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Influence of repeated dinoprost treatment on ovarian activity in cycling dairy cows

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ABSTRACT

To study the ovarian response to the long-term effect of $PGF_{2\alpha}$, 16 cows were treated with 25 mg tromethamine dinoprost (Pronalgon F; Pfizer, Tokyo, Japan) for 21 days after natural ovulation. Five control cows were treated with sterile physiological saline. The follicle and corpus luteum (CL) development were monitored using a real-time ultrasound instrument. In addition, the plasma concentration of progesterone (P_4) was determined. In nine of the 16 Pronalgon-treated cows, the first dominant follicle (1st DF), second dominant follicle (2nd DF), and third dominant follicle ovulated consecutively (group A). In five cows, the 1st and 2nd DFs ovulated consecutively (group B). The developing CL started to regress approximately 5 days after each ovulation without maturation in groups A and B. In the two remaining Pronalgon-treated cows, there was no further ovulation after natural ovulation (group C). In one cow in group C, the 1st DF became atretic and the 2nd DF became cystic with the diameter of the cystic follicle reaching 31.2 mm on Day 30. In another cow, the 1st DF became cystic with a diameter of 30.9 mm on Day 18. Although P_4 began to increase after each ovulation in all of the Pronalgon-treated cows, it decreased immediately after each ovulation without a large increase, peaking at approximately 1 ng/mL. Furthermore, the number of days when P_4 was >1 ng/mL from natural ovulation to Day 21 was 2.6 ± 0.7 days, which was significantly less than that in the control cows (16.0 ± 0.6 days). These results indicate that the long-term effect of $PGF_{2\alpha}$ has an important role in ovulation of all dominant follicles and might induce cystic ovaries in cows.

1. Introduction

The beginning of normal ovarian cyclic activity is one of the most important events for dairy cows to regain maximum breeding potential after parturition. However, ovarian disturbance is highly prevalent during this period. The most common disturbances are delayed cyclicity or ovulation [1], prolonged luteal phase [1,2], and cystic ovaries [3]. Uterine abnormalities affect ovarian functions and induce a prolonged luteal phase [2,4,5]. When ovulation occurs before the uterus has expelled all of the exudates and debris, a heavy growth of bacteria such as *Trueperella pyogenes* occurs and the corpus luteum (CL) is retained for a long period [5–7]. An exudative purulent response is generated in the endometrium, and the ability of the uterus to produce or transport sufficient amounts of $PGF_{2\alpha}$ is compromised [5,6,8]. Conversely, secretion of less progesterone (P_4) as a result of postpartum uterine infection might contribute to the early demise of the CL [9,10]. Kaneko et al. reported that repeated infusions of *T. pyogenes* into the uterus of normally cycling cows caused luteal regression or shortened luteal lifespan, and induced ovulation of dominant follicles which normally become atretic [11]. The increased ratio of $PGF_{2\alpha}$ after the infusion has been shown to play an important role in the regression of the CL [12]. However, the levels of PGFM ranged from 36 pg/mL to 1800 pg/mL in that study, and the high level of PGFM was not maintained for long. In postpartum cows

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with endometritis, PGFM levels are very high (ng/mL) [13] and last for a longer period. To clarify the ovarian response to the long-term effect of high levels of $PGF_{2\alpha}$, cows were treated with 25 mg of tromethamine dinoprost (Pronalgon F; Pfizer, Tokyo, Japan) every day for 21 days in this study. The follicle and CL development were monitored using a real-time ultrasound instrument and the plasma concentration of P_4 was determined.

2. Materials and methods

2.1. Animals

Twenty-one Holstein cows ≥4 years old (5.1 ± 0.4; mean ± SEM) were used. Animals were housed in a tie-stall barn at Azabu University. None of these cows had been inseminated after the last parturition because they were kept for educational purposes. Parities of the cows were unknown and ≥2 years had passed since the last parturition for all cows. None had been milked and all had exhibited ≥2 normal estrous cycles before the start of the study. Rectal examination of all cows confirmed that there were no clinical abnormalities of the uterus or abnormal vulval discharge. All experiments were carried out with approval from the ethics committee of Azabu University.

