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Characteristics of urethral and epididymal semen collected from domestic cats—A retrospective study of 214 cases



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ABSTRACT

This study was designed to describe and compare basic semen characteristics and sperm motility parameters obtained via computer-assisted sperm analysis (CASA) in feline semen collected from the urethra and epididymis, on the basis of large, unselected population of domestic cats. The semen collected from 214 males was subjected for routine semen assessment and CASA evaluation. Semen collected by urethral catheterization (CT) and by epididymal slicing (EP) has comparable characteristics according to total sperm count (47.7 ± 42.1 and 52.9 ± 45.0), subjective motility (71.1 ± 17.0 and 69.3 ± 13.9), viability (74.9 ± 13.4 and 76.7 ± 10.6), and morphology (52.6 ± 19.0 and 47.2 ± 17.4). The study of a large feline population confirmed a high incidence of teratospermy in cats, which negatively affects sperm motility parameters assessed by CASA. A lack of a correlation between CT and EP semen for total sperm count and viability, as well as occasional gross differences between the morphology of CT and EP semen of the same cat suggests that many factors may affect sperm cells, and the fertility and/or infertility of patients should not be assessed after examining only one sample. Additionally, technical problems with assessment of EP samples (understated results) suggest that CT semen is more appropriate for an analysis by CASA than EP.

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1. Introduction

Recently, artificial reproductive techniques (ARTs) have become of great interest in the field of feline reproduction for a number of reasons. The first is the development of feline breeding and an increasing necessity of veterinary management and supervision over reproduction, as well as a demand for the introduction of ART in pedigree cats on the level, which is nowadays seen in dogs. The second one is a potential role of ART in rescue programs of endangered felines via establishing banks of genetic resources of semen, oocytes, and embryos. The domestic cat is considered a

model animal for the extrapolation of knowledge to threatened species [1].

Before implementing most of ART, semen has to be collected. Until a few years ago, three methods applicable in domestic cats were possible: using an artificial vagina (AV), electroejaculation (EE), and slicing of the epididymis (EP) (reviewed by [2]). The artificial vagina cannot be widely used as it requires a trained tomcat and a teaser female in estrus. Electroejaculation is restricted in many countries and may be questionable from the ethical point of view. Epididymis slicing is easy to perform, but the main disadvantage is that it can be used only once in each male. Additionally, separation of sperm cells from the tissue debris is time consuming and impractical. In 2006, Zambelli et al. [3] proposed a practical method of semen collection—urethral catheterization (CT) after

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medetomidine administration. Medetomidine induces the contraction of the ductus deferens and transport of sperm cells from the epididymis to the urethra from where it can be collected by a urethral catheter. The main advantages of this method are that it is repeatable, nontraumatic, and no special equipment or training is required. Therefore, it can be useful for not only ART but also the assessment of male fertility and/or infertility in clinical conditions.

Only a few authors described the quality and *in vitro* fertilizing ability of urethral semen in comparison with electroejaculated [4] and epididymal [5] semen. To the authors' knowledge, there are no comprehensive, wide studies on the basis of large clinical materials collected over several years. Also, the use of computer-assisted sperm analysis (CASA) is rather poorly documented in cats [6], and there are no studies in which CASAs were used to examine urethral semen.

Therefore, the aim of this study was to describe and compare detailed characteristics of sperm cells collected from the urethra and epididymis of 214 individuals assessed over 3 years in our laboratory. The second aim of the study was to describe sperm motility parameters obtained *via* CASA in a large population of unselected, domestic cats to define normal values of these parameters for urethral semen in cats.

2. Materials and methods

All laboratory chemicals and reagents used in this study were purchased from Sigma–Aldrich Poland. TRIS buffer used as a semen extender for both urethral and EP semen contained 3.02% (w:v) TRIS, 1.35% (w:v) citric acid, 1.25% (w:v) fructose, in bidistilled water; pH 6.5.

