Biochemical and Biophysical Research Communications 464 (2015) 467–472

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Antioxidative effects of magnetized extender containing bovine serum albumin on sperm oxidative stress during long-term liquid preservation of boar semen

Sang-Hee Lee, Choon-Keun Park*

*College of Animal Life Sciences, Kangwon National University, Chuncheon 200-701, Republic of Korea*

ARTICLE INFO

*Article history:*
Received 12 June 2015
Accepted 24 June 2015
Available online 2 July 2015

*Keywords:*
Magnetized water
Liquid preservation
Boar sperm
Antioxidative effects

ABSTRACT

Magnetized water is defined as water that has passed through a magnet and shows increased permeability into cells and electron-donating characteristics. These attributes can protect against membrane damage and remove reactive oxygen species (ROS) in mammalian cells. We explored the effects of improved magnetized semen extenders containing bovine serum albumin (BSA) as antioxidants on apoptosis in boar sperm. Ejaculated semen was diluted in magnetized extender (0G and 6000G) with or without BSA (0G + BSA and 6000G + BSA), and sperm were analyzed based on viability, acrosome reaction, and $H_2O_2$ level of live sperm using flow cytometry. Sperm were then preserved for 11 days at 18 °C. We found that viability was significantly higher in 6000G + BSA than under the other treatments ($P < 0.05$). The acrosome reaction was significantly lower in the 6000G + BSA group compared with the other treatments ($P < 0.05$). Live sperm with high intracellular $H_2O_2$ level were significantly lower in the 6000G + BSA group than under other treatments ($P < 0.05$). Based on our results, magnetized extenders have antioxidative effects on the liquid preservation of boar sperm.



## 1. Introduction

Artificial insemination (AI) is an important reproductive technique for the production of domestic animals; accordingly, sperm control is important for generating high-capacity animals and successful AI [1]. Pig ejaculate (200–500 ml semen) can be preserved over 14 days at 15–20 °C [2]. However, sperm cannot divide (mitosis) compared with other somatic cells (such as epithelial and fibroblast cells) and are sensitive to exterior damage, such as temperature, storage period, components of the extender, and other physiological factors [3,4]. Thus, understanding sperm physiology can improve conception rate and piglet capacity.

Androhep® and Modena is used for dilution of boar semen, diluted boar semen is utilized to preservation of semen and AI [5]. The extenders contain various components for sperm metabolism; of these, bovine serum albumin (BSA) increases sperm motility, viability, and capacitation during fertilization in the female reproductive tract [6]. In addition, BSA in semen extender effuses cholesterol of the plasma membrane in sperm, which increases the levels of reactive oxygen species (ROS) [7]. Consequentially, suitable ROS levels in sperm lead to an acrosome reaction, increased intracellular calcium ions, hyperactivation, and capacitation, which assist in successful fertilization [8], however, excessive ROS levels can damage organelles, membranes, and DNA of sperm, resulting in a low conception rate [8,9]. Thus, the addition of BSA in semen extender during short-term (<4 days) liquid preservation is beneficial for sperm fertilization. However, BSA in semen extender during long-term (>7 days) liquid preservation results in the release of large amounts of cholesterol from the plasma membrane, which generates excessive ROS and has a negative effect on fertilization [10]. Antioxidants decrease boar sperm ROS levels during liquid preservation [11,12], ROS generated by BSA in boar semen extender during long-term liquid preservation has not been explored.

Magnetized water is generated by passing water through a general or electro-magnet, and it shows high electronic donor characteristics and a stable structure compared with general water [13]. In addition, magnetized cell culture medium shows decreased

* Corresponding author. Animal Biotechnology Program, Division of Applied Animal Science, College of Animal Life Science, Kangwon National University, Dongsangdae 1-#205, Chuncheon 200-701, Republic of Korea. Tel.: +82 332508689; fax: +82 332595574.
*E-mail address:* parkck@kangwon.ac.kr (C.-K. Park).

http://dx.doi.org/10.1016/j.bbrc.2015.06.159
0006-291X/

intracellular ROS levels and apoptosis and increased antioxidant levels in porcine cumulus cell–oocyte complexes [14]. Previous studies reported that magnetized semen extender without BSA has beneficial effects on plasma, acrosome, and mitochondrial membranes during liquid preservation [15]. The aim of this study was to investigate the effect of improved magnetized semen extender containing BSA on the viability, acrosome reaction, and antioxidant ability of boar sperm.

## 2. Materials and methods

### 2.1. Chemicals

Unless otherwise indicated, all reagents used in this study were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA).

