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In vitro fertilization using frozen-thawed feline epididymal spermatozoa from corpus and cauda regions

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ABSTRACT

Epididymal sperm preservation offers a potential for rescuing genetic material from endangered or valuable animals after injury or death. Spermatozoa from corpus, as well as from cauda, have the capability to be motile and to undergo capacitation and can thus potentially be preserved for assisted reproductive technologies. In the present study, feline frozen-thawed epididymal spermatozoa from corpus and cauda regions were investigated for their ability to fertilize homologous oocytes and further embryo development *in vitro*. Epididymal spermatozoa from corpus and cauda of seven cats were cryopreserved and used for IVF. Cumulus–oocyte complexes ($n = 419$) were obtained from female cats after routine spaying. Frozen-thawed corpus epididymal spermatozoa showed similar properties of acrosome integrity, membrane integrity, and chromatin integrity as frozen-thawed spermatozoa from cauda except corpus spermatozoa showed lower motility ($P < 0.05$). The fertilizing capacity of frozen-thawed corpus epididymal spermatozoa was confirmed by similar number of embryos developing to the two- and four-cell stages compared with sperm from cauda (32.03% vs. 33.33%). However, oocytes fertilized with corpus spermatozoa had lower potential to develop to the blastocyst stage (6.79%) and had lower cell numbers compared to oocytes fertilized with cauda spermatozoa (14.08%). In conclusion, spermatozoa from corpus epididymis had a similar capability to fertilize homologous oocytes *in vitro* as sperm from cauda but resulted in fewer embryos developing to the blastocyst stage compared to spermatozoa from the cauda.

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1. Introduction

Many of the world's wild feline species are threatened by poaching and habitat loss. Therefore, many of them are classified as endangered. Some species are approaching critically low numbers (such as the Iberian lynx and the flat-headed cat; data from International Union for Conservation of Nature red list of threatened species 2015). Therefore, to rescue them from extinction, gene banks are created with the aim of preserving genetic materials, increasing genetic variation, and avoiding inbreeding within small populations. The unexpected death of an animal leads to loss of genetic material. In this case, epididymal sperm collection techniques and sperm cryopreservation can be used to avoid this loss [1].

Epididymal sperm preservation could be a way to preserve genetic material from dead animals. Many previous studies have demonstrated that it is possible to preserve spermatozoa from the cauda epididymidis. The spermatozoa from cauda are mostly mature, have ability to undergo capacitation, and are able to fertilize by IVF and artificial insemination (AI) [2–4]. Therefore, currently cauda epididymal spermatozoa

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are usually preserved for assisted reproductive technology such as AI or IVF. In humans, there are some studies which reported successful IVF results using spermatozoa from corpus epididymis [5], whereas there are, to the authors' knowledge, no such reports in the feline. Our aim was to better understand the physiology and capability of feline epididymal spermatozoa from corpus and the possibility of preserving them for future use in assisted reproductive technology. Therefore, we evaluated the IVF and further embryo development ability *in vitro* of frozen-thawed spermatozoa from corpus compared to cauda epididymidis. In this study, domestic cats were used as a model for wild felids.

2. Materials and methods

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA); the sigma product number is given within brackets unless otherwise indicated. All cumulus–oocyte complexes (COCs) and oocyte/embryo handling were done on heated surfaces at 38 °C.

2.1. Oocyte recovery

Ovaries were obtained from 48 female cats which had undergone normal ovariohysterectomy from veterinary clinics. The ovaries were put in NaCl 0.9% isotonic solution and transported in a cold box at 4 °C to the laboratory as soon as possible. Oocyte recovery of all batches was done within 24 hours after castration. In brief, the ovaries were washed in NaCl (0.9% isotonic solution) and placed in washing medium consisting of HEPES-TCM 199 (He-199; M2520), 0.36-mM $C_3H_3NaO_3$ (P-3662), 2.2-mM $C_6H_{10}CaO_6$ (L-4388), 1-mM L-glutamine (G-8520), 1-mg/mL BSA (A-3311), and 50-μg/mL gentamycin (G-1264). Cumulus–oocyte complexes were recovered by ovarian slicing in washing medium and morphologically classified at ×40 magnification using a stereomicroscope. Only COCs grade I and II were selected for IVM [6]. In brief, grade I COCs were typified by the oocytes being fully surrounded with more than five layers of compact cumulus cells and containing a homogeneous dark cytoplasm, whereas grade II COCs had homogeneous cytoplasm surrounded with fewer layers of compact cumulus cells. Grade III COCs had a complete corona radiata but only partial layers of cumulus cells [6].

