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Effects of Bovine Somatotropin (bST) Administration Combined with Controlled Internal Drug Release (CIDR) on Embryo Quality and Pregnancy of Hanwoo (Korean Native Beef Cattle) during Commercial Embryo Transfer Program

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ABSTRACT : Effects of recombinant bovine somatotropin (bST) on plasma hormonal concentration, embryo quality, and pregnancy rate were examined during the superovulation and synchronization treatment in donor and recipient cows. Hanwoos (Korean native beef cattle) were treated with controlled internal drug release (CIDR) combined with bST (CIDR+bST) or without bST (CIDR) as donor cows. The embryos recovered from donors were transferred into Holstein recipient heifers treated with bST (CIDR+bST) or without bST (CIDR) for synchronization. The correlation between IGF-I and P4 showed a positive pattern in the CIDR+bST group ($r = 0.44$, $p<0.01$), but a negative pattern was shown in the CIDR group ($r = -0.59$, $p<0.02$) at day 7 of estrous cycles. Although the number of recovered, transferable, and degenerated embryos was not different, quantities of grade 1 (excellent) embryos in CIDR+bST group were significantly higher than those of the CIDR group ($p<0.01$). The pregnancy rate was higher in the CIDR+bST recipient group compared to CIDR group ($p<0.05$), when the embryos were recovered from the donors treated with CIDR. However, the pregnancy was maintained highly in both recipient groups, when the embryos were produced by CIDR+bST treated donors. It can be concluded that bST administration combined with CIDR is an effective method for superovulation and synchronization treatment to stabilize plasma hormonal levels, to obtain excellent quality of embryos, and to get higher pregnancy rate. (**Key Words :** Bovine Somatotropin, Embryo Transfer Program, Corpus Luteum, Plasma Hormonal Level, Pregnancy, bST (Bovine Somatotropin), CIDR (Controlled Internal Drug Release), CL (Corpus Luteum), D (Donor), R (Recipient))

INTRODUCTION

Embryo transfer in cattle was one of the most important techniques for reproduction and breeding. The production of Hanwoo (Korean native beef cattle) from Holstein dairy heifers has been used to control the quantity of them. However, there are some problems such as high expense and low pregnancy rate. To overcome these problems, we need to improve the efficiency of *in vivo* embryo production from donor Hanwoos and to increase the pregnancy rate in recipient Holstein cows.

The factors affecting superovulation treatment in donor cows were the differences in nutrition, body condition score (BCS), species, and individual response to gonadotropins (Shea et al., 1984), kinds of (Staigmiller et al., 1992), treatment timing (Goulding et al., 1990), and number of treatments (Bo et al., 1994). Among these factors, the use of gonadotropins was one of the most effective methods to improve the efficiency of superovulation treatment. Recently superovulation treatments were developed such as, controlled internal drug release (CIDR) devices (Lafri et al., 2002; Sartori et al., 2003; Zi et al., 2005), progesterone releasing intravaginal device (PRID) (Alnimer and Lubbadeh, 2003; Nasser et al., 2004), and GnRH (Davis et al., 2003), to increase the efficiency of a synchronized estrus and a production of high quality embryos.

The CIDR device is used to induce estrous cyclicity in anestrus cows and prepubertal heifers (Xu et al., 2000; Lucy

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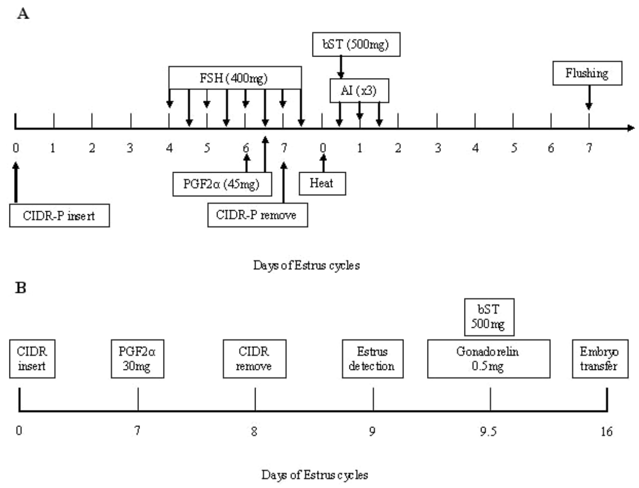

Figure 1. The schematic protocols for superovulation treatment (A) in donor Hanwoo cows and synchronization treatment (B) in recipient Holstein cows.

et al., 2001), to synchronize the return to estrus of previously inseminated cattle (Macmillan and Burke, 1996; Chenault et al., 2003a), and to use one component of treatment regimens administered to synchronize estrus of cattle (Xu et al., 1996; Ryan et al., 1999). Chenault et al. (2003b) reported that the CIDR inserts improved synchrony of returns to estrus but didn't affect conception rate or pregnancy rate to artificial insemination (AI) during the resynchronized period.

