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Milk progesterone on day 5 following insemination in the dairy cow: associated metabolic variables and reproductive consequences

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Abstract

Despite the importance of progesterone on the fertility of lactating dairy cows, the factors that affect post ovulatory progesterone concentration are still unclear. Thus, the aim of the present study was to identify factors associated with the post ovulatory progesterone rise following 1st insemination in lactating dairy cows. Data collected across a number of complimentary studies were compiled to produce a single database of 168 lactating Holstein Friesian dairy cows maintained under commercial conditions. In all animals a number of variables were measured during the insemination period and related to milk progesterone measured on day 5 following 1st artificial insemination (AI). Overall, 44% of cows conceived to 1st AI and while mean day 5 progesterone was not significantly higher in these cows, there was a significant quadratic relationship between milk progesterone concentration and conception rate. While a number of variables showed some association with progesterone concentration, the only variable showing a strong and repeatable relationship was plasma leptin concentration. We conclude that adequate but not excessive progesterone levels on day 5 bring about a better fertility, and plasma leptin concentration may be a much better indicator of metabolic status in lactating dairy cows.

Keywords: Conception rate, dairy cow, leptin, metabolic variables, milk progesterone
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Introduction

It is now well established that failure of the corpus luteum to produce sufficient progesterone represents a major cause of early embryo loss and reproductive problems in the modern dairy cow. During early pregnancy, progesterone influences the secretory function of the uterus, including nutrients, growth factors, immunosuppressive agents, enzymes, ions, and steroids that are essential for embryo development (Graham & Clarke, 1997).

Further studies on the role of progesterone during early pregnancy in the cow have now identified the close link between maternal progesterone concentration after mating and early embryo development (Mann & Lamming, 2001; Green *et al*., 2005; Mann *et al*., 2006; McNeill *et al*., 2006; Morris & Diskin, 2008), as well as the outcome of pregnancy (Larson *et al*., 1997; Starbuck *et al*., 2001; Hommeida *et al*., 2004; Bisinotto *et al*., 2010; Kafi & Mirzaei, 2010). In both lactating (Starbuck *et al*., 2001) and non-lactating dairy cows (Mann & Lamming, 2001), a poor post-ovulatory rise in progesterone, and more specifically, poor progesterone concentrations on day 5 after mating, has been associated with poor embryo development and low pregnancy rates. Stronge *et al*. (2005) found a linear and quadratic relationship between the concentration of milk progesterone on days 5 - 7 following insemination and also between the rate of increase in progesterone between days 4 and 7 and embryo survival. Green *et al*. (2005) demonstrated that embryo developmental capacity of dairy cows is closely related to both plasma and luteal progesterone concentrations as early as day 5 post-AI. McNeill *et al*. (2006) also found a similar linear and quadratic relationship between the concentration of milk progesterone and embryo survival on days 4 to 6 after ovulation and embryo survival, and progesterone

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concentration on days 4 and 5 were highly predictive of concentrations on days 7 and 6, respectively. This provides evidence that progesterone production is key to the success of early pregnancy in the dairy cow and milk progesterone concentration on day 5 following mating can be used to monitor reproductive function during the post-partum period. Despite the importance of progesterone, many questions remain unanswered surrounding the way in which level of progesterone secretion is controlled during this important time. Studies using milk progesterone analysis to monitor luteal activity have suggested associations between factors such as milk yield and body condition score (BCS) and progesterone concentrations (Mann *et al*., 2005; Bech-Sa`bat *et al*., 2008). However, the mechanisms regulating post-ovulatory progesterone concentration are complex and require further study. Therefore, in the present study, we have investigated the relationship between milk progesterone on day 5 following insemination and a variety of metabolic variables as well as measures of reproductive performance in lactating dairy cows.

