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Comparison of Motility, Acrosome, Viability and ATP of Boar Sperm with or without Cold Shock Resistance in Liquid Semen at 17°C and 4°C, and Frozen-thawed Semen

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ABSTRACT : This study was designed to analyze boar sperm to compare motility, acrosome morphology, viability and ATP by various preservation methods between Duroc boar A with cold shock resistance sperm and Duroc boar B with cold shock sensitivity sperm. Semen volume, sperm concentration, motility and normal acrosome between Duroc boar A and B did not show any differences within 2 h after collection. There were no differences in sperm motility and normal acrosome between boar A and B at 1 day of preservation at 17°C and 4°C, respectively. However, sperm motility and normal acrosome from 2 day of preservation at 17°C and 4°C, respectively, were higher for boar A than boar B. The frozen-thawed sperm motility and normal acrosome were higher for boar A than boar B. The sperm viability and ATP concentration according to storage period of liquid semen at 17°C and 4°C were higher for boar A than boar B. Also, the sperm viability and ATP concentration of frozen-thawed semen were higher for boar A than boar B. In conclusion, we found out that the original quality of boar semen with cold shock resistance sperm played an important role. (**Key Words :** Boar, Liquid Semen, Motility, Acrosome, ATP)

INTRODUCTION

The artificial insemination stations should have some indication of the fertilizing capacity of the semen and they need to monitor the performance of the boars prior to and during their service. For research on sperm physiology and semen preservation, it is also important to be able to study the viability of the sperm and their fertilizing ability *in vitro*. *In vitro* assessment methods pertain to criteria of the sperm that are believed to be relevant for the fertilizing ability of the sperm. Examples of such criteria are motility, acrosome integrity, membrane integrity, stress sensitivity, rate of metabolic fluxes, metabolite concentrations, and various parameters that relate to sperm-oocyte interaction. The simple visual estimation of sperm motility remains a useful tool for routine semen assessment for research purposes and in the artificial insemination industry. The percentage of cells with an intact acrosome is regarded as an important semen quality parameter (Pursel et al., 1972a). Also other features with respect to the acrosome and acrosome reaction are used to measure semen quality or to predict boar ability.

Freshly ejaculated boar sperm in the whole ejaculate will not survive even slow cooling below 15°C, but they acquire resistance to cooling stress upon incubation (Pursel and Park, 1985). Our understanding of these phenomena is growing as a result of studies of sperm from species both susceptible and resistant to cold shock. The organelles most frequently damaged by cold shock are the plasmal membrane, the acrosome and the mitochondria. The most obvious sign of cold shock injury to sperm is an irreversible loss of motility.

The selection of superior boars for use in artificial insemination programs using fresh, liquid and frozen semen requires use of objective, well-validated laboratory tests on large numbers of sperm (Oh et al., 2006). Such methods would also be valuable for assessing accurately the damage caused during various preservation procedures. Previous studies on qualitative assessment of preserved boar semen were based on sperm motility after storage. The acrosome was of vital importance for fertilization and boars with high numbers of abnormal acrosomes in the native semen were

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sterile or subfertile (Bane, 1961; Anderson, 1974). Evaluation of acrosome morphology has been widely used as a viability indicator in the development of freezing methods (Pursel and Johnson, 1971; Westendorf et al., 1975). Evaluation of sperm viability after staining with fluorescent dyes has become a valid assessment method during the past 10 years. SYBR-14 is most effective when used in conjunction with a counter-stain, such as propidium iodide (PI) or Hoechst 33258, that identifies dead sperm (Garner and Johnson, 1995). With the development of an automated bioluminescence procedure for measuring ATP concentration in sperm, Johnson et al. (1981) reported the ATP concentration in fresh and frozen boar sperm being used in a field trial designed to compare fertility in fresh and frozen semen.

In this study, we analyzed boar sperm to compare sperm motility, acrosome morphology, viability and ATP by various preservation methods between Duroc boar A with cold shock resistance sperm and Duroc boar B with cold shock sensitivity sperm.

MATERIALS AND METHODS

Semen collection

Semen was collected from August 2005 to December 2006 from adult boars 15-22 months of age. Boars were housed at pig farm of Chungnam National University in Daejeon. Semen was collected one time per week from each of two Duroc boars with or without cold shock resistance. The sperm with greater than 70% motility and normal acrosome at 2 day preservation of liquid semen at 4°C were defined as cold shock resistance boar (Boar A) and the sperm with lower than 60% motility and normal acrosome at 2 day preservation of liquid semen at 4°C were defined as cold shock sensitivity boar (Boar B). The sperm-rich fraction of ejaculate was collected into an insulated vacuum bottle. The sperm-rich fractions of ejaculates with greater than 85% motile sperm and normal acrosome were used.