Recombinant bovine somatotropin (bST) has been developed as a commercially available recombinant hormone for use in dairy herds due to its ability to increase milk production. Its effect on reproduction must also be considered. In studies conducted using lactating dairy cows treated with bST, researchers have reported a decrease in reproductive performance (Moallem et al., 1997). Additional experiments have found conception rates to be decreased by as much as 16.9% with bST treatment (Luna-Dominguez et al., 2000). However, there is evidence that bST may offer some benefits when used in conjunction with ovulation synchronization. The administration of bST increased pregnancy rates following the synchronized service (Moreira et al., 2001; 2002; Santos et al., 2004). However, we know very little about how bST is regulated in reproduction.

The present study was performed to determine the effects of bST treatment combined with CIDR on the reproductive variables compared to CIDR only treatment.

MATERIALS AND METHODS

Donors and superovulation treatment

This study was performed on four dairy farms located in Kyeonggi province in central Korea. Hanwoos were maintained in free-stall facilities and fed a total mixed ration. Donors were selected ranging from 4 to 7 parities and averaging 70±10 days postpartum. The protocol for superovulation treatment was followed (Figure 1A). Briefly, all cows received a CIDR device containing 1.9 g of progesterone (InterAg, Hamilton, New Zealand) at random stages of the estrous cycle (day 0). On the morning of day 4 and then twice daily until the afternoon of day 7, donor cows received a sequence of eight of the same doses (2.5 ml/injection) of follicle-stimulating hormone (FSH, 20 mg/ml im; Folltropin-V, Vetrepharm, Belleville, Canada). On the morning and afternoon of day 6, donor cows were treated twice (22.5 mg/each) with prostaglandin F2α (PGF2α, im; Lutalyse, Pharmacia, Kalamazoo, MI, USA). Progesterone implants were removed in the morning of day 7. After day 8, cows were observed for estrous behavior and inseminated at 12 h after the onset of estrus and every 12 h for 3 times. Hanwoo semen was used at random to inseminate donor cows.

Donor cows were assigned randomly to a non-treated control group or to receive a single treatment of recombinant bST (Boostin-S, 500 mg, via SC route, LG Life Sciences, Ltd., Seoul, Korea) at the initiation of AI. Treatments were assigned so that 12 cows received bST (CIDR+bST) and 5 cows served as controls (CIDR). After initial assignment, treatments were alternated for every experimental donor; therefore, each donor served as experimental units for CIDR or CIDR+bST treatment.

Donor cows had to be superovulated and flushed a minimum of two times to be included in the experiment. At approximately 7 days after the first insemination, embryos were non-surgically recovered from donor cows. Immediately following embryo recovery, embryos were counted and the transferable embryos were morphologically scored for quality, color, and developmental stage (stereomicroscope, 90× magnifications). Embryos were classified according to stage of development and quality according to the standards adopted by the International Embryo Transfer Society (Stringfellow and Seidel, 1990). Because differences among stages of embryonic development can be subtle, embryo classification was performed by two investigators. Classification of embryos determined to be in grade 1 (excellent), grade 2 (good), grade 3 (fair), and grade 4 (degenerated).

Synchronization treatment for recipients and embryo transfer

Holstein heifers were used as recipients. The synchronization protocol for recipients is shown in Figure 1B. At approximately 12 h after estrus was detected, heifers were assigned randomly to receive bST (CIDR+bST, n = 64) or serve as controls (CIDR, n = 50). Recombinant bovine somatotropin (Boostin-S) was a generous gift from

