Theriogenology 82 (2014) 1273–1280

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of dilution rate on feline urethral sperm motility, viability, and DNA integrity

Sylwia Prochowska, Wojciech Niżański*, Małgorzata Ochota, Agnieszka Partyka

Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Wrocław, Poland

ARTICLE INFO

Article history:
Received 13 January 2014
Received in revised form 19 August 2014
Accepted 19 August 2014

Keywords:
Cat semen
Sperm concentration
Dilution effect
Flow cytometry

ABSTRACT

This study was designed to investigate if the characteristics of feline urethral sperm can be affected by high dilution in an artificial medium. The semen collected by urethral catheterization from eight male cats was evaluated for sperm concentration and motility and subsequently diluted with a TRIS-based extender to the concentration of spermatozoa 10×10^6/mL, 5×10^6/mL, and 1×10^6/mL. Immediately after the extension samples were assessed for motility, cell viability using SYBR-14 and propidium iodide, acrosome integrity using lectin from *Arachis hypogaea Alexa Fluor 488 Conjugate*, and propidium iodide and chromatin status by acridine orange. Compared with 10×10^6/mL dilution rate, spermatozoa diluted to 1×10^6 sperm/mL had a significantly lower proportion of motile (31.1% ± 19.8 and 0.7% ± 1.6, respectively, $P < 0.05$) and viable spermatozoa (88.3% ± 3.1 and 69.1% ± 12.8, respectively, $P < 0.01$). There was no dilution-related difference in the acrosome integrity (76.7% ± 11.9 vs. 75.9% ± 10.6) and chromatin status (defragmentation index, 3.3% ± 0.97 vs. 3.4% ± 1.7). These results indicate that feline urethral semen is susceptible to high dilution rate, and some sperm characteristics can be artifactually changed by semen dilution. It also suggests the potential role of seminal plasma in maintaining sperm motility and viability in high dilution rates.

1. Introduction

The handling of semen and its processing associated with assisted reproductive technologies (ART) very often includes extension of sperm, as in the case of preparing spermatozoa for cryopreservation, IVF, flow cytometric assessment, or cell sorting by chromosomal sex. The latter two require high dilution, which may be detrimental for the motility and viability of spermatozoa, as is the case in bovine [1,2] and ram semen [3]. This phenomenon has been described as the "dilution effect" [4].

It is thought that the dilution effect is connected with a partial or even complete removal of some essential seminal plasma (SP) compounds that protect the sperm from damage, thereby contributing to the deleterious effects of semen dilution. Although the role of SP is ambiguous and many authors reported both its beneficial and detrimental influence on sperm characteristics [5–7], the presence of SP seems to play a crucial role in the case of excessive semen dilution. The evidence in support of this hypothesis is that the re-addition of SP or its compounds to the highly diluted sperm can prevent or reduce its damage in sheep [3,8,9], rabbits [5], cattle [2,9], and pigs [9–12].

Articles dedicated to feline sperm dilution are scarce. Howard et al. [13] showed that even a twofold dilution of electroejaculated feline semen resulted in a decrease of sperm motility. To the authors' knowledge, no other information on this topic is available, although the semen in this species is characterized by a high concentration of sperm cells and an extremely low volume of ejaculate

* Corresponding author. Tel.: +48 71 3205315; fax: +48 71 3205306.
E-mail address: wojciech.nizanski@up.wroc.pl (W. Niżański).

0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.08.012

ranging from 10 μL [14] to 200 μL [15]. Therefore, high dilution rates of semen are usually necessary before sperm cell assessment, especially in semen collected by urethral catheterization [14]. The dilution of semen is also necessary before artificial insemination—insemination dose for intrauterine insemination of queens varies from 4.2×10^6 to 10×10^6 of sperm cells deposited in 30 μL [16] up to 200 μL [17,18].