### 2.2. Magnetic equipment

Magnet connection device for flowing water was designed by computer aided design (Fig. 1A). Two neodymium magnet of 4000 G (G) were installed for production magnetized semen extender on magnet connection device (Fig. 1B). And magnetic field in magnet connection device (between two 4000G neodymium magnets) was controlled on 6000G (Fig. 1C). All magnetic field was measured using tesla meter (Tesla meter TM-701, Kanetec, Japan).

### 2.3. Sample collection and magnetic treatment 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). The semen was collected by gloved-hand methods from five pigs once a week and filtered through gauze to remove gel particles. Samples from the fresh semen were evaluated for viability and morphology of the spermatozoa and treated semen extender as described by Lee and Park [15]. Ejaculated boar sperm was used with more than 70% viability and 20% acrosome reaction. Semen extender (modified-modena B; glucose 30.0 g/l, EDTA 2.25 g/l, sodium citrate 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 and BSA 0.04 g/l) was passed through magnetic equipment for 10 min and 100 rpm using peristaltic pump (BT100-2J, Longerpump, China) before 2 h dilution with semen (Fig. 1D).

### 2.4. Semen dilution and preservation

Experiment groups were divided into extender (0G), magnetized extender (6000G) excluding BSA, extender (0G + BSA) and magnetized extender (6000G + BSA) including BSA. Final semen concentration adjusted until $3 \times 10^7$ sperm/ml using semen extender of treatment groups and preserved for 11 days (collection time, 1 day) at 18 °C. Sperm characteristics were each checked at 1 (fresh), 4, 7 and 11 days during liquid preservation.

### 2.5. Analysis of viability and acrosome reaction

Sperm ability and acrosome reaction were measured using methods previously described [15]. The SYBR-14 (Invitrogen, Eugene, OR, USA) and propidium iodide (PI) used to detect live sperm as green fluorescence and dead sperm as red fluorescence. To detect acrosome reaction of boar sperm was used peanut agglutinin conjugated with phycoerythrin and PI double stain methods [16]. Stained sperm sample ($1 \times 10^6$ sperm/ml) were incubated for 10 min at 38 °C in dark room. After incubation, stained 10,000 count sperm were measured using flow cytometry (FACSCaliber, BD, USA) and viability (Supplementary Fig. 1A) and acrosome reaction (Supplementary Fig. 1B) were analyzed using dot-plot method (CELLQuest, version 6.0 software, BD, USA).

### 2.6. Measurement of intracellular hydrogen peroxide ($H_2O_2$)

5-(and-6)-carboxy-2′,7′-dichlorofluorescein diacetate (Carboxy-DCFDA; Invitrogen), mixed isomers respectively, as described

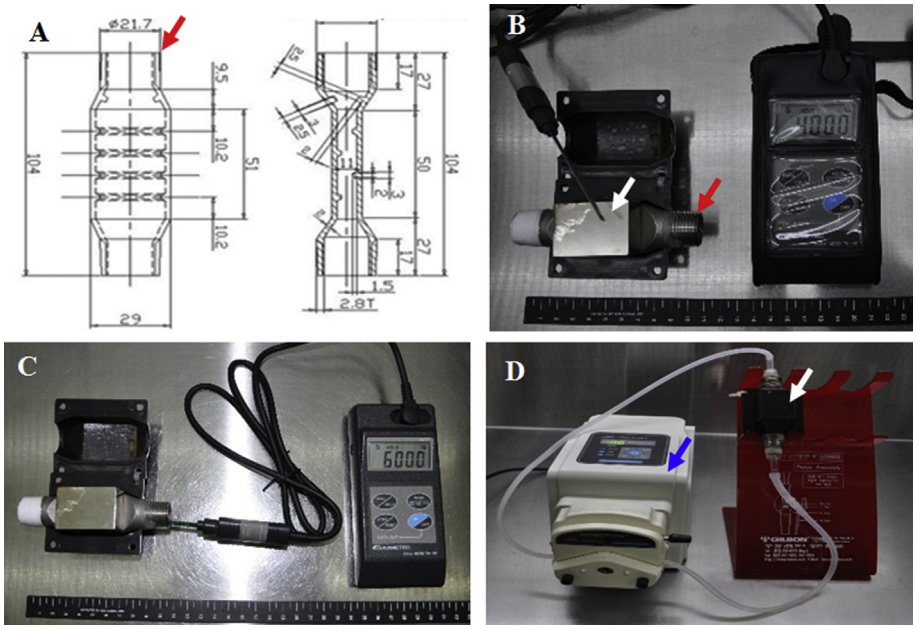


**Fig. 1.** Image of magnetic equipment, A, designed connection device (red arrows); B, 4000G neodymium magnet (white arrow); C, controlled magnetic field between 4000 G neodymium magnets; D, magnetic equipment system for production of magnetized semen diluter using peristaltic pump (blue arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

