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Relationship between Estrous Expression Rate, BCS and Transferable Embryos in Holstein Donor Cows

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ABSTRACT

This research was investigated the relationship between the number of the transferable embryos and estrus expression rate, BCS (Body Condition Score), which affect the nutritional state of the cow, in Holstein donor cows. CIDRs were inserted into the vaginas of twenty two head of Holstein cows, regardless of estrous cycle. Superovulation was induced using folliclar stimulating hormone (FSH). For artificial insemination, donor cows were injected with $PGF_{2\alpha}$ and estrus was checked about 48 hours after the injection. Then they were treated with 4 straws of semen 3 times, with 12-hour intervals. Embryos were collected by a non-surgical method 7 days after the first artificial insemination. When BCS was ≦2.5, the total number of collected ova was 7.3 + 1.9, which is significantly lower ($p<0.05$) than the numbers 15.4 + 2.8 and 15.4 + 2.1 that were obtained when BCSs were 2.75 and ≧3.0, respectively. Whereas the numbers of transferable embryos were 5.2 + 1.4 when BCS was ≦2.5, which was smaller than the numbers 6.0 + 2.1 and 8.5 + 1.8 that were obtained when BCSs were 2.75 and ≧3.0, respectively; however, the differences were not significant. As for estrus induction rate, the cow groups whose BCSs were 2.75 and ≧3.0 showed 100.0% and 95.0%, respectively. Whereas the cow group whose BCS was ≦2.5 showed 57.1%, and the differences were significant ($p<0.05$). As for estrous expression rate, the cow groups whose BCSs were ≦2.5, 2.75 and ≧3.0 showed 100.0%, 100.0% and 85.7%, respectively; however, the differences were not significant. According to the result of this research, it is considered that the total number of collected ova and the number of transferable embryos will be affected by the nutritional state before and after *in vivo* embryo production and superovulation treatment, and that although the mechanism is not clear, poor stockbreeding management and nutritional level would cause the decrease of ovum recovery rate and the number of transferable embryos in high-producing cows. On the other hand, diverse researches on the superovulation treatment method that is suitable for high-producing Holstein donor cows would contribute to preventing ovarian cyclicity disorder, as well as to the early multiplication of cows with superior genes by increasing the utilization value of donor cows.

(Key words : Holstein donors, transferable embryo, BCS, estrus expression)

INTRODUCTION

Of the various fields of domestic dairy industry, animal improvement is still in a poor environment compared to the countries with an advanced dairy industry. Although many researches have recently been conducted, the selection and utilization of superior genetic resources still need much improvement. Development of high-producing Holstein cows has resulted in the increase of milk production, yet reproductive efficiency is decreasing every year (Roche *et al*., 2000; Lucy, 2001). The phenomena that are currently appearing in high-producing Holstein cow include delayed return to estrus, estrous expression disorder, and the continuous fall in conception rate (Wiltbank *et al*., 2006). Because of physiological and environmental changes, there are increasing incidences of non-estrus, weak estrus and silent estrus. In addition, estrous expression is decreasing and estrus duration too, thus making it difficult to predict ovulation time (Kaim *et al*., 2003). Many problems related reproduction are emerging, such as ovulation delay and the fall in conception (Pankowski *et al*., 1995; Austin *et al*., 1999).

Body Condition Score (BCS) is widely used as a general method for evaluating the nutritional management of cows. According to a research with both Holstein cows and buffalos on the postpartum resumption of ovarian cyclicity by BCS

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(score range: 1～5), the earliest resumption (29.33 days) was observed when BCS was 3.5～4.0; whereas the latest resumption, when BCS was 1.5 (Alapati *et al*., 2010). There are reports that low BCS affected reproductive performance (Markusfeld *et al*., 1997) and caused delayed resumption of ovarian activity (Butler and Smith, 1989; Senatore *et al*., 1996; De Vries *et al*., 1998). In addition, it has been reported that in cows, hereditary low BCS caused delayed return of postpartum estrus during early milking period, as well as the fall in conception rate (Bastin *et al*., 2010) and delayed first ovulation (Beam and Butler, 1999). Thus BCS is recently attracting interests as an important factor for estrous expression. On the other hand, Blood urea nitrogen (BUN) is a substance produced in the liver during the detoxification process of ammonia, and BUN concentration reflects the amount and degradability of the proteins taken, as well as the balance of protein and energy (Ferguson *et al*., 1993). However, BUN concentration depends to some extent on reproductive performance and on stock breeding management for cows (Carroll *et al*., 1988).

