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**Effect of pre-mating supplementation with
monopropylene glycol on reproductive
performance of dairy cows**

A field trial

A thesis presented in partial fulfillment of the requirements for the degree of

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Dedicated to my sister, Carolina

Abstract

Tuñon, G. (2005) Efficacy of pre-mating supplementation with monopropylene glycol on reproductive performance of dairy cows. A field trial. *MSc Thesis. Massey University, Palmerston North, New Zealand.*

Low body condition score (BCS) at calving is associated with extended time to resume cycling in dairy cows. Prolonged postpartum anoestrous intervals (PPAI) are the major source of infertility in New Zealand dairy cows. Postpartum supplementation with monopropylene glycol (MPG), under controlled experimental conditions, was previously shown to reduce PPAI in heifers. This experiment tested this treatment in four large commercial dairy herds. During the 6-week period preceding the planned start of mating (PSM; Week 0), cows were drenched either once (MPGx1) or twice (MPGx2) daily with 200 ml MPG, or served as untreated controls (Con). A total of 2,122 cows were included in the analysis. Analyses were confined to the group of cows that were anoestrus at Week - 6 or calved between Week - 6 and Week -4 relative to PSM ($n = 684$ to 714 per treatment). None of the four variables: oestrous behaviour during the treatment period, anoestrous rate one week before PSM, 3-week submission rate or 3-week pregnancy rate were affected by MPG. However, MPG did increase the 6-week and final pregnancy rates ($P < 0.005$), with MPGx1 having higher values (74.6% and 92.1%) than MPGx2 (69.1% and 88.5%) and Controls (67.7% and 88.1%), respectively. MPG-treated cows also produced more milk protein than the control-cows, by 0.01 and 0.017 kg of milk protein per day for MPGx1 and MPGx2, respectively ($P = 0.02$), evidence of a metabolic effect of MPG. Recent studies suggest that diets that are optimal for follicle growth are not necessarily optimal for oocyte quality and subsequent embryo survival. It is hypothesised that MPGx1 had positive effects on the follicle/oocyte through gonadotrophin-independent mechanisms and that MPGx2 had negative effects on the embryo. Probably the gonadotrophin-dependent effects necessary for ovulation could not be exerted in the present study because MPG was not administered for sufficient time to effect the LH surge. Both change in body condition score between Week - 6 and Week - 1, and proportion of cows that were anoestrus at Week -1, were influenced by herd, age and time of calving ($P < 0.05$) but not by treatment ($P > 0.1$). Treatment did not influence the proportion of anoestrous cows, treated with progesterone, which were inseminated after oestrus detection ($n = 263$).

Change in BCS affected both milk yield and pregnancy rates, with cows that gained BCS producing less milk ($P = 0.01$) but showing higher 6-week pregnancy rates ($P < 0.05$). This study highlighted that key factors influencing the percentage of anoestrus at PSM are cow age, time of calving and management of the herd.

Key words: monopropylene glycol; anoestrus; body condition score; pregnancy; dairy cow.

Foreword

The presence of anoestrous cows at the start of the breeding season is a major problem that impairs the reproductive performance of dairy cows in seasonal-calving herds. While these cows can be induced to cycle by hormonal treatments, such treatments cannot eliminate the problems caused by anoestrus. The New Zealand dairy industry urgently needs a solution for the anoestrus problem, a solution that respects the clean, green image of the New Zealand milk in the world market, respects the welfare of the animals, and is feasible. This study and similar previous studies analysing the production and reproduction performance of dairy systems in New Zealand, suggest that there is scope for improvements in all areas to achieve better reproduction without sacrificing the survival of the cows. Logical solutions come from: i) selection of animals that suit the pastoral system, with appropriate emphasis on fertility; ii) effective nutritional management to achieve optimal body condition score (BCS) at calving and maintain high levels of milksolids production and pasture management; iii) effective health management; and iv) decrease error levels in all the areas in which there is human intervention (i.e. oestrous detection). Improving reproductive performance would have a significant impact on milk production (by better fitting the herd's demand to the supply of pasture, by achieving more days in milk, and by an increased ability to selectively cull low producing cows), the rate of genetic gain (by increased ability to selectively rear calves only from high genetic merit cows), and farm costs (by reducing the costs for breeding, induction, anoestrous treatments, and rearing replacements). Manipulation of nutrition seems to be the solution, since hormonal treatments are being highly discouraged by the dairy industry. This thesis is about an on-farm test of a non-hormonal intervention to enhance reproduction of dairy cows.

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List of abbreviations

AI	Artificial insemination
BCS	Body condition score
CIDR	Controlled internal drug release
CL	Corpus luteum
DM	Dry matter
DMI	Dry matter intake
E2	Oestrogen
ELISA	Enzyme-linked immunosorbant assay
FSH	Follicle stimulating hormone
G	Gram
GH	Growth hormone
GHR	Growth hormone receptor
GnRH	Gonadotrophin releasing hormone
H	Hour
ha	Hectare
HF	Holstein Friesian
hp	Horse power
IFN- τ	Interferon tau
IGF	Insulin-like growth factor
IGF-1	Insulin-like growth factor-one
IGBP	Insulin-like growth factor binding proteins
IU	International unit
kg	Kilogram
L	Litre
LH	Luteinising hormone
LIC	Livestock Improvement Corporation
ME	Metabolisable energy
MHz	Mega Hertz
MJ	Mega Joule
ml	Millilitre

mm	Millimetre
MPG	Monopropylene glycol
M	Metre
NEB	Negative energy balance
ng	Nanogram
OAD	Once a day
ODB	Oestradiol benzoate
OTR	Oxytocin receptor
P4	Progesterone
PGF ₂ α	Prostaglandin F ₂ α
PPAI	Postpartum anoestrous interval
PSC	Planned start of calving
PSM	Planned start of mating
RFM	Retained foetal membranes
sem	Standard error of the mean
sec	Second
T	Tonne
TMR	Total mixed ration
TG	Triglyceride
μl	Microlitre
VFA	Volatile fatty acid
wk	Week

Introduction

On the spring-calving pastoral dairy farm, the majority of the cows calve during late winter-early spring. This is planned to match maximum requirements of early lactation with the period when pasture growth is highest. The reliance on pasture limits the length of lactation, because / unless supplements are used / the herd is dried off when pasture growth decreases (i.e. in an occasional dry summer, or in every year, in late autumn-winter). This match between animal requirements and seasonal growth of pasture also means that a 365-day calving interval is essential in this type of system. The pregnancy lasts for about 282 days. Therefore, cows have about 80 days to recover from pregnancy, start cycling again, and conceive. The challenge of the seasonal pastoral dairy farms is therefore to achieve excellent reproductive management in order to get as many cows pregnant as possible, as soon as possible after calving, because the cows that fail to become pregnant during the period of mating are systematically culled from the herd.

The postpartum anoestrous interval (PPAI) is the normal physiological period of time from calving to resumption of oestrous activity (McDougall, 1994). The length of the PPAI is affected mainly by farm management, genetic background, and most importantly, by nutrition. The relationship between infertility and poor nutrition was recognised a while ago. Even Aristotle (384-322 BC) wrote that nutrition was the most important environmental factor controlling conception (Cited in Medvei, 1982). Indeed, cows calving in poor body condition score (BCS) have prolonged PPAI and increased chances of being anoestrous at the planned start of mating (PSM). Anoestrus during the

period of mating is the major factor affecting fertility in the New Zealand dairy herd (Macmillan, 1980).

Hormonal interventions, such as induction of premature parturition or use of progesterone treatment to effect oestrus, are used in the seasonal system to increase the compactness of the mating period and to overcome the spreading out of calving patterns. However, the New Zealand Dairy Industry is now discouraging these practices. Thus, enhancing management in all the areas of the system, and achieving optimum BCS at calving would overcome the problem in the long term, and reduce the need for the treatments described above. In addition, there is an urgent need to improve, in the short term, the reproductive performance of New Zealand dairy cows.

Chapter 1

Literature Review

1.1 The New Zealand dairy industry

There are more than 3.8 million dairy cows in New Zealand. During the last 30 years the number of herds has decreased from 29,000 to about 12,000 but the average herd size has increased from 68 to about 300 cows (Holmes, 2002a). During the season 2003/04, dairy companies processed 1.25 billion kg of milksolids (LIC, 2003/04). However, New Zealand has a small domestic market (4 million people), therefore, 95% of the milk produced is exported (Statistics-NZ, 2000), and New Zealand milk comprises more than 40% of the milk exported worldwide (IFCN, 2004). The government does not subsidise dairying, therefore, producing at low cost is the means by which New Zealand dairy farmers compete in the international milk commodity market (Holmes *et al.*, 2002b). The milk price determines the type of feed that can profitably be used (Holmes, 1999); in other words, low milk prices require low-cost feeding systems.

1.2 Key features of the New Zealand seasonal pastoral dairy system

1.2.1 A low input system

The majority of dairy farms in New Zealand are low input, pastoral systems (Bryant and Trigg, 1982), partly because the relatively low price received for milk forces New Zealand dairy farmers to rely on grazed pasture as the main feed source for their cows (Holmes *et al.*, 1987; Deane, 1993). Temperate climatic conditions and potentially good soil fertility enable year-round pasture growth (McCall and Clark, 1999) and the

average pasture production in New Zealand is 10 to 16 t DM per ha per year, of which about 60 to 85% is harvested by animals (Holmes, 2000). However, all-pasture systems impose a limited feed supply (Clark *et al.*, 2001), so cows must be dried off at times when pasture growth is low (i.e. dry summer and winter). The resultant short lactation lengths are a weakness of the pastoral system (Zwald *et al.*, 2001). Some farmers do use extra feed to increase the length of lactations and, hence, milksolids production per cow/ha (McCallum *et al.*, 1995; Pinares and Holmes, 1996; Deane, 1999; Penno, 2001a). Various types of supplements are used, such as turnips as a summer crop (Harris *et al.*, 1998) and maize silage (Penno *et al.*, 1999). However, high pasture utilisation remains a crucial feature of efficient seasonal pastoral systems and care is needed not to increase yields per cow at the expense of profit per hectare.

1.2.2 Balance between feed demand and feed supply

Maintaining the balance between feed demand and feed supply is a key to the seasonal pastoral system. Effective management matches the feed requirements of the herd with the feed available to maximise productivity of both stock and pasture, month by month throughout the year (Holmes and Macmillan, 1982). Movement of pasture, in conserved forms, from spring to summer or winter, whilst ensuring that peak lactation coincides with the period of fastest growth for as long as possible, should achieve the highest possible biological efficiency with the 'lowest' possible cost of production (Holmes *et al.*, 1987).

1.2.3 Stocking rate

High pasture utilisation through use of appropriate stocking rates is an important aspect of the pastoral dairy system (Holmes and Macmillan, 1982). The choice of the stocking rate can strongly influence the productivity and profitability of a grazing system (White, 1987; Penno and Kolver, 2000), as relatively high stocking rates mean that level of intake per cow is constrained (Poppi *et al.*, 1987), but wastage of herbage is reduced (L'Huillier, 1987).

A useful measure of stocking density is the comparative stocking rate, which is the ratio of total herd liveweight to total feed supply. This gives a measure of the potential feed demand of a herd. It is more accurate than simply considering the number of cows per ha, because it takes into account differences in sizes between animals and the use of supplementary feeds (Penno, 1999). Moreover, changing the number of cows farmed or changing the feed supply can change the comparative stocking rate (Penno, 2003). With the type of cow currently used in New Zealand, the economically optimum comparative stocking rate appears to lie between 80 and 90 kg liveweight/t dry matter (DM) (Macdonald *et al.*, 2001).

1.2.4 Seasonal calving

Seasonal production is another key to the pastoral dairy system (Holmes and Macmillan, 1982). More than 90% of the New Zealand dairy herds have a single period of calving that is concentrated in late winter and early spring (Holmes and Macmillan, 1982; Macmillan, 1998). Dates of calving and drying-off are chosen to match herd

requirements with patterns of pasture growth. Figure 1.1 illustrates one possible match of the curves of pasture growth and feed consumption of a herd with 2.5 cows per ha, during a year with average pasture growth, with no supplementary feeds added to the system (Holmes, 2000).

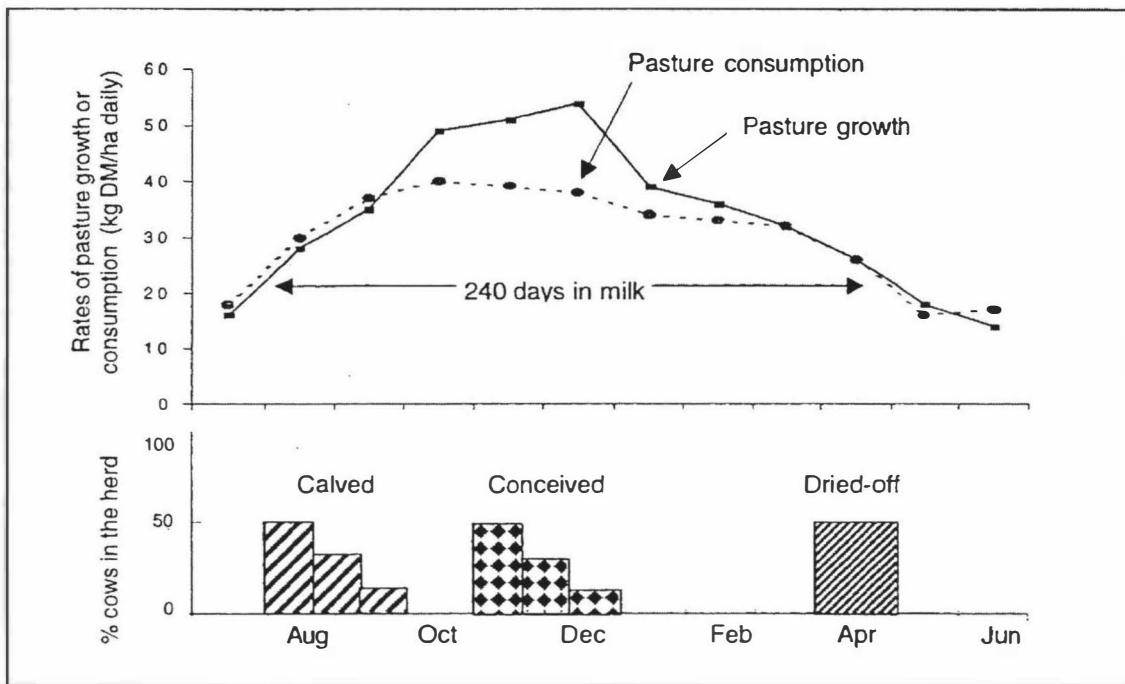


Figure 1.1: The seasonal pattern of calving and drying-off, and the synchrony between feed requirements and pasture growth, for a farm with moist summer/cool winter, with 2.5 cows per hectare and no supplements (Holmes, 2000).

1.2.4.1 Dates of calving

The date of the start of calving of a herd is essentially a feeding decision, made 282 days in advance (Holmes *et al.*, 1987). The entire herd is usually dried off over a short period of time, before the winter period of slow pasture growth. Therefore, cows which calve early in the calving season normally have longer lactations than do those which

calve later (Holmes and Macmillan, 1982). Up to one fourth of a herd may calve late, during the 6-week period before the planned start of mating (PSM) (Macmillan *et al.*, 1990). Late calving cows are typically well fed in early lactation but have shorter lactations and often conceive late in the mating season (Garcia *et al.*, 1998). These cows are also more likely to be anoestrous at the PSM (82 days after the start of calving), and therefore less likely to conceive by the end of mating (Harris, 1989).

1.2.4.2 Calving pattern

A compact calving pattern at the appropriate time is the main reproductive target for the success of the seasonal dairy farm. To achieve compact calving patterns, the seasonal pastoral dairy system requires highly efficient reproductive performance (Holmes *et al.*, 2002a). Poor reproductive performance often means that the pattern of calving is not compact. Additionally, a spread out calving pattern is likely to cause farmers to extend the mating period in order to reduce the number of empty cows. However, this will further extend future calving periods and the spread of calving (McGowan, 1981). The calving pattern of the current season results from the success of the previous mating period, and it has a strong influence on the success of the following mating period (Macmillan *et al.*, 1990). In most herds, more than half the herd calve within a period of 14 to 28 days (Holmes and Macmillan, 1982; Macmillan, 1998). The timing and the spread of calving affect: i) the rate of increase of feed requirements that occurs before the peak in pasture production, ii) the amount of pasture the herd will consume at any time during early spring, iii) the amount of pasture available for silage, iv) the supplements required and v) the average length of lactation (Bryant and Trigg, 1982).

1.2.5 Reproductive management of the seasonal pastoral dairy system

The seasonal system requires that cows are fertile in order to achieve high pregnancy rates in a short period of time (Holmes, 2001). The system, therefore requires: i) a concentrated conception pattern, allowing a single, seasonally concentrated calving period; ii) minimising the percentage of cows which either conceive late in the mating season and must therefore be culled, induced to calve or be late calvers with shortened lactations; or which fail to conceive and therefore increase the percentage of involuntary culling; iii) production of sufficient high breeding value replacement heifer calves to maintain or increase herd size; and iv) optimum rearing of replacement heifers to be mated and to conceive at 15 months of age and consequently calve at 24 months of age (Macmillan, 1998).

The need to calve every 365 days imposes a constraint on the seasonal system (Holmes *et al.*, 2002a). In New Zealand, 368 days is the average interval between successive calvings (Montgomerie, 2003). As commented in 1.2.4.1, the farmer plans the start of the mating season depending on the time when the herd is to start calving. The seasonal breeding programme starts 282 days before the planned start of calving (PSC) because this is the average gestation length of mature cows in New Zealand herds (Macmillan, 1998). Therefore, the cows have 83 days to get back in calf after calving to maintain the yearly interval between calvings (Xu and Burton, 1996).

Cows are usually inseminated over a period of three to eight weeks after the PSM. This is followed by a period of natural mating, which usually lasts at least six weeks (Vishwanath, 2002). Bull mating, or natural mating, in dairy herds has therefore

largely been relegated to mating cows that have failed to conceive to artificial insemination (AI), mating cows of low genetic merit, or mating virgin heifers (Parkinson and Vermunt, 2000; Parkinson, 2004). For most dairy herds in New Zealand, AI is used solely to generate heifer replacements. After enough cows have conceived for this requirement, natural mating is used to breed the remaining cows in the herd (Xu and Burton, 2003).

Accurate and thorough detection of oestrus is an important requirement for effective breeding management. Lack of precision in detection of oestrus results in decreased submission rates and delayed conception dates, even when cows are fertile and cycling. Detection of oestrous cows may become more difficult with increased herd size and extended mating periods. Tailpainting is a “detection aid” that allows farmers to identify animals in oestrus during the milking process (Williamson *et al.*, 1972; Macmillan, 1998).

1.2.5.1 Reproductive targets

The submission rate is an important reproductive parameter. The 3-week submission rate is calculated as the number of cows detected in oestrus and inseminated during the first three weeks of the period of AI, expressed as a percentage of the total number of cows in the herd to be mated (Macmillan, 1974; Macmillan *et al.*, 1990). A 3-week submission rate of 90% is desired for optimal reproductive performance (Macmillan, 1998; Anon, 2003b; Xu and Burton, 2003).

The non-return rate at 49 days is the percentage of cows that have been inseminated in the first 3 weeks and have not been detected on oestrus again by Day 49 after the PSM. The target non-return rate is 70% (Anon, 2003b). This measure gives the earliest indication of conception rates (Macmillan, 1998).

Conception rate is also an important determinant of the reproductive performance of the herd. It is calculated as the number of cows that conceive, expressed as a percentage of the number of matings that were used. Three main physiological processes influence the conception rate of a dairy herd: i) the quality of the oocyte released from the ovary and its ability to support normal embryonic development post-fertilisation (Ferguson, 1996); ii) the availability of capacitated sperm to fertilise the ovum before it degenerates (Vishwanath *et al.*, 1996); and iii) a successful maternal recognition of pregnancy (Thatcher *et al.*, 1989). Additionally, the efficiency of oestrus detection, the fertility of the semen, the fertility of the cow and the skills of the AI technician or bull, can all affect each of the above-mentioned three stages (Vishwanath *et al.*, 1996). The target is 65% conception rate during the first three weeks of the AI period (Xu and Burton, 1996; Verkerk, 2003). Pregnancy rate is the number of cows that become pregnant in a certain period of time. The combination of submission and conception rates will dictate the achieved pregnancy rate (Holmes *et al.*, 1987).