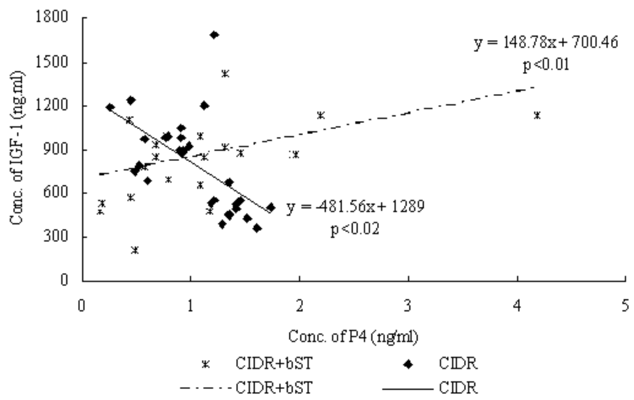

Figure 2. The correlation between plasma IGF-I and P4 levels in both CIDR and CIDR+bST treatment groups. CIDR: n = 28; CIDR+bST: n = 32.

the LG Life Sciences, Ltd. (Seoul, Korea).

At approximately 7 days after estrus, a single fresh transferable embryo was transferred non-surgically into each recipient heifer. Embryos transferred were assigned randomly to the available group of recipients in an attempt to keep the number of experimental units similar among treatment groups. Therefore, four groups were formed: the embryo produced from CIDR-donors (CIDR-D) were transferred to CIDR-recipient (CIDR-R) (CIDR-D/CIDR-R; n = 23) or CIDR+bST-recipient (CIDR+bST-R) (CIDR-D/CIDR+bST-R; n = 35) and the embryo by CIDR+bST-donors (CIDR+bST-D) were delivered to CIDR-R (CIDR+bST-D/CIDR-R; n = 27) or CIDR+bST-R (CIDR+bST-D/CIDR+bST-R; n = 29).

Plasma hormonal assay

The blood samples, about 10 ml, were drawn from the jugular vein in recipients at 7, 14, and 21 days after estrus detection (day 9 = day 0, Figure 1B) directly into silicone-coated test tubes. Occasional samples were missed, we eliminated the samples. After about 3 h, to allow the blood to clot, the samples were centrifuged at 2,500 g for 25 min and the serum obtained was frozen (-80°C) until assayed. Serum concentrations of IGF-1 and P4 were determined by radioimmunoassay (RIA) using commercial kits (Bouty Techno Genetics, Milan, Italy) validated for bovine sera. The intra- and inter-assay coefficients of variation for the IGF-1 and P4 assays were 6.1 and 9.9% and 4.1 and 6.1%, respectively.

Ultrasound scanning

Ovaries were monitored daily by ultrasonography under optimized conditions to determine corpus luteum (CL) size with a Sono 600 linear-array real-time B-mode ultrasound scanner equipped with a 5-MHz rectal transducer (Medison Co. Ltd., Seoul, Korea). After fecal material was removed from the rectum, the ultrasound probe was inserted into the

Table 1. Effects of bST treatment on the size of corpus luteum at day-7 of estrus cycles in recipient heifers

Treatment	No. of recipients examined	Size of corpus luteum	
		Diameter* (mm)	Tissue area** (mm^2)
CIDR	16	21.5±1.1	373.9±39.4
CIDR+bST	16	20.6±0.9	340.9±39.4

The results were expressed as mean±standard deviation (SD).
* Determined by CL length+CL width/2.
** Calculated using a formula (CL length×0.5×CL width×0.5×3.14).

rectum with lubricant to cover the transducer for better contact to obtain images, and the probe was moved along the dorsal surface of the uterine horns; then the probe was moved laterally and positioned adjacent to each ovary for examination, and each ovary was scanned in several positions by moving the probe along its surface to monitor the CL. All ultrasound examinations were performed by the same operator. The diameter of CL was determined by CL length+CL width/2. And CL area was calculated using a formula (CL length×0.5×CL width×0.5×3.14; Kastelic et al., 1990).

Pregnancy diagnosis

Pregnancy diagnosis was determined at 50-70 days after embryo transfer by using both ultrasonography and rectal palpation. Pregnancy rate was defined as the percentage of cows confirmed pregnant at the single pregnancy diagnosis.

Statistical analysis

Data were analyzed by Chi-square test for grade of embryos recovered and two-sample *t*-test statistic for number of embryos, size of CL, and pregnancy in different classes. Plasma IGF-I and P4 levels for differences between CIDR and CIDR+bST groups were analyzed by repeat measures of analysis of variance (ANOVA) procedure of SAS program (Release 9.1, Cary, NC, USA: Inst. Inc.; 2002). Pearson's correlation coefficient was performed to analyze the correlation. A probability level of $p<0.05$ was considered significant.

