# Timed Artificial Insemination or Embryo Transfer using CIDR, Estradiol Benzoate and Prostaglandin $F_{2\alpha}$ for the Rebreeding of Korean Native Donor Cattle

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## ABSTRACT

This study compared the pregnancy rates of Korean native donor cattle after either a timed artificial insemination (TAI) or embryo transfer (TET) following the synchronization of ovulation using a controlled internal drug release (CIDR) device together with estradiol benzoate (EB) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Fifty five cows and 8 heifers which had been previously used for embryo production were assigned to two treatments: (1) Thirty-two cattle received a CIDR device and 2 mg EB (Day 0), 25 mg $PGF_{2\alpha}$ injection at the time of CIDR removal on Day 7, and 1 mg EB injection on Day 8. All of the cattle received a TAI 30 h (Day 9) after the second EB injection (TAI group). (2) Thirty-one cattle received the same hormonal treatments as in the TAI group. The cattle with corpus luteum (CL) received a TET on Day 16 using frozen-thawed embryos (TET group). Ultrasonographic observations demonstrated that the proportion of cattle with synchronized ovulation on Day 10 and the concomitant formation of new CL on Day 13 did not differ between groups ($p>0.05$); the overall mean rates were 65.1 and 73.0%, respectively. The conception and pregnancy rates did not differ ($p>0.05$) between the TAI (12.5% and 12.5%) and TET groups (13.0% and 9.7%), respectively. We conclude that the pregnancy rate following TAI or TET in Korean native donor cattle was poor, which might be due in part to a poor synchrony of ovulation and concomitant CL formation.

(Key words : CIDR, EB, $PGF_{2\alpha}$, Korean native donor cows, synchronization of ovulation)

## INTRODUCTION

Embryo production by superovulation has been used worldwide for the improvement of genetic gain in cattle (Nicholas, 1996). Genetically superior donor cattle receive repeated superovulation treatments and rebreeding in order to become pregnant and produce embryos. Thus, a reduction in the number of days in which donor cattle are not pregnant (open days) is important for the maximal use of superior genetic resources. After embryo collection by uterine flushing, donor cattle are commonly treated with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in preparation for the next superovulatory treatment or rebreeding. In the latter case the estrus signs of the donor cattle are observed, and they can be rebred following the detection of estrus. If donor cattle do not exhibit signs of estrus or the person managing the cattle fails to detect these signs, rebreeding could be delayed.

The Ovsynch protocol involves injections of gonadotrophin-releasing hormone (GnRH), $PGF_{2\alpha}$ and GnRH and can precisely control the time of ovulation; this protocol has been developed to increase fertility, regardless of the exhibition of an estrous response in the animal (Pursley *et al.*, 1995). Moreover, it has been shown that administration of GnRH or estradiol, at random stages of the estrous cycle effectively induces the emergence of a new follicular wave following ovulation or the atresia of a dominant follicle present at the time of treatment (Bo *et al.*, 1995; Pursley *et al.*, 1995). Subsequent synchronized ovulation following the second administration of GnRH or estradiol allows cows to ovulate at a planned time (Kim *et al.*, 2007). Various protocols that synchronize ovulation by inducing the emergence of a new follicular wave have been used to increase the fertility of normal cycling or infertile cows (Pursley *et al.*, 1995; Yamada *et al.*, 1999; Bartolome *et al.*, 2000; Kim *et al.*, 2006], as well as to improve the superovulation of bovine donor cattle (Bo *et al.*, 1996; Andrade *et al.*, 2002). The protocols allow cows to receive timed artificial insemination (TAI) or embryo transfer (TET) without a decrease in their preg-

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nancy rate irrespective of the estrus response. Furthermore, these TAI or TET regimens increased the pregnancy rate in repeat breeder cattle (Kasimanickam *et al.*, 2005; Takahashi *et al.*, 2006). We hypothesized that the TAI or TET protocol might help to shorten the rebreeding interval in Korean native donor cattle. We therefore evaluated the effects of TAI or TET following the synchronization of ovulation using a controlled internal drug release (CIDR) device, estradiol benzoate (EB) and $PGF_{2\alpha}$ on the pregnancy rates of Korean native donor cattle with highly extended open days.