Final pregnancy rate, or in-calf rate, is defined as the percentage of cows in a herd that have conceived by the end of the breeding season. It represents the overall reproductive efficiency after the period of artificial and natural mating. This is related to the final empty rate (final in-calf rate + empty rate = 100%) (Xu and Burton, 2003). The target is 93% after 12 to 16 weeks of mating (Anon,

2003b). However, increasingly, farmers are now targeting short mating periods (e.g. 10 weeks), with a high total pregnancy rate (over 90%) (Xu and Burton, 1996). A compact pattern of conception early in the mating season is the result of achieving all the above-mentioned targets. Table 1.1 shows targets for high reproductive performance within the seasonal pastoral system.

Table 1.1: Reproductive targets for optimum performance that New Zealand dairy farmers use to monitor their herds (Anon, 2003b).

Reproductive parameters	Targets
Days PSC to mid point of calving	14 days
4-week calving rate	70 %
8-week calving rate	95 %
Inductions	5 %
3-week submission rate	> 90 %
Cows cycling before PSM	> 70 %
Non-cyclers treated	< 20 %
Days of AI mating period	42 days
Days of natural mating	42 days
Number of bulls used	1:30 empty cows
Cows confirmed as not in calf	7 %

As Table 1.1 shows, treating less than 20% of the herd for anoestrus and inducing parturition of less than 5% of the herds are targets for effective reproductive management. Dairy farmers have adopted induction with corticosteroid treatments as a management tool to induce parturition in seasonal systems (Day, 1977). Currently, anoestrous treatments and inductions are used in about 10% and 8% of the national herd, respectively (Verkerk, 2003).

The most commonly used and successful approach for treatment of anoestrous dairy cows during the mating period involves the use of an intravaginal controlled internal drug release (CIDR) device (to provide progesterone priming for 6 or more days), followed by an injection of 1 mg oestradiol-benzoate (ODB) 24 hr after device removal (Macmillan, 1998). Progesterone priming increased the proportion of ovulating cows expressing behavioural oestrus (83% vs. 37% for controls) (McDougall, 1994). The intravaginal device allows a single hormonal treatment, which minimises the requirement for repeated handling of animals and avoids the problems associated with the feeding of oral progestogens or injecting progesterone (Rhodes *et al.*, 2003). Progesterone treatments are usually followed by normal length oestrous cycles (McDougall, 2001b). Figure 1.2 illustrates the proposed effect of the treatment with progesterone on hormone concentrations and ovarian follicular development.

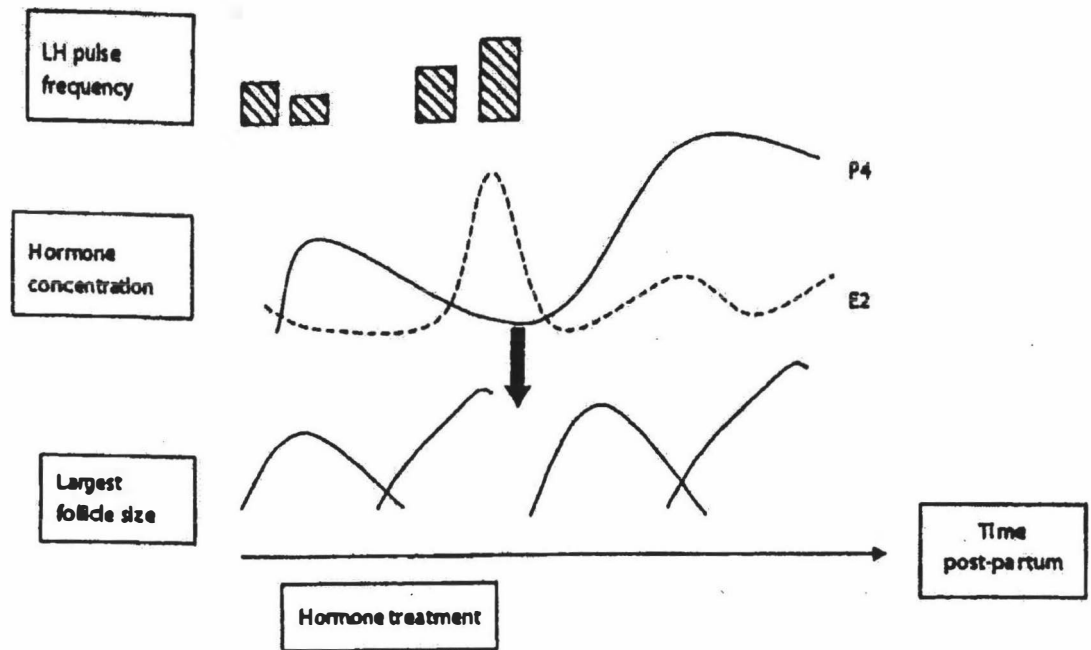


Figure 1.2: Schematic diagram demonstrating the proposed effect in anovulatory anoestrous cows of progesterone treatment, followed by oestradiol, on the development of the largest ovarian follicles and associated changes in circulating concentrations of oestradiol (E2) and progesterone (P4), and frequency of pulsatile LH release. Ovulation with oestrus is indicated by the solid arrow (Rhodes *et al.*, 2003).

However, CIDR treatments are not always followed by normal oestrous cycles. On average, 6% of the cows treated with progesterone are detected to be in oestrus but do not ovulate (false oestrus), 7% of cows ovulate without showing behavioural oestrus, and 5% of cows neither show oestrus nor ovulate (Rhodes *et al.*, 1998a). Another scenario of irregular fertility is given by cows which do not return to oestrus after AI, thus appear to have become pregnant, but they are not. Cavallieri *et al.* (2000) identified a group of cows that were retrospectively diagnosed as pregnant to their first AI and were not re-submitted for second AI within 25 days, but at the end were not pregnant. The term 'phantom cow' has been assigned to describe this population. Lean *et al.* (2003) considered phantom cows to

be relatively common in commercial herds and to be another cause of decreased fertility.

1.2.5.2 Declining fertility

Dairy cow fertility has been declining internationally, especially in the Holstein-Friesian breed (Lucy, 2001a). In New Zealand, selective pressure within the seasonal system has resulted in higher fertility than in other countries, because cows which do not conceive within the mating period are culled (Verkerk *et al.*, 2000b). Conception rate, measured as non-return rate to first service in New Zealand, ranges from 52 to 60% (Xu and Burton, 2003) and is higher than is observed in other countries (Macmillan *et al.*, 1996). In the USA conception rates have declined from 66% to 40% since 1950 (Beam and Butler, 1999). However, the fertility of the New Zealand dairy herds is also declining. In 1989, failure to conceive was the second largest cause of removal of cows from New Zealand dairy herds, at 1.4% to 3.2% of cows in the herd per annum, depending on cow age (Harris, 1989). Currently, about 43% of the cows which are culled are removed owing to reproductive failure (Verkerk *et al.*, 2000b). Submission rates to AI during the first 21 days of mating have also declined by 0.5% per year, from 93.5% to 82.1% in HF cows in the 23 years from 1973 to 1996 (Verkerk, 2003).

An antagonistic relationship appears to exist between milk production and several fertility traits (Beam and Butler, 1999). The importation of cows from the USA and Holland that have been highly selected for high yields of milk and protein have had a negative impact on herd reproductive performance (Xu and Burton, 2000). Genetic correlations between milk yield and fertility are negative. Therefore,

fertility declines with increasing genetic merit for yield (Pryce *et al.*, 2004). Since the 1960s, Holstein bulls of USA origin have been introduced into New Zealand, because of the increased ability of these lines to produce large volumes of milk (Verkerk, 2000). This has resulted in increased production gains in the National herd (see 1.1). However, this increase in milk production may have occurred at the expense of reproduction (Lucy, 2003).

In the pastoral system, however, there is inconsistent evidence for the relationship between milk production and reproduction traits (submission and conception rates). Fulkerson (1984) found a positive relationship between milk production and reproduction. Villa-Godoy *et al.* (1988) found no relationship these two variables. Recently, Buckley (2003) found that higher milk yield was associated with a lower likelihood of pregnancy to first service but cows reaching peak milk yields earlier tended to have higher pregnancy to first service. This seems to be a critical point to analyse when comparing the two types of system in terms of the effects of milk production on reproduction.

The increasing average size of the herds may be placing additional stress on the cows and on the farmers, causing a decrease in reproductive performance. In a large-scale field trial, Xu and Burton (2000) found that increased herd size had a negative effect on most parameters of reproductive performance. Logically, high levels of management skills are needed with larger herds, and the risks associated with large herds may be greater than for herds with reduced numbers of animals. This pressure may be particularly important for the youngest cows of the herd (Verkerk *et al.*, 2000a). Large herds also create difficulties for detecting oestrus accurately (Verkerk *et al.*,

2000a), and, hence, getting cows in calf (Garnsworthy and Webb, 2000; Montgomerie, 2003).

A failure to maintain adequate BCS in modern, higher-yielding cows, which lose more BCS in early lactation, may be affecting fertility in the seasonal pastoral dairy system. Moreover, hormonal treatments might have compounded the situation since they may have allowed subfertile cows to remain in the herd from year to year, instead of being culled for low fertility (Verkerk, 2003). Nonetheless, erroneous detection of oestrus, fertilisation failure, embryo mortality (Xu and Burton, 1996) and anoestrus (Macmillan, 1980) are the main four sources of conception failure that have been identified in New Zealand dairy farms.

1.3 The physiology of reproduction

1.3.1 The hypothalamic-pituitary-ovarian axis

The ovaries, the endocrine glands and the brain all interact in the hypothalamic-pituitary-ovarian axis. The hypothalamus acts as a pacemaker at the centre of the axis, producing gonadotrophin-releasing hormone (GnRH) (Thiery and Martin, 1991). This hormone stimulates the pituitary to secrete the gonadotrophins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Nett, 1987). Follicle development in the ovaries is regulated directly by the gonadotrophins (Lamming *et al.*, 1981) and also indirectly through a range of local growth factor systems (Lucy *et al.*, 1992a). Ovulation occurs when both the LH surge mechanism and the positive feedback of oestradiol are

established. A corpus luteum (CL) is created after ovulation from the remains of the follicle. The CL produces progesterone, which inhibits high-level LH secretion, and consequently, follicles growing in the background cannot ovulate (Figure 1.3). If the cow does not become pregnant, prostaglandin F_{2α} (PGF_{2α}), secreted from the endometrium lyses the CL and a new cycle starts (Ginther, 1974; Thatcher *et al.*, 1984).

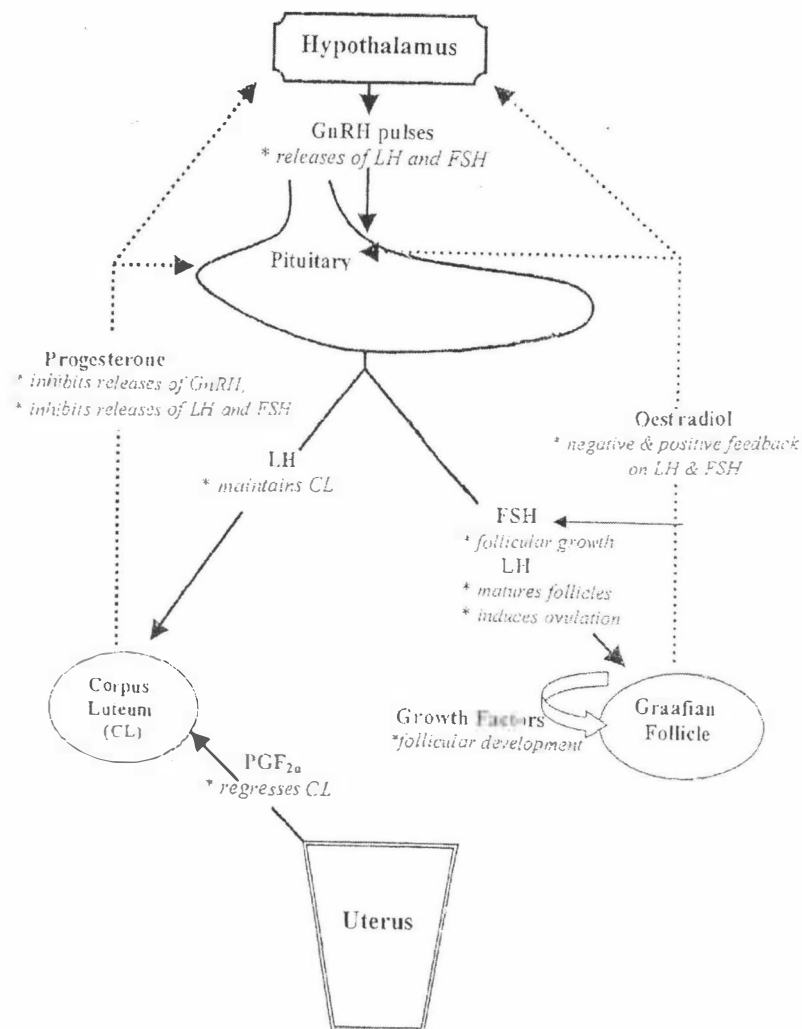


Figure 1.3: The hormonal interactions of the hypothalamic-pituitary-gonadal axis controlling reproductive activity in cows (adapted from Thiengtham, 2003).

1.3.1.1 The oestrous cycle

The oestrous cycle is a sequence of hormonal events, with each step in the sequence initiating the next hormonal change (Rajakoski, 1960). The oestrous cycle of the cow is typically around 21 days in length with 60 to 70% of animals exhibiting oestrus at intervals of between 17 and 25 days (Peters and Ball, 1987). A follicular phase, characterised by development of follicles within the ovaries, and a luteal phase, in which the CL secretes progesterone after ovulation, both occur during each oestrous cycle (Figure 1.4).

High plasma concentrations of progesterone occur during the luteal phase of the oestrous cycle. Progesterone concentrations are baseline during oestrus, but increase rapidly 3 to 5 days after ovulation, as the developing CL becomes functional. Progesterone concentrations plateau and remain elevated until the time when the CL begins to regress, after which they rapidly return to basal values (McLeod and Phillips, 1998). After a period of progesterone priming, the endometrium starts to become responsive to oxytocin, and to gain the capacity to secrete PGF₂α (Vallet *et al.*, 1989). Luteal (and, possibly, pituitary) oxytocin, released concurrently with progesterone episodes, elicits uterine secretion of PGF₂α, which, in turn, stimulates secretion of oxytocin (Flint and Sheldrick, 1985). Episodes of PGF₂α secretion generated by this mechanism cause luteal regression. As progesterone concentration declines, LH secretion is released from its negative feed back effects, so increases, causing the initiation of pre-ovulatory follicle growth. Rising oestrogen concentrations also promote greater expression of oxytocin receptors (OT-R), PGF₂α secretion and, hence, hasten the destruction of the CL (Goding, 1974).

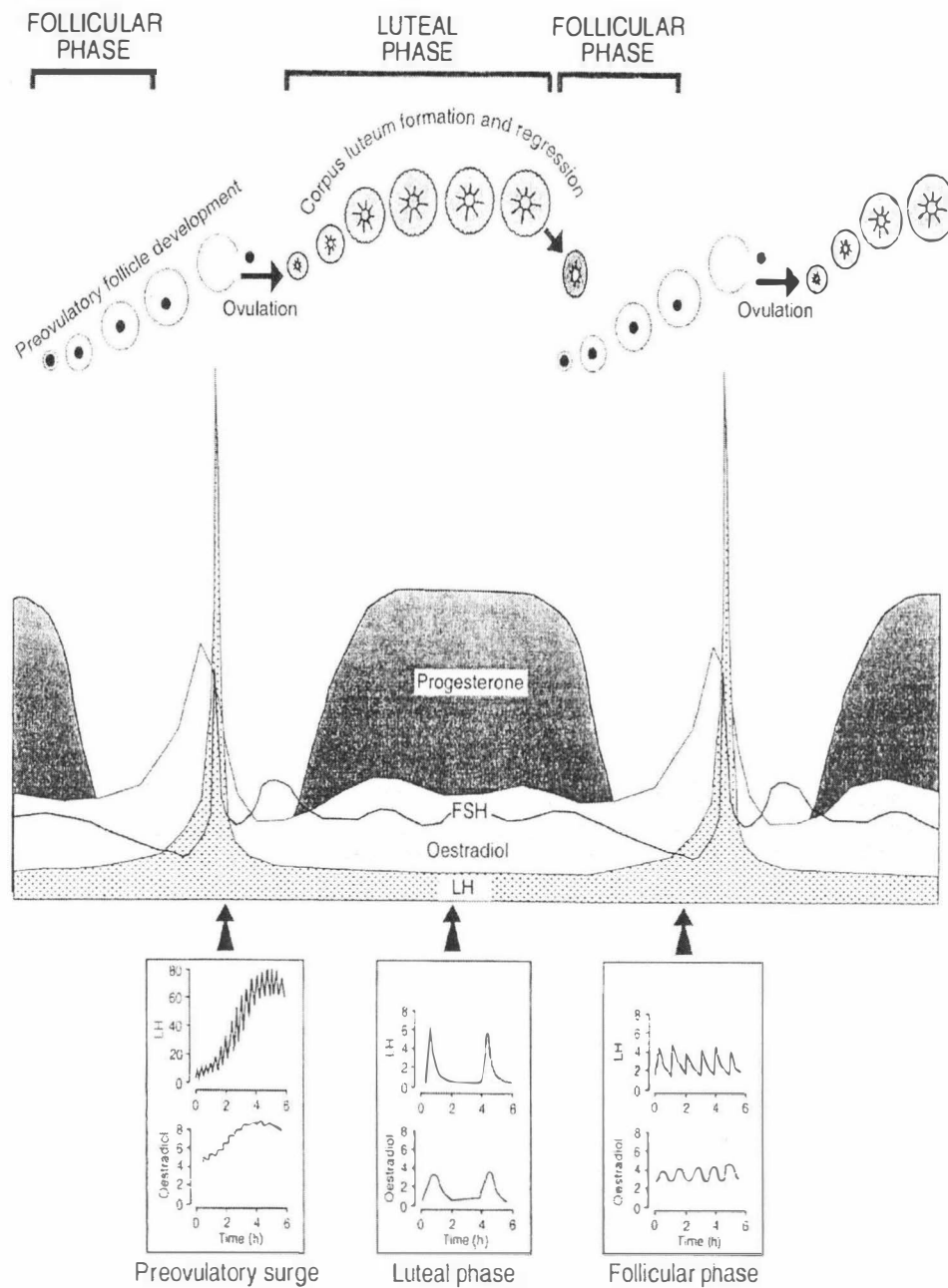


Figure 1.4: Hormonal patterns of the oestrous cycle showing the dynamics of hormone release during preovulatory follicle development (follicular phase) and when the corpus luteum is functional (luteal phase). Also shown (inserts) are the changes in secretion patterns of LH and oestradiol at specific stages of the cycle (McLeod and Phillips, 1998).

1.3.1.2 Follicular development and follicular waves

Ovarian follicular development occurs in waves, during phases of recruitment, selection, dominance and atresia (Savio *et al.*, 1990; Ginther *et al.*, 2003). Most cows have 2 (or 3) follicular waves per oestrous cycle (Lucy *et al.*, 1992b), with follicular recruitment occurring every 8 to 10 days. Follicles are initially recruited from the growing pool at about 3 to 5 mm diameter, under the control of FSH (Mihm and Austin, 2002) (see Figure 1.5). One of this group of follicles rapidly emerges as the dominant follicle (>7 to 9 mm in diameter) (Ginther *et al.*, 1996). Inhibins and activins are present in the ovary and stimulate development of the dominant follicle, but inhibit the growth of subordinate follicles. Dominant follicles have increased responsiveness to FSH, allowed by high levels of activin (Mihm and Austin, 2002; Figure 1.6).