2.1. Animals

The study was performed from January 2011 to June 2014 on 214 privately owned domestic shorthair tomcats between 8 months and 6 years of age (mean 16 months, median 12 months). All cats were presented for a routine orchietomy at the owner's request at the Department of Reproduction and Farm Animal Clinic, Faculty of Veterinary Medicine in Wrocław, Poland. All procedures were performed with the agreement of the Second Local Ethical Committee in Wrocław.

2.2. Semen collection

After a general clinical examination, each healthy cat subjected for castration was sedated with medetomidine hydrochloride intramuscular (im) at 100 µg/kg of body weight (Sedator 1.0 mg/mL; Novartis, Poland). After 10 – minutes, urethral semen was collected by urethral catheterization (CT), as previously described by Zambelli et al. [4]. Briefly, a tomcat urinary catheter with its tip cut to get a shorter, open-ended catheter was inserted approximately 9 cm into the urethra, taking care not to reach the bladder. Subsequently, the catheter was removed from the urethra, and the volume of the obtained semen was quickly assessed on the basis of the values measured beforehand on the same type of catheter. Subsequently, the sperm

sample was placed in an Eppendorf tube containing 200 µL of prewarmed semen extender. In case of obtaining a full catheter of materials, the cat was immediately catheterized a second time. The second sample was added to the same tube as the first and was evaluated as a pooled sample.

Immediately after CT semen collection, an injection of ketamine im at 5 mg/kg of body weight (VetKetam 100 mg/mL, Vet-Agro, Poland) was given, and an orchietomy was performed. Meloxicam subcutaneous 0.3 mg/kg of body weight (Metacam, Boehringer Ingelheim Vetmedica, Germany) was administered to reduce postoperative pain, and a mixture of benzathine benzylpenicillin 100,000 intrauterine/mL, procaine benzylpenicillin 100,000 intrauterine/mL, and dihydrostreptomycin sulfate 200 mg/mL im 1 mL/10 kg of body weight (Shotapen L.A., Virbac, France) was given as an infection prophylaxis. Within 5 to 10 minutes after the removal of the testes, epididymal spermatozoa were collected by epididymal slicing (EP) [7]. The caudae epididymis were dissected from the testes and cleaned from connective tissues and visible blood vessels. The caudae epididymis were then placed in 1 mL of prewarmed semen extender in a glass Petri dish and minced using a scalpel blade. After a 10-minute incubation, the epididymal tissues were removed, and a suspension of spermatozoa was filtered (CellTrics 30 µm, Partec) into an Eppendorf tube. CT and EP semen were kept under the same conditions and underwent the same assessment procedures.

2.3. Semen assessment

2.3.1. Sperm concentration and total sperm count

To evaluate sperm concentration, a 10-µL aliquot of the sample was diluted in 200 µL of distilled water, and cells were counted in 80 squares of the Thoma chamber. Counting was repeated in a second chamber, and the mean value was calculated. The total sperm count was calculated on the basis of the sperm concentration and volume.

2.3.2. Sperm motility

Each sperm sample was assessed for subjective motility just after collection. Ten microliters of the specimen was placed on a prewarmed slide, and the percentage of motile spermatozoa (MOT) was estimated subjectively under a contrast phase microscope (magnification × 200) by two independent persons.

In second step, semen samples were assessed using a computer-assisted sperm analyzer (CASA-system, HTM-IVOS, 12.3D, Hamilton-Thorne Biosciences, MA, USA). The procedure and software settings were set according to Nizański et al. [8], with an adjustment for cats. The software settings are summarized in Table 1. An aliquot of 4 µL of specimen was mounted on a prewarmed Leja counting chamber (Leja Products B.V., Holland) and placed in the stage warmer set at 39 °C. Five randomly selected microscopic fields were scanned. The playback function was used after every scan to ensure that all spermatozoa were identified correctly, and their trajectory was reconstructed properly. In the case of false results (erythrocytes, epithelial cells, or other debris and contaminants counted as sperm cells), which could not be corrected, the sperm samples

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