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Pentoxifylline effects on capacitation and fertility of stallion epididymal sperm

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ABSTRACT

The aims of this study were to determinate whether pentoxifylline (PTX) increases the motion parameters of fresh and frozen-thawed equine epididymal spermatozoa, to evaluate the tyrosine phosphorylation of frozen-thawed epididymal sperm in the presence of PTX and to determine whether the PTX-treatment of stallion epididymal sperm prior to freezing improves the fertility response of mares to a reduced number of spermatozoa per insemination dose. Fifty epididymis were flushed with a skim milk based extender with or without PTX. The pre-treatment with PTX enhanced the sperm motility after being harvested ($P < 0.05$); however the freeze-thaw process did not alter the sperm kinematics between control and treated samples ($P > 0.05$). Plasma membrane integrity did not differ between control and PTX group after recovery and after thawing ($P > 0.05$), as observed in tyrosine phosphorylation, which the PTX treatment did not alter the percentage of tail-associated immunofluorescence of cryopreserved epididymal sperm ($P > 0.05$). For the fertility trial, different insemination groups were tested: 800×10^6 epididymal sperm (C800); 100×10^6 epididymal sperm (C100); 100×10^6 epididymal sperm recovered in an extender containing PTX (PTX100). The conception rates for C800; C100 and PTX100 were 68.7% (11/16); 31.5% (5/16) and 50% (8/16), respectively. The conception rate did not differ among groups ($P > 0.05$), however, a low number of animals was used in this study. A trend toward significance ($P = 0.07$) was observed between C800 and C100 groups. In conclusion, PTX has no deleterious effect on sperm motility, viability and capacitation of cryopreserved stallion epididymal sperm. The conventional artificial insemination with 100×10^6 sperm recovered with PTX ensures acceptable conception rates and maximize the limited number of doses of cryopreserved stallion epididymal sperm.

1. Introduction

The recovery of epididymal sperm might be the last chance to preserve the genetic material of endangered species or in case of unexpected injury of valuable sires, which will end their breeding career or cause death. Thus, many studies investigating different transportation and storage methods (Monteiro et al., 2013; Stawicky et al.,

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2016), collection techniques (Cary et al., 2004; Bruemmer, 2006), flushing extenders (Papa et al., 2008; Guasti et al., 2013) have been performed in order to improve recovery and fertility rates of cauda epididymal sperm.

After collection, the epididymal sperm may remain immotile due to the quiescent state in the cauda epididymis (Turner and Reich, 1985). The quiescence has been reported in the rat, mouse, hamster (Morton et al., 1978) and bull spermatozoa (Cascieri et al., 1976) and may be dependent of some factors in the epididymal fluid *e.g.* proteins (Usselman and Cone, 1983), ions (Wong et al., 1983), pH (Acott and Carr, 1984) and cyclic AMP (Luconi et al., 2005).

Pentoxifylline (PTX) is a phosphodiesterase inhibitor of the methylxanthine group inhibiting the breakdown of cyclic adenosine monophosaphate (cAMP) (Tash, 1990). In sperm, cAMP has been shown to activate protein kinase (PKA) regulating protein tyrosine phosphorylation, which is an important regulatory pathway in modulating the events associated to capacitation (Naz and Rajesh, 2004). PTX is routinely used in assisted reproductive technology (ART) in humans (Carrel and Aston, 2013); in order to stimulate tail movements in immotile testicular spermatozoa and in ejaculated spermatozoa with very low motility sperm motility (Nassar et al., 1999) and it has been shown to improve the fertilization rates (Kovacic et al., 2006).

In equine-ejaculated spermatozoa, the presence of PTX enhances the quality of chilled and frozen semen (Goulart et al., 2004; Stephens et al., 2013). Therefore, the addition of PTX to epididymal sperm is assumed to improve the motility of the quiescent sperm cells and the fertility rate. However, in a previous study from our laboratory, treatment with equine epididymal sperm with PTX did not affect the post-thaw motility (Guasti et al., 2013).

