on superovulatory responses in CIDR-treated Korean native cows. Forty-two cows, at random stages of the estrous cycle, received a CIDR device (CIDR™; InterAg, Hamilton, New Zealand), 1 mg estradiol benzoate (SY Esrone; Samyang, Seoul, Korea) and 50 mg progesterone (SY Ovaron; Samyang); gonadotropin treatment began 4 days later. Cows were divided into 2 groups based on the dose and numbers of days of treatment with porcine FSH (pFSH): T1 group ($n = 20$): a total of 28 mg pFSH (recommended dose of Antorin®; Kawasaki Pharmaceutical, Tokyo, Japan) was given in twice daily IM injections in decreasing doses over 4 days (5, 5, 4, 4, 3, 3, 2, and 2 mg); and T2 group ($n = 22$): a total of 24 mg pFSH given in twice daily decreasing doses over 3 days (5, 5, 4, 4, 3, and 3 mg). Otherwise, all cows received the same treatments. Twenty-five and 15 mg dinoprost ($PGF_{2\alpha}$; Lutalyse; Pharmacia & Upjohn, Puurs, Belgium) were given with the 5th and 6th injections of pFSH, respectively. CIDR devices were withdrawn with the 6th pFSH injection, and the cows received 100 μg Gonadorelin (GnRH; Fertagyl; Intervet, Boxmeer, The Netherlands) 36 h after CIDR device removal. Cows were artificially inseminated using commercial semen from 4 Korean native bulls twice, at 48 and 60 h after CIDR device removal, and embryos were recovered 6 or 7 days after the 2nd insemination. The number of CL was counted on the day of embryo recovery by transrectal ultrasonography (Sonovet 600 with 5.0 MHz linear-array transducer; Medison Co., Ltd., Seoul, Korea). The recovered embryos were evaluated according to the IETS Manual for stage of development and quality. All data between groups were compared using Student's *t*-test. The numbers of CL (9.7 ± 1.1 vs. 9.4 ± 1.3), total ova/embryos (7.2 ± 1.1 vs. 6.3 ± 1.4), transferable embryos (4.4 ± 1.0 vs. 3.6 ± 0.9), degenerate embryos (0.9 ± 0.3 vs. 1.3 ± 0.4), and unfertilized ova (2.0 ± 0.6 vs. 1.5 ± 0.5) did not differ between groups (T1 vs. T2), respectively ($P > 0.05$). Data indicate that the reduced dose (24 vs. 28 mg) and numbers of treatments (6 vs. 8) of pFSH for superstimulation of Korean native cows does not affect the embryo yield.

391 EFFECTS OF SEASON AND LACTATION STATUS ON EMBRYO PRODUCTION IN HOLSTEIN COWS

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The aim of this study was to evaluate effects of season and lactation status on embryo production in Holstein donors ($n = 51$). Data from 195 embryo collections (94 from lactating cows and 101 from non-lactating cows), at a dairy farm located in Descalvado, Sao Paulo, Brazil, in 2005, were analyzed. The superstimulation protocol included two 3-mg norgestomet ear implants (Crestar®; Intervet, Sao Paulo, Brazil) and an injection of 3 mg of estradiol benzoate IM (EB) on random days of the estrous cycle. Four days later, 500 IU FSH IM (Pluset®; Calier, Buenos Aires, Argentina) were divided into 8 decreasing doses given 12 h apart. With the seventh FSH injection, 0.530 mg IM of sodium cloprostenol ($PGF_{2\alpha}$; Ciosin®; Coopers-Brazil, Sao Paulo, Brazil) was given, and implants were removed with the eighth FSH injection. Twelve hours later, 250 μg IM of gonadorelin (Fertagyl®; Intervet) was given, followed by two AI 12 and 24 h later. Ova/embryos were recovered 6.5 days after the first insemination. The total number of ova/embryos, IETS grades 1, 2, and 3 (viable) and grade 4 plus unfertilized (non-viable), were analyzed by General Linear Model (GLM). Effects of donor, lactation status (lactating or non-lactating), sire, season (1: January through March, $n = 56$; 2: April through June, $n = 37$; 3: July through September, $n = 50$; 4: October through December, $n = 52$), and their interactions were included in the model. There was an effect of donor on all outcome variables ($P < 0.01$). There was an effect of season on the total number of ova/embryos (1: 10.2 ± 1.2; 2: 6.6 ± 1.0; 3: 10.0 ± 1.1; 4: 11.2 ± 1.2; $P < 0.02$) and of viable embryos (1: 3.7 ± 0.5; 2: 2.4 ± 0.5; 3: 5.9 ± 0.9; 4: 4.4 ± 0.7; $P < 0.001$). Lactation status influenced the total number of ova/embryos (lactating: 10.9 ± 0.9 vs. non-lactating: 8.7 ± 0.7; $P < 0.001$) and the number of non-viable embryos (lactating: 6.7 ± 0.8 vs. non-lactating: 4.4 ± 0.5; $P < 0.01$). However, there was no effect of lactation status on the number of viable embryos (lactating: 4.14 ± 0.5 vs. non-lactating: 4.29 ± 0.5; $P > 0.10$). In summary, the number of viable embryos in Holstein cows was influenced by season and donor, but not by lactation status.

