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Short communication: Follicle superstimulation before ovum pick-up for in vitro embryo production in Holstein cows

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ABSTRACT

The objective was to evaluate in vitro embryo production (IVEP) in nonlactating Holstein cows after ovarian superstimulation. Cows were randomly assigned in a crossover design to 1 of 2 groups: control (n = 35), which was not synchronized and not treated with hormones before ovum pick-up (OPU), or hormone-treated (n = 35), in which wave emergence was synchronized and animals treated with porcine (p)-FSH in the presence of norgestomet before OPU. In the hormone-treated group, all follicles ≥7 mm in diameter were aspirated for synchronization of wave emergence and cows received a norgestomet ear implant. After 36 h, treatment with p-FSH (6 doses of 40 mg each, 12 h apart, i.m.) started. Ovum pick-up from follicles >2 mm in diameter was performed 44 h after the last p-FSH (coasting). Then, IVEP was performed. The total number of cumulus-oocyte complexes recovered (16.0 vs. 20.5 ± 2.2) and number of grades I to III (viable) oocytes (10.7 vs. 12.3 ± 1.6) did not differ between hormone-treated and control groups Additionally, no differences were found in the number of blastocysts per cow per OPU (3.0 vs. 2.6 ± 0.5) or in blastocyst rates (17.1 vs. 12.2 ± 2.4%) between hormone-treated and control, respectively. Thus, in this study, ovarian follicle superstimulation with p-FSH followed by coasting in nonlactating Holstein cows that had synchronization of wave emergence and progestin supplementation did not improve oocyte quality or IVEP compared to no hormonal treatment.
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Short Communication

In dairy cows, embryo transfer (**ET**) is commonly used as a tool for genetic improvement. In addition, ET can be used to enhance reproductive efficiency of herds particularly in cows in specific physiological conditions such as heat stress (Demetrio et al., 2006; Stewart et al., 2011). Embryo production by multiple ovulation and ET is widely used around the world, but the variability in response to the superstimulatory treatments remains an important limitation (Galli et al., 2003). An alternative reproductive biotechnology for embryo production is in vitro embryo production (**IVEP**). This biotechnology allows production of a large number of embryos from high genetic potential donors during a shortened period (Pontes et al., 2009) and allows the use of sex-sorted sperm without substantially compromising embryo production (Zhang et al., 2003; Carvalho et al., 2010). In North America, use of IVEP has dramatically increased during the last few years (Perry, 2015) probably because of the improvements in genetics, reproductive efficiency, and sex ratio of offspring that can be achieved with this technique. In Brazil, IVEP has been primarily used for *Bos indicus* beef cattle because of their greater antral follicle counts and therefore greater oocyte yield per collection than observed in *Bos taurus* (Sartori et al., 2016). However, use of IVEP has recently increased dramatically in dairy cattle in Brazil and other Latin American countries.

The successful implementation of an IVEP program depends on many factors including genetics of the donor, vacuum pressure during aspiration, needle type during ovum pick-up (**OPU**), and number of follicles present (Bols et al., 1997; Ward et al., 2000). These factors may influence the quality of oocytes collected, as determined visually, or the success of in vitro maturation and fertilization of oocytes or subsequent in vitro embryo development, as determined by number, quality, and stage of the resulting embryos. In addition, some studies have indicated that stage of the follicular wave or follicle size may affect the success of IVEP after OPU (Hendriksen et al., 2004; Machatkova et al., 2004). For example, in cows on a random day of the estrous cycle, oocytes were more likely to be classified as healthy if they were from larger (>8 mm; 70%) than medium (6 to 8 mm; 33%) or smaller (<6 mm; 12%) follicles (Carolan et al., 1996). Furthermore, cows that were treated with FSH had a greater proportion of follicles with intermediate diameters (>6 and <10 mm) compared with cows from the control group (Goodhand et al., 1999). Thus, it follows logically that hormonal treatments during growth of the follicular wave before OPU could be used to presynchronize follicles to a more ideal stage for IVEP.

