J. Emb. Trans. (2012) Vol. 27, No. 4, pp. 229～235
http://dx.doi.org/10.12750/JET.2012.27.4.229

Relationship between Transferable Embryos and Major Metabolite Concentrations in Holstein Donor Cows

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ABSTRACT

This research was investigated the relationship, in high-producing Holstein donor cows, between the number of the transferable embryos and the blood serum concentrations of Blood Urea Nitrogen (BUN), glucose and cholesterol, which affect the nutritional state of cows. CIDRs were inserted into the vaginas of twenty two heads 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. The total numbers of ova collected from 3 experimental groups whose blood BUN concentrations were <10 mg/dl, 11～18 mg/dl and ≥19 mg/dl were 8.9, 12.5 and 19.0, respectively; whereas the numbers of transferable embryos were 5.8 + 1.9, 7.9 + 2.8 and 5.2 + 1.4, respectively. When glucose concentration was <60 mg/dl, the total number of collected ova was 9.9, which was smaller than when the concentration was 60～70 mg/dl or ≥70 mg/dl. When glucose concentration was 60～70 mg/dl, the number of transferable embryos was 7.1 + 2.4, which was slightly larger than the numbers 6.4 + 2.1 and 6.1 + 1.7 that were obtained when the concentrations were <60 mg/dl and ≥70 mg/dl, respectively ; however, the differences were not significant (p>0.05). When cholesterol concentrations were <150 mg/dl, 150～200 mg/dl and ≥200 mg/dl, the total numbers of collected ova were 11.2, 11.3 and 8.6, respectively. Whereas the numbers of transferable embryos were 7.1 + 2.1, 7.3 + 1.9 and 5.6 + 1.3, respectively ; however, the differences were again not significant (p>0.05). The result of this research showed no significant difference in ovum recovery rate and the number of transferable embryos according to major metabolite concentrations in high-producing Holstein donor cows. However, it is considered that the failure of maintaining proper nutritional status would cause the fall in *in vivo* embryo productivity.

(Key words : Holstein donors, transferable embryo, BUN, glucose, cholesterol)

INTRODUCTION

With the development of high-producing Holstein cows, as well as with the changes in environmental factors, there are increasing incidences of weak estrus and silent estrus, and the reproductive efficiency and conception rate of both domestic and foreign Holstein cows have been decreasing every year (Roche *et al*., 2000; Lucy, 2001). A general method currently utilized for improving high-producing Holstein cows is artificial insemination, but this method has the weak points of being applicable only to superior donor cows and of slow improvement speed. In Korea, with the development of technologies for *in vitro* fertilized (IVF) embryo production and culture, there are active practices of IVF embryo transfer. However, IVF embryos show lower conception rate than *in vivo* produced (IVP) embryos. On the other hand, the production and transfer of IVF embryos are not actively practiced with domestic Holstein cows, although actively practiced in Korean native cattle (Hanwoo). Researches, both domestic and foreign, on the technologies for *in vivo* derived embryo production and for superovulation treatment are actively going on. However, there have been insufficient researches on superovulation treatment method and *in vivo* embryo production with high-producing Holstein cows.

Embryo transfer technology, along with *in vivo* derived embryo production can reinforce the competitiveness and productivity of farmhouses by improving the ability and reproductive efficiency of farm animals. The reason is that it can shorten

