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Optimizing production of in vivo-matured oocytes from superstimulated Holstein cows for in vitro production of embryos using X-sorted sperm

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ABSTRACT

The present study aimed to establish an efficient system for the production of female embryos from dairy cows by in vitro fertilization (IVF) using X-sorted sperm and in vivo-matured oocytes collected by ovum pick up (OPU). Nonlactating Holstein cows (n = 36) were administered a controlled intravaginal progesterone-releasing (controlled internal drug release) device (d 0), underwent dominant follicle ablation (DFA) or ovulation by administration of 100 μg of GnRH on d 5, and were superstimulated with FSH and $PGF_{2\alpha}$, following standard procedures. Controlled internal drug release devices were removed on the evening of d 8 or on the morning of d 9, depending on the experiment. For LH surge induction, 200 μg of GnRH was administered on the morning of d 10 (0 h). In experiment 1, the peak (48.1%) of ovulating follicles was detected at 29 to 32 h after GnRH injection (0 h), and the range in the timing of the initiation of ovulation was less by timing from GnRH administration (30.0 ± 2.8 h) rather than by timing the onset of estrus (32.7 ± 4.7 h). Only 0.9% of total ovulated follicles were recorded before 26 h after GnRH injection. Therefore, OPU was carried out at 26 h and IVF occurred at 30 h after GnRH in experiments 2 and 3. In experiment 2, 83.3 ± 10.8% of oocytes with expanded cumulus cells had extruded the first polar body at 30 h after GnRH injection. The aim of experiment 3 was to compare the effect of either DFA or GnRH-induced LH surge before superstimulation on the efficiency of embryo production by IVF following superstimulation. Progesterone concentrations from d 10 to 12 in the DFA group were lower than those in the GnRH group. A greater proportion of recovered oocytes with expanded cumulus cells from ≥8-mm follicles was observed in the DFA group than in the GnRH group (95.9 and 77.4%, respectively). Blastocyst rates in the DFA and GnRH groups (58.0 and 52.8%, respectively) did not differ from those of oocytes collected from nonstimulated OPU and matured in vitro (49.9%). However, the proportion of high-quality blastocysts was higher in the DFA group compared with the GnRH group (54.9 vs. 21.5%). Our results demonstrate that high rates of good-quality blastocysts can be produced by IVF with X-sorted frozen sperm using in vivo-matured oocytes collected by OPU from cows after DFA and superstimulation combined with ovulation induction.

Key words: in vivo-matured oocyte, sex-sorted sperm, in vitro fertilization, superstimulation

INTRODUCTION

The ability to produce calves of the desired sex is an attractive reproductive technology for dairy farmers wishing to breed replacement heifers. The potential benefits of using sexed semen have recently been outlined (Hutchinson et al., 2013). Two broad approaches to sex selection in cattle have been used: sexing embryos before transfer or the use of sex-sorted sperm to bias the sex ratio at fertilization. A variety of PCR-based techniques are available for sexing biopsies taken from embryos (Bondioli et al., 1989; Hasler et al., 2002). However, in most cases, this requires specialized equipment such as a micromanipulator and related PCR machines (Hasler et al., 2002). Moreover, embryo biopsy requires skill, increases the time required for

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processing, and potentially reduces the viability of the embryo (Thibier and Nibart, 1995; Hasler et al., 2002; Hirayama et al., 2004).

Currently, sexed sperm is commercially available and represents an attractive alternative to embryo sexing (Seidel, 2003; Garner and Seidel, 2008; DeJarnette et al., 2009). The accuracy of the sexing procedure has been reported to be close to 90% (Seidel, 1999; Garner and Seidel, 2008; Schenk et al., 2009). Despite the potential use of this technology, fertility rates after AI with sexed sperm are lower than those achieved with conventional unsorted semen, possibly due to DNA damage caused to sperm by the sorting process, reduced sperm viability, and low sperm numbers per insemination (DeJarnette et al., 2008; Schenk et al., 2009; DeJarnette et al., 2011).

