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Hormonal treatments for increasing the oocyte and embryo production in an OPU–IVP system

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Abstract

The objective was to enhance the inherent developmental ability of bovine oocytes retrieved by ultrasound-guided transvaginal aspiration. Various hormonal regimes were utilized to produce partially matured oocytes *in vivo*, in order to improve embryo development following IVF. In the first experiment, a two-by-two factorial design was used with FSH (multiple versus single dose) and im administration of LH (yes versus no) 6 h prior to OPU. In all protocols (which lasted for nine consecutive weeks), ovarian stimulation was performed in the presence of a CIDR. One FSH administration was adequate for ovarian stimulation (9.33 ± 0.7 and 10.14 ± 0.7 follicles per cow per OPU session); however, multiple injections increased ($P < 0.05$) follicular response (12.97 ± 0.7 and 13.97 ± 0.7). In the second experiment, a two-by-two factorial design was used to compare the effects, during ovarian stimulation, of the presence or absence of CIDR, and iv treatment with LH 6 h prior to OPU (yes versus no), on oocyte competence (judged by blastocyst development rates following IVF). Presence of CIDR during superstimulation had no effect on the follicular response. Administration of LH 6 h prior to OPU increased ($P < 0.05$) the oocytes of higher morphological grades, and in the absence of a CIDR, improved ($P < 0.05$) blastocyst development rate. Treatment with LH, 6 h prior to OPU without the use of CIDR during ovarian stimulation, resulted in 2.89 ± 0.4 blastocysts per cow per OPU session as compared to 1.56 ± 0.4, 1.56 ± 0.4 and 1.33 ± 0.4 for all other groups. In conclusion, compared to single administration, multiple FSH administration increased ($P < 0.05$) available follicles for aspiration. Moreover, when ovarian stimulation in the absence of CIDR was followed by administration of LH 6 h prior to OPU, it increased ($P < 0.05$) the number of blastocysts per OPU session.

Keywords: Bovine; OPU; Single FSH; CIDR; LH administration

1. Introduction

The technique of ultrasound-guided transvaginal follicular aspiration for ovum pickup (OPU), coupled with *in vitro* production of embryos, has been shown to be a feasible and practical alternative to the conventional multiple ovulation and embryo transfer (MOET) program [1,2], and is being increasingly used for large-scale commercial applications worldwide [3–6].

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During the follicular wave during the estrous cycle in the cow, the period of follicular growth and dominance prior to ovulation is critical for the developmental potential of the oocyte [7,8]. Ultrastructural and functional modulations occurring in the oocyte prior to the LH surge are termed as ''pre-maturation'' or ''oocyte capacitation,'' as they impart to the oocyte its full developmental capacity [8,9]. The final phase of oocyte maturation within the ovulatory follicle is stimulated by the LH surge, producing a haploid secondary oocyte, capable of fertilization and initial embryonic development [8]. However, in an OPU–IVP system, oocytes recovered are from 3 to 8 mm follicles, and hence do not experience the preovulatory LH surge and subsequent follicular microenvironment [9].

A possible way to increase the developmental competence of these oocytes would be to modify the superovulatory regimen to permit oocytes to complete their final maturation following rapid follicular growth [10]. Manipulation of follicular development with an FSH starvation period following ovarian stimulation, and administration of LH 6 h before OPU, produced developmentally highly competent oocytes resulting in very high *in vitro* embryo production rates [11].

In order to induce ovarian stimulation prior to OPU, the timing of exogenous gonadotropin administration in relation to the estrous cycle stage is crucial. The relationship between the stage of ovarian follicle growth and follicle dominance to the superstimulatory response remains controversial [12–14]. To prevent stress due to repeated animal handling for multiple FSH treatments for superovulation, a few studies have tested the efficacy of single-dose FSH administration in MOET [15–17]. However, there is a paucity of data on the efficacy of ovarian stimulation by a single dose of FSH before oocyte retrieval.

We aimed to address these three issues, namely: the influence of progesterone on ovarian stimulation, the effectiveness of a single administration of FSH, and the effect of administered LH on oocyte quality. Two experiments were planned with the following objectives: (1) to evaluate single versus multiple administration of FSH in inducing follicular response for oocyte retrieval by OPU; (2) to compare the ovarian stimulatory response in the presence versus absence of an exogenous progesterone source; and (3) to evaluate the influence of LH administered 6 h prior to OPU on the developmental competence of the retrieved oocytes.

2. Materials and methods

2.1. Experimental animals

These experiments were conducted at Trans Ova Genetics, Sioux Center, Iowa, which houses a large recipient herd for ''in clinic'' embryo transfer work. From this herd, cyclic Angus cross cows were selected and were maintained in a separate holding pen throughout the experimental period. All animal-related procedures and protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Connecticut, as well as at Trans Ova Genetics.

2.2. Experiment 1: experimental design and treatment protocols

Sixteen normal, cyclic, approximately 4-year-old Angus cross cows were selected based on transrectal palpation of their ovaries. All cows were nonlactating and, on a body condition scale (BCS) of 1–10, had an average score of 6. They were fed corn silage, wet distillers grain (10% fat, 30% protein) and a blend of alfalfa hay, grass hay, and corn stock in a total mixed ration (TMR) yielding an approximate daily dry matter intake of 25 lbs with 10–12% protein. The selected cows were randomly allocated to one of four treatment groups (four cows per group). Each group was allotted a different treatment protocol, designed within the time frame of 1 week, which was repeated for 9 consecutive weeks. The treatment groups, their codes, treatment protocols, and experimental design are explained below, and are summarized in Fig. 1.

The objective of this experiment was to compare the effect of multiple im FSH treatments (three times, 24 h apart) with a single administration of the same dose of FSH, in inducing ovarian stimulation prior to OPU. The total dose of FSH in each treatment group was 200 mg (50% of the dose used in classical MOET programs). As the protocols were based on a weekly schedule and were going to be repeated for nine consecutive weeks, we wanted ovaries to be adequately stimulated without risking hyperstimulation due to a higher dose of FSH. As the animals were subjected to weekly dominant follicle removal (DFR), as well as OPU (every 3 or 4 days), there was no possibility of dominant follicle formation, ovulation, or subsequent luteal tissue formation. Hence, an exogenous progesterone supplement, in the form of a CIDR (a progesterone releasing intravaginal device, containing 1.38 g progesterone, InterAg, Hamilton, New Zealand, marketed by Pfizer) was inserted every week. This was designed to mimic a