Effect of premating supplementation with monopropylene glycol on reproductive performance of dairy cows*

Tuñon, G.[1,2]; Parkinson, T.J.[2]; Holmes, C.W.[2]; Chagas, L.M.[2]

[1] Massey University, Turitea, P.O BOX 11 222, Palmerston North, New Zealand.
[2] Dexcel Limited, P.O BOX 3 221, Hamilton, New Zealand.
E–mail: larinconada@copetel.com.ar

Abstract

Tuñon, G.; Parkinson, T.J.; Holmes, C.W.; Chagas, L.M.: ***Effect of premating supplementation with monopropylene glycol on reproductive performance of dairy cows.*** *Rev. vet. 17: 1, 11–19, 2006.* 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. A total of 2,122 cows were included in the analysis (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 control (67.7% and 88.1%), respectively. MPG 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 (BCS) 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). 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: dairy cow, monopropylene glycol, pregnancy, anoestrus, body condition.

Resumen

Tuñon, G.; Parkinson, T.J.; Holmes, C.W.; Chagas, L.M.: ***Efecto de la suplementación preparto con monopropilenglicol en la performance reproductiva de vacas lecheras.*** *Rev. vet. 17: 1, 11–19, 2006.* Los intervalos de anestro postparto prolongados (APP) son la mayor causa de infertilidad para las vacas lecheras de Nueva Zelanda. Se afirma que la suplementación postparto con monopropilenglicol (MPG), bajo condiciones experimentales controladas, sería capaz de reducir el intervalo APP en vacas jóvenes. El presente ensayo tuvo como objetivo probar este efecto en cuatro tambos comerciales, utilizando un total de 2.122 vacas (n = 684 a 714 por tratamiento). Durante el período de seis semanas que precede al comienzo del servicio (CS, semana 0), las vacas fueron suplementadas una (MPGx1) o dos (MPGx2) veces por día con 200 ml de MPG, o fueron usadas como vacas controles sin tratar. Ninguna de las cuatro variables consideradas: comportamiento estral durante el período de tratamiento, porcentaje de anestro una semana antes del CS, porcentaje de presentacion a servicio a las tres semanas o porcentaje de preñez a las tres semanas, fueron afectadas por MPG. Por otro

Recibido: 15 abril 2006 / Aceptado: 22 mayo 2006

*Fertility project, Dexcel Limited, presented in the Conference of the New Zealand Society of Animal Production in Hamilton, New Zealand. Published in the Proceedings of the Conference 2004. Funded by Dairy Insight New Zealand.

lado, MPG aumentó el porcentaje de preñez a las seis semanas y al final del servicio (p<0,005), mostrando MPGx1 mayores valores (74,6% y 92,1%) que MPGx2 (69,1% y 88,5%) y controles (67,7% y 88,1%), respectivamente. Las vacas tratadas con MPG también produjeron mayores cantidades de proteína láctea que las vacas controles, con valores de 0,01 y 0,017 kg/día de proteína para MPGx1 y MPGx2, respectivamente (p=0,02), evidenciando un efecto metabólico del MPG. Estudios recientes sugieren que las dietas que son benéficas para el desarrollo folicular no necesariamente lo son para la calidad del oocito y la subsecuente supervivencia del embrión. Se presume que MPGx1 tuvo efectos positivos sobre el folículo/oocito a través de mecanismos gonadotropina–independientes y que MPGx2 tuvo efectos negativos sobre el embrión. Probablemente los efectos gonadotropina–dependientes necesarios para la ovulación no ocurrieron en el presente estudio porque MPG no fue administrado durante el tiempo necesario para provocar el pico de LH. El cambio en la condición corporal (CC) entre las semanas –6 y –1, y el porcentaje de vacas que estaban en anestro en la semana –1, fueron influenciados por las variables: rodeo, edad y momento del parto (p<0,05), pero no por el tratamiento (p>0,1). El cambio de CC afectó la producción de leche y el porcentaje de preñez: vacas que ganaron CC produjeron menos leche (p=0,01) y mostraron mayor porcentaje de preñez a las seis semanas (p<0,05). Se concluye que la edad de la vaca, el momento del parto y el manejo del rodeo, son factores que influencian el porcentaje de anestro del rodeo en el momento del servicio.

