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Research Article

Use of Spermac® Staining Technique in the Determination of Acrosomal Defects in Cat Semen

Alper BARAN, B. Evrim ŞAHİN, Mithat EVECEN, Kamber DEMİR, İ. Kamuran İLERİ
Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, İstanbul University, 34320, İstanbul - TURKEY

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Abstract: The types and rates of acrosome and other (head, mid-piece and tail) abnormal spermatozoon types were determined by the Spermac® staining technique.

Semen from 5 stray tom cats under the same management conditions was used. Semen collection was performed under general anesthesia by electro-ejaculator once a week for 5 weeks. After ejaculation the semen was diluted by 100 µl of 0.9% NaCl solution and stained with Spermac® stain for morphological evaluation. The morphological criteria were acrosome, other (head, mid-piece and tail) and total morphological defect rates, and were 9.20 ± 2.55%, 9.20 ± 3.49% and 18.40 ± 4.79%, respectively.

It was concluded at the end of the study that the Spermac® staining technique could provide a detailed observation of cat spermatozoa, especially the acrosomal region, and could be employed in the determination of acrosomal defects.

Key Words: Cat, spermatozoa, morphology, Spermac® stain, defect

Kedi Spermasında Akrozomal Bozuklukların Tespitinde Spermac® Boyama Tekniğinin Kullanılması

Özet: Çalışmada, Spermac boyama tekniği kullanılarak kedi spermasında saptanan akrozom ve diğer (baş, orta-kısım ve kuyruk) anormal spermatozoon tip ve oranlarının belirlenmesine çalışıldı.

Aynı bakım ve beslenme şartlarında barındırılan beş erkek sokak kedisinin sperması kullanıldı. Sperma genel anestezi altında elektro-ejakülatör ile haftada bir kez beş hafta süre ile alındı. Ejakülasyon sonrası sperma 100 µl % 0,9 NaCl solüsyonu ile sulandırıldı ve morfolojik inceleme için Spermac® Boya ile boyandı. Morfolojik özellikler, akrozom, diğer (baş, orta-kısım ve kuyruk) ve toplam morfolojik bozukluk oranları sırasıyla; % 9,20±2,55, % 9,20±3,49 ve % 18,40±4,79 olarak bulundu.

Çalışmanın sonunda, spermac boyama tekniğinin kedi spermatozoasının özellikle akrozomal bölgesini detaylı bir şekilde görebilme olanağı sağladığı ve özellikle akrozom bozukluklarının belirlenmesinde yararlı olacağı kanısına varıldı.

Anahtar Sözcükler: Kedi, spermatozoa, morfoloji, Spermac® boya, bozukluk

Introduction

The first artificial insemination studies were conducted by Sojka et al. (1) in 1970. Domestic cats have been successfully used in the experimental stages of biotechnological studies (IVF, ICSI and cloning) and represent a model for endangered cat species (2). There is an urgent need in Turkey to study how to preserve the genetic material of native Van and Angora cats. Studies on spermatological characteristics and artificial insemination are limited in number (3).

The success of artificial insemination is directly related to the quality of the collected semen. Microscopic morphological examination of spermatozoa is one of the most important tests to evaluate the potential fertility of semen. Morphologically defective spermatozoa have no fertilizing ability (4). The abnormal spermatozoa rates in the semen of some domestic and wild cat species are relatively high (5).

There are few studies on the spermatozoa morphology of the domestic cat (6-9). Toms with a normal spermatozoa rate higher than 60% are considered normospermic, and those with a rate lower than 40% are considered teratospermic (10). The influence of abnormal spermatozoa morphology on fertility in nature is not known (6).

The head of the cat spermatozoon is 6.5 µm long and 3 µm wide, the spermatozoon having an average total length of 55-65 µm (11). The most frequently observed morphological defect types in cat semen are classified as follows (7):

a) Acrosomal abnormalities (knobbed acrosome, swollen acrosome, acrosome with abnormal borders),

b) Head abnormalities (detached head, narrow head, macrocephalic head, undeveloped head),

c) Mid-piece abnormalities (double mid-piece and mid-piece defect), and

d) Tail abnormalities (coiled tail, bent tail).

Total morphological defect rates recorded by researchers in cat semen using various staining (carbol-fuchsin, eosin-nigrosin and papanicolaou) and fixation (formol saline fixed solution) techniques are 2-29% (5,8,10,12-15).

