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Role of residual cytoplasm on oxidative status during sperm maturation in dogs

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ABSTRACT

During maturation sperm cells acquire their fertilizing ability; however, this event also produces reactive oxygen species and as such local antioxidant protection is required. Thirteen epididymis from dogs were used, and sperm samples were collected from different segments of the epididymis (i.e. caput, corpus and cauda). The samples were evaluated for motility and vigor, permeability of plasma membrane, presence of cytoplasmic droplet, acrosome integrity and the activity of the antioxidant enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD). Samples collected from the cauda showed higher motility, vigor and lower permeability of plasma and acrosomal membranes in relation to the corpus and caput, indicating different levels of maturity. The enzyme activity remained unchanged in the samples. A significant positive correlation was observed in the epididymal caput, between distal cytoplasmic droplet and SOD, and a negative correlation, in the epididymal cauda, between proximal cytoplasmic droplet and GPx, indicating the physiological need of the specific antioxidants in these segments.

1. Introduction

During spermatogenesis in mammals, most of the cytoplasm is degraded by Sertoli cells, leaving only a small residue in spermatozoa known as the cytoplasmic droplet (Cooper, 2011). While transiting through the epididymis, sperm cytoplasmic droplet migrates from the proximal area of the mid piece to the distal area as part of the final sperm maturation process (Zhang et al., 2003). In addition to the migration of the cytoplasmic droplet in the epididymis, the spermatozoa undergo other morphological and functional changes, such as a gain of motility and the ability to recognize and fertilize to the oocyte (Jervis and Robaire, 2001).

These structural and functional modifications may, however, cause increased sperm susceptibility to attack by the reactive oxygen species (ROS) present in the epididymal lumen (Irvine et al., 2000). Nevertheless, for the occurrence of sperm capacitation, the ROS generated and the antioxidant mechanisms must be balanced (de Lamirande et al., 1997). When an imbalance occurs between antioxidant capacity and ROS production, oxidative stress occurs (Kashou et al., 2013). Spermatozoa are particularly susceptible to oxidative stress due to reduced cytoplasm content and, ultimately, a limited amount of enzymatic antioxidant (Aitken and Krausz, 2001; Aitken and Sawyer, 2003). Thus, spermatozoa are extremely dependent upon antioxidant protection provided by the reproductive tract environment. According to several

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authors, glutathione peroxidase (GPx) and superoxide dismutase (SOD) are involved in the complex maturation process along the epididymal transit, by the maintenance of sperm motility, membrane integrity, DNA integrity, counter acting against lipid peroxidation (Alvarez and Storey, 1989; Kobayashi et al., 1991; Rejraji et al., 2002; Roca et al., 2005; Drevet, 2006; Kasimanickam et al., 2006; Noblanc et al., 2012).

Therefore, it is expected that the presence of these antioxidants in the epididymal lumen is crucial for the reduction of oxidative stress damage to spermatozoa and for providing necessary modifications during the sperm maturation process. In a previous study, Angrimani et al. (2014) highlighted the enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD) as antioxidants of great relevance in the canine epididymal lumen. However, even with the protective role of antioxidants in the epididymal fluid, the modifications that occur in the sperm cell and the long period of transit through the epididymis predispose the spermatozoa to oxidative stress.

Although the cytoplasmic droplet is considered a defect in ejaculated sperm, studies have suggested an antioxidant role during sperm transit through the epididymis in cattle, which could contribute to oxidative homeostasis (Nichi et al., 2007). On the other hand, Thundathil et al. (2001) reported a pro-oxidative function of the proximal cytoplasmic droplet in spermatozoa found in the epididymis of men. Therefore, the exact role of the cytoplasmic droplet on the oxidative physiology of the epididymal fluid and ejaculate, as well as the antioxidant environment in the canine epididymis, remains unclear.

Dogs are considered the experimental model of choice for endangered species and humans (Kirchhoff, 2002; Thomassen and Farstad, 2009). Furthermore, canine reproduction studies contribute to the physiological knowledge of these species, which may allow for the development of new biotechnologies and aid the treatment of diseases related to sperm maturation (Ma et al., 2013). In this context, the objective of this study was to evaluate the enzymatic activity of the antioxidants during structural and functional changes in epididymal spermatozoa, especially with regard to the possible role of cytoplasmic droplets during this process.

2. Materials and methods

2.1. Animals and sample collection

Thirteen healthy mature dogs (aged from 1 to 6 years) of distinct breeds and body weights were used. The current study was approved by the Bioethics Committee of the School of Veterinary Medicine and Animal Science – University of São Paulo (protocol number 2277/2011). Unless otherwise stated, all chemicals and reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Samples were collected from the epididymis after bilateral orchiectomy. Since surgical procedure was not performed in the same location as the sperm analysis, epididymides were stored at 5 °C for not less than 13 h and for a maximum of 24 h. According to Hori et al. (2009), no effect of epididymal storage was observed on sperm viability if testicles were kept under 4 °C over a period of 24 h. Epididymal sperm were collected by incisions (<1 mm) in the caput, corpus and cauda epididymides with a scalpel blade. Dissection was performed carefully to avoid sectioning of blood vessels. The flowing epididymal fluid was collected with an automatic pipette, and the total volume of the epididymal sample was resuspended in 300 μL of PBS medium (phosphate buffered saline). Therefore, the dilution rate varied according to epididymal size, similar to a previously described protocol (Kaabi et al., 2003). To calculate the epididymal fluid dilution factor, tubes containing solely the PBS medium were weighted before (tube + PBS) and after the addition of the epididymal sample (tube + PBS + epididymal fluid). Because of the small volume recovered, the experimental samples from the left and right epididymides of the same animal were pooled.

2.2. Immediate sperm evaluation

Samples from the epididymal segments (i.e., cauda, corpus and caput) were immediately evaluated for sperm percentage motility (%) and vigor (arbitrary scale from 0 to 5) using a drop of semen placed on a pre-warmed glass slide with coverslip. Evaluation was performed under light microscopy (Nikon, Eclipse E200, Japan) at 400× magnification.

To evaluate plasma membrane permeability and the presence of cytoplasmic droplets, Eosin/Nigrosin stain was used. In brief, 5 μL of semen and 5 μL of the previously prepared stain were placed in a pre-warmed glass slide. The sperm smear was evaluated under light microscopy (Nikon, Eclipse E200, Japan) at 1000× magnification. We considered damaged sperm (membrane lesion) as pink colored cells, while intact sperm (membrane integrity) presented no stain (Lagergren, 1953). Sperm cytoplasmic droplet was classified as proximal, distal and no-droplet, considering the presence and position of the droplet in the sperm mid piece (Barth and Oko, 1989).

Sperm acrosome integrity was assessed using the protocol described by Pope et al. (1991), adapted for dogs. Briefly, 5 μL of semen was mixed with 5 μL of Fast Green/Rose Bengal stain in a pre-warmed glass slide. Smears were evaluated under light microscopy (Nikon, Eclipse E200, Japan) at 1000× magnification. If the sperm acrosomal region stained in purple or darker than the post-acrosomal area, spermatozoa acrosome was considered as intact. Whenever the acrosomal region remained unstained or brighter than the post-acrosomal area, acrosome was considered as damaged. The percentage of stained sperm was analyzed through optical microscopy by counting 200 cells.

2.3. Antioxidant enzymes activity assessment

The activity of the enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) was determined in all samples collected from the caput, corpus and cauda epididymides.

The activity of SOD was measured by the indirect reduction of cytochrome C by the superoxide anion (O_2^-), which was generated by the xanthine/xanthine oxidase system. The reaction medium contained cytochrome C