Palabras clave: vaca lechera, monopropilenglicol, preñez, anestro, condición corporal.

INTRODUCTION

The postpartum anoestrous interval (PPAI) is the normal physiological period of time from calving to resumption of oestrous activity [32]. Prolonged PPAI is a major cause of infertility in the seasonal pastoral dairy system [27]. In the seasonal system, prolonged PPAI leads to spread patterns of conception and consequently spread patterns of calving. 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. Cows calving in poor body condition score (BCS) have prolonged PPAI and increased chances of being anoestrous at the planned start of mating (PSM) [34, 14].

Cows that are nutritionally compromised have low concentrations of metabolites and metabolic hormones in their blood [7, 26]. Low insulin availability during early lactation causes a decrease in the responsiveness of ovarian follicles to gonadotrophin stimulation [6]. Several studies have proven that administration of MPG increases glucose and insulin [8, 10, 15, 42]. Supplementation with MPG reduced PPAI [8, 10]. These studies suggest that supplementation with MPG may improve reproductive performance.

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 [8]. 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. 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 artificial insemination (AI).

MATERIALS AND METHODS

Trial design and treatments. Four herds, which differed in size, age composition and breed structure (n = 2,629 cows), were selected on the basis that the cows were predominantly in poor BCS at calving. The calving periods in the farms ranged from July to September and pasture was the main feed for all the herds. The experiment was designed to measure the effects of MPG, given by drenching, on the fertility of the 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. The period of six weeks before the PSM for each farm was termed the "treatment period" (Figure 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.

Allocation of cows to treatment groups. A total of 2,414 cows were randomly allocated to the three treatment groups within each farm according to initial BCS, age and expected calving date. Ear tags identified all cows and coloured collars identified treatment groups. 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.

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 1). 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.

MPG drenching and herd test. The treatment period started at week –6 (six weeks before PSM; Figure 1). Drenching was done during milkings. 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).

Body condition score and detection of oestrus during the treatment period. The BCS of each cow was visually assessed prepartum and at weeks –6 and –1 (Figure 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) [29]. 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.

Identification and treatment of anoestrous cows. Seven to 10 days before the PSM, cows with no recorded oestrus were presented for veterinary inspection. Cows with ovaries that were assessed to be inactive (n = 687) received an intravaginal device containing 1.38 g of progesterone (controlled internal drug release, CIDR; Pfizer Animal Health, Auckland, New Zealand) for 8 days, with an intramuscular injection of 2 mg of oestradiol benzoate, 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.

Insemination and pregnancy diagnoses. The cows were inseminated using fresh semen. Data from the pregnancy diagnoses, done in January and March, 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 [38].

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 pregnancy rate, 6– week pregnancy rate, change in BCS between Week –6 to Week –1, and milk production. Linear models were used to analyse the data from yields of milksolids, fat and protein. Generalised linear models with a binomial error distribution and logit link were used to analyse the proportions of anoestrous cows 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 data were analysed using Genstat [12].

RESULTS

The following results will be based on the group of 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; total n = 1,996).