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the period required for establishing the basis of superior seed farm animals and for their multiplication, while distributing their genetic abilities rapidly and widely. Embryo transfer technology can effectively multiply farm animals with superior genetic characters, by collecting many embryos from female farm animals with such characters and transferring them to other female farm animals to produce their young. This technology allows the fast production of a large number of their young that have same characters, and so it can be utilized very usefully for improving the abilities of farm animals (Smith, 1984; Christensen, 1991). In Korea, embryo transfer technology was first introduced in the 1980s, and since then there has been a great improvement of its efficiency. For the steady securing of many embryos with superior abilities, diverse researches on superovulation treatment technology are also being conducted. MOET (multiple ovulation and embryo transfer) has been used for cow embryo transfer to produce superior cows, in an effort to improve farm animals (Seidel, 1981; Smith, 1988; Son *et al*., 2000). Superovulation treatment technology -a method for the maximal production of transferable embryos in donor cows with superior genetic abilities-is under active research world widely. However, in the case of high-producing Holstein cows, there has been almost no improvement toward a stable system for normal embryo production. One of the reasons is that many restrictions related to milk production delays the establishment of a systematic technology. Other reasons include that individual animals show big differences in ovarian reaction to superovulation according to hormone administration, and that many factors affecting embryo production are involved in a complicated manner (Armstrong, 1993).

The factors affecting superovulation treatment and the number of transferable embryos in 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 stimulation hormone (Elsden *et al*., 1978; Goulding *et al*., 1991; Staigmiller *et al*., 1992), dosage of administrated hormones (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 cows on superovulation treatment and on 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).

The nutritional status of Holstein cows exerts a very important effect on the number of collected transferable embryos after superovulation treatment (Ferguson *et al*., 1993). Many researches are going on to improve the efficiency of superovulation treatment by increasing Holstein donor cow's intake of proteins, lipids, vitamins and minerals; however, no accurate mechanism has yet been known. There was a report that vitamin supply tended to increase the number of transferable embryos at the time of *in vivo* embryo production (Velazquez, 2011). There has not been sufficient research on the effect of the supply condition of vitamin, as well as of protein and lipid, on the efficiency of transferable embryo production. Therefore active researches would be necessary in this area to enable systematic nutritional design, so that larger numbers of transferable embryos can be obtained from donor cows. An energy unbalance after *in vivo* embryo production would result in the prolongation of ovulation interval, and could be the cause of diestrus as well as sterility.

The status of energy intake is reflected by glucose concentration. Glucose concentration is low when energy intake is not sufficient, although sometimes high because of excessive intake of concentrated feed or because of stress. A remarkably low concentration indicates severe lack of energy, which exerts an effect on diseases, such as ketosis and reproductive disorder (Ferguson *et al*., 1993). Cholesterol concentration reflects comprehensive nutritional status as well as liver function. Cholesterol concentration becomes low when liver function is deteriorated or when nutrition intake is insufficient, but becomes high when lipid intake is excessive. Total cholesterol concentration has the efficacy of regulating a role that is important for ovarian steroid production on long-term basis (Pradhan *et al*., 2008). Whereas 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). The improvement of reproductive efficiency requires the improvement of estrus detection rate, the efficient maintenance of the length of postpartum period until the day of the first insemination, and high conception rate (Pelssier, 1976). However, the efficiency depends to some extent on the reproductive performance, and also on stockbreeding manage-

ment for cows (Carroll *et al*., 1988). Nevertheless, there has been insufficient research about the effect of stockbreeding management condition on the reproductive efficiency of cows, as well as about the relationship between transferable embryo production and the concentrations of major metabolites, such as BUN, glucose and total cholesterol. 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 cows. Accordingly, in an effort to establish an efficient embryo production system for Holstein cows, this research aimed to find out the relationship between the number of transferable embryos and the concentrations of BUN, glucose and total cholesterol 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 twenty two 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, InterAg, 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 28AU of FSH (Antorin, 2AU=1ml, 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 15mg 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 code1 (excellent or good) orcode 2 (fair); and (2) a group of non - transferable embryos, if they were evaluated as code3 (poor) or code4 (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 and Biochemical Analysis

Twenty two head of Holstein donor cows were used for biochemical analysis. Beginning from 1 month before the insertion of CIDR, blood was collected and analyzed weekly 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, were immediately carried to the laboratory, and then serum was separated within 3 hours by centrifugation for 15 minutes at 3000 rpm. The serum samples were stored at −20℃ prior to analysis. The concentrations of BUN, glucose and total cholesterol were analyzed using an automated biochemical analyzer [7180, Kawasaki Mitaka Co., Ltd., Japan].