Determination of semen volume, sperm concentration, motility and acrosome

Semen volumes were determined with a graduated cylinder. Sperm concentrations were estimated by a hemocytometer. The percentage of motile sperm was estimated at 38.5°C by light microscope at 250× (Pursel and Park, 1985). Sperm were fixed with 1% glutaraldehyde in Beltsville thawing solution (BTS; 3.71 g glucose, 0.60 g tri-sodium citrate, 1.25 g ethylenediamine tetraacetic acid, 1.25 g sodium bicarbonate, 0.75 g potassium chloride and 100.0 ml distilled water) to examine acrosome morphology. One hundred sperm per sample were evaluated by phase contrast microscopy at 1,000×. Acrosomes were differentially categorized into four morphological classes: normal apical ridge (NAR), damaged apical ridge (DAR), missing apical ridge (MAR) and loose acrosomal cap (LAC) as described by Pursel et al. (1972a). The sperm-rich fractions of ejaculates with >85% motile sperm and normal apical ridge (NAR) acrosome were used in the experiments.

Short term liquid semen processing

Semen was slowly cooled to room temperature (20 to 23°C) by 2 h after collection. Semen was diluted with BTS diluent to a final concentration of 35×10^6 sperm/ml in 100 ml of BTS diluent. The diluted semen was stored in Cool&Warm box (Micom Control System, Cason, Korea) at 17°C for 4 days.

Long term liquid semen processing

Semen was slowly cooled to room temperature (20 to 23°C) by 2 h after collection. Semen was transferred into 15 ml tubes, centrifuged at room temperature for 10 min at 800×g, and supernatant solution was poured off. The concentrated sperm was resuspended with 10 ml of LEN (11.0 g lactose hydrate, 20.0 ml egg yolk, 0.05 g N-acetyl-D-glucosamine and 100.0 ml distilled water) diluent to provide 1.0×10^9 sperm/ml at room temperature. The resuspended semen was cooled in a refrigerator to 4°C and preserved for 5 days (Park et al., 2004).

Frozen semen processing

The sperm-rich fraction of ejaculate was collected into an insulated vacuum bottle. Semen was slowly cooled to room temperature (20 to 23°C) by 2 h after collection. Semen was transferred into 15 ml tubes, centrifuged at room temperature for 10 min at 800×g and the supernatant solution was poured off. One volume of concentrated sperm was resuspended with 1 volume of lactose, egg yolk and N-acetyl-D-glucosamine (LEN) diluent (the first diluent to provide 1.0×10^9 sperm/ml) at room temperature (Yi et al., 2002).

Semen was cooled in a refrigerator to 4°C over 2 h periods and 1 volume a LEN+4% glycerol diluent (the second diluent) was added to 1 volume of cooled semen. Straws (Minitüb Gmbh, Landshut, Germany) were immediately filled with 5 ml of semen and steel or glass balls were used to seal the ends of the straws. The air bubble was adjusted to the center of the straws and the straws were horizontally placed on an aluminum rack and set into a liquid nitrogen tank containing liquid nitrogen (LN). The straws were situated 5 cm above the LN, and kept at that level for 20 min before the straws were transferred into LN storage. Straws were thawed in 52°C water bath for 40 sec, which brings the temperature of the sperm to 15°C.

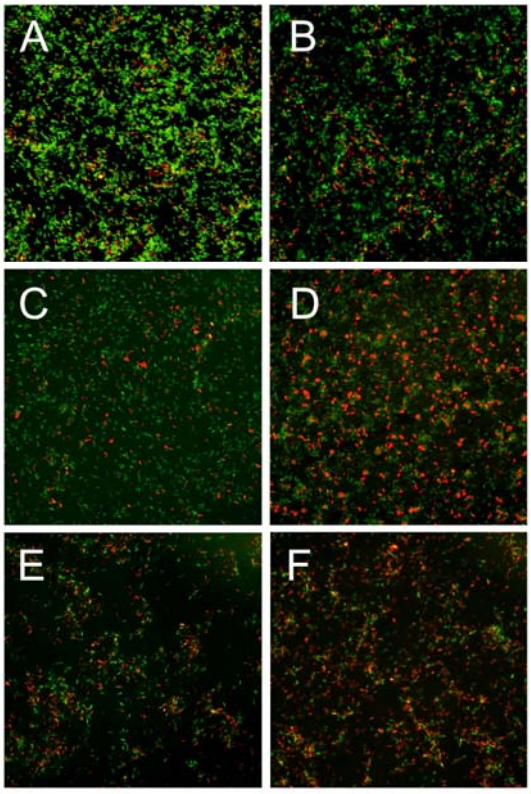

Figure 1. Sperm were fluorescently stained through use of a combination of SYBR-14 and PI. A: Liquid semen of Duroc boar A at 17°C, B: Liquid semen of Duroc boar B at 17°C, C: Liquid semen of Duroc boar A at 4°C, D: Liquid semen of Duroc boar B at 4°C, E: Frozen-thawed sperm of Duroc boar A, and F: Frozen-thawed sperm of Duroc boar B. Liquid and frozen-thawed sperm were stained at 2 day of preservation after thawing, respectively.

