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Effective embryo production from Holstein cows treated with gonadotropin-releasing hormone during early lactation

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ABSTRACT

The low efficiency of embryo production in Holstein cows during early lactation presents many challenges for animal production. To improve its efficiency, the outcomes of single GnRH injections 48 hours before each of three cycles of ovum pick up (OPU; weeks 2, 4, and 6) were compared with three cycles of unstimulated OPU (controls; weeks 1, 3, and 5) in 35 Holstein cows during 6 weeks of early lactation (40–80 days postpartum). More total follicle numbers (19.5 vs. 16.0; $P < 0.05$) but fewer dominant follicles (0.5 vs. 1.4; $P < 0.01$) were observed by ultrasound, and more cumulus–oocyte complexes were collected in a single OPU in the treatment cycles compared with controls (15.3 vs. 11.5; $P < 0.05$). The numbers of morphologically "good" cumulus–oocyte complexes graded A and B in the stimulated OPUs were significantly greater than in controls (2.8 vs. 1.7 and 5.8 vs. 4.2, respectively; $P < 0.05$). Significantly, more oocytes stained positively with brilliant cresyl blue after GnRH treatment compared with the control cycles (13.7 vs. 9.6; $P < 0.05$). After *in vitro* fertilization, embryos in the treatment cycles had improved development ($P < 0.01$) during each developmental stage compared with the controls (9.0 vs. 6.2 two-cell embryos; 4.7 vs. 3.0 four-cell embryos; 3.3 vs. 2.0 morulae; and 3.0 vs. 1.7 blastocysts, respectively). Moreover, there was no significant difference in pregnancy rate of the recipient cows after embryo transfer (57.1% vs. 42.1%; $P > 0.05$) no matter if the embryos came from the GnRH-treated cycles or not. Thus, GnRH-stimulated OPUs improved the efficiency of embryo production in Holstein cows during early lactation. This novel method for *in vitro* embryo production should benefit the dairy industry.

1. Introduction

In the dairy industry, embryos can be produced either *in vivo* or *in vitro*. *In vivo*–fertilized embryos are produced by superovulation combined with artificial insemination. However, this is difficult to implement during peak lactation because of poor sensitivity to exogenous hormones, such as FSH [1]. At present, the production of *in vivo*–fertilized embryos in dairy cows is mainly carried out when they are not lactating or on heifers, which are generally dedicated for embryo production. Thus, the major means for bovine embryo production has changed from *in vivo* to *in vitro* methods [2]. *In vitro* embryo production (IVP), using ovum pick up (OPU) coupled with *in vitro* fertilization (IVF), is believed to be more efficient and requires fewer spermatozoa [3,4].

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Many modifications have been developed with the aim of improving the overall efficiency of IVP [5–7]. It is believed that the use of gonadotropins helps to increase the size of follicles that can be selected by inexperienced operators and increase in blastocysts rates and viability [8,9]. However, there are many uncertainties affecting the efficiency of IVF-derived embryo production during the FSH refractory period of early lactation. In fact, the Ovsynch protocol successfully resets a new wave of follicular development using GnRH administration even during the FSH-insensitive early postpartum phase [10]. If the injection of GnRH could increase the number of follicles observed by ultrasound, then the efficient production of IVF-derived embryos should be achievable. It was also reported that the numbers of cumulus–oocyte complexes (COCs) were correlated with the number of days postpartum before an OPU [11].

With the specific objective of finding an effective embryo production method during early lactation, we examined the efficiency of conducting OPUs in lactating Holstein cows after GnRH treatment and evaluated the subsequent efficacy of IVP.

2. Materials and methods

2.1. Animals and experimental design

All lactating Holstein cows used in this study were handled in accordance with the regulations of Hiroshima Prefecture Livestock Technology Research Center for animal experiments. Cycles of OPU were conducted once a week for 6 weeks on 35 cows beginning at 40 days postpartum. All cows received a single dose of GnRH i.m. (200 μg; Fertirelin acetate; Daiichi Seiyaku Co., Ltd., Tokyo, Japan) 48 hours before follicle aspiration in weeks 2, 4, and 6, whereas control OPUs with no stimulation were carried out in weeks 1, 3, and 5 (Fig. 1).

