Semen Characteristics in Bulls Produced by Embryo Transfer in Hanwoo (Korean native cattle) of Hoengseong Area

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ABSTRACT

The aim of this study was to investigate the individual difference of semen characteristics in bulls produced by embryo transfer (ET) in Hanwoo. A total of 5 bulls (Bull No. 0846, 3010, 8777, 8778 and 8807) which had been produced by ET were examined. Semen was collected with artificial vagina method and were replicated three to four times for each bulls. Extended semen was evaluated survival rate, mitocondrial activity, abnormal spermatozoa and stability of the sperm membrane. Acrosome reaction and acrosome status of sperm was measured by coomassie brilliant blue (CBB) staining and chlortetracycline (CTC) assay, respectively. As a result, the rates of survival in semen of bull No. 0846, 3010 and 8777 were significantly ($p<0.05$) higher than that of bull No. 8778 and 8807. Acrosome reaction rate in bull No. 8778 was significantly lower ($p<0.05$) than that of the other semens, but membrane stability was not different among the semen of bull No. 0846, 3010 and 8777. Also the mitocondrial function, acrosome status and morphological abnormality were not significantly different among bulls. Therefore, bull No. 0846 can be consider as the highest reproductive ability male animal on basis of semen characteristics.

(Key words : Korean native cattle, bull, semen, embryo transfer, sperm characteristics)

INTRODUCTION

The use of embryo transfer (ET) technologies could enhance cattle production at several levels in both dairy and beef industries (Hossein-Zadeh, 2010). However, research on semen characteristics in hanwoo produced by ET was not sufficient. The selection of breeding bull by fertility were required to improve the pregnancy rate (Clay *et al.*, 2004). Because, the increase of calving rate following reproductive techniques such as AI and ET can lead to an amount of disadvantage of cost and effort (Lee *et al.*, 2010).

And the evaluation of semen is an important tool in determining the breeding potential of a bull (Bhattacharyya *et al.*, 2009). In order to be able to obtain better fertilization results, it is necessary to assess the quality of sperm just before their use in assisted reproductive techniques, such as artificial insemination (AI) and *in vitro* fertilization (IVF) (Silva and Gadella, 2006), because the conception rate with AI depends on the characteristics of the semen provided by AI centres (Druet *et al.*, 2008).

The objective high-throughput multi-parameter sperm assessments provide statistically stronger data which will be required for future studies to be able to get correlations between sperm quality parameters and fertility results (Silva and Gadella, 2006). The quality of the semen depends on different factors such as the number of motile spermatozoa, sperm motility, sperm viability, abnormal spermatozoids and also some genetic factors associated to the bull (Druet *et al.*, 2008).

Thus, the objectives of the present study were to investigate the individual difference of semen characteristics in bulls produced by ET in Hanwoo of Hoengseong area.

MATERIALS AND METHODS

1. Semen Collection

A total of 5 bulls which were produced by ET were examined (Bull No. 0846, 3010, 8777, 8778 and 8807). Semens were collected at Hoengseong Livestock Cooperation and were

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collected with artificial vagina method. The age and weight of bulls was 28 to 32 month and 700 to 850 kg. The internal temperature of the artificial vagina was maintained at 36 to 40℃ (average, 38℃). The fresh collected semen was diluted in a 1:1 ratio (v/v semen:extender) with TRILADYL® (KRUUSE, Cat. no. 340244) one of the semen extenders. Then, bovine semens were transferred to the laboratory at 5℃ within 2 h for experiments. Semen collections were replicated three to four times for each bulls and a total of 900 to 1,200 sperms were counted for analysis of semen characteristics.

2. Analysis of Semen Characteristics

Survival rates of sperms were measured by SYBR-14/PI staining as described previously (Lee *et al.*, 2005). Both SYBR-14 and PI were obtained from the live or dead sperm viability kit (L-7011, Molecular Probes, Inc., Eugene, OR). The stock solutions were first prepared as 0.02 mM SYBR-14 in DMSO and 2.4 mM PI in D_2O, respectively. The staining solution contained 0.187 μM SYBR-14 and 38.4 μM PI. Fifty microliters of semen was incubated with the staining solution at 37℃ for 15 min under lightproof conditions. The stained spermatozoa were then examined with filter under a fluorescent microscope. Survival rate of sperm was examined through the use of two stains to quantify the green population of spermatozoa with SYBR-14 and those dead spermatozoa that stained red with PI (Thomas *et al.*, 1997).