Flow cytometry, which is widely used in andrology laboratories, is a valuable tool for the assessment of semen [19]. One of the advantages of flow cytometry is the possibility to assess a large number (>10,000) of spermatozoa in a short period of time. However, it requires a relatively large sample volume. This problem is solved by sample dilution. In cats, the assessment of semen by flow cytometry is rarely used most likely because of a low ejaculate volume and because a small overall number of spermatozoa can be obtained. Depending on the collection method, the total sperm count varies from about 20×10^6 in the case of electroejaculation [20] and urethral catheterization [14] to 80.1×10^6 in the case of an artificial vagina [21]. Such a small number of spermatozoa per ejaculate is a limiting factor with reference to the parallel assessment of many sperm characteristics or to combine flow cytometry sperm evaluation with other procedures (e.g., cryopreservation or artificial insemination). The possibility of assessing highly diluted samples (1×10^6 cells/mL, as recommended by manufacturers of flow cytometers and fluorescent dyes) through flow cytometry may enable a simultaneous broad sperm evaluation and its use for ART. It could also widen the spectrum of the assessed sperm parameters. However, due to the fact that extreme dilution may influence semen characteristics, there is a theoretical risk of an artifactual change of the results of the semen evaluation. Consequently, the laboratory assessment of the fertilizing potential of a particular male may be erroneous.

Urethral catheterization is a novel method of semen collection in cats, easy to introduce in everyday practice. In comparison with ejaculated spermatozoa (obtained *via* an artificial vagina or electroejaculation), semen collected using this method is characterized by a lower volume, higher sperm concentration, and a minimal presence of SP [14]. Thus, it very often requires a high dilution, but it has not been checked if such extensions may affect the sperm characteristics. Therefore, the aim of this study was to assess the influence of high sample dilution on spermatozoal motility, viability, acrosome integrity, and chromatin status in the feline urethral semen.

2. Materials and methods

2.1. Animals

Spermatozoa were collected from eight privately owned male cats scheduled for routine castration procedures at the Department of Reproduction and Farm Animal Clinic of the Wroclaw University of Environmental and Life Sciences (Poland). All tomcats were clinically healthy Domestic Shorthair cats, aged between 8 and 36 months. All procedures were performed with the consent of the Second Local Ethical Committee in Wroclaw.

2.2. Semen collection

Tomcats presented for routine orchidectomy were anesthetized using medetomidine hydrochloride im 80 μg/kg of body weight (Sedator 1.0 mg/mL, Novartis, Poland) combined with ketamine im 5 mg/kg of body weight (VetKetam 100 mg/mL, VetAgro, Poland). To reduce the postoperative pain, an injection of meloxicam sc 0.3 mg/kg of body weight (Metacam 5 mg/mL, Boehringer Ingelheim Vetmedica, Germany) was given before the anesthesia. After the orchidectomy, the cats were given a mixture of benzathine benzylpenicillin 100,000 IU/mL, procaine benzylpenicillin 100,000 IU/mL, and dihydrostreptomycin sulfate 200 mg/mL im 1 mL/10 kg of body weight (Shotapen L.A., Virbac, France). The urethral semen was collected as previously described by Zambelli et al. [14]. 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 for the purpose of collecting a semen sample. Immediately after successful sample collection, it was placed in a prewarmed Eppendorf tube containing 200 μL of semen extender based on TRIS buffer ((3.02%, wt/vol) TRIS (Sigma–Aldrich, Poland), 1.35% (wt/vol) citric acid (Sigma–Aldrich), 1.25% (wt/vol) fructose (Sigma–Aldrich), in bi-distilled water; pH 6.5).

2.3. Experimental design and semen evaluation

Each sperm sample was assessed for sperm motility and sperm concentration immediately after collection. The sample was then divided into three aliquots and a semen extender was added to reach the final concentration of spermatozoa 10×10^6/mL, 5×10^6/mL, and 1×10^6/mL. The 1×10^6/mL dilution rate was chosen as recommended by manufacturers of flow cytometers and fluorescent dyes. The 10×10^6/mL was the highest possible concentration to prepare, according to the amount of sperm obtainable from a cat that allows the creation of three dilutions for comparison. The concentration 5×10^6/mL was the value in between. Immediately after dilution, each sample was assessed for sperm motility and then prepared for a flow cytometric evaluation of viability, acrosome integrity, and chromatin status.

2.3.1. Sperm motility and concentration

In order to assess the motility, 10 μL of a sperm sample (raw semen and a sample from each of the investigated dilutions) was placed on a prewarmed slide and the percentage of motile sperm was subjectively estimated under a contrast-phase microscope at a ×200 magnification by three independent researchers and the mean value was calculated.

To evaluate sperm concentration, a 10-μL aliquot of the semen 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.

2.3.2. Flow cytometry assessment

Measurements were carried out on a FACSCalibur (Becton Dickinson, San Jose, CA, USA) flow cytometer. The fluorescent probes were excited by an Argon ion 488 nm laser. Detection