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

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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

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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 3×10^7 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 (1×10^6 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

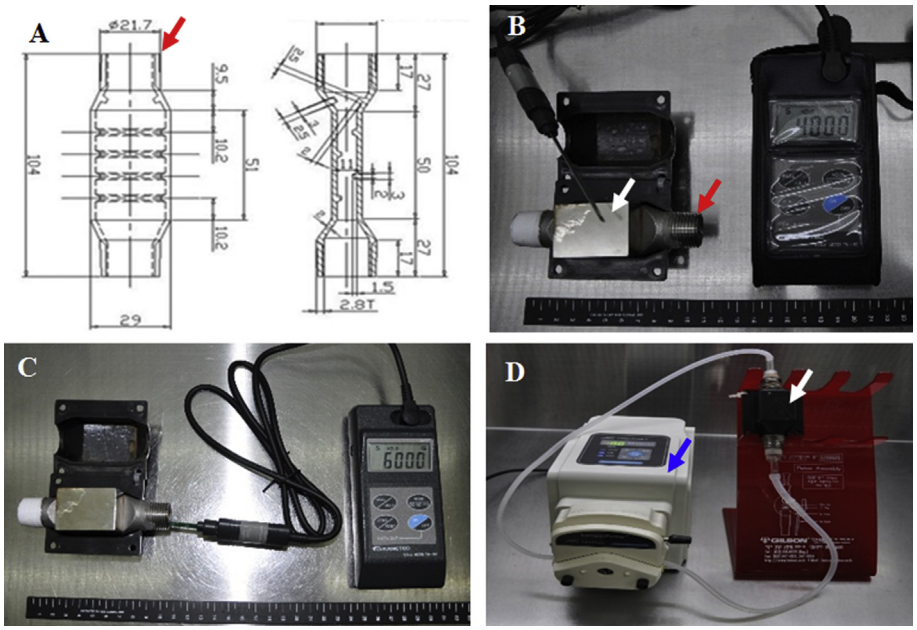

Fig. 1. Image of magnetic equipment, A, designed connection device (red arrows); B, 4000G neodymium magnet (white arrow); C, controlled magnetic field between 4000 G neodymium magnets; D, magnetic equipment system for production of magnetized semen diluter using peristaltic pump (blue arrow). (For interpretation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

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