

Veterinary and Comparative Biomedical Research

SHORT COMMUNICATION

Epididymal Sperm Characteristics of Iranian Short-Haired Cats

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Online ISSN: 3060-7663

<https://doi.org/10.22103/vcbr.2025.25378.1073>

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Article History

Received: 2 June 2025
Revised: 2 July 2025
Accepted: 8 August 2025
Published: 19 August 2025

Keywords

Epididymal Sperm Quality
Iranian Short-Haired Cats
Motility
Viability
Morphology

Running Title

Epididymal Sperm of Short-Haired Cats

Abstract

The aim of this study was to assess epididymal sperm parameters in Iranian short-haired cats. The study examined the epididymal sperm quality in seven Iranian short-haired cats (aged 1–4 years) undergoing routine orchiectomy at the Veterinary Hospital of Veterinary Faculty, Shahrekord University, Shahrekord, Iran, between January and March 2024. Sperm samples were collected from the tail of the epididymis in normal saline, filtered, and evaluated for one hour. Motility was assessed using light microscopy, morphology was evaluated using Giemsa staining, and viability was determined using Eosin-Nigrosin staining. The mean sperm concentration was 20.6 million/mL, with progressive motility of 57.0% and viability of 67.1%. Morphologically, 65.9 % of sperm were normal, with defects in 6.9% of the head, 13.5% of the body, 10.6% of the tail, and 3.1% showing cytoplasmic droplets. The mean and standard deviation (μm) for sperm head length, width, and perimeter were 4.39 ± 0.06 , 2.20 ± 0.04 , and 12.71 ± 0.18 , respectively. The sperm head area was $9.70 \pm 0.25 \mu\text{m}^2$, and the sperm tail length was $28.11 \pm 0.88 \mu\text{m}$. Testicular weights averaged 1.02 g (left) and 1.038 g (right). A positive correlation between motility and viability was observed ($r = 0.68$, $p < 0.01$). These findings provide valuable information on epididymal sperm in Iranian short-haired cats, with implications for feline breeding and conservation. Further research is needed to investigate factors such as acrosome integrity and environmental influences on sperm quality.

How to cite this article: Ahmad Reza Borzouei, Sajjad Nekoei Shahraki, Farid Barati. Epididymal Sperm characteristics of Iranian Short-Haired Cats. *Veterinary and Comparative Biomedical Research*, 2025, 2(2): 1 – 4. <http://doi: 10.22103/vcbr.2025.25378.1073>



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Introduction

The domestic cat (*Felis catus*) is a seasonal breeder with high prolificacy that has generated significant scientific interest in using assisted reproductive techniques (ARTs) to preserve the genetic diversity of wild cats. Given the decline in genetic diversity and population size of wild felids, effective reproductive interventions are crucial (1). The domestic cat serves as an established model for optimizing assisted ARTs that may support conservation strategies in wild feline species (2). For this purpose, epididymal sperm is a valuable source for ART in felines (3). The epididymis is a critical reservoir of male genetic material for endangered animals that have died unexpectedly, both in the wild and in captivity (4). Sperm retrieval from the epididymis is important for the application of ARTs due to their unique properties, such as prolonged viability when refrigerated and their capacity to produce viable embryos through ARTs (1).

Morphological examination of sperm is a valuable tool for determining the quality of cat sperm for cryopreservation and/or artificial insemination (5). Sperm morphometry is one of the important indicators of sperm quality that may influence fertilization success (6). In a small group of studies in colony cats, it was found that males with less than 40% morphologically normal sperm had lower zona pellucida penetration rates (7). Abnormal sperm tails are likely to negatively impact fertility; however, they may not be as significant a defect as abnormal sperm heads (7).

The dynamic nature of sperm morphology and its relationship to reproductive outcomes highlight the importance of ongoing research in this area. By improving our understanding of epididymal sperm characteristics, efforts can be directed towards enhancing reproductive performance not only in domestic shorthair cats but also in conservation efforts for endangered feline species. This

study aimed to evaluate characteristics of the epididymal sperm of Iranian short-haired cats in Shahrekord, Iran.

