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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Electron Microscopic and Morphometric Analysis of Wistar Rat Sperm

Emre DEMİRCİ*
Kastamonu Provincial Directorate of Agriculture and Forestry, Saraçlar District Kastamonu Provincial Directorate of Agriculture and Forestry Annex Building Headquarters/Kastamonu, Türkiye

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*: https://orcid.org/0000-0002-3558-1760

***Corresponding author's:**
Emre DEMİRCİ
Kastamonu Provincial Directorate of Agriculture and Forestry, Saraçlar District Kastamonu Provincial Directorate of Agriculture and Forestry Annex Building Headquarters/Kastamonu, Turkey
✉: d_emre_67@hotmail.com

Abstract: The aim of the study is to image and morphologically examine epididymal sperm in Wistar rats with scanning electron microscopy (SEM). The material of the study was epididymal sperm taken from three Wistar Rats. Routine fixation was performed on the obtained epididymal sperm using 2.5% glutaraldehyde solution. Morphometric measurements of sperm were carried out using scanning electron microscopy. The study focused on examining various regions of the sperm, including the head, midpiece, and the remainder of the flagellar segment. Measurements were taken into account in semen that preserved its integrity in different fields. Measurements of these regions were made with the ImageJ program. As a result of the measurements, it was determined that the average head length of rat semen was 7.821 ± 0.844 µm, the average total flagellum length was 168.549 ± 9.19 µm and the average flagellum/head ratio was 21.550 and the flagellum was quite long. It was observed that the measurements of the rat sperm used in the study were consistent with the measurements of other rat sperm. When the studies were examined, it was concluded that rat sperm has an extremely long flagellum compared to its body size. The morphological differences of the sperms revealed will be a guide for experimental studies.

Keywords: Electron microscope, morphology, rat sperm.

Wistar Sıçan Sperminin Elektron Mikroskobik ve Morfometrik Analizi

***Sorumlu yazar:**
Emre DEMİRCİ
Kastamonu İl Tarım ve Orman Müdürlüğü, Saraçlar Mahallesi Kastamonu İl Tarım ve Orman Müdürlüğü Ek Bina, Merkez, Kastamonu, Türkiye
✉: d_emre_67@hotmail.com

Öz Wistar sıçanlarda epididimal spermin taramalı elektron mikroskobu (SEM) ile görüntülenmesi ve morfolojik incelenmesi çalışmanın amacını oluşturmaktadır. Çalışmanın materyalini üç Wistar Sıçanından alınan epididimal spermalar oluşturdu. Elde edilen epididimal spermalara % 2,5 glutaraldehit solüsyonu kullanılarak rutin fiksasyon işlemi yapıldı. Spermaların morfometrik ölçümleri Taramalı elektron mikroskobu kullanılarak gerçekleştirildi. Çalışma, baş, orta parça ve flagellar bölümün kalan kısmı dahil olmak üzere spermin çeşitli bölgelerinin incelenmesine odaklanmıştır. Farklı sahalarda bütünlüğünü korumuş spermalarda ölçümler dikkate alındı. Bu bölgelerin ölçümleri İmageJ programı ile yapıldı. Yapılan ölçümler Sonucunda sıçan spermasının baş uzunluğunun ortalama 7,821 ± 0,844 µm total flagellum uzunluğunun ortalama 168,549 ± 9,19 µm ve ortalama flagellum/baş oranının 21,550 olduğu ve flagellumun oldukça uzun olduğu belirlendi. Araştırmada kullanılan sıçan sperminin ölçümlerinin diğer sıçan spermlerinin ölçümleriyle tutarlı olduğu görüldü. Yapılan çalışmalar incelendiğinde, sıçan spermasının vücut boyutuna göre son derece uzun bir kamçıya sahip olduğu sonucuna varıldı. Ortaya konulan spermaların morfolojik farklılıkları yapılacak deneysel çalışmalara bir rehber olacaktır.

Anahtar kelimeler: Elektron mikroskop, morfoloji, sıçan sperma.