## MATERIALS AND METHODS

### 1. Animals

This study was performed at the Animal Genetic Resources Station, National Institute of Animal Science located in Namwon City, Korea. Fifty five non-suckled Korean native cows or 8 heifers, which had been used for embryo production using superovulation treatment, with an average parity of 2.2±0.2 (Means±SEM) and a body condition score of 3.9±0.1 (1 to 5 point scale; Edmonson *et al.*, 1989) were used for this study. The postpartum interval for the cows was 856±247 days and the age of the heifers was 50.5±8.9 mon, indicating highly extended open days.

The reproductive tract of each cow was examined to check its normality by rectal palpation and ultrasonography (Sonoace 600 with a 5.0 MHz linear-array transducer; Medison, Korea) prior to experimentation.

### 2. The Experimental Design

Sixty-three Korean native donor cattle were randomly assigned to two treatment groups with equivalent parity and body condition scores: (1) Thirty-two cattle, at random stages of the estrous cycle, received a CIDR device containing 1.9 g of progesterone (P4) (CIDR$^{TM}$, InterAg, Hamilton, New Zealand) and 2 mg EB (SY Esrone, Samyang, Seoul, Korea) (Day 0), an injection of 25 mg $PGF_{2\alpha}$ (Lutalyse, Pharmacia & Upjohn, Puurs, Belgium) at the time of the removal of the CIDR on Day 7, and 1 mg EB injection on Day 8. All of the cattle received TAI 30 h (Day 9) after the second EB injection using Korean native bull semen of known fertility (TAI group). (2) Thirty-one cattle, at random stages of the estrous cycle, received the same hormonal treatments as in the TAI protocol. The cattle with corpus luteum (CL) received TET on Day 16 using frozen-thawed bovine embryos collected from Korean native donors (TET group). A detailed description of the embryo production, embryo freezing/thawing and ET methods is described below. All hormone injections were administered intramuscularly (im).

The diagnosis of pregnancy was determined 60 days after AI or ET using both ultrasonography and rectal palpation. Conception rates were determined for all of the cattle that received AI or ET in both of the groups. Pregnancy rates were defined as the percentage of pregnant cattle in the total number of cattle that were treated.

### 3. Embryo Production, Freezing/thawing and Transfer

A Korean native cow received twice superovulation treatments for embryo production at forty-nine days interval. The donor cow during mid-cycle was superovulated with 28 mg of porcine follicle stimulating hormone (FSH) (Antrin-R10, Kawasaki Mitaka Pharmaceutical Co., Tokyo, Japan) in twice daily im injections, with a gradual decrease in the hormone concentration over 4 days (5, 5, 4, 4, 3, 3, 2, 2 mg). On the 5th and 6th injections of FSH, 25 mg and 15 mg $PGF_{2\alpha}$ were administered, respectively. The cows received 200 $\mu$g gonadorelin (GnRH, Fertagyl®, Intervet, Boxmeer, Holland) 12 h after the final (8th) injection of FSH. The cows were artificially inseminated using commercial semen from a Korean native bull 24 h and 36 h after the final injection of FSH. The embryos were recovered 7 days after the first 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 (Wright, 1998) for the stage of development and quality. The morulae and blastocysts that were rated 1 or 2 in quality were equilibrated in 1.8 M ethylene glycol in D-PBS supplemented with 0.5% bovine serum albumin (IFP9620, Research Institute for the Functional Peptides, Japan) for 10 min and were individually loaded into 0.25 ml straws (IMV, L'Aigle, France). The straws were placed into a chamber of a programmable freezer (CL863, CryoLogic, Australia) that was precooled to −7℃. After 3 min, the straws were seeded, maintained for another 7 min, and then cooled at a rate of 0.3℃/min to −35℃ before being immersed in liquid nitrogen. The straws were thawed for 10 sec in air followed by immersion for 20 sec in a 37℃ water bath.