by research [17]. Carboxy-DCFDA was added for detecting intracellular $H_2O_2$ in preserved semen sample ($3.0 \times 10^7$ sperm/ml). To simultaneously differentiate living from dead cells, PI was added in semen sample, were incubated for 60 min at 38 °C. After incubation, stained 10,000 count sperm were measured using flow cytometry. Intracellular $H_2O_2$ assay was used to measure high oxidative stress induced sperm using dot-pot method (Supplementary Fig. 1C).

### 2.7. Statistical analysis

Data were analyzed using Statistical Analysis System software (SAS®, version 9.2). Unless otherwise stated, data are presented as means ± standard error mean (SEM). Differences were considered to be significant when their probability of occurring by chance was less than 5% ($P < 0.05$). Treatment groups were compared for differences though use of Duncan's modified multiple range test. All data obtained were analyzed using one-way ANOVA.

## 3. Results

### 3.1. Changes in sperm viability during liquid preservation

The effect of magnetized extender containing BSA on sperm viability is shown in Fig. 2. Viability showed no significant difference among treatment groups until 4 days of liquid preservation. However, viability was higher in the groups containing BSA (0G + BSA and 6000G + BSA) compared with the other groups (0G and 6000G) at days 7 and 11. In addition, viability of the 6000G group (day 7, 76.1 ± 1.2%; day 11, 73.1 ± 0.6%) was significantly higher than that of the 0G group (day 7, 69.1 ± 0.8%; day 11, 70.3 ± 0.4%) at days 7 and 11 ($P < 0.05$), and there were no differences in viability between the BSA treatment groups (0G + BSA and 6000G + BSA) at days 7 and 11 of liquid preservation. However, viability of the 6000G + BSA group (day 7, 80.8 ± 0.7%; day 11, 78.1 ± 0.4%) was significantly higher than that of the other treatment groups at days 7 and 11 of liquid preservation ($P < 0.05$).

### 3.2. Changes in the acrosome reaction in sperm during preservation

Fig. 3 shows the effect of magnetized semen extender containing BSA on the boar acrosome reaction during liquid preservation. The acrosome reaction showed no significant differences among groups until day 4, and there was no difference between the BSA treatment groups (0G + BSA and 6000G + BSA) at days 7 and 11 of liquid preservation. However, the acrosome reaction was significantly lower in the 6000G (day 7, 31.8 ± 0.7%; day 11, 39.04 ± 1.5%) than 0G group (day 7, 32.1 ± 0.9%; day 11, 44.9 ± 2.3%) during liquid preservation ($P < 0.05$).

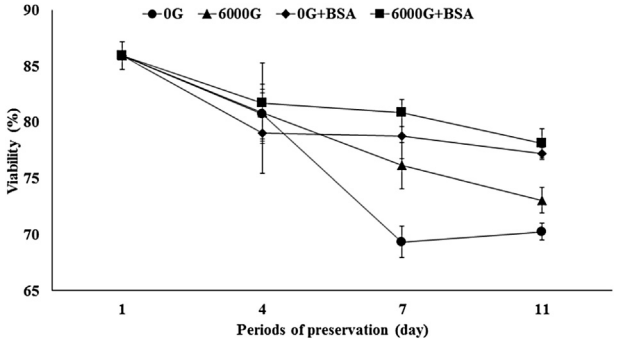


**Fig. 2.** Changes in viability viability during long-term (11 days) liquid preservation in diluted boar semen with semen extender (●, 0G), magnetized semen extender (▲, 6000G), excluding BSA, semen extender (◆, 0G + BSA) and magnetized semen extender (■, 6000G + BSA) including BSA.