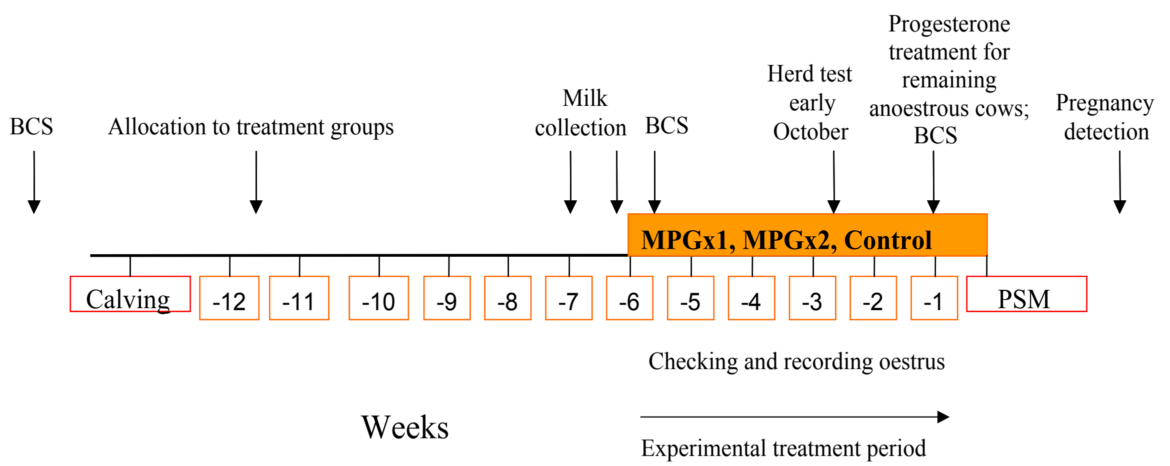

Figure 1. Time sequence of events; treatment period is the time when MPG was given. The BCS of the cows was assessed before calving, by week –6 and by week –1. Cows which were still anoestrous in week –6 and cows which calved during the first two weeks of the treatment period were included in the analysis.

Cows which resumed oestrous activity during the treatment period, and before the planned start of mating. 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). The onset of oestrous activity was also 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. 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).

Younger and later calving cows had the highest (p<0.001) anoestrous rates at week –1; 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 2).

Table 1. 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 ± 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).

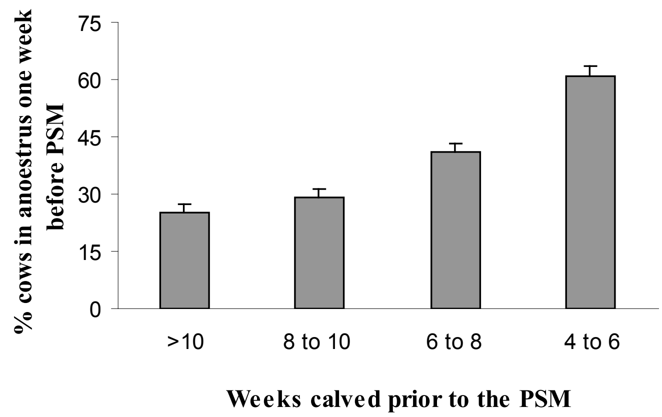

Figure 2. The rates of anoestrus in groups of cows that had calved at different times prior to the planned start of mating (PSM).

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 1) than for other groups.

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. Final pregnancy rate was also lower for 2 year–old cows (83%) than for mature cows (92%; p<0.001). 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. 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).

Yields of milksolids, protein and fat. 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.

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). 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.

DISCUSSION

The rationale of the present study was based on outcomes of a previous study [8] that demonstrated that MPG given post–calving 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.

Recent studies support the idea that the hormonal environment during early lactation differs between high– and low–yielding cows [4, 6, 19]. 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 [19, 26]. Most experiments testing effects of MPG upon reproduction were done with high–yielding cows, which are in contrasting difference with the type of cow used in the present experiment. The genetic background of the animals was not analysed. However, it would be wise to take this differences into account when analyzing the results, since, some of the differences in the responses among trials could have been due to the different types of cows used in them.

The effects of MPG on reproductive performance. 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. The 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* [43]. Guitierrez [16] reported that granullosa cells in the follicle were critically dependent on the presence of physiological concentrations of insulin. In humans, for instance, the increased circulating insulin in women with diabetes augments the action of LH upon theca cells [44]. 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.

Research from UK [1, 11] 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 [2] 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 [1, 2, 31]. 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 [11] to be detrimental to oocyte quality.

Several factors may influence the effectiveness that increases in insulin have upon fertility. Garnsworthy [11] suggested that the changes at the follicular level required for ovulation may require long–term increases in basal concentrations of insulin, rather that transient peaks of insulin release. If so, this would contradict the view of Miyoshi [35] of the means by which MPG influences reproduction (i.e. by inducing an episode of insulin release). Nevertheless, Mann [30] 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 [18], who showed that alterations in the premating diet influenced follicular/oocyte characteristics, without altering gonadotrophin secretion *per se*. For example, Gutierrez [16] and Gong [14] 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 [8] 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 2).

