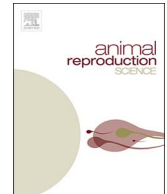




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Review article

Influence of cooling temperature in sperm subpopulations of domestic cats

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ABSTRACT

The objective of this study was to identify and compare domestic feline sperm subpopulations, chilled at -1°C for 24 and 48 h, as well as to analyze the sperm frequency in different subpopulations. Ten adult cats were used. Sperm collection was performed using electroejaculation (EEJ). Spermatic kinetics were evaluated using a computerized system at three moments: fresh, 24 and 48 h after refrigeration. The ejaculates were divided into a group refrigerated at -1°C ($n = 5$), and a group refrigerated at 4°C ($n = 5$). A total of 1560 spermatozoa were analyzed individually, and the sperm subpopulations were identified using multivariate statistics. Three spermatic subpopulations were defined using prior analysis of the hierarchical dendrogram. A principal components analysis (PCA) identified the existence of three groups with higher iterations at the three moments: PC1 (VAP, VCL, VSL, ALH, SVI), PC2 (STR, LIN, WOB and SMI) and PC3 (BCF). Subpopulation 1, after 48 h of refrigeration at -1°C , and subpopulation 3, after 24 h of refrigeration at 4°C , maintained their sperm quality, which allowed us to characterize the groups of spermatozoa that were resistant to cryopreservation. The present study identified three well defined ejaculate spermatozoa subpopulations, with proportional distributions between the groups and two refrigeration resistant subpopulations.

1. Introduction

The use of computer-assisted sperm analysis (CASA) allows for the identification of sperm cell kinetics and the individual record of their trajectories, including the detection of subtle changes in velocity parameters over different experimental conditions (Kraemer et al., 1998; Ferreira, 2000). However, there is an underutilization of this analysis, as mean motility and kinetic parameter values assume that the ejaculate is uniform, causing loss of important information related to the reproductive system (Amann and Graham, 1993; Martínez-Pastor et al., 2011).

Different sperm populations with specific movements, which have been studied with CASA system to improve the understanding of cell characteristics and the existence of well-defined spermatozoa subpopulations within mammalian ejaculates, have been accepted by scientific community (Martínez-Pastor et al., 2011; Contri et al., 2012). The heterogeneity of spermatozoa inside ejaculate can guarantee a greater fertilization potential (Curry, 2000). Therefore, it is possible to propose that some individuals, even with similar mean motility values, may be more fertile because they contain different subpopulations in their ejaculate (Holt and Van

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Parameters	Valores
Chamber Type	Cell-Vu®
Temperature, Set (°C)	37
Frames Acquired	30
Frame Rate (Hz)	60
Number of frames	5
Minimum Contrast:	40
Minimum Cell Size (pixels)	4
Straightness (STR), Threshold (%)	80
VAP cutoff (µm/s)	30
Prog. Min VAP (µm/s)	70
VSL cutoff (µm/s)	20
Cell Intensity	50
Magnification	1,89

Fig. 1. Setup used in HTR-IVOS 14.0 for evaluation of cats spermatozoa.

Look, 2004).

To identify all relevant information, studies have used multivariable statistical analyses, including principal component analysis (PCA) and clustering statistical procedures (*cluster*) to identify and monitor the behavior of sperm subpopulations against stress, such as cryopreservation.

The standard distributions of sperm subpopulations vary when they are subjected to temperature changes (Martinez et al., 2006; Dorado et al., 2011a, 2011b) and can lead to a marked reduction in fertility. Spermatozoa that were refrigerated for 24 h presented different behavior when compared with fresh spermatozoa, which described changes in their non-progressive trajectories (Dorado et al., 2011a, 2011b; Buranaamnuay, 2017). The identification of subpopulations with spermatozoa capable of surviving and maintaining progressive movements after a certain period of cooling becomes relevant in the study of reproduction biotechnology, as they may indicate the sample quality (Davis et al., 1995; Peña et al., 2012; Niżański et al., 2016).

The maintenance structure of a global subpopulation of cryopreservation resistant spermatozoa may be important in maintaining the overall function of ejaculate when challenged (Flores et al., 2008). Therefore, the objective of this study was to identify and compare ejaculated subpopulations of domestic cats, collected by electroejaculation, and refrigerated at -1°C for 24 and 48 h.

2. Material and methods

2.1. Ethical aspects

This study was approved by the Ethical Commission on the Use of Institutional Animals – CEUA number 7099.2015.53.

2.2. Animals

Ten adult, mongrel, semi-domiciled cats, in good nutritional status and weighing 2–6 kg, were used. Although male fertility status was unknown, all of them had both palpable testicles in the scrotum and evident penile spines.

2.3. Experimental design

The ejaculate was collected using electroejaculation (EEJ) and diluted in an ACP-117® extender, composed of coconut water (ACP-Biotechnology, Fortaleza, Brazil). Analysis of the spermatid kinetics was performed by a computer system at three moments: fresh, 24 h after cooling and 48 h after cooling at one of two temperatures: -1°C ($n = 5$) and 4°C ($n = 5$).

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