In the case of Holstein cows, research on *in vivo* embryo production technology has been insufficient compared to Korean native cattle (Hanwoo). Therefore, based on basic technologies for *in vivo* embryo production and embryo transfer, active research would have to be carried out with high - producing Holstein cows to improve the collection of *in vivo* embryos and the number of transferable embryos. The bovine embryo transfer technology that began to be used recently is being recognized as a very proper method for such a purpose in the domestic dairy farm environment. The reason is that it allows not only the establishment of the basis of superior seed animals and the shortening of the period required for multiplication, but also the fast distribution of the genetic abilities of superior seed animals. Multiple ovulation and embryo transfer (MOET) has been used for bovine embryo transfer to produce superior cows, in an effort to improve farm animals (Seidel, 1981; Smith, 1988; Son *et al*., 2000). For the successful practice of MOET, it is of primary importance to select superior donor cows and collect high quality embryos by superovulation treatment. An important factor of embryo transfer technology is to produce as many transferable embryos as possible in donor cows with superior genetic abilities. The production of their young allows efficient multiplication of cows with superior genetic characters, and a large number of their young with same characters can be produced in a short time. Thus MOET can be used very effectively for the improvement of farm animals (Smith, 1984; Christensen, 1991). However, there has been almost no improvement in pursuit of a stable system for embryo production. One reason is that individual animals show big differences in ovarian reaction to superovulation according to hormone administration. Another reason is that many factors affecting embryo production are involved in a complicated way (Armstrong, 1993).

The factors affecting the superovulation treatment and transferable embryo of individual donor cows are: postpartum treatment time, difference by season (Lim *et al*., 2009), difference in ovarian reaction (Shea *et al*., 1984), stockbreeding management condition (Bader *et al*., 2005), kind of follicular stimulating hormone (Elsden *et al*., 1978; Goulding *et al*., 1991; Staigmiller *et al*., 1992), dosage of hormone (Donaldson, 1984; Pawlyshyn *et al*., 1986), superovulation treatment method (Takedomi *et al*., 1992; Yamamoto *et al*., 1994; Lim *et al*., 1998), age of donor cow (Hasler *et al*., 1981; Donaldson, 1984), breeding experience (Isogai *et al*., 1993), repeated superovulation treatments (Donoidson and Perry, 1983; Warfeld *et al*., 1986; Almeida, 1987), and season (Shea *et al*., 1984; Bastidas and Randel, 1987). Since the mid-1990s, there also have been many domestic researches with the cow on superovulation treatment and the number of transferable embryos (Son *et al*., 1997; Kim *et al*., 1997; Lim *et al*., 1998; Son *et al*., 2006; Son *et al*., 2010).

For the practical utilization of embryo transfer technology, it would be the most basic and very important task to artificially and stably produce many transferable embryos in high-producing Holstein cow. Accordingly, in an effort to establish an efficient embryo production system for high-producing Holstein cows, this research aimed to elucidate the relationship between the number of transferable embryos, BCS, and BUN concentration according to nutritional management, upon analyzing a set of performance data obtained from National Institute of Animal Science.

MATERIALS AND METHODS

1. Experimental Cows

The experimental cows used for this research were 22 head of Holstein heifers owned by the Department of Dairy Science, National Institute of Animal Science, which had been selected as high-producing cows with superior characters.

2. Donor Cow, Superovulation Treatment, and Artificial Insemination

Progesterone releasing intravaginal devices (CIDR-plus, In-

terAg, New Zealand) were inserted into the vaginas of the experimental donor cows, regardless of their estrous cycle. From the 4th days after that, each of them was administered with 28 AU of FSH (Antorin, 2 AU=1 ml, Kawasaki Mitaka, Japan) over a 4 day period by intramuscular injection. On the 7th day after the insertion of CIDRs, 25 mg of $PGF_{2\alpha}$ (Lutalyse™, Phamacia Co., Belgium) was administered in the morning and 15 mg in the afternoon, with a 12-hour interval, and the CIDRs were removed. For artificial insemination, they were injected with $PGF_{2\alpha}$ and estrus was checked about 48 hours after the injection. Then they were treated with 4 straws of semen 3 times, with 12-hour intervals. 100 μg of Gonadolerin (GnRH, Fertagyl®, Intervet, Holland) was administered by intramuscular injection after the first artificial insemination.

3. Collection, Evaluation and Ultrasonic Test of Embryos

Embryos were collected on the 7th day after artificial insemination, while the infusion fluid was collected by a non - surgical method using Embryo Collection Medium (Agtech, Biolife™, USA). Collected embryos were classified - according to the criteria set by 'Manual of the International Embryo Transfer Society' (Stringfellow and Seidel, 1998) - into 2 groups: (1) a group of transferable embryos, if they were evaluated as code 1 (excellent or good) or code 2 (fair); and (2) a group of non - transferable embryos, if they were evaluated as code 3 (poor) or code 4 (dead or degenerating). In order to find out the number of corpus luteum in the donor cows that had received superovulation treatment, ovarian ultrasonic examination was carried out using Sonoace 600 with a 5.0 MHz linear array transducer (Medison Co., Led., Seoul, Korea).

