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Improvement of liquid stored boar semen quality by removing low molecular weight proteins and supplementation with α-tocopherol

M. Zakošek Pipan[a,*], J. Mrkun[a], A. Nemec Svete[b], P. Zrimšek[c]

[a] Clinic for Reproduction and Large Animals, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia
[b] Small Animal Clinic, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia
[c] Institute for Preclinical Sciences, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, Ljubljana, Slovenia

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ABSTRACT

Seminal plasma contains low-molecular weight components that can exert a harmful effect on sperm function. We have evaluated the effects of removing low-molecular weight components from seminal plasma and adding α-tocopherol on boar semen quality after 72 h of liquid storage. Semen was evaluated on the basis of motility, morphology, acrosome integrity, plasma membrane modifications, mitochondrial activity, DNA fragmentation and lipid peroxidation. Thiobarbituric acid reactive substances (TBARS), 8-isoprostane, and antioxidant status (total antioxidant capacity (TAC) and superoxide dismutase activity (SOD)) were measured in seminal plasma.

Removal of low-molecular weight components from seminal plasma, together with the addition of α-tocopherol, kept the lipid peroxidation and mitochondrial activity and DNA fragmentation at the same level as in native semen samples. Dialysing semen and adding 200 μM of α-tocopherol led to higher progressive motility, a higher proportion of morphologically normal spermatozoa and a significantly lower level of acrosomal reacted spermatozoa compared to non-dialyzed semen samples after 72 h of storage. In conclusion, liquid stored boar semen was better preserved, and oxidative stress in the semen was reduced when semen was dialyzed and α-tocopherol was added prior to storage.

1. Introduction

Evaluation of ejaculate is economically important in breeding management. Quantitative and qualitative sperm parameters of an individual boar are assessed to clarify whether the semen comply with the minimum requirements for successful fertilization (Rodríguez-Martinez, 2003). However, fertilization success cannot be attributed solely to the absolute number of viable, motile, morphologically normal spermatozoa inseminated into the female but, more importantly, to their functional competence (Petrunkina et al., 2007). After liquid preservation, boar spermatozoa show morpho-functional changes that resemble the natural ageing process. The duration of storage is the most important factor influencing the severity of these changes (Lange-Consiglio et al., 2013; De Ambrogi et al., 2006). There is some evidence that seminal plasma contains low-molecular weight components which can be deleterious to sperm function (Fraser et al., 2007). Moreover, some of the bacteria present in raw semen produce toxins that can also have a detrimental effect on semen quality (Kuster and Althouse, 2016). The elimination of low-molecular weight components (including toxins) can thus minimize the detrimental effect associated with the dilution and storage of boar semen. However, low molecular weight components in seminal plasma also include some antioxidants, and their removal could contribute to oxidative

* Corresponding author.
E-mail address: maja.zakosekpipan@vf.uni-lj.si (M. Zakošek Pipan).

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stress.

In ejaculate, there are different mechanisms that can protect spermatozoa against oxidative stress. Boar ejaculate is endowed with high quantities of superoxide dismutase (SOD), which catalyses the dismutation of both extracellular and intracellular superoxide anions to hydrogen peroxide (H_2O_2) and oxygen, and is therefore important in preventing lipid peroxidation of the plasma membrane (Kowalowka et al., 2008; Perumal, 2014).

On the other hand, boar semen contains an extremely low level of catalase, which converts H_2O_2 to water and oxygen. For this reason, boar semen often contains a high level of H_2O_2 (Kowalowka et al., 2008), which is responsible for much of the damage to the cell structure and function of boar spermatozoa (Kim et al., 2011). Seminal plasma also contains, in addition to antioxidant enzymes, low molecular weight, non-enzymatic antioxidants such as L-glutathione (GSH), L-ergothioneine (ERT), α-tocopherol and others (Ishii et al., 2005), that help to protect against reactive oxygen species (ROS) and can counterbalance the lower amount of antioxidant catalase. Specifically, vitamin E (α-tocopherol) can break the covalent links formed by ROS between fatty acid side chains in membrane lipids and is one of the major membrane protectants against ROS and lipid peroxidation (Jeong et al., 2009). The addition of vitamin E to diluted semen reduces malondialdehyde (MDA) production in boar semen stored at 15 °C for 72 h (Mendez et al., 2013).