Kaya (12) examined the semen of 8 Angora cats in Hancock solution and gave the results as; 2.47 ± 0.18% acrosome, 16.86 ± 2.86% other (mid-piece and tail) and 26.48 ± 1.29% total morphological defects. Wood et al. (16) employed the triple staining technique and determined an 89.6% intact acrosome rate.

The Spermac® staining technique has been used successfully in bull, ram, stallion, boar, dog and human semen (fresh or diluted) in recent years (17,18). Spermac®, which is a metachromatic stain, is a rapid, easy and reliable staining technique and is used to observe different levels of acrosome defects (17). Schäfer and Holzman (9) used the Spermac® staining technique in cat semen and reported that it had no adverse effect on acrosomal integrity.

This study was carried out to evaluate acrosomal and other abnormal spermatozoon types and rates using the Spermac® staining technique.

Materials and Methods

Five stray cats aged 2-3 (average 3.0-4.5 kg live weight each) served as semen donors. The cats were kept in 60 x 90 x 120 (h) cm stainless steel cages and received 12 h daylight per day. Cats consumed 65-70 g commercial cat food (IAMS Company, Ohio, USA) daily and were given drinking water ad libitum.

Semen Collection by Electro-Ejaculator

Semen was collected from the toms by means of a specially adapted electro-ejaculator (P-T Electronics, Model 302, Boring, Oregon, USA) (Figure 1). A combination of xylazine (2 mg/kg body weight, s.c., Rompun®, Bayer, İstanbul, Turkey) and ketamine HCl (5 mg/kg body weight, i.m., Ketalar®, Eczacıbaşı, İstanbul, Turkey) was employed in anesthetizing the toms. Anesthetized toms were placed horizontally and a lubricated rectal probe (P-T Electronics, Boring, Oregon, USA), which was 1 cm thick, 12 cm long and had 3 electrodes (1.5 mm thick and 3 cm long each), was placed into the rectum (average 9 cm). Electric pulses were administered following the Platz and Seager (13) procedure (3 s pulse, 2 s interval). The set of administrations was between 2 and 7 v and each set contained 60 pulses with a total of 180 electric pulses. Before the electrical stimulation, an Eppendorf tube was placed over the penis. Each tom gave a total of 5 ejaculates throughout the study with a once a week schedule (total 25 ejaculates).

Determination of Abnormal Spermatozoa Rate

Semen was diluted with 100 µl of 0.9% NaCl after collection. Morphological defects were determined by staining with Spermac® (Stain Enterprise, P.O. Box 12421, 0110, Onderstepoort, Republic of South Africa) stain kit (Figure 2). A drop of semen was placed on a glass slide and a thin smear was prepared and air-dried for 3 min. The slide was then fixed for 5 min and washed with distilled water 5-6 times. Excess water was removed with a piece of filter paper and the slide was placed into stain solution A for 1-2 min before being totally dried. This procedure was repeated for solutions B and C. Finally, the slide was air dried.

The smears were stained with Spermac®, and 200 spermatozoa were evaluated for abnormal acrosome, head, mid-piece and tail forms under a light microscope at x1000 magnification according to the method described by Axner et al. (7).

Under the microscope the acrosome, mid-piece and tail, the head's post-acrosomal region, and the equatorial segment were green, red and light green, respectively (Figure 3a).

The acrosome, other and total morphological defect mean values were evaluated by the SPPS program and ANOVA (one-way).