The abnormal spermatozoa of extended semen was evaluated by using rose-bengal staining. Semen samples were permanently mounted to the slide glass and stained with 0.5 μl rose-bengal. The examination of abnormal sperm was conducted under the phase contrast microscope.

Acrosome status were examined by chlortetracycline (CTC) assay. The three fluorescent staining patterns identified were: F (an uncapacitated spermatozoa), with uniform fluorescence over the whole sperm head; B (a capacitated spermatozoa), with a fluorescence-free band in the post-acrosome region; and AR (an acrosome-reacted spermatozoa), with almost no fluorescence over the sperm head, except for a thin band of fluorescence in the equatorial segment (Abeydeera *et al.*, 1997).

Acrosome reaction was evaluated by coomassie brilliant blue (CBB) staining methods. The CBB G250 staining were applied to evaluate the bovine sperm rates of acrosome reaction (Lu *et al.*, 2002). The smear of spermatozoa before and after capacitation and induced acrosome reaction were stained with 0.05% CBB G250 respectively, and visualized with light microscopy. The acrosome reaction of spermatozoa were calculated.

Sperm membrane stability was assessed using hypoosmotic swelling test (HOST; Garde *et al.*, 1998). The test was conducted by adding 10 μl of semen to 2 ml of HOST solution (100m osm kg^{-1}) with incubated at 37℃ for 1 h. Following incubation, sperm swelling was estimated by examining a drop of sample on a glass slide covered with a cover slip. A total of 300 sperms were counted using a warm stage (37℃) under a phase contrast microscope at 400×magnification.

To evaluate the sperm mitochondrial activity, stock solutions of 0.53 mM Rhodamine 123 (R8004, Sigma Aldrich. St, Louis, MO, USA) were prepared in DMSO and 2.99 mM propidium iodide (PI) (P-4170, Sigma, Deisenhofen, Germany) in PBS. The final staining solution contained 2 μl of Rhodamine 123 solution and 2 μl PI solution/ml PBS. Diluted semen was stained with 500 μl of the final staining solution of Rhodamine 123. The samples were incubated at 38℃ for 5min before examination. The changes in the mitochondrial activity were measured depending on the greed fluorescent of Rhodamine 123.

3. Statistical Analysis

Statistics were performed with the analysis of variance (ANOVA) using SAS (version 9.1, SAS Institute Inc., Cary, NC, USA). Values in each variate were subjected to Duncan's modified multiple range test. Differences were considered significant when $p<0.05$.

RESULTS

Viability and characteristics of semen from bulls which were produced by ET in Hanwoo are shown in Table 1. Ratios of survival in semen of 0846, 3010 and 8777 were significantly ($p<0.05$) higher than that of 8778 and 8807 in bull number. The highest rate of membrane stability was obtained from semen of bull No. 8777 (56.2 ± 11.6), but membrane stability rates was not significantly different among the group of 0846, 3010 and 8777. On the other hand, ratio of mitochondrial function, morphological abnormality were not significantly different among groups.

Table 2 shows the changes of CTC patterns and acrosome reaction in bulls which were produced by ET in Hanwoo. Acrosome reaction rates of bull No. 8778 was significantly ($p<0.05$) lower than the other semens which was not significantly different. The acrosome status were not significantly different for all of the bulls.

Table 1. Viability and characteristics of semen from bulls that produced by ET in Hanwoo

Bull no.	Survival rate (%)	Mitocondrial function (%)	Morphological abnormality (%)	Membrane stability (%)
0846	76.3 ± 2.1[a]	97.1 ± 0.9	3.6 ± 1.0	46.2 ± 3.5[ab]
3010	70.7 ± 8.1[a]	95.7 ± 2.1	6.7 ± 2.3	36.2 ± 5.0[abc]
8777	72.3 ± 3.8[a]	97.7 ± 1.2	3.1 ± 1.8	56.2 ± 11.6[a]
8778	28.4 ± 12.9[b]	98.6 ± 0.6	5.6 ± 0.4	21.4 ± 6.8[c]
8807	46.2 ± 9.2[b]	97.4 ± 0.5	5.0 ± 0.6	23.8 ± 10.8[bc]

Values with different superscripts differ significantly ($p<0.05$).

