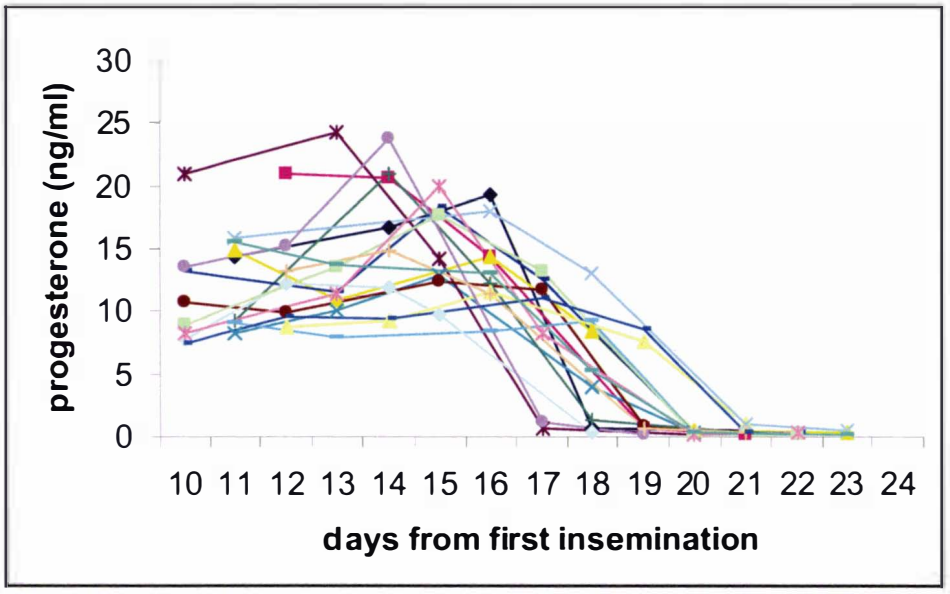


Figure 7-4: Progesterone concentrations from Day 10 post-insemination in overseas animals that failed to conceive to first insemination. Each line represents one animal.

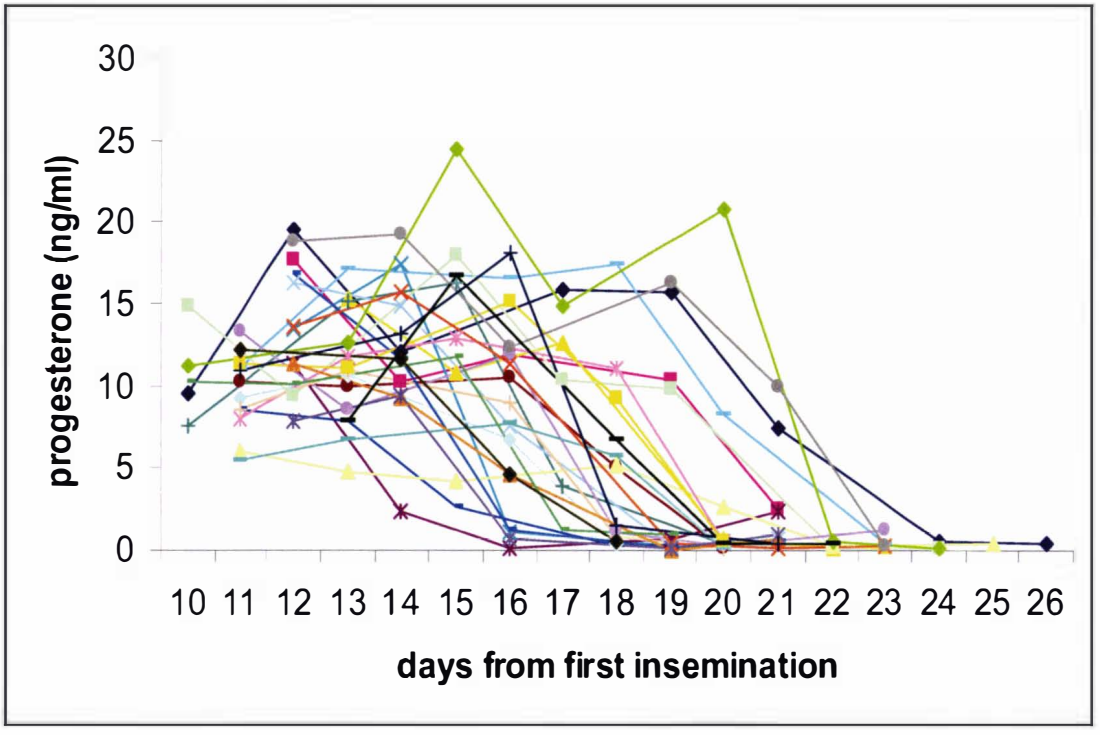


Figure 7-5: Milk progesterone concentrations in cows that failed to conceive to first insemination, in relation to second insemination, by strain (—♦— New Zealand high non-pregnant; —■— overseas non-pregnant). Only animals that had a second insemination could be included in this dataset.

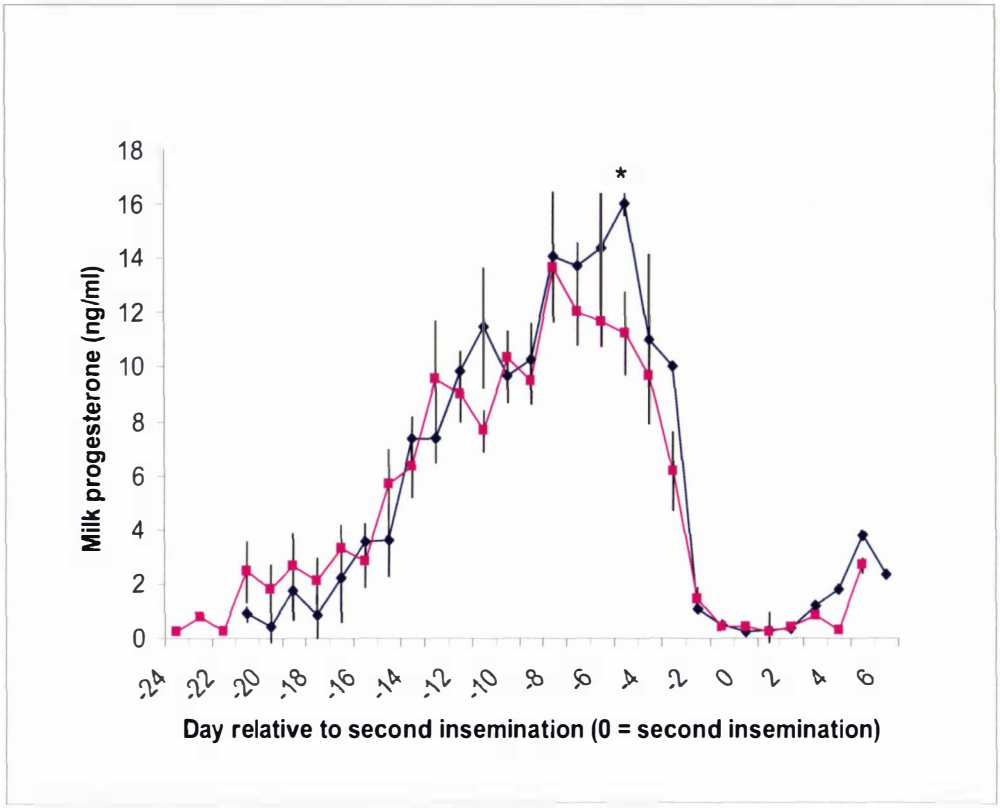
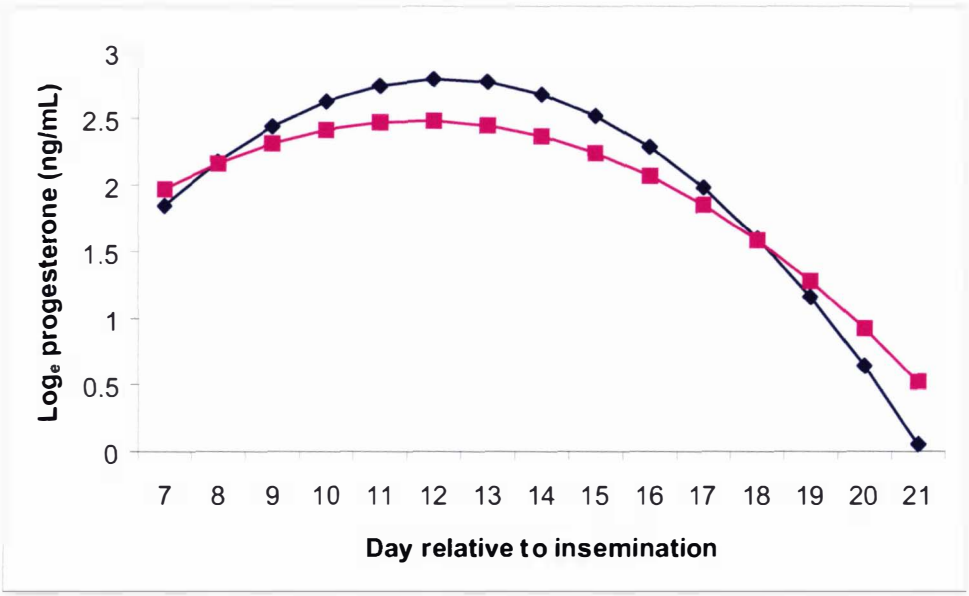


Figure 7-6: Fitted quadratic regression curves for $\log_e$ milk progesterone concentrations in non-pregnant cows, by strain (—♦— New Zealand high non-pregnant; —■— overseas non-pregnant).



7.5 Discussion

Results from the current study suggest that first service conception rate tends to be lower in spontaneously ovulating OS cows than NZH cows. There were no differences in milk progesterone concentrations between OS and NZH strains that conceived. However, there was evidence to suggest that luteal regression occurred earlier in some OS than NZH cows, and that the rate of luteal regression during luteolysis is reduced in OS compared to NZH cows that failed to conceive.