To increase the production of calves of the desired sex above that achievable with routine AI, multiple ovulation embryo transfer can be combined with AI with sex-sorted semen (Hayakawa et al., 2009; Soares et al., 2011). However, whether this potential is achievable in commercial practice has been questioned, particularly in cows, because of reduced fertilization rates when using low doses of X-sorted semen (Hayakawa et al., 2009; Peippo et al., 2009; Larson et al., 2010).

Ovum pick up (**OPU**) coupled with in vitro fertilization (**IVF**) has been reported to be an efficient method of embryo production compared with multiple ovulation embryo transfer, and requires far fewer sperm than required for AI (Merton et al., 2003; Imai et al., 2006). However, blastocyst developmental rates using oocytes recovered from small follicles and matured in vitro are typically low (<30 to 40%; Lonergan et al., 2003a,b). In contrast, blastocyst development from in vivo-matured oocytes is consistently higher than that from in vitro-matured oocytes (Greve et al., 1987; Marquant-Le Guienne et al., 1989; Dieleman et al., 2002; Rizos et al., 2002). Thus, IVF of in vivo-matured oocytes with sex-sorted sperm may be one way of maximizing the number of transferable female embryos in applied reproductive programs in dairy cattle.

The objective of this study was to establish an efficient program for the production of female embryos in dairy cattle by IVF using in vivo-matured oocytes collected by OPU and sex-sorted sperm. To achieve this, the specific aims were to (1) determine the optimum timing of aspiration of in vivo-matured oocytes from cows after superstimulation combined with LH surge induction using GnRH, (2) assess the morphology of aspirated oocytes, and (3) examine embryo production following IVF of in vivo-matured oocytes with X-sorted sperm following dominant follicle removal before superstimulation.

MATERIALS AND METHODS

The overall scheme for the collection of in vivo-matured oocytes using superstimulation and OPU and subsequent embryo production by IVF is illustrated in Figure 1.

Animal Management

The use of animals was subject to the regulations set out by the Law for the Humane Treatment and Management of Animals (Law No. 105, 1973) and notification no. 6 and no. 22 of the Japanese Guidelines for Animal Care and Use. All experimental procedures involving animals were approved by the Ethics Committee for Care and Use of Experimental Animals [National Livestock Breeding Center (NLBC), Fukushima, Japan].

Nonlactating Holstein cows were used (n = 39; parity = 2.5 ± 0.9; BCS at start of experiment = 3.39 ± 0.52). All cows were housed in a loose barn and fed based on the Japanese Feeding Standard for Dairy Cattle (National Agricultural Research Organization, 2006). The BCS was assessed on a 5-point scale (with 1 being extremely thin and 5 being extremely fat) with 0.25 increments (Ferguson et al., 1994).

Superstimulation

Donor cows received an intravaginal progesterone-releasing device [controlled internal drug release, (**CIDR**); Pfizer, Tokyo, Japan; CIDR insertion = d 0]. Before FSH administration in experiments 1 and 2, all follicles ≥8 mm in diameter were aspirated (dominant follicle ablation, **DFA**) on d 5. In experiment 3, a crossover design was used; half of the animals underwent DFA and half received 100 μg of a GnRH analog (fertirelin acetate, Supolnen; Kyoritsu Seiyaku Co., Tokyo, Japan) to induce dominant follicle ovulation on d 5, and cows were subsequently switched to the opposite treatment. A total of 30 armour units (AU) of FSH (Antrin R10; Kyoritsu Seiyaku Co.) was administered twice daily for 4 d in decreasing doses (6, 6, 4, 4, 3, 3, 2, and 2 AU, respectively) from the evening of d 6 to the morning of d 10 to stimulate follicular growth. All cows received 0.225 mg of $PGF_{2\alpha}$ (D-cloprostenol, Dalmazin; Kyoritsu Seiyaku Co.) at CIDR removal on the evening of d 8 (in experiment 3, CIDR removal was delayed until the morning of d 9 to avoid ovulation of FSH-stimulated follicles before OPU). Gonadotropin-releasing hormone (200 μg; 0 h) was administered to induce the LH surge of growing follicles on the morning of d 10. All drugs were administered i.m. into the ischiorectal fossa.