Assessment of Sperm Characteristics in Fresh and Frozen Semen of Miniature-Pig*

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ABSTRACT

The present study was conducted to assess sperm characteristics in miniature-pig. The semen samples were transported to the laboratory at 17℃ within 3 hours after collection. The extended semen was stored at 17℃, and sperm quality was evaluated at 0, 1, 3, 5 and 7 days after storage. The semen volume of miniature-pig (62±22㎖) was significantly ($p<0.05$) lower than that of Duroc (155±25㎖) and Yorkshire (154±23㎖). Significant differences were also observed in sperm concentrations. During 3 days of storage, sperm viability did not differ among miniature-pig, Duroc and Yorkshire. However, the viability was significantly ($p<0.05$) lower in miniature-pig than in Duroc and Yorkshire semen after Day 3 of storage. In abnormality, acrosome intactness and intensity, there were no differences among miniature-pig, Duroc and Yorkshire semen. On the other hand, the viability of frozen-thawed sperm in miniature-pig was significantly ($p<0.05$) lower than in that of Duroc and Yorkshire. This study also examined CTC patterns in frozen-thawed spermatozoa. The rates of AR pattern were higher in miniature-pig than in Duroc and Yorkshire. However, no difference was found in F, B and AR patterns. The results of present study suggest that further research is necessary to develop of semen extender and freezing methods to improve sperm quality in miniature-pig.

(Key words : Miniature-pig, Semen characteristics, Frozen-thawing, CTC pattern)

INTRODUCTION

As science moves closer to using pig organs for human transplants, many experts study that pig organs could be made available to people. Many research teams announced that they have cloned piglets that lack one of two genes that prompt the human immune system to reject swine tissue. The next step is breeding or cloning that would eliminate the gene entirely from a strain of pigs.

Outside the country, first cloned pigs has been reproduce at Virginia USA on March 2000. Specific Pathogen Free (SPF) pigs has been reproduce a research team of Missouri University USA on september 2001. Gal Transferase (GT) knock-out pig has been reproduce at PPL corporation on July 2002. The transgenic cloned pig has been reproduce at Seoul National University on september 2003 in our country. The study of stem cell is medical treats for diseases intractable and negative. Study of bio-organ transplantation is give that motive for growth industry to next-generation.

Artificial insemination (AI) in pigs has attained wide acceptability throughout the world with its use increasing greatly over the past 25 years. However, the AI of cryopreserved boar semen is still limited, mainly due to the generally lower farrowing rate and little size. The precesses of cryopreservation cause serve damage or death to about 60% of the cells (Courtens and Paguignon, 1985). To improve boar semen cryopreservation, knowledge is needed regarding the physical and biochemical properties of spermatozoa and how these attributes are affected during the stages of cryopreservation (Gilmore et al., 1996). However, little information exists fresh and frozen-thawed semen characteristics in miniature-pig for production of bio-organs. The present study was carried out to assess sperm characteristics between miniature-pig and commecial pigs.

MATERIALS AND METHODS

Semen Collection and Processing

Boar (Duroc and Yorkshire) and miniature-pig semen

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were collected using a gloved-hand technique and filtered through cotton gauze to remove the gel particles. The fresh collected semen was extended with same volume of extenders (Byu-Ri, Sperm Gene). After maintenance at room temperature for 20 min, the semen samples were transported to the laboratory at 17℃ with in 2 hours. The extended semen was stored at 17℃, and was used for examination of semen characteristics at 0, 1, 3, 5 and 7 days of storage.

Semen Evaluation

Sperm characteristics of boar semen stored at 17℃ was evaluated at days 0, 1, 3, 5 and 7 of storage (Johnson et al., 2002), respectively. For examination, semen volumes were determined by an electron weighing beam. Sperm concentrations were estimated by a hemocytometer. Sperm viability was determined using by Makler Counting Chamber staining. Sperm abnormality was determined by Rose-Bengal staining methods. Sperm acrosome intactness was determines using by Coomassie Briliant Blue staining (Huo et al., 2002). Sperm intensity was determines by hypoosmotic swelling test (Neild et al., 1999). In another experiments, the frozen semen was evaluated by same methods described above (Maxwell and Salamon, 1979).

