The Effects of Consecutive Superovulation in Hanwoo and Holstein Cattle

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한우와 젖소에서 연속적인 다배란 처리의 효과

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SUMMARY

본 연구는 한우와 홀스타인 젖소에서 연속적인 다배란 처리 시 효율성 및 부작용에 대한 문제점을 연구하고자 다른 종간 다배란 처리효과를 비교, 조사하였다. 연속적인 다배란 처리를 위하여 estradiol benzoate(EB)와 CIDR plus, FSH 호르몬을 이용하여 한우에서는 17두, 젖소에서는 9두를 2회 이상 반복 이용하였다. 다난포 발생을 위해서는 FSH 호르몬을 이용하고 호르몬 처리 동기화를 위해서는 CIDR plus 기구를 질 내 삽입하였으며, 주요 조사 항목으로는 번식기관의 문제점, 배란 효율, 회수된 수정란 수, 우수 수정란 수 등을 분류하고, 종합적으로 종간 연속적인 다배란 효과를 비교, 조사하였다. 한우에서는 17두 중 4두(23.5%)에서 자궁경관의 협착이 발생하였고, 자궁 내에 손상이 발생하여 채란이 되지 않는 경우도 4두(23.5%)의 발생이 조사되었으나, 상대적으로 젖소에서는 9두 중 4두에서 일시적으로 배란이 전혀 일어나지 않는 경우가 있었다. 연속 처리 후 채란된 수정란의 수를 종합해 보면, 젖소에서는 평균 4.3±3.4개, 한우에서는 2.7±2.5개였으며, 종간 호르몬 효율과 연관된 배란율 조사에서는 한우에서는 89.7%, 젖소에서는 80.4%의 성적을 보였다. 또한, 3회까지 연속 다배란 처리를 한 결과, 한우나 젖소에서 채란된 수정란의 수는 비슷한 수준으로 채란되었다. 이와 같은 성적을 종합해 볼 때 EB제나 CIDR plus, FSH를 이용하여 1개월 주기로 연속적인 수정란 채란을 위한 다배란 처리가 소의 종에 무관하게 가능하다고 사료되지만 일시적으로 배란이 일어나지 않는 소에 대한 문제나 번식기관의 손상, 채란되는 수정란의 수가 적다는 것에 더 많은 연구가 필요하다고 사료된다.

(Key words : consecutive superovulation, EB, CIDR plus, Hanwoo, Holstein)

INTRODUCTION

Numerous studies have been published on superovulation in heifers and cows using porcine or equine pituitary FSH (Sartori *et al.*, 2003; Danner *et al.*, 1979; Schams *et al.*, 1978), or pregnant mare serum gonadotrophin (PMSG) (Saumande, 1981; Newcomb *et al.*, 1979). It is generally accepted that the success of superovulation is affected by various factors such as the individual response, physiological state at the time of treatment, age, and breeds of the animal, the season, and the number of consecutive stimulations. Further, it is accepted that the type of gonadotrophin used and the ratio of its effective FSH- and LH-like biological activities on ovarian structures play a very important role (Gordon, 1975). Although studies on superovulation in cattle have progressed in recent years and satisfactory results have been obtained, the variability of individual response to superovulation still limits the advantages of embryo transfer (Saumande *et al.*, 1978). In the present work, to determine whether FSH and β-estradiol 3-benzoate (EB) treatment could enhance embryo production in elite cattle, the effect of superovulation on different breeds of cows and their embryo-production rate was studied by using the consecutive superovulation method.

MATERIALS AND METHODS

Seventeen Hanwoo heifers (age, 18~24 months) and nine Holstein heifers (H) (age, 18~25 months) were used. All the animals were clinically normal, with regular estrous cycle.

1. Hormone Treatments

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The heifers were treated on Day 3～19 of the cycle (Day 0 = day of estrus) with an CIDR insertion for 6 days into the vaginal 24 hrs later injection of 2.5 mg EB (β-Estradiol 3-benzoate, i.m.), followed 144 h (6 days), 50 mg FSH equivalent injection were done for superovulation treatment. Each animal was superovulated twice daily, with a total dose of 400 mg FSH (porcine Folltropin-V®, Bioniche Life Sciences Inc., Canada) with the constantly divided doses over 4 days. On the third day after the first injection of FSH, a 25 mg $PGF_{2\alpha}$ (Lutalyse™, Dinoprost tromethamine, Pharmacia & Upjohn Co., Belgium) was administered i.m. to all the animals to induce estrus. After 48 h, at estrus, the animals received two sperm straws and an injection of 200 μg GnRH (Gonadon™, Gonadorelin, Dongbang Co., Korea). After 7 days, all the animals were examined to determine the number of corpus lutea (CL) and non-ovulating follicles under epidural anesthesia by a skilled veterinarian. All the heifers were artificially inseminated, and the fertilized eggs were collected non-surgically on day 7.

