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Progesterone causes metabolic changes involving aminotransferases and creatine kinase in cryopreserved bovine spermatozoa

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ABSTRACT

Progesterone (P4) is capable of inducing acrosome reaction in many species. The objective of this study was to determine the activity of enzymes involved in metabolism that contribute to the redox state and supply energy for acrosome reaction in cryopreserved bull spermatozoa. To accomplish this aim, acrosome reaction was induced by P4 in capacitated and non-capacitated samples. Alanine and aspartate aminotransferases (ALT, AST) and creatine kinase (CK) activities were measured spectrophotometrically at 340 nm after acrosome reaction with P4. Oxygen consumption was measured polarographically. ALT and AST activities increased by the addition of P4 capacitated and non-capacitated samples. P4 addition provoked an increase in CK activity in non-capacitated spermatozoa compared to heparin capacitated spermatozoa with or without P4 addition. P4 increased oxygen consumption, the percentage of acrosome reacted spermatozoa as well as the absence of acrosome integrity in both capacitated and non-capacitated bovine spermatozoa, but oxygen consumption in P4 samples was significantly lower than in heparin capacitated spermatozoa ($P < 0.05$). Acrosome reaction induction by P4 required different creatine kinase activity with the same oxygen consumption and transaminases level to maintain oxidative metabolism and redox state through reducing equivalents transfer between cytosolic and mitochondrial compartment. In conclusion, P4 induces a lower oxidative metabolism during acrosome reaction in bovine cryopreserved spermatozoa, compared to heparin induced capacitation process.

1. Introduction

Spermatozoa exhibit a great versatility in their metabolism (Ferramosca and Zara, 2014), being able to activate different metabolic pathways to obtain energy. Noteworthy, the preferred pathway seems to be highly species specific (Piomboni et al., 2012) and also dependent on the substrates available in the female genital tracts (Storey, 2008).

Progesterone (P4) is present in high concentrations in follicular fluid as well as in the cumulus matrix of the oocyte (Osman et al., 1989). It has been observed that free P4 is capable of inducing (AR) in human (Meizel and Turner, 1991), pig (Melendrez et al., 1994) and mouse (Roldán et al., 1994) capacitated spermatozoa. P4 also induces AR in both capacitated (Ryu et al., 2014) and non-capacitated bovine spermatozoa by enhancing intracellular calcium and activating protein kinase C (Córdoba and Beconi,

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2001). Glycosaminoglycan heparin promotes capacitation and metabolic changes in order to provide energy and adequate redox state in bovine spermatozoa (Satorre and Córdoba, 2010). However, little is known about cryopreserved bovine sperm metabolism during capacitation and AR *in vitro*.

Sperm metabolism involves distinct shuttles (lactate/pyruvate, aspartate/malate, glycerol 3-phosphate and creatine/creatine phosphate) for the transport of reducing equivalents into the mitochondrial matrix (Piomboni et al., 2012; Córdoba et al., 2008). Lactate/pyruvate shuttle between cytosol and mitochondria in spermatozoa requires: (a) lactate dehydrogenase (LDH-X), an enzyme which is present in both cytosol and the mitochondria matrix; and (b) an aminotransferase that can supply substrates for LDH-X (Blanco, 1980; Córdoba et al., 2007).

Various alpha-ketoacids and alpha-hydroxyacids are substrates for LDH-X although it has a higher affinity for lactate and pyruvate (Trinchero et al., 1993). Alanine aminotransferase and aspartate aminotransferase are present in the majority of mammalian tissues and they have a key role in amino acid metabolism (Torchinsky, 1989). Malate/aspartate shuttle requires the cooperation of mitochondrial and cytosolic aspartate aminotransferase and malate dehydrogenase in order to transport reducing equivalents across the mitochondrial membranes (Setoyama et al., 1990).