The sperm–oocyte interaction depends on a series of events that occur in the female reproductive tract, which comprises the membrane-architectural and metabolic changes in spermatozoa (Yanagimachi, 1994). These maturational changes correspond to sperm capacitation, in which only capacitated sperm can bind to the zona pellucida (Gadella et al., 2001). The seminal plasma (SP) proteins are also described to be involved in sperm capacitation (Topfer-Petersen et al., 2005). The major stallion SP proteins (HSP-1 and HSP-2) are associated to sperm surface during ejaculation and their heparin-binding properties indicate a potential role in modulation of capacitation (Topfer-Petersen et al., 2005). However, the epididymal sperm is not exposed to seminal plasma and the capacitation process may be affected by the absence of these proteins.

The optimization of the use of cryopreserved epididymal sperm from a particular stallion is critical since only a limited amount of genetic material is available. Using a reduced number of spermatozoa per insemination may be an alternative to maximize the efficiency of this technique and to increase the fertility rate in the field. The hysteroscopic insemination of 150×10^6 fresh epididymal spermatozoa resulted in pregnancy rates per cycle of 45% (Morris et al., 2002) and in the same study, higher pregnancy rates were obtained from hysteroscopic insemination (9/51, 18%) compared to the ones from conventional insemination (1/13, 8%) of 200×10^6 frozen–thawed epididymal spermatozoa. However, the hysteroscopic insemination requires expensive equipment and special-trained personnel, thus, is not readily available to the practitioner in the field. Therefore, strategies to improve the fertilizing ability and the optimization of stallion epididymal sperm should also be developed considering its applicability in the field through conventional artificial insemination.

The effect of pentoxifylline on capacitation and fertility of stallion epididymal spermatozoa has not been reported previously. The purpose of this study were: first, to determinate whether PTX increases the motion parameters of fresh and frozen-thawed equine epididymal spermatozoa; second, to evaluate the tyrosine phosphorylation of frozen-thawed epididymal sperm in the presence of PTX; and third, to determine whether the PTX-treatment of stallion epididymal sperm prior to freezing improves the fertility response of mares to a reduced number of spermatozoa per insemination dose.

2. Materials and methods

2.1. Animals

A total of 25 stallions (experiment I and II: 23 stallions and experiment III: 2 stallions) between 3 and 5 years old were used. The fertility trial was conducted with 16 healthy mares between 4 and 15 years old with proven fertility. Experimental procedures were performed in accordance with the Institutional Ethics Committee of School of Veterinary Medicine and Animal Science (FMVZ), São Paulo State University (UNESP), concerning the protection of animals used in scientific experimentation.

2.2. Orchiectomy

Before castration, three ejaculates from each stallion were collected at 48-hr intervals to deplete epididymal sperm reserves. Then, after clinical exam, the stallions were submitted to bilateral orchiectomy using emasculator in standing position, under neuroleptic analgesia with 1% acepromazine (0.04 mg/kg) and after 15–20 min 10% xylazine (0.5 mg/kg), both intravenously (IV). The regional anesthesia was performed by injecting 10 mL of 2% lidocaine with epinephrine under the scrotal skin along the incision line using a 21-gauge 1.25 in. needle. An incision was made through the skin and fascia to expose the common vaginal tunic. The ligament of the epididymal cauda was detached, the testis was pushed dorsally, and the emasculator was positioned perpendicular to the spermatic cord and kept clamped for 2 min. The testis was removed, cleaned with lactated ringer's solution (LRS) and the deferent duct was tied with a silk thread. The testis of each side was identified, placed in plastic bags containing 40 mL of LRS and stored in semen transport containers at 5 °C until arrival at the laboratory (approximately 5 h).

After orchiectomy, tetanus (Lyophilized Anti-Tetanus Serum 5000 UI, Lema Injex Biologic, SP, Brazil) and antibiotic prophylaxis (benzilpenicilin benzatin 9,000,000 UI/per animal; Pentabiotic Veterinary Reinforced, Fort Dodge, SP, Brazil) were adopted. The horses were kept together in