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Evaluation of models to induce low progesterone during the early luteal phase in cattle

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Abstract

Two experiments were designed to evaluate models for generation of low circulating progesterone concentrations during early pregnancy in cattle. In experiment 1 17 crossbred heifers were assigned to either prostaglandin F2α (PG) injections on Days 3, 3.5 and 4 (PG3; n=9) or controls (n = 8). Blood samples were collected from heifers from Days 1 to 9 for progesterone assay. Progesterone concentrations were decreased (P<0.03) between 18 and 48 h after first PG injection in heifers assigned to PG3 compared with controls. In Experiment 2, 39 crossbred heifers detected in estrus were inseminated (Day 0) and assigned to either: i) PG injection on Days 3, 3.5 and 4 (PG3; n=10), ii) PG injection on Days 3, 3.5, 4 and 4.5 (PG4; n=10), iii) Progesterone Releasing Intravaginal Device (PRID) insertion on Day 4.5 with PG injection on Days 5 and 6 (PRID+PG; n=10), or iv) control (n = 9). Blood samples were collected daily until Day 15 and conceptus survival rate was determined at slaughter on Day 16. Progesterone concentrations during the sampling period in the PG3 and PG4 groups did not differ, but were less than controls (P<0.01). After an initial peak, progesterone concentrations in the PRID+PG group were similar to controls. More heifers in the PG4 group (6/10) had complete luteal regression than in the PG3 group (3/10). Conceptus survival rate on Day 16 did not differ between groups. There was a significant correlation between progesterone concentration on Days 5 and 6 and conceptus size on Day 16 (P<0.03). In summary, treatment with PG on Days 3, 3.5 and 4 post-estrus appeared to provide the best model to induce reduced circulating progesterone concentrations during the early luteal phase in cattle.

1. Introduction

Progesterone is an essential hormone for the establishment and maintenance of pregnancy in mammals [1]. In cattle, 40% of conception loss occurs in the period from Days 8-16 of pregnancy (Day 0 = ovulation) [2]; a substantial proportion of this loss may be attributable to inadequate circulating progesterone concentrations and the subsequent downstream consequences on endometrial gene expression [3] and histotroph secretion into the uterine lumen [4]. The concentrations of circulating progesterone during early pregnancy have a significant effect on the survival of the embryo/conceptus [5,6]. Low concentrations of progesterone on Days 3-8 of pregnancy result in smaller embryos at later stages of the preimplantation period [7], with a lower potential to produce sufficient interferon-τ or other pregnancy specific factors to override the default luteolytic mechanisms in cattle. A delay in the post-ovulatory rise of progesterone has been associated with a decreased pregnancy rate in dairy cows and beef heifers [6,8,9].

Several studies have reported a positive association between elevated progesterone in the early post conception period and an advancement of conceptus elongation in ruminants [10-12]. However, in order to truly test the importance of progesterone for embryo survival, a model in which low progesterone concentrations can be maintained is required. This, however, is fraught with difficulty due to the necessity to maintain progesterone concentrations above a threshold below which pregnancy would be terminated. The two main approaches to achieve low progesterone concentrations *in vivo* are by surgical removal of the corpus luteum (CL) bearing ovary or the CL itself [13], or pharmacological manipulation of the CL, each method possibly followed by supplementation with the desired progesterone concentration from an exogenous source. Surgical intervention has an associated risk [14] such as hemorrhage, adhesions and peritonitis [15] which may have a negative impact on the fertility of the animal. In addition, surgery may cause stress which could lead to the release of stress-induced mediators that may affect early embryo survival [16].