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 result of embryo collection after the superovulration of experimental cows was shown in Table 1. The total numbers of collected ova from the group of less than 10 corpus luteum and the group of 10 or more corpus luteum were 7.8 and 12.7, respectively; while the numbers of transferable embryos were 5.4 and 10, respectively. Greve *et al*. (1983) reported that after the superovulation treatment of donor Korean cows, the average number of corpus luteum of was 9, the average total number of collected ova was 7, and the average number of transferable embryos was 4.

In the present research, the average total number of collected

Table 1. Effect of corpus luteum (CL) number on *in vivo* Holstein embryo productivity

No. of CL	No. of cows	Embryo yield (per head)	
		No. of total embryo	No. of transferable embryo
< 10	7	7.8 + 1.8	5.4 + 1.3
≥ 10	15	12.7 + 2.7	8.1 + 3.4

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

ova and the average number of transferable embryos were all shown to be larger compared to the results of Greve *et al*. (1983). Such a difference is considered due to the difference in superovulation treatment method and stock breeding management.

BUN concentration is used as an index for judging the suitability of stock breeding management. BUN concentration, in general, increases for several weeks after delivery and then remains constant at a proper level (Carroll *et al*., 1988; Canfield *et al*., 1990; Park *et al*., 1997). Table 2 showed that the effect of BUN concentration on the number of transferable embryos in 3 groups of donor cows whose BUN concentrations were <10, 11～18 and ≥19 mg/dl, respectively. The total numbers of embryo collected from the 3 treatment groups whose BUN concentrations were <10 mg/dl, 11～18 mg/dl and ≥19 mg/dl were 8.9, 12.5 and 19.0, respectively; whereas the numbers of transferable embryos were 5.8 + 1.9, 7.9 + 2.8 and 5.2 + 1.4, respectively. Lee *et al*. (2012) reported that the ratio of transferable embryos was high when Milk Urea Nitrogen (MUN) concentration was 12～18 mg/dl. This result shows a similar trend when compared to the result of the present research, which showed that the number of transferable embryos was large when BUN concentration was 11～18 mg/dl. There was no significant difference in the number of transferable embryos according to BUN concentration. However, the cows with a proper range (11～18 mg/dl) of BUN concentration had larger numbers of corpus luteum, of collected ova and of transferable embryos. BUN concentration reflects the amount and degradability of the proteins taken, as well as the balance of protein and energy (Ferguson *et al*., 1993). For this reason it has been utilized as an index for judging the suitability of nutritional management. General interpretation is that BUN concentration lower than reference value mostly indicates insufficient feed intake, and that BUN concentration higher than reference is caused by the lack of energy in feed. Thus it is possible to deduce the current energy level of cows by measuring BUN concentration. It has been reported that low BUN concentration affected not only the number of collected ova and the number of transferable embryos, but also estrous expression rate, reproductive efficiency and conception rate (Ferguson *et al*., 1993; Butler *et al*., 1996; Park *et al*., 1997).

Table 3 showed the effect of glucose concentration on the number of transferable embryos. When glucose concentration was <60 mg/dl, the total number of collected embryo was 9.9, which was smaller than when it was 60～70 mg/dl or ≥70 mg/dl. When glucose concentration was 60～70 mg/dl, the number of transferable embryos was 7.1 + 2.4, which was larger than the numbers 6.4 + 2.1 and 6.1 + 1.7 that were obtained when the concentrations were <60 mg/dl and ≥70 mg/dl, respectively; however, the differences were not significant. Glucose concentration reflects the status of energy intake, and low glucose concentration is caused by severe lack of energy. Sometimes high glucose concentration is observed because of

