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Impact of seminal plasma superoxide dismutase and glutathione peroxidase on cryopreserved buffalo spermatozoa

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ABSTRACT

Fifty semen samples were collected from sixteen buffalo-bulls (4–10 years old) and evaluated before cryopreservation. The activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-*S*-transferase (GST) as well as the levels of glutathione (GSH) and malondialdehyde (MDA) were assayed in the seminal plasma before freezing. Aspartate aminotransferase (AST) activity and cholesterol content were assayed in seminal plasma before freezing and after thawing. Results revealed the presence of SOD and GPx activities (0.07 ± 0.01 U/ml and 14.59 ± 0.50 nmol/min/ml, respectively) in buffaloes' seminal plasma. SOD activity was positively correlated with both of GSH level and GST activity in seminal plasma, and showed an inverse relationship with both cholesterol efflux and post-thaw abnormal tails of buffalo spermatozoa. A positive correlation was found between GPx activity in seminal plasma and abnormal tails and an inverse relationship with both post-thaw viability indices and increased motility in response to PTx. GST activity showed a positive correlation with the increased motility after addition of PTx and negative correlations with both of cholesterol level and AST activity. MDA levels were negatively correlated with motility after addition of PTx and positive correlations with both post-thaw abnormal acrosomes and tails. Buffalo seminal plasma contains high activities of SOD, GPx and GST enzymes and GSH levels that have an influence on the functional competence of cryopreserved spermatozoa.

1. Introduction

There is growing evidence that oxidative stress significantly impairs sperm functions (Ochsendorf et al., 1998). The role of reactive oxygen species (ROS) in pathophysiological functions of spermatozoa has been defined for many years (Bilodeau et al., 2000; Chatterjee and Gagnon, 2001). Due to their high content of polyunsaturated fatty acids, spermatozoa are susceptible to damage induced by reactive oxygen species (de Lamirande and Gagnon, 1995; McCarthy et al., 2010). The process of peroxidation is accompanied by extensive structural alterations, particularly in the acrosomal region of the sperm cell, a rapid and irreversible loss of motility, a profound change in metabolism, and a high rate of leakage of intracellular sperm constituents (Jones and Mann, 1977). Bovine spermatozoa are poorly adapted to metabolize the toxic hydrogen peroxide (Bilodeau et al., 2000) and frozen–thawed bull spermatozoa are more easily peroxidized than fresh

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spermatozoa (Trinchero et al., 1990; Sariözkan et al., 2009). This is associated with production of oxygen free radicals that leads to a reduction in sperm function following cryopreservation (Chatterjee and Gagnon, 2001). One of the main antioxidant enzymes involved in ROS detoxification in bovine semen is SOD (Bilodeau et al., 2000).

Mammalian semen is endowed with high levels of ROS scavengers including GPx (Li, 1975; Brown et al., 1977; Atig et al., 2012) and previous investigations indicated that cryopreservation could reduce the activity of GPx in semen with a subsequent decrease in the longevity and fertilizing ability of spermatozoa (Hammerstedt, 1993; Atig et al., 2012).

The present study was undertaken to determine the presence of SOD, GPx, GSH and GST in buffalo semen and their influence on the motility, viability, membrane integrity and morphologic abnormalities of cryopreserved spermatozoa.

2. Materials and methods

2.1. Animals

Sixteen adult buffalo-bulls (4–11 years old) from Abassia center for frozen buffalo semen, belonging to the General Organization for Veterinary Services, Ministry of Agriculture, Cairo, were used in this study. Semen collection was performed according to artificial insemination standard procedures. In most experiments, the first and the second ejaculates from each animal were pooled but in some the first or the second ejaculate was used separately.