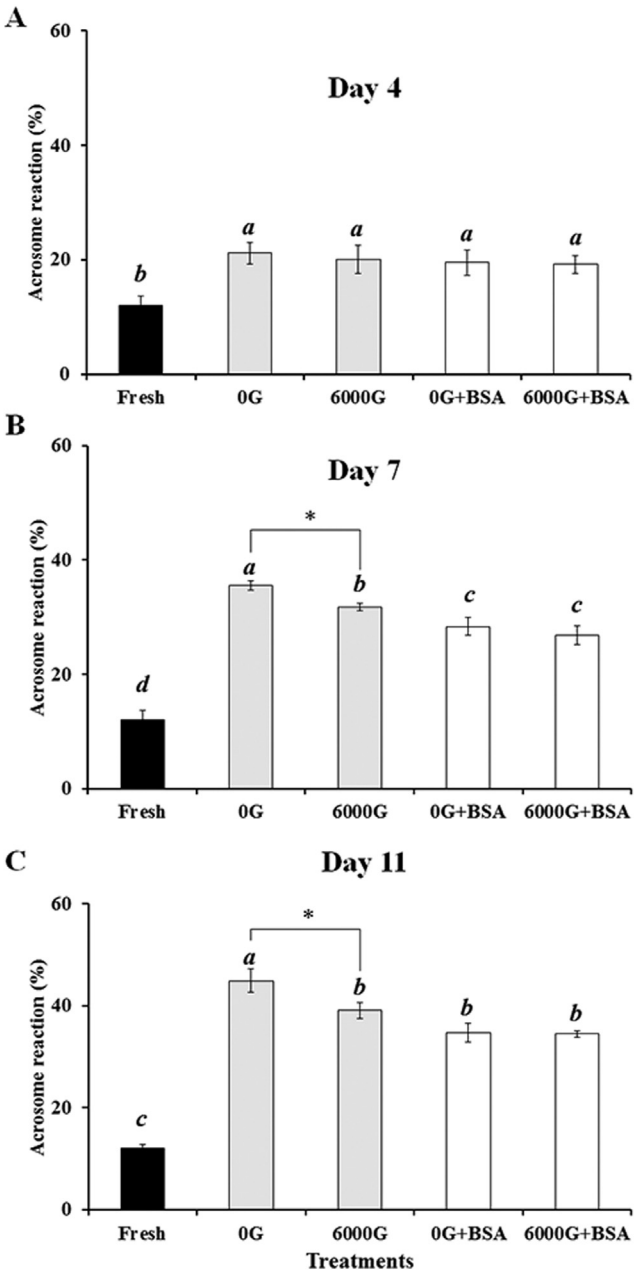


**Fig. 3.** Changes of acrosome reaction in diluted sperm with magnetized semen extender containing of BSA during long-term (11 days) liquid preservation in pigs. The periods of storage were day 1 (Fresh), day 4 (A), day 7 (B), and day 11 (C). Similarly shaded bars with different letters are significantly different within histogram ($P < 0.05$).

### 3.3. Intracellular $H_2O_2$ level in sperm during preservation

The antioxidant effect of magnetized extender on sperm oxidative stress from BSA was shown in Fig. 4. The ratio of live sperm with high intracellular $H_2O_2$ level was significantly increased in the 0G + BSA group (day 4, 12.6 ± 0.9%; day 7, 15.5 ± 0.7%; and day 11, 22.2 ± 2.0%) compared with the other groups during liquid preservation ($P < 0.05$). Especially, live sperm with high

