Prediction of pregnancy following insemination of dairy cows

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Abstract

Plasma samples from dairy cows across two seasons (warm and cold) were re-assayed utilizing a more sensitive and uniform procedure in order to assess the use of progesterone and PGFM concentrations on the day of AI (Day 0) to day 8 in predicting future pregnancy status of the cow (pregnant vs. open, maintenance vs. loss of pregnancy). The effect of season on progesterone and PGFM serum levels was also assessed. Progesterone concentrations on the day of AI did not differ between cows inseminated during different seasons, nor did they differ between cows of different pregnancy statuses. PGFM concentrations on the day of AI also did not display any differences in cows of various pregnancy statuses, but there appeared to be a seasonal effect, with cold season cows maintaining higher PGFM levels than warm season cows. These results were unexpected, but do suggest that PGFM and progesterone levels on the day of AI may not be the best predictive factor for establishment of pregnancy.

Introduction

In the dairy cow, the production of progesterone from a corpus luteum initiates maternal recognition of the conceptus and maintenance of pregnancy, resulting in development of the uterine endometrium to sustain the embryo (Sreenan et al., 2001). It is believed that a faster post-ovulatory rise in progesterone days 1-5 after AI increases the likelihood of the cow becoming pregnant (Ahmad et al., 1996; Butler et al., 1996). Similarly, data collected from the Multistate Hatch Project NE- 1007 found that cows with plasma progesterone levels slightly above normal on the day of AI were less likely to become pregnant than cows with lower plasma progesterone levels, suggesting that residual plasma progesterone at AI may be used as a predictive factor in determining whether or not the cow will become pregnant.

In contrast to progesterone's role in maintaining pregnancy, uterine prostaglandin $F_{2\alpha}$ (PGF) functions to terminate pregnancy by initiating luteolysis. The timing of large losses of early embryos from days 4-9 after AI corresponds to the timing of increased uterine PGF concentrations in cows with inadequate progesterone levels, suggesting that a slower rise in progesterone after AI may allow for increased uterine PGF release and accumulation, which is detrimental to the embryo (Inskeep, 2004). Prior to ovulation, the uterus releases PGF, causing luteolysis of the previous corpus luteum. Once the corpus luteum has regressed, development and ovulation of the next oocyte is permitted. However, the regressed corpus luteum may still be the source of an embryotixic factor (Inskeep, 2004). Neither this embryotoxic factor nor its source has yet to be identified, although it appears to work in conjunction with uterine PGF. PGF may be directly measured by its plasma metabolite, PGFM.

By re-assaying plasma samples from Multistate Hatch Project NE- 1007, we sought to compare pregnant and non-pregnant cows for two seasons for progesterone and PGFM (after eliminating any non-pregnant cow with progesterone greater than 0.7 ng/ml on day of AI, and any cows that appeared to be non-ovulating due to maintenance of progesterone levels below 1 ng/ml on day 5 post-AI). Using statistical analysis, we will compare the effect of season on progesterone and on PGFM. If season is not significant, we will compare the levels of progesterone and PGFM in pregnant vs. non-pregnant, and maintenance of pregnant vs. pregnant and subsequent loss of the conceptus.

Methods and Materials

Plasma samples collected from cows (open, maintained pregnancy, and lost pregnancy) on days 0-8 after AI from the NE-1007 project were re-assayed for plasma progesterone and PGFM. The assay was made more sensitive and uniform by reassaying all samples with one set of reagents, and by using a larger sample volume. The procedure called for a radioimmunoassay using ^{125}I. Data from the re-assayed plasma samples were used to examine the interference of low plasma progesterone at AI on pregnancy outcome, and to compare PGFM levels between pregnant and non-pregnant cows.

Using an analysis of variance ($p = 0.05$), we examined the levels of progesterone and PGFM on the day of AI to determine whether or not they were affected by season, and if they affected future pregnancy status. If there was no seasonal effect, PGFM and progesterone would be entered into a repeated measures analysis of variance ($p = 0.05$) to examine whether their concentrations during days 0-8 after AI were influenced by season, and if they influenced future pregnancy status.

Results

Progesterone Concentration on the Day of AI- For progesterone on the Day of AI, pregnant cows appeared to have slightly higher progesterone levels than non-pregnant cows, and cold season animals had higher concentrations than warm season animals. Upon ANOVA testing, pregnancy status, season, and pregnancy status + season yielded no significant differences.

