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Lack of complete regression of the Day 5 corpus luteum after one or two doses of $PGF_{2\alpha}$ in nonlactating Holstein cows

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ABSTRACT

The early corpus luteum (CL) (before Day 6) does not regress after a single $PGF_{2\alpha}$ treatment. We hypothesized that increasing $PGF_{2\alpha}$ dose or number of treatments would allow regression of the early CL (Day 5). Nonlactating Holstein cows (N = 22) were synchronized using the Ovsynch protocol. On Day 5 (Day 0 = second GnRH treatment), cows were assigned to: (1) control (N = 5): no further treatment; (2) 1PGF (N = 6): one dose of 25 mg $PGF_{2\alpha}$; (3) 2PGF (N = 5): two doses of 25 mg $PGF_{2\alpha}$ (50 mg) given 8 hours apart (second $PGF_{2\alpha}$ on Day 5 at the same time as the other $PGF_{2\alpha}$ treatments); (4) DPGF (N = 6): double dose of 25 mg $PGF_{2\alpha}$ (50 mg) given on Day 5. Blood samples were collected to monitor progesterone (P4) profiles in two periods. In the first period (0 to 24 hours), there were effects of treatment ($P = 0.01$), time ($P < 0.01$), and an interaction of treatment and time ($P = 0.02$). Group 1PGF versus control was different only at 12 hours ($P = 0.02$). Cows treated with DPGF were different than control at 4 hours ($P = 0.04$), 12 hours ($P < 0.01$), and 24 hours ($P < 0.01$). Only cows treated with 2PGF had lower P4 than control during the entire period and low P4 (0.37 ± 0.17 ng/mL) at 24 hours, usually indicative of luteolysis. In the second period (Day 5 to 15 of the cycle), there were effects of treatment ($P < 0.01$), time ($P < 0.01$), and interaction of treatment and time ($P = 0.002$). Group 1PGF was not different than control from Day 5 to 13 and P4 was greater than control on Day 14 ($P = 0.01$) and 15 ($P < 0.01$). Circulating P4 in DPGF cows was lower than control from Day 7 ($P = 0.05$) through 12 ($P < 0.01$). Likewise, there were differences between control and 2PGF from Day 7 to 13, but not on Day 14 and 15. On Day 15, all $PGF_{2\alpha}$-treated groups had circulating P4 consistent with an active CL. Ultrasound evaluation confirmed that no CL from any group completely regressed during the experiment and no new ovulations occurred to account for functional CL later in cycle. In summary, a double dose of $PGF_{2\alpha}$ (twice on Day 5 or 8 hours apart) can dramatically decrease P4, consistent with classical definitions of luteolysis; however, these CL recover and become fully functional. Thus, the Day 5 CL of mature Holstein cows do not regress even to two doses of $PGF_{2\alpha}$.

1. Introduction

Synchronization of ovulation programs in cattle allow for use of fixed time artificial insemination (TAI), decrease the time necessary to breed cows, and reduce or eliminate dependence of reproductive management on detection of estrus [1,2]. Ovsynch-based TAI programs rely on GnRH

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and $PGF_{2\alpha}$ to synchronize follicle wave emergence, CL regression, and ovulation [1]. Recent reports have highlighted the importance of reducing the period of follicle dominance to improve embryo quality and fertility results to TAI procedures [3–5]. Thus, reduction in the period of follicle dominance by altering the interval from the first GnRH treatment to $PGF_{2\alpha}$ treatment from 7 to 5 days might improve fertility of cattle.

However, reducing the time of follicular development from 7 to 5 days leads to the requirement of giving two $PGF_{2\alpha}$ treatments to induce an acceptable luteal regression to decrease progesterone (P4) concentrations near the time of AI sufficiently to optimize fertility [4,6]. Previous studies reported CL regression of more than 90% in cows given a single luteolytic dose of $PGF_{2\alpha}$ after Day 8 of the estrous cycle [7,8]. Conversely, a single treatment with the recommended dose of $PGF_{2\alpha}$ in the first 4 days of the cycle is unable to induce luteolysis [7,9,10]. On Day 5 of the estrous cycle a single dose of $PGF_{2\alpha}$ did not induce complete luteolysis because cows did not show estrus during the week after $PGF_{2\alpha}$ treatment and had a normal length of estrous cycle; however, some reduction in mean P4 concentrations was observed after $PGF_{2\alpha}$ treatment [8,9]. The cellular and molecular mechanisms that prevent or impair luteolysis in the early CL are currently being investigated in a number of laboratories [11–19].