The frozen-thawed embryos were directly transferred into the uterine horn that was ipsilateral to the ovary containing the CL on Day 16.

### 4. Ovarian Ultrasound Scanning

The ovaries of each cow or heifer were examined by transrectal ultrasonography before the insertion of the CIDR device and the EB injection (Day 0), before the injection of $PGF_{2\alpha}$ and the removal of the device (Day 7), and 24 h (Day 8), 77 h (Day 10) and 144 h (Day 13) after the $PGF_{2\alpha}$ injection in order to observe the changes in ovarian structure (follicles and CL). All visible follicles (antral diameter $\geq$ 4 mm) and CL were measured for each cow or heifer. The ovaries of the cattle were assessed via ultrasonography to detect the preovulatory follicles on Days 7 and 8. The subsequent ovulation and concomitant formation of new CL were diagnosed when the preovulatory follicle had disappeared by Day 10 (53 h after 2nd EB injection) and was confirmed by the appearance of new CL on Day 13 (Cartmill *et al.*, 2001; Lamb *et al.*, 2001; Sellars *et al.*, 2006).

### 5. Serum P4 Assay

Blood samples were collected from the tail vein on Day 0, Days 7 to 10, and Day 13 for the analysis of serum P4 concentrations from each cow or heifer. After 2 h at 5℃, the samples were centrifuged at 2500 × g for 10 min, and the sera were collected, immediately frozen, and thereafter kept at −20 ℃ until the assay. P4 concentrations were determined by fluoroimmunoassay (1234 Delfia Fluorometer, Wallac Inc., Turku, Finland). The intra- and interassay coefficients of variation for the serum P4 analyses were 8.7, 8.5, 6.7, 8.5, 11.5, and 9.6%, and 4.0, 5.4, 6.6, 6.9, 10.5, and 8.1% for the standard concentrations of 0, 0.31, 1.26, 3.14, 12.6, and 37.7 ng/ml, respectively.

### 6. Statistical Analyses

Statistical analyses were performed using SAS (1999). Data from the proportion of cattle with synchronized ovulation on Day 10, the concomitant formation of new CL on Day 13, a P4 concentration of less than 1.0 ng/ml (failure to form a new CL) on Day 13, and the conception and pregnancy rates between the TAI and TET groups were compared using the chi-square test or Fisher's exact test. The mean serum P4 concentrations during the treatments were compared between the groups using the Student's *t*-test. A probability level of $p>0.05$ was considered to be significant.

## RESULTS

The ultrasonographic observations demonstrated that the proportion of cattle with synchronized ovulation on Day 10 and the concomitant formation of new CL on Day 13 did not differ between the TAI (59.4, 71.9%) and TET (71.0, 74.2%) groups ($p>0.05$); the combined mean rates were 65.1 and 73.0%, respectively (Table 1). The mean serum P4 concentrations on Day 0, Days 7 to 10, and Day 13 did not differ between groups ($p>0.05$, Fig. 1). The overall mean serum P4 concentrations on Day 0 (9.0±1.4 ng/ml) decreased slightly in both of the groups until Day 7 (7.0±0.7 ng/ml), dramatically decreased on Day 8 (1.5±0.1 ng/ml), maintained a level below 1.0 ng/ml until Day 10, and subsequently increased on Day 13 (1.3±0.1 ng/ ml). The proportion of cows or heifers with serum P4 concentrations that were less than 1.0 ng/ml on Day 13 did not differ between the groups ($p>0.05$); the combined mean rate was 30.2% (Table 1). The conception and pregnancy rates did not differ ($p>0.05$) between the TAI (12.5, 12.5%) and TET groups (13.0, 9.7%) (Table 1).