7.5.1 Reproductive performance

Conception to first service tended to be higher in the subset of NZH strain animals used in the current trial. It was therefore important to examine factors that could have affected reproductive performance. Calving date has a significant effect on reproductive performance, with earlier calving animals more likely to become pregnant early in the mating period (Morton, 2000a). However, mean calving date and calving spread was similar for all groups, suggesting that calving date effects were unlikely to explain the differences in conception rate between the two strains. PPAI was significantly shorter in the OS than NZH strain, (as reported in Chapter 6), in an expanded population of Strain Trial cows. A shorter PPAI increases the interval from first luteal activity to first insemination and allows more oestrous cycles prior to the planned start of mating, which has previously been shown to be beneficial to first service conception rate (Macmillan and Clayton, 1980). On average, the OS strain had the opportunity to have more oestrous cycles prior to first insemination than the NZH strain. Thus, an advantage in terms of calving date, or the number of oestrous cycles prior to insemination cannot explain the tendency for a higher first service conception rate in the NZH strain in the present trial. In fact, it might have been expected that first service conception rate would be higher in the OS strain.

It has been suggested that oestrous cycle length can influence conception rates; Royal *et al.* (2000) reported that interovulatory intervals immediately preceding insemination of shorter than 18 days and longer than 24 days resulted in lower pregnancy rates to first service. Variation in luteal phase length accounts for most of the variation in oestrous cycle length (Chapter 5), thus the length of the luteal phase in the cycle preceding

insemination was compared between strains and between cows that conceived or failed to conceive, but no differences were found. However, for animals that failed to conceive, the average inter-service interval was the same across strains, but there were more intervals outside the normal range in the OS strain, including five extended interservice intervals, that may have indicated embryonic mortality. The interservice interval can be considered as an indicator of oestrous cycle length, indicating more variability in the length of the oestrous cycle in OS cows. Previous research into strain differences has identified that OS animals have longer inter-ovulatory intervals than NZ strains. In yearling heifers in the Strain Trial (Chapter 5) OS animals had significantly longer interovulatory intervals than their NZH counterparts. Likewise, a study by Fahey *et al.* (2003) reported that OS animals had significantly longer interovulatory intervals than NZ animals, despite all cows having two waves of follicular development per cycle. Royal *et al.* (2000) reported that the proportion of animals with an interovulatory interval of greater than 24 days had increased significantly between the late 1970s/early 1980s and the mid to late 1990s, coincident with increases in both Holstein genetics and milk production. A further project could investigate inter-ovulatory intervals prior to, and following, all inseminations in Strain Trial animals to determine whether the difference between strains that was indicated in this trial is in fact a real phenomenon.

7.5.2 Progesterone profiles

In the present trial milk progesterone profiles differed between pregnant and non-pregnant animals from 16 days after insemination, indicating that in some animals luteal regression had been initiated prior to the maternal recognition of pregnancy (Northey and French, 1980). The divergence in progesterone concentrations between pregnant and non-pregnant cows occurred later than some other researchers have reported. Butler *et al.* (1996) and Erb *et al.* (1976) found cows that conceived had significantly higher progesterone concentrations from 4 and 6 days after insemination respectively. Others have failed to find a difference in milk progesterone concentrations between pregnant and non-pregnant dairy cattle until Day 10 post-insemination or later (Bulman and Lamming, 1978; Lamming *et al.*, 1989; Verkerk and Macmillan, 1998; do Carmo Feliciano *et al.*, 2003). Northey and French (1980) reported a decline in milk progesterone concentrations from Day 15 in non-bred control animals, or animals that had embryos removed at Days 13 or 15, whilst in non-bred heifers in Chapter 5

progesterone concentrations also began to decline around Day 15. At Day 16 luteal regression had been initiated in non-pregnant animals in the present trial, as evidenced by a decline in milk progesterone concentrations, whilst progesterone concentrations were maintained in pregnant animals.

A key focus of this trial was to determine whether progesterone concentrations were different between NZ and OS cows that failed to conceive. Previous research in NZ with cows that differed genetically for live weight, and also for the proportion of OS genetics found that milk progesterone concentrations declined earlier in cows with a high proportion of OS genetics that failed to conceive (Thiengtham *et al.*, 2002). Milk progesterone concentrations were significantly lower at Day 16 in non-pregnant cows with a high proportion of OS genetics than in non-pregnant cows with a lower proportion of OS genetics (Thiengtham *et al.*, 2002). Likewise, in cyclic postpartum cows plasma progesterone concentrations were significantly higher in NZ than OS cows four days prior to the LH surge (Meier *et al.*, 2002). This is similar to the findings of the current trial, in which non-pregnant OS cows had significantly lower milk progesterone concentrations than non-pregnant NZH cows on Day 16 after insemination. When the data were compared in relation to second insemination, a similar pattern was observed, with milk progesterone concentrations tending to be higher in the NZH strain five days prior to second insemination. The raw data revealed that there was significant variation in the decline in progesterone concentrations, with more variation in the OS than NZH strain. There is a group of OS animals where progesterone concentrations are declining earlier than the maternal recognition of pregnancy. Clearly, pregnancy cannot be established in cows where progesterone concentrations decline before the maternal recognition of pregnancy.

Furthermore, although luteal regression appeared to be initiated earlier in some of the OS than NZH cows, the process of luteal regression was actually slower in OS than NZH cows. This was shown by analysis of the rates of decline of progesterone concentrations for individual cows. Thus, luteal regression begins around a day earlier in OS cows even though oestrous cycle lengths are similar between the strains (the current study) or slightly longer (1.3 days; (Fahey *et al.*, 2003)). However, in trials in which Bilby (1998), found a larger difference in oestrous cycle length (OS 3.5 days

longer) the regression of the CL occurred later in OS cows and the rate of luteal regression was reduced in OS, compared to NZ cows.

Bilby (1998) also reported greater persistence of dominant follicles in OS cows, which he postulated could be a mechanism to explain this reduced fertility. However, Fahey *et al.* (2003) found no difference in the interval from emergence of the ovulatory follicle to ovulation between NZ and OS strains. Perhaps it is not just the interval from emergence to ovulation, but rather the endocrine environment in which the ovulatory follicle develops that has negative effects on fertility. It has previously been demonstrated that follicles whose duration of dominance is extended by administration of sub-luteal concentrations of progesterone are associated with a reduction in fertility (Mihm *et al.*, 1994). Perhaps the reduced rate of progesterone decline that has been demonstrated in OS strain has a negative impact on the quality of the oocyte that ovulates, either via gonadotropin, or via local intra-ovarian effects, with resulting negative effects on fertility. Declining progesterone concentrations lead to an increase in LH pulse frequency that promotes follicular maturation. The earlier decline in progesterone concentration, combined with a slower decline in progesterone concentrations could potentially expose the preovulatory follicle in OS animals to a higher LH pulse frequency over a longer period than in the NZ cows, and produce an extended period of dominance. A slow increase in LH pulse frequency could disrupt the association between the resumption of meiosis and the LH surge and ovulation, leading to the ovulation of an over-mature follicle (Mattheij *et al.*, 1994).

The question that arises from such considerations, is what causes the earlier, slower decline in progesterone concentrations that has been demonstrated in non-pregnant and also in cycling animals in the current and previous trials (Meier *et al.*, 2002; Thiengtham *et al.*, 2002). The similarity of findings between non-pregnant and cycling animals suggests that this is not due to the presence of an embryo, thus a failure of maternal recognition of pregnancy seems unlikely to be the predominant cause. Two possible alternative causes of the earlier decline in progesterone concentrations are a) luteal inadequacy, or b) the premature induction of luteal regression. If progesterone concentrations are taken to indicate luteal adequacy, then luteal inadequacy seems an unlikely cause of the earlier decline in progesterone concentrations in the current trial. There were two animals with luteal inadequacy as identified by progesterone

concentrations, but both of these animals were excluded from the analysis of milk progesterone profiles. In the remainder of animals progesterone concentrations were not different until the time of onset of luteal regression, which seemed to be occurring just prior to the normal time of maternal recognition of pregnancy. In those animals where luteal regression was initiated prior to or at Day 15, even a strong IFN-τ signal from an embryo at the normal time of maternal recognition of pregnancy would be to no effect, and the embryo would be lost. Thus, the premature induction of luteal regression needs to be considered. Luteal regression involves the interplay of reproductive steroids, oxytocin and $PGF_2\alpha$ (McCracken *et al.*, 1999). Although the current data indicates luteal regression begins earlier and is more protracted in OS than NZH animals further investigation of progesterone secretion and the timing and duration of luteolysis is required, to enable more definite conclusions to be drawn and to elucidate the mechanisms operating. A focus of future experiments should be frequent sample collection around the time of luteal regression to quantify the pattern of $PGF_2\ \alpha$ secretion.

7.6 Conclusions

In conclusion, the data presented here indicate that the conception rate to first service in a group of cyclic OS animals was lower than cyclic NZH animals, despite having a similar mean calving date, and earlier onset of postpartum luteal activity. Differences in milk progesterone concentrations between pregnant and non-pregnant cows were not present until the time of luteal regression in non-pregnant cows. The earlier, and slower decline in progesterone concentrations in non-pregnant OS cows is in agreement with previous data, and suggests that luteal regression is being initiated earlier in OS cows. Of particular significance is finding that in some OS cows luteal regression was initiated prior to the normal timing of maternal recognition of pregnancy.

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8 *An oestradiol challenge in New Zealand or overseas Holstein-Friesian dairy cattle, fed either pasture or a total mixed ration.*

8.1 Abstract

The re-establishment of the hypothalamic-pituitary responsiveness to oestradiol is an important step in the resumption of ovulatory activity in the postpartum period. Delayed resumption of ovulatory activity is the major form of infertility in the New Zealand dairy industry. An oestradiol challenge was conducted in New Zealand (NZ) or overseas (OS) Holstein Friesian dairy cattle fed either total mixed ration, or pasture, to investigate the responsiveness of the hypothalamic-pituitary ovarian axis to oestradiol in the early postpartum period. After ultrasound scanning of the ovaries oestradiol benzoate (0.75mg/500 kg live weight) was administered at Day 17 or Day 18 postpartum and blood samples collected at 4 hourly intervals for 48 hours. There was no difference ($p>0.05$) in the number of animals that ovulated prior to challenge between the strains or between the feeding treatments. More pasture than TMR fed cows exhibited a luteinizing hormone (LH) surge in response to oestradiol (χ^2, $p<0.05$). There was no effect of strain on whether cows responded to oestradiol with an LH surge. The single biggest follicle was larger in cows that ovulated than cows that did not ovulate in response to the LH surge ($p=0.0052$). The effect of strain approached significance ($p<0.1$), with NZ cows tending to have greater total LH release than OS cows. In cows that were defined as having an LH surge, peak LH did not differ between strains or feeding treatments ($p>0.05$). Time to peak LH concentration was not affected by strain (OS, 23 vs. NZ 29 hours after oestradiol benzoate). The results from the present trial suggest that differences in postpartum anovulatory intervals between NZ and OS Holstein Friesians are not due to differences in the resumption of oestradiol positive feedback in the hypothalamo-pituitary ovarian axis at day 18 postpartum.