Table 1: Progesterone on the day of AI

	Pregnant		Non-pregnant	
Season	N	Progesterone (ng/ml±SE)	N	Progesterone (ng/ml±SE)
Cold	25	0.1551 ± 0.0267	38	0.1871 ± 0.0217
Warm	23	0.1374 ± 0.0279	42	0.1627 ± 0.0206

PGFM Concentration on the Day of AI- For PGFM on the day of AI, non-pregnant cows appeared to have higher PGFM levels than pregnant cows, but after statistical analysis, pregnancy status of the cows was found to be insignificant. However, there were differences in PGFM concentrations between cows in the warm and cold season. Cold cows maintained a much higher PGFM concentration than warm cows. ANOVA testing revealed that season had a significant effect on PGFM ($p < 0.0001$).

Table 2: PGFM on the Day of AI.

	Pregnant		Non-pregnant	
Season	N	PGFM (ng/ml±SE)	N	PGFM (ng/ml±SE)
Cold	10	91.24 ± 13.038[a]	11	101.62 ± 12.441[a]
Warm	13	39.58 ± 11.444[b]	12	47.18 ± 11.912[b]

[a,b] Means are significantly different, $P = 0.0001$

Progesterone Concentrations Post-AI (Day of AI-Day 8)- For cows of various pregnancy status (Pregnant vs. Open) there appeared to be little deviation in progesterone concentrations during the first few days post-AI. ANOVA testing yielded an insignificant effect of pregnancy status on progesterone concentration during early pregnancy.

Table 3: Progesterone Post-AI by Pregnancy Status

Pregnancy Status	N	Progesterone (ng/ml±SE)
Pregnant	432	1.357162 ± 9.218541 E -2
Open	729	1.325582 ± 7.096436 E -2

For cows in different seasons, warm season cows appeared to have a slightly elevated concentration of progesterone than cold season cows, although standard errors remained relatively the same. After repeated measures ANOVA testing, season was found to have a significant effect on progesterone concentration, with warm season cows maintaining higher concentrations ($p<0.05$).

Table 4: Progesterone Post-AI by Season

Season	N	Progesterone (ng/ml±SE)
Cold	576	1.223619 ± 7.98349 E −2 [a]
Warm	585	1.459126 ± 7.921841 E -2 [b]

[a,b] Means are significantly different, $P < 0.05$.

For cows by pregnancy status and day, there is a rapid rise in progesterone concentrations occurring about two days after AI. There was no significant difference in progesterone concentrations on the day of AI between cows that lost their conceptus and those that maintained their pregnancy. This trend appeared to carry over into the days post-AI, as there appeared to be little difference between progesterone concentrations in cows of different pregnancy status.

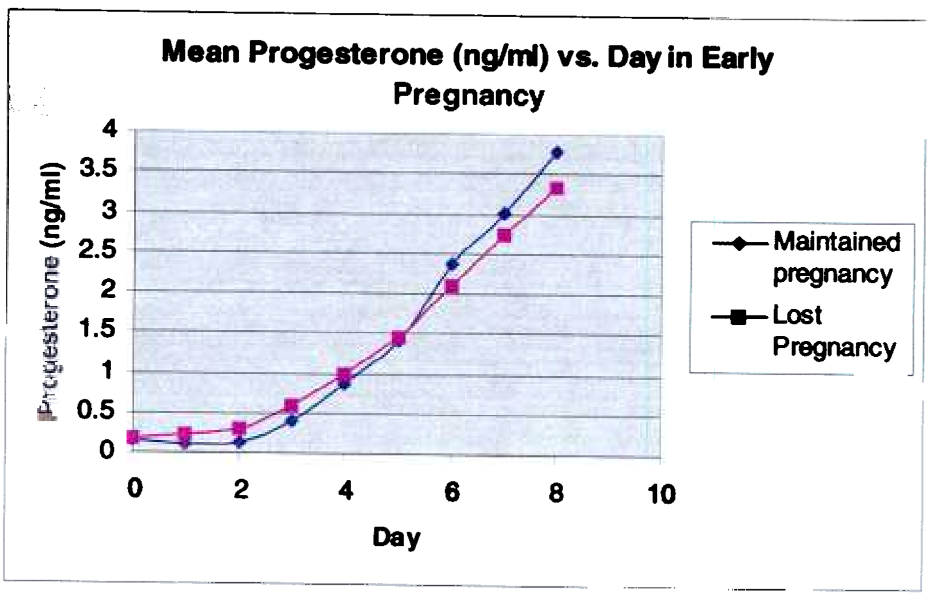


Figure 1: Pregnancy Status by Day
St Err of cows that maintained their pregnancy (n=48) = 0.08 ng/ml
St Err of cows that lost their conceptus (n=81) = 0.15 ng/ml