Freezing and Thawing

Semen samples were processed using the straw freezing procedure described by Westendorf et al. (1975) with minor modifications indicated on the following (Einarsson, 1973). Diluted semen was placed at 15℃ for 2 h and later centrifuged at 800×g for 10 min. The supernatant was discarded and the semen pellet was re-suspended with lactose-egg yolk extender (LEY, 80㎖ of 11% lactose and 20㎖ egg yolk) to provide 1.5×10^9 spermatozoa/㎖. After further cooling to 5℃ over a 2 h, two parts of LEY-extender semen were mixed with LEY extender with 1.5% Orvus Es Paste and 9% glycerol. The final concentrations of semen to be frozen was 1.0×10^9 spermatozoa/㎖ and 3% glycerol. The diluted and cooled semen was loaded into 0.5㎖ straws and placed in liquid nitrogen vapor approximately 8㎝ above the level of the liquid nitrogen for 10 min. The straws were then stored in liquid nitrogen until thawing.

Thawing of frozen-semen was done after storage in liquid nitrogen. The 0.5㎖ straw was thawed in a water bath at 37℃ for 30 sec, respectively (Larsson and Einarsson, 1975; Selles et al., 2003).

Statistical Analysis

Data are presented as mean ± standard deviatin (S.D). Statistical analyes were conducted using SAS Version 8.01 for Windows (SAS Institute, 1990). Statistical significance was regarded when $p<0.05$. Multiple comparisons were made with the protected Fisher's least significant difference (LSD) test. The Duncan's multiple range test was used to determine the significance of difference between the mean.

RESULTS AND DISCUSSION

Comparison of fresh semen were determined semen volumes and sperm concentrations between miniature-pig (n=4) and boars (Duroc ; n=13 and Yorkshire ; n=9). As shown in Table 1, the semen volume of miniature-pig (62±22㎖) was significantly ($p<0.05$) lower than in Duroc (155 ±25㎖) and Yorkshire (154±23㎖). Significant differences were also observed sperm concentrations. However, sperm volumes and concentrations did not significantly differ between Duroc and Yorkshire. In this study, we found semen volumes and sperm concentrations that affected by pigs size.

Leman and Rodeffer (1976) reported that puberty occurred between 5 and 8 months, and the number of sperm and the volume of ejaculate increase until the boar reaches 18 months (Leman and Rodeffer, 1976). Diehl et al. (1979) reported that the number of sperm and the volume of semen was $30\sim60\times10^9$ sperm and 150~200㎖, respectively. Hafez (1987) reported that semen volume and sperm concentration were 150~200㎖ and $2\sim3\times10^8$ per ㎖, respectively. On the other hand, Von Rohloff (1973) reported that there were no differences of semen volume between 1 and 4 years of age in boars. There were significant between - breed differences in sperm production and generally the larger breeds. Such as the Yorkshire and Large White tended to produce a greater volume of semen per ejaculate and greater numbers of sperm cells over a period, although it is not clear how mature size affects sperm concentration. In this study, our results indicate that breed size affected semen volumes and sperm concentrations.

Comparison of sperm viability in miniature-pig and boars (Duroc and Yorkshire) semen is shown in Fig. 1. At Day 0, approximately 95% of the sperm were viable,

Table 1. Comparison of semen volumes and sperm concentrations in fresh semen of miniature-pig, Duroc and Yorkshire (mean±S.D)

	Semen Volume. (㎖)	Sperm concentrations (cells /㎖)
Duroc (n=13)	155 ± 25[a]	5.6×10^9 ± 1.8[a]
Yorkshire (n=9)	154 ± 23[a]	6.0×10^9 ± 1.1[a]
Miniature-pig (n=4)	62 ± 22[b]	2.5×10^7 ± 2.1[b]

[a,b] Values with different superscripts within the same column are significantly different ($p<0.05$).

but viability decreased to 64% at Day 3. Sperm viability was significantly ($p<0.05$) lower in miniature-pig than in Duroc and Yorkshire after Day 3 of storage. During the first 3 days of storage, however, viability did not differ significantly between Duroc and Yorkshire.

Comparison of sperm abnormality in miniature-pig and Boars (Duroc and Yorkshire) semen is shown in Fig. 2. There were 5, 7, 10, 15 and 15% for abnormality spermatozoa in miniature-pig on Day 0, 1, 3, 5 and 7 of storage. The percentage of sperm abnormality spermatozoa remained less than 10% during the frist 3 days of storage. During the storage of spermatozoa, sperm abnormality did not differ significantly among miniature-pig, Duroc and Yorkshire.

