The Effect of Reduced Dose and Number of Treatments of FSH on Superovulatory Response in CIDR-Treated Korean Native Cows

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Abstract. The objective of this study was to investigate the effect of dosage and number of days of follicle stimulating hormone (FSH) treatment on superovulatory response in controlled internal drug release (CIDR)-treated Korean native cows. Forty cows underwent two superovulatory treatments with a crossover design. Cows, at random stages of the estrous cycle, received a CIDR together with injections of 1 mg estradiol benzoate and 50 mg progesterone, and gonadotropin treatment began 4 days later. The cows were divided into 2 groups based on the dosage and number of days of treatment with porcine FSH; a total of 28 mg FSH was given in twice daily intramuscular injections in decreasing doses over 4 days (5, 5, 4, 3, 2 and 2 mg; T1 group, n=20) or a total of 24 mg FSH was given in twice daily decreasing doses over 3 days (5, 5, 4, 3 and 3 mg; T2 group, n=20). This was followed by the alternate treatment in the subsequent superovulation. The cows were treated identically in all other respects. PGF$_2$alpha (25 mg and 15 mg) was given with the 5th and 6th injections of FSH, CIDR were withdrawn at the 6th FSH injection and the cows received 200 µg GnRH 36 h after CIDR withdrawal. The cows were artificially inseminated twice, at 48 and 60 h after CIDR withdrawal, using commercial semen from four Korean native bulls, and embryos were recovered 6 or 7 days after the 2nd insemination. The numbers of corpora lutea (CL; 7.9 ± 1.0 vs. 8.3 ± 1.1) and large follicles (1.2 ± 0.2 vs. 1.3 ± 0.3) present at the time embryo recovery, as detected by ultrasonography, did not differ between the T1 and T2 groups (P>0.05). Similarly, the numbers of total ova/embryos (6.2 ± 0.9 vs. 6.4 ± 1.1), transferable embryos (3.4 ± 0.8 vs. 3.2 ± 0.7), degenerate embryos (0.8 ± 0.2 vs. 1.0 ± 0.3) and unfertilized ova (2.1 ± 0.5 vs. 2.2 ± 0.5) did not differ between the groups (P>0.05). These data indicate that a reduced dose (24 vs. 28 mg) and number of treatments (6 vs. 8) of FSH for superovulation of CIDR-treated Korean native cows does not affect the embryo yield.

Key words: Controlled internal drug release (CIDR), Dosage, Follicle stimulating hormone (FSH), Korean native cow, Superovulation

Superovulation has been used worldwide to produce valuable bovine embryos for breeding. Superovulation treatments consist of administration of gonadotropins and luteolytic agents. Two different types of gonadotropin have generally been used for bovine superovulation, follicle stimulating hormone (FSH) and equine chorionic gonadotrophin (eCG). A single injection of eCG (2,500 IU) is sufficient to induce superovulation...
because of its long biological half-life (40 h; [1]). But the long half-life of eCG causes continued ovarian stimulation, unovulated follicles and abnormal endocrine profiles, resulting in reduced embryo quality [2–4]. In contrast, due to its short biological half-life (5 h or less), FSH is usually administered in multiple injections (8 to 10 times) twice daily over 4 to 5 days with a total dose of 28 to 50 mg [5–10]. Some previous reports on superovulation using FSH yielded more transferable embryos compared to eCG [4, 6, 11]; however, Goulding et al. [12] found no differences between FSH- and eCG-treated cows. Although multiple injections of FSH may produce more viable embryos, the multiple injections require more labor and expense and are also more stressful to the donor animals.

To avoid discomfort due to multiple FSH injections, various studies have tried to reduce the number of days of FSH treatment. A single injection of FSH dissolved in polyvinylpyrrolidone (PVP) for superovulation has been studied [13], but, the efficiency of superovulation using a single injection compared to multiple injections with FSH is controversial, producing results that are less effective [7, 14] or similar [15, 16] to multiple injection approaches. Alternatively, Sugamo and Watanabe [17] showed that administration of 16 mg FSH-R over 3 days was sufficient to induce superovulation in Japanese Black cattle. Martens et al. [18] demonstrated that four injections of FSH can lead to superovulation results comparable with those reached with eight injections on four consecutive days; however, the proportion of transferable embryos was reduced. Further studies on reducing the number of treatments without decreasing embryo yield for practical embryo production are warranted. At the same time, reducing the total dose of FSH without decreasing embryo yield might be more economical. Therefore, the objective of this study was to investigate the effect of dose (24 vs. 28 mg) and number (6 vs. 8) of days of FSH treatment on superovulatory response in controlled internal drug release (CIDR)-treated Korean native cows.

