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Length of the follicular growing phase and oocyte competence in beef heifers

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ABSTRACT

We tested the hypotheses that extending the duration of follicular growth by superstimulation increases oocyte competence, and that FSH starvation at the end of superstimulatory treatment decreases oocyte competence. Heifers were randomly assigned to three groups: short FSH, FSH starvation, and long FSH (N = 8 per group). At 5 to 8 days after ovulation, follicle ablation was performed, and a progesterone-releasing device (CIDR) was placed intravaginally. Short FSH and FSH starvation groups were given eight doses of FSH im at 12-hour intervals, and the long FSH group was given 14 doses. $PGF_{2\alpha}$ was administered twice (12 hours apart) and the CIDR was removed on Day 3 (Day 0 = wave emergence) in the short FSH group, and on Day 6 in the other two groups. Heifers were given LH 24 hours after CIDR removal and cumulus-oocyte complexes (COC) were collected 24 hours later. The COC were matured *in vitro* for 6 hours and fertilized *in vitro*; embryos were cultured for 10 days. A greater number of follicles ≥9 mm were detected in the long FSH group than in the FSH starvation and short FSH groups (25.4 ± 5.3, 11.0 ± 2.1, 10.6 ± 2.3, respectively; $P < 0.03$). A greater proportion of expanded COC were collected from the long FSH than from the FSH starvation group ($P < 0.001$), and the short FSH group was intermediate (93%, 54%, and 74%, respectively). The FSH starvation group had a greater proportion of poor quality oocytes than the short and long FSH groups (70%, 45%, and 33%, respectively; $P < 0.001$) and cleavage rate was lower (22%, 54%, and 56%, respectively; $P = 0.003$). The proportion of oocytes that developed into embryos (morulae and blastocysts on Day 9 after IVF) was also lower in the FSH starvation group than in the short and long FSH groups, (5% vs. 25% and 37%; $P = 0.04$); the latter two groups did not differ. The long FSH treatment resulted in 2.5 and 3.4 times more transferable embryos per animal (morulae and blastocysts) at Day 9 after IVF than the short FSH and FSH starvation groups (5.6, 2.5, and 1.7 embryos per heifer respectively; $P = 0.04$). In conclusion, extending the standard superstimulation protocol by 3 days enhanced the ovarian response to FSH treatment, and a period of FSH starvation after superstimulatory treatment compromised oocyte quality and the fertilization process.

1. Introduction

Most cows have two or three follicular waves during an interovulatory interval [1,2]. The luteal phase and the interovulatory interval are 2 to 3 days shorter, the duration of dominance of the first follicular wave is 2 to 3 days longer, and the interval from emergence of the final wave to ovulation is 3 days longer (9 vs. 6 days) in two- wave than in three-wave cycles [3–5]. Furthermore, the ovulatory follicle grows 3 days longer in a high progesterone environment (before luteolysis) in a two-wave cycle and ultimately attains a greater preovulatory diameter than in

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a three-wave cycle [6]. However, the interval of follicle growth under a low-progesterone environment (i.e., after luteolysis) is similar between two- and three-wave cycles (i.e., 3 days) [5]. Until recently, the relationship between the number of waves in a cycle and fertility has been contradictory [7–11]. However, in a recent study involving a large number of cows (N = 365) [6], there was no difference in pregnancy rate between those given short progesterone exposure during the growing phase of the ovulatory follicle (analogous to a three-wave cycle) versus those given longer progesterone exposure (analogous to a two-wave cycle). These results did not support the notion that oocytes from dominant follicles of two-wave cycles are aged and therefore less fertile than those from three-wave cycles.

A superstimulation model has also been used to examine the effects of progesterone and the duration of the follicular growing phase on oocyte competence [12,13]. Progesterone-induced prolongation of the follicular growing phase (analogous to a two-wave cycle) resulted in a greater proportion of follicles that reached ovulatory size [12,13]. Moreover, there was no difference between longer and shorter progesterone exposure (analogous to two- and three-wave cycles, respectively) on developmental competence of oocytes matured and fertilized *in vivo* [12].

It was suggested that increasing the interval from the end of FSH treatments to removal of a progesterone-releasing device (i.e., a period of FSH starvation) might be beneficial for oocyte maturation [14–16]. A 48-hour interval of FSH starvation at the end of superstimulatory treatment was associated with increased in number of embryos produced *in vitro* [14]. However, 144 hours of FSH starvation after superstimulatory treatment (in a low progesterone environment) resulted in 100% ovulation failure [17], and 84 hours of FSH starvation (luteal-phase levels of progesterone for 60 hours followed by low progesterone) resulted in 50% ovulation failure [12]. The effects of FSH starvation on the oocyte itself could not be assessed in the latter two studies.