392 CHARACTERISTICS OF THE SUPEROVULATORY RESPONSE IN GYR (*BOS INDICUS*) CATTLE

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Gyr is the most important zebu breed for dairy herds in Brazil and in other tropical countries. Superovulatory responses in this breed have been shown to be lower than in European or beef zebu breeds such as Nelore and Brahman. The aims of this study were to investigate the effect of the presence of a dominant follicle on the superovulatory response, and to determine endocrine patterns in superstimulated and non-superstimulated Gyr cows. The first experiment was designed to evaluate the effect of the dominant follicle on embryo yield. Multiparous, non-lactating Gyr cows were treated with a conventional superovulation (SOV) protocol [300 IU of Pluset (Serono, Roma, Italy) in 8 decreasing doses] starting on either Day 10 (G10, $n = 14$) or Day 8 (G8, $n = 16$) of the estrous cycle or 48 h after dominant follicle removal by ultrasound-guided follicle aspiration (G48, $n = 10$). Ovarian follicle populations were monitored daily by ultrasonography. Data were analyzed by ANOVA, and means were compared by Tukey's test. Dominant follicle removal resulted in a larger number of small follicles before SOV (27.1 ± 2.7 vs. 14.7 ± 1.5 and 13.1 ± 1.2; $P < 0.05$), but the number of follicles reaching a diameter larger than 9 mm after superstimulation (17.4 ± 1.3 vs. 14.4 ± 2.0 and 11.4 ± 2.0; $P > 0.05$), and the number of viable (IETS grades 1 and 2) embryos (3.1 ± 0.8 vs. 3.0 ± 0.7 and 3.3 ± 0.8; $P > 0.05$) did not differ from G10 and G8 groups, respectively. There was great variation in superovulatory response, and the Pearson correlation between follicle numbers at the time of initiating superstimulatory treatments and response was low ($r = 0.49$; $P > 0.05$). In the second experiment, endocrine patterns in superstimulated ($n = 32$) and non-superstimulated ($n = 24$) Gyr cows