4. Blood Collection, BCS Measurement, and BUN Analysis

The BCS of 22 head of experimental cows was measured 3 times over a 2 month period before embryos were collected, and the average values were used for analysis. 22 head of Holstein donor cows were involved in biochemical analysis. Beginning from 1 month before the insertion of CIDR, blood was collected and analyzed semiweekly until the day of insemination. Blood collection was carried out between 10～11 a.m. using 15 ml tubes without heparin or EDTA. Blood samples of about 10 ml each, collected from the jugular vein, was immediately carried to the laboratory, and then serum was separated within 3 hours by centrifugation for 15 minutes at 3,000 rpm. The serum samples were stored at −20℃ prior to analysis. BUN concentration was analyzed using an automated biochemical analyzer (7180, Kawasaki Mitaka Co., Ltd., Japan). Progesterone concentration was analyzed using an Immulite 1000 (NJ 07836, Simens Healthcare Diagnostics Inc. USA).

5. Statistical Analysis

Statistical significance was considered at $p<0.05$. All the statistical analyses were performed using Chi-square test of SAS program.

RESULTS AND DISCUSSIONS

The effect of BCS on the number of transferable embryos in Holstein donor cows was shown in Table 1. When BCS was ≦2.5, the total number of collected ova was 7.3 + 1.9, which was significantly lower ($p<0.05$) than the numbers 15.4 + 2.8 and 15.4 + 2.1 that were obtained when BCSs were 2.75 and ≧3.0, respectively. BCS is widely used as a means of evaluating not only the number of collected ova but also the nutritional status of cows, and low scores have been reported to exert bad effect on reproductive efficiency (Markusfeld *et al*., 1997). The number of transferable embryos was 5.2 + 1.4 when BCS was ≦2.5, which was again lower than the numbers 6.0 + 2.1 and 8.5 + 1.8 that were obtained when BCSs were 2.75 and ≧ 3.0; however, the differences were not significant. Although the result of the present research showed no significant difference in the number of transferable embryos according to BCS level, it did showed a significant difference in the number of collected embryo. It is therefore considered important to regularly and carefully check BCS, which can be done by the naked eye, and accordingly to maintain good nutritional status.

Table 2 showed the relationship between BCS and estrus induction rate, estrous expression and rate of mounting. A total of 22 heads of experimental cows were subjected to superovu-

Table 1. Effect of BCS concentration on *in vivo* Holstein embryo productivity

BCS	No. of cows	Embryo yield (per head)	
		No. of total ova	No. of transferable embryos
≦2.5	7	7.3 + 1.9*	5.2 + 1.4
2.75	7	15.4 + 2.8	6.0 + 2.1
≧3.0	8	15.4 + 2.1	8.5 + 1.8

* Different superscripts in the same column are significantly different ($p<0.05$).

Table 2. Relationship between BCS and estrous detection in Holstein donors

BCS	No. of cows	Estrous inducement rate	Estrous expression rate	Rate of mounting
≦ 2.5	7	57.1 (4/7)*	100.0 (4/4)	100.0 (4/4)
2.75	7	100.0 (7/7)	100.0 (7/7)	85.7 (6/7)
≧ 3.0	8	87.5 (7/8)	85.7 (6/7)	85.7 (6/7)
Total	22	81.8 (18/22)	94.4 (17/18)	88.9 (16/18)

* Different superscripts in the same column are significantly different ($p<0.05$).

lation treatment and artificial insemination. Then estrus was induced in 18 of them within 50 days. Of the 18 cows, estrous expression was shown by 4 cows in one group, 7 cows in another group and 6 cows in the other group. Estrus induction rates were 100.0% and 95.0% among the groups whose BCSs were 2.75 and ≧3.0, respectively; while it was 57.1% among the group whose BCS was ≦2.5 and the differences were significant ($p<0.05$). Estrus expression rates were 100.0%, 100.0% and 85.7% among the groups whose BCSs were ≦ 2.5, 2.75 and ≧ 3.0, respectively; however, the differences were not significant. Rates of mounting w.ere 85.7%, 85.7% and 100.0% among the groups whose BCS were 2.75, ≧3.0 and ≦2.5, respectively; however, the differences were not significant. The result of this research showed that estrus induction rate was significantly low among the cow group whose BCS was ≦2.5 compared to the other cow groups. And it is considered that the fall in BCS below 2.5 would cause not only the fall in reproductive efficiency but also delayed return to estrus after *in vivo* embryo production in high - producing Holstein donor cows. It is therefore very important to provide very careful stockbreeding management, in order to prevent the fall in reproductive efficiency (Lowman, 1985).