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8.2 Introduction

Ovarian follicular development begins early in the postpartum period. Initially, it starts as the development of follicular waves with large follicles (>8mm diameter) present by Day 7 postpartum in acyclic beef cows (Spicer *et al.*, 1986). Savio *et al.* (1990) reported that 3/22 well fed Friesian dairy cows ovulated before Day 10 postpartum and that the interval from calving to detection of the first dominant follicle (defined as a follicle ≥10mm, in the absence of other large follicles) was 11.6 ± 8.9 days for the remaining 19 animals. Over half the animals ovulated the first dominant follicle (14/19). In pasture-fed New Zealand dairy cattle, the first follicle >9mm diameter was detected 10.3 ± 0.7 (range 6 to 17) days postpartum. In 12% of animals this follicle ovulated (McDougall, 1994).

Early in the post-partum period the pituitary is relatively refractory to GnRH stimulation, probably due to low pituitary concentrations of LH (Fernandes *et al.*, 1978). During late gestation, high concentrations of oestrogen and progesterone inhibit pituitary LH synthesis, resulting in low concentrations of LH in the pituitary immediately after parturition (Jolly, 1993). However, reproductive steroid concentrations fall rapidly at parturition, allowing pituitary LH synthesis to resume. Consequently whilst the release of LH from the pituitary in response to GnRH is negligible prior to Day 10 postpartum (Schallenberger *et al.*, 1978), after Day 10 postpartum significant LH release occurred by 2-3 hours after GnRH administration. By Day 20 the pituitary response, in terms of peak response had returned to normal levels (Schallenberger *et al.*, 1978).

An extended postpartum anovulatory period is the major form of infertility in the New Zealand dairy industry (Macmillan, 2002). Previous trial work has identified that New Zealand cows take longer to resume ovulatory activity in the postpartum period than OS animals (Verkerk *et al.*, 2000; McNaughton *et al.*, 2003). The recovery of the stimulatory ability of oestradiol-17β is essential to end the postpartum anoestrous period (Schallenberger and Prokopp, 1985), as during the follicular phase of the oestrous cycle, it is increasing concentrations of oestradiol that trigger the GnRH surge, which in turn triggers the LH surge and ovulation (Kesner *et al.*, 1981; Moenter *et al.*, 1990). Oestradiol feeds back to the hypothalamus to induce further GnRH release (Moenter,

Brand and Karsch, 1992) and also stimulates the responsiveness of the pituitary to GnRH (Kesner *et al.*, 1981; Caraty *et al.*, 1995).

Administration of GnRH, and subsequent sampling to measure plasma LH levels test the ability of the pituitary gland to release LH in response to GnRH. Whilst oestradiol administration tests the ability of the hypothalamus to respond to oestradiol and release a GnRH surge, then the ability of the pituitary to respond to GnRH and release LH and FSH. The current study will investigate whether the LH response to oestradiol differs between New Zealand and OS origin dairy cattle, fed either pasture or a total mixed ration, using an oestradiol challenge. We postulated that the earlier postpartum ovulatory activity in OS animals could be due to the earlier restoration of a positive feedback back interaction between oestradiol and LH.

8.3 Materials and Methods

This study was approved by the Ruakura Animal Ethics Committee, approval 3958.

8.3.1 Animals

A total of 49 Holstein Friesian cows of either 100% OS genetic origin (North American and Dutch), or New Zealand genetic origin (less than 12.5% OS genetics) were used in this trial. Cows were selected to have equivalent breeding worth, at the initiation of the trial. The OS foundation animals were imported into New Zealand by Holland Genetics Ltd as embryos, the male calves being used in the Livestock Improvement Corporation sire proving scheme. Animals were fed either a generous allowance of pasture, or a total mixed ration (TMR), according to an existing experimental protocol (Kolver *et al.*, 2000) in a 2 x 2 factorial design. Pasture-fed animals were offered an allowance of >60kg DM/cow/day, with grass silage during summer to maintain targeted pasture residuals. New Zealand and OS animals grazed the same paddock, but were separated by a temporary fence. Total mixed ration was mixed to resemble a typical North American or European diet and fed to achieve a 10% refusal rate, equivalent to *ad libitum* intake. The nutrient composition of the TMR and pasture diets, over the lactation are given in Table 8-1. Cows fed TMR were confined to a feed pad during July, August and September, or rotated around three small loafing paddocks the remainder of the time (Kolver *et al.*, 2000). There were 12 animals in each group except the OS TMR group, which had 13 animals.

Table 8-1: Mean annual nutrient composition of the grass and TMR diets over a complete lactation, from Kolver *et al.* (2002)

	Grass	TMR
ME (MJME/kg DM)	11.7	11.8
Crude Protein (% DM)	26.9	18.2
Degradable protein (% protein)	57.8	56.2
Soluble protein (% protein)	30.8	33.1
Neutral detergent fibre (% DM)	42.5	34.3
Acid detergent fibre (% DM)	23.3	21.0
Non-fibre carbohydrate (% DM)	16.1	36.3
Fat (% DM)	4.2	6.7
Ash (% DM)	10.3	7.9

8.3.2 Procedures

On the morning of Day 16 or 17 postpartum all animals were given an intramuscular injection of 5mg prostaglandin $F_{2\alpha}$ (Lutalyse, Pharmacia, New Zealand). Twenty-four hours later, animals were scanned via transrectal ultrasonography. Follicle numbers and sizes were recorded. Live weight was recorded and animals returned to their herds. At 2-pm, a coccygeal blood sample was collected (Time 0) and oestradiol benzoate (Cidirol, Bomac Laboratories Ltd, NZ) administered (0.75mg oestradiol benzoate / 500kg live weight). Blood samples were collected via coccygeal venipuncture at 8, 16, 20, 24, 28, 32, 36, 40 and 48 h after oestradiol benzoate administration, as per the sample collection procedure described in 3.3.1. Plasma was analysed for concentrations of LH (all samples) and oestradiol (samples 0 to 5 only), insulin, IGF-I and progesterone (Time 0 only). Serum, collected at Time 0 was analysed for non-esterified fatty acids (NEFA), β-hydroxybutyrate (BOH), urea and cholesterol.

At a minimum of 6 days after the challenge animals were scanned once via transrectal ultrasonography to identify whether a corpus luteum was present. A progesterone releasing intra-vaginal device (CIDR; Pharmacia, New Zealand) was administered to each cow soon after this, as part of the protocol of another experiment.

8.3.3 Hormone Assays

Luteinizing hormone

Luteinizing hormone concentrations were determined using a double antibody radioimmunoassay, as described in 3.4.2. Inter-assay coefficients of variation for bovine plasma pools of 12.44, 3.45, 1.58 and 0.48ng/ml were 12.3, 0, 5.6 and 5.65% respectively. Intra-assay coefficients of variation for the same plasma pools were 11.4, 5.21, 5.6 and 15.1% respectively. The minimum detectable concentration across two assays was 0.08 ng/ml.

Oestradiol

Oestradiol concentrations in bovine plasma were determined by a modification of the method using the Serono Estradiol MAIA assay kit (Biodata S.p.A., Montecelio, Italy), as described in 3.4.1. Inter-assay coefficients of variation for bovine plasma pools of 11.02, 4.07, 0.61pg/ml were 10.2, 7.3, 5.9% respectively. Intra-assay coefficients of variation for the same plasma pools were 28.1, 17.1, and 13.8% respectively. The minimum detectable concentration across two assays was 0.58 pg/ml.

Progesterone

Progesterone concentrations were determined in the Time 0 sample; using the Coat-a-Count radioimmunoassay kit (DPC, California), see 3.4.7. Intra-assay coefficients of variation for plasma pools of 5.36, 3.59 and 0.47 ng/ml were 13.5, 8.02 and 12.2% respectively. The minimum detectable concentration across two assays was 0.032 ng/ml.

Insulin-like growth factor-1

Samples were analysed at the University of Western Australia, Perth, in a double antibody radioimmunoassay (see 3.4.3 for further details).

Intra-assay co-efficients of variation for plasma pools of 3.2 ng/ml and 10.7 ng/ml and 30.4 ng/ml were 8.3, 6.8 and 6.2% respectively. The minimum detectable concentration was 0.95 ng/ml.

Metabolite concentrations

Concentrations of metabolites were determined at Alpha Scientific Ltd, Hamilton, New Zealand using enzyme immunoassay, read on a spectrophotometeric auto-analyser (Hitachi 717, Hitachi Ltd, Tokyo, Japan). Intra-assay coefficients of variation were less than 5%. See 3.5 for further analysis details.

8.3.4 Definitions

An LH surge was defined as having occurred when the peak LH concentration was greater than the mean concentration (calculated from all 10 values per cow) plus 2 standard deviations of the mean. The peak value was then examined and, if it was not above 2ng/ml, no surge was considered to have occurred, even if the first condition had been reached. Basal LH was defined as the average of the first two LH samples at 0 and 8 h. Total LH release was the sum of LH concentrations in all samples. Peak LH release was defined as the single highest LH concentration measured and was only recorded in cows that met the criteria for having an LH surge.

8.3.5 Analysis of data

Continuous variables were analysed using analysis of variance in the mixed model procedure of SAS. The models used included the fixed effects of strain, feed and the interaction of these, age and whether animals had a single or double ovulation. Sire, nested within strain was tested as a random effect, but excluded if found to have no effect on the model. Categorical data were tested using chi-squared test.

8.4 Results

There was no difference in the number of animals that ovulated prior to challenge between the strains 8 OS, vs. 8 NZ, or between the feeding treatments 8 TMR vs. 8 Grass. Two of the OS that ovulated prior to challenge had double ovulations. The single biggest follicle present at oestradiol challenge was smaller in cows that had already ovulated compared to anovulatory animals (8.1 vs. 14.2 mm; $p<0.0001$).