were compared. Blood samples were collected on Day 14 of the estrous cycle or after 4 days of FSH treatment when follicular fluid was also obtained from both groups by ultrasound-guided follicle aspiration. Plasma and follicular fluid samples were stored at −20°C until assay for progesterone (P_4), androstenedione (A_2), and estradiol (E_2) by RIA, using commercial kits (MedLab, Auckland, New Zealand, and Diagnostic Systems Laboratories, Inc., Webster, MN, USA). Mean plasma E_2 concentrations did not differ between FSH-treated and control cows (3.7 ± 0.4 vs. 3.2 ± 0.4 ng mL^{-1}; $P > 0.05$). Intrafollicular concentrations of E_2, A_2, and P_4 in FSH-stimulated follicles (mean size of 14.0 ± 1.2 mm) were 193.5 ± 83.0, 55.7 ± 17.0, and 54.8 ± 28.1 ng mL^{-1}, respectively, lower ($P < 0.05$) than those found in non-superstimulated growing dominant (mean size of 11.6 ± 0.5 mm) follicles (501.2 ± 83.8, 122.2 ± 22.5, and 97.0 ± 21.9 ng mL^{-1}, respectively), but similar to concentrations in non-superstimulated, non-dominant (mean size of 7.2 ± 0.4) follicles (152.6 ± 99.2, 37.7 ± 20.3, and 37.3 ± 6.8 ng mL^{-1} for E_2, A_2, and P_4, respectively). Results suggest that factors other than follicle dominance adversely affect superovulatory responses in the Gyr breed, and that follicular steroidogenesis may also be adversely affected following treatment with exogenous FSH. Results also failed to support the use of ultrasonography to predict superovulatory response in the Gyr breed.

393 A STUDY OF THE USE OF SEXED SEMEN IN SUPERSTIMULATED HOLSTEIN COWS

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udy was designed to investigate the use of sexed semen in superstimulated Holstein cows. Non-lactating Holstein cows ($n = 107$; those that did not superovulate were excluded) were superstimulated and inseminated with frozen–thawed, sexed semen (at 1 insemination dose) or control semen (at 2 different insemination doses) in 3 groups. Cows received a Cue-Mate (Bioniche Animal Health, Beijing, China) and were injected with 5 mg of estradiol-17β plus 100 mg of progesterone on Day 0. Cows were treated from Days 4 to 7 with decreasing doses of FSH for a total of 300 mg Folltropin-V (Bioniche; 50, 50, 40, 40, 30, 30, 30, and 30 mg) at 12 h intervals. On Day 7, Cue-Mates were removed and cows were injected twice with 0.4 mg cloprostenol (PGF; Institute of Family Planning, Shanghai, China). Bilateral deep uterine horn AI was performed 12 and 24 h after the onset of estrus on Day 9 (Group A: 4×10^6 sexed, frozen–thawed sperm per insemination from 1 of 3 bulls; Group B: 4×10^6 non-sexed, frozen–thawed sperm, or Group C: 10×10^6 non-sexed, frozen–thawed sperm per insemination from 1 of 3 bulls). Cows were assigned to balanced insemination groups at random. Embryos were collected and evaluated on Day 16. Group A had a significantly lower fertilization and usable embryo (IETS grades 1 and 2) rate than the other 2 groups (Table 1). Although the rate of usable embryos between Groups B and C did not differ, the fertilization rate in Group C was significantly higher, suggesting that 4 million sperm in Group B was an inadequate insemination dose for superovulated cows. The significantly lower number of usable embryos in Group A suggests that the use of sexed sperm in superovulation and embryo transfer may not be economically feasible. More research is required to optimize and standardize bovine superstimulation and AI with sexed semen.

Table 1. Embryo production following insemination of superstimulated Holstein cows with frozen–thawed sexed (Group A) or unsexed (Groups B and C) semen

Group	No. donors	No. total embryos	Rate of fertilized ova	Rate of usable embryos	Mean no. usable embryos/donor
A	51	444	57.4% (255/444)[a]	45.3% (201/444)[a]	3.9 ± 3.21[a]
B	21	193	75.6% (146/193)[b]	56.0% (108/193)[b]	5.1 ± 1.29[ab]
C	35	308	88.6% (273/308)[c]	64.3% (198/308)[b]	5.7 ± 1.89[b]

[a–c]Values with different superscripts within a column differ significantly ($P < 0.05$).