The second approach involves pharmacological manipulation of the CL by administration of prostaglandin F2α (PGF2α). Regression of the CL by the administration of PGF2α can only be accomplished reliably after Day 4 post-ovulation [17], circulating progesterone concentrations then decrease within 24-48 h [18] due to CL regression. However, when PGF2α is administered during early CL development (Day 3-onwards), function may be sufficiently compromised to result in lower concentrations of progesterone [19,20]. Negative effects of the administration of PGF2α on embryo development have been reported in the literature, however, these effects generally occur when the embryo is exposed to PGF2α from day 5 onwards [21-23]. Therefore early administration of PGF2α generates a potential model for reduced progesterone that involves only endogenously produced progesterone simulating naturally occurring low progesterone as occurs in the high yielding dairy cow due to ovarian dysfunction [24,25] or increased steroid metabolism [26,27]. Alternatively, the CL may be regressed completely with PGF2α administration on Day 5 followed by progesterone supplementation from an exogenous source [28]. This leads to an initial peak in progesterone followed by a more flat progesterone curve than is seen in high yielding dairy cows with low fertility that is related to the absence of a CL [29]. When both methods to create low circulating progesterone concentrations (CL impairment and CL regression + supplementation) were compared, a difference in follicular development and steroidgenesis of granulosa and theca cells was found suggesting that the low progesterone achieved by exogenous progesterone does not mimic the natural low progesterone concentration as seen, for example, in high yielding dairy cows [19].

We have recently shown that elevation of progesterone concentrations in beef heifers from Day 3 of the estrous cycle results in dramatic changes in the transcriptional profile of the endometrium [3] and has consequences for the developing conceptus in terms of advancement of elongation [12]. The objective of this study was to determine whether the method described in the study conducted by Shaham-Albalancy et al [19] could be optimized and used to develop a model where the rise in progesterone concentrations is delayed in beef heifers that could, in the future, be used to study the consequences of low progesterone on the endometrium, thereby improving our knowledge of the causes of early embryo mortality without the many confounding factors when using post partum lactating dairy cows.

2. Materials and Methods

2.1 Experiment 1

The aim of this experiment was to study the effect of administration of PGF2α on Days 3, 3.5 and 4 of the estrous cycle on circulating progesterone concentrations in beef heifers.

2.1.1. Animal management and treatments

Twenty three commercial cross bred beef heifers of similar average age (2.2± 0.23 years) and weight (484±8.58 kg) were housed in straw-bedded pens under the same management conditions. All heifers had *ad libitum* access to a diet consisting of grass silage and maize silage in a 1:1 ratio with 2 kg of concentrates per heifer per day.

Estrous cycles of heifers were synchronized with a Controlled Internal Drug Release (CIDR 1.9g, Pfizer UK) device containing progesterone intravaginally for 8 days with an injection of PGF2α analogue (Prosolvin, Intervet Ireland Ltd.) given on Day 7. Heifers were checked for signs of estrus 4 times per day commencing 36 h after CIDR withdrawal and only those recorded in standing estrus (= Day 0; n = 17) within 36 to 54 h after withdrawal were included in the experiment. These heifers were randomly assigned to one of two groups (i) PGF2α administration on Days 3, 3.5 and 4 (PG3, n=9) or (ii) controls (n=8) i.e. heifers with normal circulating concentrations of progesterone. The dose of PGF2α was 2 ml (equivalent to 15 mg of luprostenol – the recommended dose for luteolysis) per injection.

Blood samples were collected from all heifers via jugular venipuncture for subsequent measurement of progesterone on Days 1, 6 and 9 after onset of estrus. To fully characterize the effect of treatment on progesterone concentrations, blood was collected every 6 h from the first PG injection on Day 3 until 30 h after the last PG injection on Day 4. Blood samples were stored at room temperature for 1 h and at 4°C for a further 16 h. Serum was decanted after centrifugation for 20 minutes at 1600 x g and stored at -20°C until subsequent analysis. Serum progesterone concentrations were measured using a time-resolved fluorescentimmunoassay (FIA) with an AutoDELFIA™ Progesterone kit (Perkin Elmer, Wallac Oy, Turku, Finland) as previously used by Carter et al. (2008). All samples were assayed within a single assay. The sensitivity of the assay was 0.01 ng/ml. The intra-assay coefficients of variation (CV) were 4.8, 4.0 and 3.0% for high, medium and low progesterone quality control sera, respectively. The quality control sera had progesterone concentrations of 0.29 ng/ml (low), 1.4 ng/ml (medium) and 1.8 ng/ml (high). The assay was validated by ensuring diluted serum samples were parallel to the standard curve and the progesterone antibody did not cross-react with related progestagens.