Table 8-2: Luteinizing hormone response (in terms of numbers of animals) of different genotypes and feeding treatments to oestradiol benzoate (0.75 mg/500kg live weight).

	Surge	No surge
OS TMR	5	8
OS Grass	10	2
NZ TMR	7	5
NZ Grass	10	2

Treatments: overseas (OS), New Zealand (NZ), total mixed ration (TMR).

Cows fed grass were more likely than TMR-fed cows to exhibit an LH surge in response to oestradiol (χ^2, $p<0.05$). There was no effect of strain on whether cows responded to oestradiol with an LH surge (see Table 8-2).

Of the 17 animals that did not respond with an LH surge, four of these had a CL visible at the initial scan and plasma progesterone concentrations of greater than 1 ng/ml at oestradiol administration, a further two animals had progesterone concentrations of greater than 0.5 ng/ml. Five animals had a CL present (as detected by ultrasonography), but low progesterone concentrations.

A further five animals that did not exhibit an LH surge had a CL present six days after the challenge when examined via ultrasound. Concentrations of oestradiol in the Time 0 samples for these animals were elevated. One animal did not respond at all, and had no CL present either at the initial scan, or when scanned six days after challenge.

Of the 32 animals that exhibited an LH surge, 22 formed a CL and seven did not form a CL and had no existing CL. Three animals had an existing CL and exhibited an LH surge; two of these animals had plasma progesterone concentrations of 0.5 and 8ng/ml at the time of oestradiol administration.

The single biggest follicle (or two follicles where both ovulated) was larger in cows that ovulated than cows that did not ovulate in response to the LH surge ($p=0.0052$), this effect remained significant even when two possibly cystic follicles were excluded from the analysis ($p=0.0140$). Peak LH concentrations were not different between cows that ovulated and those that did not ovulate.

Table 8-3 details the distribution of double ovulations, either before or after oestradiol challenge, and which strain and feeding level cows belonged to. All double ovulations were in cows that were 5-years-old, except for 2, 3-year-old OS TMR cows. Cows that had double ovulations had significantly higher milk yields around the challenge (combined yield from 2 days before to 3 days after the challenge) than 3, 4 and 5 year old cows that did not ovulate ($P=0.038$), or cows that had a single ovulation ($P=0.035$). There was no difference in average yield around the challenge between cows that had single ovulation, and cows that did not ovulate.

Table 8-3: The distribution of double ovulations, by strain and feeding treatment and whether the double ovulation occurred before or as a result of the oestradiol challenge. † No LH surge, but 2 corpora lutea were present at scanning 6 days after challenge

	Pre challenge	Result of challenge
OS G	1	1
OS TMR	1	4
NZ G		
NZ TMR	1†	

Treatments: Overseas grass-fed (OSG), Overseas total mixed ration-fed (OS TMR), New Zealand grass-fed (NZ G) and New Zealand total mixed ration-fed (NZ TMR).

8.4.1 Luteinizing hormone concentrations

There was no strain or feeding effect on basal LH concentrations. When all cows were included, total LH release tended to be greater in NZ cows (NZ 13.28 vs. OS 10.7; P=0.09). Feed has a significant effect on total LH release; grass 14.1 vs. TMR 9.89 (P<0.01). A breakdown of total LH release, by strain and by feeding system is found in Table 8-4.

Table 8-4: Total luteinizing hormone (LH) release in response to 0.75 mg oestradiol benzoate/500 kg live weight, by strain and by feeding system in all cows that were challenged. Differing superscripts indicate a significant difference within a column.

	Total LH release (ng/ml)
NZ Grass	14.9^{a}
NZ TMR	11.6^{ab}
OS Grass	13.3^{a}
OS TMR	8.1^{b}

Treatments: Overseas grass-fed (OSG), Overseas total mixed ration-fed (OS TMR), New Zealand grass-fed (NZ G) and New Zealand total mixed ration-fed (NZ TMR).

<u>In cows with an LH surge only</u>

Total LH release was lower in 4-year-old cows than in 3- and 5-year-old cows (P<0.05). The effect of strain approached significance (P<0.1), with NZ cows tending to have greater total LH release than OS cows.

Peak LH did not differ between strains or feeding treatments (Figure 8-1), but was significantly affected by age (Figure 8-2; P<0.01). Two and three year old animals had higher (P<0.05) peak LH concentrations than 4 and 5 year old animals (Figure 8-2).

Time to peak LH concentration was not affected by strain (OS, 23 vs. NZ 29 hours after oestradiol benzoate).

Figure 8-1: Peak luteinizing hormone (LH) release, by strain and by feeding system in cows that exhibited an LH surge. Overseas (OS) or New Zealand (NZ) fed either grass or total mixed ration (TMR).

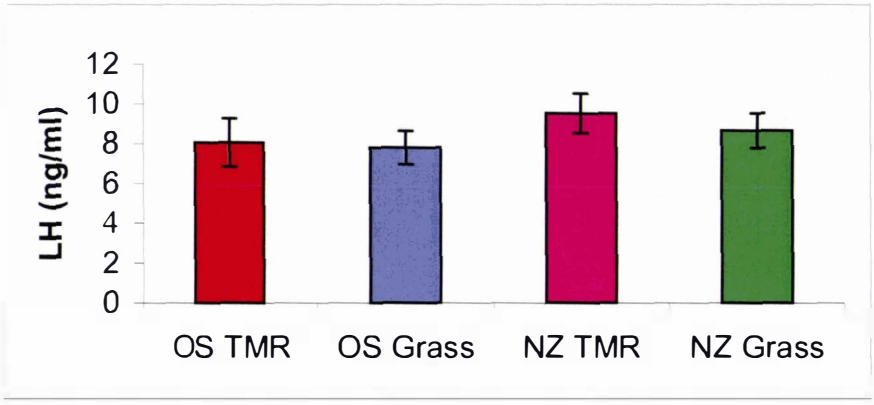
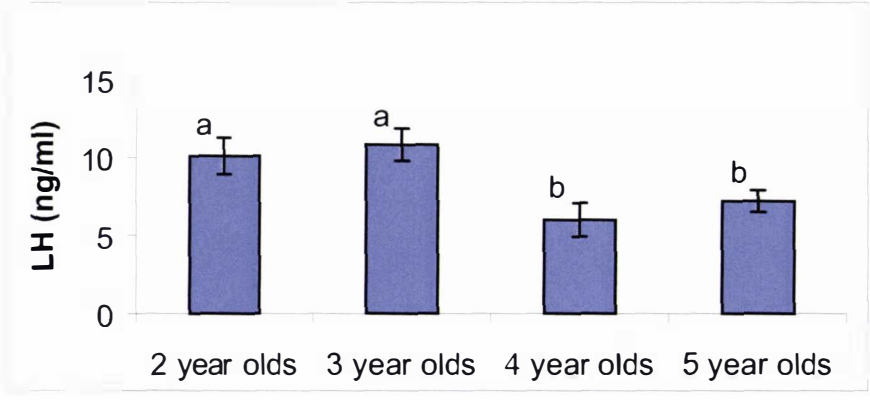


Figure 8-2: Peak LH release, by age, in cows that exhibited a luteinizing hormone surge.



Area under the curve was not different between strains or feeding treatments.
Age had a significant effect on area under the curve ($P<0.05$). The 4-year-old cows had a smaller area under the curve than 3 and 5 year old cows, see Figure 8-3.

Concentrations of the metabolic hormone IGF-1 and various metabolites, by strain and feeding system are detailed in Table 8-5. Concentrations of IGF-1 were not different between cows that had ovulated before challenge compared to cows that had not ovulated (5.1 ± 1.0 vs. 5.7 ± 0.6; $p=0.6$), but IGF-1 concentrations tended to be different

between cows that did or did not have an LH surge in response to oestradiol (5.0 ± 0.6 vs. 6.8 ± 0.9; p=0.09).

Figure 8-3: Area under the curve, by age, for animals that exhibited a luteinizing hormone surge.

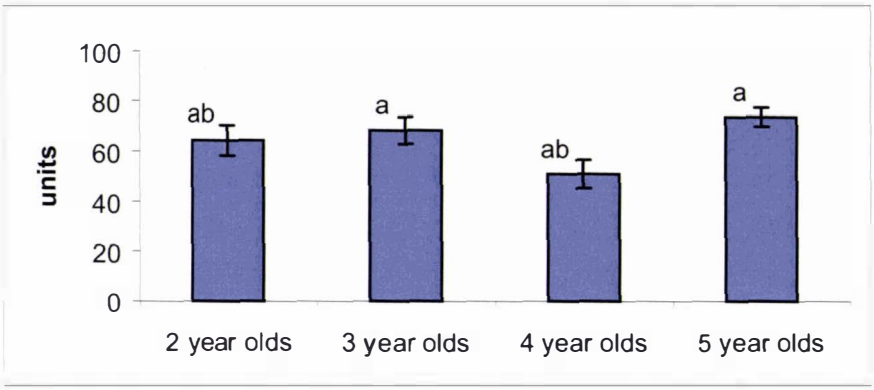


Table 8-5: Concentrations of insulin like growth factor-1 (IGF-1) in plasma and of non-esterified fatty acids (NEFA), cholesterol, urea and β-hydroxybutyrate (BOH) in serum of cows.

	NZ G	NZ TMR	OS G	OS TMR	Genotype effect	Diet effect
IGF-1	3.4 ± 1.0	7.3 ± 1.0	4.4 ± 1.0	7.2 ± 0.9	NS	P<0.01
NEFA	0.42 ± 0.1	0.52 ± 0.1	0.38 ± 0.1	0.46 ± 0.1	NS	NS
Cholesterol	3.1 ± 0.2	3.4 ± 0.2	2.4 ± 0.2	3.0 ± 0.1	P<0.01	P<0.01
Urea	7.4 ± 0.5	5.8 ± 0.5	7.1 ± 0.5	6.9 ± 0.5	NS	P=0.08
BOH	0.7 ± 0.2	1.4 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	P<0.05	NS

Treatments: New Zealand (NZ) or overseas (OS) genotype, fed either grass (G) or a total mixed ration (TMR).