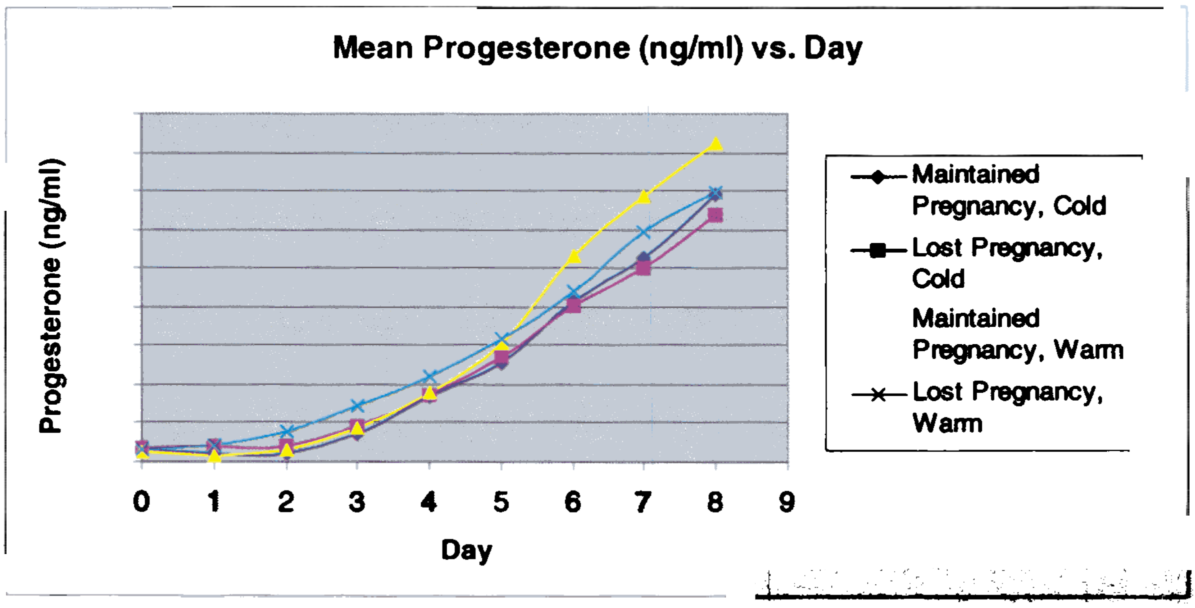
The rise in progesterone begins to increase most rapidly two days after AI. All pregnant cows appear to maintain similar concentrations of progesterone in the first few days post-AI, regardless of subsequent pregnancy loss status.

Comparing cows by season and day, we also saw the steady increase in progesterone concentrations following AI. However, warm season cows were found to be maintaining significantly higher concentrations of progesterone than cold season cows ($p<0.0001$)

Table 5: Progesterone (ng/ml) Season by Day

Season	Day								
	0 (AI)	1	2	3	4	5	6	7	8
Cold	0.16997	0.15254	0.15833	0.41987	0.86180	1.31613	2.03800	2.56923	3.32671
Warm	0.15005	0.14788	0.26743	0.57055	1.00588	1.56066	2.43025	3.19136	3.80807

Comparing cows by pregnancy status and season, we observed the steady increase in progesterone concentrations accompanying AI. There did not appear to be large differences in the rise of progesterone between maintained + cold, lost + cold, maintained + warm, and maintained + cold, although warm cows of either pregnancy status appeared to maintain slightly higher progesterone. After statistical analysis, differences between the four groups of cows were found to be insignificant.



The rise in progesterone concentration begins to increase most rapidly at day 2 after AI. Progesterone levels appear to be relatively similar between all four categories of cows.

Progesterone Concentration According to Pregnancy Loss Status during Early Pregnancy-

Comparing pregnant cows that maintained their pregnancy with those that underwent early embryo loss, it appeared that cows that maintained pregnancy had a somewhat higher progesterone concentration. However, upon repeated measures ANOVA testing, progesterone concentrations between cows that maintained vs. those that lost their pregnancies were found to be insignificant.