Lack of complete luteolysis has been shown to reduce fertility in timed AI protocols that are used in dairy cows [4,6,20–22], dairy heifers [23], and beef cows [5,24], using either commercially available $PGF_{2\alpha}$ analogue, dinoprost tromethamine [6,20,23], or cloprostenol sodium [4,6,22,25]. A number of laboratories are investigating the development of practical methods to induce complete luteolysis of CL during timed AI protocols including: larger doses of dinoprost or cloprostenol [6,25–27] or two doses of dinoprost or cloprostenol given with varying intervals between the two $PGF_{2\alpha}$ treatments [4,6,21,28]. Thus, a greater understanding of regression of the early CL in response to $PGF_{2\alpha}$ treatment is of interest from a physiological and practical perspective. Unfortunately, previous studies have failed to provide a complete P4 profile after $PGF_{2\alpha}$ treatment of early CL. In addition, experiments using double doses of $PGF_{2\alpha}$ were generally confounded by application of a second dose of $PGF_{2\alpha}$ at a time that was later than Day 5, usually Day 6 [4–6]. However, the Day 6 CL can regress in response to a single $PGF_{2\alpha}$ treatment, even without previous $PGF_{2\alpha}$ treatments. Thus, the objective of the present study was to clearly evaluate the P4 profile after single and double treatments of $PGF_{2\alpha}$ in cows with a CL on Day 5 of a synchronized cycle. The specific hypothesis for this study was that increasing the $PGF_{2\alpha}$ dose or the number of $PGF_{2\alpha}$ treatments would produce complete luteolysis of the Day 5 CL.

2. Materials and methods

2.1. Animals and treatments

This experiment was conducted in cool weather (November to January) using nonlactating multiparous ($N = 22$) Holstein cows housed in a tie-stall facility at the University of Wisconsin-Madison. During the study period, cows were fed a total mixed ration (TMR) diet once a day with ad libitum access to feed and water. The diet was formulated to meet or exceed requirements for nonlactating dairy cows. All procedures were approved by the Animal Care and Use Committee for the College of Agriculture and Life Sciences of the University of Wisconsin-Madison.

Cows were blocked according to parity and randomly assigned to one of four treatments (see Fig. 1). All cows had their estrous cycles synchronized using Ovsynch [1]: GnRH treatment (100 μg im; Cystorelin, Merial Ltd., Duluth, GA, USA) followed 7 days later by $PGF_{2\alpha}$ treatment (25 mg im; Pfizer Animal Health, New York, NY, USA) and finally 2 days later, a second GnRH treatment (100 μg im). All treatments were done in relation to the final GnRH treatment, in other words, Day 5 was 5 days after the second GnRH treatment (Day 0). Cows that ovulated in response to the final GnRH treatment were randomly assigned to one of four treatment groups. Control cows were treated im with a saline solution on Day 5 (7 to 8:30 AM). Cows in the single $PGF_{2\alpha}$ treatment group (1PGF) were treated im with a full dose (25 mg) of $PGF_{2\alpha}$ on Day 5 (7–8:30 AM). Cows in the two $PGF_{2\alpha}$ treatment group (2PGF) were treated im with two doses (25 mg and 25 mg) of $PGF_{2\alpha}$ 8 hours apart, with the first dose given 8 hours before Day 5 (approximately 11:00 PM on Day 4; i.e., Day 4.7), and the second treatment on Day 5 (7–8 AM). Cows in the double $PGF_{2\alpha}$ treatment group (DPGF) were treated im with a double dose (50 mg) of $PGF_{2\alpha}$ on Day 5 (7–8:30 AM).

2.2. Ovarian ultrasonography and blood sampling

During the Ovsynch protocol that was used to synchronize the cows for the experiment, transrectal ultrasonography (US) was used to assure that all cows had a CL at the time of $PGF_{2\alpha}$ treatment and a regressed CL and a large preovulatory follicle at the time of the second GnRH treatment. To determine ovulation after the second GnRH treatment and assure the presence of a new, functional CL, all cows were evaluated at 22 and 48 hours after the second GnRH treatment. Ovulation in response to GnRH was defined as disappearance of one or more preovulatory follicles ($\geq$9 mm) that were present on the ovaries at the time of the second GnRH treatment and 22 hours later but absent at 48 hours after the GnRH treatment. Cows were also evaluated using US during the experimental protocol on Days 5, 6, 7, 11, 12, and 14. All US examinations were performed using a Sonovet 2000 (Universal Medical System, Bedford Hills, NY, USA) with a 7.5-MHz linear array transducer.

Blood samples were collected via puncture of the median caudal vein or artery with 8-mL evacuated tubes for collection of serum (Vacutainer; Becton, Dickinson and Co., Franklin Lakes, NJ, USA). Samples were collected before treatment on Day 5 and at 2, 4, 6, 12, and 24 hours after treatment. In addition, blood samples were collected on Day 7, 8, 11, 12, 13, 14, and 15. Within 2 hours after collection, samples were centrifuged (3000 × g) at