## DISCUSSION

The delayed rebreeding of superior donor cattle following embryo production leads to economic losses. We therefore tried to shorten the rebreeding interval after embryo production using TAI or TET protocols in Korean native donor cattle with highly extended open days. In this study the synchronization of ovulation was not successful (overall mean rate of 65.1%), which ultimately resulted in equally poor pregnancy rates after both TAI and TET (12.5 vs. 9.7%).

The proportion of cattle with synchronized ovulation in both groups of our study (59.4～71.0%) was lower than that reported for previously studies (85～100%; Pursley *et al.*, 1995; Kim *et al.*, 2005; Kim *et al.*, 2006; Sellars *et al.*, 2006; Kim and Kim, 2007). In addition, the proportion of cows with concomitant CL formation following the ovulation of preovulatory follicles (71.9～74.2%) was also lower than a previous study (Son *et al.*, 2007) in which they found that all thirteen dairy cows showed ovulation of their preovulatory follicles and a concomitant formation of new CL using the same synchronization of ovulation protocol that we used in this study. Bo *et al.* (2005) and Moreno *et al.* (2003) also demonstrated slightly higher rates of concomitant formation of new CL (82.9～85.8%) than we observed in this study, following the synchronization of ovulation using a P4 releasing device (DIB), EB and equine chorionic gonadotrophin (eCG) using crossbred beef cows. The general change in the pattern of the mean serum P4 concen-

Table 1. Synchronized ovulation, concomitant formation of new CL, serum progesterone concentrations and conception and pregnancy rates following TAI or TET after the synchronization of ovulation protocol using a CIDR device, EB and $PGF_{2\alpha}$ in Korean native donor cattle

| Variables | Treatment group* | | |
|---|---|---|---|
| | TAI | TET | Combined |
| Proportion of cattle with synchronized ovulation on Day 10 (%) | 19/32 (59.4) | 22/31 (71.0) | 41/63 (65.1) |
| Proportion of cattle with the concomitant formation of new CL on Day 13 (%) | 23/32 (71.9) | 23/31 (74.2) | 46/63 (73.0) |
| Proportion of cattle with serum progesterone concentrations less than 1.0 ng/ml on Day 13 (%) | 9/32 (28.1) | 10/31 (32.3) | 19/63 (30.2) |
| Conception rate (%) | 4/32 (12.5) | 3/23 (13.0) | 7/55 (12.7) |
| Pregnancy rate (%) | 4/32 (12.5) | 3/31 ( 9.7) | 7/63 (11.1) |

* TAI: cows or heifers received a CIDR device and 2 mg EB (Day 0), an injection of 25 mg $PGF_{2\alpha}$ at the time of CIDR removal on Day 7, and 1 mg EB injection on Day 8. All of the cattle received TAI 30 h (Day 9) after the second EB injection. TET: cows or heifers received the same hormonal treatments as in the TAI protocol. The cattle with CL received TET on Day 16.

