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Progesterone supplementation in the early luteal phase after artificial insemination improves conception rates in high-producing dairy cows

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ABSTRACT

This study examines the possible effects on the reproductive performance of high-producing dairy cows of progesterone (P4) given in the early luteal phase (1.55 g of P4), from Days 3 to 5 post-artificial insemination (AI) as compared with the time of pregnancy recognition, from Days 15 to 17 post-AI. Cows in their third day post-AI were alternately assigned on a weekly rotational basis to the following groups: control, no treatment (C: n = 351), P4 treatment started 15 days after AI (P4-D15: n = 261), or P4 treatment started 3 days after AI (P4-D3: n = 203). Based on odds ratios, cows in P4-D3 were 1.71 times more likely to conceive than control cows (P = 0.004), whereas cows in P4-D15 showed a 1.4-fold greater risk approaching significance of becoming pregnant compared with control cows (P = 0.06). Differences were not observed between treatments. In nonpregnant cows, the given treatment (D3 vs. D15) had no effect on subsequent return to estrus or AI interval and neither were any effects of treatment observed on early fetal loss rates. In contrast, in pregnant cows, the relative risk of twin pregnancy was 2.5 times higher for those in P4-D15 (P = 0.02) than the remaining cows. These findings indicate the efficacy of P4 supplementation after AI. However, when given at the time of pregnancy recognition rather than in the early luteal phase, this treatment increases the twin pregnancy rate.

1. Introduction

Embryo mortality may be the main limiting factor for the efficiency of dairy systems [1–3]. Most conception losses in dairy cows occur during the first 3 weeks of pregnancy [1,4,5] and lower conception rates have been associated with lower progesterone (P4) concentrations during the luteal phase subsequent to artificial insemination (AI) [6–8], which is a situation commonly observed in high-producing dairy cows [9,10]. To reduce embryo losses, the benefits of supplementing cows with P4 after AI have been extensively explored. However, the results of individual studies have revealed results varying widely from beneficial effects to markedly reduced pregnancy rates. In a recent meta-analysis of 84 treatments involving data from 19,040 cows, the efficacy of P4 supplementation early post-AI at improving pregnancy rates was assessed [11]. Results indicated that P4 supplementation between Days 3 to 7 post-AI was beneficial but treatment earlier or later than this was not [11].

In a previous study, we observed beneficial effects of P4 supplementation at the time of pregnancy recognition, from Day 15 to 17 post-AI, on the reproductive performance of high-producing dairy cows [12]. The objective of the present study was to compare the effects of P4 supplementation in the early luteal phase (from Day 3 to 5 post-AI) or at

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pregnancy recognition (from Day 15 to 17 post-AI) on the reproductive performance of high-producing dairy cows.

2. Material and methods

2.1. Cattle and herd management

This study was performed on a commercial Holstein-Friesian dairy herd in northeastern Spain. This same herd was used in a prior study of the efficacy of P4 supplementation from Day 15 to 17 post-AI [12]. During the study period (November 2014 to December 2015), the mean number of lactating cows in the herd was 880 and mean annual milk production was 11,990 kg per cow. The mean annual culling rate was 30%. Cows were grouped according to age (primiparous vs. multiparous), milked three times daily, and fed complete rations. Dry cows were kept in a separate group and transferred to a "parturition group" 7 to 25 days before parturition depending on their body condition score [13,14] and on whether or not they were carrying twins [15]. An early postpartum, or "fresh cow," group was established for postpartum daily checks and nutrition controls 7 to 20 days postpartum. All cows were artificially inseminated, and the herd was maintained on a weekly reproductive health program, as described elsewhere [16].

