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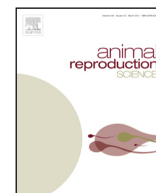

Inter- and intra-individual variability of total antioxidant capacity of bovine seminal plasma and relationships with sperm quality before and after cryopreservation

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ABSTRACT

The aim of the present study was to investigate inter- and intra-individual variability of total antioxidant capacity (TAC) in seminal plasma of bulls. In addition, relationships between TAC and glutathione peroxidase (GPx), superoxide dismutase activities (SOD), and parameters of sperm quality, respectively, were examined. Eight consecutive ejaculates were collected from nine Holstein–Friesian bulls. The percentage of plasma membrane and acrosome intact (PMAI) sperm was measured by using the FITC-PNA/PI assay, the amount of membrane lipid peroxidation (LPO) without and with stimulation(s) of LPO was quantified by using the BODIPY assay before cryopreservation and immediately (0 h) as well as 3 h after thawing. The percentage of sperm with a greater DNA fragmentation index was measured by the sperm chromatin structure assay. The amount of TAC differed ($P < 0.0001$) between bulls but not ($P > 0.05$) between ejaculates within bulls. The amounts of TAC were not related ($P > 0.05$) to amounts of SOD and GPx, but were negatively associated with LPO 0 h ($r = -0.85$; $P \leq 0.01$). The amounts of SOD showed positive relationships with LPO 0 h ($r = 0.71$; $P \leq 0.05$) and LPO 3 h ($r = 0.80$; $P \leq 0.05$). In conclusion, total antioxidant activity varied among bulls, but not between ejaculates within bulls. While the amounts of antioxidative enzyme GPx was not related to sperm quality and SOD was positively related with lipid peroxidation after thawing of sperm, whereas total antioxidative capacity was negatively correlated with lipid peroxidation of cryopreserved sperm.

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1. Introduction

Bull semen has been cryopreserved for more than a half century for artificial insemination. However, cryopreservation is detrimental to sperm, as it increases generation of reactive oxygen species (ROS) and decreases antioxidant concentrations. This imbalance between production of ROS and cellular antioxidants is defined as oxidative stress (Aitken and Baker, 2004). As sperm cell plasma membranes have high concentrations of polyunsaturated fatty acids, mammalian sperm are particularly susceptible to free radical attack and consequently lipid peroxidation (Aitken and Baker, 2004; Ashworth et al., 1995). Membrane lipid peroxidation (LPO) can alter membrane fluidity and these alterations have deleterious effects on sperm function, e.g., ability to fuse with oocytes (Ball, 2008; Storey, 1997). Bull sperm have limited endogenous antioxidants;

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the main antioxidant source for protection against ROS is the seminal plasma (Bilodeau et al., 2000; Dawra and Sharma, 1985; Dawra et al., 1984), which contains both enzymatic ROS scavengers, including superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), as well as low molecular weight scavengers (i.e., vitamin E, selenium, ascorbic acid, and vitamin A) (Agarwal et al., 2005; Agarwal and Saleh, 2002; Sikka et al., 1995). The low molecular weight scavengers from seminal plasma appeared as important if not more important than high molecular components (Alvarez and Storey, 1989).

On the basis of the above considerations, it could be interesting not only to measure enzymatic ROS scavengers, but also the total non-enzymatic antioxidants of seminal plasma. For the determination of these non-enzymatic agents an assay for measurements of the total antioxidant capacity has been used. Using this assay, it could be demonstrated that seminal plasma from fertile control men had a greater antioxidant capacity compared with seminal plasma from sub-fertile men. This fact as well as an inverse relationship observed between antioxidant capacity and lipid peroxidation potential indicates that this assay might be valuable for the evaluation of bull sperm quality and fertility.

The quality of cryopreserved semen does not only differ between bulls, but had great variation between ejaculates within bulls (Fischer et al., 2010). However, to the best of our knowledge there is no information about the inter- and intra-individual variability of antioxidants in bull semen. Therefore, the aim of the present study was to determine inter- and intra-individual variability of several enzymatic scavengers and total antioxidant capacity and its relationships with bull sperm quality before and after cryopreservation.