Material and Methods

Data from a total of 8 groups of cows spread across 6 herds investigating the relationships between metabolic parameters and reproductive performance in Holstein-Friesian dairy cows in the UK were collated into a single dataset of 168 cows. The cows were spring calving and multiparous, parity ranged from 2 to 6 and were randomly assigned to each group. In all studies, no experimental treatments had been imposed on the animals which were all lactating Holstein Friesian cows maintained within a range of commercial herd environments. Cows were fed various diets comprising a total mixed ration based on grass and maize silage with additional feedstuffs as well as concentrates fed according to yield. Daily feed allowances were adjusted for each cow to at least 110% of intake, to ensure *ad libitum* feeding. All work was conducted under the Animals (Scientific Procedures) Act 1986 with local ethical approval.

Depending on the study, blood samples were collected by venipuncture into heparinised tubes at various 1 to 4 week intervals during the post-partum period and kept on ice until centrifuged and plasma harvested and stored at -20 °C. In the present study, a mean value was determined for each parameter from samples collected between weeks 7 and 15 post-partum (the time period during which 1st AI occurred). In addition, animals were condition scored (on a 1 - 5 scale) and milk yield recorded at various time-points post-partum. Once again, a mean value was determined for each animal based on those measurements made between weeks 7 and 15 post-partum. Cows were artificially inseminated at observed oestrus according to routine farm practice with semen from bulls selected by the farm following a voluntary waiting period of around 7 weeks. In all animals, a milk sample was collected on day 5 following oestrus (day 0) associated with 1st insemination with cows inseminated on the day of oestrus or the following morning.

All samples were analysed for leptin by radioimmunoassay as described by Blache *et al*. (2000) with inter- and intra-assay coefficients of variation of 13.1 and 8.5% respectively and a sensitivity of 0.2 ng/ml. Milk progesterone was measured by radioimmunoassay (Lamming & Bulman, 1976) with a sensitivity of 1 ng/ml and intra- and inter-assay coefficients of variation of 11.3 and 14.2%, respectively. These two analyses were compared and show no differences in the determination of progesterone concentrations of the same samples. In Groups 1 to 4, plasma concentrations of urea were measured by an enzymatic glutamate dehydrogenase method (Randox Laboratories Ltd, Crumlin, Co. Antrim, UK) with an inter-assay coefficient of variation of 10.5%. Plasma concentrations of β-hydroxybutyrate (BHB) were measured using a commercially available (β-hydroxybutyrate procedure 310-UV, Sigma Diagnostics, Fancy Road, Poole, Dorset, UK) with an inter-assay coefficient of variation of 4.8%. In studies 5 - 8, plasma BHB and urea concentrations were measured on an OPERA (Operationally Enhanced Random Access) analyser (Bayer, Newbury, Berks, UK) using kinetic enzymatic kits (Randox Laboratories Ltd, Co. Antrim, UK). Assay sensitivity was 0.1 mmol/l for both methodologies.

All statistical analyses were performed using GenStat Version 15.0 (VSN international, UK). A summary of variables across the 8 groups of experimental animals is shown in Table 1 with data expressed as mean ± SEM. In all analyses Group was included as a main factor to ensure that any differences between animal Groups were fully accounted for in the analytical model. Relationships between variables were determined by linear regression analysis, while logistic regression was used to evaluate the relationship between each variables and subsequent conception. For the relationship between conception rate and milk progesterone concentration, further analysis was performed with milk progesterone categorised into incremental 3 ng/ml concentration intervals (<3; 3 - 6; 6 - 9; 9 - 12; 12 - 15; >15 ng/ml) to fully assess the quadratic regression relationship, as Starbuck *et al*. (2001) who reported increased embryo survival at progesterone concentration above 3 ng/ml, declining at concentrations greater than 9 ng/ml. Generalized Linear Models (GLM) regression analysis (accumulated analysis) was used to evaluate the factors that affected milk progesterone secretion.

