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Sperm quality and oxidative status as affected by homogenization of liquid-stored boar semen diluted in short- and long-term extenders

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ABSTRACT

Homogenization of diluted boar semen during storage has for a long time been regarded as beneficial. Recent studies indicated an adverse effect of homogenization on sperm quality for yet unknown reasons. This study aimed to verify the effect of homogenization on sperm parameters and to elucidate the impact of oxidative stress. Twenty-one normospermic ejaculates (21 boars) were diluted with Androstar® Plus (AND) and Beltsville Thawing Solution (BTS). Semen doses were submitted to no-homogenization (NoHom) or twice-a-day manual homogenization (2xHom) during storage at 17 °C for 168 h. NoHom and 2xHom were similar ($P > 0.05$) for both short- and long-term extenders with respect to motility and kinematics parameters (CASA system), membrane viability (SYBR-14/PI), acrosome integrity, lipid peroxidation, protein oxidation, intracellular reactive oxygen species, sulfhydryl content, and total radical-trapping antioxidant potential. 2xHom reduced sperm motility and motion kinematics (VCL, VSL, VAP, BCF, and ALH) following the thermoresistance test and presented with a slight increase in pH along the storage ($P = 0.05$) as compared to NoHom. Furthermore, 2xHom semen doses presented with a constant SOD and GSH-Px activity during storage whereas enzymatic activity increased for NoHom at the end of the storage. These findings confirm that homogenization of semen doses is detrimental to sperm quality. Moreover, it is shown that the effect of homogenization is unlikely to be primarily related to oxidative stress. Homogenization is not recommended for storage of liquid boar semen for up to 168 h in both short- and long-term extenders.

1. Introduction

Liquid-storage of boar semen using extenders is a standard protocol followed for sperm preservation and artificial insemination (Riesenbeck, 2011). It is well established that storage of extended semen at 15–20 °C is limited to three

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to 10 days, which constitutes a challenge for sperm quality maintenance (Johnson et al., 2000). At this temperature range, raised sperm metabolic rate and ineffective inhibition of bacteria growth are observed when compared to storage at lower temperatures (Althouse et al., 1998; Casas and Althouse, 2013). Also, longer sperm life beyond this storage period is a functional restriction for currently available extenders. At the same time, the current trend to reduce the number of spermatozoa per insemination dose, to incorporate methods for bacterial control and to prolong semen storage time demands a strict quality assurance during semen storage (Riesenbeck et al., 2015).

Liquid-storage of boar semen is influenced by several factors such as type of extender (Dubé et al., 2004; Vyt et al., 2004; Waterhouse et al., 2004; Pinart et al., 2015), dilution rate (Schulze et al., 2015a), temperature stability inside the storage unit (Young et al., 2005), presence of air in the semen dose (Vyt et al., 2007; Ribeiro et al., 2016), as well as sperm sedimentation (Rodríguez-Gil and Rigau, 1995; Simmet et al., 1998; Schulze et al., 2015b). Resting semen doses during storage allows spermatozoa, microorganisms and other particles to be gradually deposited at the bottom as sediment (Belstra, 2007). In order to promote the resuspension of sperm cells within the extender, manual homogenization of semen doses is widely practiced and recommended. Nevertheless, its consequences on sperm quality and semen preservation have not been completely elucidated.

Previous research on the effect of homogenization of boar semen doses had analyzed parameters of sperm quality along storage without homogenization as well as with either manual or automatic homogenization systems. Interestingly, the results diverge among studies. The early study from Rodríguez-Gil and Rigau (1995) determined that slight agitation of semen doses using an automated system that prevents sedimentation improves sperm quality. Contrastingly, Simmet et al. (1998) and recent research from Schulze et al. (2015b) substantiated a detrimental effect of either once- or twice-a-day manual rotation or automatic rotation on sperm quality. Despite the evidences that homogenization adversely affects semen preservation, its underlying mechanism remains undetermined. The first attempt to address further explanation was made by Schulze et al. (2015b), as the authors hypothesized a negative effect of the pH variation induced by semen rotation. However, as the authors had proven the hypothesis was wrong, the oxidative stress was proposed as a possible mechanism warranting further investigation.

Oxidative stress in sperm cells is induced by an imbalance between reactive oxygen species (ROS) production and antioxidant defense. The spermatozoa spontaneously produce a variety of ROS primarily in the mitochondria, including superoxide anion (O_2^-), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) (Aitken, 1995; Guthrie et al., 2008). Boar seminal plasma counteracts towards neutralizing the effects of ROS with radical-scavenging agents and enzymatic antioxidant systems, the latter mainly composed of the enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (Aitken, 1995; Strzeżek et al., 1999). The balance between pro-oxidant and antioxidant systems is regarded as efficient under normal basal conditions or in the absence of an additional ROS-generating system. Indeed, ROS formation is low in viable fresh boar sperm (Guthrie and Welch, 2006). However, liquid preservation of boar semen is considered to disrupt the balance by increasing ROS formation and consequently ROS-mediated damages to spermatozoa (Cerolini et al., 2000; Kumaresan et al., 2009). As homogenization takes place during semen storage, it is appealing to investigate to what extent the homogenization process can shift the redox status of liquid-stored boar semen.

The present study aimed to clarify whether the daily manual homogenization of liquid-stored boar semen is detrimental to sperm quality and whether the oxidative stress is the primary underlying mechanism. Our study evaluated the effect of homogenization on sperm parameters and on oxidative status during storage considering the impact of different types of extenders (short- and long-term) and prolonged storage time (168 h) for the purpose of comparison.

2. Material and methods

2.1. Animals and facilities

The study was conducted with approval from the Federal University of Rio Grande do Sul's Ethical Committee on Animal Experimentation (project #28591). One normospermic ejaculate from each of the 21 crossbred PIC® Génétiporc boars (Piétrain × Duroc) obtained from a commercial boar stud was used in the study. The boars were healthy, mature (average age: 14.5 ± 1.8 months old), and were housed in individual crates in a temperature-controlled barn (16–18 °C). All boars had *ad libitum* access to water and were fed a commercial corn-soybean meal diet. The boars were routinely used for production of semen doses with an average interval of 6.1 ± 0.9 days between semen collections.

2.2. Semen collection and processing

Ejaculates were collected using a semi-automatic collection system (Equittec®, Marau, RS, Brazil) within a pre-warmed (38 °C) collection cup equipped with a filter for removal of the gel fraction. Raw semen was weighed and evaluated for motility and sperm concentration by using a computer-assisted semen analysis system (CASA system; AndroVision®, Minitüb GmbH, Tiefenbach, Germany). Only normospermic ejaculates that fulfilled the criteria of having at least 70% total sperm motility and minimum 75% morphologically normal spermatozoa, were used for the study.

Semen was diluted in a split sample design in Androstar® Plus (AND, Minitüb GmbH, Tiefenbach, Germany) and Beltsville Thawing Solution (BTS, Minitüb GmbH, Tiefenbach, Germany) by using the isothermic dilution (34 °C) methodology. Insemination doses were produced with 1.5×10^9 spermatozoa and stored in 60 mL tubes (QuickTip Flexitube® Minitüb GmbH, Tiefenbach, Germany) with a total volume of 55 mL. Extended semen doses were placed in ventilated air-conditioned boxes at 22 °C for 3 h to ensure temperature stabilization and