8.5 Discussion

The results of the present study suggest there is no difference in the time course of the restoration of the positive feedback response of the hypothalamo-pituitary axis during the post partum period between NZ and OS origin Holstein Friesian cattle fed either pasture or a total mixed ration. The restoration of the positive feedback response during the postpartum period is not responsible for the differences in postpartum anovulatory intervals seen between NZ and OS strains.

Initially it was planned to challenge animals at around Day 9 or 10 postpartum, to minimize the number of postpartum ovulations prior to challenge. However, locating the ovaries in such early postpartum animals proved to be impossible in some cases. Therefore, to preserve animal numbers, it was decided to delay challenging animals until Day 17 or 18, when the ovaries could be readily scanned, and to give an injection of prostaglandin $F_{2\alpha}$ to all animals 24 hours before challenge.

The responses to oestradiol in the present trial are similar to those previously reported in postpartum cattle. Schallenberger and Prokopp (1985) reported a gradual recovery of the stimulatory effect of an intramuscular 1mg dose of oestradiol-17β on LH release in the early postpartum cow. However, no LH surge occurred before 10 days postpartum. Positive feedback of oestradiol on LH was prevented when progesterone concentrations were >0.5ng/ml (Schallenberger and Prokopp, 1985) or >0.3 ng/ml (Alam and Dobson, 1987). In the present trial six of the cows that failed to exhibit an LH response to oestradiol had progesterone concentrations of >0.5 ng/ml at the time of oestradiol administration, thus it is likely that their failure to respond can be explained by the action of progesterone in inhibiting the LH surge (Kesner *et al.*, 1982). The LH surge response in the two animals that had progesterone concentrations of >0.5ng/ml was surprising. The progesterone concentration in one of these animals was between 0.5 and1ng/ml, and may have been declining as a result of the prostaglandin $F_{2\alpha}$. It is unclear why we saw elevated progesterone and an LH response in the second animal.

In the study of Alam and Dobson (1987) five cows were treated with 20 μg GnRH, i.v. then 4 h later with 1mg ODB i.m. on Days 1, 7, 14, 21, 28, 35, 42 and 49 postpartum. All cows responded to an injection of GnRH with LH release, however, two of the cows failed to respond to ODB five times out of the eight times these two cows were challenged between days 28 and 49 postpartum. Both of these animals were in poor body condition. Likewise, Schallenberger and Prokopp (1985) reported that some cows failed to exhibit an LH response to oestradiol until after Day 25 postpartum. Reasons for the lack of response were not suggested. There was only 1 animal in the current trial that failed to respond at all (no CL present prior to challenge, and no LH surge or CL present after challenge). This animal was in good condition (as determined by condition score data) at the time of challenge. Reasons for the non-response are not clear.

Total LH released tended to be greater in NZ than in OS animals, most likely because more NZ animals had an LH surge in response to oestradiol (17 vs. 15). More grass than TMR fed cows had an LH surge, leading to greater LH release in grass than TMR fed cows. Animals that had previously ovulated tended not to respond to oestradiol administration, probably due to elevated progesterone concentrations that inhibit the LH surge (Kesner *et al.*, 1982). There was no difference between feeding treatments in the proportion of animals that ovulated before challenge. Prostaglandin $F_{2\alpha}$ was given 24 hours prior to oestradiol administration, in an attempt to ensure progesterone concentrations did not interfere with the action of oestradiol on the hypothalamus. However, prostaglandin $F_{2\alpha}$ is not effective when administered before Day 5 of the oestrous cycle (Rowson *et al.*, 1972; Henricks *et al.*, 1974). Thus, some of the animals that had recently ovulated would have had increasing progesterone concentrations at the time of oestradiol administration.

The other group of animals that did not respond to oestradiol with a measurable LH surge were animals in which the LH surge had likely occurred just before or around oestradiol administration and was missed by the 8-hourly sampling regime during the first 16 hours of the trial. These animals were all TMR cows. The proportion of TMR cows that ovulated around the time of challenge may be related to energy balance being more positive in the TMR fed cows, as there is a relationship between energy balance and timing of first postpartum ovulation (Canfield and Butler, 1991; Beam and Butler, 1998). Serum cholesterol concentrations are positively associated with energy balance (Lean *et al.*, 1992) and concentrations of IGF-1 are greater in cows in positive than in negative energy balance (Spicer *et al.*, 1990). Both cholesterol and IGF-1 concentrations were significantly higher in the TMR-fed cows, indicative of more positive energy balance than their pasture-fed contemporaries.

The five TMR-fed cows that apparently ovulated around the start of the oestradiol challenge without an LH surge being measured probably contributed to the higher proportion of grass-fed cows that exhibited an LH surge in response to oestradiol challenge.

In cows that did exhibit an LH surge, there was no difference in peak LH concentrations between NZ and OS animals, or the time from oestradiol administration to peak LH concentrations, but total LH release tended to be higher in NZ animals.

There was a significant age effect on total LH release and area under the curve. Four-year-old animals had significantly lower total LH release and a smaller area under the curve than 3- and 5-year-old animals. However, peak LH release was lower in 4- and 5-year-olds than in 2- and 3-year-old animals. If the reduced LH release had been consistent in the 4- and 5-year-old animals a milk production effect may have been indicated. However, this was not the case. The reasons for the age effect are not clear.

The number of ovulations before challenge is similar to what would be expected for a mixed age herd. Stage of follicular development can affect the response of an animal to oestradiol benzoate (ODB) and may explain why seven animals exhibited an LH surge, but apparently failed to ovulate. Follicle size was greater in cows that ovulated, compared to cows that did not ovulate in the present trial. McDougall (1994) found that early postpartum cows treated with 0.5 mg ODB were more likely to ovulate if the dominant follicle had ceased growing than if the dominant follicle was still growing. Fewer cows ovulated a younger, smaller dominant follicle, than a mature, larger dominant follicle in response to ODB (Burke *et al.*, 2001) . Sartori *et al.* (2001) found that follicles less than 10mm did not ovulate in response to a high dose of exogenous LH. An important step in the maturation of a follicle is the acquisition of LH receptors in granulosa cells (Ireland and Roche, 1982), which allows follicles to respond to LH pulses and grow beyond 9mm (Webb *et al.*, 1999). In the current trial the dominant follicle may have recently emerged in some animals and not yet acquired sufficient LH receptors to allow it to ovulate in response to the induced LH surge. Therefore, whilst the pituitary was capable of responding to oestradiol positive feedback with an LH surge, not all animals had follicles at a suitable developmental stage to ovulate in response to this surge.

The largest follicle present at the pre-challenge ultrasonography was smaller in cows that had ovulated prior to challenge than in cows that had not ovulated before the challenge and affected the likelihood of ovulation. A proportion of cows that had not ovulated at the initial scan went on to ovulate, apparently without an LH surge. A large

pre-ovulatory follicle was probably present in these cows, increasing the average follicle diameter in the anovulatory group. The animals that had ovulated before the challenge tended to have recently ovulated (corpus haemorhagicum, or an early corpus luteum visible by ultrasonography), with few, if medium and large follicles present. During the early oestrous cycle (up to day 4) there are few, if any follicles above 5mm (Savio *et al.*, 1988).

The finding that milk production was greater in cows that had double, compared to single ovulations is of interest, although the numbers in this trial were only small. Twinning is generally considered an undesirable trait in dairy cattle, due to an increase in reproductive problems following twin pregnancies (Kinsel, 1998). Previous work has suggested that milk yield is one of the main risk factors for double ovulation (Wiltbank *et al.*, 2000) and twinning (Kinsel, 1998). A double ovulation rate of 14.1% was reported in 199 cows in an Ovsynch® protocol, with a nearly 3 fold greater incidence of double ovulations in high producing cows (Fricke and Wiltbank, 1999). The current trial data does not necessarily suggest that high milk production is causing an increase in the rate of double ovulations. However, a mechanism by which increased milk production could lead to an increase in double ovulations, through alterations in steroid metabolism has been suggested (Wiltbank *et al.*, 2000). The rate of twinning in NZ and Australia is low, current reports suggest around 1.3 – 1.5% of births are twins (McDougall and Murray, 2000; Morton, 2000a). The rate of twinning in the USA seems to be increasing. A large data set of over 50, 000 lactations in the USA reported a twinning rate of 1.4% in 1983 and 2.4% by 1993, on the same farms (Kinsel, 1998). The results of the current study are consistent with the hypothesis that milk production and rate of twinning are related.

It is possible that the challenge was not conducted early enough in the postpartum period to detect a difference in response between the strains. However, an oestradiol challenge conducted with animals bred for either light or heavy mature live weight, that also differ in the proportion of OS genetics (70% in heavy line and 15 % in light line), failed to identify a difference in response between the strains at days 7, 14, 21 and 28 postpartum (Thiengtham *et al.*, 2003).

8.6 Conclusions

The results from the present trial suggest that differences in postpartum anovulatory intervals between NZ and OS Holstein Friesians are not due to differences in the resumption of oestradiol positive feedback in the hypothalamo-pituitary ovarian axis at day 18 postpartum.

8.7 References

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9 *General Discussion*

9.1 Introduction

The primary aims of this thesis were firstly to compare the reproductive performance and physiology of New Zealand Holstein-Friesians of high and low genetic merit, with overseas high genetic merit Holstein-Friesian dairy cattle in a pasture-based dairy production system, and secondly to establish whether OS animals can achieve reasonable reproductive outcomes in a pasture-based system. The inclusion of the two New Zealand strains of differing breeding worth allowed the effect of 25-30 years of genetic selection for milk production within a pastoral-based system on reproductive characteristics to be determined. The New Zealand high (NZH) strain was representative of high genetic merit dairy cattle in the late 1990s, whilst the New Zealand low strain (NZL) were representative of high genetic merit dairy cattle of the 1970s, having been bred from semen stored from bulls that had been used widely for artificial insemination during this time. The OS animals used in the present trial had undergone some selection for reproductive fitness in pasture-based dairying system as the dams of strain trial animals had to have conceived at least once in New Zealand to be eligible for selection as a dam. This is the first investigation to examine specifically for the effects of genetic origin on reproductive performance and reproductive physiology in a pasture-based dairy production system in New Zealand.