2.1.2. Statistical analyses

Total area under the curve (AUC) was calculated for progesterone concentrations of each individual heifer in each treatment group. Three separate AUC were calculated for the first 18 h after the first PGF2α injection (Day 3), the time period between 24 and 42 h after the first PGF2α injection (Day 4) and a combined AUC for Day 5, 6 and 9 (48-150 h after the first PGF2α injection), respectively. Differences between treatment groups were analysed using ANOVA with Bonferroni for multiple variance using SPSS for Windows.

2.2. Experiment 2

The aim of this experiment was to compare 3 methods for creating a low progesterone environment post insemination and to examine the consequences of this treatment on conceptus survival rate.

2.2.1. Animal management and treatments

Forty-five commercial cross bred beef heifers were used (approximately 2.3±0.26 years old with an average weight of 523±5.05 kg). All heifers were housed in straw-bedded pens under the same management conditions and had *ad libitum* access to a diet consisting of grass silage and maize silage in a 1:1 ratio with 4 kg concentrates per heifer per day. The estrous cycles of all heifers were synchronized using the same protocol as described for Experiment 1 and heifers were detected in estrus (n=39) as previously described. Heifers were inseminated with frozen-thawed semen from a single ejaculate of a fertile bull 12-18 h after they were first detected in standing estrus. Following insemination, heifers were randomly assigned to 1 of 4 treatment groups: (i) PG injection on Days 3, 3.5 and 4, as in Experiment 1 (PG3, n=10), (ii) PG injection on Days 3, 3.5, 4 and 4.5 (PG4, n=10), (iii) Progesterone Releasing Intravaginal Device (PRID; 1.55 g, Ceva Animal Health Limited, UK) insertion on Day 4.5, PG injection on Day 5 and 6 (PRID + PG, n=10) and (iv) control (n = 9).

Daily blood samples were collected from all heifers via jugular venipuncture for subsequent measurement of progesterone from Days 1 to 15. A time line for the treatments and blood sampling is shown in Figure 1. Blood samples were stored at room temperature for 1 h and at 4°C for a further 16 h. Serum was decanted after centrifugation at 1600 x g for 20 minutes and stored at -20°C until subsequent analysis. Progesterone concentrations were measured as previously described for Experiment 1. The sensitivity of the assay was 0.01 ng/ml. The inter-assay CVs were 8.2%, 3.3% and 4.0% for high, medium and low progesterone quality control serum pools, respectively. The intra-assay CVs (n=3) were 4.0, 3.4 and 8.4% for the same quality control sera. Pregnancy status was determined following slaughter on Day 16 by flushing the uterus with 20 ml of 10 mM Tris (pH 7.2) (Sigma, Dublin, Ireland). The presence of a conceptus was determined using a stereomicroscope and conceptus length was measured in a petri dish over a transparent graduated grid (1 mm graduations). All CL were dissected out of the ovary and weighed.

2.2.2. Statistical analysis

Area under the curve (AUC) was calculated for progesterone concentrations of each individual heifer in each treatment group. Separate AUCs were calculated for the time period Days 3 to 6, the time period between Days 6 and 11 and the time period for Days 11 to 15, The differences in AUC and CL weights were analysed using ANOVA with Bonferroni for multiple comparisons using SPSS for Windows. Regression analysis was used to characterize the relationship between progesterone concentration on Days 5 to 8 and conceptus length.

3. Results

3.1. Experiment 1

The mean progesterone profiles (± SE) for all heifers are shown in Figure 2. Based on the progesterone profiles of the individual heifers, the CL was not affected in 2 of the treated heifers (22%), it regressed in 1 (11%) and its progesterone secreting capacity was reduced in 6 others (67%). Progesterone concentrations were lower ($P<0.03$) in treated heifers between 24 and 48 h after the first PG injection compared with the control heifers

3.2. Experiment 2

3.2.1. Progesterone concentrations

Administration of 3 or 4 injections of PG beginning on Day 3 significantly reduced serum progesterone concentrations (P<0.01), but concentrations were not different between the heifers allocated to PG3 or PG4 groups.