Materials and Methods

Between January and March 2024, seven Iranian shorthair cats (aged 1–4 years) were presented for routine orchiectomy at the Shahrekord University Veterinary Clinic in Iran. The cats were clinically healthy, with no history of reproductive or systemic diseases as confirmed by physical examination. This study was conducted with ethical approval from Shahrekord University, ensuring compliance with animal welfare standards.

After orchiectomy, the testes were immediately placed in a sterile container and transported to the laboratory at 4°C to prevent thermal damage to the sperm. The epididymis was separated, sliced with a sterile scalpel, and placed in 2 ml of heparinized (5 IU/mL) normal saline for 15 minutes (pH 7.4, osmolality 300 mOsm/kg) at 37°C. The tissue was then filtered to isolate sperm. Samples were processed within one hour of collection to ensure accuracy.

A total of 14 microscope slides were prepared (two slides for each cat: one for each staining method). Measurements were taken on each slide, analyzing a total of 1,400 sperm (200 sperm per cat).

Sperm concentration was estimated using a Neubauer slide under light microscopy (×400) with a dilution rate of 1:20 in 1% formal saline solution (0.9% NaCl). Eosin-Nigrosin staining (5 µl of sperm mixed with 10 µl of a stain consisting of 5% eosin and 10% nigrosin in a ratio of 1:4) was applied to assess sperm viability. Giemsa stain was used to evaluate sperm morphology and morphometry. Image J software was used for morphometric measurements. Sperm motility and progressive motility were estimated using a warmed slide under light microscopy. Statistical analysis was performed using the Univariate Normal Plot procedure in SAS software.