The Dexcel Strain Trial was conceived principally as a production trial, to compare the productive performance of Holstein-Friesians of either New Zealand or overseas genetic origin, in a range of pastoral dairying systems that could typically be found in New Zealand. This trial was developed in response to the realisation that the proportion of overseas genetics within the New Zealand Holstein-Friesian population was increasing significantly (Harris and Kolver, 2001), and that the overseas Holstein-Friesian had been selected in a very different production system (Garrick and Lopez-Villalobos, 1998). As a result the trial design (in terms of group, genetic merit, feeding level, and reproductive management) was fixed, so there was little flexibility to alter the management of these animals, particularly the lactating cows.

9.2 Puberty in Strain Trial heifers

In Chapter 4, difference in live weight at puberty was found between all of the strains, and the importance of live weight, rather than age, in determining the timing of the onset of puberty was confirmed. Such differences in live weight at puberty between the strains clearly reflect the difference in mature live weight between the strains, as puberty seems to have been reached at around 40 to 50% of mature live weight in predominantly pasture-raised heifers in both the current study and those of Garcia-Muniz (1998) and McGrath *et al.* (2001). A difference in live weight at puberty between the New Zealand strains was not expected, as the live weight breeding values (which are an estimate of mature live weight) were similar for New Zealand strains. However, when the live weights of lactating cows were compared, taking into account variation in condition score (Appendix D – Live Weight Measurements), the difference in live weight between the two New Zealand strains persisted, suggesting the difference in live weight at puberty represented a difference in mature live weight, rather than an earlier onset of puberty. Moreover, the live weight breeding values (an estimate of mature live weight) may not have been accurate for the NZL strain, as the information used to generate these values is based on relative information, which would only have been available for distant relatives of the NZL sires, as live weight breeding values were not introduced until 1996. With this single caveat, however, live weight breeding values were found to be a useful predictor of live weight at puberty. It is feasible that such breeding values could be used by farmers to estimate target live weights for their replacement heifers on the basis of the relationship between live weight breeding value and live weight at puberty that was established in this study. The reproductive performance of heifers in the current study was high and was not different between the three strains, which concurs with previous reports of high reproductive performance in nulliparous heifers (Butler and Smith, 1989; Pursley *et al.*, 1997; Pryce *et al.*, 1999).

9.3 Heifer oestrous cycle characteristics

Chapter 5 compared the endocrinology of the oestrous cycle in nulliparous heifers of the three strains. Although oestrous cycle length was found to be significantly longer in the OS strain, due to a longer luteal phase, there was no effect on reproductive performance in heifers in the strain trial (Figure 5-1 and Figure 5-2). Mean plasma progesterone concentrations were significantly greater in the NZL than the NZH and OS from Days 6

to 14 of the oestrous cycle. Interestingly, this is in agreement with measurements from lactating animals that have been selected for milk production where progesterone concentrations were lower than in control (unselected) animals (Lucy and Crooker, 1999). However, in heifers from the same selection line there were no differences in progesterone concentrations (Weber *et al.*, 2003). There were differences in the metabolic hormones leptin and IGF-1, which were both higher in the NZH strain, despite similar body condition scores and growth rates during the trial. It was curious to note that there were differences in the leptin/body condition score relationship between strains, but this may merely mean that body condition scoring may not provide an accurate measure of fat depots in rapidly growing animals. The number of heifers used in the current study (12 per strain) was not sufficient to allow the effect on fertility measures such as conception rates to be documented, and even when reproductive performance was compared in all heifers from the strains (Chapter 4) no difference in the pattern of conception could be determined (Figure 4-3 and Figure 4-4).

9.4 Reproductive performance of lactating cows

In Chapter 6 the reproductive performance of the three strains of lactating cows was compared, and metabolic hormones were measured during the early postpartum period. The most significant finding from this experiment was that NZH have a greater PPAI than either NZL or OS strains. A previous study found differences in PPAI (Verkerk *et al.*, 2000) and final pregnancy rates in a small group of cows that had been imported into NZ as embryos (Kolver, 2001) compared to cows of New Zealand origin. The difference in PPAI between the NZH and NZL strain probably represents the effect of selection for milk production (genetic merit as measured by Breeding Worth), such a view is in agreement with Fulkerson *et al.* (2001) and Lucy & Crooker (1999), who also found differences in PPAI in animals that differed in genetic merit for milk production. The difference in PPAI between the NZH and OS strains most probably represents a genotype effect, which warrants further investigation to determine the mechanisms, which are likely to involve an effect on LH secretion. For example Thiengtham *et al.* (2003) noted that mean LH concentrations and LH pulse amplitude were significantly higher in cows selected for heavier mature live weights (and with a greater proportion of OS genetics) on Days 14, 21, 28 and 35 postpartum than in cows selected for light mature live weight. These differences in LH secretion in the postpartum period were

accompanied by earlier postpartum ovulation in the heavy strain (Thiengtham *et al.*, 2003). A lower LH pulse frequency in the early postpartum period has been associated with a longer postpartum period (Peters *et al.*, 1981; Chagas, 2003).

Body condition score was an important predictor of the length of the PPAI in the current study, as it was in the studies of McGowan (1981) and Burke *et al.* (1995). It was clear that OS strain animals ovulated earlier in the postpartum period despite losing more body condition (in Year 1 of the trial). *A priori* this could be seen to be an advantage within the New Zealand system, where extended PPAI is the major cause of infertility (Macmillan, 2002). However, very early postpartum ovulation (before Day 21) has been associated with adverse reproductive outcomes (Smith and Wallace, 1998), and was associated with reduced 6-week in-calf rates in the current trial.

After calving at similar BCS, the OS animals mobilised more body condition than the NZ strains, (particularly NZL) and maintained a lower body condition score throughout lactation. As animals were dried off according to BCS, this contributed to the shorter lactation lengths in the OS strain (see Appendix C – Milk Yields). Body condition score was negatively related to PPAI in this, and other studies (McGowan, 1981; Burke *et al.*, 1995; Pryce *et al.*, 2001; Chagas, 2003). Body condition score is under a degree of genetic control and has been genetically correlated with reproductive traits, such as PPAI, calving interval and first service conception rate in a number of studies (Pryce *et al.*, 2001; Veerkamp *et al.*, 2001; Pryce *et al.*, 2002). Selection for increased milk production and an increase in Holstein percentage has been associated with a decline in average BCS (Pryce *et al.*, 2001; Veerkamp *et al.*, 2001) and the same trend emerged in the current study. The InCalf study in Australia identified pre-calving BCS as an important factor affecting reproductive performance especially 3-week submission rates, first service conception rates and 6-week in-calf rates. Pre-calving BCS was more important than body condition change in early lactation (Morton, 2000b). Some of the effect of pre-calving BCS on reproductive performance may be mediated through reduced levels of anoestrus (through effects on PPAI), leading to increased submission rates. In the current study the differences in PPAI did not translate into differences in pregnancy rates, probably due to the aggressive treatment of extended PPAI with CIDR devices (Pharmacia, Auckland). The possible effects of anoestrus treatment on

reproductive outcomes must be borne in mind when considering conception and pregnancy rates.

Differences in metabolic hormone concentrations between the strains were mainly small and inconsistent, although there were effects of year and age. Concentrations of GH could be related to changes in body condition score during the early postpartum period. Six and 12-week pregnancy rates were only different between the strains in Year 1 of the trial, and suggest that OS animals are able to successfully reproduce in seasonal, pastoral feeding systems as 2 and 3-year-olds. Final pregnancy rates after a 12-week breeding period were lower in the OS than NZH or NZL strains in Year 1, but not in Year 2. This was predominantly a sire effect (7 out of 11 non-pregnant animals had the same sire) and illustrates the importance of the inclusion of fertility in the selection index for NZ dairy cattle. The inclusion of fertility in the breeding worth index should allow bulls that sire daughters that are efficient producers of milk whilst remaining fertile, to be selected regardless of their genetic origin. Calving OS animals at similar BCS to NZ strains in the current trial seems to have had a protective effect on reproductive performance during the first two years of the Strain Trial. Whether this effect will continue as Strain Trial animals reach maturity, and milk production increases remains to be seen. Reproductive performance and survival of mature animals (4-, 5- and 6-year-olds) needs to be compared before definitive conclusions on the effect of OS genetics on reproductive performance in pastured-based dairying systems can be drawn.

9.5 Post-insemination progesterone secretion

Chapter 7 focussed on progesterone secretion following first insemination in NZH and OS strains as cows with low progesterone concentrations 5 days after insemination have been shown to have severely reduced conception rates compared to cows that have high progesterone concentrations at this time (Mann *et al.*, 2003). Furthermore, progesterone concentrations during the early luteal phase are important in controlling the development of the embryo and its ability to release IFN-τ (Mann *et al.*, 1998). First service conception rates tended ($p=0.067$) to be lower in spontaneously ovulating OS compared to NZH animals; and such trends have also been shown by Fulkerson *et al.* (2001) and Snijders *et al.* (2001), who reported differences in first service conception

rates between animals that differed in genetic merit and the proportion of OS genetics, or for animals who differed in the proportion of OS genetics, but had equivalent genetic merit Garcia-Muniz (1998). Yet, this is not a simple effect of breeding worth, as the genetic merit for milk production and actual milk production was similar between OS and NZH strains in the current study. Previous research has documented an earlier decline in progesterone in OS than NZ cows (Meier *et al.*, 2002; Thiengtham *et al.*, 2002), and the results in the current study were consistent with this. In most animals, progesterone profiles following first insemination were not different in OS and NZH cows, but in some OS cows luteolysis was initiated prior to maternal recognition of pregnancy, so that even if a strong IFN-τ signal were delivered from an embryo could not prevent luteal regression. This finding warrants further investigation of the regulation of $PGF_2\alpha$ release and of maternal recognition of pregnancy in the two strains.

9.6 Postpartum hypothalamo-pituitary axis responsiveness

In Chapter 8 the positive feedback response of the hypothalamo-pituitary axis to oestradiol was investigated in New Zealand and OS cows fed either total mixed ration or pasture. In animals that exhibited an LH surge there were no differences in oestradiol-induced LH secretion between the strains or feeding treatments, which agrees with results from a similar study in New Zealand (Thiengtham *et al.*, 2003). Animals that failed to exhibit a positive feedback response either had elevated progesterone concentrations, due to a previous ovulation, or ovulated spontaneously around the time of oestradiol challenge. It was concluded, that by Day 18 postpartum the positive feedback response of the hypothalamo-pituitary axis was restored to the same degree in both strains. The restoration of positive feedback response during the postpartum period is not responsible for the differences in PPAI seen between the NZ and OS strains.