Fluorescent staining to assess sperm viability

Sperm viability was assessed using SYBR-14 and propidium iodide (PI) kit according to the manufacturer's protocol (Live/Dead Sperm Viability Kit, Molecular Probes, OR, USA). One μl SYBR-14 of 1 mM solution in dimethylsulphoxide (DMSO) was diluted with 49 μl HEPES-buffered saline solution (10 mM HEPES, 150 mM NaCl, pH 7.4) containing 10% bovine serum albumin (A8022, Sigma, USA). Five μl SYBR-14 was added into 1 ml sperm samples containing 2×10^7 sperm/ml in HEPES-buffered saline solution. Sperm samples were incubated for 10 min at 38.5°C. After then, 5 μl PI was added into the samples and sperm were incubated for 10 min at 38.5°C. Stained sperm cells were placed on microscope slides and covered with coverslips. One hundred sperm cells per slide were examined in random fields, using an epifluorescence microscope (Olympus BX51, Korea) equipped with blue and green excitations for SYBR-14 and PI, respectively. The nucleus of the SYBR-14-stained cells fluoresced bright green while the dead sperm nuclei exhibited red fluorescence (PI). The fluorescent staining of sperm was monitored and photographed by DP controller (Olympus) equipped with fluorescent image analyzer (Olympus DP70; Figure 1).

Measurement of adenosine triphosphate (ATP)

Semen was adjusted to concentration of 2×10^7 sperm and washed with 10 ml HEPES-buffered Tyrode lactate medium containing 0.1% (w/v) polyvinyl alcohol (PVA) at room temperature, twice (120×g, 10 min). After washing, the supernatant were discarded and resuspended with buffer (50 mM Tricine, 10 mM $MgSO_4$, 2 mM EDTA, pH 7.8) (Ford and Leach, 1998). The buffers were heated for 10 min before the samples were added. Sperm samples were boiled in the water bath for 3 min at 100°C, transferred to an ice bath, and centrifuged at 5,000×g for 30 min at 4°C. The supernatant was used for determination of total adenosine triphosphate (ATP). ATP was determined using a luciferase reaction kit according to the manufacturer's protocol (Enliten® ATP Assay System, Promega, Madison, WI, USA). Standards were prepared from ATP standard (F203A, Promega) using serial dilutions to obtain concentrations of 1×10^{-7}, 5×10^{-8}, 1×10^{-8}, 5×10^{-9}, 1×10^{-9}, 5×10^{-10} and 1×10^{-10}. Aliquots of the ATP stock solution were stored at -20°C until use and standard curve dilutions were prepared for each assay. Bioluminescence was measured with a HTS multi label reader (Perkin Elmer Inc., Boston, MA, USA) after addition of 50 μl sample and 50 μl luciferin-luciferase reagent.

Experimental design

Experiment 1 was carried out to investigate semen characteristics of sperm rich fractions of Duroc boar A and B.

Experiment 2 was conducted to investigate sperm motility, NAR acrosome, sperm viability and ATP according to storage period of liquid semen at 17°C. Diluent was based on BTS diluent and the liquid semen was stored for 4 days at 17°C.

Experiment 3 was carried out to examine sperm motility, NAR acrosome, sperm viability and ATP according to storage period of liquid semen at 4°C. Diluent was based on LEN diluent and the liquid semen was stored for 5 days at 4°C.

Experiment 4 was performed to examine post-thawed sperm motility, NAR acrosome, sperm viability and ATP. Diluent was based on LEN diluent. The 5 ml maxi-straws were stored in liquid nitrogen at -196°C. Straws were thawed in 52°C water bath for 40 sec, and diluted with BTS at room temperature.

For determination of sperm motility, a test tube containing 5 ml of semen at room temperature was warmed up to 38.5°C in a water bath for 30 min.