Table 2. Changes of CTC patterns and acrosome reaction in bulls that produced by ET in Hanwoo

Bull no.	Acrosome reaction (%)	Acrosome status (%)		
		F	B	AR
0846	89.5 ± 5.7[a]	44.6 ± 2.4	49.1 ± 1.0	6.3 ± 2.7
3010	59.9 ± 21.1[ab]	35.5 ± 10.0	58.2 ± 9.6	6.3 ± 1.2
8777	57.7 ± 20.9[ab]	53.0 ± 0.7	44.6 ± 0.6	2.4 ± 1.0
8778	27.2 ± 10.7[b]	37.8 ± 11.0	54.9 ± 11.4	7.3 ± 2.1
8807	67.0 ± 22.2[ab]	35.1 ± 5.7	58.3 ± 7.3	6.6 ± 1.9

Values with different superscripts differ significantly ($p<0.05$). F: an uncapacitated spermatozoa, B: a capaciated spermatozoa, AR: an acrosome reacted spermatozoa.

DISCUSSION

The purpose of this study was to analyze sperm characteristics of bulls produced by ET. The survival rate, mitocondrial function, morphological abnormality and membrane stability of the semen of each bulls are shown in Table 1. Sperm viability in bull No. 0846 was revealed significantly ($p<0.05$) highest in survival rate. Also, there is a difference in membrane stability of bull sperm. However, the characteristic of semen did not differ significantly in mitocondrial function and morphological abnormality. This result indicate that the survival rate of bull No. 0846 maintained better motility than other four bulls.

In the CTC fluorescence assay, CTC binds the sperm plasma membrane in a Ca^{2+} and Mg^{2+} and forming fluorescent complexes of CTC with those ions (Chandler *et al.*, 1978). These principles are suitable to decide pattern of uncapacitated, capacitated or acrosome reacted spermatozoa. However, rate of acrosome reacted spermatozoa after acrosome reaction treatments are not discriminated. Therefore acrosome reaction was evaluated by CBB staining methods. As a results, the CTC patterns was similar in the all bulls, but the rate of acrosome-reacted spermatozoa of bull No. 0846 was higher than any other bulls. The results of this data also revealed that, the patterns of acrosome integrity were not significantly different in all groups, but the highest acrosome reaction after treatment was obtained in bull No. 0846. Also, acrosome reaction showed a wide variation by individual.

For a successful conception, the fertilizing sperm should have functional competent membranes, organelles and an intact haploid genome (Silva *et al.*, 2006). Pregnancy rate has been studied in several countries, including Brazil (Sá Filho *et al.*, 2009), Japan (Long *et al.*, 2010) and USA (Larson *et al.*, 2008). Previous studies reported that the conception rate in lactating cows after Ovsynch is 40% and 50% after CIDR-Ovsynch (Stevenson *et al.*, 2006). In order to be able to obtain better fertilization results, it is necessary to assess the quality of sperm just before their use in assisted reproductive techniques, such as artificial insemination (AI) and *in vitro* fertilization (IVF) (Silva and Gadella, 2006).

Cryopreservation exposes sperm to mechanical and anisosmotic stresses (Hammerstedt *et al.*, 1990; Medeiros *et al.*, 2002) that reduce cell survival and alter surviving sperm function (Curry, 2000), in turn, reducing cell longevity and fertility compared with fresh sperm. The governmental institution supplies the frozen semen of Korean proven bull which were able to use for control group. On the other hand, fresh semen were evaluated in this study. Therefore, more researches would be needed to compare the Korean proven bull and Hanwoo which were produced in Hoengseong area by ET.

In conclusion, bull of 0846 can be considered as the highest reproductive ability male animal on basis of semen characteristics. Moreover, the obtaining by multi-parameter sperm assessments may lead to applications, such as selecting top male animals regarding to high quality sperm for breeding programs as a proven bull.

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