Creatine kinase (CK) shuttle is a source of extramitochondrial ATP and responsible to transfer energy from mitochondria to cytosol (Kaldis et al., 1996; Wallimann et al., 2011). In bovine spermatozoa CK is an enzyme involved in supporting cytosolic ATP changes during heparin capacitation (Córdoba et al., 2008). Furthermore, CK activity is indicative of normal spermatogenesis and maturation and a predictor of the fertilizing potential in human sperm (Huszar et al., 1997; Durutovic et al., 2013). In cryopreserved bovine sperm, capacitation requires equilibrium between oxidative damage susceptibility and reactive oxygen species level and CK activity is associated with redox state variation and energy sources (Córdoba et al., 2008).

Pursuant to our previous studies on the respiratory burst generated with heparin in bovine cryopreserved spermatozoa (Córdoba et al., 2006) and sperm metabolism during capacitation (Córdoba et al., 2005, 2007, 2008), the purpose of the present study was to determine alanine aminotransferase, aspartate aminotransferase and CK activities in AR induced by P4 in capacitated and non-capacitated bovine sperm.

2. Materials and methods

2.1. Materials

All chemicals used were purchased from Sigma Chemical Company (St Louis, MO, USA).

2.2. Semen collection and cryopreservation

Semen from three 4–5 year-old Holstein bulls of proven fertility was collected using an artificial vagina. Bulls were routinely collected to provide semen for artificial insemination and were maintained under uniform nutritional and management conditions throughout the study. Progressive motility exceeded 70% in all ejaculates. Once a week, two ejaculates were obtained from each bull, they were pooled and diluted in a buffer containing 0.20 M Tris, 0.06 M citrate, 0.13 M glycine, 0.06 M fructose, 20% egg yolk and 7% glycerol (2:1 ratio) to reach a final concentration of $3.0–4.5 \times 10^7$ spermatozoa/ml. The diluted semen was slow cooled (1 °C/min) up to 5 °C and after equilibration it was frozen in vapors of liquid nitrogen (−120 °C) for a period of 10 min and then straws were dipped in nitrogen for storage.

2.3. Sperm suspension

Frozen pooled semen were thawed at 37 °C and incubated for 10 min in modified Tyrode's medium (TALP medium) (100 mM NaCl, 3 mM KCl, 0.3 mM NaH_2PO_4, 10 mM $NaHCO_3$, 40 mM Hepes, 1.5 mM $MgSO_4$, 22 mM sodium lactate and 1.2 mM sodium pyruvate). Vigor and percentage of cells with progressive motility were evaluated at 38 °C using a common optical microscope at 10× magnification after 10 min of thawing. Sperm concentration was determined by hematocytometry in a Neubauer chamber. After progressive motility and vigor evaluation, samples were centrifuged at 600×*g* for 5 min and resuspended in TALP medium with $CaCl_2$ (2.1 mM) and BSA (6 mg/ml), and then incubated with capacitation and acrosome reaction inductors or in the presence of no additional compounds (controls).

2.4. Induction of sperm capacitation and acrosome reaction in vitro

Sperm suspension was incubated for 15 min at 38 °C with heparin (60 μg/ml) (Fukui et al., 1990; Córdoba et al., 1997). After capacitation, sperm suspensions were subjected to the effect of a water-soluble progesterone solution (1 μmol/l) at 38 °C for 10 min to induce acrosome reaction (Córdoba and Beconi, 2001).

2.5. Evaluation of acrosome reaction

Acrosome-reacted spermatozoa were determined by the epifluorescence chlortetracycline technique (CTC) in an epifluorescence microscope (Jenamed 2, Carl Zeiss, Jena, Germany) (Córdoba et al., 1997; Beorlegui et al., 1997). Three different CTC patterns were recognized: (F) non-capacitated spermatozoa, with a fluorescent head; (B) capacitated spermatozoa, with a non-fluorescent band in the post-acrosomal region and (AR) spermatozoa with reacted acrosome, with low fluorescence in the whole cell except for a band in the equatorial segment.

2.6. Evaluation of sperm viability and acrosome integrity

Live spermatozoa percentages were determined by vital trypan blue stain using differential interference contrast (DIC) microscopy (Jenamed 2, Carl Zeiss, Jena, Germany). An aliquot of sperm suspension from each different treatment was incubated with an equal volume of 0.25% (w/v)