9.7 Conclusions

9.7.1 Suitability of overseas genetics in New Zealand farming systems

The results from the current trial suggest that there is significant variation both between and within strains of Holstein Friesian dairy cattle in aspects of reproductive performance that are important in seasonal dairy production systems (for example, live

weight at puberty onset and postpartum onset of ovulatory activity). In the case of differences of live weight at puberty, genetic information that is readily available to the farmer can be used to determine suitable target live weights to achieve good reproductive performance in all Holstein Friesian strains. Genetic evaluation of bulls to be used in New Zealand is normally based on progeny test information, from daughters milked in a large number of herds across New Zealand. This method of bull selection should ensure that the most suitable genetics for the New Zealand dairy system are selected, regardless of whether those genetics originated in New Zealand or the USA. The current trial has demonstrated that some animals of predominantly OS genetic origin can perform well in the New Zealand production system. Provided bulls are tested under New Zealand production systems before they are widely used commercially there is no reason to recommend against the use of OS Holstein Friesian genetics.

9.7.2 Summary of main points

- Heifers of New Zealand genetic origin weigh less at the onset of puberty than heifers of overseas genetic origin. Selection for milk production over the past 30 years has lead to an increase in live weight at puberty (and mature live weight) in animals of New Zealand genetic origin (Table 4-3 and Table 4-4)

- Genetic information, in the form of live weight breeding values, can be used to predict live weight at puberty.

- Oestrous cycle lengths were shorter in heifers of New Zealand genetic origin than in heifers of overseas genetic origin, due to differences in luteal phase length between the strains (Figure 5-2). Despite this difference, and despite small differences in endocrine parameters, there was no difference in the reproductive performance of heifers between the three strains.

- Body condition score at calving is an important predictor of length of the postpartum anovulatory interval (PPAI).

- Calving animals in good body condition (at least score 5 for cows, and score 5.5 for heifers on the New Zealand scale) minimises the need for anoestrus treatment.

- Body condition score loss was greatest in the OS strain, and OS animals maintained lower BCS during lactation (Figure 6-3 and Figure 6-4).

- Despite differences in PPAI, there was no difference in conception rates and 6- and 12-week in-calf rates between the three strains when anoestrus was treated aggressively (Table 6-6).

- The in-calf rate of OS cows in the first two years of the Strain Trial was not different to NZ cows (Table 6-6).

- The point at which progesterone concentrations declined following insemination in non-pregnant cows was more variable in the OS strain, and in some animals progesterone concentrations were declining prior to maternal recognition of pregnancy (Figure 7-3).

- There was no difference in hypothalamo-pituitary axis responsiveness to oestradiol at Day 18 postpartum between NZ and OS cows.

The findings presented in this thesis have revealed subtle differences between current high genetic merit New Zealand, 1970s high merit New Zealand and current high genetic merit overseas origin Holstein Friesian dairy cattle. Data from lactating animals suggested no differences in reproductive performance between the strains, but only included data from 2 and 3-year-old animals, and were confounded by the effects of anoestrus treatments, therefore it is difficult to draw firm conclusions on the effect of OS genetics on reproductive performance. Based on current results the use of OS genetics in New Zealand would not appear to have adverse effects on reproductive performance. Further investigation into the differences between the strains should focus on the control of body condition mobilisation and the control of luteolysis.

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10 Appendices

10.1 Appendix A - Iodination Procedures

10.1.1 Iodogen iodination procedure

1. Tap the vial to ensure all Growth Hormone (GH) is at the bottom.
2. Add the GH to the iodogen vial
3. Add 20µl 0.5M PO4.
4. Add 0.6mCi (6µl) I125. Deglove*.
5. Mix with gentle agitation.
6. Incubate for 15 minutes mixing gently at 5-minute intervals.
7. After 15 mins transfer the contents of the iodogen vial to a separate vial containing 200ul of 0.05M PO4 buffer.
8. Add 200ul of Elution Buffer.
9. Gently mix well.
10. Transfer a 10µl aliquot to a specific activity (SA) tube and add 490µl elution buffer.
11. Add the remainder of iodinated protein to the column and elute in 1.0ml fractions per 20 minutes*. Run the column overnight collecting approximately 80 fractions.
12. In the first 2 hours check to see the column is stable and the tubes on the fraction collector are aligned and moving as they should be.
13. While waiting to check on (l) ensure all equipment and fume cupboards are in a clean safe condition ready for the next user.

Iodination Components

PO_4 Stock Solution (0.5M PO_4)

7.80g NaH2PO4 . 2H2 O in 100 mls distilled water. Name 'A'.
35.51g Na_2HPO4 anhydrous in 500 ml distilled water. Name 'B'.
(or 44.5g $Na_2HPO_4 . 2H_2O$)

Add 82mls of 'A' to the 500mls of 'B' , adjust to pH 7.4
Store as 40ml aliquots in screw top jars at -20°C.
This becomes the PO_4 stock solution.

0.05 M PO_4

Take Phosphate stock solution and dilute 1:10 in DI water.
Make up 100mls for iodination.

Elution Buffer (200mls)

20mls Phosphate stock solution
0.4 g BSA
0.2g NaN_3

DI water
Adjust pH to 7.4

Make up to 200mls

Iodogen

Iodogen (1, 3, 4, 6-tetrachloro-3α, 6α diphenyl glycoluril; Pierce and Warriner, NZ Ltd; 100ul of a 100umol/l solution in dichloromethane) is added to a polypropylene vial and the solvent evaporated.

Precipitation Buffer

2mls of Stock PO_4
1.861g EDTA (disodium,dihydrate)
1g BSA
0.1g Sodium Azide
Make up to 100mls with Milli Q water

TCA

10% Tricloroacetic acid
Make up to 200mls in deionised water.

Label (I^{125})

0.5millicuries

10.1.2 Chloramine T iodination method

Iodination takes place in the vial that the LH is stored.

1. Tap the vial to ensure all LH is at the bottom.
2. Add 10µl 0.5M PO4.
3. Add 0.5mCi (5µl) I125. Deglove*.
4. Mix with gentle agitation.
5. Add 5µl of Chloromine T.
6. Gently agitate for 60 seconds (Prepare for addition of sodium metabisulphate during this 60 seconds.)
7. Add 10µl of Metabisulphate.
8. Agitate to mix well.
9. Add 500µl of elution buffer. Mix well.
10. Transfer a 10µl aliquot to a specific activity (SA) tube and add 490µl elution buffer.
11. Add the remainder of iodinated protein to the column and elute in 1.0ml fractions per 20 minutes. Run the column overnight collecting around 80 fractions.
12. In the first 2 hours check to see the column is stable and the tubes on the fraction collector are aligned and moving as they should be.

Iodination components

PO_4 Stock Solution (0.5M PO_4)

7.80g $NaH_2PO_4 .2H_2O$ in 100 mls distilled water. Name 'A'.
35.51g Na_2HPO4 anhydrous in 500 ml distilled water. Name 'B'.
(or 44.5g $Na_2HPO_4 . 2H_2O$)

Add 82mls of 'A' to the 500mls of 'B' , adjust to pH 7.4
Store as 40ml aliquots in screw top jars at -20°C.
This becomes the PO_4 stock solution.

0.05 M PO_4

Take PO_4 stock solution and dilute 1:10.
Make up 100mls for iodination.
Prepare fresh on the day of Iodination. Do not add azide.

Elution Buffer (200mls)

20mls PO_4 stock solution
0.4 g BSA
0.2g NaN_3
Adjust pH to 7.4
Make up to 200mls

Chloramine T

40mg Chloramine T	or	16mg Chloramine T
25ml 0.05M PO_4 buffer		10mls 0.05M PO_4 buffer

Make up immediately prior to use.

Sodium Metabisulphite

40mg sodium metabisulphite	or	16mg sodium metabisulphite
25mls of 0.05M PO_4 buffer		10mls 0.05M PO_4 buffer

Make up immediately prior to use.

Precipitation Buffer

2mls of Stock PO_4
1.861g EDTA
1g BSA
0.1g Sodium Azide
Make up to 100mls with Milli Q water

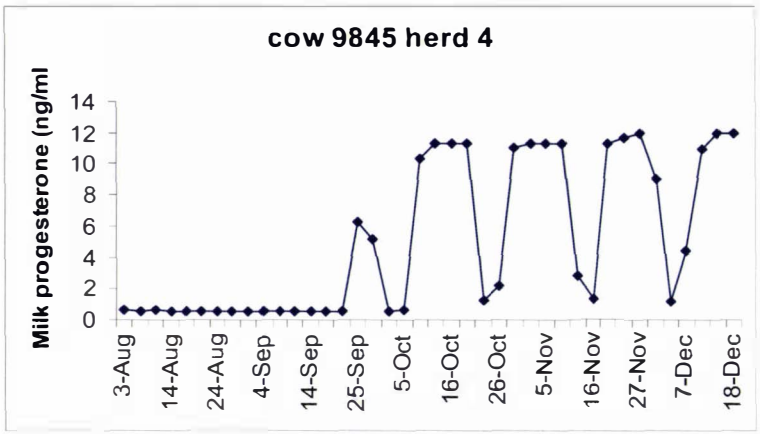
Label (I^{125})