Table 6: Progesterone during Early Pregnancy by Pregnancy Loss Status

Pregnancy Loss Status	N	Progesterone (ng/ml±SE)
Maintained Pregnancy	333	1.37636 ± 6.688936 E -2
Lost Pregnancy	99	1.286248 ± 0.1226766

Comparing pregnant cows by warm and cold season, warm season cows displayed much more elevated progesterone levels than cold season cows. After repeated measures ANOVA testing, season was found to have a significant effect on early progesterone concentration ($p<0.04$). All pregnant cows in the warm season had higher progesterone, although we did not distinguish between pregnancy loss status.

Table 7: Progesterone During Early Pregnancy for All Pregnant Cows by Season

Season	N	Progesterone (ng/ml±SE)
Cold	225	1.179006 ± 8.137442 E −2 [a]
Warm	207	1.483603 ± 0.0848387 [b]

[a,b] Means are significantly different, $P < 0.04$

PGFM Concentrations Post-AI (Day of AI-Day 7)- For cows of various pregnancy status (Pregnant vs. Open) there appeared to be little deviation in PGFM concentrations during the first few days post-AI, although open cows seemed to have a slightly higher level of PGFM. Repeated measures ANOVA testing yielded an insignificant effect of pregnancy status on PGFM concentration during early pregnancy.

Table 8: PGFM Post-AI by Pregnancy Status

Pregnancy Status	N	PGFM (ng/ml±SE)
Pregnant	198	61.92581 ± 7.635195
Open	183	64.71516 ± 7.94195

For cows in different seasons (warm vs. cold), cold season cows appeared to have slightly elevated concentrations of PGFM than warm season cows, although standard errors remained relatively the same. After repeated measures ANOVA testing, season was found to have a significant effect on PGFM levels, with cold season cows maintaining higher concentrations ($p<0.03$).

Table 9: PGFM Post-AI by Season

Season	N	PGFM (ng/ml±SE)
Cold	181	75.72205 ± 7.985708 [a]
Warm	200	50.91893 ± 7.596923 [b]

[a,b] Means are significantly different, $P < 0.03$.

Mean PGFM across seasons by day, regardless of pregnancy status was graphed and analyzed. It appears there is a seasonal effect on PGFM levels in the days after AI, with cold season yielding higher concentrations. After testing with repeated measures ANOVA, season was found to be a significant influence ($p = 0.009$).

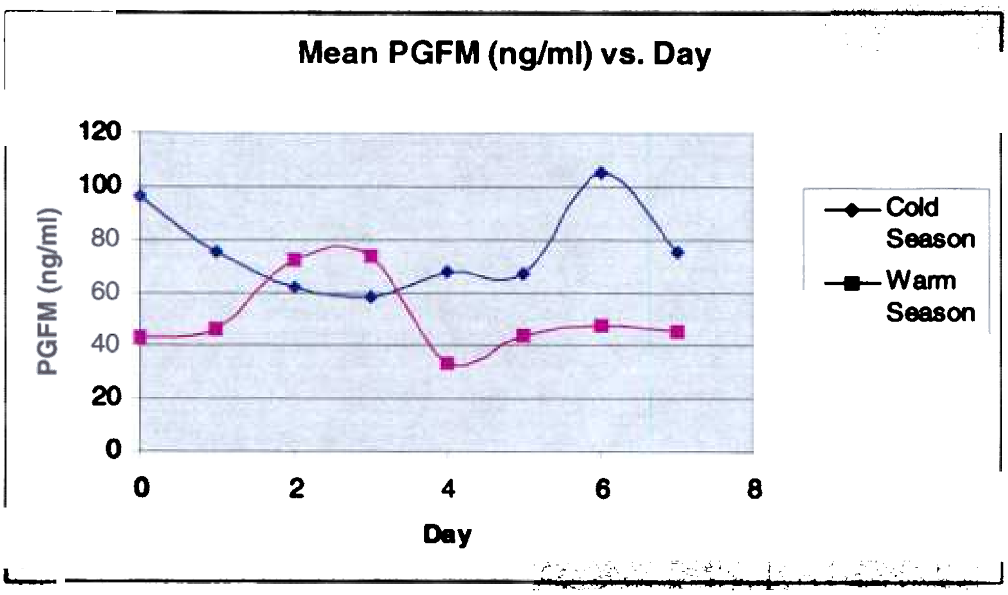


Figure 3: PGFM concentration by Season and Day Post-AI
St. Err Cold Season (n= 23)=11.41
St. Err Warm Season (n= 25)=10.94

The pattern of PGFM concentrations is highly variable between cold and warm season cows, with cold season cows maintaining what appear to be higher levels.