Table 2. Some differences between the previous MPG trial 2002 (research farmlet; 8) 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 [41]. 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 [21]. Chagas [8], 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 [8] 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 [8] 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.

Two caveats exist to this argument. Firstly, Chagas [8] 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 resistent to its effects.

The second caveat, which is indeed a substantial caveat, is that the numbers of animals used in Chagas [8] 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 [8] 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 [22]. Herd level decisions, such as stocking rate, rotation length, supplements, breed and culling strategies add background noise [31]. Likewise, health management cannot be controlled when performing field trials [32]. The mean size of the four herds enrolled in this study (n = 604) was exactly twice the national average (n = 302) [23] and several studies [6] 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 [25, 28]. The herds contained significant numbers of thin cows (overall mean BCS = 4.4) before the planned start of calving 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.

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 [36, 37]. 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 [8]. 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.

Effects of MPG on milk yield and condition score. One litre of MPG has approximately 24 megajoules of metabolisable energy (MJME) [35], which is two times the energy content of 1 kg DM of high quality pasture [17]. 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 [17, 20]. 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.

Nonetheless, the dose–dependent increase in milk protein yield with MPG supplementation is evidence of MPG having some metabolic effects in the cows. The conversion of MPG into propionate in the liver results in an increase in blood glucose [15, 42]. Increasing availability of glucose to the mammary gland results in increased protein synthesis and protein yield, due to a reduced need to break down amino acids for gluconeogenesis [9]. 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.

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 [33, 39, 40]. As expected, these factors affected reproductive performance of the cows in the present study. A higher proportion of first calving heifers than older cows were anoestrous at week –1. 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%).

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. Late calving is also a major contributor to poor in–calf rates in New Zealand dairy herds [32]. 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.

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 [24] observed that cows losing 1 unit or more BCS (5–point scale) during early lactation are at greatest risk for low fertility [3, 5, 13], with conception rates of 38% to 17%, respectively.

Summary and conclusions. The experiment reported in this trial was undertaken to investigate the hypothesis, based on the work of Chagas [8], 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.

It is not certain whether the reasons for the discrepancy between the present results and those of Chagas [8] 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 [8] 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 dairy cow.

Acknowledgements. Massive thanks to the Ministry of Foreign Affairs of New Zealand, which granted me the scholarship to accomplish a Masters program at Massey University. Thanks to my supervisors Tim Parkinson, Colin Holmes and Lucia Chagas. Viliami Taufa and Chris Burke had great input in the confection of this paper. Thanks also to the farmers involved in the trial. Barbara Dow helped with the data analysis, as well as Nicolas Lopez Villalobos. I am also grateful to Chris Glassey, Kevin Macdonald, Gwyneth Verkerk, John Roche, Garry Waghorn, Eric Kolver, Jo Smith, for their suggestions during the trial. Javier Baudracco and Federico Riet helped to prepare this paper for the NZSAP conference. Thanks Professor Coppo in Corrientes, Argentina.