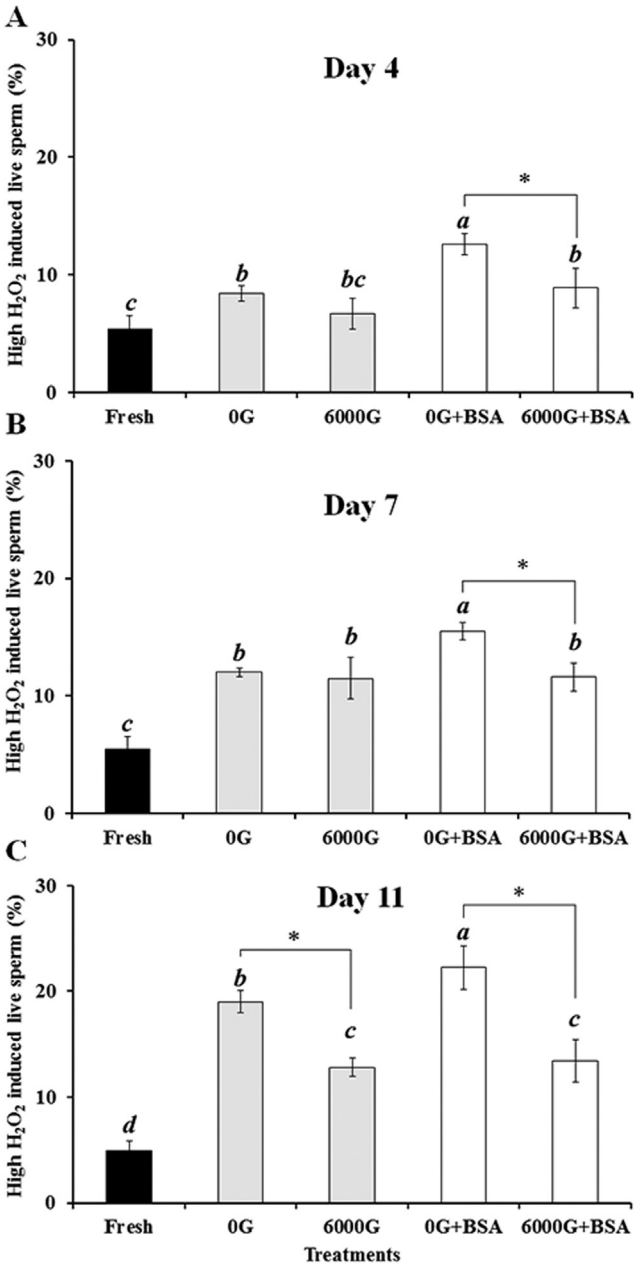


**Fig. 4.** Changes of live sperm with intracellular $H_2O_2$ level in diluted with magnetized semen extender containing of BSA during long-term (11 days) liquid preservation in pigs. The periods of storage were day 1 (Fresh), day 4 (A), day 7 (B), and day 11 (C). Similarly shaded bars with different letters are significantly different within histogram ($p < 0.05$).

intracellular $H_2O_2$ level was significantly lower in the 6000G + BSA (day 4, 8.9 ± 1.7%; day 7, 11.6 ± 1.2%; and day 11 13.4 ± 2.0%) than 0G + BSA group at days 4, 7, and 11, and was significantly decreased in the 6000G (12.8 ± 0.9%) compared with the 0G group (19.0 ± 1.0%) at day 11 of liquid preservation ($P < 0.05$). Thus, magnetized semen extender groups showed significantly decreased $H_2O_2$ level of live sperm at day 11 of liquid preservation ($P < 0.05$).

## 4. Discussion

AI contributes to genetic improvements, which are applied extensively in the domestic industry worldwide [1]. The development of AI techniques accelerates andrology such as sperm physiology, storage methods, cryopreservation, and our understanding of the composition of seminal plasma for successful fertilization [11,15,18]. Of these, seminal plasma is a component of semen that does not contain sperm and that supplies the necessary nutrients to sperm for metabolic activity and transports fluids to oocytes in the female genital tract [19]. Seminal plasma in ejaculated semen contains various factors involved in sperm maturation for successful fertilization; however, components involved in capacitation increase calcium ions in sperm cells [20]. Capacitated sperm show hyperactivation, acrosome reactions, and enzymatic reactions; eventually, sperm die spontaneously during fertilization [21]. Thus, understanding the relationship between sperm and seminal plasma, such as their metabolic activity and suitable environments, is important to preserve spermatozoa for extended periods.

Boar semen extenders are diluted with ejaculated semen for AI for many sows and to preserve semen during storage, which accomplishes basic functions such as providing nutrients for sperm metabolism, stabilizing sperm membranes, preventing capacitation, neutralizing metabolic waste products, maintaining osmotic equilibrium, and retarding bacterial growth during storage [22]. Therefore, boar semen extender must supply energy (e.g., glucose, lactose and sodium citrate), a pH buffering system (e.g. Tris, HEPES, sodium bicarbonate, pH 6.8–7.8), a plasma membrane stabilizer (BSA, ethylenediaminetetraacetic acid), and antibiotics to prevent bacterial growth (e.g., gentamycin, penicillin) [23]. In addition, boar semen extenders are classified according to storage period; beltsville thawing solution [24] and Illinois variable temperature [25] are frequently used for short-term (1–3 days) storage. In addition, MR-A® [26], ZORPVA [27], Reading [28], Androhep® [29] and Modena [30] are diluted with boar semen for long-term (>7 day) liquid preservation. These solutions have different ingredients, capacities to maintain viability, and acrosome reactions [27].