2. Embryo Recovery

The embryos were recovered non-surgically from the 2 breeds on day 7 (day 0 = day of artificial insemination (AI)). Each uterine horn was flushed by using a three-way tubing system with the animals under epidural anesthesia (5 ml Lidocaine). A silastic catheter was inserted at the bifurcation of the uterine horns to introduce the flushing medium (PBS supplemented with 5% fetal calf serum, Sigma, St. Louis, USA). After embryos recovery, cows were infused into uterus with 40 ml of 2% iodine solution (Betadine, Korea Pharma, Korea) and received a 25 mg $PGF_{2\alpha}$ i.m. injection for repeated superovulatory treatments. Repeated superovulatory treatments were the same protocol as the first superovulation. The cervical stenosis was defined which when catheters insertion's difficult at embryo collection, and uterine damage was defined by epithelial cell's present after embryo collection.

3. Statistical Analysis

Differences in means were tested for statistical significance by student's t-test and Fisher's PLSD and were considered to be significant at $p<0.05$. The data are presented as means±S.D.

RESULTS AND DISCUSSION

The ovarian quiescence rate by consecutive hormone treatment was lower in Hanwoos (5.9%) than in Holsteins (44.4%). However, cervical stenosis (23.5%) and uterine damage (23.5 %) occurred only in the Hanwoos (Table 1).

Significant differences ($p<0.05$) were observed between the two breeds of heifers with regard to the number of non-ovulating follicles at each ovarian stage (CL detection vs no CL detection) during the consecutive hormone treatments. The ovulation rate at the CL stage was 98.6% in the Hanwoos and 88.0 % in the Holstein heifers. The ovulation rate of the non-CL group was 84.4% in the Hanwoos and 75.0% in the Holstein heifers. The number of follicle development, ovulation and non-ovulation were no significantly different in heifers with repeated hormone treatment methods (Table 2).

Although there were no significant differences among the cows with regard to the number of developing and ovulating

Table 1. The side-effect in reproductive organs by consecutive hormone treatment

Breeds	No. of heifers	Cervical stenosis (%)	Uterine damage (%)	Non-ovulating (%)
Hanwoo	17	4 (23.5)	4 (23.5)	1 (5.9)[a]
Holstein	9	–	–	4 (44.4)[b]

[a,b] $p<0.05$.

Table 2. The number of developing follicles in each the ovarian stage in the consecutive hormone treatment

Breeds	No. of heifers	Ovarian stage	No. of developing follicles (mean±S.E.M)	No. of ovulating follicles (%)	No. of non-ovulating follicles (%)
Hanwoo	18	CL* stage	141 (9.4±4.0)	139 (98.6)	2 (1.4)
	10	Non-CL	70 (7.0±3.2)	59 (84.4)	11 (15.7)
Holstein	9	CL stage	108 (12.0±4.2)	95 (88.0)	13 (12.0)
	11	Non-CL	140 (12.7±6.3)	105 (75.0)	35 (25.0)

* CL: Corpus luteum.

follicles, 89.7% in the Hanwoos and 80.4% in the Holstein heifers, during the consecutive hormone treatments, we found to solve about non-ovulation follicle in 10.3% in Hanwoo and 19.6% in Holstein (Table 3).

The number of grade 1 embryos that were produced by the consecutive hormone treatments was 1.9±2.0 in the Hanwoos and 2.1±2.4 in the Holstein heifers, and the number of embryos recovered from the Hanwoos and Holstein heifers was 2.7±2.5 and 4.3±3.4, respectively, but no significantly different in heifers (Table 4).

The number of embryo production by repeated collection times was 2.7 (17 heads) ; first, 2.9 (14 heads) ; second, 2.3 (8 heads) ; third times in Hanwoo and 2.1 (9 heads) ; first, 5.4 (9 heads) ; second, 8.5 (2 heads) ; third times in Holstein, respectively, but no significantly different in heifers (Table 5).