Area under the curve (AUC) for progesterone from the heifers that received the PRID + PG treatment was different (P<0.01) between Days 3 to 6 compared to all other treatments (PG3, PG4 and controls). AUC for progesterone concentrations from both the heifers that received the PG3 and PG4 treatments were different from Days 6 to 11 (P<0.03) compared with the PRID + PG treatment and controls. From Days 11 to 15, progesterone concentrations were different (P<0.03) between all 3 treatment groups and the controls.

Based on the progesterone profiles of the individual heifers, the progesterone secretory capacity of the CL was not affected in 1 heifer (10%), it was reduced in 6 others (60%) and the CL regressed in 3 (30%) heifers in the PG3 group. In the PG4 group the CL was not affected in 1 heifer (10%), was reduced in 3 heifers (30%) with the remaining 6 heifers exhibited a regressed CL (60%).

3.1.2. Conceptus survival rates

Administration of 3 or 4 injections of PG did not have an effect on conceptus survival as measured by conceptus recovery on Day 16, with a conceptus survival rate of 25% in both the PG3 and PG4 group, a 40% survival rate in the PRID + PG group and a 22% survival rate in the control group. The mean length of all recovered conceptuses was 40.5 ± 10.9 mm. In both the PG3 and the PG4 group only one heifer (10%) had a conceptus that was judged to be impaired in development based on length (2 mm in the PG3 group and 4 mm in the PG4 group respectively) compared with the conceptuses in the PRID + PG and control group which had an average length of 66mm. There was a significant relationship ($P<0.03$) between conceptus size and progesterone concentrations on Days 5 and 6, but not on Days 7 and 8 (Table 1).

3.1.3. CL weight

There was no difference ($P>0.05$) in CL weight between the 2 groups that received the PGF2α injections and the control group (Table 2). In 7/10 heifers in the PRID + PG group the CL completely regressed after the 2 PG injections on Days 5 + 6, 1 heifer had a small CL and the remaining 2 heifers had a CL of normal weight. The weight of the CL in heifers in the PRID + PG group that did not regress tended to be lower ($p=0.08$) than that of the controls.

4. Discussion

The aim of this study was to develop a low progesterone model in beef heifers that could be used to study the effects of low progesterone on endometrial gene expression and conceptus development in the absence of the many confounding effects associated with early post partum dairy cows. Experiment 1 shows that it was possible to decrease peripheral progesterone concentrations in heifers by administering 3 injections of PGF2α on Days 3, 3.5 and 4 similar to that reported by Shaham-Albalancy et al [19] and Beal et al. [19,20]. The potential direct negative effect on the embryo with this type of treatment has only been reported when PGF2α was administered from day 4 onwards [22,23]. The administration of the 3 injections lead to decreased progesterone concentrations in the treated heifers, but it was felt that further analysis of different types of CL manipulation was warranted in order to allow us to determine an optimum model for reduced progesterone that may be capable of supporting conceptus development.

The increase in circulating progesterone concentrations was delayed after administration of both 3 and 4 injections of PGF2α for those where the CL did not regress (60% in PG3; 30% in PG4). The progesterone curves generated by these treatments were similar to those reported by Rosenberg et al [30], where plasma concentrations in the midluteal phase before insemination (Day 10-14 of the cycle) were lower in cows that failed to conceive compared with those that did conceive. However, in both treatment groups that received the PG injections to impair the progesterone output from the CL, some heifers (30% in PG3; 60% in PG4) were seen in standing estrus indicating that the injections had resulted in complete CL regression. This was also apparent from the progesterone concentrations of these heifers.

