Establishment of critical timing of progesterone supplementation on corpus luteum and embryo development in beef heifers

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ABSTRACT

Optimal concentrations of progesterone (P4) during early pregnancy are a major determinant of embryo survival in cattle. This study examined the effects of P4 supplementation, following a period of induced low P4, on corpus luteum (CL) development, circulating P4 concentrations and embryo development. A total of 107 beef heifers from one herd were used in the study. Following AI (Day 0) at a synchronized oestrus, heifers were randomly assigned to 1 of 5 treatment groups: (1) no subsequent treatment (control; n = 15); (2) administration of a synthetic prostaglandin $F_{2\alpha}$ analogue (PG) on Days 3, 3.5 and 4 to induce low P4 and no further treatment (LP4; n = 23); administration of PG as above followed by P4 supplementation via insertion of a Controlled Internal Drug Release device from (3) Day 4–7 (P4 d4-7; n = 22); (4) Day 4–10 (P4 d4-10; n = 23); or (5) Day 7–10 (P4 d7-10; n = 24). Embryo and CL characteristics were determined following slaughter on Day 16. Embryo size was greater in heifers administered P4 d4-7 and P4 d4-10 in comparison with LP4. Corpus luteum area was significantly reduced in heifers administered P4 d4-7 and P4 d4-10 up to Day 7, and Day 10 (P4 d4-10), in comparison with LP4 heifers. Initiation of supplemental P4 on Day 7 had no effect on CL area or embryo size in comparison with LP4 heifers. Concentrations of P4 were less in P4 d4-7, P4 d7-10 and P4 d4-10 on Day 15 in comparison with LP4 heifers. The results of the study indicate an initiation and duration effect of supplemental P4 on circulating P4, embryo and CL development following induction of LP4 on Day 3 and 4.

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1. Introduction

Successful establishment of pregnancy depends on appropriate signalling among the corpus luteum (CL), uterus and developing embryo. Progesterone (P4) has a key role in embryo development as it is involved in a cascade of molecular, biochemical and morphological events involved in the establishment and maintenance of pregnancy (Spencer et al., 2004). Increasing concentrations of P4 support the secretory function of the uterine endometrium that sustains conceptus elongation and implantation in ruminants (Niswender et al., 2000; Spencer et al., 2007). There is a positive relationship between early luteal phase concentration of P4 and concep-

tus interferon-tau (IFN-τ) synthesis (Kerbler et al., 1997), and development (Mann and Lamming 2001). However, elevating concentrations of P4 in the early luteal phase does not affect the proportion of embryos reaching the blastocyst stage (Carter et al., 2010). The timing of the increase in concentration of P4 in the early luteal phase affects the timing of the down-regulation of its receptor in the luminal and glandular epithelium, which is a critical checkpoint for the establishment of pregnancy in all species studied (Okumu et al., 2010; Bazer et al., 2011).

Progesterone supplementation conducted in the early luteal phase has yielded varying results in terms of pregnancy survival rate in both cows and heifers. Administration of exogenous P4 to beef cows on Day 1–4 post AI advanced conceptus elongation on Day 14 (Garrett et al., 1988a, 1988b). Furthermore, an increase in P/AI was achieved in lactating dairy cows when P4 was supplemented in the luteal phase (Robinson et al., 1989; Mann et al., 2001; Larson et al., 2007). However, in other studies, a negative impact of supplemental P4 on P/AI has been recorded in heifers (Van Cleeff et al., 1991; Lynch et al., 1999). Moreover, evidence from this laboratory has shown that supplementation of P4 on Days 4 through 9 decreased P/AI in lactating Holstein Friesian cows (Parr et al., 2014). Inconsistency in results between studies may be a consequence of animals with low compared with high endogenous P4 concentrations responding differently to exogenous P4 supplementation as some evidence exists for both a linear and quadratic relationship between P4 and pregnancy rate with a slight decrease in pregnancy rate at highest P4 concentrations (Stronge et al., 2005; Parr et al., 2012). An alternative hypothesis, supported by data in the literature, is that administration of P4 early in the oestrous cycle may compromise CL function, ultimately leading to luteolysis and embryo loss (Burke et al., 1994; Ginther 1970; Macmillan and Peterson, 1993; Van Cleeff et al., 1996).