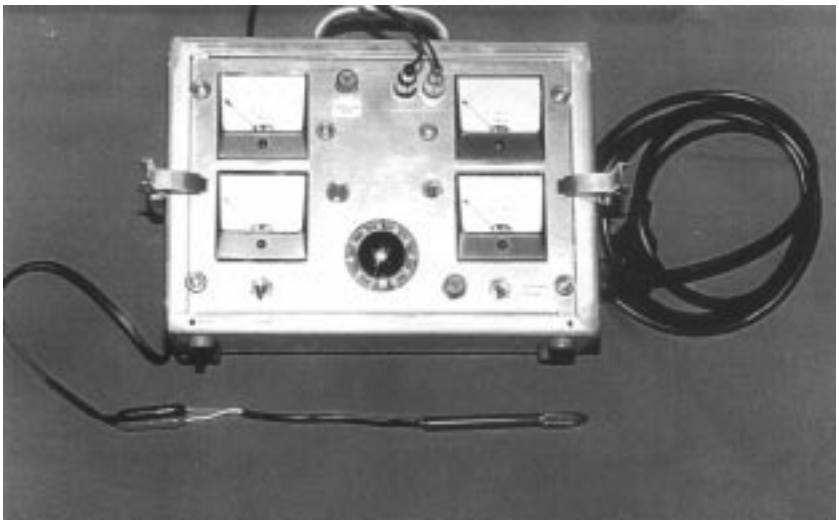

Figure 1. Electro-ejaculator and bipolar rectal probe (P-T Electronics, Model 302, Boring, Oregon, USA).

Figure 2. Spermac® stain kits (Fixative, solutions A, B, C, Stain Enterprises, P.O. Box 12421, 0110, Onderstepoort, Republic of South Africa).

Results

The mean values of morphological defects are presented in the Table.

The mean total morphological defect rate in the study was 18.40 ± 4.79%, while the acrosomal defect rate was 9.20 ± 2.55% (Table). The most observed acrosomal defect types were swollen acrosome (Figure 3b), lost acrosome (Figure 4a) and dissolved acrosome (Figure 4b).

The total other morphological defect (head, mid-piece and tail) rate average was 9.20 ± 3.49% (Table). The most frequently seen head defects were a microcephalic (Figure 5a), a narrowed (Figure 5b-i) and a macrocephalic (Figure 6a) head. Mid-piece defect types were thickening (Figure 5b-ii), a swollen (Figure 6b-i) and a double (Figure 6a) mid-piece. The tail defects were generally coiled (Figure 6b-ii) and rarely double (Figure 6a) tails.

Table. Abnormal morphology rates in fresh semen (n = 25).

Acrosome (%) $\bar{X} \pm S\bar{x}$	Other (head-midpiece and tail) (%) $\bar{X} \pm S\bar{x}$	Total (%) $\bar{X} \pm S\bar{x}$
9.20 ± 2.55	9.20 ± 3.49	18.4 ± 4.79

Means ± SD (standard deviation)

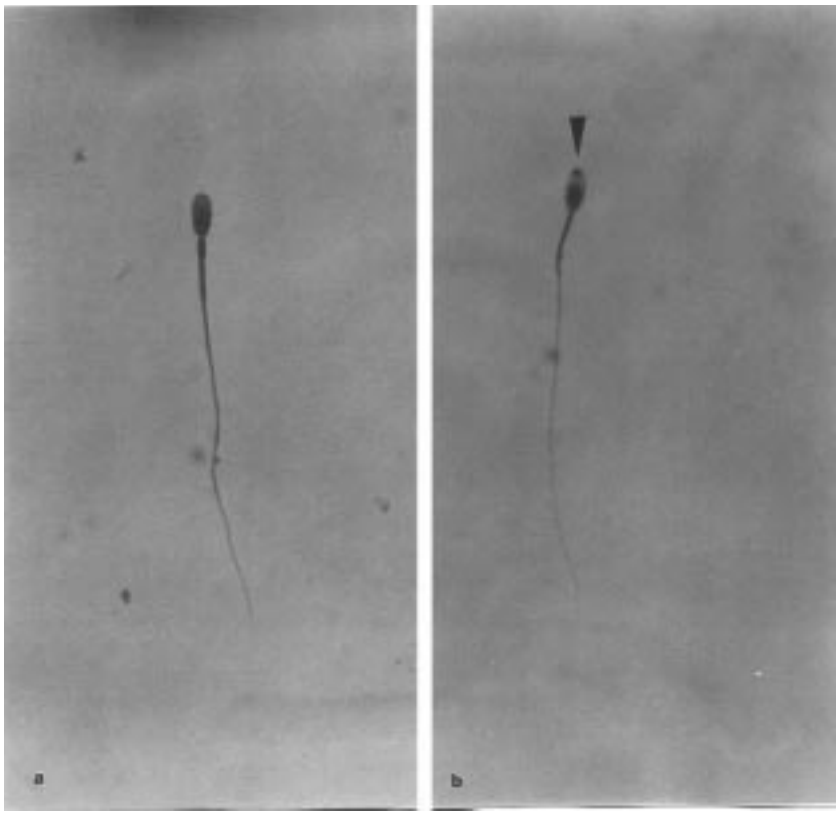

Figure 3. Normal cat spermatozoa and acrosomal abnormality.
a) Normal cat spermatozoa,
b) Swollen acrosome (light microscopy, x1000 magnification).