Nevertheless, removal of low molecular weight antioxidants in seminal plasma improved semen quality after 72 h of storage at 15–17 °C, but it also decreased the TAC of liquid stored boar semen and increased lipid peroxidation (Zakošek Pipan et al., 2014). The aim of our study was to evaluate whether adding an antioxidant, α-tocopherol, to dialyzed semen could protect sperm against oxidative damage and better preserve the quality of liquid stored boar semen.

2. Materials and methods

2.1. Semen samples

Twenty ejaculates, obtained from eight mature and healthy boars of different breeds (2 Slovenian landrace line 11, 1 Slovenian landrace line 55, 2 Slovenian large white, 2 Pietrain, and 1 Hybrid line (54)) aged from 12 to 24 months, were included in the study. Whole ejaculates lacking the gel fraction were collected by the gloved-hand technique during routine farm operations at a local AI centre (Agriculture and Forestry Institute Ptuj, Ptuj, Slovenia). The collection frequency of each boar was at intervals of 2–3 days. Following collection, the filtered semen of each the ejaculate was extended with Beltsville Thawing Solution (BTS, Truadeco, Netherlands) at a ratio of 1:2. Diluted samples were delivered to the laboratory in a thermo stabile box, and the initial analysis was performed within two hours after collection.

2.2. Sample preparation

After the initial evaluation (Section 2.3), each diluted ejaculate that fulfilled the study requirements (> 70% total motility, > 35% progressive motility, < 30% of morphologically abnormal spermatozoa, < 20% proximal and distal cytoplasmic droplets) was divided into two aliquots. The first aliquot was dialyzed against BTS twice for a total period of 3 h (first dialysis 1 h, second dialysis two hours; ratio of semen to dialysate, 1:50) using semi-permeable cellulose tubing of 12–14 kDa molecular weight cut-off (Visking Dialysis Tubing, Serva Electrophoresis, Heidelberg, Germany). During this step, semen was kept at room temperature (20 °C +/ −1 °C) in order to decrease chilling injury.

Each non-dialyzed and dialyzed semen sample was further divided into two aliquots. 200 μM of α-tocopherol was added to both the dialyzed and the non-dialyzed aliquot. The second non-dialyzed and dialyzed aliquots served as controls. The final dilution of the ejaculates in all of the semen samples was 100×10^6 spermatozoa per ml. These samples were then stored in closed plastic containers in a thermal box at 15–17 °C with constant gentle agitation for 72 h. To reduce clumping of sperm cells during storage, semen samples were constantly gently agitated (Rodríguez-Gil and Rigau, 1995).

2.3. Basic semen parameters

Semen parameters were determined in the first hour following collection. Sperm motility was analysed using assisted semen analysis (Hamilton Thorne IVOS 10.2; Hamilton Thorne Research, MA, USA) with a Makler counting chamber (Sefi Medical Instruments, Israel). The concentration was measured with a photometer (Photometer SDM 5, Minitüb, Germany). The morphology of 200 spermatozoa was assessed in diluted semen samples after eosin-nigrosin staining (Morphology stain, Society for Theriogenology). Semen analysis of dialyzed and non-dialyzed semen samples with or without α-tocopherol was carried out on the day of collection (day 0) and after 72 h of semen preservation (Fig. 1).

2.4. Capacitation and acrosome reaction

Chlortetracycline (CTC) staining analysis was used to identify the progress of capacitation and acrosome reaction of boar spermatozoa. A CTC solution was prepared by adding 500 μM CTC to a buffer containing 130 mM NaCl, 20 mM Tris (Trizma base; Sigma) and 5 mM L-cysteine, then passed through a 0.22 μm filter (Millex-GV, low solute binding, Milli-pore SLGV02565). The pH was adjusted to 7.8, and the solution was protected from light and held at 5 °C until using. Before addition to the spermatozoa, the solution was warmed to room temperature.

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