Table 2. Effect of BUN concentration on *in vivo* Holstein embryo productivity

BUN concentration (mg/dl)	No. of cows	Embryo yield (per head)	
		No. of total embryo	No. of transferable embryo
< 10	4	8.9 + 1.7	5.8 + 1.9
11～18	12	12.5 + 3.1	7.9 + 2.8
≥ 19	6	9.0 + 2.4	5.2 + 1.4

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

Table 3. Effect of glucose concentration on *in vivo* Holstein embryo productivity

Glucose concentration (mg/dl)	No. of cows	Embryo yield (per head)	
		No. of total embryo	No. of transferable embryo
< 60	5	9.9 + 2.9	6.4 + 2.1
60～70	11	10.9 + 3.3	7.1 + 2.4
≥ 70	6	10.6 + 2.9	6.1 + 1.7

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

excessive intake of concentrated feed or because of stress. Glucose concentration is low when energy intake is not sufficient, although sometimes high because of excessive intake of concentrated feed or because of stress. A remarkably low concentration indicates severe lack of energy, which exerts an effect on diseases, such as ketosis and reproductive disorder (Ferguson *et al*., 1993). High milk producing groups tend to show high glucose concentrations, thus reflecting sufficient energy intake. In this research, however, such an effect is thought not to be great when it is taken into consideration that the experimental cows were heifers.

Table 4 showed the relationship between cholesterol concentration and the total number of collected ova and the number of transferable embryos. There was no significant difference in the total number of collected ova and the number of transferable embryos according to cholesterol concentration. When cholesterol concentrations were <150 mg/dl, 150~200 mg/dl and ≥200 mg/dl, the total numbers of collected ova were 11.2, 11.3 and 8.6, respectively; whereas the numbers of transferable embryos were 7.1 + 2.1, 7.3 + 1.9 and 5.6 + 1.3, respectively. Cholesterol concentration reflects the comprehensive nutritional status, as well as the normal or abnormal liver function, of donor cow. It becomes low when liver function is deteriorated or when nutrition intake is insufficient, whereas it becomes high when lipid intake is excessive (Pradhan *et al*., 2008). In the case of dairy cows, high milk producing cow groups tend to show higher cholesterol concentration. However, in the case of dry period or of heifers, which are the subjects of the present research, they show no big difference.

Compared to the research on the relationship between BUN concentration and the number of transferable embryos, there has been insufficient research on the relationship between major metabolite concentrations and the number of transferable embryos. In the case of Holstein heifers, high level of protein intake causes the increase of milk production. However, BUN concentration increases at the same time, and this in turn can cause the decrease of reproductive efficiency as well as the change of uterine environment (Theera *et al*., 2011). According to the result of the present research, it is considered that the nutritional level before and after the superovulation treatment of high-producing Holstein donor cows affect the total number of collected ova as well as the number of transferable embryos, although the mechanism and cause are not clear. And nutritional unbalance and the low concentrations of such major metabolites would cause the decrease of ovum recovery rate as well as the decrease of the number of transferable embryos. Therefore, if proper nutritional status can be maintained through diversified researches on superovulation treatment method and by nutritional management, it is considered possible to enhance the utilization value of donor cows by increasing ovum recovery rate and the number of transferable embryos, as well as by preventing ovarian cyclicity disorder after *in vivo* embryo production. Further, efficient production of superior embryos of high-producing Holstein donor cows could also be widely utilized for the improvement of high-producing Holstein cows, through the earlier multiplication of cows with superior genetic abilities as well as the improvement of embryo transfer technology.

Table 4. Effect of cholesterol concentration on *in vivo* Holstein embryo productivity

Cholesterol concentration (mg/dl)	No. of cows	Embryo yield (per head)	
		No. of total embryo	No. of transferable embryo
< 150	11	11.2 + 2.5	7.1 + 2.1
150~200	6	11.3 + 3.3	7.3 + 1.9
≥ 200	5	8.6 + 3.1	5.6 + 1.3

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

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(received: 2012. 10. 18 / revised: 2012. 10. 19 / accepted: 2012. 10. 30)