Materials and Methods

Animals

This study was performed from May to December 2006 at the Animal Genetic Resources Station (National Institute of Animal Science, Namwon, Jeonbuk, Korea). Forty non-suckled Korean native cows with an average body weight of 428.5 ± 6.4 Kg (mean ± SEM) and a body condition score of 2.8 ± 0.1 (1 to 5 point scale; [19]) were subjected to clinical gynecological examinations prior to experimentation. All experiments were performed with the approval of the Animal Ethics Committee at the College of Veterinary Medicine, Chungbuk National University (Cheongju, Chungbuk, Korea).

Superovulation and embryo recovery

Korean native cows, at random stages of the estrous cycle, received a CIDR containing 1.9 g progesterone (EAZI-BREED™ 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 after CIDR implantation. The cows were divided into 2 groups based on the dosage and number of days of treatment with porcine FSH (FSH, Antrin-R10; Kawasaki Mitaka Pharmaceutical, Tokyo, Japan). A total of 28 mg FSH (the current standard) was given in twice daily intramascular (im) injections in decreasing doses over 4 days (5, 5, 4, 4, 3, 3, 2 and 2 mg; T1 group, n=20) or a total of 24 mg FSH was given in twice daily decreasing doses over 3 days (5, 5, 4, 4, 3 and 3 mg; T2 group, n=20). All cows received the same treatment in all other respects. At the time of the 5th and 6th injections of FSH, 25 mg and 15 mg PGF2α (Lutalyse; Pharmacia & Upjohn, Puurs, Belgium) were administered, respectively. CIDRs were withdrawn at the time of the 6th FSH injection, and the cows received 200 µg Gonadorelin (GnRH, Fertagyl®; Intervet, Boxmeer, Netherlands) 36 h after CIDR withdrawal. At 48 and 60 h post CIDR withdrawal, the cows were artificially inseminated using commercial semen from four Korean native bulls; the semen was equally distributed among the two experimental groups. Embryos were recovered 6 or 7 days after the second insemination by flushing the uterus with Dulbecco’s phosphate buffered saline (D-PBS, Gibco) supplemented with 0.1% polyvinyl alcohol (PVA, Sigma). The recovered embryos were evaluated according to the International Embryo Transfer Society Manual [20] by stage of development and quality. The number of total ova/embryos included the unfertilized ova plus all embryos. Transferable embryos included morulae
and blastocysts rated 1 or 2 in quality.

**Estrus synchronization and second superovulation**

Ten min after uterine flushing, the cows were treated with PGF2α (25 mg, im) in preparation for a second superovulatory treatment. Forty-two days after the first superovulatory treatment, all 40 cows that underwent the first superovulatory treatment were superovulated using the same protocol as the first superovulation. The cows assigned to a total of 28 mg FSH over 4 days in the first replicate were assigned to a total of 24 mg FSH over 3 days in the second replicate and vice versa. Otherwise, all cows received the same treatments used in the first replicate.

**Ultrasound scanning**

The ovaries of each cow were examined at embryo recovery by transrectal ultrasonography (Sonoace 600 with a 5.0 MHz linear array transducer; Medison, Seoul, Korea). Examination involved counting the number of corpora lutea (CL) and large follicles (≥10 mm in diameters).

**Statistical analyses**

Statistical analyses were performed using SAS [21]. The numbers of CL, large follicles, total ova/embryos, transferable embryos, degenerate embryos, and unfertilized ova in the first and second superovulation treatments were compared by the Student’s t-test for the T1 and T2 groups, and the combined data, comprising those of the first and second superovulatory treatment, of the T1 and T2 groups was compared by the Student’s t-test or Wilcoxon-rank-sum test. A value of P<0.05 was considered significant.