Table 1. Comparison of Duroc boar A and B on semen characteristics of sperm rich fractions

Duroc boar	Volume of sperm rich fraction (ml)[1]	Sperm concentration ($\times10^8$/ml)[1]	Motility (%)[1]	Normal acrosome (%)[1]
A[2]	58.2±9.3	9.3±1.3	91.0±1.0	94.4±2.6
B[2]	55.2±7.3	11.7±2.6	89.0±2.5	92.2±2.5

[1] Means±SE for six ejaculates from Duroc boar A and B, respectively.
[2] A: cold shock resistance boar, B: cold shock sensitivity boar.

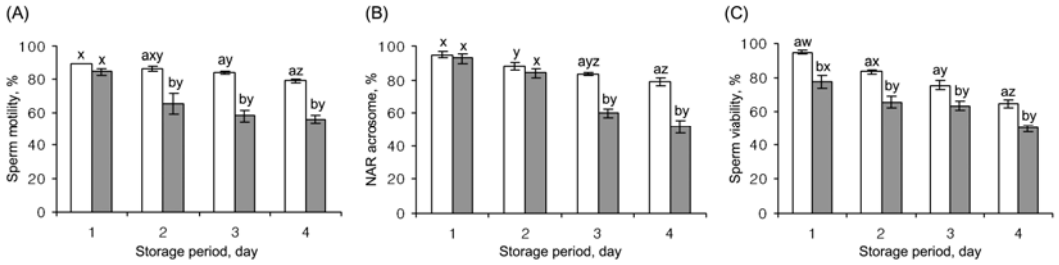

Figure 2. Comparison of Duroc boar A (□) and B (■) on sperm motility (A), NAR acrosome (B) and sperm viability (C) according to storage period of liquid semen at 17°C. Experiments were repeated 6 times. Graph showing mean percentages±SE. Superscripts a and b in figures denote a significant difference at $p<0.05$ between Duroc boar A and B. Superscripts w, x, y and z in figures denote a significant difference at $p<0.05$ among storage periods in boar A and B, respectively.

Statistical analysis

Analyses of variance (ANOVA) were carried out using the SAS package (Cary, NC) in a completely randomized design. Duncan's multiple range test and Student's t-test were used to compare mean values of individual treatment, when the F-value was significant ($p<0.05$).

RESULTS

Comparison of Duroc boar A and B on semen characteristics

Semen volume, sperm concentration, motility and normal acrosome between Duroc boar A with cold shock resistance sperm and Duroc boar B with cold sensitivity sperm did not show any differences as shown in Table 1.

Comparison of Duroc boar A and B on sperm motility, NAR acrosome, sperm viability and ATP according to storage period of liquid semen at 17°C

As shown in Figure 2A, there was no difference on sperm motility between Duroc boar A and B at 1 day of preservation at 17°C. But sperm motility showed differences between Duroc boar A and B from 2 to 4 day of preservation. The sperm of boar B with cold shock sensitivity showed more rapid reduction of motility than that of boar A with cold shock resistance from 2 day of preservation period. As shown in Figure 2B, there were no differences on the percentages of NAR acrosome between boar A and B from 1 to 2 day of preservation at 17°C. However, the percentages of NAR acrosome showed differences between boar A and B from 3 to 4 day of preservation. The destruction of acrosomes of boar B with cold shock sensitivity was higher than that of boar A with cold shock resistance from 3 day of preservation period. The sperm viability according to storage period of liquid semen at 17°C using SYBR-14 and PI showed significant differences between boar A with cold shock resistance sperm and boar B with cold shock sensitivity sperm as shown in Figure 2C. Sperm ATP concentration according to storage period of liquid semen at 17°C was higher on boar A than on boar B (Table 2).

Comparison of Duroc boar A and B on sperm motility, NAR acrosome, sperm viability and ATP according to storage period of liquid semen at 4°C

As shown in Figure 3A, there was no difference on sperm motility between boar A and B at 1 day of preservation at 4°C. But sperm motility showed differences

Table 2. Comparison of Duroc boar A and B on sperm ATP ($\mu M/2\times10^7$ sperm) according to storage period of liquid semen at 17°C

Duroc boar	Storage period (day)[1]			
	1	2	3	4
A[2]	2.26 ± 0.01^{aw}	0.99 ± 0.01^{ax}	0.81 ± 0.11^{ay}	0.74 ± 0.01^{az}
B[2]	1.57 ± 0.01^{bw}	0.48 ± 0.01^{bx}	0.20 ± 0.00^{by}	0.11 ± 0.00^{bz}

[1] Experiments were repeated 6 times. [2] A: cold shock resistance boar, B: cold shock sensitivity boar.
[a, b] Means±SE in the same column with different letters differ significantly ($p<0.05$).
[w, x, y, z] Means±SE in the same row with different letters differ significantly ($p<0.05$).