INTRODUCTION

Biomedical and genetic investigations have long relied on the use of experimental animals. Rats, as subjects in experimental research, have proven to be valuable models for studying both human and animal disorders (Lazar et al., 2005; Tesson et al., 2005). Sperm morphological characteristics such as head shape and flagellar length vary among mammals. Generally, the width and length of the sperm head vary depending on changes in the size and organization of the acrosome and nucleus (Mortimer, 2018). Research suggests that, unlike other mammalian species, rats do not have a well-known method for freezing and storing sperm, mainly due to the sensitivity of the membrane integrity and mitochondrial membrane to the freezing procedure (Nakatsukasa et al., 2001; Yamashiro et al., 2007; Varisli et al., 2013). The cryopreservation and storage of rat embryos, spermatozoa, oocytes, and reproductive organs play a crucial role in conserving rat species and facilitating the development of new rat species (Oh et al., 1998). Spermatozoa cryopreservation is a more convenient and cost-effective technique than embryo cryopreservation. It plays a crucial role in preserving current and future rat strains (Nakatsukasa et al., 2003). Compared to sperm from other laboratory animals, such as mice, it has been observed that rat sperm does not have acceptable levels of motility and fertility rates (Yamashiro et al., 2007; Varisli et al., 2013). Rat spermatozoa exhibit heightened sensitivity to aberrant circumstances such as centrifugation, chilling, and osmotic stress (Nakatsukasa et al., 2001; Varisli et al., 2009). The cryogenic properties can be influenced by the morphology and dimensions of the rat spermatozoa head. Spermatozoa from many animal species exhibit distinct variations in terms of size, shape, membrane phospholipids, and metabolism. When comparing the spermatozoa of experimental animals, particularly rodents, to those of domestic animals, it is seen that they exhibit distinct traits such as a lengthy tail, specific head shape, and unique membrane composition in contrast to mammals (Devireddy et al., 1999; Holt, 2000).

The sperm flagellum can be functionally divided into two parts. The midpiece includes the neck region, and the principal piece includes the tip (Commins, 1985). The neck region lacks many structural features and protein compositions and connects with internal structures in the midpiece (Avidor-Reiss et al., 2015; Wooley et al., 2008). The midpiece comprises bundled mitochondria surrounded by the cell's cytoskeleton (Lindemann & Lesich, 2016). The principal piece contains a fibrous sheath surrounding the cell's cytoskeleton (Eddy et al., 2003). Mitochondria in the midpiece of sperm serve as a source of adenosine triphosphate (ATP). It generates the energy in the principal piece that drives sperm propulsion.

The lengths of the midpiece and principal piece of sperm may be critically important in determining sperm swimming speed and fertilization rate. The objective of this work was to observe rat spermatozoa, which possess a distinct sperm morphology compared to other animals, using a SEM, and to elucidate the architecture of the head, neck, and tail.

MATERIAL AND METHOD

Animals: Epididymides of Wistar rats were subjected to standard care conditions without manipulation. In our study, three male Wistar rats aged 10-12 weeks with an average weight of 236.66 ± 12.47 g were used for epididymal sperm collection. Procedures of that study were approved by the Local Ethics Committee of Kastamonu University (dated: 13.11.2023 and numbered: E-16498365-000-2300125782).

Collection of Epididymal Sperm Samples: The epididymides were separated from the testes of the rats. Each epididymis was thoroughly minced in a petri dish containing physiological saline solution (0.9% NaCl) using a scalpel and scissors to allow spermatozoa to pass into the solution.

Determining the sample size: A power analysis with 95% power and 0.5 effect size revealed the necessity for a minimum sample size of > 44. Literature review conducted prior to the study indicated variability in the required number of experimental animals for detecting sperm morphometry in rat studies (Dibal et al., 2020; Gu et al., 2019). Therefore, in this study, sperm samples were obtained from 3 healthy male Wistar rats, and 30 measurements were taken for each animal, resulting in a total of 90 measurements (n = 3 (experimental animals) × 30 (measurements)). Measurement results were presented as mean value ± standard deviation.

