PEER REVIEWED

Impact of a Progesterone-Releasing Intravaginal Device on Plasma Progesterone Levels in Lactating Dairy Cattle

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Abstract

Plasma progesterone (P4) levels were assessed by radioimmunoassay in lactating dairy cows treated with a progesterone-releasing intravaginal device (PRID). Group A cows (N = 6) received no treatment. PRIDs were administered to Group B1 (N = 3) on day 25 after the first observed estrus (Day 0) and to Group B2 (N = 3) on day 31, and were withdrawn 14 days after administration. Blood was collected daily from day 0 until 56 hours after withdrawal of the PRID (Group B), or on alternate days until a second estrus was observed (Group A). The area under the P4 curve (AUC) did not differ for Groups A and B pre-insertion of the PRID, but was significantly greater for Group B post-insertion. The AUC in Group B cows was significantly greater post-insertion than pre-insertion, but AUC did not differ in Group A cows for these study days. Maximum P4 was significantly greater in Group B1 post-insertion than pre-insertion. Results of this study are consistent with clinical studies demonstrating the efficacy of the PRID in estrus synchronization and treatment of infertility, and support the hypothesis that these effects are due, at least in part, to modulation of circulating progesterone levels.

Résumé

Le niveau de progestérone (P4) dans le plasma a été évalué par dosage radio-immunologique chez des vaches laitières traitées avec un dispositif intravaginal libérant de la progestérone. Les vaches du groupe A ne recevaient pas de traitement (N = 6). Les dispositifs ont été mis en place chez les vaches du groupe B1 (N = 3) 25 jours après le début du premier œstrus observé (jour 0) et 31 jours après chez les vaches du groupe B2 (N = 3). Les dispositifs ont été laissés en place pendant 14 jours. Des échantillons sanguins étaient prélevés quotidiennement et ce jusqu'à 56 heures suivant le retrait du dispositif dans le groupe B ou à tous les deux jours et ce jusqu'à l'apparition du second œstrus dans le groupe A. L'aire sous la courbe de P4 était similaire dans tous les groupes avant la mise en place des dispositifs mais était significativement plus élevée dans le groupe B suivant l'implantation. L'aire sous la courbe chez les vaches du groupe B était significativement plus élevée après l'implantation qu'avant. Une telle différence n'était pas observée chez les vaches du groupe A. La concentration maximum de P4 était significativement plus élevée dans le groupe B1 après l'implantation. Les résultats de cette étude sont compatibles avec les études cliniques démontrant l'efficacité des implants dans la synchronisation des oestrus et le traitement de l'infertilité. Ces résultats appuient aussi l'hypothèse selon laquelle ces effets sont causés, au moins en partie, par la modulation des niveaux circulants de progestérone.

Introduction

Numerous studies have reported the association between pre-ovulatory progesterone concentrations and subsequent fertility in dairy cows.[10,13,15] The same mechanism is believed to be responsible for the effect of either endogenous or exogenous progesterone on reproductive performance. The progesterone-releasing intravaginal device[a] (PRID) is a hormone delivery system consisting of a coiled silicone rubber cylinder impregnated with 1.55 grams of progesterone (P4). This device has proven effective in treatment of anestrus in dairy cattle,[7,13] as a component of estrus synchronization regimes[3,8] and in treatment of cystic ovarian disease.[2,22] In addition, cows treated with P4 post-insemination had fewer days open, and embryonic and early fetal losses were lower, compared to untreated cows.[11,19,20]

Although clinical effects of the PRID are believed to result from increased levels of circulating progesterone, there have been, to our knowledge, no studies on the effect of PRID administration on sequential plasma progesterone levels. The objective of this study was to evaluate the influence of the PRID on plasma progesterone levels in lactating dairy cattle, with the intention of elucidating the physiologic mechanism underlying its clinical effectiveness.

