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The ability of feline spermatozoa in different epididymal regions to undergo capacitation and acrosome reaction

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ABSTRACT

The sperm maturation process that occurs in the epididymis is a necessary process for spermatozoa to acquire motility and the ability to undergo capacitation, which is an important key for fertilization. The aim of this study was to evaluate the ability of feline spermatozoa from different regions of the epididymis to undergo capacitation and acrosome reaction. Experiment I: epididymal spermatozoa from caput, corpus and cauda regions were placed in phosphate buffered saline (control medium) and *in vitro* fertilization medium (capacitating conditions). Sperm motility, motility patterns, plasma membrane integrity and tyrosine phosphorylation were evaluated at time 0 and 60 min after incubation. Experiment II: spermatozoa were treated with 2 μM of calcium ionophore (A23187) to induce the acrosome reaction and acrosome reaction was evaluated. The results showed a significant effect of region with a higher percentage of tyrosine phosphorylation in spermatozoa from the cauda than in the caput or corpus regions ($P = 0.0061$; $P = 0.0088$). Spermatozoa from corpus and cauda showed higher values in the majority of the measured motility parameters than spermatozoa from the caput ($P < 0.0001$). Spermatozoa from all epididymal regions can undergo the acrosome reaction *in vitro* in response to induction by calcium ionophore with no difference between regions ($P > 0.05$). Spermatozoa from all epididymal regions were able to undergo capacitation. Higher percentage of tyrosine phosphorylation in spermatozoa from the cauda reflect that they more easily underwent capacitation compared to spermatozoa from caput and corpus which required more time of incubation for capacitation. In conclusion feline epididymal spermatozoa from all regions can undergo capacitation and acrosome reaction *in vitro* and do not require incubation under capacitating conditions.

1. Introduction

Many of the world's wild feline species are threatened by poaching and habitat loss. Although there have been attempts to preserve and protect these species in the wild, their numbers continue to decrease. Genetic management is a key tool in ensuring the continued survival of endangered feline species. Gene banks are ideally created before the number of individuals, and thereby the genetic variation in the species, have reached critically low

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limits (Pukazhenthi et al., 2006). Preservation of epididymal spermatozoa from endangered or valuable individuals maintains genetic diversity that would otherwise be lost. After an animal dies, it is possible to rescue genetic material in the form of spermatozoa from the epididymis of males (Cocchia et al., 2010). Epididymal spermatozoa, similar to ejaculated spermatozoa, can be preserved for assisted reproductive technology; artificial insemination (AI), *in vitro* fertilization (IVF) (Tsutsui et al., 2003) and intracytoplasmic sperm injection (ICSI) (Bogliolo et al., 2001).

Currently epididymal spermatozoa are only collected from the cauda for preservation in many species including felids (Martinez-Pastor et al., 2006; Tajik et al., 2007; Cocchia et al., 2010; Toyonaga et al., 2011). However, the number of feline spermatozoa that can be collected from the cauda is usually low and varies in number of spermatozoa from 20 to 60×10^6 spermatozoa (Mota and Ramalho-Santos, 2006; Tsutsui, 2006), often not exceeding the number required for one insemination with current technology (Axner, 2008). If spermatozoa collected from caput and corpus have similar properties as spermatozoa from the cauda, the total number of spermatozoa that can be collected and saved from one individual could be increased by adding spermatozoa from these sites. An increased number of preserved spermatozoa would in turn increase the chance to produce offspring.

