

ABSTRACTS FOR POSTER PRESENTATION

Superovulation

335 EFFECT OF PATERNAL LINE OF DONOR ON SUPEROVULATORY RESPONSE AND EMBRYO QUALITY IN JAPANESE BLACK HEIFERS

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The objective of this study was to investigate the effect of paternal line of donor on superovulatory response and embryo quality in Japanese Black cattle. Japanese Black cattle have paternal lines that can be classified into the following, according to meat quality and the growth rate: 'high marbling meat type', 'high growth rate type' and 'high marbling meat and high growth rate (intermediate type)'. We hypothesized that paternal line of donor may affect superovulatory response. One hundred and forty superstimulations were performed on 56 Japanese Black heifers, and data on superovulatory response and embryo collection were analyzed. Japanese Black donors used in this study were from the following 4 paternal lines: Kedaka (large-framed with high growth rate; $n = 9$, 30 times), Fujiyoshi (intermediate type; $n = 22$, 47 times), Shigekane (high marbling meat type; $n = 13$, 40 times), and Tajiri (high marbling meat type and small-frame with slow growth rate; $n = 12$, 23 times). Donors were synchronized using a CIDR-B (EAZI-BREED, InterAg, NZ) for 8 to 12 days, and 500 μg PGF analog (Resipron-C; Teikoku Zouki, Japan or Clopromate-C; Sumitomo, Japan) administered at CIDR removal. Superstimulation was initiated on Day 10 (Day 0 = day of estrus) of the synchronized cycle; FSH (Antrin R10; Denka, Japan) was administered twice daily for 4 days with decreasing doses for a total of 28 Armour units FSH. PGF analog was administered in the morning on the last day of FSH treatment. Donors were given 50 or 100 μg GnRH analog (Sporunen, Denka, or Conceral, Takeda Schering prau, Japan) at the time of estrus detection, and were inseminated 12 h after the onset of estrus. Embryos were recovered on Day 7 after AI. The numbers of CL and follicles were recorded with transrectal ultrasonography immediately after embryo recovery. Embryos were classified according to the IETS Manual. Data were analyzed by Kruskal-Wallis test combined with Scheffe's multiple comparison test. There were no significant differences in CL numbers among paternal lines: Kedaka (9.5 ± 4.2), Fujiyoshi (8.5 ± 5.1), Shigekane (7.8 ± 5.0) and Tajiri (8.6 ± 4.1). Mean number of recovered ova/embryos per donor was significantly ($P < 0.05$) higher in the Kedaka line (8.4 ± 5.1) than in the Fujiyoshi (4.9 ± 4.1) and Shigekane (5.5 ± 7.0) lines. The percentages of viable embryos was greater ($P < 0.05$) in the Fujiyoshi (73.7%) and Shigekane lines (62.8%) than in the Tajiri line (50.9%), whereas the percentage of unfertilized oocytes was significantly ($P < 0.05$) higher in the Tajiri line (41.5%) than in the Fujiyoshi (17.5%) or Shigekane (26.1%) lines. The percentages of freezeable and transferable embryos were not significantly different among paternal lines. Data suggest that the paternal line had an effect on the superstimulatory response in Japanese Black heifers, and especially the number and percentage of viable embryos per donor.