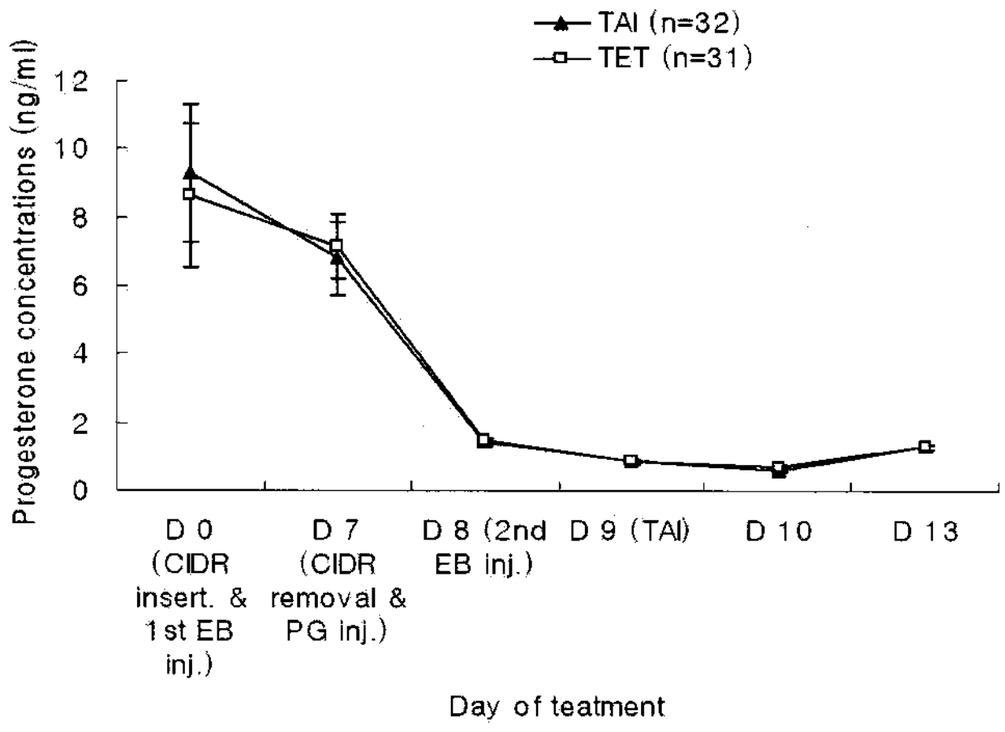


Fig. 1. Serum progesterone concentrations (mean±SEM) on Day 0, Days 7 to 10, and Day 13 in TAI and TET groups. No significance differences in progesterone concentration were observed between the groups ($p$>0.05).

trations that were measured in both groups of this study (Fig. 1) showed that the endocrine system was properly regulated during the synchronization of ovulation. In accordance with our ultrasonographical observations, 30.2% of the treated cattle had low serum P4 concentration that were less than 1.0 ng/ml on Day 13, indicating the failure to form a new concomitant CL.

The pregnancy rates after both TAI and TET in this study were poor (9.7～12.5%). The pregnancy rate after TAI was lower than that reported by Kasimanickam *et al.* (2005), who described a 23.8% pregnancy rate after TAI following the Ovsynch regimen in lactating dairy cows. Moreover, the pregnancy rate after TET in this study was lower than other studies that used for beef recipients (42.4～63.4%; Moreno *et al.*, 2003; Nasser *et al.*, 2004; Bo *et al.*, 2005). Al-Katanami *et al.* (2002) demonstrated that the pregnancy rates following TAI and TETs (with fresh or vitrified *in vitro* produced embryos) using the Ovsynch protocol in lactating dairy cows under heat stress conditions were 6.2, 19.0 and 6.5%, respectively. They showed that TET (using fresh *in vitro* produced embryos) can improve the pregnancy rate to more than that observed following TAI. Similarly, Son *et al.* (2007) also reported that the pregnancy rate was significantly higher in the TET group (with frozen-thawed *in vivo* produced embryos) compared with the TAI group using the same synchronization of ovulation protocol that we used in this study. We have not clarified the reason for the disparity in the results between the studies. Taken together, our ultrasonographical and endocrine results indicate that the poor pregnancy rates following TAI or TET in this study might be due in part to a poor synchrony of ovulation and concomitant CL formation. In addition, we suspect that the donor cattle were not provided with suitable nutrition and this, together with repeated superovulation treatments and/ or long open days without rebreeding, might be also possible causes for the low fertility we observed.

In conclusion, the protocol we used for the synchronization of ovulation in Korean native donor cattle was not successful in view of the poor synchrony of ovulation and concomitant CL formation rates, which ultimately resulted in low preg-

nancy rates following both TAI and TET. Additional research into protocols that would decrease the interval between rebreeding following embryo production is needed in Korean native donor cattle.

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(접수일: 2008. 3. 25 / 채택일: 2008. 6. 20)