Nevertheless, use of follicular aspiration or treatment with estradiol benzoate had no significant effect on oocyte number or quality, cleavage rate, or blastocyst rate or number (Ramos et al., 2010). Similarly, oocytes collected from cows at different phases of the estrous cycle had similar developmental capacity (de Wit et al., 2000). However, in another study, greater blastocyst rates were found for oocytes collected from follicles in the growth rather than dominance phase of a follicular wave (Machatkova et al., 2004). In contrast, although superstimulation with FSH increased the number of large, healthy follicles in the growth phase, FSH treatment reduced the blastocyst development rates in oocytes collected from these follicles (Blondin et al., 1996). Nonetheless, Blondin et al. (2002) suggested that superstimulation should be combined with a period of "coasting" (time between last FSH and OPU) before OPU. Coasting for 48 h produced more follicles of 5 to 10 mm compared with only 33 h of coasting. Further, addition of an LH surge 6 h before OPU increased blastocyst production in oocytes collected after 33 h of coasting but not in oocytes collected after 48 h of coasting (Blondin et al., 2002). Although blastocyst rates were exceedingly high in this study, no comparisons were made to oocytes from cows without FSH superstimulation. Several other studies have evaluated the role of FSH and subsequent coasting on oocyte quality and blastocyst production, with generally positive effects in Holstein cattle (Nivet et al., 2012) but no significant effects on IVEP success in Nelore cattle (Monteiro et al., 2010).

The substantial increase in IVEP during the last few years, particularly in North and South America, makes evaluation of the effects of superstimulation on IVEP success of substantial practical interest. In addition, superstimulation has become routine for many IVEP practitioners in the United States, but it has been used very little in South America where a substantial number of the global IVEP embryos are produced (Blondin, 2015). The experimental design and statistical power for evaluating this practical question is important because of the substantial variability between cows in antral follicle numbers and potentially in developmental capacity of oocytes collected from different cows. Therefore, the objective of the present study was to evaluate IVEP in nonlactating Holstein cows treated with a protocol very similar to that described by Nivet et al. (2012) in direct comparison to performing OPU in cows not submitted to any synchronization or hormonal treatment. A crossover experimental design was used so that each individual cow was subjected to both treatments to increase statistical power of the study. Based on previous research, our hypothesis was that Holstein cattle treated with FSH after follicle wave synchronization and with a norgestomet implant and then having a coasting period of 44 h before OPU would have greater blastocyst production than cows that had OPU without synchronization or hormone treatment.

Thirty-five nonlactating multiparous Holstein cows [5.8 ± 1.78 (mean ± SD) years old and BCS of 3.2 ± 0.30 (mean ± SD)] were used in the study performed in Brazil. Cows were housed in free-stall barns equipped with sprinklers and fans and were fed ad libitum a TMR-based diet of corn silage and corn and soybean meal-based concentrate with minerals and vitamins, which was balanced to meet or exceed the nutritional requirements of nonlactating dairy cows (NRC, 2001).

Cows were randomly assigned in a crossover design to the following experimental groups: control (n = 35), in which cows were not synchronized and not treated with hormones before OPU, or hormone-treated (n = 35), in which, 36 h after OPU for follicle wave synchronization and insertion of a norgestomet implant (Crestar, MSD, São Paulo, Brazil), cows were treated with porcine (**p**)-FSH (Folltropin-V, Bioniche Animal Health, ON, Canada) and were subjected to OPU sessions. Fourteen days were allowed between periods. In the hormone-treated group, all ovarian follicles ≥7 mm in diameter were aspirated for synchronization of follicle wave emergence and cows received a norgestomet ear implant. Thirty-six hours later, the treatment with 240 mg of p-FSH i.m. was started, which was administered in 6 doses of 40 mg each, 12 h apart. The OPU was performed 44 h after the last p-FSH (coasting), when the implant was removed (Figure 1).

Before the OPU sessions, cows had their perineal area cleaned with water and 70% ethanol. Epidural anesthesia was performed with 5 mL of 2% lidocaine hydrochloride (Xylestesin, Cristália, Itapira, Brazil). All follicles >2 mm in diameter were aspirated by ul-