2.2. Ultrasound scanning

Changes in the follicle and the CL were monitored daily between 9:00 AM and 12:00 PM using a real-time ultrasonograph (Model HS-2100V; Honda Electronics Co., Ltd., Aichi, Japan) equipped with a 10-MHz transrectal linear transducer for 30 days. Natural ovulation (Day 0) was defined when the dominant follicle present in the ovary at estrus was confirmed to have disappeared using transrectal palpation and real-time ultrasonography. The CL area was recorded using the area-measuring function of the ultrasonograph. When a central cavity was present in the CL, the area of the cavity was subtracted from the total area [14]. In this study, the dominant follicles that grew during the 30-day observation period were named as follows, irrespective of whether the previous follicle had ovulated: first dominant follicle (1st DF), follicles that grew after natural ovulation; second dominant follicle (2nd DF), follicles that grew after the 1st DF growth; and third dominant follicle, follicles that grew after the 2nd DF growth.

2.3. Treatment with tromethamine dinoprost

Cows were randomly allocated into tromethamine dinoprost (Pronalgon F) treatment or control groups. Sixteen cows were treated with Pronalgon F (5 mL im) daily from Day 1 to Day 21. The five remaining control cows were given 5 mL im sterile physiological saline.

2.4. Blood sampling

Blood samples were collected each day from the tail vein using a vacuum-type heparinized tube. Plasma was separated using centrifugation (2000 × *g* for 10 minutes) and stored at −80 °C until the determination of P_4 concentration.

2.5. Hormone analysis

Concentrations of P_4 were analyzed in blood samples collected from all cows on Days 1 to 30. Progesterone was measured using radioimmunoassay without extraction using a commercial kit (Diagnostic Products, Los Angeles, CA, USA). Cross-reactivity of the anti-P_4 antibody for progesterone, 5α-pregnane-3-20-dione, 17α-hydroxyprogesterone, 5β-pregnan-3-20-dione, 20α-dihydroprogesterone, testosterone, 5β-pregnane-3α-ol-20-one, androstenediol, and 17β-estradiol were 100%, 9%, 3.4%, 3.2%, 0.2%, 0.1%, 0.05%, <0.05%, and <0.05%, respectively. Intra- and interassay coefficients of variation were 8.8% and 9.7%, respectively, and the detection limit was 0.02 ng/mL.

2.6. Data analysis

We compared the number of days when P_4 was >1 ng/mL from Day 1 to Day 21 between the control group and the dinoprost-injected group using Student *t* tests. Values are presented as mean ± SEM and P values <0.05 were considered statistically significant.

3. Results

3.1. Changes in ovaries

In the five control cows, the 1st DF that developed after natural ovulation became atretic without ovulation, and the 2nd DF ovulated on Day 22, Day 23 (N = 2), or Day 24 (N = 2). The number of ovulations during the 30-day observation period was one. After natural ovulation, the CL developed normally and matured. A representative time course for the development of the follicles and the CL in the control cows is shown in Figure 1 (control). In nine of the 15 Pronalgon F-treated cows, ovulation of the dominant follicles occurred consecutively (group A). The number of ovulations during the 30-day observation period was three. A representative time course for the development of the follicles in group A is shown in Figure 1 (group A). In five other Pronalgon F-treated cows, ovulation of the 1st and 2nd DFs occurred consecutively (group B). The number of ovulations during the 30-day observation period was two. A representative time course for the development of the follicles in Group B is shown in Figure 1 (group B). In the two remaining Pronalgon F-treated cows, there were no further ovulations after natural ovulation (group C). In one of these cows, the 1st DF became atretic, and the 2nd DF became cystic, reaching a diameter of 31.2 mm on Day 30 (Fig. 1 [group C-1]). In the other cow, the 1st DF also became cystic, reaching a diameter of 30.9 mm on Day 18 (Fig. 1-[Group C-2]). In all Pronalgon F-treated cows, the developing CL after natural ovulation, the 1st DF ovulation, and the 2nd DF ovulation started to regress approximately 5 days after each ovulation without maturing (Fig. 1).