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Osmotic tolerance of feline epididymal spermatozoa

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ABSTRACT

During the cryopreservation process, spermatozoa are exposed to hypertonic solutions contributed by the high concentration of cryoprotectant. During addition and removal of cryoprotectant the spermatozoa are subjected to a substantial osmotic stress. Spermatozoa of different species and different stages of maturation may have different susceptibility to osmotic stress depending on the biology of the cell membrane and this will affect their tolerance to the freezing-thawing stress. The aims of this study were to determine the osmotic tolerance limits for motility, membrane integrity and mitochondrial membrane potential of feline epididymal spermatozoa and to study the effect of osmotic stress on the feline spermatozoa of different epididymal regions. Epididymal spermatozoa from three regions (caput, corpus and cauda) were pre-exposed to various osmolalities (75, 300, 600, 900, 1200 mOsm) in a single step for 10 min and returned to 300 mOsm afterward. Percentage of motile spermatozoa was measured subjectively and membrane integrity (SYBR-14 positive cells) was evaluated prior to and after exposure to different osmolalities. The mitochondrial membrane potential (JC1) of spermatozoa were evaluated using flow cytometer and compared between epididymal regions (caput, corpus and cauda). All the parameters were compared using a mixed procedure. The percentage of motile epididymal spermatozoa decreased significantly when spermatozoa were exposed to 75 mOsm and 600 mOsm. Epididymal spermatozoa showed signs of damage when pre-exposed to 900 and 1200 mOsm and returned to isotonic condition as significant reduction of membrane integrity and mitochondrial membrane potential were observed ($P < 0.05$). The plasma membrane of spermatozoa from the cauda epididymal region showed higher susceptibility to osmotic stress than the other regions as demonstrated by a significant difference between regions after return to isotonicity from 900 mOsm ($P > 0.01$) and a difference between caput and corpus after return from 1200 mOsm ($P < 0.05$). The corpus and cauda epididymal spermatozoa had higher percentage of spermatozoa with high mitochondrial membrane potential than those from caput when exposed to 75, 300 and 600 mOsm ($P < 0.05$). In conclusion, a single step exposure to hypertonic solution of greater than 600 mOsm prior to return to isotonic condition can cause severe damage to sperm membrane and mitochondrial membrane potential compared to non-returning (exposure to various osmolality but not returned to isotonic condition). Changes in osmolality impacted mostly on sperm motility. Spermatozoa from cauda epididymis were more

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susceptible to osmotic stress compared to those from corpus and caput indicating that the maturation changes in the sperm membrane during passage through the epididymis increase susceptibility to the osmotic changes that may occur during sperm cryopreservation.

1. Introduction

Preservation of feline epididymal sperm is currently a subject of interest with the purpose to rescue genetic material from threatened and endangered wild felids species in which the genetic materials could be lost by unexpected death of the animals (Cocchia et al., 2010). Epididymal spermatozoa, similar to ejaculated spermatozoa, can be used by assisted reproductive technologies (ARTs) such as; artificial insemination (AI), *in vitro* fertilization (IVF) (Tsutsui et al., 2003; Tebet et al., 2006) and intra-cytoplasmic sperm injection (ICSI) to produce offspring (Bogliolo et al., 2001).

Currently, spermatozoa from corpus region of epididymis have been proved to have similar abilities as spermatozoa from cauda, capability to undergo capacitation, fertilize oocytes *in vitro* and to be able to survive cryopreservation (Kunkitti et al., 2015; Kunkitti et al., 2016a; Kunkitti et al., 2016b). However, up to 40–60% of feline epididymal sperm motility is lost in the cryopreservation process (Kunkitti et al., 2016b). In order to facilitate improvement in cryopreservation of feline epididymal sperm, a better understanding of basic cryobiological properties of the cells are needed, including their tolerance limits and the effects of osmotic stress.

During the cryopreservation process, spermatozoa are exposed to high concentrations of cryoprotectants (CPAs) which equilibrate the spermatozoa and minimise ice crystal formation within the cells. However, addition and removal of CPAs subjects the spermatozoa to a substantial osmotic stress. Before freezing, high concentrations of CPAs are added to the sperm suspension. Spermatozoa are exposed to a hyperosmotic environment and left frozen in high concentration of CPAs (Guthrie et al., 2002). In the thawing process, the sperm suspensions in hypertonic solution are diluted and returned to near isotonic conditions. The rapid osmolality changes throughout the process can lead to loss of functional integrity, such as sperm plasma membrane integrity, DNA integrity and motility. (Watson, 2000; Ball and Vo, 2001)

Spermatozoa from different regions of the epididymis differ in their degree of maturation and may have different susceptibility for osmotic stress depending on the composition of the cell membrane and this will affect their tolerance for the freezing-thawing stress. The objectives of this study were to evaluate the osmotic tolerance limits for motility, membrane integrity and mitochondrial membrane potential of feline epididymal spermatozoa and to determine the effect of osmotic stress on the epididymal spermatozoa of different epididymal regions. The results contributed to a better understanding of basic physiological properties of epididymal spermatozoa from different regions which may be helpful for designing cryopreservation protocols for epididymal sperm.

2. Materials and methods

2.1. Experimental design

Epididymal spermatozoa from three different regions (caput, corpus and cauda) were exposed to solutions of various osmolalities (75, 300, 600, 900, 1200 mOsm) in a single step for 10 min and then returned to 300 mOsm. Percentage of motile spermatozoa and sperm membrane integrity (SYBR-14/PI) was evaluated after exposure and after returning to 300 mOsm (isotonic condition). The mitochondrial membrane potential (JC1) were evaluated by flow cytometry after the return to isotonic condition. The experiment was performed according to Thailand regulations.

2.2. Animals

The study included epididymal spermatozoa from 55 privately owned domestic male cats of various breeds and ages. All cats were subjected to routine castration by closed-technique at the Veterinary Public Health of Bangkok, Thailand. After testes and epididymides were removed from the cats, they were immediately kept in 0.9% (w/v) normal saline solution supplemented with penicillin-streptomycin at room temperature until epididymal spermatozoa were transferred to the laboratory. The experiment was performed within 6 h after the testes were removed from the cats.

2.3. Epididymal sperm recovery

The epididymides were dissected free from blood vessels and connective tissues. Each epididymis was divided into three regions; caput, corpus and cauda. The epididymides from 5 cats were randomly pooled as one replicate in order to increase the sperm numbers and also to reduce individual variations. A total of 11 replicates were performed in this study. To collect spermatozoa from epididymides, the tissue segment of each region was transversely cut into small pieces and placed in 2 mL 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 for 10 min. After 10 min, the tissues were removed. Sperm concentration and total sperm number were evaluated.