BSA is a macromolecular complex protein isolated from bovine plasma that has been implicated in ROS activity, due to its function as a membrane stabilizer [31]. Sperm bound with BSA improve the sperm-oocyte fusion rate; moreover, the addition of BSA to semen extender improves motility and prevents lipid peroxidation in rabbits [32]. Johnson et al. [23] suggested that BSA is too large to transport intracellularly across the plasma membrane; BSA in the plasma membrane may protect against oxidative stress on the extracellular side. Waberski et al. [33] reported that BSA improves the motility of sperm for 6 days, and that the addition of BSA to semen extender for a 3- or 5-day liquid preservation period increases fertility rate [34]. In this study, supplementation of BSA in semen extender increased sperm ability and decreased acrosome reactions during the storage periods. However, BSA induced temporary lipid fluidity in the sperm membrane [10]. Thus, BSA in semen extender improves fertilization during short-term liquid preservation; however, intracellular oxidative stress of boar sperm was increased during long-term liquid storage over 6 days, which decreased the fertilizing ability. Nevertheless, our understanding of BSA signaling under oxidative stress in boar sperm remains limited.

Optimal ROS levels are important for sperm maturation in testis and capacitation in the female reproductive tract during fertilization [35]. ROS mediate lipid redistribution (which destabilizes the plasma membrane), and capacitated sperm can penetrate oocytes by acrosomyl exocytosis (acrosome) [36]. However, excessive ROS levels can be generated via endogenous antioxidant responses, influencing membrane damage and apoptosis, lowering mitochondrial membrane potential, and potentially resulting in loss of DNA and sperm motility [37,38]. Low $H_2O_2$ levels in sperm maintain capacitation and acrosome reactions, whereas high $H_2O_2$ levels

induce rapid capacitation processes, which affect homeostatic mechanisms such as phospholipid peroxidation [39], thus, excessive ROS production causes infertility [40].

Magnetized water has stronger electronic donor, hydrogen-bond, and electric conduction characteristics, as well as reduced formation of ice-crystals compared with normal water [41,42]. Thus, magnetized water is used to reduce oxides ($SiO_2$, $Fe_2O_3$, CaO, MgO, $SO_3$, $Na_2O$, $K_2O$, CuO, $Mn_2O_3$, ZnO and $CO_2$) and limit corrosion in pipes [43]. However, biological applications of magnetized water have not been examined. From a biological aspect, magnetized water has numerous applications. Supplementation with magnetized water has an anti-aging effect [44] and improves blood glucose and lymphocytes [45] in mice. In addition, growth is enhanced by magnetized water in plants [46]. Previous studies explored magnetized semen diluters excluding BSA and showed that they protect against sperm membrane damage and maintain fertility during long-term liquid preservation [15].

In this study, live sperm with high intracellular $H_2O_2$ level increased in the BSA-treated groups during the preservation period. These results suggest that BSA cannot prevent oxidative stress, leading to lipid fluidity of the sperm membrane, during long-term liquid preservation. However, magnetized semen extender significantly decreased intracellular $H_2O_2$ levels after 7 and 11 days of liquid preservation. Thus, ROS levels induced by sperm metabolism in BSA-containing semen extender decreased in the presence of stable magnetic water during long-term liquid preservation. Kim et al. [14] reported that magnetized culture medium reduced intracellular ROS levels in porcine cumulus cell–oocyte complexes. Thus, magnetized semen extender is associated with oxidative stress during sperm metabolism throughout liquid storage periods.

Our results are beneficial with regard to long-term (>7 days) liquid boar semen preservation and reveal a novel aspect of cell storage based on magnetized water. In addition, magnetized water may be used in animal cell culture systems for the production of low-oxidative stress extender or medium.

## Acknowledgments

This work was supported by Grant No. IPET31060-05-1-CG000 from Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET). And this study was also supported partially by 2014 Research Grant from Kangwon National University (No. 12140209), College of animal life sciences, Division of applied animal science, Republic of Korea.

## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.159.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.06.159.