RESULTS

The differences of plasma IGF-I and P4 levels were not significant between CIDR and CIDR+bST in recipient heifers (data not shown). Interestingly, the correlation between plasma IGF-I and P4 levels of recipient heifers at day 7 of estrous cycles showed definitely different patterns between the two groups (Figure 2). The correlation was positive in the CIDR+bST (n = 32, r = 0.44, $p<0.01$) and negative in the CIDR (n = 28, r = -0.59, $p<0.02$).

The effects of bST treatment on the size of CL at day 7 of estrous cycles in recipient cows are shown in Table 1. Although the diameter and area of CL in CIDR group were

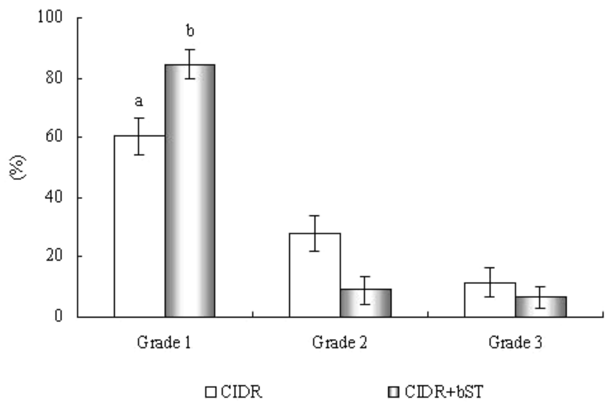

Figure 3. Effects of bST treatment on the quality of transferable embryos recovered from donor cows. [a, b] $p<0.01$.

Table 2. Effects of the bST treatment on the quantity and quality of embryos recovered from donor cows

Treatment	No. of donors[1]	No. of embryos		
		Recovered	Transferable*	Degenerated**
CIDR	5	9.5±1.9	6.9±1.6	2.6±0.7
CIDR+bST	12	13.1±2.1	9.6±1.8	3.5±0.8

The results were expressed as mean±SD.
[1] Korean native beef cattle (Hanwoo) were used as donors.
* Including grade 1 (excellent), 2 (good), and 3 (fair) embryos.
** Including unfertilized oocytes and degenerating embryos.

slightly bigger than those of CIDR+bST group, the differences were not statistically significant.

The effects of bST treatment on the quantity and quality of embryos recovered from donor cows are shown in Table 2. The numbers of recovered, transferable, and degenerated embryos were not statistically different between CIDR and CIDR+bST groups. Among transferable embryos, the percentage of grade 1 (excellent) embryos in CIDR+bST group was significantly higher compared to CIDR group ($p<0.01$) (Figure 3).

The pregnancy rate of CIDR-D/CIDR+bST-R was significantly higher than that of CIDR-D/CIDR-R ($p<0.05$, Figure 4). When the embryos collected from donors with CIDR+bST group were transferred, the pregnancy rates were high in both recipient groups.

DISCUSSION

During superovulation treatment and embryo transfer periods, plasma hormones, such as P4 and IGF-I, are prerequisite for the regulation of circumstances of uterus. It was reported that bST treatment affected the concentration of plasma P4 and IGF-I (Morales-Roura et al., 2001; Bilby et al., 2004). The production and maintenance of P4 is very important, otherwise PGF2α secretion from endometrium, luteolysis and initiation of folliculogenesis will happen. A higher concentration of P4 in blood was associated with a

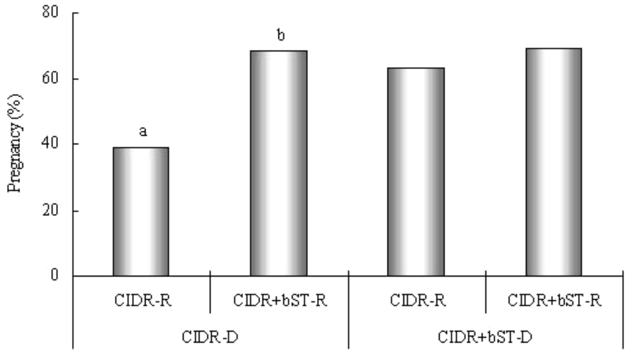

Figure 4. Pregnancy rate according to the treatment with or without bST in donor or recipient cows. D: donor; R: recipient. [a, b] $p<0.05$.

higher pregnancy rate (Marques et al., 2003). And IGF-I is an anabolic polypeptide involved in reproductive functions in several species. It is involved in the regulation of bovine ovarian function (Woad et al., 2000). IGF-I receptor is expressed in the uterus during the estrous cycle and early pregnancy (Robinson et al., 2000), suggesting that IGF-I might be important in the regulation of uterine function in cattle.