The dominant follicle prevents the growth of other follicles, because of the rise in its oestrogen and inhibin secretion, and so continues to develop whilst the subordinate follicles become atretic and regress (Fortune, 1994). The dominant follicle grows to 14 to 20 mm diameter and maintains its size for a period of 5 to 7 days, and can ovulate only when the endocrine environment is suitable, otherwise it too regresses and a new follicular wave begins (D'Occhio and Aspden, 1999). The dominant follicle can be recognised by increasing oestrogen synthesis that occurs in the face of a decline in FSH support (Savio *et al.*, 1990).

Atresia of the dominant follicle enables an increase in circulating FSH concentrations as inhibin negative feedback is decreased. The increase in blood FSH concentrations initiates the recruitment phase of the next wave. Luteal regression, or

progesterone withdrawal is followed by rapid maturation (growth and oestradiol synthesis) of the dominant follicles via increased LH secretion. A high frequency of LH episodes is essential for the follicular development, ovulation and luteal function in the ovary (Savio *et al.*, 1990). The increase in oestradiol triggers an LH surge that causes ovulation of the dominant follicle (Fortune, 1994).

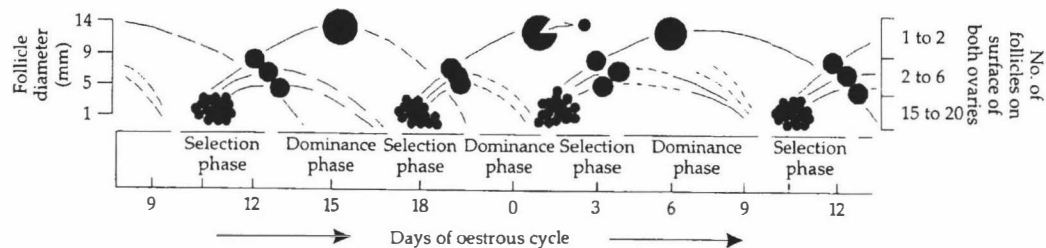


Figure 1.5: The waves of follicular development throughout the oestrous cycle in cattle. Day 0 = oestrus (adapted from Roche, 1996).

Follicular dominance is determined by many other factors apart from gonadotrophins, such as the insulin-like growth factor (IGF) system. Insulin growth factor-1 (IGF-1) increases ovarian sensitivity to FSH and LH resulting in stimulation of steroidogenic function, and increased proliferation, differentiation and survival of granulosa cells (Lucy, 2000). Figure 1.6 shows a model proposed by Mihm and Austin (2002), based upon the interrelationships between activins, IGF-1 and gonadotrophins. The presence of IGF-binding proteins 1 to 5 (IGFBP1-5), binding proteases and IGF receptors influence the bioavailability and bioactivity of the IGF-1, which in turn stimulates follicular proliferation and steroidogenesis (Monget *et al.*, 2002).

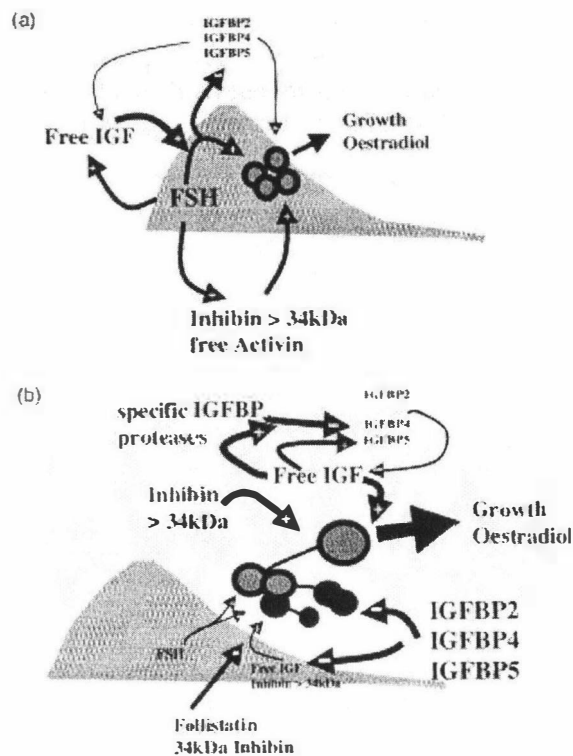


Figure 1.6: A model of the actions of proteins belonging to the inhibin and IGF systems in follicular development in cattle (a) during the emergence of the dominant follicle and (b) after selection of the dominant follicle (Mihm and Austin, 2002).

1.3.1.3 During pregnancy

As the cow conceives, the presence of a viable, developing embryo prevents regression of the CL, inhibiting the resumption of oestrous cycles. The bovine conceptus produces an antiluteolytic protein, interferon- τ (IFN- τ), whose maximum secretion occurs between Days 16 and 19 of gestation. Its secretion continues until about Day 38 of gestation (Noakes *et al.*, 2001). Bovine IFN- τ inhibits the OTR expression and stimulates the synthesis of proteins that inhibit one or several steps of the cascade for the synthesis of PGF2 α in the endometrium (Binelli *et al.*, 2001; Demmers *et al.*, 2001). Progesterone concentrations remain high throughout pregnancy, as a prerequisite for the

maintenance of pregnancy. As a consequence of such prolonged, high concentrations of progesterone, ovarian activity decreases as progesterone inhibits GnRH secretion (Wathes and Lamming, 1995), and the activity of the pituitary declines. In addition, the amount of LH in the pituitary, LH release in response to either oestrogen or GnRH, and the bioactivity of LH all decline (Jolly, 1993).

1.3.2 Physiology of the postpartum period

After parturition, cows are infertile for a variable period of time (Short *et al.*, 1990). During the postpartum period, a series of physical, endocrine and histological changes occur, which all lead to uterine repair, ovulation and resumption of oestrous cycles, and finally, to conception (Ferguson, 1996). Three processes are identifiable during the postpartum period. Firstly, the uterus involutes by Days 30 to 40 postpartum (Fonseca *et al.*, 1983). Secondly, the hypothalamus and pituitary recover from the effects of the previous pregnancy, and FSH and LH secretion release resumes. Finally, there is a resumption of follicular development, follicular waves and the development of dominant follicles, and the re-establishment of the LH surge mechanism (Lamming *et al.*, 1981).

1.3.2.1 Uterine involution

Immediately after calving, the size of the uterus begins to decrease and all the tissues associated with pregnancy are remodelled. As commented above, all these events normally take place within the first 40 days after calving (Fonseca *et al.*, 1983). Periparturient diseases, including dystocia, retained foetal membranes (RFM),

endometritis and recumbency reduce the rate of involution. This in turn delays the resumption of ovarian activity (Griffin *et al.*, 1974), increasing the intervals from calving to first oestrus, calving to conception and calving to calving (McDougall, 2001a).

A substantial release of PGF₂ α occurs initially during the period of uterine involution. In cows with retained foetal membranes (RFM), a second pulsatile release of PGF₂ α is seen concurrently with the growth and final elimination of bacteria. The duration of these prostaglandin releases are negatively correlated to uterine involution in normal cows and positively correlated in cows with RFM/endometritis respectively. As long as the release is maintained, the cow is unable to ovulate (Kindahl *et al.*, 1999). However, in a normal uterine involution, delaying first service beyond 40 days postpartum will benefit conception rate (Ferguson, 1996).

1.3.2.2 Resumption of follicular activity

The resumption of follicular activity after calving is crucial for fertility (Butler and Smith, 1989). An early resumption of ovulatory cycles after parturition means that the animal has increased chances of having multiple oestrous cycles occur prior to mating. The first wave of follicular development can be initiated within a week after calving, in response to the elevation in plasma FSH concentrations that occurs within the first three to five days postpartum (Butler and Smith, 1989). The LH pulse frequency increases until a regular episodic pattern of pulsatile LH release effects an increase in basal plasma LH concentrations (Butler and Smith, 1989). A pulsatile pattern of LH is initiated early in the postpartum period, but the preovulatory LH surge mechanism is

not restored until later on (Lamming *et al.*, 1981). Follicular development might result in: i) failure of the dominant follicle to ovulate, with the follicle then becoming cystic. This situation could prolong the interval to first ovulation to 40 to 50 days (Butler, 2000), ii) non-ovulation of the first dominant follicle followed by turnover and a new follicular wave; or iii) ovulation of the first dominant follicle after a period of 14 to 21 days (Lucy, 2001a).

1.3.2.3 *The first ovulation postpartum*

The first ovulation after calving occurs once the normal functions of the hypothalamic-pituitary-ovarian axis have recovered. Most New Zealand dairy cows resume cycling between 30 to 52 days after calving (McDougall *et al.*, 1995a; McDougall *et al.*, 1995b; Verkerk *et al.*, 2000b). The first ovulation is not normally accompanied by behavioural oestrus; the interval from calving to first oestrus is generally two to three weeks longer than the interval from calving to first ovulation (McDougall, 1994). Therefore, the PPAI is the period of time between the day of calving and the resumption of ovulatory cycles but can be defined as the interval from calving to first detected oestrus (McDougall, 1994).

Prolonged PPAI is a major cause of infertility in the seasonal pastoral dairy system (Macmillan and Lean, 1996; Xu and Burton, 1996). Cows that were anoestrous at the PSM have lower 3-week submission rates than do cows that were already cycling. Therefore, the former cows conceive later, and consequently are due to calve later during the following season, which may result in reduced lactations (Macmillan *et al.*, 1995a).

Identification of cows that are anoestrous one week before the PSM, and treatment to initiate ovarian activity in these animals with hormonal treatments have been recommended to prevent the adverse effects of prolonged PPAI (Macmillan, 1997). In the seasonal system, prolonged PPAI leads to spread patterns of conception and consequently spread patterns of calving. A high proportion of anoestrous cows at the start of mating necessitates an increased number of interventions to offset the aforementioned inefficiencies of having a spread out conception pattern. The main factors affecting the duration of the postpartum period are age or parity, season and periparturient disease, and most importantly, nutrition before or after calving (Short *et al.*, 1990; Rhodes *et al.*, 2003).

1.4 Effect of nutrition on reproduction

Nutritional effects on reproduction are mediated via a complex interplay between feed intake, body reserves and nutrient partitioning (Short *et al.*, 1990). Around calving, dry matter intake (DMI) decreases substantially (Bertics *et al.*, 1992). Subsequently, DMI does not increase rapidly enough to meet the demands of milk production in early lactation (Ulyatt and Waghorn, 1993). Thus, the metabolic status of the cow shifts from accumulation of nutrients (i.e. during pregnancy) to their rapid mobilisation in order to cope with the sudden onset of high milk production (i.e. during early lactation) (Bauman and Currie, 1980; Vandehaar *et al.*, 1999). Therefore, as body reserves are mobilised to provide energy resources to maintain lactation, cows generally lose liveweight during this period (Butler and Smith, 1989).

Nutrition may act at the ovarian level, affecting follicle growth, oocyte maturation and early embryo development. The nutritional status of the animal may send signals to the reproductive axis, which provide the link between nutrition and reproduction. In turn, these signals may act either directly or by modulating the effect of gonadotrophins on the ovary (Gong, 2002). All external and internal inputs ultimately converge into a final common pathway that controls the secretion of the neurohormone GnRH (Lucy, 2004; Figure 1.7).

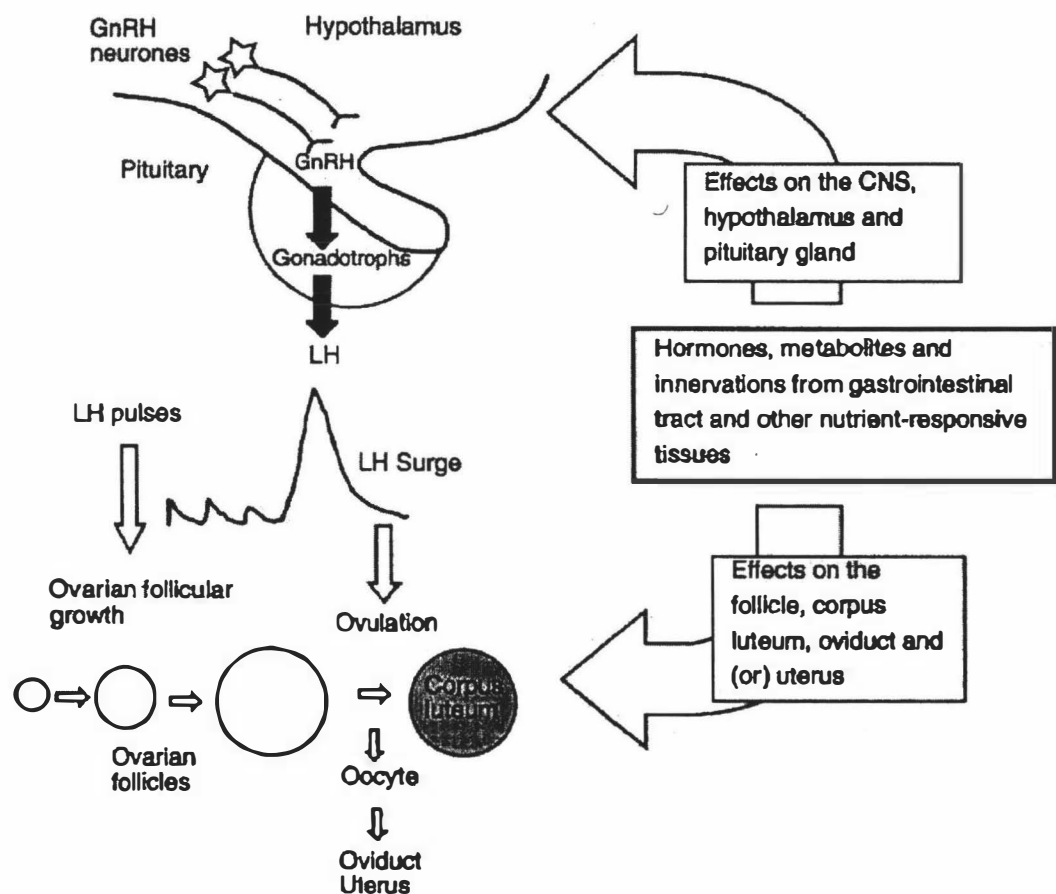


Figure 1.7: Conceptual model for the mechanisms through which nutrition affects reproduction in postpartum cows (Lucy, 2004).

1.4.1 Negative energy balance

Overall energy balance is the result of the difference between the amount of megajoules (MJ) of metabolisable energy (ME) eaten per day and the MJME that is expended on metabolic activities (Butler and Smith, 1989). Cows usually undergo a period of negative energy balance (NEB) after calving, which generally reaches nadir between two (Butler *et al.*, 1981; Butler and Smith, 1989) to three weeks (De Vries *et al.*, 1999; Jorritsma *et al.*, 2005) after calving. The extent of the NEB is associated with the genetic merit for milk production (Berry *et al.*, 2002) and the energy intake (Staples *et al.*, 1990; Macmillan and Lean, 1996), both affecting the interaction between homeostatic and homeorhetic mechanisms within the animal (Bauman and Currie, 1980). These factors determine the level of metabolic stress of the animal during early lactation. NEB has been associated with: i) high concentrations of non-esterified fatty acids (NEFA) and ketone bodies due to fat mobilisation (Studer *et al.*, 1993) and with ii) low glucose concentrations in blood due to insufficient gluconeogenesis (Grummer, 1995).

Negative energy balance leads to depressed GnRH in the hypothalamic centers which exist in the brain (Butler and Smith, 1989; Jolly *et al.*, 1995). This state of undernutrition therefore impairs LH concentration and pulse frequency secretion, and deters ovulation (Butler and Smith, 1989; Canfield and Butler, 1990; Staples *et al.*, 1990; Lucy *et al.*, 1992b). Consequently, activities that lead to energy restrictions and extended/deep NEB have been associated with prolonged PPAI. For instance, prolonged PPAI arises in situations of low peripartum nutrition (McGowan, 1981), low BCS at calving (Grainger *et al.*, 1982), reduced per cow level of intake due to high stocking

rates in the seasonal pastoral farm (McDougall, 1994), and high milk yields per cow (Macmillan *et al.*, 1996). Cows that are nutritionally compromised have low concentrations of metabolites and metabolic hormones in their blood (Lucy, 2004b).

1.4.2 Hormone interactions

Insulin, growth hormone (GH) and IGF-1 have distinct individual effects on the reproductive axis but they interact in a signalling network, influencing the ovarian follicle growth and development during the early postpartum period (Gong, 2002). Insulin is directly modulated by blood glucose concentrations and stimulates lipogenesis and uptake of glucose by all tissues (Malaisse, 1972). Low concentrations of insulin in plasma lead to increased gluconeogenesis and ketogenesis (production of acetoacetate and β -hydroxybutyrate) in the liver (Vernon, 1989). GH is a pituitary hormone that controls many aspects of animal growth and nutrient metabolism (Gluckman *et al.*, 1987), and also affects reproduction (Lucy, 2000). Finally, IGF-1, as described in 1.3.1.2, causes growth, differentiation and survival of follicular cells, acting synergistically with the gonadotrophins (Lucy, 2000). These three hormones are closely associated with nutrition-induced alterations in energy (Gong, 2002; Butler *et al.*, 2003).

Plasma insulin concentrations decline steadily from about three weeks before calving and then remain at low levels postpartum (Doepel *et al.*, 2002). Likewise, before calving, blood concentrations of GH are low (Lucy, 2000) but circulating concentrations of IGF-1 are high (Spicer *et al.*, 1990). This situation persists for several weeks after calving. As the lactation progresses, the circulating concentrations of GH

gradually decline, and the concentration of insulin and IGF-1 gradually increases (Lucy, 2000).

Insulin is pivotal for reproduction because it regulates the metabolism of glucose within the hypothalamus and modulates hypothalamic GnRH output (Butler and Smith, 1989). Insulin also increases IGF-1 synthesis. When concentrations of both IGF-1 and insulin are low, the follicle does not produce adequate levels of oestradiol or grow to a size that is able to trigger the LH surge and ovulation (Spicer and Echtenkamp, 1995; Beam and Butler, 1999). Thus, insulin and IGF-1 influence GnRH and LH secretion, through the GnRH neurones, the upstream neuronal pathways that regulate these neurones, or the pituitary gonadotroph (Butler *et al.*, 2003; Lucy, 2003). Therefore, lower insulin availability during early lactation causes a decrease in the responsiveness of ovarian follicles to gonadotrophin stimulation (Butler, 2000).

In summary, ovarian function is controlled primarily by the hypothalamo-pituitary-ovarian axis. However, GH, insulin, and IGF-1 play important roles in the control of follicular development in the dairy cow (Lucy *et al.*, 1999). Metabolic hormones can act either directly to control gonadotrophin-independent stages of follicle development, or in synergy with gonadotrophins to modulate follicular recruitment and final development and maturation of preovulatory follicles (Gong, 2002).

1.4.2.1 Effects of NEB on days to ovulation

The mechanisms through which NEB inhibits follicular development are complex (Lucy, 2001a). As commented above, extreme NEB postpartum heavily influences LH pulse frequency and, hence, the interval to first ovulation (Lamming *et al.*, 1981; Beam and Butler, 1999; Butler, 2000; Gong, 2002). Even underfed cows develop follicles after calving, but the critical factor is the reappearance of the LH surge mechanism that is required to cause ovulation. Thus, the timing of first ovulation depends on the duration and the depth of the period of NEB (Staples *et al.*, 1990). LH is considered as the most important candidate to link NEB with days to first ovulation (Canfield and Butler, 1990). As soon as cows pass the nadir of NEB and begin to recover toward a positive energy balance, LH pulsatility is increased (Lucy, 2003) and ovulation may occur (Macmillan *et al.*, 1996). The rise in concentration of IGF-1 and insulin improves the responsiveness of the dominant follicle to LH and oestrogen productions (Butler, 2000; Figure 1.8).