## References

[1] J. Roca, I. Parrilla, H. Rodriguez-Martinez, M.A. Gil, C. Cuello, J.M. Vazquez, E.A. Martinez, Approaches towards efficient use of boar semen in the pig industry, Reprod. Domest. Anim. 46 (2011) 79–83.
[2] L.A. Johnson, K.F. Weitze, P. Fiser, W.M.C. Maxwell, Storage of boar semen, Anim. Reprod. Sci. 62 (2000) 143–172.
[3] L. Aghajanova, S. Shen, A.M. Rojas, S.J. Fisher, J.C. Irwin, L.C. Giudice, Comparative transcriptome analysis of human trophectoderm and embryonic stem cell-derived trophoblasts reveal key participants in early implantation, Biol. Reprod. 86 (2012) 1–21.
[4] G.B. Boe-Hansen, A.K. Ersbøll, T. Greve, P. Christensen, Increasing storage time of extended boar semen reduces sperm DNA integrity, Theriogenology 63 (2005) 2006–2019.
[5] M.J. Estienne, A.F. Harper, J.L. Day, Characteristics of sperm motility in boar semen diluted in different extenders and stored for seven days at 18 degrees C, Reprod. Biol. 7 (2007) 221–231.
[6] C.E. Green, P.F. Watson, Comparison of the capacitation-like state of cooled boar spermatozoa with true capacitation, Reproduction 122 (2001) 889–898.
[7] L. Radomil, M.J. Pettitt, K.M. Merkies, K.D. Hickey, M.M. Buhr, Stress and dietary factors modify boar sperm for processing, Reprod. Domest. Anim. 46 (Suppl. 2) (2011) 39–44.
[8] B.J. Awda, M. Mackenzie-Bell, M.M. Buhr, Reactive oxygen species and boar sperm function, Biol. Reprod. 81 (2009) 553–561.
[9] P.F.N. Silva, B.M. Gadella, Detection of damage in mammalian sperm cells, Theriogenology 65 (2006) 958–978.
[10] M. Buhr, Preservation of boar sperm alters membrane molecular dynamics, in: Proc. 2nd Int. Conf. Boar Semen Preserv., Beltsville, 1990, pp. 81–93.
[11] H. Funahashi, T. Sano, Select antioxidants improve the function of extended boar semen stored at 10 °C, Theriogenology 63 (2005) 1605–1616.
[12] R. Toniolli, J. Bussière, M. Courot, M. Magistrini, Y. Combarnous, Effect of indole-3-acetic acid (plant auxin) on the preservation at 15 °C of boar semen for artificial insemination, Reprod. Nutr. Dev. 36 (1996) 503–511.
[13] S. Tigrek, F. Barnes, Water structures and effects of electric and magnetic fields, in: Giuliani, Soffritti, Fidenza (Eds.), Non-Thermal Effects and Mechanisms of Interaction between Electromagnetic Fields and Living Matter, an ICEMS Monograph, Bologna Italy, 2010, pp. 25–50.
[14] Y.-J. Kim, S.-H. Lee, S.-J. Jung, C.-K. Park, Effects of magnetized medium on *in vitro* maturation of porcine cumulus cell-oocyte complexes, J. Magn. 19 (2014) 241–247.
[15] S.-H. Lee, C.-K. Park, Effect of magnetized extender on sperm membrane integrity and development of oocytes *in vitro* fertilized with liquid storage boar semen, Anim. Reprod. Sci. 154 (2015) 86–94.
[16] K.Z. Papaioannou, R.P. Murphy, R.S. Monks, N. Hynes, M.P. Ryan, M.P. Boland, J.F. Roche, Assessment of viability and mitochondrial function of equine spermatozoa using double staining and flow cytometry, Theriogenology 48 (1997) 299–312.
[17] H.D. Guthrie, G.R. Welch, Determination of intracellular reactive oxygen species and high mitochondrial membrane potential in Percoll-treated viable boar sperm using fluorescence-activated flow cytometry, J. Anim. Sci. 84 (2006) 2089–2100.
[18] J.L. Bailey, C. Lessard, J. Jacques, C. Brèque, I. Dobrinski, W. Zeng, H.L. Galantino-Homer, Cryopreservation of boar semen and its future importance to the industry, Theriogenology 70 (2008) 1251–1259.
[19] A. López Rodríguez, T. Rijsselaere, J. Beek, P. Vyt, A. Van Soom, D. Maes, Boar seminal plasma components and their relation with semen quality, Syst. Biol. Reprod. Med. 59 (2013) 5–12.
[20] R.A. Harrison, B.M. Gadella, Bicarbonate-induced membrane processing in sperm capacitation, Theriogenology 63 (2005) 342–351.
[21] S. Suarez, H.C. Ho, Hyperactivated motility in sperm, Reprod. Domest. Anim. 38 (2003) 119–124.
[22] W.L. Flowers, Management of boars for efficient semen production, J. Reprod. Fertil. Suppl. 52 (1997) 67–78.
[23] L. Johnson, K. Weitze, P. Fiser, W. Maxwell, Storage of boar semen, Anim. Reprod. Sci. 62 (2000) 143–172.
[24] V.G. Pursel, L.A. Johnson, Freezing of boar spermatozoa: fertilizing capacity with concentrated semen and a new thawing procedure, J. Anim. Sci. 40 (1975) 99–102.
[25] D. Mesnil, D. Buisson, L. Dauzier, Improvement of practical use of preservation techniques for boar semen by saturation of the diluent with carbon dioxide, Ann. Zootech. Suppl. 8 (1959) 81–96.
[26] J.E. Rodríguez-Gil, T. Rigau, Effects of slight agitation on the quality of refrigerated boar sperm, Anim. Reprod. Sci. 39 (1995) 141–146.
[27] J. Gadea, Review: Semen extenders used in the artificial insemination of swine, Span. J. Agric. Res. 1 (2003) 17–27.
[28] S.G. Revell, C.E. Glossop, A long-time ambient temperature diluent for boar semen, Anim. Sci. 48 (1989) 579–584.
[29] H. Paulenz, E. Kommisrud, P. Hofmo, Effect of long-term storage at different temperatures on the quality of liquid boar semen, Reprod. Domest. Anim. 35 (2000) 83–87.
[30] L.A. Johnson, J.G. Aalbers, H.G. Grooten, Artificial insemination of swine: fecundity of boar semen stored in beltsville ts (bts), modified modena (mm), or mr-a and inseminated on one, three and four days after collection12, Reprod. Domest. Anim. 23 (1988) 49–55.
[31] M. Blank, L. Soo, J.S. Britten, Adsorption of albumin on rabbit sperm membranes, J. Membr. Biol. 29 (1976) 401–409.
[32] J.G. Alvarez, B.T. Storey, Spontaneous lipid peroxidation in rabbit epididymal spermatozoa: its effect on sperm motility, Biol. Reprod. 27 (1982) 1102–1108.
[33] D. Waberski, K. Weitze, D. Rath, H. Sallmann, Contents-effect of bovine serum-albumin and zwitterionic buffers on stored liquid boar semen, Zuchthyg. Reprod. Domest. Anim. 24 (1989) 128–133.
[34] D. Waberski, S. Meding, G. Dirksen, K. Weitze, C. Leiding, R. Hahn, Fertility of long-term-stored boar semen: Influence of extender (Androhep and Kiev),

