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Effect of exogenous progesterone supplementation in the early luteal phase post-insemination on pregnancy per artificial insemination in Holstein–Friesian cows

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ABSTRACT

One of the main determining factors of pregnancy per artificial insemination (P/AI) is an optimum concentration of progesterone (P4) in the early luteal phase. This study examined the effects of P4 supplementation on P/AI in lactating Holstein–Friesian cows. A total of 453 cows in 8 spring-calving herds were used in the study. Following AI, cows were randomly assigned to 1 of 2 treatment groups: (1) no subsequent treatment (control; $n = 221$); (2) insertion of a Controlled Internal Drug Release device (CIDR) from day 4 to day 9 post-estrus (supplemented; $n = 232$). Pregnancy per AI was determined by transrectal ultrasonography at day 30 following AI. Insertion of a CIDR increased concentrations of milk P4 in supplemented cows by 4.78 ng/mL between day 4 and 4.5 in comparison with a 0.55 ng/mL increase in control cows. Progesterone supplementation from day 4 to 9 after AI decreased P/AI by 12 percentage points (56 vs 44%). There was a positive linear and quadratic relationship between P/AI and milk concentration of P4 on day 4 post-estrus in control cows. An optimum concentration of 2.5 ng/mL on day 4 was calculated from the logistic regression curve to achieve a probability of P/AI of 65%. When both treatments groups were included in the analysis, there was no association between P/AI and concentrations of P4 on day 4. The results of the study indicate that supplementation with P4 initiated in the early luteal phase had a negative effect on P/AI in dairy cows.

1. Introduction

Early pregnancy loss in cows is a growing concern for an ever-expanding dairy industry. In Europe, for example, removal of milk quotas in 2015, which have restricted milk production since their introduction in 1984, is predicted to lead to a significant increase in milk production. Whilst fertilisation rates are high (>80%) in dairy cows (Sartori et al., 2009), a significant amount of pregnancy loss occurs, the majority before day 16 post-insemination (Sreenan et al., 2001). Indeed, recent studies suggest that in lactating dairy cows up to 50% of embryos fail to survive to day 7 (Cerri et al., 2009a,b,c). Progesterone (P4) plays a central role in pregnancy per insemination (P/AI) as it is involved in a cascade of biochemical, molecular and morphological events in the establishment and maintenance of pregnancy

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(Spencer et al., 2004). There is evidence in dairy cows (Stronge et al., 2005; McNeill et al., 2006) dairy heifers (Parr et al., 2012) and beef heifers (Diskin et al., 2006) that as concentrations of P4 increase during the early luteal phase that P/AI initially increases, then plateaus before declining at the highest concentrations. Supplementation of P4 during the early post-ovulatory rise in endogenous P4 enhances development and interferon-τ production of the embryo (Mann et al., 2006). It was concluded from that study that the timing and/or magnitude of the post-ovulatory rise in P4 is more important than the final concentration of P4 achieved later in the luteal phase. Low circulating concentrations of P4 in the early post-ovulatory period have been shown to have a negative impact on embryo development (Mann and Lamming, 2001), conceptus elongation (Forde et al., 2011) and the endometrial transcriptome (Forde et al., 2012) compared with animals with normal or high circulating concentrations of P4. A large proportion of dairy cows have inadequate concentrations of milk P4 on days 5–7 to maintain embryo survival (Stronge et al., 2005). Despite this, P4 supplementation studies carried out in the early luteal phase have yielded variable results in terms of pregnancy rate, with supplementation of P4 increasing P/AI in some studies (Johnson et al., 1958; Robinson et al., 1989), but having a negative effect in others (Van Cleeff et al., 1996). Some of the discrepancy between different studies may be related to the timing and duration of supplemental P4 treatment as well as to the mode of attaining elevated P4 (e.g., by providing exogenous P4 in the form of P4 injections (Johnson et al., 1958), or via a P4-containing vaginal insert (Robinson et al., 1989; Macmillan and Peterson, 1993; Stevenson et al., 2007), or by altering the endogenous P4-producing capacity of the CL by manipulating the size of the ovulatory follicle (Inskeep, 2004) or by administering luteotrophic hormones such as human chorionic gonadotrophin (Santos et al., 2001).

There is no evidence in the literature to identify the effects of supplementation of exogenous P4 on P/AI in dairy cows categorised as having low, medium and high milk concentrations of P4 on days 3 and 4 post-oestrus. Therefore, the objectives of this study were to: (1) determine if supplementation of exogenous P4 would increase P/AI in lactating dairy cows; (2) quantify the relationship between P/AI and concentrations of milk P4 on days 3 and 4; and (3) determine the concentrations of milk P4 on days 3 and 4 necessary to maximise the probability of P/AI in dairy cows.

2. Materials and methods

2.1. Animals and management

All experimental procedures involving cows were licensed by the Department of Health and Children, Ireland. Protocols were in accord 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.

This study was carried out over a 3-month period and involved 8 spring-calving dairy herds in Ireland with a total of 453 Holstein–Friesian cows enrolled in the study. Parity ranged from 1 to 10 and 305 days milk yield for all herds was 7549 ± 1524 kg. The mean number of days in milk (DIM) on the day of breeding was 78 ± 6.60 days. Cows were randomly allocated to each treatment group which allowed a balance of parity, and milk production to be maintained between the cows in both treatments. All cows were kept full time on predominately ryegrass pasture for the duration of the study and were supplemented with an average of 1–2 kg of a 14–16% protein concentrate feedstuff per cow per day. Pastures typically received nitrogenous fertilizer (30–35 kg N/ha) at 20–30 days intervals.

2.2. Estrus detection, AI and determination of pregnancy per AI

The experimental design is illustrated in Fig. 1. In each herd, cows were monitored for signs of estrus at 0700 h, 1300 h, 1700 h and 2100 h. Only cows displaying standing estrus were artificially inseminated (AI) using frozen thawed semen. All inseminations took place within 12 h of standing estrus. Day 0 was designated as the day of standing estrus. All cows enrolled in the study were receiving their first AI post-partum.

Pregnancy was determined by ultrasound scanning of the uterus using an ALOKA SSD 500 V scanner with a 7.5-MHz transducer (Aloka Ltd., Tokyo, Japan) at day 30 following AI. A positive pregnancy diagnosis was based on the presence of an apparently viable embryo with a visible heartbeat and clear amniotic fluid.

2.3. Supplementation of progesterone

All cows were randomly allocated to one of two treatments, cows which received exogenous P4 (supplemented) and cows which received no treatment (control). Exogenous P4 was administered using a Controlled Internal Drug Release device (Eazi-Breed CIDR 1.38 g P4 Pfizer Animal Health, UK). The device was inserted after the collection of milk samples during the morning milking on day 4 and removed on the morning of day 9 post-estrus.

2.4. Milk sampling and progesterone assay

Milk samples, representative of the entire milking were collected during the morning milking on day 0, morning and evening milking on days 3 and 4 from all cows and the morning milking of days 5–7 (day 0 = day of standing estrus) from a random subset of 30 cows (15 cows from each treatment). Samples were collected into 30 mL plastic containers containing a Lactab Mark III preservative tablet (Thompson and Capper Ltd., Cheshire, England). Following collection, all samples were agitated to ensure mixture with the Lactab and were stored at −20 °C until assaying.

Concentrations of P4 were measured by radioimmunoassay (Coat-A-Count Progesterone In Vitro Diagnostic Test Kit™, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) as previously described by Forde et al. (2011), adapted and validated for whole milk as the sample matrix. Briefly 100 μL of milk or standard was added to the antibody coated tubes, 750 μL radiolabeled P4 tracer was