The PRID + PG treatment led to a flat progesterone curve after an initial peak in progesterone concentrations on the day of administration. These concentrations were similar to those reported in other studies where constant low progesterone concentrations was related to low fertility [31]. The progesterone profiles of the PRID + PG group were not the same as those seen in dairy cows during the early luteal phase [29], especially as progesterone concentrations initially peaked after the PRID insertion, which would also be the case when a used progesterone device was used[32]. Prostaglandin injections on Days 3, 3.5 and 4 led to a model of low progesterone that does not require the exogenous supplementation of progesterone and therefore provided a progesterone profile that was similar to those of cows with poor embryo survival associated with low progesterone concentrations in early pregnancy. Various studies have shown that it is the timing of the post-ovulatory rise rather than actual progesterone concentrations that has the major negative effect on embryo development [7,33]. Even a one day delay in this post-ovulatory rise results in smaller embryos that secrete less interferon t on day 16 [8]. The optimum chosen method of those tested to create a low progesterone model was 3 injections of PG on Days 3, 3.5 and 4. As there was no reduction in CL weights it implies that injections with PGF2α led to reduced progesterone output without altering CL size.

Conceptus survival rate was low in all groups in Experiment 2, but all low progesterone models were able to maintain pregnancy until slaughter on Day 16 in at least some heifers. The low progesterone concentrations were associated with smaller conceptus size (<5 mm vs average size of 40.5 mm) in 2 of the pregnant heifers, suggesting that conceptus elongation was compromised in these heifers. This was similar to what occurs in high yielding dairy cows [31,34]; however low numbers of conceptuses recovered in this study precludes any further conclusions.

There was a significant relationship between conceptus size and progesterone concentrations on Days 5 and 6, but not on Days 7 and 8. This would imply conceptus elongation is dependent in part on circulating progesterone concentrations before Day 7, which is also supported by previous studies [10-12].

In summary we have developed a low progesterone model using 3 injections of PGF2α on Days 3, 3.5 and 4 of pregnancy. The advantages of this model are that it simulates the low progesterone concentrations found in high yielding dairy cows without the many confounding factors that can be present in these cows. In addition while low progesterone concentrations were achieved in this model, we showed that maintenance of pregnancy, albeit in a small number of heifers, was possible using this model and thus it will be an extremely useful tool in elucidating low progesterone contributions to infertility.

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Table 1: Correlation between progesterone concentration on Days 5, 6, 7, and 8 and conceptus size at slaughter on Day 16. This correlation was significant for Days 5 and 6, but not for Days 7 and 8.

		Progesterone concentration (ng/ml)			
		Day 5	Day 6	Day 7	Day 8
Conceptus size	r^2	0.554	0.480	0.191	0.010
(mm)	p	0.0136	0.0264	0.261	0.7802

Table 2: Mean ± S.E. corpora lutea (CL) weights (g) and progesterone (P4 in ng/ml) concentrations for all heifers. All CL were weighed after slaughter on Day 16.

	All heifers			Pregnant heifers			Non-Pregnant heifers		
	n	Mean CL weight	Mean P4 [conc]	n	Mean CL weight	Mean P4 [conc]	n	Mean CL weight	Mean P4 [conc]
PG3	10	5.53 ± 0.60[b]	0.45 ± 0.07	2	5.51 ± 0.53	0.69 ± 0.16	8	5.60 ± 3.63	0.39 ± 0.08
PG4	10	5.56 ± 0.82[b]	0.44 ± 0.09	2	5.32 ± 1.08	0.98 ± 0.21	8	6.07 ± 1.07	0.30 ± 0.08
PRID + PG	10	3.12 ± 0.40[a]	0.94 ± 0.14	4	3.81 ± 0.05	0.72 ± 0.17	6	1.73 ± 0	1.09 ± 0.14
Control	9	7.02 ± 0.54[b]	1.28 ± 0.22	2	6.76 ± 0.73	1.21 ± 0.21	7	6.30 ± 0.08	1.30 ± 0.22

There was no significant difference in CL weight between the PG3 and PG4 group when compared to the controls, the CL weight in the PRID + PG group was significantly reduced after the PGF2α injections on day 5 and 6 as expected.