336 EFFECT OF AN INDIVIDUALLY ADAPTED SUPERSTIMULATION PROTOCOL FOR OPU IN COWS

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The aim of this experiment was to apply an ovarian superstimulation protocol to OPU, with attention to the individual responses of each cow, and to show effects on follicle numbers and diameters. Ten non-lactating dairy cows were stimulated with pFSH and submitted to OPU-IVF 6 times at 2-week intervals. On Day 0 of each 2-week period, the dominant follicle was punctured and a progestin ear implant was inserted. Starting on Day 2, animals were treated with 6 equal doses of pFSH, administered twice daily over 3 days. On Day 7, 48 h after the last pFSH injection, follicle diameters were measured by ultrasound and animals were submitted to OPU (OPU-IVF results are not shown here). The administered dose of pFSH for the first OPU session was 30 mg per animal (stimufol, ULg-FMV, Liège, Belgium). In the following OPU sessions, pFSH doses were individually adapted to the percentage of follicles larger than 11 mm, present during the previous OPU session. If the number of follicles larger than 11 mm was between 1% and 20%, total dose remained unchanged; if between 20% and 40%, total dose was reduced by 5 mg; and if more than 40%, total dose was reduced by 10 mg. If no follicles larger than 11 mm (0%) were present, total dose was increased by 5 mg. Only one animal received the initial pFSH dose for all 6 OPU sessions for a total of 180 mg. All other animals received more or less than 180 mg, ranging from 135 to 230 mg. For interpretation of the effects of pFSH dose on follicular diameter, data were grouped in pairs (before and after changing the dose of pFSH). This resulted in 3 groups: Fixed pFSH dose—pFSH dose was unchanged in the 2 sessions (of that pair); reduced pFSH dose—pFSH dose was reduced in the second session (of that pair); and increased pFSH dose—pFSH dose was increased in the second session (of that pair). No changes in response were found when pFSH dose remained unchanged. However, the number of small-sized follicles increased and the number of large-sized follicles decreased when dose of pFSH was reduced. The number of larger-sized follicles increased when pFSH dose was increased. The total number of follicles remained unchanged regardless of pFSH dose. Overall, the mean number of punctured follicles per session was 11.9 ± 7.7 with 16% of the follicles over 11 mm. In conclusion, subtle changes in dose of pFSH influenced follicle sizes but not follicle numbers.

Table 1. Effect of fixed, reduced or increased doses of pFSH on follicular diameters (mean number \pm SD)

| Doses of pFSH | | Fixed | Reduced | Increased |
|----------------------|--------|----------------|-----------------|-----------------|
| No. of pairs | | 24 | 16 | 20 |
| Follicles of 2–7 mm | before | 5.6 ± 3.8 | 2.1 ± 1.8^a | 7.5 ± 6.1 |
| | after | 5.0 ± 5.3 | 5.1 ± 4.8^b | 5.7 ± 4.2 |
| Follicles of 7–11 mm | before | 5.2 ± 5.1 | 3.7 ± 4.3 | 5.1 ± 4.5 |
| | after | 5.4 ± 5.2 | 4.1 ± 3.9 | 4.1 ± 4.3 |
| Follicles > 11 mm | before | 1.5 ± 1.2 | 4.3 ± 3.2^a | 0.4 ± 0.7^a |
| | after | 1.8 ± 2.3 | 1.4 ± 1.7^b | 2.1 ± 2.9^b |
| Total follicles | before | 12.3 ± 7.2 | 10.1 ± 8.3 | 12.7 ± 8.5 |
| | after | 12.2 ± 8.8 | 10.3 ± 5.9 | 11.9 ± 7.6 |

^{ab}Different superscripts within columns indicate significant differences ($P < 0.05$).

337 THE ONSET AND DURATION OF OVULATION IN DAIRY COWS SUPEROVULATED FOLLOWING SYNCHRONIZATION OF FOLLICLE WAVE WITH CIDR AND ESTRADIOL BENZOATE