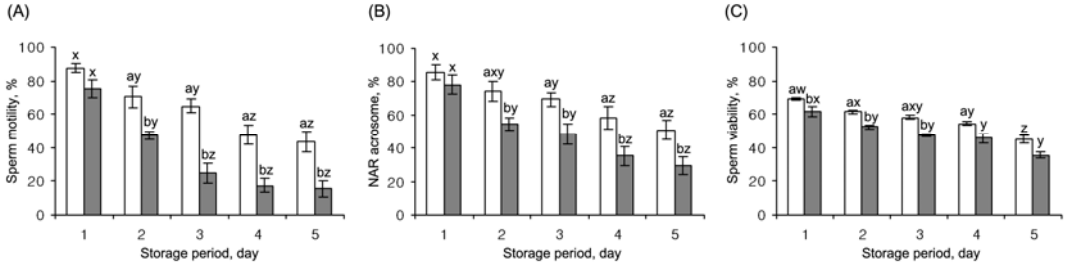

Figure 3. Comparison of Duroc boar A (□) and B (■) on sperm motility (A), NAR acrosome (B) and sperm viability (C) according to storage period of liquid semen at 4°C. Experiments were repeated 6 times. Graph showing mean percentages±SE. Superscripts a and b in figures denote a significant difference at p<0.05 between Duroc boar A and B. Superscripts w, x, y and z in figures denote a significant difference at p<0.05 among storage periods in boar A and B, respectively.

Table 3. Comparison of Duroc boar A and B on sperm ATP (μM/2×10^7 sperm) according to storage period of liquid semen at 4°C

Duroc boar	Storage period (day)[1]				
	1	2	3	4	5
A[2]	2.26±0.01^{ax}	1.30±0.02^{ay}	1.01±0.03^{ayz}	0.88±0.03^{az}	0.60±0.01^{az}
B[2]	1.89±0.04^{bw}	0.85±0.05^{bx}	0.74±0.00^{bxy}	0.45±0.00^{byz}	0.42±0.01^{bz}

[1] Experiments were repeated 6 times. [2] A: cold shock resistance boar, B: cold shock sensitivity boar.
[a, b] Means±SE in the same column with different letters differ significantly (p<0.05).
[w, x, y, z] Means±SE in the same row with different letters differ significantly (p<0.05).

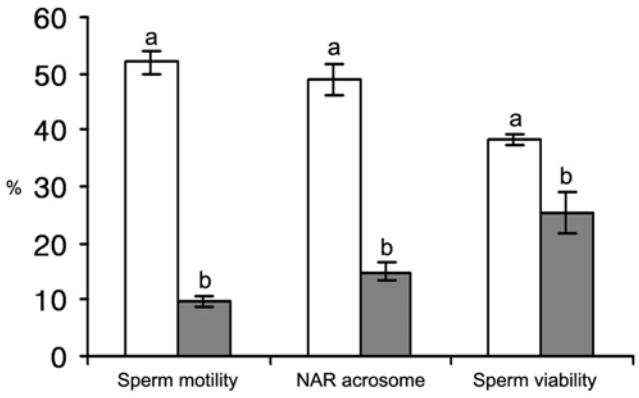

Figure 4. Comparison of Duroc boar A (□) and B (■) on frozen-thawed sperm motility, NAR acrosome and sperm viability in 5 ml maxi-straw. Experiments were repeated 6 times. Graph showing mean percentages±SE. Superscripts a and b in figures denote a significant difference at p<0.05 between Duroc boar A and B.

between boar A and B from 2 to 5 day of preservation. Especially the reduction of motility was significant from 2 day of preservation on boar B with cold shock sensitivity sperm. As shown in Figure 3B, the percentages of NAR acrosome were not different between boar A and B at 1 day of preservation at 4°C. However, the percentages of NAR acrosome showed differences between boar A and B from 2 to 5 day of preservation. Especially the percentages of NAR acrosome of boar B with cold shock sensitivity sperm was significantly declined from 2 day of preservation. As shown in Figure 3C, the significant differences between boar A and B were found on sperm viability according to storage period of liquid semen at 4°C using SYBR-14 and PI. Sperm ATP concentration according to storage period of liquid semen at 4°C was higher on boar A than on boar B (Table 3).

Table 4. Comparison of Duroc boar A and B on ATP of frozen-thawed sperm

Duroc boar	Sperm ATP (μM/2×10^7 sperm)[1]
A[2]	0.85±0.11^{a}
B[2]	0.49±0.38^{b}

[1] Experiments were repeated 6 times.
[2] A: cold shock resistance boar, B: cold shock sensitivity boar.
[a, b] Means±SE in the same column with different letters differ significantly (p<0.05).