2.2. Detection of estrus, insemination, pregnancy diagnosis, and pregnancy loss

Estrus was detected using a pedometer system (AfiFarm System; SAE Afikim). Walking activity values were recorded at the milking parlor (three times daily) and analyzed automatically using a herd management computer program. A walking activity greater than 80% of the mean activity recorded in the previous 2 days was taken as the lower limit for a cow to be considered in estrus. Since this herd was observed in a previous study to show a very significant positive relationship between increased walking activity and fertility provided this increase was 80% to 993% [16], values lower than 80% were not considered as estrous signs. Prior individual information concerning estrus detection was also taken into account. For example, if a cow normally showed a 400% increase in activity but showed an increase of around 120% during its two last estrous periods, the cow was explored for possible conditions other than estrus such as acute lameness or a change in location.

Estrus was confirmed by palpation per rectum in cows deemed to be in estrus using the pedometer system described above, and the animals were inseminated at this time. Only cows showing spontaneous estrous signs with strong uterine contractility (determined by uterine tone) and copious transparent vaginal fluid were inseminated. If a cow returned to estrus, its status was confirmed by examination per rectum, and the animal was recorded as nonpregnant. In the remaining cows, pregnancy diagnosis was performed by ultrasound 28 to 34 days post-AI and confirmed 58 to 64 days post-AI. Since management and cow-related factors of a noninfectious nature have been extensively linked to late embryonic or early fetal loss in our geographical area [2,15], pregnancy loss was recorded when the 58 to 64 day-diagnosis proved negative.

2.3. Experimental design

All procedures were approved by the Ethics Committee on Animal Experimentation of the University of Lleida (license numbers CEEA.09–01/12 and CEEA.09–01/13).

Cows in their third day post-AI were alternately assigned on a weekly rotational basis to the groups: control, no treatment (C: n = 351), P4 treatment at the time of pregnancy recognition, from Days 15 to 17 post-AI (P4-D15: n = 261), or P4 treatment in the early luteal phase, from Days 3 to 5 post-AI (P4-D3: n = 203). Thus, cows reaching their third day post-AI on Friday through Sunday, Monday through Tuesday or Wednesday through Thursday were included in the groups C, P4-D15, or P4-D3, respectively. Animals in the two P4 groups were fitted with a P4-releasing intravaginal device (PRID; PRID DELTA, containing 1.55 g of P4; CEVA Salud Animal, Barcelona, Spain) for 3 days. Only healthy cows with no signs of mastitis, lameness, or digestive disorders were included in the study. Cows were included only once in the experiment.

To establish the possible effects of each treatment on plasma P4 concentrations, plasma samples were collected on Days 5 and 17 post-AI from a subset of 12 multiparous cows of each group. Only cows diagnosed pregnant on Day 28 post-AI with one single embryo and its corresponding CL were included in this analysis.

Since unexpectedly P4-D15 treatment was related to a 2.5 times higher risk of twin pregnancies, the sex of the twins was also registered.

2.4. Blood sampling and P4 determinations

Blood samples and ultrasonography were performed in each multiparous cow on Days 5 and 17 post-AI. Blood samples were collected from the coccygeal vein into two heparinized vacuum tubes (BD VacutainerTM, Becton-Dickenson and Company, Plymouth, UK), centrifuged within 20 minutes after collection (10 minutes, $\times$1600*g*) and the plasma stored at -20 °C until analysis. Samples from the first 12 diagnosed pregnant cows with one embryo and its corresponding CL were analyzed.

A commercial ELISA kit was used to determined plasma P4 concentrations (Ridgeway Scientific, Alvington, Gloucestershire, UK). The sensitivity of the assay was 0.15 ng/mL. Samples were evaluated in duplicate and all samples were analyzed in one assay (intraassay coefficient of variation, 6%).

2.5. Data collection and statistical analysis

The following data were recorded for each animal: parturition and AI dates; parity (primiparous vs. multiparous); previous retained placenta (fetal membranes retained longer than 12 hours after parturition) or metritis (diagnosed during the first or second week postpartum in animals with no history of retained placenta); insemination number; repeat breeding syndrome (cows undergoing more than three AI); treatment (C vs. P4-D15 and P4-D3); milk production at AI (mean production during the 3 days before estrus behavior; low producers$<$ 40 kg vs. high producers $\geq$40 kg); days in milk at AI (DIM; $<$90 days