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Determining the optimal time for AI of superovulated cattle is important for yielding acceptable fertilization rates. The objectives of this study were to determine the interval to onset, and the duration of ovulation in dairy cows superstimulated with FSH following synchronization of follicular wave emergence with a CIDR and estradiol benzoate (EB). Holstein ($n = 8$) and Jersey ($n = 1$) cows received an intravaginal progesterone insert (CIDR-B, InterAg, Hamilton, New Zealand) combined with an injection of 2 mg EB at a random stage of the estrous cycle (Day 0). Superstimulatory treatments were initiated on Day 6 with a total dose of 24 or 36 mg FSH (Antrin, Denka, Kawasaki, Japan) via twice-daily i.m. injections in decreasing doses for 4 days. At 72 h after the first FSH injection, 30 mg PGF (Pronalgon F, Pharmacia, Tsukuba, Japan) was administered, and CIDR-B were removed at 80 h. Transrectal ultrasonography of ovaries was performed at 3-h intervals from 40 to 77 h after the PGF injection. The numbers of ovulations were counted as previously described (Purwantara *et al.* 1994 Anim. Reprod. Sci. 37, 1–5). All detected follicles were counted and classified as small, medium, or large (diameter 2–4 mm, 5–9 mm, or ≥ 10 mm, respectively). The number of ovulations recorded after each scanning was defined as the change in the number of large- and medium-sized follicles observed since the previous scanning. Data were analyzed using ANOVA. Results are presented in Table 1. There were two patterns of ovulations: one was a concentrated pattern of ovulations (ovulatory period < 10 h); the other was a prolonged pattern of ovulations (ovulatory period > 10 h). In cows with a high superovulatory response (≥ 14 ovulations), the onset of ovulation was earlier ($P < 0.05$) and the duration of the ovulatory period was longer ($P < 0.05$) than in cows with lower (≤ 8 ovulations) responses. In conclusion, concentrated and prolonged ovulation patterns were seen in dairy cows superstimulated after synchronization of the follicle wave with a CIDR and EB. In cows with a large number of follicles, the onset of ovulation tended to be earlier and the duration of the ovulatory period longer than in cows with fewer follicles.

Table 1. The onset and duration of ovulation in dairy cows superstimulated following synchronization of the follicle wave with a CIDR and EB

| Ovulation patterns | No. of animals | Onset of ovulation (h) | Completion of ovulation (h) | Ovulatory period (h) | No. of follicles | No. of ovulations |
|--------------------|----------------|-------------------------|-----------------------------|-------------------------|------------------|--------------------------|
| Concentrated | 4 | 61.0 ± 9.5 ^a | 68.0 ± 8.3 | 7.0 ± 2.5 ^a | 17.3 ± 9.1 | 7.3 ± 1.0 ^a |
| Prolonged | 5 | 42.4 ± 3.3 ^b | 57.4 ± 5.8 | 15.0 ± 3.7 ^b | 28.4 ± 10.7 | 24.0 ± 10.3 ^b |
| Average | | 50.7 ± 11.6 | 62.1 ± 8.6 | 11.4 ± 5.2 | 23.4 ± 11.1 | 16.6 ± 11.5 |

^{a,b}Means within the same column with different superscripts differ significantly ($P < 0.05$).

338 EFFECT OF TRACE MINERAL NUTRITION ON FOLLICULAR RESPONSE, OVULATION, AND EMBRYO PRODUCTION IN SUPEROVULATED ANGUS HEIFERS

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We determined whether trace mineral supplementation prior to embryo collection affected embryo production and quality. Twenty half-sibling, Angus heifers originating from a common herd were assigned to three treatment groups using a 3×3 latin square design and three rotations of the treatments: (1) heifers received no added mineral to their diet (Control; $n = 53$); (2) heifers received a commercially available organic mineral supplement (Organic; Albion Cattle Breeder Pak, Des Moines, IA, USA; $n = 52$); and (3) heifers received an isomineral, all inorganic mineral supplement (Inorganic; Inorganic Breeder Pak, Albion, Des Moines, IA, USA; $n = 55$). All heifers had ad libitum access to hay and were fed a supplement containing corn and soybean meal. Heifers received a 25-mg injection of PGF on Day -23 at which point individual feeding of the corn/soybean/mineral supplement was initiated and fed at recommended levels until the day of embryo collection. All heifers were monitored for signs of estrus, but regardless, all heifers received a 1-mg injection of estradiol cypionate (ECP; Pharmacia, Kalamazoo, MI, USA) and a CIDR (Pharmacia) on Day -16. From Day -12 to Day -8 heifers received 29 mg of follicle stimulating hormone (pFSH, batch 9109, Sioux Biochemical, Sioux Center, IA, USA) in a twice daily decreasing dose schedule. On Day -9 heifers received two 5-mg injections of PGF (AM and PM) and the CIDR was removed in the PM. All heifers were inseminated artificially at 36, 48, and 60 h after CIDR removal. On Day 0, embryos were recovered using a nonsurgical procedure and were evaluated under a stereomicroscope. Heifers were given a 45-day adaption period of no mineral supplementation before initiating a new treatment as above. On Days -12, -7, and 0, ovaries were scanned via transrectal ultrasound to determine the presence and number of follicles and CL in each ovary. There were no treatment differences in the number of heifers with a CL on Day -12, the total number of follicles on Day -7, or the total numbers of CL and unovulated follicles on Day 0. Although the total number of recovered ova/embryos were similar among groups (4.2 ± 0.6 , 3.6 ± 0.6 , and 3.3 ± 0.6 for Control, Inorganic, and Organic heifers, respectively), the number of unfertilized oocytes was greater ($P < 0.05$) in Inorganic (2.3 ± 0.4) than in Organic (0.8 ± 0.4) heifers, whereas Control heifers were intermediate (1.3 ± 0.4). In addition, Control heifers had a greater ($P < 0.10$) number of degenerate embryos (0.9 ± 0.2) than Organic (0.3 ± 0.2) or Inorganic (0.3 ± 0.2) heifers. Organic heifers produced a greater number ($P < 0.10$) of transferable embryos (2.2 ± 0.4) than Inorganic heifers (1.1 ± 0.4), with Control heifers intermediate (2.0 ± 0.4). We conclude that heifer and mineral rotation accounted for the greatest differences in embryo production and quality. However, feeding an Organic mineral tended to increase the production of transferable embryos in purebred Angus heifers.