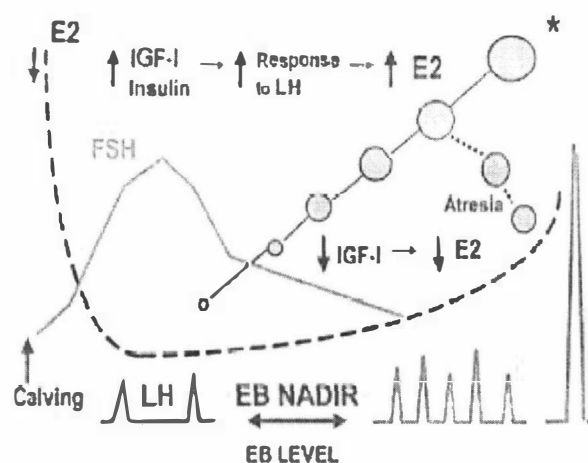


Figure 1.8: A schematic model describing dominant follicle development circles and function in relation to changing metabolic and reproductive hormones, and energy balance, during the first follicular wave postpartum in dairy cows (Beam and Butler, 1999).

The NEB status may affect the follicle with LH and IGF-1 as mediators. Cows in NEB have fewer episodes of LH and lower plasma concentrations of IGF-1 and insulin. During NEB, ovarian responsiveness to LH stimulation is reduced. During the first two weeks postpartum, the concentrations of IGF-1 were 40 to 50% higher in those cows in which the dominant follicle would subsequently ovulate, than in cows in which the follicles would not subsequently ovulate (Lucy, 2000). Cows respond to NEB by trying to increase feed intake and/or mobilising more body reserves. Therefore, neither changes in body weight nor milk yield are as sensitive as energy balance in predicting the impact on days to ovulation (Butler and Smith, 1989).

1.4.3 Body condition score and reproductive performance

Fertility and BCS are closely related (Veerkamp *et al.*, 1994; Gong, 2002). Measuring energy balance is not easy and liveweight alone is not a good indicator of body reserves (Staples *et al.*, 1990). This has led to the development of the BCS as a strategic tool. The use of BCS is a simple, quick and repeatable method to assess cows' subcutaneous fat (Garnsworthy, 1988), and hence, indirectly, energy balance. Internationally, 1 to 5 (Wildman *et al.*, 1982) or 1 to 8 (Earle, 1976) scales are commonly used. However, in New Zealand, a 1 to 10 scale (1 = thin, 10 = fat; Macdonald and Roche (2004) is used. One condition score unit represents approximately 35 kg liveweight on the 1 to 10 scale used in New Zealand (Macdonald and Macmillan, 1993).

BCS is maximum around calving in most cows, to decline during the early lactation period and then recover in late lactation or early in the dry period (Butler,

2000). Loss of BCS is associated with the NEB, which, as mentioned above, delays the initiation of ovarian activity postpartum. Cows with excessive BCS loss during early lactation may then show impaired reproductive performance. Indeed, cows losing 1 unit of BCS during early lactation (equivalent to 70 kg liveweight, on a 1 to 5 scale) may have conception rates of only 17% to 38% (Butler, 2000). Domecq *et al.* (1997) and Loeffler *et al.* (1999) reported similar results, and showed that cows losing < 1 or > 1 unit of BCS (on the 1 to 5 scale) had first service conception rates of 53% and 17%, respectively.

However, targeted BCS at calving seems to be more significant than the future change in BCS for reproductive efficiency (Morton, 2000b). In the New Zealand scale (1 to 10) the target values for BCS at calving are 5 BCS for mature cows and 5.5 BCS for heifers (Anon, 2003b) and cows that calve in poor BCS are likely to undergo prolonged PPAI (Grainger *et al.*, 1982; Burke *et al.*, 1995). In effect, cows diagnosed as anoestrous usually have lower BCS than do their cycling herd mates (McDougall and Rhodes, 2004). Heifers fed to calve at poor BCS had lower LH pulse frequencies than did those fed to calve at optimum BCS (Chagas *et al.*, 2001). Grainger *et al.* (1982) found that for each unit of BCS lower at calving (on a 1 to 8 scale), the interval to first ovulation and oestrus is delayed by 5 days. The BCS of the cows can be managed strategically to enhance reproductive performance. This should commence before or at drying-off at the latest, in order to attain targets at calving (Grainger and McGowan, 1982).

Previous paragraphs highlighted the importance of achieving optimal BCS at calving. However, BCS which is too high can be detrimental. Higher than optimum

BCS at calving results in a larger BCS decline postpartum, in turn associated with lower first service conception rates (Curtis and Lean, 1998). Higher than optimum BCS at calving also predispose to metabolic problems due to excessive mobilisation of NEFA, which leads to accumulation of triglycerides (TG) in the liver (Grummer, 1993), dystocia and RFM (Morton, 2000b), and also to milk fever and ketosis (Curtis and Lean, 1998). Nevertheless, higher than optimum BCS is not usually a problem of the seasonal pastoral system. Conversely, the constraints and the type of cows used in the pastoral system usually result in cows calving in suboptimal BCS, with prolonged PPAI and, as a result, decreased fertility.

1.4.4 Solutions for declining fertility

Improving reproductive performance enhances milk production (reduced culling of low producers and increased days in milk), increases genetic gain (calves reared from the best cows), and decreases farm costs (reduced costs for breeding, induction or parturition, anoestrus treatment, and rearing replacements) (Xu and Burton, 2000). Calving inductions might not be a viable option to use to overcome decreasing reproductive performance in the near future. The New Zealand dairy industry is working towards zero inductions and fewer hormonal treatments, while preserving a low cost, export-driven, “clean-green” image (Holmes and Burke, 2003). Indeed, the percentage of dairy herds not inducing in New Zealand have increased from 17% in 1999/00 to 46% in 2003/04, while the percentage of cows induced decreased from 9% to 8% during the same period. In addition, the percentage of herds not using CIDRs increased from 21% to 39% and the percentage of cows treated with CIDRs decreased

from 11% to 8%, in the same period as the inductions (C. Glassey, personal communication).

The use of crossbreeding, identification and marketing of short gestation length bulls, and treatment of anoestrous cows at the PSM may improve reproductive performance (Verkerk *et al.*, 2000a; Verkerk, 2003). In the long term, incorporating fertility traits into the selection index will result in improved fertility of the New Zealand dairy herd (Lopez Villalobos *et al.*, 2004). A further index of reproductive success, such as days to first mating from the PSM or calves born to AI, is also being considered, but it will take some years before changes in the national breeding objectives will have an impact at herd level (Harris and Winkelman, 2000). Meanwhile, new solutions to increase the reproductive performance of dairy cattle must be found (Verkerk, 2000). It remains crucial for the seasonal system to have the cows cycling when the mating period starts, and, clearly, hormonal interventions are being discouraged.

Therefore, a non-invasive and non-hormonal method for decreasing the chances of the cows being anoestrous at PSM is urgently needed. As explained in 1.4.2, insulin can have positive effects on the reproductive axis. Manipulation of insulin concentration may have the greatest potential for enhancing ovarian function of cows (Garnsworthy, 2004). Propionate is the main source of glucose in cows; so increased production of propionate in the rumen can increase blood glucose and, in turn, stimulate insulin release.

1.5 Monopropylene glycol (MPG) as a stimulant of ovarian function

Monopropylene glycol is a glucogenic precursor. A role of MPG in manipulating glucose concentration levels was firstly identified from studies of ketosis, which is common metabolic disorder of dairy cows, defined as a lack of glucose. In order to increase the molar percentage of ruminal propionate in ketotic dairy cattle, oral administration of MPG has been used as a gluconeogenic precursor (Emery *et al.*, 1967; Christensen *et al.*, 1997). The potential use of MPG as a tool to enhance reproduction arose after Bertics *et al.* (1992) suggested that the increase in glucose resulting from MPG administration would increase insulin. Several studies have proven that administration of MPG reduces plasma NEFA and increases glucose and insulin (Studer *et al.*, 1993; Grummer, 1994; Formigoni *et al.*, 1996; Miyoshi *et al.*, 2001). MPG may stimulate insulin secretion directly (Studer *et al.*, 1993). MPG may also stimulate pancreatic insulin secretion indirectly, through the propionate that comes from the MPG degradation in the rumen (Grovm, 1995).

MPG is highly digestible. MPG rate of disappearance from the rumen is almost 50% within 1 to 2 hours, and 80 to 90% within 3 hours (Emery *et al.*, 1964; Emery *et al.*, 1967; Clapperton and Czerkawski, 1972). MPG disappears from the rumen via absorption, fermentation or direct passage to the intestine, and less than 0.1% of the MPG administered may be detected in faeces (Emery *et al.*, 1964; Emery *et al.*, 1967).

Propionate is the major end product of MPG fermentation (Emery *et al.*, 1964; Clapperton and Czerkawski, 1972; Czerkawski and Breckenridge, 1973). After oral administration, a portion of the MPG is metabolised to propionate (Emery *et al.*, 1964),

propionate is absorbed directly from the rumen (Schultz, 1952) and is transported to the liver through the portal system. In the liver, propionate is transformed into pyruvate and thence, eventually to glucose (Van Soest, 1994).

However, it remains unclear how much of the MPG is degraded in rumen and how much escapes the rumen with no alteration. Emery *et al.* (1967), Clapperton and Czerkawski (1972) and Hidalgo *et al.* (2004) concluded that MPG was predominantly absorbed from the rumen without alteration to be converted to glucose by the liver, primarily via the lactaldehyde pathway and subsequent oxidation to lactate. However, Kristensen *et al.* (2002) suggested that, under normal rumen conditions, MPG is predominantly metabolised in the rumen, because a significantly higher proportion of propionate in rumen volatile fatty acids (VFA) after MPG administration was indicative of substantial intraruminal metabolism of MPG. Therefore, whether MPG is absorbed directly from the rumen, or fermented in the rumen to propionate, is not well established (Nielsen and Ingvarsen, 2004).

The quality of the diet might influence the rate of rumen metabolism of the MPG. For example, feeds with a high forage/concentrate ratio result in less conversion of MPG to propionate, compared with feeds with a low forage/concentrate ratio (Czerkawski and Breckenridge, 1973). Moreover, molassed sugar beet pulp induced the conversion of MPG to propionate, probably because the highly digestible fibre in this feed stimulated the growth of certain microbes with the ability to rapidly ferment MPG to propionate (Czerkawski and Breckenridge, 1973). Therefore, dietary components and the method of MPG administration have an effect on the fate of MPG in the rumen. It is possible that, in total mixed ration (TMR) diets, MPG would take a longer time to

metabolise than when MPG is administered as a drench. Under diets with restricted energy intake, such as pasture-based diets in late winter-early spring, MPG drenched to cows could therefore leave the rumen without much alteration, as postulated by Emery *et al.* (1964) and Clapperton and Czerkawski (1972).

Nonetheless, administration of MPG results in an increase in insulin within a period of 90 minutes (Miyoshi *et al.*, 2001), although the mechanism by which MPG induces this episode in insulin release remains unclear. These authors postulated that the effects of MPG were mediated primarily by inducing an insulin spike.

Previous studies have shown positive effects of MPG on reproduction. Supplementation with MPG reduced PPAI (Formigoni *et al.*, 1996; Chagas, 2003). MPG supplementation during NEB altered luteal function of the first oestrous cycle after calving (Miyoshi *et al.*, 2001) and reduced the PPAI (Formigoni *et al.*, 1996). These studies suggest that supplementation with MPG may improve reproductive performance, preventing high levels of NEFA and low levels of insulin and glucose during the postpartum period. Figure 1.9 illustrates one hypothesis of how MPG, drenched orally, enhances ovarian function.

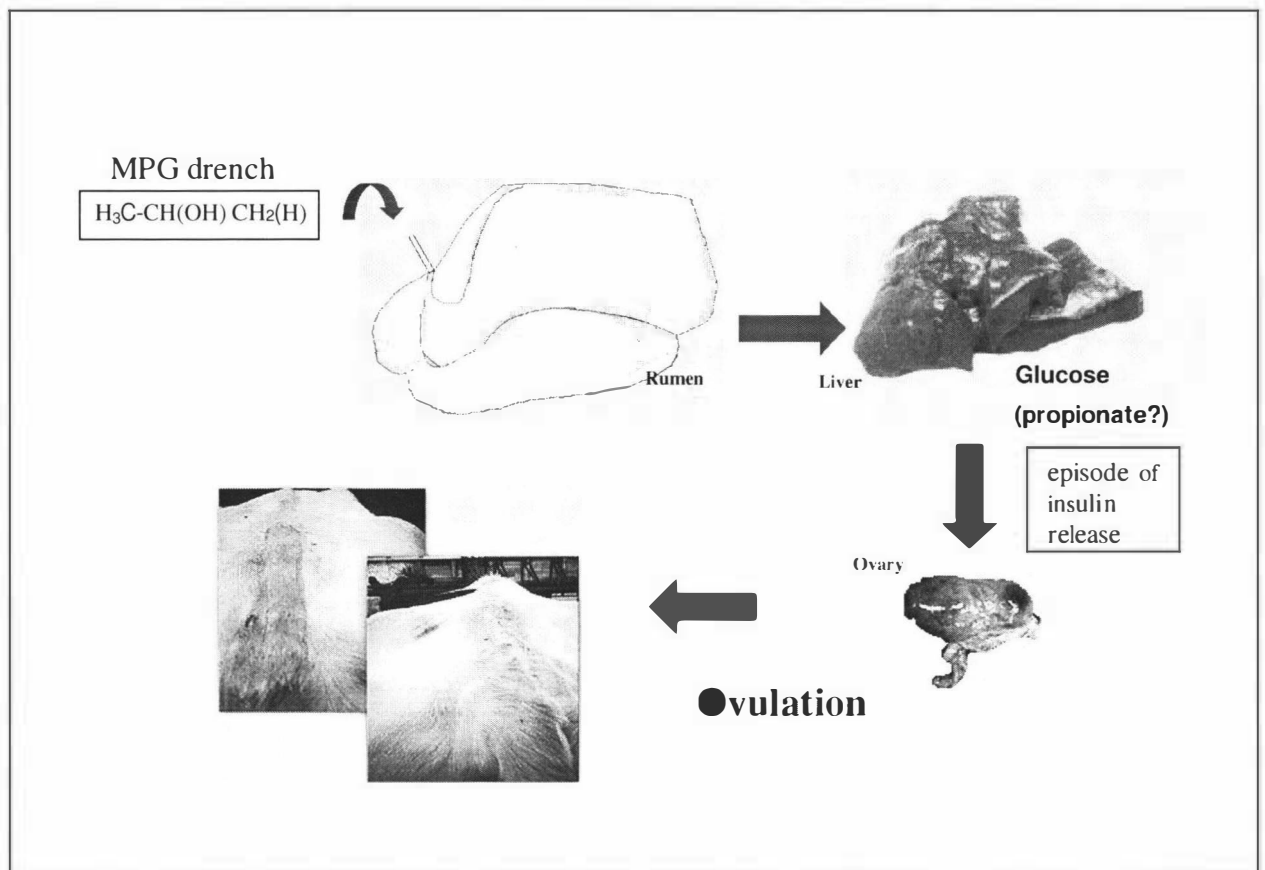


Figure 1.9: Hypothesis of MPG action.

It becomes critical to test the potential benefits of MPG supplementation of dairy herds but under farming conditions, even when management practices of the 'real world' (i.e. oestrous detection, calving pattern and nutritional management) may interact with experimental interventions (McDougall and Rhodes, 2004).

1.6 The rationale of the present experiment

The rationale for the present experiment is based on the results of a previous trial with a small number of first calved heifers, which showed that MPG supplementation for 13 weeks improved reproductive performance (Chagas, 2003). In the present experiment, MPG supplementation was given to a larger number of mixed age cows milked in four commercial herds, in order to test its effect more rigorously. The outcomes of this large-scale experiment would be used as a model to understand the PPAI of dairy cows.

It is postulated that cows that are in thin body condition at calving will resume cycling activity earlier after calving if they are given a small dose of a glucogenic substance (in this case MPG) during the 6-week period before mating, than will cows that do not receive the treatment. As a consequence, shorter PPAIs would lead to higher submission rates, and therefore higher conception rates to AI. This may in turn increase length of lactation, decrease involuntary culling rate and increase profits.

Chapter 2

Materials and Methods

2.1 The farms and the cows

The experiment was carried out in the Waikato area of New Zealand from July 2003 to March 2004. Four herds, which differed in size, age composition and breed structure ($n = 2629$ cows), were selected on the basis of the following criteria: firstly that the cows were predominantly in poor body condition at calving (see Figure 3.8); and secondly, that the herd managers were willing to cooperate with the researchers to collect the required reproductive performance data. All animal experimentation was performed prior approval n° 4,629, from the Ruakura Animal Ethics Committee. The calving periods in the farms ranged from July to September. Pasture was the main feed for all the herds. Estimated annual pasture production ranged from 9.5 to 16 t DM/ha, and supplementary feeds accounted for less than 10% of the diet. Details of the 4 farms are given in Table 2.1 (see Appendix for more details).

Table 2.1: Study farms: size, feed management, animals and calving pattern.

	Farm #1	Farm #2	Farm #3	Farm #4
Effective farm area (ha)	270	168	220	240
Pasture grown (tDM per ha)	13	16	9.5	10
Extra feed (tDM per ha)	0.3	1	0.8	0.7
Total feed available (tDM per ha)	13.3	17	10.3	10.7
Cows in trial (enrolled in July)	640	515	609	650
Stocking rate	2.6	3.1	2.6	2.7
Cow liveweight (kg)	450	500	450	470
Liveweight per ha (kg)	1,067	1,571	1,186	1,289
Planned start of calving	27 July	24 July	30 July	25 July
Date of mid-point of calving	26 Aug	10 Aug	12 Aug	12 Aug
Planned start of mating	23 Oct	17 Oct	23 Oct	20 Oct

2.2 Trial design and treatments

The experiment was designed to measure the effects of MPG, given by drenching, on the fertility of cows which were still anoestrous six weeks before the start of mating. Three treatments were applied during the 6-week period preceding the PSM: i) MPGx1, in which cows were drenched with 200 ml MPG once daily during morning milking; ii) MPGx2, in which cows were drenched with 200 ml twice daily during morning and afternoon milking (a total of 400 ml/day), or iii) Control, in which cows were untreated and received no MPG. Table 2.2 shows detail of amount, frequency and timing of the drenching for each treatment group. The period of six weeks before the PSM for each farm was termed the “treatment period” (Figure 2.1). The PSM is designated Week 0, and the treatment period was therefore designated as Week-6 to Week 0. The cows that calved after the start of treatment period received less than six weeks of treatment.

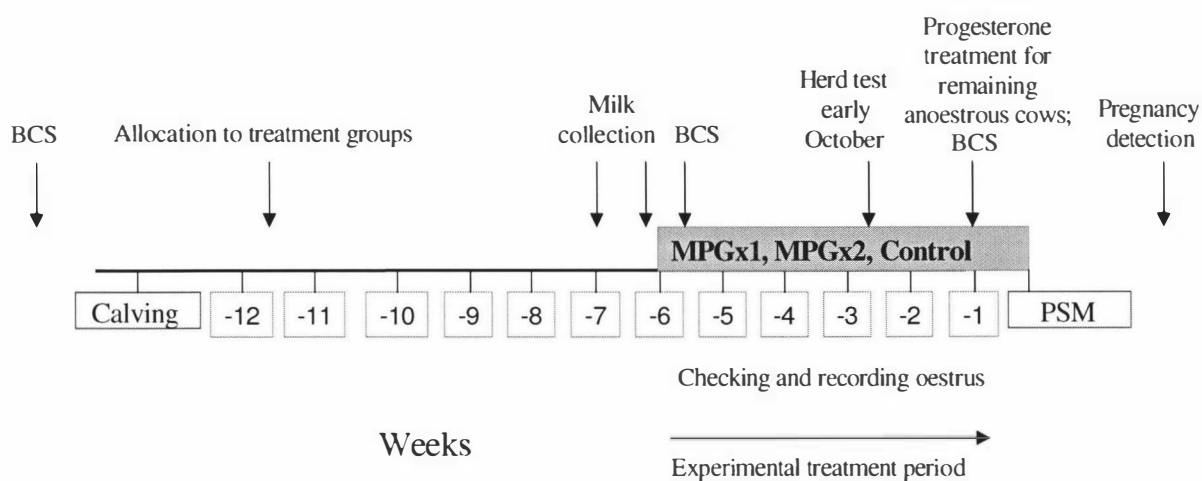


Figure 2.1: Time sequence of events; treatment period is the time when MPG was given. The BCS of the cows was assessed before calving, by Week -6 and by Week -1. Cows which were still anoestrous in Week -6 and cows which calved during the first two weeks of the treatment period were included in the analysis.