2.2. Semen evaluation and processing

Fifty semen samples were collected in this study. Immediately after collection, semen samples were evaluated using conventional methods. An aliquot of one ml of each semen sample was centrifuged at 1500*g* for 5 min and the supernatant fluid (seminal plasma) was collected, immediately frozen and stored at −20 °C until analysis of SOD, GPx and GST activities and GSH and MDA levels. Following initial evaluation, semen samples were prepared for dilution in Laiciphos P488®-egg yolk–glycerol diluent (Sudheer, 2000). They were diluted (one-step dilution) to a final concentration of $50–60 \times 10^6$ sperm/ml diluent and cooled down to 5 °C within 1 h (Talevi et al., 1994). After cooling, the diluted semen was packaged in 0.5 ml straws during the equilibration period (4 h). Semen of four straws (2 ml) was centrifuged at 1500*g* for 10 min and the supernatant fluid was frozen at −20 °C pending analysis for cholesterol levels and AST activity before freezing. After equilibration, semen straws were frozen on liquid nitrogen vapor at about −80 to −120 °C for 10 min, after which the straws were immersed into liquid nitrogen at −196 °C (Sansone et al., 2000).

2.3. Post-thaw sperm incubation

Twenty-four hours after freezing, straws were thawed in a water bath at 40 °C for 30 s and then transferred to a water bath (30 °C) and incubated for 4 h. Sperm motility (percentage) was evaluated every hour and viability indices (analysis of motility at different times) were computed (Milovanov et al., 1964) using the following equation:

$$\mathrm{VI} = \Sigma \left[M \times \left(\frac{T - R}{2} \right) \right]$$

where; VI is the viability index, Σ is a sign for the sum total, M is the percentage of sperm motility, T is the time of next determination of motility and R is the time of previous determination of motility.

Immediately after thawing, smears stained with nigrosin-eosin stain (Dott and Foster, 1972) were examined for sperm abnormalities. The percentage of spermatozoa with abnormal acrosomes was recorded in other smears of thawed semen stained by Fast green FCF according to the method of Wells and Awa (1970). An aliquot (2 ml) of thawed semen was removed just after thawing and centrifuged at 1500*g* for 10 min. The supernatant was stored at −20 °C until analysis of cholesterol levels and AST activity. Following incubation for 4 h, a split sample of thawed semen was diluted (1:1) with tris-based buffer supplemented with 5 mM pentoxifylline (PTx) and sperm motility was determined after 15 min. Subsequently, the rate of increase in sperm motility was calculated.

2.4. Determination of antioxidants activities in seminal plasma

Buffalo-bulls' seminal plasma antioxidants activities and other parameters were determined by ELISA (Absorbance Microplate Reader ELx 800™ BioTek®, USA; Microplate Strip Washer ELx 50™ BioTek®, USA) using commercial kits. The antioxidants were:

(1) SOD (U/ml; Cayman Chemical Company, USA, Catalog No. 706002), the coefficients of variance of the intra- and inter-assay were 3.2% and 3.7%, respectively.
(2) GPx (nmol/min/ml; Cayman Chemical Company, USA, Catalog No. 703102), the coefficients of variance of the intra- and inter-assay were 5.7% and 7.2%, respectively.
(3) GST (nmol/min/ml; Cayman Chemical Company, USA, Catalog No. 703302), the coefficients of variance of the intra- and inter-assay were 3.6% and 4.8%, respectively.

The determined parameters were:

(1) GSH (μM; Cayman Chemical Company, USA, Catalog No. 703002), the coefficients of variance of the intra- and inter-assay were 1.6% and 3.6%, respectively.
(2) MDA (pmol/mg; OxiSelect™ MDA adduct ELISA kit, Cell Biolabs, Inc, USA, Catalog No. STA-332), the coefficients of variance of the intra- and inter-assay were 5.5% and 5.9%, respectively.
(3) AST (mIU/ml; CUSABIO BIOTECH CO., Ltd, Catalog No. CSB-E12708Ca), no significant cross-reactivity or interference was observed and the minimum detectable dose was typically <0.39 mIU/ml.
(4) Cholesterol (mmol/L; EnzyChrom™, BioAssay Systems, USA, Catalog No. E2CH-100), the correlation coefficient of the assay was 0.9995.