Table 1 Mean (± SEM) concentrations for the variables measured in the 8 groups of lactating Holstein Friesian dairy cows used in the current study

Variable	Group 1 (n = 13)	Group 2 (n = 19)	Group 3 (n = 27)	Group 4 (n = 37)	Group 5 (n = 46)	Group 6 (n = 5)	Group 7 (n = 8)	Group 8 (n = 13)
Conception (%)	69.2	10.5	63.0	56.8	32.6	60.0	25.0	38.5
Day 5 MP4 (ng/ml)	7.8 ± 1.62	5.5 ± 0.54	5.4 ± 0.48	10.6 ± 0.88	9.4 ± 0.69	12.9 ± 1.72	11.2 ± 1.84	5.7 ± 0.97
Day of 1^{st}AI	64.5 ± 5.08	62.4 ± 3.51	65.6 ± 3.95	80.4 ± 3.12	89.2 ± 3.45	54.4 ± 3.59	61.5 ± 7.33	65.6 ± 5.33
Milk yield (l/d)	37.7 ± 1.68	39.3 ± 1.60	29.7 ± 1.27	37.1 ± 0.99	36.3 ± 1.39	33.4 ± 3.24	32.8 ± 1.21	27.5 ± 1.49
Condition score	1.6 ± 0.19	1.2 ± 0.15	2.1 ± 0.15	1.7 ± 0.18	1.8 ± 0.15	1.8 ± 0.69	1.1 ± 0.22	2.4 ± 0.47
Plasma leptin (ng/ml)	1.7 ± 0.19	1.2 ± 0.15	3.5 ± 0.35	1.7 ± 0.18	1.8 ± 0.15	1.8 ± 0.69	1.1 ± 0.22	2.4 ± 0.47
Plasma BHB (mmol/l)	0.6 ± 0.05	0.9 ± 0.06	0.7 ± 0.05	0.7 ± 0.03	0.6 ± 0.05	0.5 ± 0.04	0.6 ± 0.05	0.6 ± 0.03
Plasma urea (mmol/l)	6.6 ± 0.43	5.8 ± 0.19	5.7 ± 0.19	6.3 ± 0.19	7.4 ± 0.27	7.10 ± 0.47	3.9 ± 0.47	5.4 ± 0.38

MP4: milk progesterone; AI: artificial insemination; BHB: β-hydroxybutyrate

Results

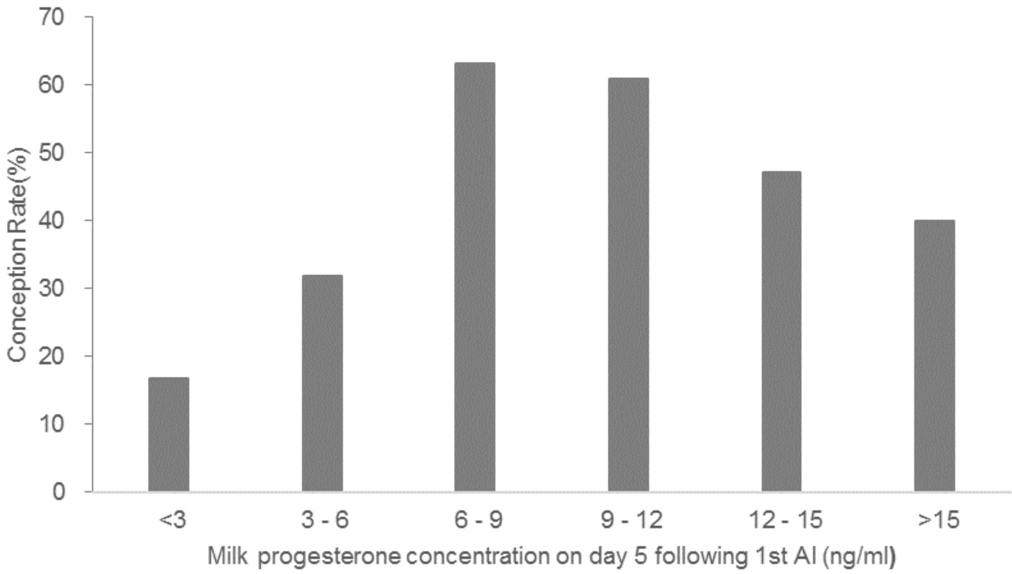
Mean ± SEM milk progesterone concentration on Day 5, milk yield, body condition score, days of first AI, plasma leptin, plasma BHB and plasma urea in cows that conceived to AI (n = 74) or did not conceive (n = 94) are shown in Table 2. Logistic regression analysis revealed no significant effect of any variable on conception rate, though milk progesterone concentration tended to have an influence (P <0.07).