2.2. Follicle aspiration

Before OPU, all cows received a single dose of Adosan (4 mL; Riken K.K., Tokyo, Japan) as an epidural anesthetic to prevent abdominal straining and to relax the rectum. This was necessary for prolonged palpation of the ovaries [12]. A real-time ultrasound scanner (ASSD-1200 type; Aloka Co., Ltd., Tokyo, Japan) and a 7.5-MHz convex array transducer (UST-M15–2290 type; Aloka) attached to a 17-Ga stainless steel aspiration needle (COVA Needle; Misawa Medical, Ibaraki, Japan) were used for each OPU. The numbers of follicles in ovaries were counted on ultrasound video images (Handycam; Hitachi Ltd., Tokyo, Japan). Follicles greater than 2 mm in diameter were punctured using an aspirator (K-MAR-5000 type; Cook Medical Technology, Eight Mile Plains, QLD, Australia) connected to the needle. The aspiration rate and vacuum pressure were 20 mL/min and 115 mm Hg, respectively. Follicular fluids were collected into 50-mL conical centrifuge tubes (Sumilon; Sumitomo Bakelite Co., Ltd., Tokyo, Japan) with collection medium maintained at 35 °C by a warmer (Model FV5; FHK, Tokyo, Japan). The collection medium consisted of lactate ringer solution (Haruzen V Zenoaq, Fukushima, Japan) supplemented with 0.3% (vol/vol) fetal calf serum (FCS; Funakoshi Co., Ltd., Tokyo, Japan), 10 U/mL heparin (Neotube Nipro, Osaka, Japan), and 0.1 mg/mL Kanamycin (Meiji Seika Pharma Co., Tokyo, Japan). All COCs were then separated on a 75-μm filter (Cellcollector; Fujihira Industrial K.K., Tokyo, Japan).

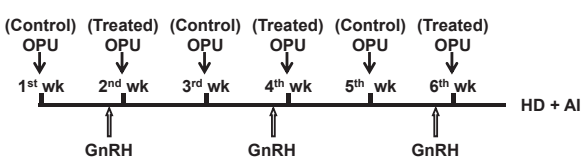

Fig. 1. Scheme of the experimental design; OPUs were conducted during 6 weeks of early lactation (40–80 days postpartum). HD, heat detection.

2.3. Oocyte evaluation

The quality of each COC was graded according to morphologic criteria as described [13]. Briefly, grade A COCs had compacted, multilayered cumulus cells and a homogeneous ooplasm, grade B had a compact cumulus cell layer with homogeneous ooplasm, grade C had a less compact cumulus cell layer with irregular ooplasm containing dark granules, and grade D had no cumulus cells or had an overexpanded cumulus cell layer and a jelly-like matrix.

Brilliant cresyl blue (BCB) staining followed the procedure described by Sugulle et al. [14] with slight modifications. Briefly, the COCs were washed three times in Dulbecco's phosphate-buffered saline (D-PBS) and exposed to 52 μM BCB in D-PBS for 30 minutes at 38.5 °C under an atmosphere of 5% CO_2 in air with 100% humidity. The COCs were then washed three times in D-PBS with 4 mg/mL BSA (Sigma–Aldrich, St. Louis, MO, USA), and examined under an inverted microscope at ×50 magnification. Oocytes were classified as BCB positive with a varying degree of blue cytoplasm or BCB negative with no blue cytoplasm.

2.4. In vitro maturation, fertilization, and embryo culture

Grade A, B, and C COCs were used for IVM according to the method described by Zhao et al. [15] with slight modification. Briefly, after three rinses in prewarmed M2 medium (M-5910, Sigma–Aldrich) with 10% FCS, COCs were cultured in medium consisting of TCM-199 medium (GIBCO 12340–030; Grand Island, NY, USA), 10% FCS, 0.12 AU/mL FSH (Kyoritu Seiyaku Co., Ltd., Tokyo, Japan), and 50 ng/mL EGF (E-1264, Sigma–Aldrich) at 38.5 °C under an atmosphere of 5% CO_2 in air with 100% humidity for 20 to 22 hours.

The IVF procedure was performed on matured oocytes as described [16]. Briefly, COCs were coincubated with frozen-thawed Percoll-separated bull spermatozoa at a concentration of 12×10^6/mL for 6 hours, and then the cumulus cells were removed. Zygotes were transferred into modified synthetic oviduct fluid (m-SOF) medium supplemented with 3 mg/mL BSA and 0.25 mg/mL linoleic acid albumin (L-8384; Sigma–Aldrich) in 100-μL droplets covered with sterile mineral oil (23306–84; Nakalai Tesque, Kyoto, Japan) and incubated for 72 hours at 38.5 °C in an