REFERENCES

1. **Armstrong DG, Gong JG, Gardner JO, Baxter G, Hogg CO, Webb R**. 2002. Steroidogenesis in bovine granullosa cells: the effect of short–term changes in dietary intake. *Reprod* 123: 371–378.
2. **Armstrong DG, Gong, JG, Webb R**. 2003. Interactions between nutrition and ovarian activity in cattle: physiological, cellular and molecular mechanisms. *Reprod Suppl* 61: 403–414.
3. **Baumann DE**. 1999. Bovine somatotropin and lactation: from basic science to commercial application. *Dom Anim Endocr* 17: 101–116.
4. **Buckley F, O´Sullivan K, Mee JF, Evans RD, Dillon P**. 2003. Relationship among milk yield, body condition, cow weight and reproduction in spring–calved Holstein–Friesians. *J Dairy Sci* 86: 2308–2319.
5. **Butler WR, Smith RD**. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *J Dairy Sci* 72: 767–783.
6. **Butler WR**. 2000. Nutritional interactions with reproductive performance in dairy cattle. *An Repr Sci* 60/61: 449–457.
7. **Butler WR**. 2005. Nutrition, negative energy balance and fertility in the postpartum dairy cow. *Cattle Pr* 13: 13–18.
8. **Chagas LM**. 2003. Propionate precursor to reduce postpartum anoestrus in heifers. *Proceedings of the Australian and New Zealand Combined Dairy Cattle Veterinarians Conference*, p. 215–220.
9. **Emery RS**. 1978. Feeding for increased milk protein. *J Dairy Sci* 61: 825–828.
10. **Formigoni AM, Cornil M, Prandi A, Mordenti A, Rossi A, Portelle D, Renaville R**. 1996. Effect of propylene glycol supplementation around parturition on milk yield, reproduction performance and some hormonal and metabolic characteristics in dairy cows. *J Dairy Res* 63: 11–24.
11. **Garnsworthy PC**. 2004. Nutritional manipulation of metabolic hormones and the implications for dairy cow fertility. *Proceedings of Reproduction x Nutrition anoestrous model workshop*, McMeekan Centre, AgResearch Ruakura, Hamilton (New Zealand).
12. **Genstat**. 1997. *Genstat 5, Release 6.1*, Lawes Educational Trust, Oxford (UK).
13. **Gillund P, Reksen O, Grohn YT, Karlberg K**. 2001. Body condition related to ketosis and reproductive performance in Norwegian dairy cattle. *J Dairy Sci* 84: 1390–1396.
14. **Gong JG, Lee WJ, Garnsworthy PC, Webb R**. 2002. Effect of dietary–induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. *Repr* 123: 419–427.
15. **Grummer RR**. 1994. Effect of propylene glycol dosage during feed restriction on metabolites in blood of prepartum Holstein Heifers. *J Dairy Sci* 77: 3618–3623.
16. **Gutierrez CG, Campbell BK, Webb R**. 1997. Development of a long–term bovine granullosa cell culture system:

induction and maintenance of oestradiol production, response to follicle stimulating hormone and morphological characteristics. *Bio Repr* 56: 608–616.