**Results**

The numbers of CL, total ova/embryos and transferable embryos were lower (P<0.01) after the second superovulatory treatment than after the first superovulatory treatment in the T1 group, while all the superovulatory responses were the same between the first and second superovulatory treatments for the T2 group (Table 1). However, the combined data, comprising those for the first and second superovulatory treatments, did not differ between the T1 and T2 groups with regard to the numbers of CL or large follicles present at the time of embryo recovery, as determined by ultrasonography (P>0.05, Table 1). Moreover, there was no difference between the groups in the numbers of total ova/embryos, transferable embryos, degenerate embryos or unfertilized ova (P>0.05, Table 1).

**Discussion**

This study evaluated the effect of dosage (24 vs. 28 mg) and number (6 vs. 8) of days of FSH treat-
ment on superovulatory responses in CIDR-treated Korean native cows. Our data showed that the numbers of CL, large follicles, total ova/embryos, transferable embryos, degenerate embryos and unfertilized ova did not differ between the two groups (24 mg/6 vs. 28 mg/8 times). Thus, these data demonstrate that a reduced dose and number of treatments of FSH for superovulation of Korean native cows does not affect embryo yield.

FSH, a major gonadotrophin used in bovine superovulation, is usually administered twice daily in multiple injections (8 to 10 times) over 4 to 5 days [5–9]. Reducing the number of FSH treatments for superovulation without decreasing the embryo yield may be less stressful for donor animals; it does reduce the labor required for bovine embryo production. Many studies aimed at reducing the required number of days of FSH treatment have been conducted, including studies that have used a single injection of FSH dissolved in PVP or a once daily FSH injection schedule over three to five days [5, 7, 13–16, 18, 22]. However, the practical applications of the studies have been limited. Our study evaluated the effect of reducing the dosage (24 vs. 28 mg) and simultaneously reducing the number (6 vs. 8) of days of twice daily FSH treatments on superovulatory responses in CIDR-treated Korean native cows. Our ultrasonographic observations showed that the numbers of CL and large follicles at embryo recovery and, in particular, the measurable embryo yield (numbers of total ova/embryos, transferable embryos, degenerate embryos and unfertilized ova) were not affected by the reduced dosage and number of treatments of FSH in this study. Looney et al. [5] showed that a once daily FSH injection schedule resulted in a superovulatory response equal to or greater than the twice daily FSH injection schedule over five consecutive days. Similarly, Martens et al. [18] found that four injections of FSH yielded a total number of ova comparable to treatments of eight injections over four consecutive days in beef cattle. However, the proportion of transferable embryos was reduced in the four FSH injection protocol, which suggests that a once daily FSH injection schedule can lead to a decrease in the number of viable embryos compared to a twice daily FSH injection schedule. Another study [22] has demonstrated that more transferable embryos are obtained following a superovulation protocol using a single injection of FSH dissolved in PVP (an average of 7.2 embryos) or a protocol of eight injections of FSH twice daily over 4 days (an average of 5.5 embryos) compared with three once daily injections of FSH for 3 days (an average of 3.0 embryos). Thus, the outcome in terms of superovulatory response as a result of reducing the number of FSH treatments has varied among these types of study. In contrast, Benyei and Barros [23] showed that the numbers of ovulations (12.3 vs. 13.4) and good quality embryos (4.8 vs. 5.3) did not differ between standard (1,000 IU of Pluset) and reduced dosages (600 IU) of FSH in a twice daily schedule over 4 days. However, this study detected a significant difference in the occurrence of large unovulated follicles, in contrast to our results. Interestingly, the numbers of CL and transferable embryos from the CIDR-treated Korean native cows in this study were in good agreement with Sugano and Watanabe’s study [17] following superovulatory treatments with a total of 16 and 24 mg FSH, respectively, given in twice daily im injections over 3 days and initiated mid-cycle in Japanese Black cattle. Furthermore, they demonstrated that the reduced dose of FSH (16 vs. 24 mg) did not affect the numbers of CL (8.7 vs. 8.2), total ova/embryos (6.4 vs. 7.2) or transferable embryos (4.6 vs. 4.4), which is consistent with the results of this study. Thus, these results show that a reduced dosage and number of treatments of FSH for superovulation does not affect the embryo yield. For practical use, however, further studies seeking more simplified superovulatory protocols without decreasing the embryo yield are warranted.

In summary, a reduced dosage (24 vs. 28 mg) and number of treatments (6 vs. 8) of FSH for superovulation of Korean native cows does not affect embryo yield and can be less stressful to donor animals and more economical for bovine embryo production.

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References