2.2. In vitro oocyte maturation

Groups of 20 to 30 COCs were cultured in 500 μL of a basic IVM medium (TCM-199 [M-2154] supplemented with 0.36-mM $C_3H_3NaO_3$, 2.2-mM $C_6H_{10}CaO_6$, 2-mM L-glutamine, 4-mg/mL BSA, 50-μg/mL gentamycin, 1.12-mM cysteine [C-7352], 25-ng/mL EGF, 5-μL/mL Suigonan [1.25 IU hCG, 2.5 IU eCG; Suigonan, MSD Animal Health, Stockholm, Sweden] and 25-μL/mL ITS [contains 1.0-mg/mL recombinant human insulin, 0.55-mg/mL human transferrin {substantially iron free}, and 0.5-μg/mL Na_2O_3SE at the 100× concentration], pH 7.4, 280–300 mOsm) at 38.5 °C in a humidified atmosphere with 5% CO_2 in air for 24 hours.

2.3. Sperm preparation

Epididymal spermatozoa were collected from seven privately owned domestic male cats, of various breeds; the average age was 14 ± 5.3 months. All cats were subjected to routine castration at local veterinary clinics and the University Animal Hospital at the Swedish University of Agricultural Sciences, Uppsala, Sweden. After castration, the testes were removed from the cats and were kept in a plastic bag in a cold box at 4 °C and transferred to the laboratory. Sperm recovery was performed within 24 hours after castration. Epididymides were dissected free from visible blood vessels and connective tissues. Tissue segments of corpus and cauda regions 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 spermatozoa from corpus and cauda were cryopreserved according to Kunkitti et al. [7]. In brief, after sperm recovery, the sample was centrifuged at 600× *g* for 6 minutes, and the supernatant was removed. Extender I (24.97-mM Tris [Merck Eurolab AB, Stockholm, Sweden], 8.84-mM $C_6H_8O_7$, 6.93-mM fructose [Kebo-Lab, Stockholm, Sweden], 3% [v:v] glycerol [Kebo-Lab], 20% [v:v] egg yolk, 0.17-mM Na-benzylpenicillin [Boehringer Ingelheim Vetmedica, Copenhagen, Denmark], 0.17-mM streptomycin sulfate in distilled water, pH 6.72, 865 mOsm) was added at room temperature to adjust the sperm concentration to 16 × 10^6 sperm/mL. The sperm sample was placed in a room temperature bench cooler that reached 4 °C in 45 to 60 minutes. The sample was diluted 1:1 with semen extender II (which had the same composition as that of extender I except containing 7% glycerol [v:v] and 1% [v:v] Equex STM paste [Nova Chemical Sales, Scituate, Inc., MA, USA] pH 6.74, 1495 mOsm) after cooling. The final sperm concentration was approximately 8 × 10^6 sperm/mL. The sperm samples were loaded into 0.25-mL straws and placed in a goblet on top of a cane. The cane was put in a canister which was then frozen by lowering it vertically, in three steps, in an Apollo SX-18 LN tank (MVE Cryogenetics B, New Prague, MN, USA) containing 15 to 18 cm of liquid nitrogen for 2, 2, and 1 minute, the top of the goblets being at 7, 13, and 20 cm below the opening of the tank, respectively. Frozen epididymal spermatozoa were thawed at 37 °C for 30 seconds and placed in 500 μL of Tyrode's HEPES medium (Tyrode's balanced salt solution [Milli-Q water supplemented with 136.89-mM NaCl, 2.68-mM KCl, 1.8-mM $CaCl_2 \cdot 2H_2O$, 1.05-mM $MgCl_2 \cdot 6H_2O$, 0.41-mM $NaH_2PO_4 \cdot H_2O$, 5.5-mM glucose, and 11.9-mM $NaHCO_3$] supplemented with 0.36-mM $C_3H_3NaO_3$, 1-mM L-glutamine, 2.2-mM $C_6H_{10}CaO_6$, 149.98-mM $NaHCO_3$, 4-g/L BSA, 50-μg/mL gentamycin, 7.53-mM HEPES [H-6147], pH 7.2, 280–300 mOsm). The sperm samples were centrifuged, and the supernatant was removed. The sperm pellet was resuspended with 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 $NaHCO_3$, 0.36-mM $C_3H_3NaO_3$, 1-mM L-glutamine, 2.2-mM $C_6H_{10}CaO_6$, 6-g/L BSA, 50-μg/mL gentamycin, pH 7.4, 280–300 mOsm). Sperm motility, membrane integrity, acrosome integrity, and chromatin integrity were evaluated before coincubation with the oocytes.

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