storage time and plasma droplets in the semen, Anim. Reprod. Sci. 36 (1994) 145–151.
[35] R.J. Aitken, P. Vernet, Maturation of redox regulatory mechanisms in the epididymis, J. Reprod. Fertil. Suppl. 53 (1998) 109–118.
[36] C. O'Flaherty, E. de Lamirande, C. Gagnon, Reactive oxygen species modulate independent protein phosphorylation pathways during human sperm capacitation, Free Radic. Biol. Med. 40 (2006) 1045–1055.
[37] S. Lopes, A. Jurisicova, J.G. Sun, R.F. Casper, Reactive oxygen species: potential cause for DNA fragmentation in human spermatozoa, Hum. Reprod. 13 (1998) 896–900.
[38] D. Sanocka, M. Kurpisz, Reactive oxygen species and sperm cells, Reprod. Biol. Endocrinol. (2004) 2–12.
[39] S. Oehninger, P. Blackmore, M. Mahony, G. Hodgen, Effects of hydrogen peroxide on human spermatozoa, J. Assis. Reprod. Gene 12 (1995) 41–47.
[40] E. de Lamirande, C. Gagnon, Impact of reactive oxygen species on spermatozoa: a balancing act between beneficial and detrimental effects, Hum. Reprod. 10 (Suppl. 1) (1995) 15–21.
[41] R. Cai, H. Yang, J. He, W. Zhu, The effects of magnetic fields on water molecular hydrogen bonds, J. Mol. Struct. 938 (2009) 15–19.
[42] P. Xu, B. Yu, Developing a new form of permeability and Kozeny–Carman constant for homogeneous porous media by means of fractal geometry, Adv. Water Resour. 31 (2008) 74–81.
[43] A. Szkatula, M. Balanda, M. Kopeć, Magnetic treatment of industrial water. Silica activation, Eur. Phys. J. AP 18 (2002) 41–49.
[44] M. Yi-long, R. Hui, R. Shu, Z. Er-kang, H. Gang, Z. Yao-wu, A study of the effect of magnetized water on enzyme activities by potentiometric enzyme electrode method, J. Tongji. Med. Univ. 12 (1992) 193–196.
[45] H.-J. Lee, M.-H. Kang, Effect of the magnetized water supplementation on blood glucose, lymphocyte DNA damage, antioxidant status, and lipid profiles in STZ-induced rats, Nutr. Res. Pract. 7 (2013) 34–42.
[46] M. Hozayn, A. Qados, A.M. Saeed, Irrigation with magnetized water enhances growth, chemical constituent and yield of chickpea (*Cicer arietinum* L.), Agric. Biol. J. North Am. 1 (2010) 671–676.