Table 2 Mean (± SEM) milk progesterone (MP4), milk yield, body condition score (BCS), days of 1^{st} AI and plasma concentrations of metabolites in pregnant and non-pregnant cows

Parameters	Pregnant (n = 74)	Non-pregnant (n = 94)	Significance
Day 5 MP4 (ng/ml)	9.1 ± 0.53	7.7 ± 0.52	0.07
Days of 1^{st} AI	74.8 ± 2.43	73.9 ± 2.49	ns
Milk yield(l/d)	34.2 ± 0.93	35.5 ± 0.82	ns
Body condition score	1.9 ± 0.05	1.9 ± 0.05	ns
Plasma leptin (ng/ml)	2.2 ± 0.18	1.8 ± 0.13	ns
Plasma BHB (mmol/l)	0.7 ± 0.04	0.7 ± 0.02	ns
Plasma urea (mmol/l)	6.2 ± 0.17	6.5 ± 0.18	ns

ns: mean value within a row did not differ significantly; MP4: milk progesterone; AI: artificial insemination; BHB: β-hydroxybutyrate

However, further analysis of the relationship between milk progesterone concentration on day 5 and the outcome of insemination revealed a significant (P <0.001) quadratic relationship with both low (< 3ng/ml and 3 - 6 ng/ml) and high progesterone (>12ng/ml) concentrations associated with reduced conception rates (Figure 1). The proportion of cows with a day 5 milk progesterone concentration of < 3ng/ml was 10.7%.



Quadratic regression revealed a significant (*P* <0.001) relationship between milk progesterone and conception rate.

Figure 1 The relationship between milk progesterone concentration (ng/ml) o on day 5 following first insemination and conception rate in lactating Holstein Friesian cows.

The relationships between milk progesterone, milk yield, BCS, days of 1st AI, plasma leptin, plasma BHB and plasma urea are shown in Table 3. Concentration of milk progesterone on Day 5 after mating was positively associated with body condition score (*P* <0.05) and days of first AI (*P* <0.05) and negatively associated with plasma BHB (*P* <0.05), plasma leptin (*P* <0.07). Plasma leptin had a significant positive relationship (*P* <0.05) with body condition score and negative relationship with milk yield (*P* <0.01). Milk yield showed a significant negative association with BCS (*P* <0.05). There was also negative correlation between plasma urea concentration and days of first AI (*P* <0.05). Contrary to expectations, there was no relationship whatsoever between milk yield and progesterone concentration (Figure 2).

Table 3 The correlation coefficients between milk progesterone, milk yield, body condition score, days of 1st artificial insemination and plasma concentrations of metabolites

Parameters	MP4	Leptin	Milk yield	BCS	AI Day	BHB
Leptin	0.144					
Milk yield	-0.045	**0.286****				
BCS	0.185	**0.235****	**-0.211****			
AI Day	**0.169***	-0.051	0.034	0.103		
BHB	**-0.173***	-0.060	0.048	-0.146	0.057	
Urea	0.109	0.018	**0.159***	0.133	**0.172***	-0.053

Mean values within a row with * was positively associated with the parameter in the first column (* *P* <0.05; ** *P* <0.01).
MP4: milk progesterone; BCS: body condition score, AI: artificial insemination; BHB: β-hydroxybutyrate

