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The tolerance of feline corpus and cauda spermatozoa to cryostress

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ABSTRACT

Epididymal sperm preservation can be used to avoid the total loss of genetic material in threatened species. Spermatozoa from the corpus, as from the cauda, are motile and can undergo capacitation. Thus, they can potentially be preserved for assisted reproductive technologies. However, cryopreservation of spermatozoa has a direct detrimental effect on sperm quality. The aim of this study was to compare the chromatin stability and the survival rate of spermatozoa from the corpus and cauda epididymis after cryopreservation. Epididymal spermatozoa were collected and cryopreserved from the corpus and cauda of 12 domestic cats. Sperm motility, progressive motility, membrane integrity, acrosome integrity, and DNA integrity were evaluated before and after freezing thawing. The average total number of spermatozoa collected from the corpus was lower ($10.2 \times 10^6 \pm 7.4$) than that from the cauda epididymis ($24.9 \times 10^6 \pm 14.4$; $P = 0.005$). The percentage of spermatozoa with intact DNA did not differ significantly whether it was collected from the corpus or cauda regions and did not decrease after freezing thawing in either region. However, motility of spermatozoa from both regions was affected by the freezing thawing process with a significant decline in motility after thaw compared with fresh spermatozoa. A significant difference in the percentage of motile sperm between the corpus and cauda was observed after the freezing thawing process ($P < 0.001$). Although sperm motility was lower in postthaw spermatozoa from the corpus epididymidis than from the cauda, the rate of the reduction did not differ between regions. This study indicates that the cryopreservation process does not have a negative effect on chromatin stability of feline epididymal spermatozoa. Spermatozoa from the corpus region have a similar freezability as spermatozoa from the cauda region. Therefore, preservation of spermatozoa from the corpus and the cauda epididymidis might be of value in preserving genetic material from endangered or valuable felids.

1. Introduction

Most of the world's wild feline species are endangered because of poaching and habitat changes or even loss. Preservation of epididymal spermatozoa offers a potentially valuable procedure to rescue genetic materials from endangered species or valuable pure breed domestic cats [1] in which the genetic materials could be lost anytime by unexpected death of the animals. Epididymal spermatozoa, similar to ejaculated spermatozoa, can be preserved for artificial insemination (AI), IVF [2], intracytoplasmic sperm injection (ICSI) [3], and other assisted reproductive technologies (ART). Through

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cryopreservation, epididymal spermatozoa can be preserved for an almost unlimited time in liquid nitrogen [4]. However, cryopreservation has also been revealed to both generate and exacerbate the extent of sperm DNA fragmentation in many species: human [5], murine [6], ovine [7], and feline [8]. Therefore, sperm DNA fragmentation is an important parameter to assess and a useful index of fertility potential [9]. Spermatozoa with severe DNA damage still have normal fertilizing ability, but a high incidence of DNA fragmentation can disturb postfertilization development of the embryo and lead to a decrease in pregnancy rates [10–13].

As sperm DNA fragmentation is known to play an important role in fertility potential, several techniques have been developed to assess it. The sperm chromatin structure assay is an Acridine Orange (AO) staining technique used to study sperm chromatin structure. The technique is based on the hypothesis that structurally abnormal sperm chromatin is more susceptible to denaturation [14]. AO-intercalated double-stranded DNA (dsDNA) emits green fluorescence, whereas denatured or single-stranded DNA (ssDNA) emits orange, yellow, or red fluorescence. Chromatin damage can be quantified by subjective evaluation using fluorescence microscopy or flow cytometric measurement of the metachromatic shift from green (dsDNA) to red (ssDNA) AO fluorescence [7].

Normally, the standard procedure of epididymal sperm collection in felids is to collect spermatozoa for preservation from only the cauda epididymidis [1,15–17]. In the domestic cat, successful use of frozen-thawed cauda epididymal spermatozoa has been reported, with a 27.3% conception rate for 11 cats after unilateral intrauterine insemination with 5×10^7 spermatozoa [2] and a 25.3% cleavage rate obtained from IVF [18]. However, the numbers of feline spermatozoa that can be collected from the cauda is comparatively low and have been reported to vary from 20 to 60×10^6 spermatozoa [19,20]. The ideal for genetic preservation of endangered species is to preserve as much genetic material as possible to optimize the chance to produce offspring. A previous study has demonstrated that the majority of spermatozoa collected from the corpus and cauda epididymidis are motile and morphologically mature [21]. Therefore, it might be possible to preserve more spermatozoa by including spermatozoa from the corpus epididymidis. As we know that spermatozoa acquire their final maturation status during their passage through the epididymis, during the maturation process, sperm's chromatin acquires its final compact status by formation of disulfide bonds in the nuclear protamines. Although evaluation of the DNA status of feline cauda epididymal spermatozoa has been described [8], to our knowledge, there has been no comparison between regions of the epididymis and between samples evaluated before and after freezing.

The aim of this study was to compare the chromatin stability and quality of spermatozoa from the corpus and cauda epididymis after freezing thawing. The results contribute to a better understanding of the potential of cryopreservation of spermatozoa from the corpus epididymis in ART such as AI or IVF. The domestic cat was used as a model to study the reproductive physiology of endangered or otherwise valuable felids.

2. Materials and methods

2.1. Experimental design

Epididymal spermatozoa from corpus and cauda were collected separately. Sperm motility, morphology, acrosome integrity, and chromatin integrity were evaluated in the fresh samples. Chromatin integrity was evaluated using AO staining and assessed with fluorescence microscopy. Each sperm sample was centrifuged in an Eppendorf tube at $700 \times g$ for 6 minutes and cooled to 4 °C after dilution with an Egg Yolk Tris extender. After cooling, sperm from corpus and cauda were frozen according to the protocol described by Axner et al. [21] and kept in a liquid nitrogen tank. After being frozen for more than a month, spermatozoa were thawed and sperm motility, membrane integrity, acrosomal status, and chromatin integrity were evaluated.

2.2. Animals

Epididymal spermatozoa were collected from 12 privately owned domestic male cats, of various breeds, aged between 6 months and 5 years. All cats were subjected to routine castration at veterinary clinics in the Uppsala region and the University Animal Hospital at the Swedish University of Agricultural Sciences, Uppsala, Sweden.

2.3. Epididymal sperm recovery

After testes and epididymides were removed from the cats, they were kept in a cold box at 4 °C and transferred to the laboratory. The experiment was performed, and spermatozoa were collected within 24 hours after the testes were removed from the cats. Epididymides were dissected free from visible blood vessels and connective tissues. The gross anatomy of the corpus and cauda regions were determined according to Axner [22], and the ultrastructure of the feline epididymal duct can be divided into 6 regions by histologic differences. Regions 1 to 4 are localized within the caput, region 5 in the corpus, and region 6 in the cauda of the feline epididymis. Corpus epididymal tissue and cauda epididymal tissue were separated.

Tissue segments from each part were transversely cut into small pieces and placed in 600 μL of warmed Tris buffer to let the spermatozoa come out. After 10-minute incubation at 38°C, the tissue was removed and an aliquot of 30 μL of fresh sperm sample was evaluated for concentration, motility, progressive motility, acrosome integrity, and chromatin integrity.

2.4. Cryopreservation media

Semen extenders and thawing media or Tris buffer were prepared according to the protocol described by Rota et al. [23]. Extender I contained 3% (w:v) Tris (Merck Eurolab AB, Stockholm, Sweden), 1.7% (w:v) citric acid (Sigma, St. Louis, USA), 1.25% (w:v) fructose (Kebo-Lab., Stockholm, Sweden),