Table 2.2: Amount, frequency and duration of MPG drenching during the treatment period.

	Control	MPGx1	MPGx2
Amount of MPG (ml/cow/day)	0	200	400
Frequency of drenching (times/day)	0	1	2
Duration of drenching (weeks)	0	6	6

2.2.1 Allocation of cows to treatment groups

Cows were randomly allocated to treatment groups within each farm according to initial BCS, age and expected calving date. Cow age was derived from farm records: the proportions of cows in different age groups are shown in Table 2.3. Some cows ($n = 215$) were excluded from the trial before the treatment period because they: i) were not pregnant and had been carried over from the previous season ($n = 82$); ii) did not have sufficient records to be allocated to a treatment group ($n = 99$); iii) were culled ($n = 17$) or iv) died during the period prior to allocation of treatment groups ($n = 17$). A total of 2,414 cows were distributed among the three treatment groups (Table 2.4). Ear tags identified all cows and coloured collars identified treatment groups.

Table 2.3: Distribution of animals on age groups for each farm enrolled in the trial.

	Farm #1	Farm #2	Farm #3	Farm #4	Total	%
2 year-olds	156	62	108	112	438	18
3year-olds	74	46	99	87	306	13
Matures	410	407	402	451	1,670	69
Total	640	515	609	650	2,414	

Table 2.4: Averages for age and expected calving date, and number of cows allocated to the 3 treatment groups

	Control	MPGx1	MPGx2
Average age (years old)	5	5	5
Average expected calving date	18 Aug	18 Aug	17 Aug
Total cows in trial	800	812	802

The majority of cows ($n = 1,941$) calved before the start of the treatment period and received the full period of treatment according to their allocated treatment. However, some cows ($n = 307$) calved after the treatment period had started. These cows did not receive their preassigned treatments until they had calved. Therefore, those cows that calved during the treatment period received less than six weeks of treatment with MPG. The spread of calving dates meant that 1,941 cows calved before the treatment period started and 307 cows calved during the treatment period. The 166 cows that calved after the PSM did not receive any treatment, but were also balanced between farms, age, BCS and expected calving date. Table 2.5 and 2.6 illustrate the groups of cows that were included in the analysis, according to treatment group and to age group.

Table 2.5: Final number of cows in each treatment.

	Control	MPGx1	MPGx2	Total
Calved by Week -7 and <i>cycling</i> when the treatment period started	48	39	39	126
Calved later than Week -4	61	52	53	166
Total cows <i>not</i> included in the analysis	109	91	92	292
Calved by Week -7 and not cycling when the treatment period started	581	622	612	1815
Calved between Week -6 and Week -4	103	102	102	307
Total cows included in the analysis	684	724	714	2122

Table 2.6: Final number of cows in each age group.

	2 year-olds	3 year-olds	Matures	Total
Calved by Week -7 and <i>cycling</i> when the treatment period started	13	12	101	126
Calved later than Week -4	19	31	116	166
Total cows <i>not</i> included in the analysis	32	43	217	292
Calved by Week -7 and not cycling when the treatment period started	354	216	1245	1815
Calved between Week -6 and Week -4	41	30	236	307
Total cows included in the analysis	395	246	1481	2122

2.2.2 Identification of cows that were in anoestrus before MPG treatment

Milk progesterone samples were used to identify cows that were in anoestrus before the treatment period started, two fore milk samples were collected from a single quarter of each cow. The samples (20 ml) were taken 7 to 10 days apart, from Week –7 to Week –6 (i.e. one week prior to start of the treatment period; Figure 2.1). The milk samples were held at 4°C until assay, which took place within 3 days of collection. Potassium dichromate (0.5 ml) was added to each tube to preserve the milk until the time of analysis (E. Merck, Dormstadt, Germany). Milk progesterone concentrations were determined using an enzyme-linked immunosorbant assay (ELISA) kit (Ridgeway Science, Gloucestershire, UK).

2.2.3 Assay details

The ELISA was validated for use in cattle using whole milk samples selected, using a random numbers table, from samples collected on a routine basis. Progesterone (0.9 ng in a 50 µl methanol) was added to aliquants (15 ml) of each sample and allowed to equilibrate at 4°C overnight (Sauer *et al.*, 1986). The intra-assay coefficient of variation was 20.5%. The sensitivity of the assay in terms of limit of detection (confidence limit) is 0.1 ng/ml (99%), the smallest quantity of progesterone measured that gives rise to a response significantly different from zero (Abraham, 1969). A concentration of progesterone in milk greater than 3.5 ng/ml was taken to be indicative of luteal activity. Therefore, those cows with progesterone concentrations lower than 3.5 ng/ml in both

milk samples were identified as being anoestrous, i.e. not cycling, at the start of the treatment period.

2.3 MPG drenching

The treatment period started at Week -6 (six weeks before PSM; Figure 2.1). The person in charge of drenching would check the list of the tag numbers of the cows to see if there were any new cows (cows were still calving during the 6-week treatment period). Each new cow would receive a treatment group collar if it belonged to Group MPGx1 or MPGx2, or a dot of paint applied to the neck if it belonged to the Control group. New collars would also be given to those cows which had lost their collars accidentally. Drenching was always done during milkings with a metered drench gun (Red Harrison manual gun, Yardmaster, Matamata, New Zealand), which had been modified to have an increased capacity of 100 ml. Single-face pumps driven by 2 hp motors were used to pump the MPG into the lines. There was a 3 sec interval between each 100 ml drench administered to each cow. The drencher also checked the collars, refreshed the paints on necks and tail of the Control cows, calibrated the drench daily, and also recorded any oestrus activity.

2.4 Herd test

A single herd test to measure the yield and composition (concentrations of fat and protein) of milk from each cow was performed in each of the four farms in early October (Week - 2) by Livestock Improvement Corporation (LIC).

2.5 Body condition score

The BCS of each cow was visually assessed prepartum and at Weeks -6 and -1 (Figure 2.1). The same operator performed all these assessments on all 4 farms after the morning milkings, using a scale of 1 to 10 (1 = emaciated and 10 = obese; Macdonald and Roche (2004).

2.6 Reproductive management

2.6.1 Detection of oestrus during the treatment period

Tailpaint was applied to cows to record oestrous behaviour before mating. During the treatment period, farm staff observed the cows for signs of behavioural oestrus, in the milking shed and twice a day in the paddocks. These were based on removal of tailpaint and overt oestrous activity. The oestrus data were collected weekly and the tailpaint was re-applied when necessary. A cow was considered to be in oestrus if it stood to be mounted (Williamson *et al.*, 1972) and/or if more than 50% of the tail paint had been removed (Macmillan *et al.*, 1988).

2.6.2 Identification and treatment of anoestrous cows

Seven to 10 days before the PSM, cows with no recorded oestrus were presented for veterinary inspection. The reproductive tract of these cows was palpated per rectum. Ovarian volumes were assessed on a size scoring system (coded 1 to 5; 1 being the

smallest), and the presence of a follicle and/or of a CL was determined. Any palpable reproductive tract pathology was noted. Table 2.7 shows subjective scores for New Zealand dairy cattle (Morris and Day, 1994). A score of 3 or more meant the ovary was active (i.e. the cow was cycling). Cows with ovaries with scores of 1 and 2 (excluding those cows that were identified as unsuitable for treatment; $n = 17$) received an intravaginal device containing 1.38 g of progesterone (CIDR; Pfizer Animal Health, Auckland, New Zealand; $n = 687$)¹ for 8 days, with an intramuscular injection of 2 mg of ODB (Intervet Ltd, Auckland, New Zealand) at device insertion and 1 mg ODB 24 hours after CIDR removal, to induce the onset of oestrus in these cows.

Table 2.7: Size scoring as an indicator of ovarian volumes assessed after palpation of the reproductive tract of the cows presented for veterinarian inspection, seven to 10 days before the planned start of mating (adapted from Morris and Day, 1994).

Ovarian volume (ml)	Ovarian size score
4.0	1
7.0	2
10.0 ₁	3
12.9	4
15.9	5

¹ Ovaries with a score of 3 and 10 ml of volume are considered to be active.

¹ Treating the anoestrous cows with progesterone was a condition *sinequan on* of the farmers involved; they wanted to ensure that these cows would have every chance of cycling. This could be one of the pitfalls of field trials; however it did not affect the outcomes (See Discussion, pp 93).

2.6.3 Insemination

Technicians from LIC inseminated the cows using fresh semen. For cows treated for anoestrus, two of the farms (Farms #1 and #2) inseminated cows 48 hours after CIDR removal, while the other two (Farms #3 and #4) inseminated cows when they were detected in oestrus.

2.6.4 Pregnancy detection

Pregnancy in all cows was diagnosed by transrectal ultrasonography using a linear array probe (7.5 mHz) with an Aloka DX210 scanner (Medtel, Auckland, New Zealand) in each of the farms. The first round of diagnoses was done in January, and this detected those pregnancies that had occurred during the 6-week period of AI. A second round of ultrasonography was carried out in March to record pregnancies that had occurred during the period of natural mating. Data from both pregnancy diagnosis series were used to provide the final in-calf rate, and also identified any pregnancies that had been lost subsequently to the first pregnancy test. Conception date was confirmed on the basis of the foetal image (Pierson and Ginther, 1984).

2.7 Analyses of data

The analysis of data includes only the cows that were diagnosed as being anoestrous at Week-6, and the group of cows that calved within the first two weeks of the treatment period (Week -6 to Week -4). The analysis did not include the cows that

were already cycling when the treatment period commenced (Week -6) nor the cows that calved later than two weeks after the period commenced (Week -4 to Week 0).

Data were analysed to test the effects of treatment on the following measurements:

- Proportion of cows remaining anoestrous at Week -1
- 3-week submission rates for all the cows included in the analysis
- Submission rates to AI for cows treated for anoestrus
- 3-week pregnancy rate
- 6- week pregnancy rate
- BCS at Week -1
- Change in BCS between Week -6 to Week -1
- Milk production

The terms submission rate, conception rate and pregnancy rate were previously defined in section 1.2.5. Linear models were used to analyse the data from yields of milksolids, fat and protein, and BCS. Generalised linear models with a binomial error distribution and logit link were used to analyse the proportions of anoestrous cows, submission rates and pregnancy rates. Calving group ($n = 5$) was defined by time of calving prior to PSM and categorised as: i) > 12 weeks, ii) 10 to 12 weeks, iii) eight to 10 weeks, iv) six to eight weeks, and v) four to six weeks. Age groups were defined as 2, 3 and ≥ 4 years old. All analyses included farm, age group, calving group, treatment and the interaction of treatment with farm, age group and calving group as fixed effects.

The least square means and their standard errors presented were predicted from the linear models fitted, with marginal weights used for other factors included in the model

as fixed effects i.e. by weighting by the number of occurrences of each level of these factors in the data. When predicted proportions were calculated for the variables analysed using generalised linear models, the averaging over the other factors was done on the scale of the original response variable; so the means presented can be interpreted as natural averages of means predicted by the fitted model. In this case the standard errors of these predicted means were calculated from the iterative models, using first-order approximations that allow for the effect of the logit link function. All data were analysed using Genstat, (1997) Release 6.1 (Lawes Educational Trust, Oxford, UK).

Chapter 3

Results

3.1 Cows that were anoestrous before the treatment period, and included in the analysis

A total of 1,815 cows calved before the start of the treatment period (Week -6). Milk samples were taken from the 1,155 cows that had calved at least 10 days before the first day of milk sample (Week -7) to know the number of cows that were in anoestrus before the MPG treatment period started. The 660 cows that calved within the 10-day period before the first day of the milk sample were assumed to be anoestrous in Week -6 and were not sampled. The progesterone content in the milk samples showed that out of the 1,155 cows sampled, 1,029 were anoestrous and only 126 cows were cycling one week before the treatment period. The cows that were anoestrous before the treatment period ($n = 1,689$) plus the cows that calved during the first three weeks of the treatment period ($n = 307$) were included in the analysis. The following results will be based on this group of animals ($n = 1,996$).

3.2 Cows which resumed oestrous activity during the treatment period, and before the planned start of mating

The percentage of cows in each group that resumed oestrous activity during the treatment period, were not significantly affected by MPG at neither 5.5 weeks ($P = 0.64$), 4.5 weeks ($P = 0.94$), 3.5 weeks ($P = 0.89$) nor 2.5 weeks ($P = 0.90$) before the PSM (Figure 3.1). No differences were found between farms in the responses to MPG treatments at the same time intervals before the PSM, although the differences approached significance ($P = 0.09$) at 4.5 weeks before the PSM.

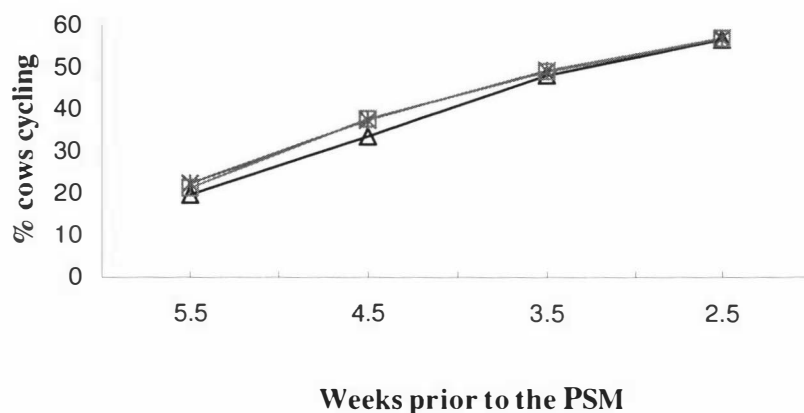


Figure 3.1: Percentages of cows in each of the three treatment groups (-△-) MPGx1, (-□-) MPGx2, (-*-) Control, which had resumed oestrous activity at different times prior to the planned start of mating (PSM).

The mature cows resumed oestrous activity earlier during the treatment period than did 2 or 3 year-old cows. At 3.5 weeks before the PSM, 52% of the mature cows were cycling, while only 37% of the 2 year-old cows and 45% of the 3 year-old cows were cycling ($P<0.001$; Figure 3.2).

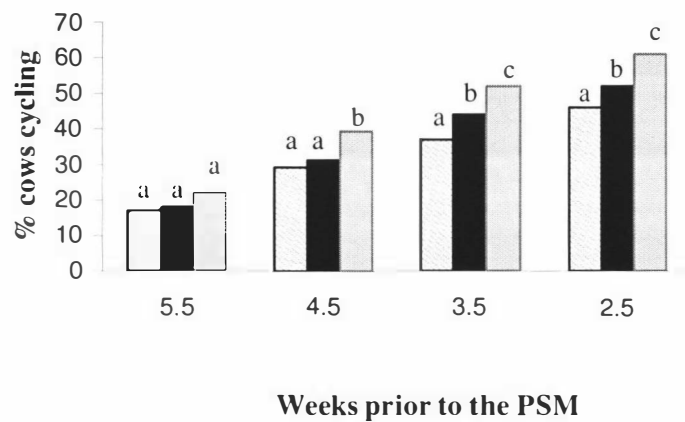





Figure 3.2: Proportions of cows in the three different age groups  2 year-old cows  3 year-old cows  mature cows, which had resumed oestrous activity at different times prior to the planned start of mating (PSM). Means within each age group with different letters ^{abc} differ significantly from each other ($P<0.05$).

The onset of oestrous activity was associated with date of calving. Of the cows that had calved by 12 weeks before the PSM (early calvers), 32% were already cycling five weeks before the PSM (Figure 3.3). By contrast, of the cows that had calved six to eight weeks before the PSM (late calvers), only 12% were cycling five weeks before the PSM ($P<0.001$).

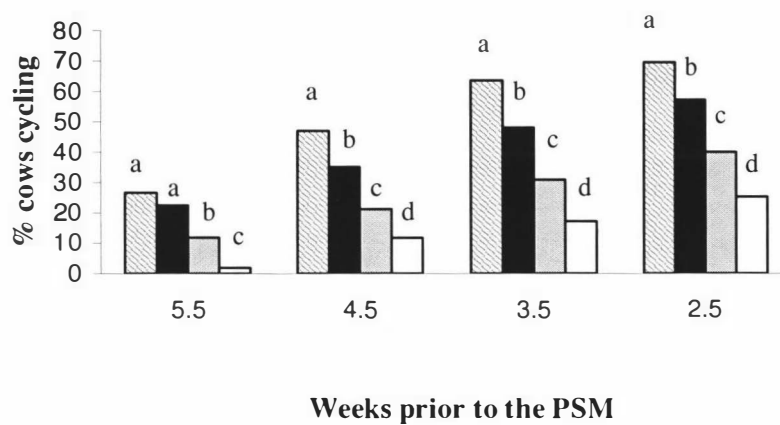






Figure 3.3: Proportion of cows in groups of cows that had calved at different times prior the planned start of mating (PSM)  10 to 12 weeks  eight to 10 weeks  six to eight weeks  four to six weeks, which had resumed oestrous activity at different times prior to the PSM. Means within each calving group with different letters ^{abc} differ significantly from each other ($P<0.05$).

3.3 Cows that remained anoestrous one week before the PSM

The percentage of cows in each group that were still anoestrous (anoestrous rate) one week before the PSM was not significantly ($P = 0.98$) affected by treatment (Table 3.1).

Table 3.1: The anoestrus rates in the three treatment groups, measured one week before the planned start of mating (PSM). Data are shown as means (\pm sem).

Treatment group	Anoestrus rate one week before the PSM (%)
Control	32 (1.6)
MPGx1 200ml	32 (1.6)
MPGx2 200 ml	31 (1.6)

Cow age ($P < 0.001$) and date of calving ($P < 0.001$) were significantly associated with the proportion of cows that were still anoestrous one week before the PSM.

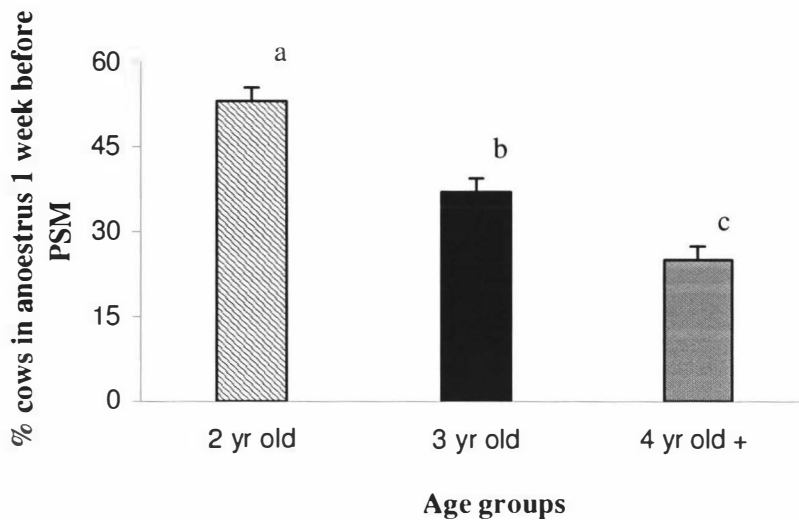


Figure 3.4: The rates of anoestrus in cows of different ages, measured one week prior to the planned start of mating (PSM). Means with with letters ^{abc} differ significantly from each other ($P<0.05$).