Comparison of sperm acrosome intactness in miniature-pig and boars (Duroc and Yorkshire) spermatozoa is shown in Fig. 3. There were 78, 75, 70, 65 and 58% for acrosome intactness spermatozoa in miniature-pig on Day 0, 1, 3, 5 and 7 of storages, and did not differ significantly among miniature-pig, Duroc and Yorkshire during the storage of spermatozoa.

Comparison of sperm intensity in miniature-pig and

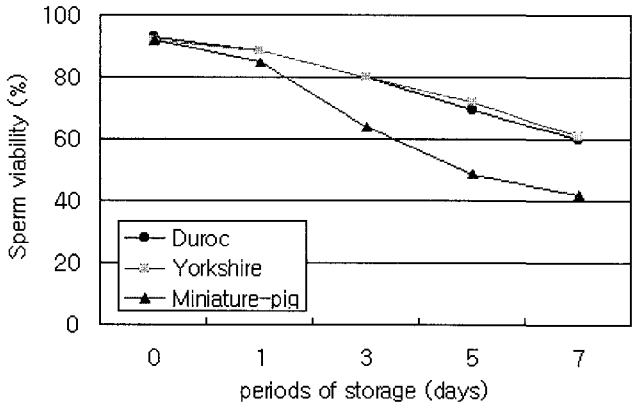

Fig. 1. Effect of storage periods on sperm viability in fresh semen in the pigs. [a,b] Values with different superscripts within the same column are significantly different ($p<0.05$).

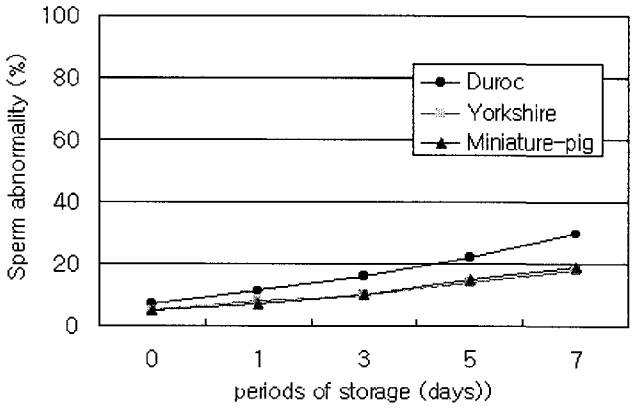

Fig. 2. Changes of sperm abnormality by storage periods of fresh semen in the pigs.

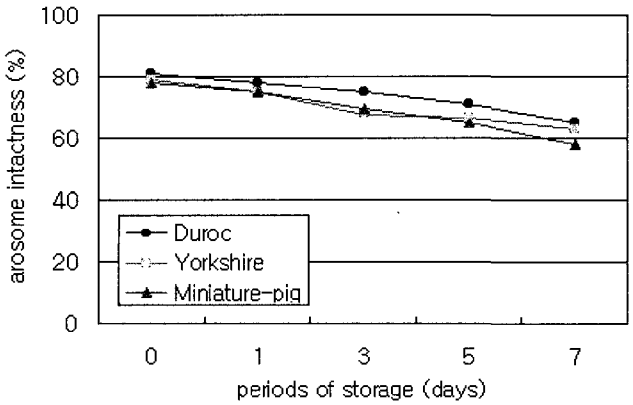

Fig. 3. Changes of crosome intactness by storage periods in the pigs.

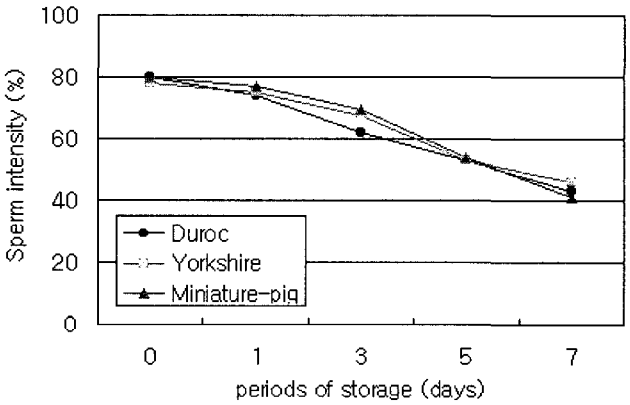

Fig. 4. Changes of sperm intensity by storage periods of fresh semen in the pigs.