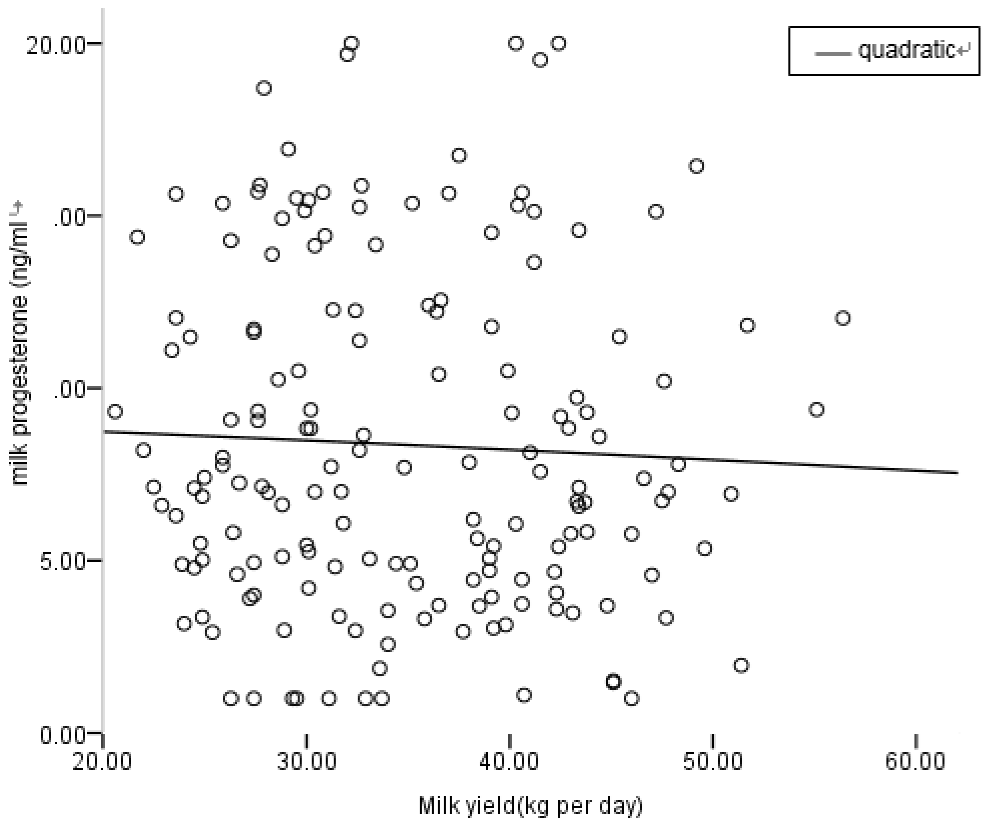


Figure 2 Quadratic relationship between milk yield (kg per day) and the concentration of progesterone (ng/ml) in milk samples collected on day 5 following first insemination in Holstein Friesian cows

The outcomes of multiple linear regression analysis of the effect of the variables measured on day 5 milk progesterone are shown in Table 4. There was a significant influence of both group (*P* <0.001) and plasma leptin concentration (*P* <0.001) on milk progesterone concentration. All the other variables had no significant influence on milk progesterone concentration.

Table 4 Accumulated regression analysis of factors affects milk progesterone production

Parameters	**Significance *P***
Group	< .001
Plasma leptin (ng/ml)	< .001
Body condition score	0.169
Milk yield (l/d)	0.144
Plasma BHB (mmol/l)	0.233
Days of 1st AI	0.324
Plasma urea (mmol/l)	0.928