339 CORRELATION BETWEEN FOLLICLE SIZE AND QUALITY OF OOCYTES FROM THE SUPEROVULATED CYNOMOLGUS MONKEY

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Variability in the superovulatory response continues to be one of the most frustrating problems with the application of assisted reproductive technologies in non-human primates. Superstimulation of donor animals with equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG) is widely used, but individual responses to these hormones vary widely. In human in vitro fertilization, follicle size is commonly used as a marker to determine the timing of hCG treatment in order to acquire oocytes matured in vivo. Incorporation of techniques designed to control follicular size in humans may improve superstimulatory response in non-human primates. In this study, we measured follicle size and examined oocyte quality in Cynomolgus Monkeys superstimulated as described below. On the first day of spontaneous menses, monkeys were treated with long-acting GnRH (Luprin; Takeda Pharm, Co. Ltd., Osaka, Japan; Day 0). A dose of 25 IU/kg/day eCG (Serotropin; Teikokuzoki Pharm, Co. Ltd., Tokyo, Japan) was administered i.m. from Day 1 to Day 9, followed by 200 IU kg⁻¹ hCG (Pubergen; Sankyo Pharm, Co. Ltd., Tokyo, Japan) i.m. on Day 10. Dominant follicle sizes were measured on Days 7 and 9 by ultrasonography. Oocytes were collected by laparoscopy in anesthetized monkeys 40 h after the hCG injection. Oocytes were classified by nuclear status. Immature oocytes at the stages of germinal vesicles (GV) and metaphase (MI) were cultured until reaching the stage of Metaphase II (MII). Matured oocytes (MII) were fertilized by ICSI and cultured for 7 days. At the end of culture, the developmental stage of oocytes was examined. The ovaries with different-sized follicles on Day 7 were divided into two groups; ovaries with large follicles (>4.5 mm) were in the first group and ovaries with small follicles (<4.0 mm) were in the second group. On Day 9, follicles in first group grew to more than 5.0 mm and follicles in second group remained less than 5.0 mm. Sixty-two percent of oocytes from follicles in first

group were at MI or MII stage, while only 15% of oocytes in second group reached the MI or MII stage. After ICSI, 42% of MII oocytes from first group developed to the blastocyst stage, while no blastocysts were observed in second group. These results suggest that the size of dominant follicle was a limiting factor for the developmental ability of oocytes in vitro. For production of *Cynomolgus* monkey blastocysts derived from ICSI, the diameter of dominant follicle was required to be at least 5 mm before hCG in order to collect MI and MII oocytes. Incorporation of hormonal treatments designed to optimize follicular size probably reduced the variability in quality of oocytes. Therefore, we expect that an adjustment of dose and duration of eCG and hCG treatment may improve developmental ability of oocytes from follicles that had not reached 5 mm.