2. Material and methods

2.1. Chemicals

Chemicals were obtained from Sigma–Aldrich Co. (Steinheim, Germany), unless otherwise indicated.

2.2. Animals

Ejaculates from nine Holstein–Friesian bulls (3.5 ± 0.8 years), housed at an artificial insemination center in Lower Saxony, Germany, were used for the studies. The bulls were reared under similar feeding and management conditions during the entire duration of the study. Bulls were fed a basal diet consisting of hay-silage (15 kg/day), concentrate (2 kg/day), and 300 g/day of a mineral feed.

2.3. Semen collection, dilution, and freezing

Semen was collected using an artificial vagina. Eight consecutive ejaculates were collected twice weekly, always between 8:00 and 11:00 pm, in May 2007. Sperm concentration (Z2™ Coulter Counter® Cell and Particle Counter, Beckman Coulter GmbH, Krefeld, Germany), volume and total sperm number of each ejaculate were determined immediately after collection.

A small proportion of each ejaculate with a total sperm number of 450×10^6 sperm was diluted to a final concentration of 60×10^6 sperms/mL in a Tris-egg yolk based extender and packaged in 30 French straws (0.25 mL) at 20 °C. Ten straws were taken for analysis of sperm quality before cooling and cryopreservation. The remaining 20 straws were slowly cooled to 5 °C over a period of 180 min and frozen to −110 °C within 390 s on racks in a freezer (Model K, Hede Nielsen, Horsens, Denmark). Frozen samples were placed directly into liquid nitrogen and stored at least 24 h until analysis. The majority of the ejaculate was retained (nothing was added) and used as a source of seminal plasma.

2.4. Handling of seminal plasma

The majority of each ejaculate was centrifuged (3000 × g, 10 min, 4 °C) and the supernatant was frozen and stored at −20 °C for at least 24 h until analysis. Samples were measured within 6 months after collection. Before measurement, frozen samples of seminal plasma were thawed at room temperature (18–22 °C) and diluted 100-fold with phosphate buffered solution (PBS; 50 mM, pH 7.4) for assessment of TAC and GPx activity, and were diluted 1000-fold for determination of superoxide dismutase activity.

2.5. Antioxidant assays

2.5.1. Total antioxidant capacity-peroxyl radical assay

Total antioxidant capacity of seminal plasma was measured using an enhanced chemiluminescence assay according to Kolettis et al. (1999). Luminol (5-amino-2.3,-dihydro-1.4-phthalazinedione) stock solution (3.1 mM dissolved in dimethyl sulfoxide (DMSO)) and para-iodophenol (40 μM dissolved in DMSO) stock solution were prepared for the signal reagent and stored at room temperature (18–22 °C). The stock solutions were protected from light. Hydrogen peroxide (8.8 M) was stored at 4 °C until analysis. Horse radish peroxidase (peroxidase type VI-A from horse radish) with 1% bovine serum albumin (BSA) was prepared as 100 U/mL aliquots and stored at 20 °C. It was thawed and diluted 1:30 in PBS before measurements. Signal reagent was prepared by adding 110 μL luminol, 10 μL para-iodophenol, and 30 μL H_2O_2 (8.8 mM) to Tris buffer (0.1 M, pH 8.0). All procedures were conducted at room temperature unless otherwise indicated. The trolox solution was prepared as 1 mM aliquots in PBS and stored at −20 °C until analysis. It was thawed before measurement and diluted further with PBS to concentrations of 3, 6, 9, 12, 15, and 25 μM.

2.5.2. Instrumentation and automated measurement of TAC

The TAC-peroxyl radical assay was modified for use in an automated 96-well microplate reader and was performed via a fully automatic multiple pipetting system (Tecan Genesis AWS 200/8, Tecan AG, Switzerland). The multiple-pipetting system was combined with a microplate reader via software (Gemini, Version 4.2.17.304, Tecan AG, Switzerland). This system automates the pipetting,

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