There is a lack of data in the literature quantifying the effects of administration of P4 to animals with a compromised CL on subsequent CL function and embryo development. Therefore, the overall aim of this study was to elucidate if the negative effects of induced low P4 (LP4) in the early luteal phase on CL and embryo development could be reversed by administration of exogenous P4 for different time periods during the luteal phase. Using a previously validated experimental model to induce low P4 (Beltman et al., 2009; Forde et al., 2012), the specific objective of the study was to quantify the effect of induced LP4 followed by supplemental P4 from Day 4–7, Day 4–10 or Day 7–10 on circulating concentrations of P4, CL size and weight, embryo recovery and embryo development on Day 16.

2. Materials and methods

2.1. Animals and management

All experimental procedures involving animals were licensed by the Department of Health and Children, Ireland. Protocols were in accordance with the Cruelty to Animals Act (Ireland 1876, as amended by European Communities regulations 2002 and 2005) and the European Community Directive 86/609/EC and were sanctioned by the Institutional Animal Research Ethics Committee of University College Dublin.

The study was conducted with three replicates involving a total of 107 reproductively normal nulliparous crossbred beef heifers from the same herd. All heifers were weighed and body condition scored (Scale 0–5; Lowman et al., 1976) at the start of each replicate and had a (mean ± s.e) weight of 511 ± 5.13 kg and body condition score of 3.52 ± 0.04. All heifers were housed in concrete slatted pens throughout the study and had *ad libitum* access to grass silage with a metabolisable energy (ME) of 11.9 MJ/kg dry matter and a crude protein (CP) content of 12%. In addition, each heifer was offered 2 kg concentrates per day (10.7 MJ/kg ME, CP 15.4%).

2.2. Oestrous synchronization and artificial insemination

Oestrous cycles were synchronized in (n = 116 heifers) by using two injections (2 ml of Estrumate equivalent of 500 μg cloprostenol) of a synthetic prostaglandin F$_{2\alpha}$ analogue (PG, Estrumate; Intervet/Schering-Plough Animal Health, Hertfordshire, UK) administered i.m. 11 days apart. All heifers were monitored for signs of oestrus five times daily, beginning 2 days after administration of the second PG injection and continuing for a further 96 h. Estrotect^{TM} patches (Dairymac Limited, Hampshire, UK) were used as an aid for oestrous detection. The standing oestrous response to the second PG injection varied from 90% to 95%. Only heifers displaying standing oestrus (n = 107, designated as Day 0) were artificially inseminated (AI) by the same experienced operator using frozen-thawed semen from a single high fertility bull. All inseminations took place within 12 h of standing oestrus.

2.3. Hormonal treatments

A schematic representation of experimental treatments and number of heifers in each treatment group is presented in Fig. 1. Following AI, heifers were randomly assigned to 1 of five groups: (1) no subsequent treatment (control; n = 15); (2) administration of PG on Days 3, 3.5 and 4, to induce low P4, with no further treatment (LP4; n = 23); (3) administration of PG as above followed by exogenous P4 supplementation by insertion of a Controlled Internal Drug Release device (CIDR) from (3) Day 4–7 (P4 D4-7; n = 22); (4) Day 4–10 (P4 D4-10; n = 23); or (5) Day 7–10 (P4 D7-10; n = 24).

2.4. Ultrasonic assessments

All heifers were ultrasonically assessed by the same operator on Days 4, 5, 7, 10, and 15 post oestrus to assess CL development using a portable Voluson I scanner (GE Healthcare, Chalfont, St. Giles, UK) with a linear (RSP6–16 MHz) transducer. Three consecutive images of the CL were taken at each time-point. Maximum diameter of the CL and any luteal cavity were recorded. The area of the CL was calculated using the formula: area = π x (radius)^2. The area of a cavity, if present, was subtracted from the