Comparison of Duroc boar A and B on frozen-thawed sperm motility, NAR acrosome, sperm viability and ATP in 5 ml maxi-straw

The frozen-thawed sperm motility, NAR acrosome and sperm viability showed significant differences between boar A with cold shock resistance sperm and boar B with cold shock sensitivity sperm as shown in Figure 4. Sperm ATP concentration of frozen-thawed semen was higher on boar A with cold shock resistance sperm than on boar B with cold shock sensitivity sperm (Table 4).

DISCUSSION

The classical microscopic measurements have been used for the evaluation of motility and acrosome integrity. This method, which is an indirect method of assessing metabolic activity, is time consuming. However, most commercial semen-processing organizations routinely use this assessment to provide the initial assessment of semen quality. The new membrane permeant nuclear stain, SYBR-

14, which brightly fluoresces the nuclei of living cells, has been used in combination with PI to determine the proportion of living sperm in semen from several different mammals and has been proven effective for assessing sperm viability (Garner et al., 1994; Garner and Johnson, 1995). The chemical energy required for sperm motility is supplied by the mitochondria in the form of adenosine triphosphate (ATP). The firefly luciferin-luciferase assay has been used to quantity ATP content in sperm from a wide variety of species, including boar (Aalbers et al., 1985), bull (Guminska et al., 1997) and human (Mendeluk et al., 1997).

In this study, we compared Duroc boar A with cold shock resistance sperm and Duroc boar B with cold shock sensitivity sperm. The sperm with greater than 70% motility and normal acrosome at 2 day preservation of liquid semen at 4°C were defined as cold shock resistance boar. The sperm with lower than 60% motility and normal acrosome at 2 day preservation of liquid semen at 4°C were defined as cold shock sensitivity boar. We analyzed fresh semen, liquid semen at 17°C and 4°C, and frozen-thawed semen to compare the motility, normal acrosome, viability, and sperm ATP between Duroc boar A and B. The semen volume, sperm concentration, motility and normal acrosome between Duroc boar A and B within 2 h after collection did not show any differences. Krider et al. (1982) reported that the semen volume of sperm-rich fraction, sperm concentration, motile sperm and morphologically normal sperm were 30-60 ml, $3\text{-}6\times10^9$ sperm/per ejaculate, 70% and 80%, respectively. In this study, the above characteristics were a little higher than those of average ejaculates in the report of Krider et al. (1982). It may be that sample size of boar, breed effect and management affected the characteristics.

Beltsville thawing solution (Pursel and Johnson, 1975) is a fairly simple diluent that is widely used for storage of boar semen for up to 3 days. De Ambrogi et al. (2006) reported that three different commercial diluents devised for short-term (BTS+) or long-term preservation (MR-A and X-Cell), were used to test whether storage of semen from four mature, fertile boars at 17°C for 96 h would affect sperm characteristics, such as motility, membrane integrity and chromatin stability. A significant decrease in motility was detected from 72 h for MR-A and BTS+ extended semen and at 96 h, also for X-Cell-extended semen. Johnson et al. (2000) set a minimum of 60% motility to give optimal fertility. In this study, this level was continued by 4 day of preservation in the boar A with cold shock resistance sperm but reached by 3 day of preservation in the boar B with cold shock sensitivity sperm. Special components have been added to boar semen diluents in an effort to prevent or retard unwanted alterations in the structure and function of plasma membranes. These components are bovine serum albumin (BSA), butylated hydroxytoluene (BHT), polyvinyl pyrrolidone (PVP-40), polyvinyl alcohol and egg yolk. It has been suggested that BSA prolongs sperm motility by replacing or maintaining the sperm cell's lipoprotein coating (Bredderman and Foote, 1971).

Immediate sperm motility has been considered as the major criterion for the quality of post thawing sperm; however, this test must be considered with reservation since Pursel et al. (1972b) showed that highly motile sperm with damaged acrosomes lost their fertilizing ability. Thus, integrity of the sperm acrosome deduced from phase contrast microscopy has been used in a number of investigations as the main laboratory test for thawed boar semen (Pursel and Johnson, 1975; Westendorf et al., 1975).

In most cases the percentage of motile sperm after freezing and thawing is less than 50%. In field fertility tests, frozen ejaculate with less than 15% (Paquignon et al., 1980) or less than 35% (Johnson et al., 1981) progressively motile sperm have been discarded. In a field trial with the Beltsville freezing method, ejaculates with a minimum of 45% NAR acrosomes after thawing were accepted (Johnson et al., 1981) but acceptable fertility has also been achieved with considerably lower percentages of NAR sperm (Schuler et al., 1979). In this study, the frozen-thawed sperm motility and normal acrosome showed significant differences between boar A with cold shock resistance sperm and boar B with cold shock sensitivity sperm. The above results mean that the original quality with cold shock resistance of the boar's semen plays a considerable role.