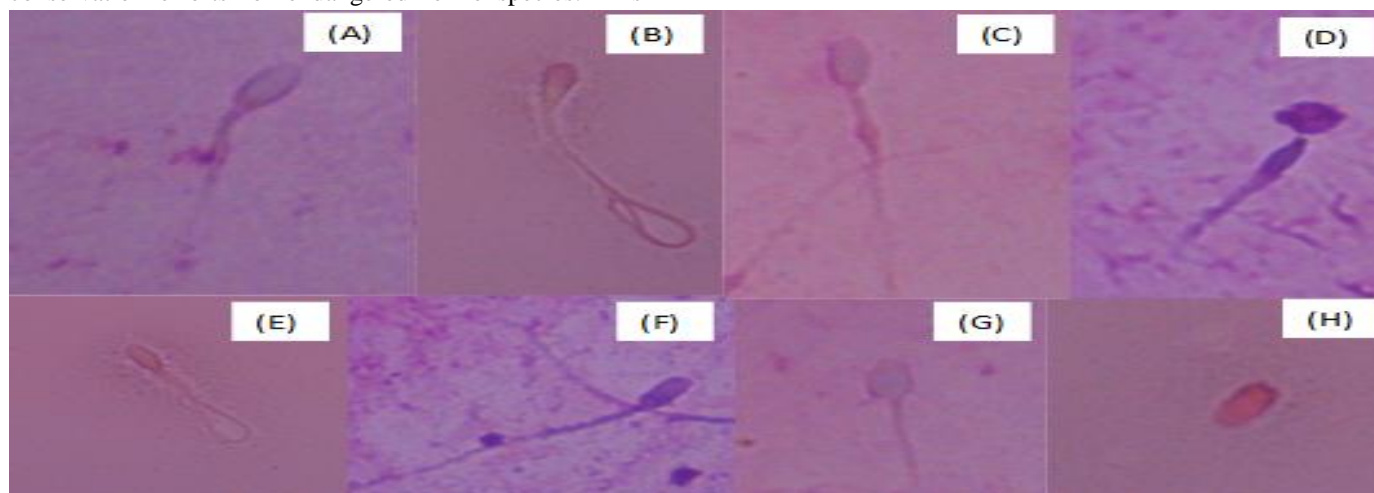


Figure 1. Morphological analysis, Giemsa staining, 81 sperm per parameter

Results

The present study provides limited data on epididymal sperm properties in Iranian short-haired cats. Of the seven cats, one cat (Cat 7, 2 years old) was azoospermic, with no sperm observed in the epididymal fluid despite repeated sampling. This finding was unexpected, as the cat's testicular size was normal (left: 1.01 g, right: 1.03 g), comparable to a previous report of 1.49 g (12), and there were no clinical signs of reproductive dysfunction. For the remaining six cats, sperm quality was assessed as follows: mean sperm concentration was 20.6 million/mL. Sperm viability and progressive motility were 67.1% and 57.0%, respectively. Morphological analysis (Giemsa staining, 81 sperm per parameter) revealed that 65.9% of sperm were normal, with 6.9% defects in the head, 13.5% in the midsection, 10.6% in the tail, and 3.1% cytoplasmic droplets (Figure 1). The mean and standard deviation (μm) for sperm head length, width, and perimeter were 4.39 ± 0.06 , 2.20 ± 0.04 , and 12.71 ± 0.18 , respectively. The sperm head area was $9.70 \pm 0.25 \mu\text{m}^2$ (Table 1). The sperm tail length averaged $28.11 \pm 0.88 \mu\text{m}$. Testis weight averaged 1.02 g (left) and 1.038 g (right).

For the remaining six cats, the mean sperm concentration, progressive motility, and viability were comparable to previous reports in domestic cats (11, 12). Morphometric parameters including sperm head length, width, and perimeter aligned with the study reported by Pintus et al. as 4.97, 2.42 and 11.97 μm , respectively with a head area of $9.46 \mu\text{m}^2$ (12), while showing some differences with the report of de Souza Barbosa et al. as follows: 5.56, 3.1, 16.16 μm and 16.94 μm^2 (11).

Table 1. Morphometry of epididymal spermatozoa of Iranian short-haired cats ($n = 6$) with normal morphology

Morphometric parameters	Mean \pm SE	Min-max
Head		
Length (μm)	4.39 ± 0.06	4.26-4.51
Width (μm)	2.19 ± 0.03	2.12-2.27
Area (μm^2)	9.7 ± 0.24	9.2-10.19
Perimeter (μm)	12.71 ± 0.17	12.35-13.06
Tail		
Length (μm)	28.1 ± 0.87	26.36-29.84

Discussion

This case series describes epididymal sperm quality in Iranian shorthair cats in Shahrekord, with notable findings of azoospermia in one cat. Azoospermia, defined as the absence of sperm in epididymal fluid, is rare in clinically healthy cats and may be associated with genetic factors, environmental stressors, or subclinical infections (8-10).

The azoospermic cat in this study had normal testicular weight, suggesting that the condition may not be related to testicular atrophy but could be due to obstruction, genetic defects, or impaired spermatogenesis, which requires further investigation.

These findings contribute to the first data on epididymal sperm characteristics in Iranian short-haired cats, a population that has not been represented in reproductive studies. However, the case of azoospermia highlights the need for further research into the prevalence and causes of infertility in this population, especially in areas with unique environmental conditions. This case series demonstrates that epididymal sperm from native short-haired cats of Shahrekord are generally suitable for processing in ART, exhibiting good motility, viability, and morphology in six out of seven cats.

Acknowledgements

The authors would like to thank the surgical staff at the Veterinary Hospital of Shahrekord University, Shahrekord, Iran.

Author Contributions

Ahmad Reza Borzouei: Case handling, investigation, methodology, writing the original draft. **Sajjad Nekoei Shahraki:** Case handling, methodology, **Farid Barati:** Conceptualization, investigation, methodology, supervision, visualization, review and editing of the draft.

Data Availability

All data are included within this published article.

Ethical Approval

This research was approved by the Ethics Committee for Animal Research at Shahrekord University, Shahrekord, Iran.

Conflict of Interest

The authors affirm that there are no competing interests.

Consent for Publication

Not applicable.

Funding

The research was financially supported by the Research Council of Shahrekord University, Shahrekord, Iran.

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