Superovulation / embryo transfer is one of the most successful reproductive techniques that have been effectively utilized for more than two decades. Nevertheless, many aspects of these techniques have not yet been scientifically optimized (Sartori *et al.*, 2003). In this study, we evaluated the effects of the embryo production and compared superovulation in different breeds of heifers after EB injection. Administration of EB induces atresia of the dominant follicles in cattle ovaries within 36 h, but delays the onset of a new wave of follicular development by 3 to 5 days (Burke *et al.*, 2003). In the present study, this effect of EB injection to delay the emergence of new follicles was used in designing the consecutive superovulation protocol. This design provided substantial comparison allowing for embryo productions of different embryo production techniques because each heifer was superovulated, and data were obtained from the two experimental groups, respectively.

The most significant result obtained from this study was that the embryo yield was substantially similar in different breeds of cows after the FSH and EB injection. In this experiment, similar results were obtained in the embryo recovery after the consecutive hormone treatments in the two breeds of cows. Some technical aspects of the superovulation method used in this study must be underscored. The protocol was used for the consecutive hormone treatment (CIDR insertion for 7 days and EB injection 24 h after CIDR insertion), with 25 mg of $PGF_{2\alpha}$ were given at 2 days after embryo collection. Researchers have previously observed that some superovulated cows do not attain complete luteolysis; lack of complete luteolysis may be a hindrance for programs that attempt to perform multiple superovulations in the same animal. Another noteworthy aspect of this study was the observed rate of ovulation of large follicles during the superovulation protocol (Hanwoos, 89.7% and Holstein heifers, 80.4%). This ovulation rate is consistent with previous reports (Kohram *et al.*, 1998; Nasser *et al.*, 1993). The reason of the failure of ovulation in some animals (Hanwoos, 10.3% and Holstein heifers, 19.6%) is unclear. Previous researchers that used vastly different superovulation stimulation protocols have also reported frequent occurrence of ovulation failures (Bo *et al.*, 1996; Nasser *et al.*, 1993); however, the rea-

Table 3. The number of developing and ovulating follicles caused by the consecutive hormone treatment

Breeds	No. of heifers	No. of developing follicles	No. of ovulating follicles (%)	No. of non-ovulating follicles (%)
Hanwoo	17	341	306 (89.7)	35 (10.3)
Holstein	9	245	197 (80.4)	48 (19.6)

Table 4. The number of embryo production by the consecutive hormone treatment

Breeds	No. of heifers	Collection times (Mean)	No. of embryos collected (Mean±S.D.)	No. of grade 1 embryos (Mean±S.D.)
Hanwoo	17	39 (2.3)	102 (2.7±2.5)	64 (1.9±2.0)
Holstein	9	20 (2.2)	85 (4.3±3.4)	41 (2.1±2.4)

Table 5. The number of embryo production by the consecutive hormone treatment

Heifers	Heads	Repeated collection times (Mean)	Repeated collection times		
			First	Second	Third
Hanwoo	17	2.3	2.7*** (1.7**) /17 H*	2.9(2.5) /14 H	2.3(1.8) /8 H
Holstein	9	2.2	2.1 (0.9) /9 H	5.4(2.4) /9 H	8.5(5.5) /2 H

H* Head, ** No. of grade 1, *** Mean no. of collected embryos.

sons of these failures remain undefined. Further, previous researchers have reported that treatment with 0.25 mg EB in heifers with a CIDR device resulted in the development of new follicular waves from 4.1 to 4.3 days after EB injection on an average (Moreno *et al.*, 2001; Bo *et al.*, 1995). Consistent with the superovulatory treatment, Lindsell *et al.* (1986) reported a better superovulatory response in Holstein heifers when FSH treatment was initiated on day 9 rather than days 3, 6, or 12 of estrous cycle. Another noteworthy aspect of the treatment with EB on day 4 was the lack of estradiol-induced CL regression in any of the heifers. Previous studies that reported estradiol-induced regression of the early CL (day 4) had made use of estradiol valerate, which has a longer half-life than EB (Pratt *et al.*, 1991; Rajamahendran and Walton, 1990). Thus, EB did not induce luteolysis in our study, possibly due to EB treatment at a time when the uterus/CL were unresponsive. It appears likely that an exogenous source of P_4 will aid most protocols by preventing premature estrous behavior and ovulation, and it may improve the synchronization of follicular waves (Bo *et al.*, 1994).

CONCLUSION

Here we reported that treatment with EB on day 7 of the vaginal insertion of the CIDR plus device improved or maintained the superovulatory response or the embryo quality in Hanwoos and Holstein heifers. But, this short term repeated induction of superovulation method needs to be improved for more embryo production of *in vivo*. Further, the results indicate that short term repeated superovulation treatment aroused damage in reproduction organ in Hanwoo and high non - ovulation rate in Holstein cows.

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