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# Effect of magnetized extender on sperm membrane integrity and development of oocytes *in vitro* fertilized with liquid storage boar semen

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ABSTRACT

The objective of this study was to evaluate the effect of a magnetized extender on sperm membrane damage and development of oocytes *in vitro* fertilized with liquid storage boar semen. Before semen dilution, extender was flowed through a neodymium magnet (0, 2000, 4000 and 6000 G) for 5 min and collected semen was preserved for 168 h at 18 °C. In results, plasma membrane integrity with live sperm was significantly higher in semen treated with extenders magnetized at 4000 G than sperm treated with extenders magnetized at 0 G during semen preservation for 120–168 h ($p < 0.05$). In addition, acrosomal membrane damage was significantly lower in semen treated with extenders magnetized at 4000 and 6000 G compared to 0 and 2000 G during semen preservation for 168 h ($p < 0.05$). And mitochondrial membrane damage with all sperm was significantly lower in semen treated with extenders magnetized at 2000 G than other groups during semen preservation for 168 h. The ability of semen to achieve successful *in vitro* fertilization was also not significantly different among the groups during preservation. However, when the semen was preserved for 168 h, the blastocyst formation rates were significantly higher at 6000 G compared to 0 and 2000 G ($p < 0.05$). In conclusion, these results suggest that highly magnetized semen extender could protect the sperm membrane from damage, and improve the ability of rates of *in vitro* blastocyst development and magnetized semen diluter is beneficial for long liquid preservation of boar semen.



## 1. Introduction

Artificial insemination (AI) is widely used by livestock producers worldwide and is a very economically important technique for this industry. Particularly in the swine livestock industry, this method has been used with great success as pigs produce a lot of piglets compared to other domestic animals (Roca et al., 2011).

Typically, boars produce more semen than other animals with amounts ranging from 200 to 500 ml (Johnson et al., 2000). Moreover, boar sperm was successfully used for AI within 72 h, with the possibility of liquid preservation for 14 days (Spinaci et al., 2010; Waberski et al., 2011). Boar sperm can be diluted with semen extender that have to preserve long time no exchange semen extender (Boe-Hansen et al., 2005).

During the liquid preservation of the boar semen, damage to plasma membrane, acrosomal membrane, mitochondrial membrane, and DNA occurs, resulting in

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gradually decreasing fertility rates of AI and *in vitro* fertilization following long time storage (Silva and Gadella, 2006). Therefore, ideal conditions for long-term preservation of boar semen were extensively researched (Funahashi and Sano, 2005; Lee et al., 2010, 2011).

The main component of semen extender is water, as cells of all aerobic organisms contain a lot of water. Magnetized water has been studied extensively and used in many industrial applications due to its unique characteristics, including better electron donor abilities, high hydrogen-bonding, increased electric conduction, formation of smaller ice crystals, reduction of oxides ($SiO_2$, $Fe_2O_3$, CaO, MgO, $SO_3$, $Na_2O$, $K_2O$, CuO, $Mn_2O_3$, ZnO and $CO_2$), and decreased corrosion in pipes (Chang and Weng, 2008; Dontas et al., 2011; Szkatula et al., 2002; Tigrek and Barnes, 2010; Zhou et al., 2000). However, limited biological applications of magnetized water have been published to date (Szkatula et al., 2002). From a biological function perspective, magnetized water has a great potential in biotechnology: high electron donor ability may be expected to possibly remove of free radicals and act as an antioxidant; formation of stabilizing cluster may lead to smaller ice crystals and improve cryo-preservation of live cells; increased absorption and permeability into cells may be beneficial in cell culture applications.

Many factors influence sperm membrane damage after ejaculation and during storage, including temperature, time, and reactive oxygen species. In order to achieve successful fertilization, it is necessary to maintain a high quality of sperm before their use in AI and *in vitro* fertilization (IVF) (Silva and Gadella, 2006). Based on its characteristics described above, we hypothesized that magnetized water will protect the sperm cell membrane during liquid storage, thus increasing its IVF ability.