AI: artificial insemination; BHB: β-hydroxybutyrate

Discussion

Progesterone (P4) is considered the gold standard for evaluation of the reproduction status for research purposes (Adriaens *et al*., 2017). A number of studies in dairy cows have reported an association between insufficient maternal progesterone production during the early pregnancy period and early embryo loss (Larson *et al*., 1997; Mann & Lamming, 2001; Starbuck *et al*. 2001; Hommeida *et al*., 2004; Green *et al*., 2005; Mann *et al*., 2006; McNeil *et al*., 2006). Progesterone concentrations on 5-10 days after insemination were higher in pregnant than non-pregnant cows in some studies (Hansel, 1981; Fonseca *et al*., 1983; Lamming *et al*., 1989; Larson *et al*., 1997; Hommeida *et al*., 2004), but not in others (Sreenan & Diskin, 1983; Nosier *et al*., 1992; Green *et al*., 2005). In the current study, the overall mean milk progesterone levels in pregnant cows did not differ significantly from non-pregnant cows (P <0.07) but there was a strong quadratic relationship with both low and high milk progesterone concentrations associated with reduced conception rates. Thus it would appear that, as well as inadequate progesterone levels, excessive progesterone levels on day 5 also present a problem. Whether these high progesterone concentrations are the result of premature development of luteal function leading to embryo–uterus asynchrony or simply reflect mistimed inseminations remains to be determined. Within the parameters of the present study, milk progesterone concentration between 6 - 12 ng/ml on Day 5 was consistent with maximal conception rate with lower or higher concentrations resulting in reduced conception rate. Similar trends in the relationship between progesterone and fertility were observed in other studies. For example, Starbuck *et al*. (2001) reported maximum conception rates at day 5 milk progesterone concentrations of 7 - 8 ng/ml while Stronge *et al*. (2005) similarly reported that maximal embryo survival with milk progesterone concentration of 7.4 ng/ml on Day 5. Cows with subnormal progesterone concentrations have a stronger luteolytic signal, which might predispose to a higher incidence of embryo loss (Mann & Lamming, 1995).

In an attempt to identify factors that affect milk progesterone concentrations, we found that plasma leptin has the most important influence on the milk progesterone concentration in dairy cows on Day 5 after mating. Previously, leptin has been shown to affect ovarian function in the cow and has been shown to have direct effects on both granulosa and theca cell function (Spicer, 2001). Nicklin *et al*. (2007) also reported the expression of leptin receptors at all stages of bovine CL, suggested that the bovine ovary is a target for leptin action, but leptin only stimulated progesterone production in the presence of IGF-1 in cultured luteal explants. Whether the association with leptin results from direct effects on the ovary or through other factors needs further study. Leptin concentrations reflect body fat levels and so higher leptin levels may reflect higher body fat reserves which could be associated with better energy status and better reproductive function. In the present study, plasma leptin showed a moderate correlation with body condition score, it was similar to other reports in dairy cows (Ehrhardt *et al*., 2000; Mann *et al*., 2005). In the past, body condition score has been used as a subjective method to assess body composition and energy balance status of dairy cattle (Dechow *et al*., 2001; Dechow *et al*., 2002; Pryce *et al*., 2001; Veerkamp *et al*., 2001). Cows that went through a period of net energy balance postpartum displayed significantly lower progesterone concentrations (Villa-Godoy *et al*.,1988; Sartori *et al*., 2004). This implies that net energy balance inhibited luteal capability for secreting progesterone, or a higher rate of metabolization in the liver of lactating cows. The significant influence of leptin on milk progesterone secretion and its correlation with BCS suggests that leptin concentration is a much better indicator of body fatness or metabolic status in lactating dairy cows.

A number of studies have reported lower progesterone concentrations in higher yielding cows and suggested increased metabolic clearance of progesterone as the principle cause of luteal inadequacy (Lopez *et al*., 2005; Wiltbank *et al*., 2014). However, in the present study, we found no relationship whatsoever between milk yield and the concentration of progesterone during a critical period of early pregnancy. It may due to the low number of animals or the variation between cows. Consequently, it is required that greater numbers of animals should be used in future experiments to draw conclusions.

Conclusion

While there was not an overall significant difference in milk progesterone concentration between those cows that conceived and those that failed to conceive there was a strong quadratic relationship with both low and high milk progesterone associated with reduced conception. The key variable associated with milk progesterone concentration appeared to be the plasma concentration of leptin with milk yield, contrary to expectations, demonstrating no relationship whatsoever.

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Authors' Contributions

LY Yan collected the data and drafted the manuscript. RS Robinson performed the statistical analysis. GE Mann designed the study and helped to draft the manuscript. ZD Shi participated in the design of the study. All authors read and approved the final manuscript.

Conflict of Interest Declaration

The authors confirm that they have no conflicts of interest to declare.

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