The objective of this study was to determine the effects of the duration of the growing phase of the ovulatory follicle and a total of 96 hours of FSH starvation on oocyte competence using a superstimulation model and IVF. We tested the hypothesis that prolongation of the growing phase of the ovulatory follicle after superstimulation will improve oocyte competence, whereas FSH starvation will adversely affect oocyte competence.

2. Materials and methods

2.1. Heifers and treatments

The experiment was conducted at the Western College of Veterinary Medicine, University of Saskatchewan, during July and August. Hereford crossbred beef heifers, weighing 346.5 ± 5.8 kg (mean ± SEM), and maintained in outdoor pens, were used. Procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the University of Saskatchewan Protocol Review Committee.

Heifers (N = 51) were given a $PGF_{2\alpha}$ analogue (500 μg cloprostenol im; Estrumate; Schering-Plough Animal Health, Pointe-Claire, Québec, Canada) to synchronize estrus, and examined daily by transrectal ultrasonography using a 7.5-MHz linear-array transducer (Aloka SSD-900; Tokyo, Japan) to detect ovulation. The first 24 heifers that ovulated after prostaglandin treatment were used. Transvaginal ultrasound-guided ablation of follicles ≥5 mm in diameter was done 5 to 8 days after ovulation to synchronize emergence of a new follicular wave [18]. A progesterone-releasing device (CIDR-B; Pfizer Canada Inc., Saint-Laurent, Québec, Canada) was placed in the vagina immediately after follicle ablation. The day of emergence of the new follicular wave (Day 0) was considered to be the day after ablation [18]. Heifers were assigned randomly to three groups to be superstimulated (N = 8 per group; Fig. 1): (1) short FSH; (2) FSH starvation; and (3) long FSH. Starting on Day 0, the short FSH group was given eight im doses of FSH (Folltropin-V; Bioniche Animal Health, Belleville Ontario, Canada) at 12-hour intervals over 4 days (Day 0.5–4; each dose equivalent to 25 mg of NIH-FSH-P1), and two luteolytic im doses of prostaglandin (500 μg cloprostenol) 12 hours apart on Day 3. The FSH starvation group was given similar treatment of eight doses of FSH; however, the two luteolytic doses of prostaglandin were given on Day 6. The long FSH group was given FSH every 12 hours for 7 days (i.e., 14 doses, each dose equivalent to 25 mg of NIH-FSH-P1; Days 0.5–7); two doses of prostaglandin were given on Day 6. The progesterone-releasing devices were removed concurrent with the second prostaglandin treatment (Day 3.5 in the short FSH group, and Day 6.5 in the FSH starvation and long FSH groups). Heifers were given 12.5 mg porcine LH (pLH) im (Lutropin-V, Bioniche Animal Health) 24 hours after progesterone-releasing device removal, and follicular aspiration was performed 24 hours after pLH treatment. Thus, the gonadotropin-free period (interval between the last FSH and pLH) was 12 hours in the short and long FSH groups and 84 hours in the FSH starvation group. The ovaries of each heifer were examined by transrectal ultrasonography 12 hours after pLH treatment to determine the number and size of follicles.

2.2. Oocyte collection and classification

Caudal epidural anesthesia was induced (2% lidocaine HCL and epinephrine USP; Bimeda-MTC Animal Health Inc., Lavaltrie, Québec, Canada) and cumulus-oocyte complexes (COC) were collected by transvaginal ultrasound-guided follicular aspiration using a 7.5-MHz convex-array transducer. Follicular contents were aspirated using a vacuum pump with a flow rate of 20 to 25 mL/min (Vacuum pump: Allied Healthcare Products, Inc., St. Louis, MO, USA) into a 70-μm embryo filter (Emcon filter; Veterinary Concepts, Spring Valley, WI, USA) containing Dulbecco's phosphate buffered saline (dPBS; Invitrogen Inc., Burlington, Ontario, Canada) with ET Surfactant (0.3% Pluronate; Bioniche Animal Health) and sodium heparin (2 IU/mL). Collection of COC was scheduled over a 12-day interval, such that one heifer from each group was represented on each collection day (i.e., three heifers per collection day). The COC were transported from the barn to the laboratory in dPBS plus 5% newborn calf serum in a portable incubator set at 37 °C.

The COC were identified by stereomicroscopy at magnification ×10, washed three times in 1× dPBS (with