Physical characteristic of magnetized water is extensively studied by many research which applied already for industry using the water characteristic. There are some research effect of magnetized water on physiological functions in organisms (Jin et al., 1994). However, magnetized water is rare researched using mammal cells. So it is need to investigate magnetized extender for biological function in mammal cells.

Therefore, this study investigated the effect of magnetized extender on sperm membrane damage, storage time, and fertility during liquid preservation in boar semen. The optimum magnetization of the semen extender was also identified.

## 2. Materials and methods

### 2.1. Semen collection and magnetization of semen extender

All procedures that involved the use of animals were approved by the Kangwon National University Institutional Animal Care and Use Committee (KIACUC-09-0139). For the experiments five ejaculates were used from each of ten boars, age of the experimental ten boars is $25.6 \pm 4.2$ months that were terminal crosses of Duroc (Gumbo, Wonju, Korea) were used semen in the study. The semen collected by gloved-hand method once a week and filtered through gauze to remove gel particles. Boar semen samples were transferred to the laboratory, which was diluted with pre-warmed (35 °C) magnetized Modena B (glucose 30.0 g/l, EDTA 2.25 g/l, sodium citrate 2.50 g/l, sodium bicarbonate 1.00 g/l, tris 5.00 g/l, citric acid 2.50 g/l, cysteine 0.05 g/l, gentamicin sulfate 0.30 g/l). Samples from the fresh semen were evaluated for viability and morphology of the spermatozoa as described by Lee et al. (2011). Ejaculated sperm was used with more than 70% plasma membrane integrity and 20% acrosome membrane damage.

### 2.2. Magnetization of semen extender

Magnet equipment was manufactured to make magnetized semen extender which was consisted of neodymium magnet (Supplementary Fig. 1A and B). Two neodymium magnet were used to manufacture magnet equipment (Supplementary Fig. 1C and D). There were spaces between the neodymium magnets to flow semen extender and center of magnetic field was measured by gauss meter (Tesla meter TM-701, KANETEC, Japan) which were 2000, 4000 and 6000 G (Supplementary Fig. 1E–G). Also, magnet equipment were had plastic pipe to flow semen extender and were put on plastic cap to protect neodymium magnet (Supplementary Fig. 2A–C). Semen extender was treated before diluting with boar semen in magnetic field by passing though neodymium magnet equipment at 0, 2000, 4000, and 6000 G (G) using peristaltic pump (BT100-2J, Longerpump, China) for 5 min and 100 rpm (Supplementary Fig. 2D). The diluted sperm ($3 \times 10^7$ sperm/ml) was preserved for 0, 48, 120 and 168 h at 18 °C.

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### 2.3. Analysis of sperm plasma membrane, acrosomal membrane, and mitochondrial membrane damage

Fluorescent staining using the LIVE/DEAD Sperm Viability Kit was used to assess boar sperm membrane integrity (Silva and Gadella, 2006). Briefly, final concentration of SYBR-14 and propidium iodide (PI) were 2.4 and 2 μM. The acrosomal membrane damage was assessed after staining the sperm with peanut agglutinin conjugated with phycoerythrin (FITC-PNA) and PI double stain methods (Papaioannou et al., 1997). Briefly, final concentration of FITC-PNA and PI were 2.4 and 2 μM. The mitochondrial damage was assessed after staining the sperm with Rhodamine123 and PI double stain methods (Fraser et al., 2002). Briefly, final concentration of Rhodamine123 and PI were 2.4 and 2 μM. Diluted semen samples ($1 \times 10^6$ sperm/ml) were incubated for 10 min at 38 °C. After incubation, stained 10,000 count sperm samples were analyzed by flow cytometry (FACSCaliber, BD, USA) using argon laser tuned to 488 nm (Fig. 1A ). Analyzed data from flow cytometry were analyzed from CELLQuest, version 6.0 software. And sperm were visualized morphology and stained dye by fluorescence microscope (BX-50,

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