Discussion

The abnormal spermatozoon rates of fresh semen were 9.20 ± 2.55% acrosome defects, 9.20 ± 3.49% other defects and 18.40 ± 4.79% total morphological defects. The acrosome defect rate was higher than the mean 2.47 ± 0.18% reported by Kaya (12) and than Long et al. (19)'s 3.1 ± 1.3% value from normospermic toms. This difference can be attributed to the different staining and fixative techniques used by these researchers. However, our mean acrosome defect rate of 9.20 ± 2.55% was similar to the value of 9% reported by Pope et al. (20).

Our mean total morphological defect level of 18.40 ± 4.79% was similar to the 16.58 ± 1.01% value reported by Kaya (12), and lower than the 29% value reported by Wildt et al. (21) and than the 25% value reported by Pukazhenthi et al. (22). Howard et al. (10) reported the abnormal spermatozoon rates of domestic and wild cats to be high. Platz and Seager (13) collected semen by electro-ejaculator and reported an abnormal spermatozoa rate of <2%. Smith (14) observed a value of 4%. Tsutsui et al. (15) collected semen with an artificial vagina and observed an abnormal spermatozoa value of 3.8 ± 1.7%, and Tanaka et al. (23) reported a

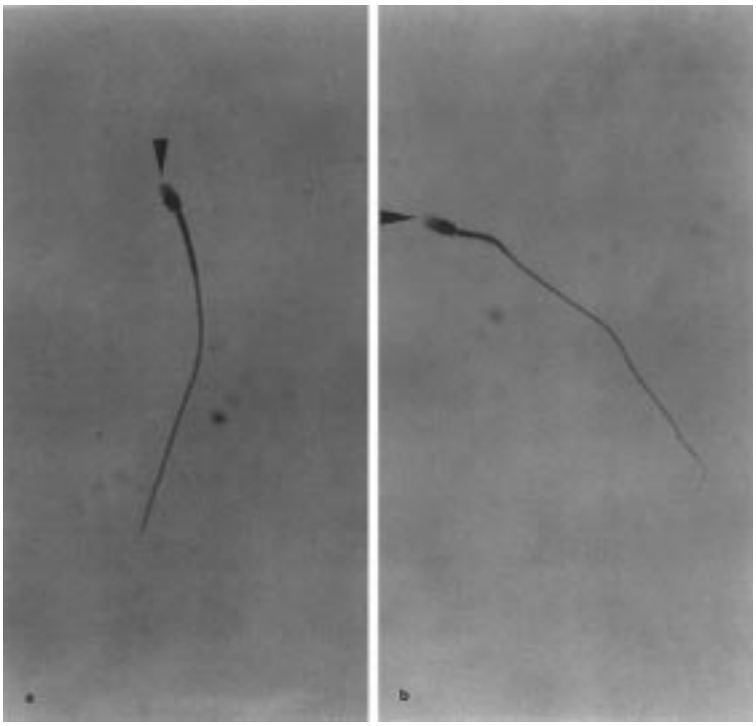

Figure 4. Acrosomal abnormalities (light microscopy, x1000 magnification).
a) Lost acrosome,
b) Dissolved acrosome.