In the present study, we measured the concentration of plasma P4 and IGF-I levels and the diameter and tissue area of corpus luteum in recipient cows treated with or without bST treatment in addition to CIDR treatment. Although the differences of the concentration of plasma IGF-I and P4 levels and CL size were not statistically significant, the plasma hormonal levels of IGF-I and P4 was higher in CIDR+bST group compared to those of CIDR group. And there were interesting correlation data. The correlation between plasma IGF-I and P4 levels of recipient heifers at day 7 of estrus cycle showed definitely different patterns in both treatment groups. The correlation showed a strong positive pattern in CIDR+bST group ($r = 0.44$, $p<0.01$), but it showed a definite negative pattern in the CIDR group ($r = -0.59$, $p<0.02$). So it can be postulated that the treatment of bST in addition to CIDR affects positively the regulation of plasma hormonal level.

Although several studies examined bST effects on pregnancy rates and plasma hormonal levels, little is known regarding the correlation between hormonal level and CL size altered by bST treatment. Lucy et al. (1995) reported that the administration of bST led to the development of heavier CL. Greater luteal mass in addition to increased steroidogenesis may lead additively to increase P4 in blood. However, there was a controversy in the effect of bST treatment on CL. Hasler et al. (2003) reported that it has failed to show a positive effect of bST treatment on the size and number of CL. The correlation between P4 concentration and a size of CL is very important, because P4 is produced mainly in CL. Like previous researches

(Spell et al., 2001; Veronesi et al., 2002), the correlation between P4 concentration and the size of CL, in this study, showed positive patterns in CIDR (r = 0.76, p = 0.04) and CIDR+bST (r = 0.58, p = 0.03) groups, respectively.

The bST treatment affected to decrease the number of unfertilized eggs and to increase the fertilizing ability (Thatcher et al., 2001). The number of growing follicles was increased when cows or heifers were treated with recombinant bST (Lucy et al., 1999, 2000) and the administration of bST in addition to gonadotropins increased the number of transferable embryos collected after superovulation (Cushman et al., 2001; Thatcher et al., 2001). The number of recovered, transferable, and degenerated embryos, in the present study, was not significantly different in both groups. The number of grade 1 embryos, however, was significantly higher in CIDR+bST group than that of CIDR group.

The effect of bST treatment on the pregnancy rate was various according to researchers. Moreira et al. (2001; 2002) reported that the administration of bST increased pregnancy rate following the synchronized service. Different researchers have found opposite effects of bST on pregnancy rate (Hasler et al., 2003). It was difficult to conclude that whether bST treatment combined with CIDR was an effective method or not in embryo transfer programs, because it had different individual responses of donor and recipient cows and the various superovulation and/or synchronization treatment protocols among research groups.

In the present study, the pregnancy rate was significantly higher in CIDR+bST group than that of CIDR group, when the embryos recovered from the donors treated with CIDR were transferred. The reason why the pregnancy rate of the recipients treated with bST was higher could be explained. The plasma hormonal regulation of IGF-I and P4 in CIDR+bST group was stabilized the uterine circumstances at the period of embryo transfer, so the environments of reproductive organs might be changed positively to help an implantation of transferred embryo. As an expectation, the pregnancy rate of the recipients treated with CIDR+bST was high, when the embryos recovered from the donors treated with CIDR+bST were transferred. However, in spite of this negative factor, the pregnancy rate of the recipients treated with CIDR was similar to the recipients treated with CIDR+bST by using the embryos recovered from the donors treated with CIDR+bST. It could be suggested that the use of the embryos recovered from the donors treated with CIDR+bST was helpful to increase the pregnancy rate regardless of the condition of the recipients, because the excellent quality of embryos was obtained from CIDR+bST group compared to CIDR group.

Based on these results, bST administration combined to CIDR is an effective method for superovulation and synchronization treatment to stabilize plasma hormonal levels, to obtain excellent quality of embryos, and to get higher pregnancy rate.

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