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Sperm evaluation and biochemical characterization of cat seminal plasma collected by electroejaculation and urethral catheterization

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Abstract

This paper aimed to evaluate cat seminal plasma protein profile (with SDS-page) and determine differences in seminal plasma composition from ejaculates obtained using urethral catheterization after pharmacological induction (UrCaPI) and electroejaculation (EE). In addition, this study evaluates whether the recovery method affected seminal plasma protein and zinc concentrations. A single ejaculation was collected from 17 mixed-breed cats by EE (5/21) or UrCaPI (12/21), while 4/21 cats underwent four sperm collections once every four days using EE and UrCaPI techniques alternately. The semen parameters evaluated were: volume, percentage of motility and progressive motility, morphology, and sperm concentration. After centrifugation, the seminal plasma obtained was stored at −80 °C and later used to measure protein and zinc concentrations, and to determine protein profile by SDS-polyacrylamide gel electrophoresis (PAGE). The results obtained indicate that cat seminal plasma protein profile is characterized by many protein bands (>30) with a molecular weight ranging from 3.5 to 200 kDa, and that the recovery method influences the seminal plasma protein profile: EE is related to the absence of two proteins (P55 and P14), and alters three protein bands (P200, P80, P28). The collection technique also affected zinc concentration (mg/dL) and protein concentration (g/dL) which were significantly higher ($P < 0.01$) in samples collected by UrCaPI; on the contrary the total Zn and protein amount/ejaculate were not significantly different in samples collected by both technique ($P < 0.05$).

Keywords: Domestic cat; Seminal plasma; Protein; Electrophoresis; Zinc

1. Introduction

Seminal plasma is the secretion of the sexual accessory glands released in the urethra at the time of ejaculation to support spermatozoa. It contains proteins, including many enzymes (acid phosphatase, alanine transaminase, alkaline phosphatase, aspartate transaminase), lipids, macroelements [sodium (Na+), potassium (K+), calcium (Ca2+), magnesium (Mg2+), phosphate (P) and chloride (Cl)] and microelements [copper (Cu), iron (Fe) and zinc (Zn)] [1]. Zn has been shown to be essential to the structure and function of a large number of macromolecules and more than 300 enzymes [2]. The metal has both catalytic and structural roles in enzymes, while in zinc finger motifs it provides a scaffold organizing protein sub-domains for interaction with either DNA or other protein [3]. Zn is present at high concentration in human seminal plasma; the mean concentration is 2 mM, 100 times higher than in serum [4]. The metal has an important role in testes development, sperm physiologic functions, and Zn deficiency causes hypogonadism [4]. A wide range of Zn

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concentrations is present in the seminal plasma of mammals, ranging from high values for boar (17.7 mg/dL) to low concentrations in fox (1.3 mg/dL) [5].

The most common techniques to collect cat semen are artificial vagina (AV) [6,7] and electroejaculation (EE) [8,9]. Recently, a new technique of urethral catheterization after pharmacological administration (UrCaPI) was described [10]. All techniques yield a good ejaculate containing spermatozoa and seminal plasma. Sperm collection with artificial vagina is an inexpensive technique which does not require physical or chemical restraint of animals, even though a teaser queen and trained tomcat are usually necessary. Electroejaculation can be performed on any male cat that can be safely anesthetized, but it requires specific equipment and is not permitted in all countries. The UrCaPI technique uses an urethral catheter to collect sperm released in the urethra in response to medetomidine administration. The UrCaPI semen samples are characterized by lower total volume and higher spermatozoa concentration (10^6/ml) compared with EE semen samples, but the total number of spermatozoa (10^6/ejaculate) is not significantly different between UrCaPI and EE semen samples [10].

The veterinary literature contains only one study on the biochemical characterization of cat seminal plasma because of the difficulty in collecting semen and the small semen volume in this species [1]. In addition, cat has been used as a model for human and nondomestic felid pathologies [11,12]. The study of seminal plasma composition is important because seminal plasma protein profile has been correlated in bovine to semen fertility [13] and freezability [14], and because studies on canine seminal plasma reported that it contains prostate and epididymidal markers [15,16]. Seminal plasma has been analyzed mainly in dog, bull, stallion and ram [13–15,17–19]. The presence of particular proteins has been associated with specific semen parameters in bulls: protein fertility-markers were detected in Holstein bulls using 2D-page [13] and differences in seminal plasma proteins were determined between bulls with high and low semen freezability [14]. A recent study reported that ram seminal plasma obtained using EE or AV did not show any significant difference in total protein content, but with EE the 2D-page protein maps displayed two additional protein spots (15 kDa, 22 kDa) and the loss of one protein (25 kDa) [19]. However the pre- and post-thaw sperm quality was not influenced by sperm collection techniques in ram [20].

SDS-page in canine seminal plasma disclosed specific protein bands as markers of prostate gland and epididymidal secretion [15,16]. The B20 band (15.6 kDa), present in high concentration in samples collected from all dogs, was identified as an arginine esterase subunit, considered a marker of normal prostate gland activity [15]. Moreover, bands B9 (42.6 kDa) and B13 (29.2 kDa) were detected as epididymidal secretion markers because of their absence in semen ejaculate samples obtained post-vasectomy [16]. Moreover, as observed in bulls [13], there was a positive correlation in dogs between semen parameters, such as sperm motility and vigor, and specific protein bands (67 kDa, 58.6 kDa) [15].

In the light of a recent report demonstrating differences in semen collected by EE or UrCaPI [10], the present study aimed to separate the proteins in cat seminal plasma using SDS-page and correlate the results to semen parameters. In addition we evaluated whether sperm collection techniques (EE versus UrCaPI) affect the protein profile and protein (g/dL; total amount/ejaculate) and zinc (mg/dL; total amount/ejaculate) concentrations of semen samples.

2. Material and methods

All the chemicals and reagents in this study were purchased from Sigma (St. Louis, MO, USA) unless otherwise stated.

2.1. Animals

Twenty-one clinically healthy, adult (age range, 1–3 years) mixed-breed male cats were enrolled in this experiment, completed from January to June 2008. The cats, privately owned or belonging to a cat pound, were brought to the Obstetrics and Gynaecology Section of the Clinical Veterinary Department to be submitted to orchiectomy. Nineteen cats had two palpably normal descended testes but two cats were cryptorchid (one bilateral inguinal and the other unilateral inguinal); these two were also included in the study.

The experiment was approved by Ethical-Scientific Committee of Alma Mater Studiorum—University of Bologna.

2.2. Semen collection and evaluation

A single ejaculate was obtained from five cats by EE and from 12 cats using UrCaPI while 16 samples were collected from four cats using both techniques alternately, once every four days. The four cats submitted to multiple collections were housed individually in boxes under natural photoperiod, with free access to food and water. For semen collection by EE or UrCaPI, the