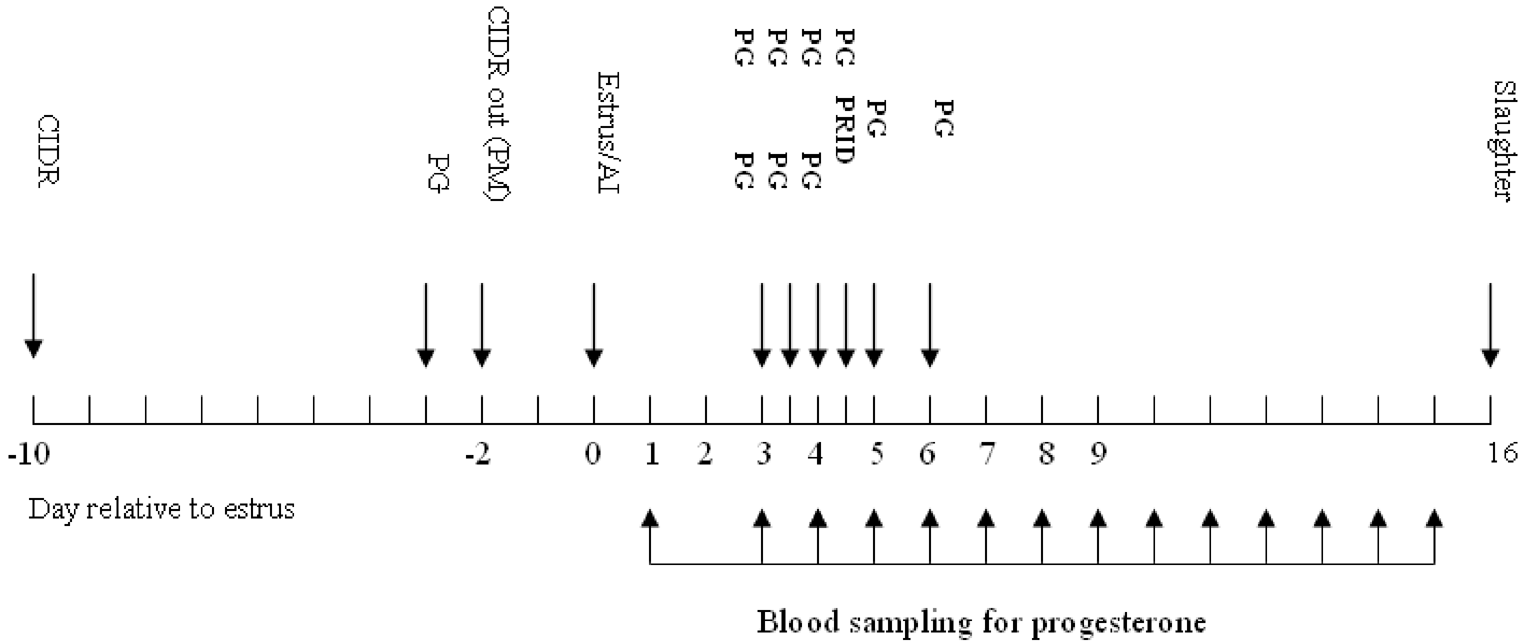

Figure 1: Experimental design of Experiment 2. Estrus was synchronized in all heifers by inserting a CIDR device for 8 days and injecting PGF2α 1 day before its removal. Heifers were assigned to one of 4 groups after insemination: (1) PGF2α injection on Days 3, 3.5, 4 (PG3, n=10), (2) PGF2α injection on Days 3, 3.5, 4 and 4.5 (PG4, n=10), (3) Progesterone Releasing Intravaginal Device (PRID;) insertion on Day 4.5, PGF2α injection on Day 5 and 6 (PRID + PG, n=10) and (4) control group (n = 9). Blood samples were collected from all heifers on Day 1 and then once daily from Day 3 until Day 15. Slaughter took place on Day 16. Key events are indicated by arrows along the timeline plot.

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