Materials and Methods

Animals

Twelve lactating, clinically healthy, mature Holstein cows (parities one to five) from a single research herd, in average or good general condition, were identified with ear tags, housed in tie stalls at the Ponsonby Dairy Research Station (University of Guelph, Ponsonby, Ontario) and fed a standard dairy ration (corn silage, haylage, mixed grains, and protein supplement). Normal estrous cycles had been observed in all animals. Before inclusion in the trial, all cows were subjected to a complete physical examination, including transrectal palpation of the genital tract. Cows were enrolled in the study at the first observed natural estrus if they were a minimum of 50 days-in-milk, with actual days-in-milk at entry ranging from 50 to 210. Cows received no hormonal treatment during the 30 days preceding the study. All animals were housed and monitored according to the guidelines of the Canadian Council on Animal Care.[14]

Experimental Design

Cows were selected to reflect a range of parities and days-in-milk, and were assigned using a formal randomization process to treatment groups A and B, with primiparous and multiparous cows balanced across treatment groups. Group A cows (controls, N = 6) received no hormonal treatment. Group B cows (N = 6) were scheduled for treatment with a PRID. All cows were released into an exercise paddock daily and observed for signs of estrus for a full estrous cycle, beginning on the day of the first observed estrus (day 0). Group A cows were similarly monitored for a second full cycle.

A PRID was inserted into the vagina of the three cows in Group B1 on day 25, and into the three cows in Group B2 on day 31. In both groups, the PRIDs were removed 14 days after insertion, day 39 for Group B1 and day 45 for Group B2.

Blood samples were collected from Group A cows at 48-hour intervals and from Group B cows at 24-hour intervals throughout the 47-day trial. The last samples were collected either when a second estrus was observed (Group A), or 56 hours after withdrawal of the PRID (Group B).

Plasma Progesterone Assay

Blood samples were collected from the coccygeal vein into 10-mL heparinized Vacutainers®[b] identified with the cow's ear tag number, and time and date of sampling. Samples were centrifuged and plasma was harvested and frozen within two to three hours of collection. Plasma samples were analysed for P4 by radioimmunoassay (RIA) using a modification of the Coat-A-Count® assay,[c] which was validated for bovine plasma using standard methods (E. James Squires, unpublished data, 1994).

All plasma samples from Group A and Group B cows were analyzed on one occasion, with assays performed in triplicate. Samples were thawed, mixed well, and assayed with the RIA using a single lot of I^{125}-labeled progesterone tracer and a single lot of antibody-coated tubes. Manufacturer's directions were followed exactly, except that 0.5 mL of labelled progesterone was used, with a total sample volume of 0.1 mL containing 50 μL of plasma with 50 μL PBS-gelatine. Two control samples, one with high and one with low P4 levels (previously assayed), were included in the analysis.

Statistical Analysis

Mean concentrations of plasma P4 for Groups A, B1 and B2 were plotted against time (study days 0 to 47). Data were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System[d] (SAS) for Personal Computers Version 6.12. For comparisons of the areas under the relevant plasma P4 curves (AUCs) and maximum P4, the data were analyzed as a split-plot design with treatment (A, B1, B2) as whole-plot factors, and time period (pre-insertion and post-insertion of the PRID) as split-plot factors. For maximum P4, which was considered to be of secondary interest, the analysis was limited to three specific contrasts: Group A cows compared to B1 and B2 cows post-insertion of the PRID; B1 cows pre- and post-insertion; and B2 cows pre- and post-insertion. Frequency distributions of the residuals from each model, as well as plots of residuals by predicted value and treatment, were prepared and examined for normality and homogeneity of variance. For all analyses, the level of significance was set at $P \leq 0.05$.

Results

Clinical and Plasma Progesterone Data

No PRIDS were lost during the trial. One cow in Group B1 was treated for clinical mastitis during days five to seven of the trial.