Fig. 1 showed the result of the analysis on the period from artificial insemination until return to estrus after the superovulation treatment of high-producing Holstein donor cows. Hormone analysis and estrus observation were carried out after

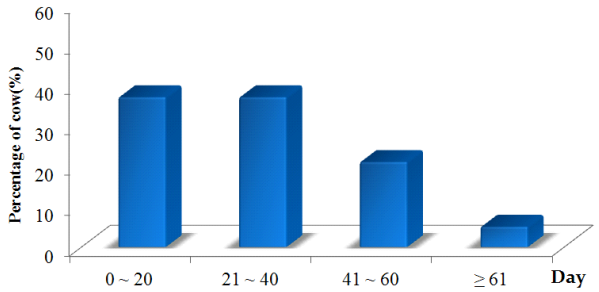

Fig. 1. Percentage of cows that resumed ovarian cyclicity after superovulation treatment in Holstein cows.

artificial insemination to analyze the time of return to estrus. According to the result, 36.4% (8/22) showed return to estrus within 20 days after artificial insemination, 36.4% (8/22) between 21 and 40 days, and 22.7% (5/22) between 41 and 60 days; while 1 cow (4.5%) did not show the resumption of ovarian cyclicity for 60 days.

BUN concentration increases for a period of time, and then remains constant at a proper level (Canfield *et al*., 1990; Park *et al*., 1997). For this reason, it is important to choose the stabilized period in order to use BUN concentration for estrous expression. In order to choose the stabilized period, BUN concentration was determined by calculating average value using the blood samples collected semiweekly from 1 month before the insertion of CIDR.

Table 3 showed the relationship between BUN concentra-

Table 3. Relationships between BUN concentration and estrous expression

BUN concentration (mg/dl)	No. of cows	Estrous inducement rate	Estrous expression rate	Rate of mounting
< 10	4	75.0 (3/4)	100.0 (3/3)	100.0 (3/3)
11 ~ 18	12	83.3 (10/12)	100.0 (10/10)	90.0 (9/10)
≧ 19	6	83.3 (5/6)	80.0 (4/5)	80.0 (4/5)
Total	22	81.8 (18/22)	94.4 (17/18)	88.9 (16/18)

Within a column, no significant differences were observed ($p>0.05$).

tion and estrous expression rate in the cow groups after superovulation treatment. As for the relationship between estrus induction rate and BUN concentration, the rates were 75.0% (3/4), 83.3% (10/12) and 83.3% (5/6) among the groups whose BUN concentrations were <10 mg/dl, 11～18 mg/dl and ≧19 mg/dl, respectively; however, the differences were not significant. As for estrous expression rate, the rates were 80.0% (4/5) among the group whose BUN concentration was ≧19 mg/dl, which was lower than the rate 100.0% among the both groups whose BUN concentrations were <10 mg/dl and 11～18 mg/dl, respectively; however, the differences were not significant. Rates of mounting were 100.0%, 90.0% and 80.0% among the groups whose BUN concentrations were <10 mg/dl, 11～18 mg/dl and ≧19 mg/dl, respectively; however the differences were again not significant. According to the result of this research, BUN concentration did not exert a significant effect on both estrus induction rate and estrous expression rate. BUN concentration has been reported to exert effect not only on estrus induction rate and estrous expression, but also on reproductive efficiency and conception rate (Freguson *et al*., 1993; Butler *et al*., 1996; Park *et al*., 1997).

As for the relationship between BCS and the number of transferable embryos in high-producing Holstein donor cows, no significant effect of BCS was observed. However, as for the relationship between BCS and the number of collected ova, the number was significantly low among the bovine group whose BCS was ≦2.5. Therefore, since embryo production is affected by donor cow's nutritional status before and after superovulation treatment, it would be necessary to provide very careful stockbreeding management so that BCS concentration does not fall below 2.5. As for the relationship between nutritional status and estrus induction rate, again the rate was low among the bovine group whose BCS concentration was ≦2.5. In addition, the bovine group whose BUN concentration was <10 mg/dl also tended to show low estrus induction rate, although the differences were not significant. From the result of this research, it is considered that the total number of collected ova and the number of transferable embryos are affected by the nutritional state before and after *in vivo* embryo production and superovulation treatment, and that, although the mechanism is not clear, poor stockbreeding management and nutritional level cause the decrease of ovum recovery rate and the number of transferable embryos in high - producing cows. On the other hand, it is expected that diversified researches on proper nutritional management, as well as on suitable superovulation treatment methods for high - producing Holstein donor cows, will not only allow the prevention of diseases, such as the disorder of ovarian cyclicity resumption, but also contribute to the early multiplication of farm animals with superior genes by increasing the utilization value of donor cows.

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