PGFM Concentration According to Pregnancy Loss Status during Early Pregnancy- Comparing pregnant cows that maintained their pregnancy with those that underwent early embryo loss, it appeared that cows that lost pregnancy had a somewhat higher PGFM concentration. However, upon repeated measures ANOVA testing, PGFM concentrations between cows that maintained vs. those that lost their pregnancies were found to be insignificant.

Table 10: PGFM during Early Pregnancy by Pregnancy Loss Status

Pregnancy Loss Status	N	PGFM (ng/ml±SE)
Maintained Pregnancy	110	60.56629 ± 12.32791
Lost Pregnancy	88	62.32443 ± 13.78302

Season was also found to have an insignificant impact on pregnancy loss status.

Discussion

Previous studies of heat stress on progesterone have yielded mixed results. Many have reported the tendency of heat stress to increase (Abilay et al., 1975; Trout et al., 1998), decrease (Jordan, 2003; Collier et al., 2006), or have no effect (Wise et al., 1988; Wolfenson et al., 1995; Wilson et al., 1998) on concentrations of progesterone during the luteal phase of the estrous cycle. In the present study, we did not find a significant difference in progesterone levels between cows in the warm season and the cold season. Wilson et al. (1998) found that serum progesterone was similar in heat stressed and thermo-neutral cows until day 16 of estrus. After day 16, progesterone concentrations in the thermo-neutral cows were lower than those of the heat stress cows, thus increasing the likelihood of pregnancy.

However, the majority of previous studies have noticed a negative effect of heat stress on reproduction and hormone concentrations. When an animal encounters environmental heat stress such as during warmer months, blood flow is diverted from the central organs to the peripheral surface of the body. Heat stress is capable of affecting the entire body, such as the metabolism of vital organs, and the dilution and concentration of blood plasma. Each of these other symptoms may affect ovarian steroid production and its concentration in the blood. The accompanying redistribution of nutrients and hormones could result in a slight decrease of reproductive function (Hansen and Are´chiga, 1999).

There also several observations indicating possible link between season and female fertility Lower progesterone concentrations and abnormal estrus ycle lengths ha been observed in Brahman heifers luri winter' short-o y thes Stahringer al 990). The

be and size of follicles per has bee nd he greate the prin than the fal suggestin that may also influence follicular populations in Lammoglia et al. 995

Unlike progesterone, the present stud found that PGFM gnificantly affected by

ld season cows vie ded higher lev PGFM than arm season cows Ou ults do not agree ith those of previous studies, which obse ed that heat and tended result higher PGFM. Heat shocks of 42 and 43' C cultured endometrial explants increased prostaglandi day of the ycle Putney al 988 Furthermore, heat stress on day of pregnancy ncreased uteri production prostag andi F2α response to oxytoc W fen al 993 Alth gh PGFM ls higher durin the season. the did not alter pregnancy success. pregnancy rates similar for the

34% in the cold season vs. % the

The lack of gnificant differences between PGFM and progesterone of of different pregnanc status was unexpected. Various stero trati udi proge ite follicles of pregnant pregnan ha been ound he differe (De los yes al 2006 Differences between pregnant who maintained pregnancies and those ho lost pregnancies be attributed to the timing of conceptus loss imilar studies ha shown what appear to be sudden bryo pregnan In tarbuck al 2004 bryonic death tended precede teal gress most that ost ptu though peripheral concentrations of progesterone appeared adequate main pregnanc

In Putney et al. 989 plasma concentration of PGFM was similar among regardless of pregnancy status Injections of ytoci increased the mean PGFM concentration. the as higher fo pregnan and pregnan ith mbry retarded de opmen than for pregnant th normal bryos Heat increased oxytoc induced PGFM concentration pregnant but had effect PGFM pregnant

Conclusion

There was no clear distinction between levels of progesterone or PGFM on the day of AI or the days following AI that presented clues to the future pregnancy statuses of cows. However, there was a difference in PGFM concentration between cows bred during the warm season and those bred during the cold season. Cows inseminated in the cold season had higher PGFM levels. While these results are unexplainable, higher PGFM concentrations during the cold season did not interfere with pregnancy rates. Therefore, early progesterone and PGFM concentrations may not be the most accurate prediction of future pregnancy status. Further research should be directed at investigating the seasonal effects on PGFM concentrations, since there is little research concerning this area.

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