Temporal patterns of plasma P4 concentration for Groups A and B are shown in Figure 1. Results for analysis of areas under the relevant plasma progesterone

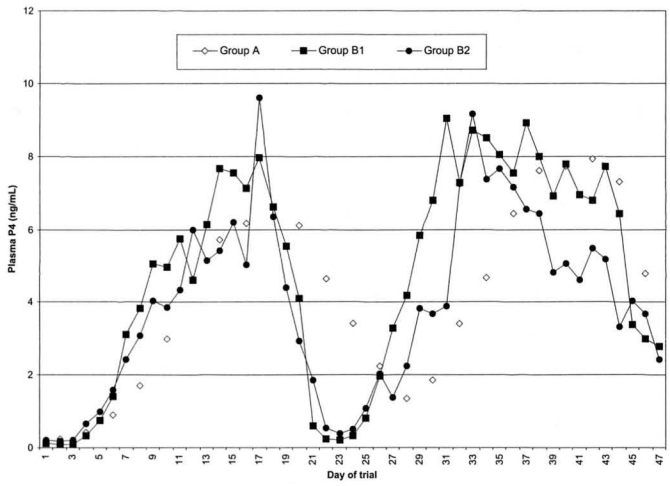

Figure 1. Plasma progesterone (P4) levels in 12 lactating dairy cows. All cows entered the study when first estrus was observed (day 0). Progesterone-releasing intravaginal devices (PRIDs) were inserted in 3 cows on day 25 (Group B1) and 3 cows on day 31 (Group B2), and were withdrawn 14 days post-insertion. PRIDs were not administered to Group A cows. Blood samples were collected from Group A cows at 48-hour intervals until a second estrus was observed and from Group B cows at 24-hour intervals until 56 h post-withdrawal of the PRID. Samples were assayed for P4 using a radioimmunoassay validated for bovine plasma.

Table 1. Area under the plasma progesterone curve (AUC) for lactating dairy cows either treated with a progesterone-releasing intravaginal device (PRID) for 14 days (Group B; N = 6), or not treated (Group A; N = 6). All cows entered the study on the day of the first observed estrus; PRIDS were administered 25 days (N = 3) or 31 days (N = 3) later. Blood samples were collected from Group A cows at 48-hr intervals until the second observed estrus and from Group B cows at 24-hour intervals until 56 h after withdrawal of the PRID. Plasma progesterone was assayed by radioimmunoassay.

Study group	AUC (mean ± SE)	
	Pre-insertion period	Post-insertion period
A	42.07 ± 13.27[a]	44.49 ± 13.27[a]
B	38.03 ± 13.27[a]	90.47 ± 13.27[b]

[a,b] Values in the same row or column with different superscripts are significantly different ($P<0.05$).

curves (AUCs) for Group A and combined Group B1 and B2 cows before and after insertion of PRIDs are shown in Table 1. The AUC for Group B cows was significantly greater post-insertion than pre-insertion ($P=0.02$). However, there was no significant difference ($P=0.90$) in the AUC for Group A cows for the study days that corresponded to the pre- and post-insertion periods for Group B cows.

There was no significant difference in the pre-insertion AUC for Group A and Group B cows ($P=0.83$); however, the AUC post-insertion was significantly greater for Group B cows than for Group A cows ($P=0.03$).

In Group B1 cows, maximum P4 concentration was significantly greater post-insertion (11.44 ng/mL) than pre-insertion (8.67 ng/mL; $P=0.03$), but was not significantly different pre-insertion (10.2 ng/mL) and post-insertion (9.8 ng/mL) in Group B2 cows ($P>.05$).

Discussion

Results of this investigation demonstrate that the PRID is effective in increasing plasma progesterone levels in lactating dairy cattle. Post-insertion AUC for treated cows (Group B; 90.47) was more than twice that of untreated cows (Group A; 44.49) on the corresponding study days, and the AUC for treated cows post-insertion (90.47) was more than twice the pre-insertion level (38.03). Additionally, maximum plasma P4 was approximately 32% greater post-insertion than pre-insertion for B1 cows (PRID inserted on day 4 of the second estrous cycle). These differences were statistically significant. Areas under the P4 curves reflect both the magnitude and the duration of elevation of plasma progesterone levels, and, by virtue of the study design, duration of progesterone secretion was the same for each cow. As the AUC represents mean P4 concentration in the plasma samples and thus reflects total plasma P4 concentration, the AUC is a plausible surrogate measure of total plasma P4. In addition, we believe the area under the P4 curve constitutes a biologically relevant measure of progesterone level in the context of this investigation. Furthermore, the post-insertion increases in both the AUC and maximum plasma P4 in treated cows are of biologically relevant magnitudes. Nevertheless, maximum plasma P4 concentration in treated cows did not exceed that reported for pregnant cows.[6]