It is known that the maturation process which occurs during sperm transport through the epididymis is essential for sperm development and for spermatozoa to acquire ability to penetrate and fertilize the female gamete (Axner, 2006). Many previous studies demonstrated that spermatozoa from the cauda epididymidis are mostly mature, have ability to undergo capacitation, acrosome reaction and are fertile when used for IVF or AI (Axner et al., 1999; Tsutsui et al., 2003; Toyonaga et al., 2011). Capacitation, a series of biological and physiological changes, is known to be an important mechanism prior to the ability of the spermatozoon to fertilize the oocyte. After sperm binding to the zona pellucida of the egg, the sperm must undergo an exocytotic process called the acrosome reaction (Ickowicz et al., 2012). This event is required for fertilization, because sperm need to get through the zona pellucida and then fertilize the egg. Nevertheless, spermatozoa from the caput and corpus epididymal regions in felid species have, to the authors' knowledge, not been tested for their ability to undergo capacitation and acrosome reaction previously.

Phosphorylation of proteins is known to play an important role in regulating numerous cellular activities as well as sperm functions (Visconti et al., 1995b; Urner and Sakkas, 2003; Nagdas et al., 2005). An increase in sperm tyrosine phosphorylation during capacitation has been shown in various species including mouse (Visconti et al., 1995b) hamster (Si and Okuno, 1999; Nagdas et al., 2005), human (Leclerc et al., 1997), bull (Galantino-Homer et al., 2004), pigs (Tardif et al., 2001; Kumaresan et al., 2012) and cat (Pukazhenthi et al., 1998). However, a study of tyrosine phosphorylation in feline epididymal spermatozoa in different regions has never been conducted.

Therefore, the aims of the present study were (1) to investigate the ability of spermatozoa from different regions of the epididymis to undergo capacitation. (2) To compare the ability of spermatozoa from different maturational status to undergo the acrosome reaction after induction with calcium ionophore A23187. The ability of spermatozoa from each part of epididymis to undergo capacitation reflects their potential for use in different assisted reproductive technologies. Domestic cats were used as a model for wild felids, and the new knowledge obtained could be beneficial for collection of genetic material from epididymis and improve conservation efforts for its wild relatives.

2. Materials and methods

2.1. Animals

The study included epididymal spermatozoa from 23 privately owned domestic male cats, various breeds, aged between 6 months and 5 years. All cats were subjected to routine castration at veterinary clinics and the University Animal Hospital at the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. After testes and epididymides were removed from the cats, they were kept in a plastic bag in a cold box at 4 °C and transferred to the laboratory. The experiment was performed within 24 h after the testes were removed from the cats.

2.2. Sperm recovery

Epididymides from the left and right side were dissected free from visible blood vessels and connective tissues. The division was done macroscopically by the same person. Each epididymis was divided into the three regions; caput, corpus and cauda, with each region being further divided into two samples. Tissue segments from each part were transversely cut into small pieces and placed in warm media (200 μl for caput and 300 μl for corpus and cauda) to let the spermatozoa come out.

2.3. Experiment I: the capability of spermatozoa from different regions of epididymis to undergo capacitation

Epididymal spermatozoa from 17 cats were used. The sperm sample from one side was placed in warm (38 °C) phosphate buffered saline (PBS) [137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4, 280 mOsm] medium as a control medium (medium without ingredients known to induce capacitation) and from the other side was placed in warm (38 °C) *in vitro* fertilization medium (IVF medium) [195.88 nM NaCl, 2.13 mM KCl, 1.43 mM $CaCl_2 \cdot 2H_2O$, 0.83 mM $MgCl_2 \cdot 6H_2O$, 0.33 mM $NaH_2PO_4 \cdot H_2O$, 4.37 mM Glucose, 9.61 mM $NaHCO_3$, 0.0036 mM Na-Pyruvate, 0.01 mM L-glutamine, 0.022 mM Ca-lactate, 6 g BSA, 50 mg Gentamycin, pH 7.4, 280–300 mOsm], composition known to induce capacitation, which had been gassed under 5% CO_2 in air at high humidity at least 20 min. The left and right side of the epididymis were alternately allocated to each of the two different media. After 10 min of incubation at 38 °C, the tissue segments were removed. Sperm motility, motility pattern, membrane integrity and tyrosine phosphorylation were evaluated in fresh (time 0) and immediately after

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