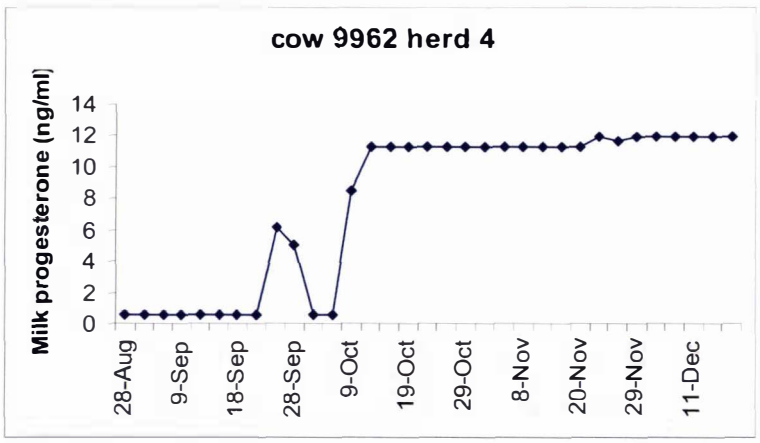
0.5 millicuries

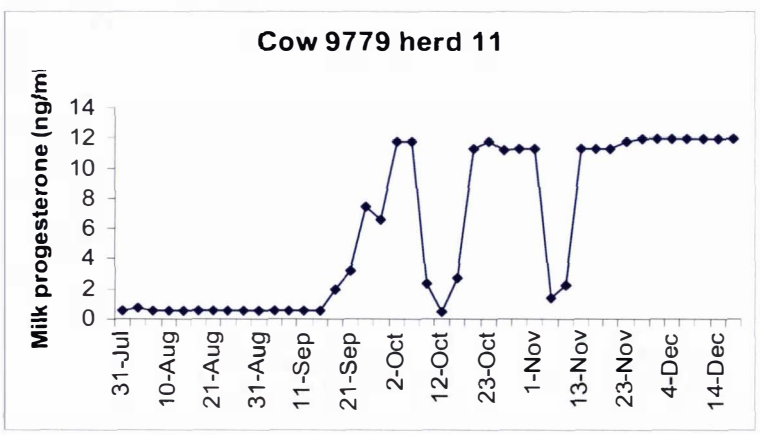
10.2 Appendix B

10.2.1 Milk progesterone profiles

The Figures below illustrate the milk progesterone profiles obtained from twice weekly sampling. The first two cows were treated with CIDR devices in late September, the first animal had not conceived before the milking sampling regime ceased, however, the other cows conceived to her first insemination (2nd October). The final cow spontaneously ovulated during September and then conceived to her second insemation (5th Nov).







10.3 Appendix C – Milk Yields

10.3.1 Milksolids yield from weeks 2 to 12 of lactation, by strain

Mean milk and milksolids yields, from weeks 2 to 12 of lactation, for cows that calved within 6-weeks of the planned start of calving in Years 1 and Years 2 of the trial, by strain. Milk and component yields were measured weekly.

	Year	NZH	NZL	OS
Milk (kg/cow)	1 - 2-year-olds	1580	1417	1654
	2 - 2-year-olds	1566	1343	1710
	2 - 3 year olds	1946	1623	2071
Milksolids (kg/cow)	1- 2-year-olds	122	106	120
	2 - 2 year olds	123	102	128
	2 - 3 year olds	153	120	153

Treatments: New Zealand high (NZH), New Zealand low (NZL) and overseas (OS)

10.3.2 Days in milk, and milk, milksolids, protein and fat yields in Year 1

Mean days in milk and milk, milksolids, protein and fat yields (kg), by strain for Year 1 of the strain trial. Milk and milk component yields were measured weekly.

	Days in milk	Milk	Milksolids	Protein	Fat
NZH	258	3853.0	306.6	131.8	174.9
NZL	268	3714.3	282.3	116.4	165.9
OS	249	3943.0	291.7	130.0	161.7

Treatments: New Zealand high (NZH), New Zealand low (NZL) and overseas (OS)

10.3.3 Days in milk, and milk, milksolids, protein and fat yields in Year 2

Mean days in milk, and milk, milksolids, protein and fat yields (kg), by strain and by age for Year 2 of the strain trial. Milk and milk component yields were measured weekly.

		Days in milk	Milk	Milksolids	Protein	Fat
NZH	2-year-olds	273	4484.6	369.4	157.6	211.7
	3-year-olds	266	5298.3	436.2	192.3	243.9
	All	267	5087.0	418.9	183.3	235.6
NZL	2-year-olds	286	4077.2	322.2	133.8	188.4
	3-year-olds	270	4540.5	351.1	149.9	201.2
	All	275	4417.0	343.4	145.6	197.8
OS	2-year-olds	263	4852.0	364.7	164.2	200.5
	3-year-olds	243	5279.3	403.0	184.6	218.4
	All	249	5515.4	391.9	178.7	213.2

Treatments: New Zealand high (NZH), New Zealand low (NZL) and overseas (OS)

10.3.4 Days in milk, and milk, milksolids, protein and fat yields, by herd in Year 1

Mean days in milk, and milk, milksolids, protein and fat yields in Year 1 of the strain trial, by herd. Milk and component yields were measured weekly.

Herd	Days in milk	Milk	Milksolids	Protein	Fat
1	267	3528.5	268.5	110.2	158.2
2	271	3853.5	289.2	120.1	169.1
3	266	3760.8	289.2	118.9	170.3
4	250	3697.3	291.4	125.8	165.6
5	259	3901.5	313.7	134.0	179.7
6	264	3859.9	308.6	131.7	179.7
7	257	3953.4	312.8	135.7	177.2
8	249	3726.8	285.9	126.0	159.9
9	239	3993.3	284.3	128.8	155.5
10	253	4053.6	298.4	132.9	165.4
11	255	3998.5	298.3	132.3	166.0

Treatments: Herds 1 – 3 are New Zealand Low, with feed allowances of 4.5, 5.5 and 6 tDM/cow respectively, herds 4-7 are New Zealand high with feed allowances of 5, 5.5, 6 and 6.5 tDM/cow respectively, and herds 8-11 overseas with feed allowances of 5.5, 6, 6.5 and 7 tDM/cow respectively. Note that all animals were 2-year-olds.

10.3.5 Days in milk, and milk, milksolids, protein and fat yields, by herd in Year 2

Mean days in milk, and milk, milksolids, protein and fat yields in Year 2 of the strain trial, by herd. Milk and component yields were measured weekly.

Herd	Days in milk	Milk	Milksolids	Protein	Fat
1	260	3912.9	310.1	127.7	182.4
2	276	4760.7	363.5	154.9	208.6
3	290	4577.3	356.5	154.1	202.4
4	243	4512.2	363.2	158.1	205.1
5	257	4942.7	398.6	170.8	227.8
6	287	5210.6	442.0	192.4	249.6
7	284	5687.5	473.0	212.2	260.8
8	231	4520.7	351.2	158.9	192.3
9	244	4891.0	370.1	168.4	201.7
10	262	5543.3	414.6	189.0	225.6
11	260	5674.7	432.0	198.5	233.5

Treatments: Herds 1 – 3 are New Zealand Low, with feed allowances of 4.5, 5.5 and 6 tDM/cow respectively, herds 4-7 are New Zealand high with feed allowances of 5, 5.5, 6 and 6.5 tDM/cow respectively, and herds 8-11 overseas with feed allowances of 5.5, 6, 6.5 and 7 tDM/cow respectively. Note that herds comprised 25% 2-year-olds and 75% 3-year-olds.

10.4 Appendix D – Live Weight Measurements

10.4.1 Mean live weights, by strain, by year and by age in Years 1 and 2

Mean live weights, by strain, year and age in Years 1 and 2 of the Strain Trial. Live weights were measured in the first week of December in each Year

	Year 1	Year 2	
	2-year-olds	2-year-olds	3-Year-olds
NZH	422.9	428.3	474.0
NZL	412.5	400.8	460.2
OS	432.7	440.9	477.6

Treatments: New Zealand high (NZH), New Zealand low (NZL) and overseas (OS)

10.4.2 Adjusted live weights, by strain, by year and by age

Lsmean live weights, by strain, year and age with condition score as a covariate, to adjust for differences in condition scores between strains. Live weights were measured in the first week of December each year.

	Year 1	Year 2	
	2-year-olds	2-year-olds	3-Year-olds
NZH	422.9	420.7	475.0
NZL	396.8	380.6	445.7
OS	441.1	446.2	490.2

Treatments: New Zealand high (NZH), New Zealand low (NZL) and overseas (OS)

10.5 Appendix E

10.5.1 Progesterone concentrations over an oestrous cycle.

Changes in lsmean progesterone concentrations over an oestrous cycle in yearling strain trial heifers, by strain and by day.

	OS	NZH	NZL
Day 1	0.2 ± 0.07	0.13 ± 0.07	0.07 ± 0.07
Day 2	0.09 ± 0.05	0.06 ± 0.05	0.16 ± 0.04
Day 3	0.35 ± 0.15	0.17 ± 0.17†	0.56 ± 0.13†
Day 4	1.13 ± 0.22	0.77 ± 0.25	1.28 ± 0.19
Day 5	2.01 ± 0.34	1.56 ± 0.39†	2.57 ± 0.31†
Day 6	3.06 ± 0.41^{a}	2.73 ± 0.46^{a}	4.41 ± 0.38^{b}
Day 7	4.03 ± 0.59^{a}	4.23 ± 0.67^{ab}	5.71 ± 0.54^{b}
Day 8	4.85 ± 0.49^{a}	4.77 ± 0.47^{a}	6.66 ± 0.49^{b}
Day 9	6.27 ± 0.66	5.46 ± 0.74†	7.41 ± 0.62†
Day 10	6.08 ± 0.59^{a}	6.32 ± 0.57^{a}	8.36 ± 0.59^{b}
Day 11	7.12 ± 0.92†	7.46 ± 1.05	9.51 ± 0.83†
Day 12	7.53 ± 0.80^{a}	8.25 ± 0.77^{ab}	10.15 ± 0.80^{b}
Day 13	8.17 ± 0.74^{ab†}	8.00 ± 0.75^{a}	10.19 ± 0.72^{b†}
Day 14	9.56 ± 0.99	9.75 ± 0.95	10.44 ± 0.99
Day 15	8.22 ± 0.94	9.01 ± 1.03	9.75 ± 0.87
Day 16	8.79 ± 0.81	8.63 ± 0.77	8.98 ± 0.81
Day 17	8.25 ± 1.21	6.98 ± 1.17	9.53 ± 1.21
Day 18	7.29 ± 1.09†	4.55 ± 1.12†	6.93 ± 1.16
Day 19	6.29 ± 1.16^{a†}	2.94 ± 1.28^{ab†}	2.42 ± 1.16^{b}
Day 20	5.67 ± 1.27	1.89 ± 1.59	1.12 ± 1.33
Day 21	4.42 ± 1.54		
Day 22	2.29 ± 0.98		

Treatments: New Zealand high (NZH), New Zealand low (NZL) and overseas (OS)

Different superscripts between columns differ significantly. † denotes approaches significance when compared to †