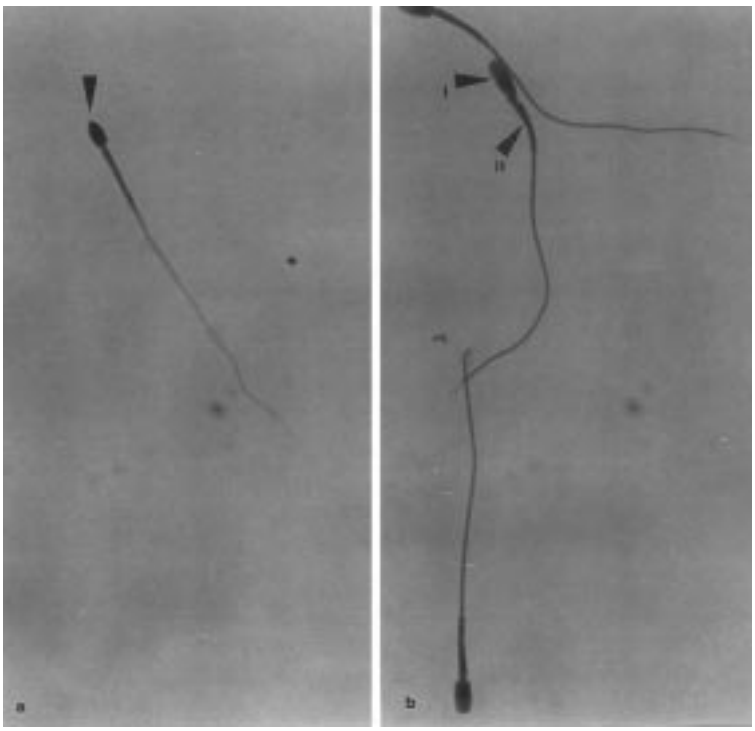

Figure 5. Head and mid-piece abnormalities (light microscopy, x1000 magnification):
a) Microcephalic head,
b-i) Narrowed head, b-ii) Mid-piece thickening.

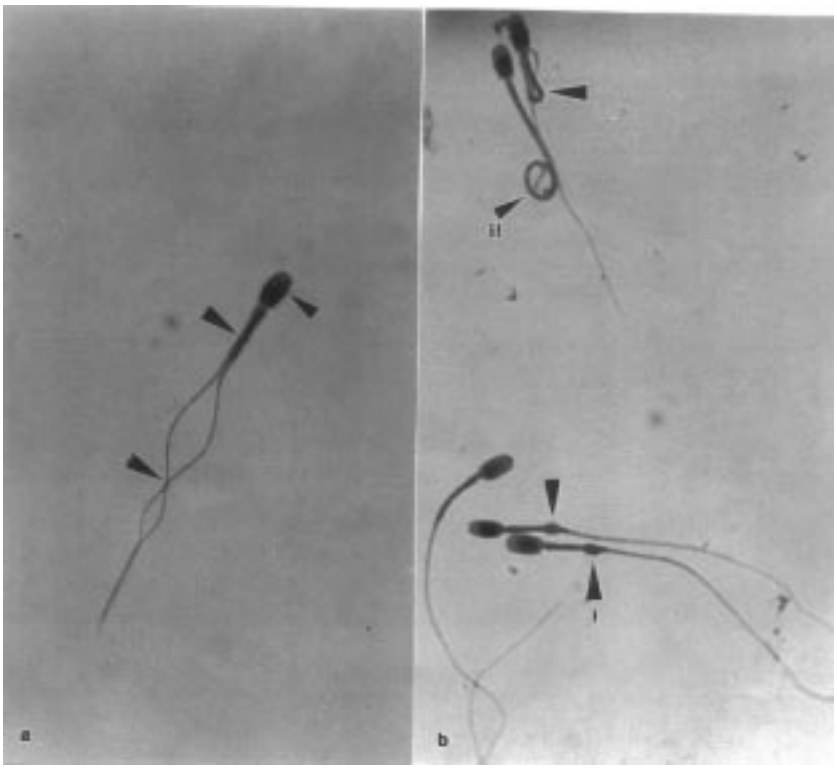

Figure 6. Head, mid-piece and tail abnormalities (light microscopy, x1000 magnification).
a) Macrocephalic, double mid-piece and double tail,
b-i) Swollen mid-piece,
b-ii) Coiled tail.

value of 8.5%, all of which were lower than the results of our study. The difference in acrosome and total morphological defect rates among the researchers may be due to the morphological evaluation techniques, the semen collection techniques and the breeds of cat used.

Schäfer and Holzman (9) compared the Spermac® staining technique and Hancock solution in their study and observed that the efficacy of Hancock solution was lower than that of Spermac® in revealing head and acrosome defect types in cat semen. These researchers also reported that the preparations with Spermac® stain contained fewer protoplasmic droplet spermatozoa, and attributed this to degeneration of the cytoplasmic droplet during staining.

The present study shows that the Spermac® staining technique possesses the advantages of being rapid, reliable and practical, and can successfully be used in the examination of acrosomal morphology in cat semen, as with that of other species. In the light of our results, this technique is suggested to be beneficial in determining acrosome defects, which are one of the leading defect types in post-thaw semen.

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