17. **Holmes CW, Brookes IM, Garrick DJ, Mackenzie DDS, Parkinson TJ, Wilson GF**. 2002. Milk production from pasture, Palmerston North, New Zealand.
18. **Hunter MG, Robinson RS, Mann GE, Webb R**. 2004. Endocrine and paracrine control of follicular development and ovulation rate in farm species. *Anim Rep Sci* 82/83: 461–477.
19. **Kolver ES, Roche JR, Veth MJD, Thorne PL, Napper AR**. 2002. Total mixed rations versus pasture diets: evidence of a genotype x diet interaction in dairy cow performance. *Proceedings of the New Zealand Society of Animal Production*, New Zealand, p. 246–251.
20. **Kolver ES**. 2003. Making money from pasture and supplements. *Proceedings of the South African large herds Conference*, Drakensberg (South Africa) 3, 64–83.
21. **Lamming GE, Wathes DC, Peters AR**. 1981. Endocrine patterns of the post–partum cow. *J Repr Fert Suppl* 30: 155–170.
22. **Lean IJ, Rabiee AR, Moss N**. 2003. A hazards analysis critical control point approach to improving reproductive performance in lactating dairy cows. *Proceedings of the Society of Dairy Cattle Veterinarians*, p. 419–444.
23. **Livestock Improvement Corporation (LIC)**. 2003/4. *New Zealand Dairy Statistics*. Hamilton (New Zealand). On line: http://www.lic.co.nz/113_7.cfm.
24. **Loeffler SH, De Vries MJ, Schukken YH, de Zeeuw AC, Dijkhuizen AA, de Graaf FM, Brand A**. 1999. Use of AI technician scores for body condition, uterine tone and uterine discharge in a model with disease and milk production parameters to predict pregnancy risk at first AI in Holstein dairy cows. *Therio* 51: 1267–1284.
25. **Lucy MC**. 2001. Reproductive physiology and management of high–yielding dairy cattle. *Proceedings of the New Zealand Society of Animal Production*, p. 120–127.
26. **Lucy MC**. 2004. Mechanisms linking the somatotropic axis with insulin: lessons from the postpartum dairy cow. *Proceedings of the New Zealand Society of Animal Prodution*, p. 19–23.
27. **Macmillan KL**. 1980. Factors influencing the interval to post–partum oestrus, conception date and empty rate in an intensively managed dairy herd. *Proceedings of the New Zealand Society of Animal Production*, p. 236–239.
28. **Macmillan KL**. 1998. Reproductive management of dairy cattle. *Proceedings of Reproductive management of grazing ruminants in New Zealand*, p. 91–112.
29. **Macdonald KA, Roche JR**. 2004. *Condition scoring made easy*, Hamilton, New Zealand.
30. **Mann GE, Green MP, Sinclair KD, Demmers KJ, Fray MD, Gutierrez CG, Garnsworthy PC, Webb R**. 2003. Effects of circulating progesterone and insulin on early embryo development in beef heifers. *Anim Repr Sci* 79: 71–79.
31. **McCaffery FH, Leask R, Riley SC, Telfer E**. 2000. Culture of bovine preantral follicles in serum–free system: markers for assessment of growth and development. *Bio Reprod* 63: 267–273.
32. **McDougall S**. 1994. Postpartum anoestrum in the pasture grazed New Zealand dairy cow. *PhD thesis*, Massey University, New Zealand.
33. **McDougall S, Burke CR, Williamson NB, Macmillan KL**. 1995. Effects of stocking rate and breed on the period of postpartum anoestrum in grazing dairy cattle. *Proceedings of the New Zealand Society of Animal Production*, p. 236–238
34. **McDougall S, Rhodes F**. 2004. Nutrition by reproduction interactions in anoestrous cows: risk factors and response to theraphy. *Proceedings of Reproduction x Nutrition anoestrous model workshop*, McMeekan Centre, AgResearch Ruakura, Hamilton (New Zealand).
35. **Miyoshi S, Pate JL, Palmquist DL**. 2001. Effects of propylene glycol drenching on energy balance, plasma glucose, plasma insulin, ovarian function and conception in dairy cows. *Anim Repr Sci* 68: 29–43.
36. **Morton J**. 2000. The InCalf project – Some risk factors for reproductive performance in Australian dairy herds. *Proceedings of the Australian and New Zealand Combined Dairy Veterinarians Conference*, p. 43–61.
37. **Morton J**. 2000. The Incalf project – Describing reproductive performance in Australian herds. *Proceedings of the Australian and New Zealand Combined Dairy Veterinarians Conference*, p. 5–22.
38. **Pierson RA, Ginther OJ**. 1984. Ultrasonography of the bovine ovary. *Therio* 21: 495–504.
39. **Rhodes FM, Clark BA, Nation D, Taufa VK, Macmillan KL, Day M, McDougall S**. 1998. Factors influencing the prevalence of postpartum anoestrus in New Zealand dairy cows. *Proceedings of the New Zealand Society of Animal Production*, p. 79–81.
40. **Rhodes FM, McDougall S, Burke CR, Verkerk GA, McMillan KL**. 2003. Invited review: Treatment of cows with an extended postpartum anoestrous interval *J Dairy Sci* 86: 1876–1894.
41. **Short RE, Bellows RA, Staigmiller RB, Berardinelli JG, Custer EE**. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. *J Anim Sci* 68: 799–816.
42. **Studer VA, Grummer RR, Bertics SJ, Reynolds CK**. 1993. Effect of prepartum propylene glycol administration on periparturient fatty liver in dairy cows. *J Dairy Sci* 76: 2931–2939.
43. **Webb R, Campbell BK, Garverick HA, Gong JG, Gutierrez CG, Armstrong DG**. 1999. Molecular mechanisms regulating follicular recruitment and selection. *J Repr Fert Suppl* 54: 33–48.
44. **Wei AY, Pritts EA**. 2003. Therapy for polycystic ovarian syndrome. *Curr Op Pharm* 3: 678–682.