Younger and later calving cows had the highest ($P<0.001$) anoestrous rates at Week -1 (Figures 3.4 and 3.5). Figure 3.4 shows that 25%, 37% and 53% of cows were in anoestrus before the PSM in the mature, 3-year old or 2-year old groups, respectively. In addition, cows that calved more than 10 weeks before the PSM had a lower rate of anoestrus ($P<0.001$) than those cows that calved closer to the PSM. Of the cows that calved only four to six weeks before the PSM, 61% were still in anoestrus by the time of mating. By contrast, only 18% were still anoestrous at that time in the group of cows that calved more than 10 weeks before PSM (Figure 3.5).

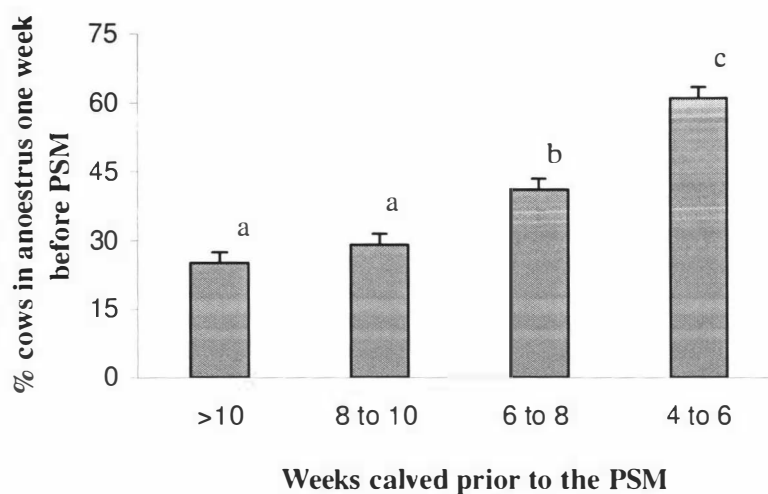


Figure 3.5: The rates of anoestrus in groups of cows that had calved at different times prior to the planned start of mating (PSM). Means with different letters ^{abc} differ significantly from each other ($P < 0.05$).

The percentages of cows that were anoestrous at Week -1 were significantly different between herds. The values were 15%, 43%, 34% and 36%, for Farm #1, Farm #2, Farm #3 and Farm #4, respectively ($P < 0.01$).

3.4 Submission rates in the three treatment groups during the first three weeks of mating

Treatment with MPG did not have a significant effect on the 3-week submission rate of the 1,996 cows included in the analysis ($P = 0.65$). However, as in the case of other reproductive variables, age and date of calving were significantly related to 3-week submission rate (Table 3.2).

Table 3.2: The 3-week submission rates of the cows included in the analysis, showing the effects of treatment, age and date of calving on this variable.

3-week submission rate (%)	
	mean (\pm sem)
Treatment group	
Control	88 (1.2)
MPGx1	87 (1.2)
MPGx2	86 (1.3)
Significance	$P = 0.65$
Age	
2-year old cows	80 (1.9) ^a
3-year old cows	91 (1.7) ^b
Mature cows	88 (0.8) ^b
Significance	$P < 0.001$
Date of calving (weeks before the planned start of mating)	
> 12	90 (1.5) ^a
12 to 10	89 (1.1) ^a
10 to 8	86 (1.6) ^a
8 to 6	85 (1.9) ^{a,b}
6 to 4	79 (2.1) ^b
Significance	$P < 0.001$

Means with different superscripts^{abc} differ significantly from each other ($P < 0.05$).

3.5 Pregnancy rates in the three treatment groups

The pregnancy rates after the first three weeks of mating were not significantly affected by treatment. However, by Week 6 (after calving), the MPGx1 group had a higher pregnancy rate than the other two groups ($P = 0.007$). The final pregnancy rate was also greater for the MPGx1 group ($P = 0.02$; Table 3.3) than for other groups.

Table 3.3: Percentage of cows confirmed as pregnant; to artificial insemination after three or six weeks of mating, and after the end of the mating season (means \pm sem).

	3-week pregnancy rate (%)	6-week pregnancy rate (%)	Final pregnancy rate (%)
Control	49.1 (1.9)	67.7 (1.7) ^a	88.1 (1.6) ^a
MPGx1	51.2 (1.9)	74.6 (1.6) ^b	92.1 (1.6) ^b
MPGx2	52.6 (1.9)	69.1 (1.7) ^a	88.5 (1.6) ^a
Significance	$P = 0.42$	$P = 0.005$	$P = 0.02$

Means with different superscripts^{abc} differ significantly from each other ($P < 0.05$).

There was an interaction between treatment and age. This meant that age groups had different responses to treatment (Table 3.4).

Table 3.4: Interaction of treatment and age on the percentage of cows confirmed as pregnant after the end of the mating season by ultrasonography ($P < 0.05$).

Final pregnancy rate			
	Control	MPGx1	MPGx2
2 year-old cows	80.9 ^a	84.9 ^b	82.1 ^a
3 year-old cows	96.5 ^a	88.6 ^b	94.4 ^a
Mature cows	88.8 ^a	94.4 ^b	88.9 ^a

Means within each age group with different superscripts ^{abc} differ significantly from each other ($P < 0.05$).

Pregnancy rate was also significantly associated with age ($P < 0.001$) and date of calving ($P < 0.001$). The percentage of cows pregnant by Week 3 and Week 6 of the period of mating was greater in older than in younger cows, as shown in Figure 3.6. Final pregnancy rate was also lower for 2 year-old cows (83%) than for mature cows (92%; $P < 0.001$).

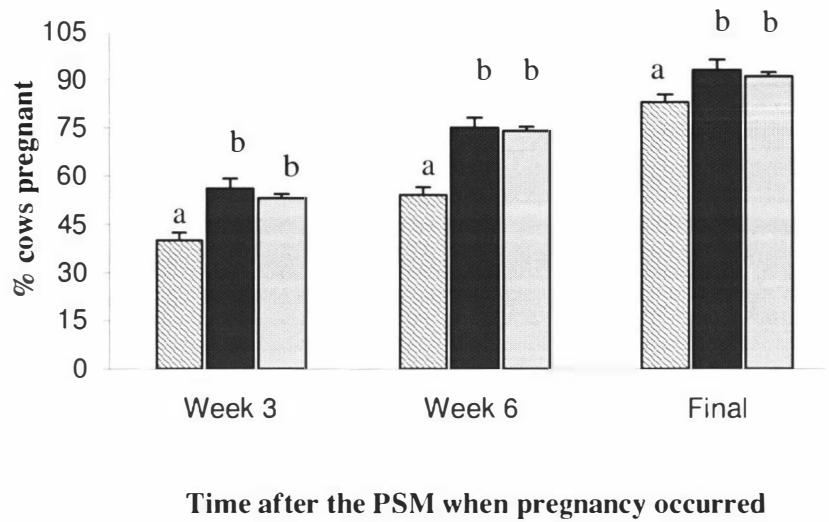



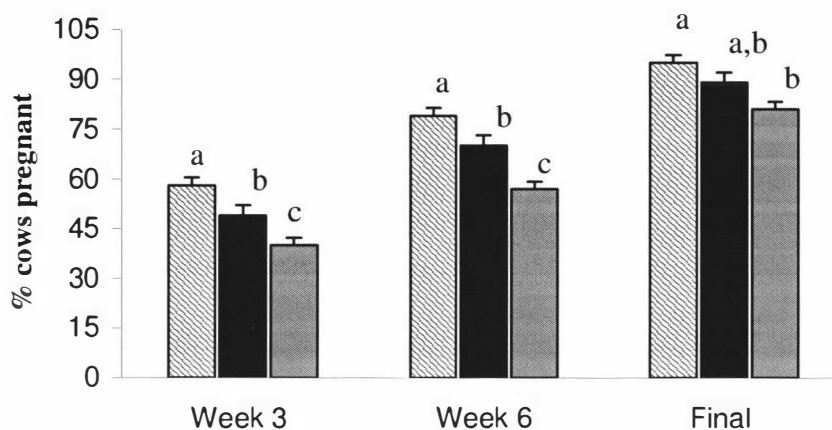





Figure 3.6: Percentage of cows in the three age groups  2 year-old cows  3 year-old cows  mature cows, which were confirmed as pregnant (by ultrasonography) to artificial insemination by three and six weeks, and after the end of the mating season. Means within each time of pregnancy detection with different letters^{abc} differ significantly from each other ($P < 0.05$).

The percentage of cows pregnant by Week 3 and Week 6 of the period of mating was also greater ($P < 0.001$) in early calvers than in late calvers (Figure 3.7).



Time after the PSM when pregnancy occurred

Figure 3.7: Percentage of cows in the three calving groups  calved > 12 weeks from the PSM  calved between eight to 10 weeks from the PSM  calved between four to six weeks from the planned start of mating (PSM), which were confirmed as pregnant to artificial insemination by three and six weeks, and after the end of the mating season by ultrasonography. Means within each period of pregnancy detection with different letters ^{abc} differ significantly from each other ($P < 0.05$).

3.6 Submission rate and pregnancy rate of non-cycling cows treated with CIDR in Week -1

A total of 694 cows were treated for anoestrus with a CIDR programme, seven days before the PSM. Following treatment, cows on two of the farms were inseminated to detected oestrus ($n = 268$) and cows on the other two farms were inseminated at a set time after CIDR removal ($n = 426$). For the first two farms, more than 90% of the cows treated for anoestrus ($n = 263$) were submitted to AI in the six days after CIDR removal, with 34% of these conceiving in this period of time. Treatment with MPG did not have a significant effect on the submission rate during the six days after CIDR removal on the two farms which mated to observed oestrous behaviour. The values were 91.7%, 90.6% and 84.6% for Control, MPGx1 and MPGx2 respectively ($P = 0.45$).

However, the values were higher for 3 year-old and mature cows than for the 2 year-old cows. Fewer 2 year-old cows (81%) than mature cows (94%) were inseminated in the six days after CIDR removal in the two herds that inseminated to detected oestrus ($P < 0.05$). No effect of herd was found for submission rate during the six days that followed CIDR removal. In the two herds that inseminated 48 hs after CIDR removal, all cows were mated in the three weeks of CIDR treatment.

In the cows given progesterone treatment (CIDRs), the two farms that used set-time insemination following device removal had slightly higher ($P = 0.092$) 3-week pregnancy rates (38% and 39%) than the two farms that inseminated on detection of oestrus (28% and 31%).

3.7 Body condition score

The pre-calving BCS averaged 4.4 units, with 62% of cows having a value of 4.5 or less (Figure 3.8).

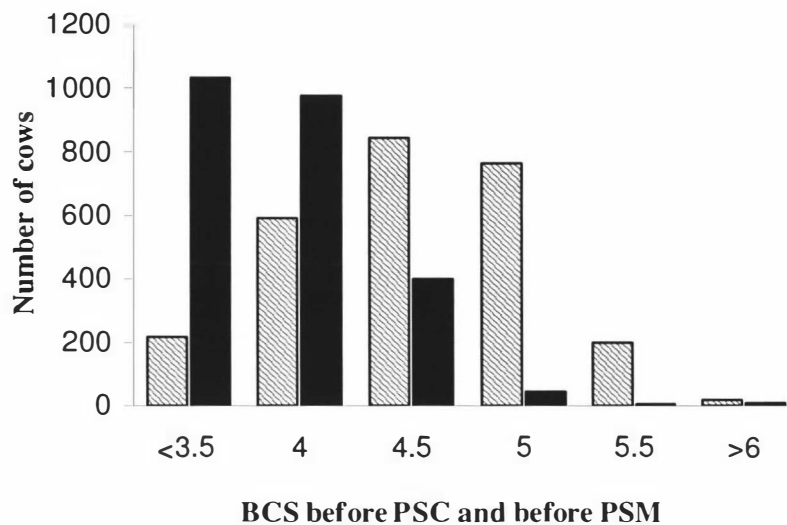


Figure 3.8: The pre-calving BCS (hatched) and the pre-mating BCS (solid black) averaged across the four herds used in the experiment.

Body condition scores for the three treatment groups in Week -6 and Week -1 are shown in Table 3.5. The average BCS of the cows increased by 0.3 units over the first five weeks of the treatment period, to be 3.9 units by one week before the PSM. The BCS recorded at Week -1, and the change in the BCS during the treatment period, were not significantly affected by MPG treatment or by age of the cows. However, the mean BCS at Week -1 and the change in BCS during the treatment period were significantly ($P<0.01$) different between farms.

Table 3.5: Body condition score (BCS) of the cows before the treatment period (Week -6), before the planned start of mating (Week -1) and the change in BCS during the treatment period in the treatment groups, in the three age groups, and in the four herds.

	BCS at Week -6	BCS at Week -1	BCS change
Treatment group			
Control	3.54	3.86	+ 0.32
MPG x1	3.53	3.85	+ 0.32
MPG x2	3.55	3.85	+ 0.30
Significance		$P = 0.70$	$P = 0.49$
Age group			
2 year-old cows	3.46	3.77 ^a	+ 0.31 ^a
3 year-old cows	3.37	3.75 ^a	+ 0.38 ^b
Mature cows	3.60	3.90 ^b	+ 0.30 ^a
Significance		$P < 0.001$	$P = 0.01$
Farm #			
1	3.71	3.97 ^a	+ 0.26 ^a
2	3.69	3.92 ^a	+ 0.33 ^b
3	3.52	3.79 ^b	+ 0.27 ^a
4	3.37	3.75 ^b	+ 0.38 ^b
Significance		$P < 0.001$	$P < 0.001$

Means with different superscripts^{abc} differ significantly from each other ($P < 0.05$).

3.8 Yields of milksolids, protein and fat

Milk production of each cow was measured after four weeks of treatment (two weeks before the PSM). Cows receiving MPG produced slightly ($P = 0.02$) more milk protein than control animals, but overall effects of MPG on yields of milksolids were not statistically significant. Milk protein yield increased with increased MPG supplementation, whereas milk fat yield tended ($P = 0.17$) to decline, so that there was no effect of treatment on total yields of milksolids as measured at the single herd test performed in early October (Table 3.6). The milksolids yields differed significantly ($P < 0.001$) between the four herds, but no interactions between herd and treatment were detected.

Table 3.6: Yields of protein and fat and total milksolids (kg per cow daily) measured at a single herd test in early October, two weeks prior to the planned start of mating, and after four weeks of the treatment.

	Protein yield (kg)	Fat yield (kg)	Milksolids yield (kg)
Treatment			
Control	0.715 ^a	0.915	1.629
MPGx1	0.725 ^{a,b}	0.907	1.633
MPGx2	0.733 ^b	0.894	1.623
Significance	$P = 0.02$	$P = 0.17$	$P = 0.94$
Age			
2 year-old cows	0.564 ^a	0.714 ^a	1.278 ^a
3 year-old cows	0.685 ^b	0.849 ^b	1.536 ^b
Mature cows	0.774 ^b	0.966 ^c	1.740 ^b
Significance	$P < 0.001$	$P < 0.001$	$P < 0.001$
Farm #			
1	0.716 ^a	0.936 ^{a,b}	1.652 ^a
2	0.914 ^b	1.068 ^a	1.982 ^a
3	0.619 ^a	0.787 ^b	1.404 ^{b,c}
4	0.665 ^a	0.839 ^b	1.503 ^c
Significance	$P < 0.001$	$P < 0.001$	$P < 0.001$

Means with different superscripts^{abc} differ significantly from each other ($P < 0.05$).

3.9 Relationship between body condition change and reproductive performance

The change in BCS during the treatment period was related to the anoestrous and pregnancy rates, and milksolids yield. Cows that lost 0.5 to 1.5 points of BCS during the 6-week period of treatment were grouped as 'loss' ($n = 137$), cows with no change in BCS were grouped as 'no change' ($n = 630$), and cows that gained BCS were grouped as 'gain' ($n = 1,068$). In Farm #2, the anoestrous rate was significantly different between the three groups ($P = 0.032$), where 68%, 48% or 30% of the cows were in anoestrus, in the "loss", "no change" or "gain" groups, respectively.

Pregnancy rate in the first three weeks of mating did not differ significantly between the three BCS groups. However, there was a significant difference between the BCS groups in pregnancy rate by Week 6 ($P < 0.05$). For cows that lost, maintained or gained BCS throughout the treatment period, 64%, 70% or 77% were pregnant by Week 6, in that order. Cows that maintained or gained BCS produced less milk than cows that lost BCS ($P = 0.01$). This difference was highly significant for Farm #4 ($P < 0.001$), where cows that lost, maintained or gained BCS from Week-6 to Week-1 produced 1.52 kg, 1.50 kg or 1.48 kg of milksolids, respectively.

Chapter 4

Discussion

Seasonal dairying systems are entirely dependent on a compact calving period that starts on the required date each year. This in turn depends on all cows conceiving in a compact mating period, 282 days earlier. Cows that are in anoestrus at the start of the mating period represent a problem to these seasonal systems; a problem that is exacerbated by the patterns of liveweight change that cows experience during the early lactation period. Modern high genetic merit cows spend much of their lactation in thin body condition and, in the seasonal pastoral system, it is often difficult to provide sufficient feed during the dry period to enable the cows to attain adequate BCS before the next calving. Consequently, many cows are still below the target BCS at calving. Thinner BCS at calving causes prolonged PPAI and therefore increased probability of being anoestrous at the PSM. This in turn increases the probability of a lower 3-week submission rate, and lower 3- and 6-week pregnancy rates, with a consequent reduction in the compactness of the subsequent calving. Hormonal treatments (e.g. CIDRs and inductions) can give a temporary solution to the deleterious effects of having spread-out calving patterns. However, the New Zealand Dairy Industry is aiming for zero inductions and lower use of CIDRs (Verkerk, 2003); therefore, prevention of the problem by short-term dietary manipulation is one of the remaining options for treatment of cows that are thin at calving.

The rationale of the present study was based on outcomes of a previous study (Chagas, 2003) that demonstrated that MPG given postcalving had a positive effect on the restoration of LH pulse frequency in heifers that had calved in suboptimal BCS. The present trial was designed to test the hypothesis that supplementing the diet of dairy cows with orally administered MPG during the 6-week period preceding the PSM would improve their reproductive performance, especially in animals that were still

anoestrous six weeks before PSM. The hypothesis was tested in 2,122 cows, in four commercial herds.

A large-scale survey by the LIC collected data from more than 100,000 New Zealand dairy cows during three consecutive seasons to benchmark reproductive performance of dairy herds (Xu and Burton, 2003). These data form a point of reference against which to compare the cows in the present study.

Table 4.1: Comparisons between the means of reproductive parameters of more than 100,000 New Zealand dairy cows during 3 consecutive seasons (Xu and Burton, 2003) and all cows in the present study.

	Xu and Burton (2003)	The present study Cows in all treatment groups; Effect of MPG	
Anoestrous at PSM %	20	32	NS
CIDRs used %	17	30	
3-week submission rate %	81	87	NS
3-week pregnancy rate %	53	51	NS
6-week pregnancy rate %	68	71	*
Final in-calf rate %	92	89	**

P<0.05; ** P<0.01 (The levels of significance or the lack of difference correspond to the comparison between MPG groups in the present study).

The 20% anoestrous rate of the LIC survey is similar to other findings (McDougall *et al.*, 1993; Xu and Burton, 1997), which show that between 15% and

27% of cows in a typical New Zealand herd are acyclic at the start of the breeding season. The 32% anoestrous rate at PSM of the MPG groups was well above the overall average for New Zealand dairy herds, but was to be expected given that they were selected to be in low BCS prior to the start of the experiment. Consequently, 30% of cows in the present study were treated for anoestrus ($n = 633$; See Table 2.6), which is almost twice the average for New Zealand dairy herds. Perhaps it was the high use of CIDRs in the present study that enabled the herds to achieve high 3-week submission rates, despite their high anoestrous rates.

4.1 The effects of MPG on reproductive performance

The analysis included the 1,815 cows that were anoestrous at the start of the treatment period, plus the 307 cows that calved within the first two weeks of the treatment period. The latter group received at least four weeks of MPG (see Table 2.5). None of the four key outcome variables (percentage of cows showing oestrous behaviour during the treatment period, anoestrous rate one week before PSM, 3-week submission rate and 3-week pregnancy rate) was affected by MPG. However, MPG did increase the 6-week and final pregnancy rates (Table 3.3), with MPGx1 (200 ml/day) having higher values than MPGx2 (400 ml/day) and Controls, for both parameters. These can be compared with results from previous studies with cows fed TMR together with the results of the two recent studies with cows on grazed pasture in New Zealand, namely that of Chagas (2003) and the present study (Table 4.2).