Tissue Culture

394 DEVELOPMENT OF A RAT ENDOMETRIAL SPHEROID AS A MODEL FOR THE ANALYSIS OF ENDOMETRIAL FUNCTIONS

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The endometrium is one of the most complex tissues; it undergoes dynamic changes in response to implantation and pregnancy processes. An *in vitro* model may provide a tool for clarifying the complex implantation process. However, there is no suitable *in vitro* model for investigation of endometrial functions. The spheroid has been utilized in cell biology research because it appears to mimic the morphology and physiology of cells in living tissues and organs, which is unlike conventional monolayer culture. Multicellular spheroids composed of normal adult cells may provide a more useful

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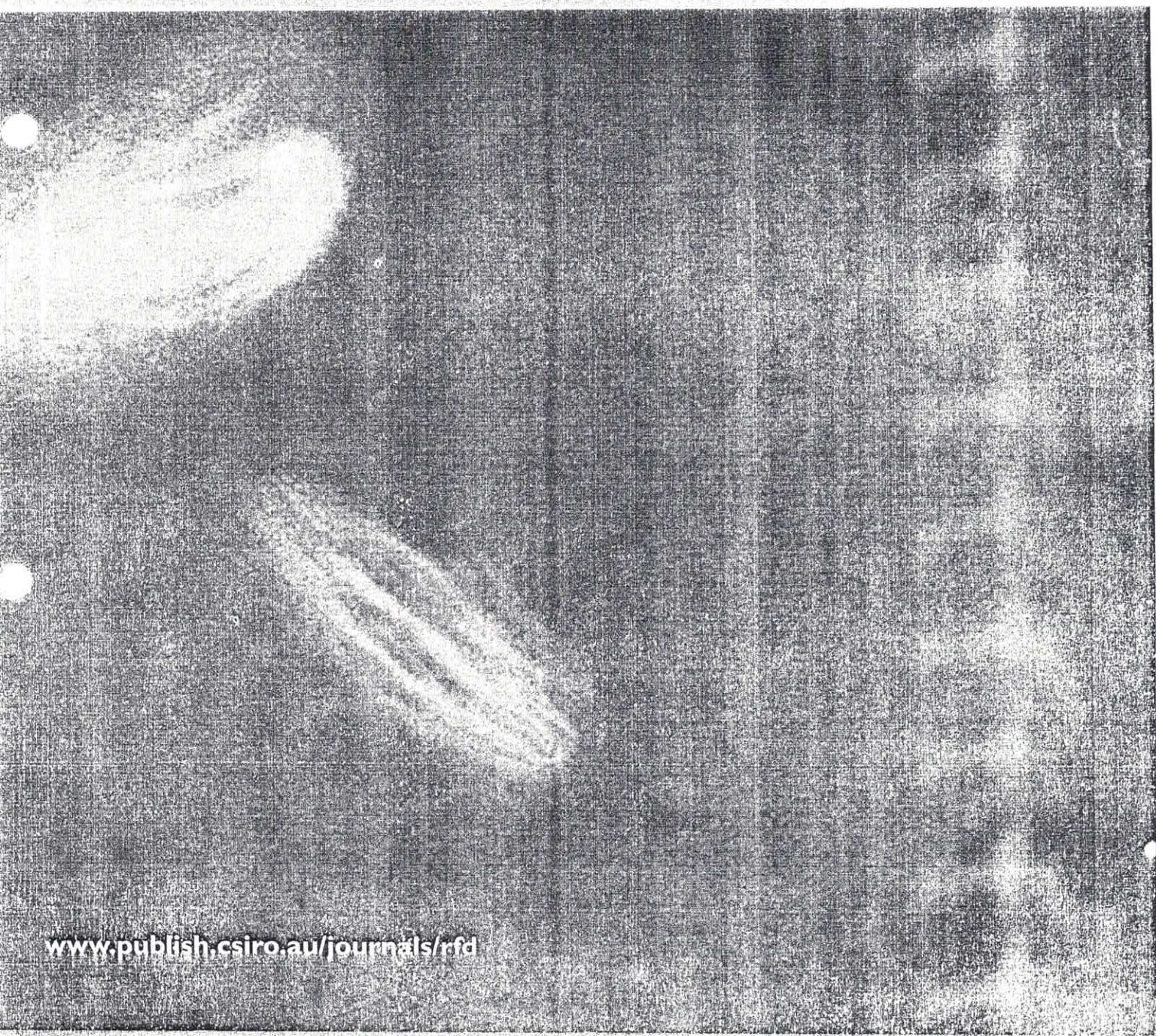

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