The PRID has demonstrated clinical efficacy in induction and synchronization of estrus, treatment of cows failing to return to estrus postpartum, treatment of cystic ovarian disease and reduction of days open in dairy cows.[2,3,7,8,11,13,19,22] Results of this study are consistent with the hypothesis that these clinical effects are mediated by an increase in circulating progesterone levels post-insertion of the PRID, and a decrease following its removal.

Progesterone, produced by the corpus luteum, regulates gonadotropin-releasing hormone release from the hypothalamus,[9] which in turn dictates luteinizing hormone (LH) pulses which support growth of the dominant follicle and its production of estradiol through to ovulation.[1] During the luteal phase, a high progesterone concentration is required to suppress LH secretion and permit development of a single dominant follicle.[11] During proestrus, progesterone concentration decreases and estradiol increases. A pre-ovulatory surge of estradiol is required to establish a subsequent cycle of normal duration.[12] In the absence of an established ovarian cycle, the hypothalamus is unresponsive to elevated estradiol, and the estradiol-induced LH surge required for ovulation and establishment of a normal luteal phase fails to occur.[21] Recent studies have reported that >25% of high-producing dairy cows fail to establish a normal ovarian cycle by 60 days postpartum,[10,17] an event associated with a greater number of days open and failure to conceive. In lactating dairy cows on a high plane of nutrition, poor reproductive efficiency may be associated with low circulating concentrations of progesterone and estradiol that are related to an increased rate of hepatic metabolism of steroids.[16] Progesterone supplementation is thought to re-establish a positive feedback mechanism between LH-releasing hormone and estradiol, and a clinical response to exogenous progesterone supplementation at the beginning of the breeding period could be expected in anestrus animals.

Delayed or insufficient progesterone production post-insemination has been associated with short luteal phases and lower conception rate. Hommeida *et al*[6] reported a positive correlation between conception rate after first insemination and total progesterone secreted in milk, which has a close correlation with plasma progesterone.[4,5] In another study, in animals synchronized with two doses of prostaglandin 14 days apart, conception rate was 36% in those with plasma progesterone <5.0 ng/mL two days before the second injection of prostaglandin, compared to a 75% conception rate in those with plasma progesterone ≥5.0 ng/mL.[13] Starbuck *et al*[18] reported that in cows confirmed pregnant five weeks post-insemination, early fetal death occurred in 50% of cases when serum progesterone was ≤2.8 ng/mL, and there was a greater risk of embryonic or early fetal death associated with the presence of multiple corpora lutea, which are believed to represent multiple ovulations.

In this study, the effect of PRID administration on plasma progesterone levels in Group B cows, as determined by pre-insertion and post-insertion AUCs and maximum P4 comparisons, is potentially confounded by time period. However, the validity of pre-insertion and post-insertion comparisons is supported both by the significantly larger AUC in the Group B cows compared to the Group A cows post-insertion, and by the absence of a significant difference in the AUC or maximum progesterone levels in Group A cows, pre-insertion and post-insertion.

Conclusions

Results of this study suggest that administration of the PRID to lactating dairy cattle results in statistically and biologically significant increases in mean and maximum plasma progesterone levels and total circulating progesterone. These results are consistent with clinical studies demonstrating the efficacy of the PRID in estrus synchronization and treatment of infertility, and support the hypothesis that these effects are due, at least in part, to modulation of circulating progesterone levels.

Endnotes

[a] Vetoquinol, Montreal, Quebec, Canada
[b] Becton Dickinson, Oakville, Ontario, Canada
[c] Inter Medico, Markham, Ontario, Canada
[d] SAS Institute, Cary, NC

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