Table 4.2: Studies that involved MPG administration and measured reproductive parameters. Parturition is considered to be Day 0.

Study	Year	Timing (days: calving=Day 0)	Experimental design	Main outcomes	Diet	Cows (n)
Formigoni <i>et al.</i>	1996	Days -10 to 12	300 ml on Days -10, 3, 6, 9 and 12	Decreased PPAI	TMR	39
Lucci <i>et al.</i>	1998	Days -10 to 16	300 ml once per day	No effect	TMR	23
Miyoshi <i>et al.</i>	2001	Days 7 to 42	500 ml once per day	Decreased PPAI	TMR	36
Melendez <i>et al.</i>	2003	Day 0	400 g at once	No effect	TMR	479
Hydalgo <i>et al.</i>	2004	For 20 days before mating	250 ml once per day	Improved pregnancy rate	TMR	275
Hoedemaker <i>et al.</i>	2004	Day -13 to Day 12	150 ml Day -13; 300 ml -12 to 0 100 ml 1 to 12	No effect	TMR	234
Chagas	2003	Days 0 to 90	250 ml twice per day	Decreased PPAI	Pasture	47
Tuñon <i>et al.</i> (Present study)	2004	28 to 42 days before mating	200 ml once per day and 200 ml twice per day	Improved conception rate	Pasture	2,122

Formigoni (1996) and Miyoshi (2001) found that first ovulation occurred earlier in the treated cows than in the control cows (i.e. MPG decreased the PPAI); but conception rates were not analysed for the small number of animals. Hydalgo *et al.* (2004), using MPG for embryo transfer, found that the group of cows treated had higher 60-day pregnancy rates than the control group. However, neither Lucci *et al.* (1998), nor Melendez *et al.* (2003) nor Hoedemaker *et al.* (2004) found any effect of MPG on reproductive performance. The latter attributed the lack of effects of MPG on reproductive variables to the fact that the herds enrolled in their study were not considered 'problem herds', but had relatively high levels of milk production, which

could only have been achieved with excellent animal and feeding management. In farms with a good nutritional program, MPG might be expected to have little or no effect, in comparison to factors such as genetics, parity, season, and disease status. By contrast, the herds used in the present study could be considered to be ‘problem’ herds due to their low BCS pre-calving and the high rate of anoestrus before mating.

These results need to be considered in terms of the rationale of this experiment and the postulated mode of action of MPG. It was postulated that administration of MPG would elicit a release of insulin in response. Insulin, in turn, would act at the pituitary level, to enhance LH pulsatility (gonadotrophin-dependent mechanisms) and also within the ovary, to enhance steroidogenesis (gonadotrophin-independent mechanisms). There are a number of pieces of evidence that support such a direct role of insulin upon reproduction. For example, insulin stimulates differentiation and steroidogenesis of bovine follicular cells *in vitro* (Webb *et al.*, 1999). Guitierrez *et al.* (1997) reported that granulosa cells in the follicle were critically dependent on the presence of physiological concentrations of insulin. In humans the increased circulating insulin in women with diabetes augments the action of LH upon theca cells (Wei and Pritts, 2003). In other words, it was postulated that, in animals that were in low BCS, a spontaneous resumption of ovarian activity and oestrous behaviour would occur in response to MPG administration, not through an effect upon energy balance, but through the manipulation of insulin concentrations induced by giving a gluconeogenic substrate.

It must be pointed out that most of the experiments described in Table 4.2 were carried in the northern hemisphere, in intensive systems (e.g. UK, USA), in which feed

intake is not restricted. By contrast, the present study and that of Chagas (2003, on which the present study bases its rationale) were carried out under pastoral conditions. The nutritional and the reproductive management of these two systems differ greatly (Macmillan *et al.*, 1996; Kolver and Muller, 1998) inasmuch as pasture-fed dairy cows can rarely consume enough energy to meet the demands of lactation. Furthermore, dairy cows from intensive systems have been highly selected for milk yield, (creating so-called 'high-yielding cows'). However, dairy cows from pastoral systems have not been subjected to such intense selection pressure for yield, resulting in cows that have relatively low yields. It may be that the differences in the responses observed between some of the different MPG trials can be explained in terms of the differences in the metabolism of these two strains of cow.

As discussed in Section 1.4.2, a series of physiological events involving GH, IGF-1 and insulin coordinate the metabolic events during early lactation (Lucy, 2004a). GH increases the synthesis and secretion of IGF-1 in the liver, and IGF-1 in turn controls GH secretion through a negative feedback loop (Baumann, 1999; Lucy, 2004b), and insulin interacts with GH to control hepatic IGF-1 production, thereby increasing plasma IGF-1 concentrations (McGuire *et al.*, 1995; Molento *et al.*, 2002).

Recent studies support the idea that the hormonal environment during early lactation differs between high- and low-yielding cows (Kolver *et al.*, 2002; Buckley *et al.*, 2003; Kolver *et al.*, 2004; Lucy, 2004b; Lucy *et al.*, 2004; Butler, 2005). High-yielding cows during early lactation have high blood GH, and low insulin concentrations; consequently, such animals are capable of very high levels of body tissue mobilisation during early lactation (Baumann, 1999; Lucy, 2004b).

In addition, recently calved high-yielding cows have very low GH-receptors (GHR) (Baumann, 1999; Lucy, 2004b). The relevance of this observation to the current study is in the recent work of Jiang *et al.* (2005), who found that in beef cows, unlike dairy cows, hepatic expression of GHR is not decreased at parturition. These differences between dairy and beef cows indicate that reduced expression of GHR in the liver of dairy cows at parturition is the result of the metabolic adaptations of the high-yielding cow. In contrast, New Zealand cows have higher GHR in the liver than the high-yielding cows, as confirmed by the results of a recent trial (G. Verkerk, personal communication). Thus, the expression of GHR in the liver is linked with the GH-IGF-1-insulin axis. That this is a dynamic situation that responds to metabolic stimuli is evident from the work of Butler and Butler (2003), who infused insulin into early postpartum dairy cows and thereby increased liver GHR. Moreover, Rhoades *et al.* (2004), using hyperinsulemic:euglycemic clamps, reported a doubling of the amount of GHR in adipose tissue when insulin was infused in early lactation. In other words, low- and high-yielding cows may respond differently to increases in circulating insulin.

The high-yielding cows that are directing most of their energy towards milk seem to have a marked degree of 'insulin resistance', because their adipose and muscular tissue appear to be less sensitive to insulin. On the other hand, glucose uptake in the mammary gland is independent of insulin. That means that cows in early lactation, especially if they are high producers, have an impaired ability to stimulate glucose uptake by adipose and muscle tissue (Cronje, 2000), whereas the mammary gland is readily able to sequester the circulating glucose for lactose production. Interestingly, similarities exist between the endocrine physiology of high-yielding cows

during early lactation and the physiology of diabetes states found in humans [Type 1 diabetes mellitus (insulin-dependent) and Type 2 diabetes mellitus (insulin resistance)] (Lucy, 2004b). The author concludes that high-yielding cows are diabetic, but with the benefit that they use all the glucose (i.e. due to its uptake into the mammary gland) that otherwise would be wasted or toxic for the organism. Conversely, low producing cows have lower blood GH and higher blood insulin concentrations. Their capacity to mobilise NEFA is smaller, and more glucose is partitioned to tissues outside the mammary gland. These features lead to lower milk production (Lucy, 2004b). If these conclusions are correct, it could be argued that the increases in insulin that are evoked by MPG administrations would have more impact in low-yielding New Zealand cows than in high-yielding cows.

In addition, rumen degradation of MPG seems to be higher when it is administered in TMR conditions (Nielsen and Ingvarsen, 2004). In intensive-TMR systems, MPG is usually mixed with the feed, which means that MPG enters the rumen at slower rate than when it is drenched. Moreover, the increased ability of animals given TMR diets to degrade MPG in the rumen, may be a result of the highly digestible fibre present in the TMR, which stimulates the growth of certain microbes that degrade the MPG (Czerkawski and Breckenridge, 1973). This degradability factor coupled with the insulin resistance factor mentioned above, means that the ability of MPG to evoke an increase in insulin might be more effective and powerful under pastoral conditions than under TMR systems.

Research from UK (Armstrong *et al.*, 2003; Garnsworthy, 2004) suggested that very high concentrations of insulin can be detrimental for the oocyte. A study with fat

beef-cross dairy heifers showed that less than 27% of the oocytes collected from heifers that had insulin concentrations of 25 international units (IU)/l developed to the blastocyst stage; on the other hand, between 40 and 52% of the oocytes survived when the insulin concentrations in their donors were between 16 to 25 IU/l (P. Garnsworthy, personal communication). Likewise, Armstrong *et al.* (2003) confirmed that diets which are optimal for follicle growth are not necessarily optimal for oocyte quality and subsequent embryo survival.

If this is so, concentrations of IGF-1 and insulin that are optimal for the growth of preantral follicles *in vitro* may in fact be detrimental to oocyte maturation. That is, to say, nutritionally-induced changes in the ovarian IGF system, coupled with changes in circulating concentrations of insulin and IGF-1 which maximise follicle recruitment, may be detrimental to the maturation of the oocyte within the growing follicle (McCaffery *et al.*, 2000; Armstrong *et al.*, 2002; Armstrong *et al.*, 2003). Whether there might have been a detrimental effect of insulin upon oocyte quality in the present study is hard to determine. Although insulin concentrations were not measured, a parallel experiment undertaken at Dexcel recorded mean insulin concentrations of only 6 IU/l after a drench of 260 ml MPG (L. Chagas, personal communication); a figure that is substantially lower than that reported by Garnsworthy (2004) to be detrimental to oocyte quality.

Several factors may influence the effectiveness that increases in insulin have upon fertility. Garnsworthy (2004) suggested that the changes at the follicular level required for ovulation may require long-term increases in basal concentrations of insulin, rather than transient peaks of insulin release. If so, this would contradict the

view of Miyoshi (2001) of the means by which MPG influences reproduction (i.e. by inducing an episode of insulin release). Nevertheless, Mann *et al.* (2003) reported that the feeding of a diet formulated to increase insulin resulted in improved early embryo development. The proportion of well-elongated embryos (empirically defined as equal or higher than 10 cm length) was higher in animals fed diets that would induce insulin secretion, resulting in a higher proportion of embryos that had undergone a sufficient degree of expansion by Day 16 to successfully prevent the initiation of luteolysis. However, in the absence of direct observations upon ovary or embryo, much of this remains conjectural. Indeed, measuring blastocyst length and follicular characteristics in response to MPG would provide very useful information for the interpretation for the effects of one or two episodes of insulin secretion on the animals in the present study.

Nonetheless, it still appears to be the case that the effect of MPG in the present study has to be understood in terms of gonadotrophin-independent mechanisms. In other words, because the effect of MPG was upon pregnancy rate, rather than anoestrus rate or the duration of PPAI, it is unlikely that its effect was upon gonadotrophins (i.e. as increased gonadotrophin secretion would have resulted in an earlier resumption of oestrous cycles). Some evidence that such a mechanism is feasible comes from the work of Hunter *et al.* (2004), who showed that alterations in the pre-mating diet influenced follicular/oocyte characteristics, without altering gonadotrophin secretion *per se*. For example, Gutierrez *et al.* (1997) and Gong (2002) showed that cows fed a diet that was twice the maintenance level had small follicles and more ovulations than did cows fed to maintenance level, despite the fact that circulating concentrations of FSH were unaffected.

There is, however, a problem with this argument, inasmuch as in a previous study of the effects of MPG in anoestrous heifers, Chagas (2003) found an earlier onset of oestrous cycles in treated compared to control animals. In the present study, MPG did not affect the oestrous activity of the animals, although the 6-week and final pregnancy rates of the MPGx1 were higher than the MPGx2 and control groups. These two studies were the only two experiments testing potential effects of MPG on reproductive performance of cows under pastoral systems. Understanding the differences between these results is therefore of some importance. There were some key differences between the studies as, in the present experiment MPG supplementation was administered to cows in commercial herds, beginning on a calendar date (i.e. six weeks before PSM), and continued for a limited duration (i.e. four to six weeks) (Table 4.3).

Table 4.3: Some differences between the previous MPG trial 2002 (research farmlet; Chagas, 2003) and the present MPG trial 2003 (commercial setting) that may have influenced the results.

	MPG 2002	MPG 2003
Age of the cows	2 year-olds	Mixed
Breed	Holstein Friesian	Mixed
Duration of the treatment	From calving to first ovulation (Up to 13 weeks)	Premating (four to six weeks)
Dosage	250 ml (twice daily)	200 ml (once or twice daily)
Level of feeding post calving	Generous	Possibly restricted
Daily milksolids yield for the 2 year-olds (kg per cow) Week 7 after calving	1.3	1.3

It may, for example, be possible to explain the difference in effects on anoestrus rates between the two experiments in the terms of the different durations of MPG administration. During the early postpartum period, LH concentrations and the frequency of LH episodes are low (Short *et al.*, 1990). However, the re-establishment of LH secretion pattern that are conducive to preovulatory follicular development is recognised as a key event in the postpartum restoration of ovarian cyclical activity (Lamming *et al.*, 1981). Chagas (2003), using twice-daily MPG administration in heifers found an increase in LH pulse frequency at Weeks 2 and 5 and an increase in the number of heifers ovulating at Week 8 postpartum. It seems that MPG did not have negative effects on the fertility of the animals in that study, even when MPG was administered from calving to mating. Furthermore, all the animals were pregnant at the end of the trial.

In the present study, the effects of MPGx1 and MPGx2 on the ovarian function were not determined. It is possible that the use of anoestrus treatments precluded the effects of MPG to be seen. Chagas (2003) measured LH frequency at Weeks 2 and 5 after calving, and found higher values for MPG-treated animals than for the other two groups. It is possible that excellent management, fully feeding after calving and the evident positive effects upon ovarian function seen in Chagas (2003) overcame potential negative effects on the oocytes. In addition, it could be argued that the prolonged period of supplementation effected some kind of resistance in the animal, which turned out, at the end, to have positive effects. Figure 4.1 summarises the key arguments in this interpretation of the results for MPGx1 and MPGx2 in the present study.

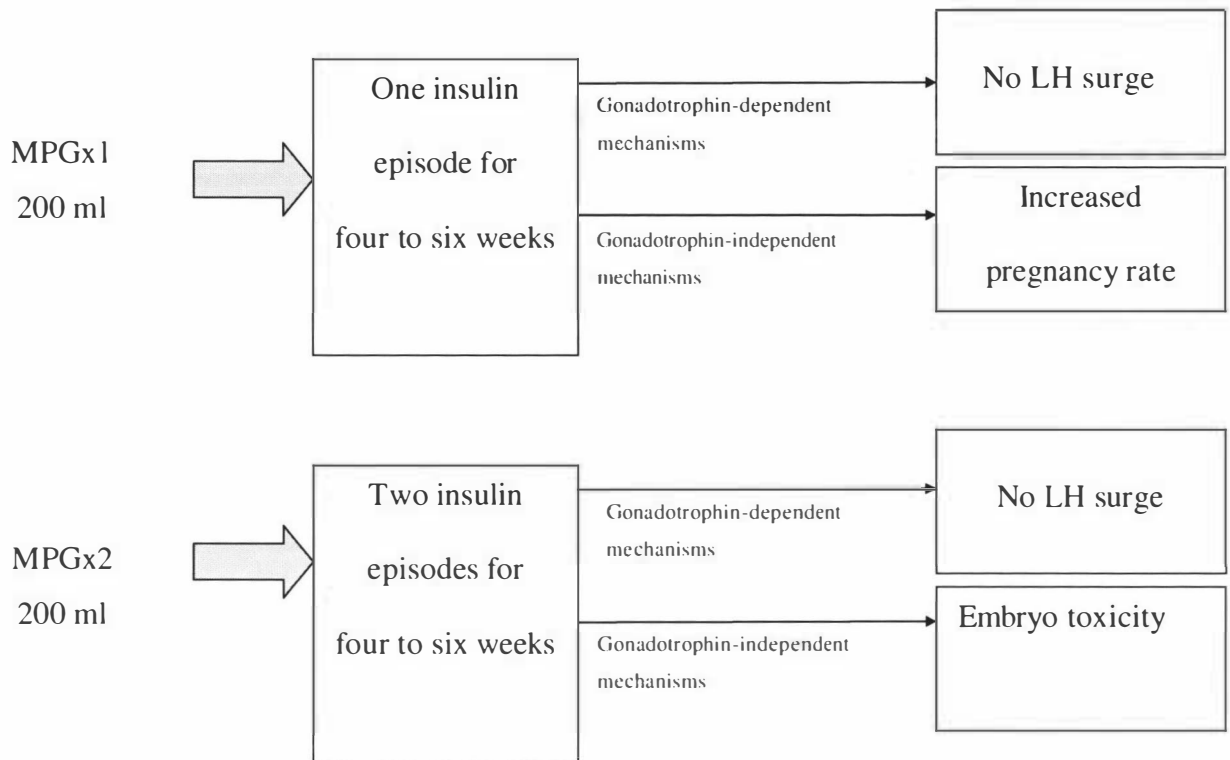


Figure 4.1: Proposed model for the action of once and twice daily MPG in the present study.

Two caveats exist to this argument. Firstly, Chagas (2003) obtained high 6-week pregnancy rates (69% and 76% for fully fed and restricted+MPG cows, respectively). Hence, whilst the higher dose of MPG in the present study failed to stimulate pregnancy rate, a slightly higher dose in the work of Chagas, substantially augmented pregnancy rate. It is hard to reconcile this with an explanation for the current experiment that invokes considerations of oocyte/embryonic toxicity. On the other hand, Chagas administered MPG for a much longer period than in the present study, and for longer than reported in any other experiments. Perhaps it might be possible to explain the higher pregnancy rate of Chagas in terms of a long-term stimulation of ovarian function, which outweighed any short-term toxic effects on the ovary. Alternatively, perhaps the cow simply became adapted to presence of MPG in its diet and, in a manner analogous to that seen with nitrate-containing diets, became resistant to its effects.

The second caveat, which is indeed a substantial caveat, is that the numbers of animals used in Chagas (2003) was small. Hence, the power of the study to detect differences in pregnancy rate was low, and, therefore, not only the magnitude but also the direction of the difference between groups could be significantly affected by the results of a very few animals. Conversely, the power of the work of Chagas (2003) to detect differences in the onset of oestrous cycles was greater than in the present study (due to the difficulties of obtaining accurate data from field observations), and, despite the large number of animals involved, many uncontrolled factors other than MPG administration could have affected the onset of oestrous cycles. Thus, large-scale experiments, such as the present study, are influenced by numerous factors that can lead to greater variability than controlled experiments. Herd level decisions, such as stocking rate, rotation length, supplements, breed and culling strategies add background noise (McDougall and Rhodes, 2004). Likewise, health management cannot be controlled when performing field trials (Lean *et al.*, 2003). The mean size of the four herds enrolled in this study ($n = 604$) was exactly twice the national average ($n = 302$; LIC, 2003/04) and several studies (e.g. Butler, 2000) have shown that increases in herd size are associated with a decrease in fertility. Oestrus detection by traditional methods of observation followed by AI may not be adequate for cows that are managed in large groups (Macmillan, 1998; Lucy, 2001b). The herds contained significant numbers of thin cows (overall mean BCS = 4.4) before the PSC and were likely to have poor reproductive performance. Equally, the poor nutritional management of the herds, as evidenced by these low condition scores, may well reflect deficiencies in other aspects of the management of the livestock.