The combination of SYBR-14 and PI effectively identified the living and dead sperm population in semen from bulls, boars, rams, rabbits, mice and men (Garner and Johnson, 1995). The living sperm, which stained green with SYBR-14, and the dead sperm, which stained red with PI, were examined through use of fluorescence microscopy. In this study, the boar A with cold shock resistance sperm showed higher sperm viability than the boar B with cold shock sensitivity sperm in the liquid semen of 17°C or 4°C and frozen sperm. The fluorescent patterns of the microscopic examinations were markedly similar in their staining patterns among the preservation conditions. The proportions of living and dead sperm in boar semen were readily identified through use of dual staining with SYBR-14 and PI and quantified through microscopic examination.

The mitochondria of mammalian sperm are restricted to the flagellar midpiece. They wrap tightly in a helical fashion around the axoneme and outer dense fibers of the flagellum and can therefore provide ATP to the dynein ATPase in the midpiece axoneme (Fawcett, 1975; Phillips, 1977). Decrease in intracellular ATP concentration has also been associated with the appearance of hyperactivated motility in rat sperm (Jeulin and Soufir, 1992). In contrast,

ATP concentration was 2-fold higher in hyperactivated than nonhyperactivated guinea-pig sperm (Mujica et al., 1994). However, glucose was present in the medium and therefore the additional ATP could have been produced by glycolysis instead of oxidative phosphorylation. Dilution before measurement may have increased the ATP content of the sperm (Kahn, 1981; Velez Cuevas, 1982). In this study, frozen-thawed semen gave significantly lower ATP values compared to fresh semen and liquid semen at 1 day of preservation at 17°C and 4°C. This agrees with results obtained for bull semen (Foulkes and MacDonald, 1979; Velez Cuevas, 1982). Differences in environment among fresh, liquid and frozen semen may have influenced the ATP content of the sperm. Storage periods of the liquid semen at 17°C and 4°C reduced the ATP content. The ATP contents were higher on boar A with cold shock resistance sperm than on boar B with cold shock sensitivity sperm.

It can be concluded that the classical microscopic measurements, fluorescent staining and firefly luciferin-luciferases assay can be used for assessments of fresh, liquid and frozen-thawed boar semen. Also, it was important that boars with cold shock resistance sperm were assessed for use in artificial insemination programs using liquid and frozen semen.

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REFERENCES

Aalbers, G. J., L. A. Johnson, E. A. Aalbers-Smit and J. H. M. Rademaker. 1985. ATP content of fresh and frozen-thawed boar semen and its relationship to sperm concentration and fertility. In: Deep freezing of boar semen (Ed. L. A. Johnson and K. Larsson), Swedish University of Agricultural Science, Uppsala, pp. 259-264.

Andersen, K. 1974. Morphological abonormalities in the acrosome and nucleus of boar spermatozoa. Nord. Vet. Med. 26:215-218.

Bane, A. 1961. Acrosomal abnormality associated with sterility in boar. In: IVth International Congress of Animal Reproduction, Hague, The Netherlands, 4:810-817.

Bredderman, P. J. and R. H. Foote. 1971. Factors stabilizing bull sperm cell volume and prolonging motility at high dilution. Exp. Cell Res. 66:458-464.

De Ambrogi, M., J. Ballester, F. Saravia, I. Caballero, A. Johannisson, M. Wallgren, M. Andersson and H. Rodriguez-Martinez. 2006. Effect of storage in short- and long-term commercial semen extenders on the motility, plasma membrane and chromatin integrity of boar spermatozoa. Int. J. Androl. 29(5):543-552.

Fawcett, D. W. 1975. The mammalian spermatozoon. Dev. Biol. 44:394-436.

Ford, S. R. and F. R. Leach. 1998. Bioluminescent assay of the adenylate energy charge. Methods Mol. Biol. 102:69-81.

Foulkes, J. A. and B. J. MacDonald. 1979. The relationship between ATP content and motility of bovine spermatozoa. Theriogenol. 11:313-319.

Garner, D. L., L. A. Johnson, S. T. Yue, B. L. Roth and R. P. Haugland. 1994. Dual DNA staining assessment of bovine sperm viability using SYBR-14 and propidium iodide. J. Androl. 16:620-629.

Garner, D. L. and L. A. Johnson. 1995. Viability assessment of mammalian sperm using SYBR-14 and propidium iodide. Biol. Reprod. 53:276-284.