Boars (Duroc and Yorkshire) spermatozoa is shown in Fig. 4. There were 80, 77, 70, 53 and 41% for intensity spermatozoa in miniature-pig on Day 0, 1, 3, 5 and 7 of storage. The percentage of intensity spermatozoa did not differ significantly from Day 0 to 7 of storage, but reached 79, 73, 62, 53 and 42% for Duroc, 80, 77, 67, 53 and 48% for Yorshire.

Comparison of sperm viability in frozen-thawed sperm among miniature-pig, Duroc and Yorkshire is shown in Fig. 5. Sperm viability was determined using Hoechst 33258 staining. The sperm viability (20%) of frozen-thawed sperm in miniature-pig was significantly ($p<0.05$) lower than in Duroc and Yorkshire. However, the viability did not differ between Duroc (51%) and Yorkshire (45%).

The slide of sample was prepared and 100 sperm per slide were counted according to the three different CTC staining patterns described by Wang et al. (1995) and Mattioli et al. (1995). Pattern F demonstrated a uniform fluorescence characterized by uncapacitated sperm, pattern B showed fluorescence only in the post acrosoma lregion (characteristic of capacitated sperm), and pattern AR sperm were very lightly fluorescent, except for a

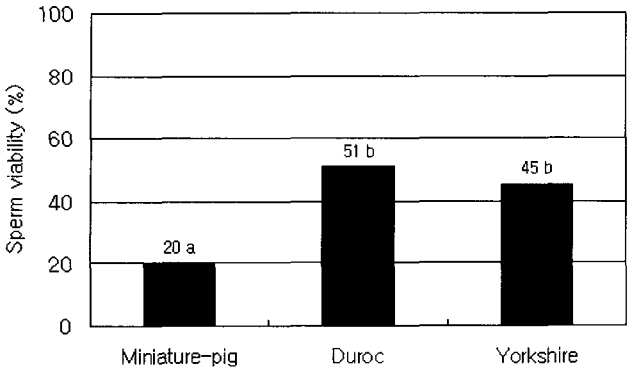

Fig. 5. Sperm viability of frozen-thawed semen in miniature-pig, Duroc and Yorkshire. ^{a,b} $p<0.05$.

band in the equatorial segment. Sperm characterized by pattern AR were considered to be acrosome reacted.

Capacitation and acrosome reaction were determined CTC pattern by CTC/Hoechst 33258 staining in frozen-thawed semen. The percentage of F patterns, corresponding to non-capacitated sperm, did not differ significantly among miniature-pig (18%), Duroc (15%) and Yorkshire (22%). The percentage of B patterns also did not differ significantly. However, F pattern in miniature-pig (44%) was slightly lower than that of Duroc (59%) and Yorkshire (47%) boars. The percentage of AR patterns in miniature-pigs was the highest, but did not differ significantly among miniature-pig, Duroc and Yorkshire (Fig. 6).

In conclusion, characteristics of semen extended did not remakable differences miniature-pig and boars. However, sperm viability of miniature-pig assessed by makler counting chamber staining was significantly lower after day 3 of storage than that of Duroc and Yorkshire. The viability in frozen-semen observed with low percentages in miniature-pig than in Duroc and Yorkshire. So, further research is necessary to develop of semen extender and freezing methods to improve sperm ability in miniature-pig.

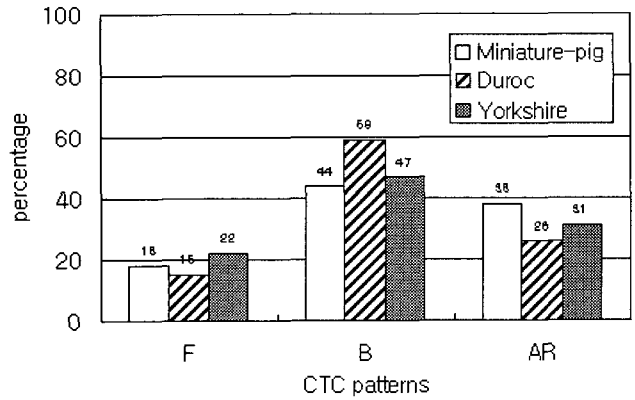

Fig. 6. CTC patterns of frozen-thawed spermatozoa in miniature-pig, Duroc and Yorkshire.

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