The extent to which the results were confounded by CIDR treatment also needs to be considered. The large number of cows (32%) that were treated with CIDRs may well have masked any effect of MPG upon the resumption of oestrous cycles and the proportion of cows that were anoestrous during the initial period of mating. That CIDR treatment did not affect the response to treatment can be inferred from the observation that there were no differences in response to CIDRs between the three treatment groups. As expected (Macmillan *et al.*, 1995b; Xu and Burton, 2003), CIDRs induced more than 90% of the cows to show oestrus within five days after treatment. Conception rates to first AI after CIDR treatment were lower for the two farms that inseminated to detected oestrus (29%) than described by Xu and Burton (2003: 39%) but were similar for the two farms that used set-time insemination (36%). Whether the lower conception rates that were achieved on the farms that inseminated to detected oestrus were indicative of poor oestrus detection is open to debate. It would be certainly be compatible with the relatively mediocre stockmanship that the poor BCS indicates. Be that as it may, it seems as if insemination at set time post CIDRs might be a good alternative for 'problem' herds.

In summary, MPG did not effect an earlier onset of oestrous activity, but MPGx1 increased 6-week and final pregnancy rate. However, MPGx2 did not. Therefore, it appears that pre-mating supplementation according to the regimen of MPGx1 had positive effects on the follicle and, hence, resulted in higher pregnancy rates. If this is true, MPG supplementation will be a very useful tool for non-invasive treatment of reproductive management, since the percentage of cows pregnant by six weeks after the PSM is a determinant of reproductive performance (Morton, 2000a,b). Why MPGx2 regimen in the present study failed to produce a higher

pregnancy rate is unclear, especially in view of the positive outcome obtained with a higher dose of MPG over a longer period in the work of Chagas (2003). The important point is, however, that in both experiments administration of MPG resulted in positive outcomes, either in terms of affecting anoestrus, or affecting pregnancy rate, or both. Hence, whilst optimisation of administration regimens (in terms of dose, frequency and duration) has yet to be finalised, it is clear that the method is of benefit in the management of cows that are at risk of nutritionally-induced infertility.

4.2 Effects of MPG on milk yield and condition score

One litre of MPG has approximately 24 MJME (Miyoshi *et al.*, 2001), which is two times the energy content of 1 kg DM of high quality pasture (Holmes *et al.*, 2002a). Therefore, daily drenches of 200 and 400 ml should provide about 5 and 10 MJME, respectively. In theory, this extra dietary energy would result in an extra 0.08 to 0.16 kg milksolids, and approximately 0.05 to 0.10 kg protein (Penno, 2001b; Holmes *et al.*, 2002a; Kolver, 2003)². However, the actual responses were only 0.010 and 0.017 kg of protein per day for MPGx1 and MPGx2, respectively ($P = 0.02$), some 20% of the response that would have been expected from changes of energy intake alone. Furthermore, fat yield decreased with MPG, albeit not significantly, by 0.08 and 0.015 kg per day with MPGx1 and MPGx2, respectively (Table 3.6). Only Laranja da Fonseca *et al.* (1998) observed an increase, albeit small, in milk production during the fourth and fifth weeks postpartum when cows were drenched with MPG. However, other studies agree with the present study (Fisher *et al.*, 1973; Studer *et al.*, 1993; Formigoni *et al.*, 1996; Juchem *et al.*, 2004) that MPG did not affect milksolids production.

Nonetheless, the dose-dependent increase in milk protein yield with MPG supplementation is evidence of MPG having some metabolic effects in the cows (Table 3.6). The conversion of MPG into propionate in the liver results in an increase in blood glucose (Studer *et al.*, 1993; Grummer, 1994). Increasing availability of glucose to the

² The potential substitution effect of the MPG, causing a reduction in the intake of forage, has to be considered here; however, as intake was not measured in this experiment, this question remains with no answer.

mammary gland results in increased protein synthesis and protein yield, due to a reduced need to break down amino acids for gluconeogenesis (Emery, 1978). Such a mechanism might explain the effect of MPG on milk protein yield. It seems probable that the effects of MPG were mediated through endocrine/metabolic processes, rather than through the addition of extra energy; the increase of energy supply to the animals was relatively short and small, and there was no change in BCS in response to MPG treatment. It is also unlikely that there was any significant change in the energy balance of MPG-treated compared to control cows.

Finally, drenching MPG was very labour demanding. Mixing MPG with the feed would be less labour intensive (Christensen *et al.*, 1997). However, doing so has been shown to reduce intake, due to reduced palatability, and thus to offset any positive effects of MPG on metabolism (Johnson, 1954; Miyoshi *et al.*, 2001; Hoedemaker *et al.*, 2004). Further, in New Zealand, concentrate feeding is generally not cost effective (Holmes, 2000). In the present experiment, the MPG was drenched. However, it is possible that measurements of intake, although not practicable, would have been helpful to determine any adverse effect of MPGx2 on intake.

4.3 Effects of age and calving date on reproductive performance

Age of cows and their date of calving are well-known to affect fertility in seasonal pastoral dairy herds (McDougall *et al.*, 1995b; Rhodes *et al.*, 1998a; Rhodes *et al.*, 2003). As expected, these factors affected reproductive performance of the cows in the present study.

Mean calving date was slightly earlier for the 2 year-olds (12/08/03) than for the 3 year-old and mature cows (19/08/03). Despite similar BCS at calving, mature cows resumed oestrous cycles earlier than did the 2 and 3 year-old cows. Thus, at 3.5 weeks before the PSM, 52% of the mature cows were cycling, whereas only 37% of the 2 year-old cows and 45% of the 3 year-old cows were cycling (Figure 3.2). A higher proportion of first calving heifers than older cows were anoestrous at Week -1 (Figure 3.4). Likewise, submission rate to AI following CIDR treatment was lower in heifers than cows, as also reported McDougall (2001b). Consequently, the pregnancy rates after three and six weeks, and the final in-calf rates were lower for the first calving heifers than for the older cows. Thus, the 2 year-old cows had the highest percentage of anoestrus before mating (29%), lowest submission rate in the first three weeks of the breeding season (78%) and the lowest final in-calf rate (89%). Xu and Burton (2000) similarly found that the youngest cows of the herd have the poorest reproductive performance, and that the highest final pregnancy rates were achieved in cows aged 4 to 6 years.

The present results highlight the effects of the higher metabolic stress experienced by the younger animals in the herd, which are still growing, producing

milk, and need to become pregnant to stay in the herd. The springtime conditions in the seasonal pastoral system of New Zealand often result in relative underfeeding of first calving animals during early lactation. Thus, the PPAI is longer in first lactation cows than mature cows, partly because of their lower DMI whilst they still have increased requirements for growth (McDougall, 1994). Consequently, a higher proportion of heifers in the herd may have adverse effects on the overall results of the next breeding season (McDougall *et al.*, 1993), which will, in turn, compound the reproductive problem. However, the 2 year-olds in the present study accounted for 20% of the cows treated for anoestrus, which is similar to the percentage reported and recommended for the average New Zealand dairy herd (Jackson, 1984; Anon, 2003a).

Late calving is also a major contributor to poor in-calf rates in New Zealand dairy herds (Xu and Burton, 2000). Late calvings lead to large decreases in both submission and conception rates (Morton, 2000a) and increased in the proportion of anoestrous cows: Xu and Burton (2003) found that the percentage of anoestrous cows was lower (22%) in early calving cows than in later calving cows (28%). Late calving leads to herds with low 6-week pregnancy rates (Morton, 2000a); a cycle which needs to be broken if large gains are to be made. Morton (2000a) suggested that a high proportion of the herd must calve in the first three weeks of the calving season if reproductive performance is to be maximised, due to the strong relationship between the herd calving pattern and reproductive management achieved in the following mating period. In the present study, about 40% of the late-calving cows (calved less than 42 days before PSM) were anoestrous in Week -1, while the corresponding value was almost 30% for cows that had calved more than 56 days before the PSM.

As expected, date of calving and age were determinant factors affecting the reproductive performance of the animals. Furthermore, the negative association of young age and late calving with fertility might have been compounded by the predominant poor condition of the cows.

4.4 Association between BCS, BCS change, reproductive performance and milk production

In the present study, cows that lost BCS during the six weeks preceding the PSM tended to produce more milk and had lower pregnancy rates than the cows that did not lose condition. There are a number of earlier reports that confirm that this is the case. Loeffler *et al.* (1999) observed that cows losing 1 unit or more BCS (5-point scale) during early lactation are at greatest risk for low fertility, with conception rates of 38% to 17%, respectively. Butler and Smith (1989) reported that cows which lost more than 1.0 unit of BCS between calving and mating achieved a lower pregnancy rate than those that lost 0.5 to 1.0 BCS units (1 to 5 scale). Likewise, Morton (2000a) found a negative relationship between BCS loss and reproduction (compared with the results of the present study in Table 4.4). Furthermore, Gillund *et al.* (2001) showed that cows that experienced marked losses in BCS during the postpartum period were only half as likely to conceive to first service as cows that experienced modest losses in BCS. In the same study, BCS loss postcalving was associated with a prolonged calving to conception interval. Pryce *et al.* (2001) agreed with these results, showing that a 1 unit increase in BCS at Week 10 of lactation was associated with a reduction in time to first service of 6.2 days and a 9% units higher conception rate to first service. Finally, Buckley *et al.* (2003) advised that loss of BCS between calving

and mating should be restricted to 0.5 units of BCS to avoid detrimental effects on reproductive performance.

Interestingly, in the present study, early calvers (i.e. those that calved in August, at least six weeks before the PSM) lost more condition than late calvers (i.e. calved in September), suggesting an inadequate feed energy intake in the early period of lactation and an inability to maintain milk production and BCS. However, MPG administration did not affect the BCS at Week -1 or the magnitude or direction of changes in BCS during the treatment period (Table 3.5).

Table 4.4: Association between body condition score (BCS) change after calving and the percentage of cows pregnant after six weeks of mating, from the Australian InCalf project, and from 'MPG trial'.

BCS change after calving ¹	6-week pregnancy rate (%)
(Morton, 2000a) (1 to 8 scale)	
Cows that lost 0.67 or more	58
Cows that lost 0.67 to 0.33	59
Cows that lost less than 0.33	61
(Tuñón <i>et al.</i> , 2004) (1 to 10 scale)	
Cows that lost 0.5 to 1.5 ($n = 137$)	64
Cows with no change ($n = 630$)	70
Cows that gained 0.5 or more ($n = 1,068$)	77

¹ Only the cows that calved before the start of the treatment period (i.e. Week -6).

Whether this reflects a genetic relationship between selection for milk and reproductive performance is a fascinating debate. Certainly a number of authors (Butler and Smith, 1989; Beam and Butler, 1999; Pryce *et al.*, 1999; Royal *et al.*, 2000; Gillund *et al.*, 2001; Gong, 2002; Buckley *et al.*, 2003) have reported negative genetic correlations between lactation and reproduction traits. On the other hand, many studies report either no relationship or a positive phenotypic relationship between milk production and reproductive parameters. Moate and Harris (1983), Fulkerson (1984), Macmillan *et al.* (1996) and Rhodes and Morgan (1999), all showed a positive relationship between milk production and reproduction (submission and conception rates) in pasture-based systems, probably as a result of better management and feeding in herds that achieve high yields (Xu and Burton, 2003). Buckley *et al.* (2003) also noted that milk production may increase due to improved environmental factors such as better nutrition, health and management, as well as genetic improvement. Other studies

found no relationship between milk production and reproduction (Villa-Godoy *et al.*, 1988). Likewise, Morton (2000a) reported no evident relationship between milk volume and reproductive performance and concluded that high-producing cows have only slightly reduced reproductive performance when compared with lower producing herd mates.

4.5 Nutritional alternatives for cows that are at risk of becoming anoestrous

Herd reproductive performance is affected by multiple factors, such as fertility is limited by more than one in most herds (Holmes, 2000; Morton, 2000a). Consequently, small improvements in several different aspects of the system will result in increased reproductive performance. Focusing on all the key details, and on the long-term day-to-day control and fine-tuning of efficiencies, will ensure that all these small individual gains in the components are combined to produce a large overall gain. Therefore, to achieve a significant improvement in reproductive performance, farmers may need to focus on a number of management issues and identify the most important limiting factors within their herds. Yet the large variation among herds in reproductive performance suggests scope for improvement through better management in the herds with poorer reproduction and, often, it is nutrition that is the most limiting factor.

Nutrition affects reproduction in numerous ways. According to Diskin *et al.* (2003), it is difficult to determine the specific functions and mechanisms by which nutrition may affect reproductive function, because there are no nutrients that are specifically required for reproduction that are not required for other normal physiological functions in the body. On the other hand, given that optimising BCS at

calving and feeding sufficient dietary energy in early lactation ensures normal resumption of ovarian function, there are a number of nutritional management practices that can help farmers minimise the adverse effects of having high numbers of cows at risk of having prolonged PPAI. Much of that can be done simply by improving management of pasture and supplement feeding (Grainger and McGowan, 1982). Likewise, dietary fat can improve the fertility of high-producing cows (Ferguson, 1996; Butler, 2005).

At a management level, strategies such as reducing milking frequency from twice to once a day (OAD) to reduce losses in cow condition and induce spontaneous resumption of oestrous cycles, are beneficial. Once a day milking is likely to result in an improved energy balance during early lactation (Holmes *et al.*, 1992), although in the work of Rhodes *et al.* (1998b), OAD milking increased the rate of spontaneous resumption of oestrous cycles in anoestrous cows but was associated with a significant decrease (-21%) in milk production. An experiment that is running now at the Waimate West Research Station in Taranaki has shown that cows milked once daily achieved 4% empty rate, compared with 12% in cows milked twice a day. In addition, the OAD cows did not need to be treated with CIDRs or to be induced (D. Dalley, personal communication).

Strategic manipulation of glucose precursors can often be a short-term solution for cows 'at risk' of having decreased fertility due to impaired nutrition. Indeed, nutritional manipulations, acting via systemic signals, may provide a non-invasive, acceptable means of maintaining, or even improving fertility outcomes (Hunter *et al.*, 2004). Research suggests that propionate precursors (manipulation of 3-carbon

substances) effect a transient increase in glucose concentrations, which in turn induces an episode of insulin release. The simplest way to stimulate insulin release is to increase propionate production in the rumen, since propionate is the main source of glucose in ruminants (Garnsworthy, 2004). MPG, meal feeding, concentrates, sugars, they all end in propionate, which in turn is converted in glucose (Garnsworthy, 2004). Fat feeding can improve the fertility of high producing cows. By pass starch and amino acids can also increase glucose.

For 50 days postpartum, Gong *et al.* (2002) fed cows diets that were formulated either to increase propionate production or not to do so, with the aim to induce a greater insulin release in response to feeding. Dietary ingredients were grass silage, whole-crop wheat silage, wheat, barley, sugar beet pulp, soya bean meal, protected fat and a mineral/vitamin supplement. The diet that maximised propionate output had a high content of barley (260 g of barley per kg DM) and resulted in increased circulating insulin concentrations in both high and low genetic merit cows. Although the high insulin diet reduced the intervals from calving to first service and to conception, the effect was not significant.

Nonetheless, the costs of implementing any treatment to increase reproductive performance should be taken into account (Short *et al.*, 1990) and treatment can only be seen as a remedy for the problem, not as a substitution for good management (Laborde, 1998). Achievement of the targeted BCS at calving is the key for optimal reproductive performance in the long term, and, whilst strategies such as the manipulation of propionate/insulin may be effective as short-term remedies for anoestrus, they probably should not be seen as long-term alternatives to proper overall feeding strategies.

Perhaps one way forward is the development of selection indices that combine all the economically important traits appropriately for a given set of local conditions and systems. The cows must be compatible with the system used; knowledge of the cows' genotype and the environment in which they are managed must be the basis to predict the phenotypic performance of dairy cattle (Buckley *et al.*, 2005), if dairying is to remain sustainable. Maybe this could even include selection for traits related to the patterns of changes in BCS, given that it is both highly heritable and highly correlated with fertility (Pryce and Harris, 2004). This will therefore increase the rate of progress that can be made in fertility.

4.6 Summary and conclusions

The experiment reported in this thesis was undertaken to investigate the hypothesis, based on the work of Chagas (2003), that administration of MPG to cows in low BCS during the early postpartum period would reduce the PPAI, reduce the proportion of cows that were anoestrous at the PSM and thereby increase pregnancy rates. It was postulated that the mode of action by which MPG would achieve this result would be by increasing the availability of gluconeogenic precursors, with consequent effects upon glucose and insulin concentrations. The result of this would be that follicle growth would resume earlier than expected in cows in poor BCS.

The results of the experiment partially substantiated this hypothesis, inasmuch as 6-week and final pregnancy rates were improved in the group that received MPG once daily. On the other hand, the group that received MPG twice daily did not exhibit an improvement in pregnancy rates, nor did either group display an earlier onset of oestrous cycles. Other results were largely as expected, namely that younger cows had higher anoestrous and lower pregnancy rates than did older cows, and cows in poorer BCS were more likely to be anoestrous and less likely to conceive than those in better condition.

It is not certain whether the reasons for the discrepancy between the present results and those of Chagas (2003) should be attributed to mechanisms of action of MPG or to differences in experimental design. It is possible to explain the differences between the studies in terms of the three actions of MPG and/or insulin within the reproductive system; namely, their effects upon gonadotrophins, their effects upon

follicular growth and their effects upon subsequent embryo survival. Alternatively, it may be that effects upon pregnancy were obscured in the work of Chagas (2003) due to small number of animals and/or that effects upon anoestrous rate were obscured in the present experiment due to other farm and management factors exerting a greater influence than did the administration of MPG.

Nonetheless, it is clear that, in the present experiment, MPG had other effects on the cows' metabolism that were not simply related to its energy content. That is, MPG-treated cows produced more protein than would have been expected on the basis of the energy content of MPG alone, suggesting that it had indeed affected the activity of insulin-responsive systems.

Many of these difficulties could be unravelled in further experiments. For example, direct measurements of circulations of insulin, glucose and gonadotrophins; this would provide information to evaluate the role of gonadotrophin-dependent mechanisms in the process. Likewise, *in vivo* assessments of follicular dynamics (e.g. through visualisation by ultrasonography or through endocrine studies) help elucidate whether the effects of MPG are mediated at the follicular level. Finally, recovery of embryos from MPG-treated cows would allow direct evaluation of whether or not there were any adverse effects of the treatment regimen upon embryonic growth and/or survival.

In conclusion, therefore, the results of this experiment confirm the notion that administering MPG in the postpartum period enhances the fertility of cows that are in poor BCS, and that it does so through a mechanism that is independent of it simply

being an energy source. As MPGx1 resulted in significant improvements in 6-week and final pregnancy rates, the use of this regimen could be advocated in herds in which fertility is limited by nutritionally-mediated postpartum anoestrus, therefore, hopefully, contributing to enhancing the reproductive performance of the New Zealand dairy cow.

Appendix

Waikato region is home to over 30% of the New Zealand's dairy farms and over 1,000,000 dairy cattle (LIC, 2003/04). The Waikato has a temperate climate. The highest mean temperatures coincide with the lowest mean rainfall (From <http://www.niwa.co.nz>).

Table 4.5: Location within Waikato (from <http://www.fallingrain.com>).

Farm #	Latitude	Longitude	Altitude
1	38°0'0S	175°11'60E	29 m
2	37°58'60S	175°45'0E	105 m
3	38°10'60	175°11'60S	44 m
4	37°41'10	175°45'0E	68 m

Table 4.6: Details of the four farms.

	Farm #1	Farm #2	Farm #3	Farm #4
Farm area	270	168	220	240
Number cows in the trial	640	515	609	650
Milking system	Rotary	Herringbone	Rotary	Rotary
Average cow liveweight (kg)	450	500	450	470
Liveweight per ha (kg)	1,067	1,571	1,186	1,289
Total pasture produced (t/ha)	12	16	9.5	10.1
Nitrogen (t extra pasture/ha)	1.2	0.7	0.8	1.4
Feed imported (t/ha)	-	0.4	-	0.7
Feed available (t/ha)	13.2	17	11.7	12.2
Young stock adjustment (t DM/ha)	1.1	0.1	0.2	0.3
Comparative stocking rate (Kg liveweight/t DM)	88	93	103	108
Milksolids production in early October (kg milksolids/cow)	1.65	1.98	1.40	1.50
Final in-calf rate (%)	86.3	92.7	88.2	91.3

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