Guminska, M., T. Kedryna, A. Laszcska, M. Godlewski and B. Szczesniak-Fabianczyk. 1997. Changes in ATP level and iron-induced ultra-weak photon emission in bull spermatozoa, caused by membrane peroxidation during thermal stress. Acta Biochim. Pol. 44:131-138.

Jeulin, C. and J-C. Soufir. 1992. Reversible intracellular ATP changes in intact rat spermatozoa and effects on flagellar sperm movement. Cell Motil. Cytoskeleton 21:201-222.

Johnson, L. A., J. G. Aalbers, C. M. T. Willems and W. Sybesma. 1981. Use of boar spermatozoa for artificial insemination. I. Fertilizing capacity of fresh and frozen spermatozoa in sows on 36 farms. J. Anim. Sci. 52:1130-1136.

Johnson, L. A., K. F. Weitze, P. Fiser and W. M. C. Maxwell. 2000. Storage of boar semen. Anim. Reprod. Sci. 62:143-172.

Kahn, W. 1981. Zur ATP- und AMP-Bestimmung mit dem Biolumineszenzverfahren im Sperma einiger Haustiere und des Menschen. Vet. Med. Diss. Munchen.

Krider, J. L., J. H. Conrad and W. E. Carroll. 1982. Swine Production. New York: McGraw-Hill Publications, pp. 179-182.

Mendeluk, G. R., M. J. Munuce, C. Carizza, M. Sardi and C. Bregni. 1997. Sperm motility and ATP content in seminal byperviscosity. Arch Androl. 39:223-227.

Mujica, A., L. Neri-Bazan, J. S. Tash and S. Uribe. 1994. Mechanism for procaine mediated hyperactivated motility in guinea pig spermatozoa. Mol. Reprod. Dev. 38:285-292.

Oh, S. H., M. T. See, T. E. Long and J. M. Galvin. 2006. Estimates of genetic correlations between production and semen traits in boar. Asian-Aust. J. Anim. Sci. 19(2):160-164.

Park, C. S., M. Y. Kim, Y. J. Yi, Y. J. Chang, S. H. Lee, J. J. Lee, M. C. Kim and D. I. Jin. 2004. Liquid boar sperm quality during storage, and *in vitro* fertilization and culture of pig oocytes. Asian-Aust. J. Anim. Sci. 17(10):1369-1373.

Paquignon, M., J. Bussière, F. Bariteau and M. Courot. 1980. Effectiveness of frozen boar semen under practical conditions of artificial insemination. Theriogenol. 14:217-226.

Phillips, D. M. 1977. Mitochondrial disposition in mammalian spermatozoa. J. Ultrastruct. Res. 2:144-154.

Pursel, V. G. and L. A. Johnson. 1971. Procedure for the preservation of boar spermatozoa by freezing. US Dept. Agric. ARS, pp. 44-227.

Pursel, V. G., L. A. Johnson and G. B. Rampacek. 1972a. Acrosome morphology of boar spermatozoa incubated before cold shock. J. Anim. Sci. 34:278-283.

Pursel, V. G., L. A. Johnson and L. L. Schulman. 1972b. Loss of boar sperm fertilizing capacity associated with altered acrosome morphology during *in vitro* storage. In: VIIth

International Congress Animal Reproduction and AI, Munich, Germany, II, pp. 1525-1600.

Pursel, V. G. and L. A. Johnson. 1975. Freezing of boar spermatozoa: fertilizing capacity with concentrated semen and a new thawing procedure. J. Anim. Sci. 40:99-102.

Pursel, V. G. and C. S. Park. 1985. Freezing and thawing procedures for boar spermatozoa. In: (Ed. L. A. Johnson and K. Lasson), Deep Freezing of Boar Semen. Uppsala, Sweden, Swedish University of Agricultural Science, pp. 147-166.

Schuler, D., U. Küpfer and F. Zimmerman. 1979. Deep freezing of boar semen: A comparison of two freezing methods. Schweiz Arch Tierheilk 121:173-178.

Velez Cuevas, G. 1982. Untersuchungen uber die Eignung des ATP- ADP- und AMP-Nachweises mit der Biolumineszenz-methode zur Bullen-spermien. Vet. Med. Diss. Munchen.

Westendorf, P., L. Richter and H. Treu. 1975. Zur Tiefgerierung von Ebersperma Labor- und Besamungsergebnisse mit dem Hüsenberger Pailletten-Verfahren. Dtsch. Tierarztl. Wschr. 82:261-300.

Yi, Y. J., Y. A. Kwon, H. J. Ko and C. S. Park. 2002. Effects of diluent component, freezing rate, thawing time and thawing temperature on acrosome morphology and motility of frozen-thawed boar sperm. Asian-Aust. J. Anim. Sci. 15(11):1553-1558.