SEM procedure: On cover glasses coated in poly-D-lysine, the sperm of three male individuals per species were plated. Next, the cover glasses were immersed in a fixation solution containing 2.5% glutaraldehyde in a 0.1 M PBS buffer with a pH of 7.4. Afterward, the sperm were treated with 1% osmium tetroxide in 0.1 M PBS buffer (pH = 7.4) to fix them. Then, they were dehydrated in a series of acetone with concentrations of 25%, 50%, 75%, 100%, and 100% for 10 minutes each. The sperm underwent the same processing method as described in a previous study (Demirci et al., 2023). The SEM pictures were analyzed using ImageJ software. Morphometric measurements in sperm morphology were measured regarding the study of Gu et al., (2019). According to Gu et al., (2019), the segmented line tool was used to measure the morphological parameters of each sperm, including head length, head width, midpiece

length, major piece length, end piece length, and diameter of 1-9 sites on the sperm.

RESULTS

Measurements were conducted on the head area, the midpiece of the flagellar section, and the remaining portion of the flagellar section. The investigation revealed that the head area had a hook-shaped structure (Figure 1).

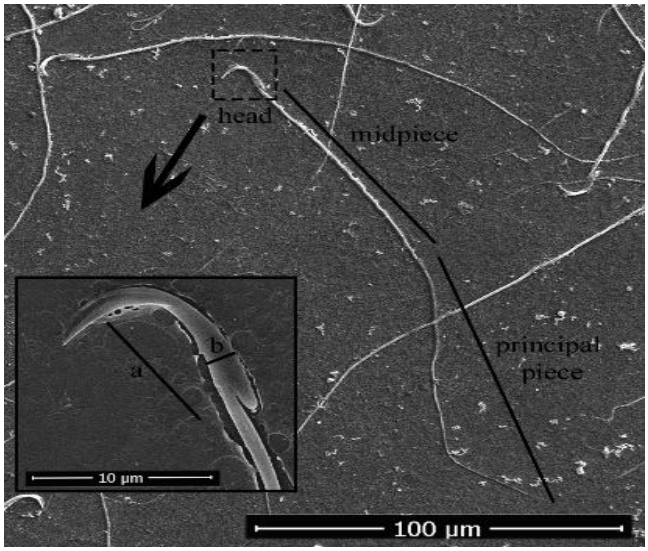

Figure 1. Scanning electron micrograph of rat sperm. **Arrow**: close-up view of the delineated squared area, **a:** Head length, **b:** Head width.

The appearance of a groove-like structure positioned above the hook form captured notice. The flagellar component lacked a jagged entrance-like feature and was attached laterally at the end of the head. The flagellar section exhibited a posterior thinning and was discerned as including two distinct components, one exhibiting a brighter hue and the other displaying a darker shade. The terminal portion of the flagellum was noticed as a slender and clearly distinguishable segment. The flagellar component appeared to be enveloped by a sheath, concealing the intertwined braid-like structure that rotated in unison. Table 1 shows the measurements of the head and flagellar regions of the sperm.

DISCUSSION

Objective and metric measures of sperm size and type categorization, obtained by statistical analysis, are highly important and effective for analyzing sperm morphology. This indicates that employing this method would be an especially accurate and precise strategy for tracking biomarkers of toxicant exposure (Davis et al., 1994).

Sperm imaging is a specialist discipline that has developed throughout time. The size of the head and tail of the sperm may vary across different mammalian species. The dimensions of the sperm head generally vary due to changes in size and the arrangement of the acrosome and nuclei (Mortimer, 2018). The sperm head contains a haploid genotype that is highly compacted. Spermatozoa are responsible for the union of the male haploid genotype with the egg during fertilization (Ward & Coffey, 1991). The hydrodynamic selection process also influences the size and form of the sperm head, aiming to maximize swimming ability and efficiency in penetrating the egg (Gage, 1998). We conducted a research where we determined the measures of both the length and width of rat spermatozoa heads. In a study conducted by Cummins, & Woodall, in 1985, the head length of the sperm of Bower's rats was 12.5 µm and the width of the sperm head was 1.70 µm; In the same study, the head length of sperm in Long-Tailed Giant Rats was 11.5 µm and the head width was 1.8 µm; In the same study, the head length of sperm in Common White and Brown rats head length respectively 11 and 1.8 µm. In their morphological study in 2019, Gu et al. reported that the head length of sperm in Norvegicus rats was 7.14 µm, the sperm head width was 1.69 µm. The data for the head length and width of sperm we obtained were 7.82 and 1.79 µm, respectively.

Table 1. Length and width measurements. Measurement results were presented as mean value ± standard deviation.

Rat	Head (µm)	Midpiece (µm)	Principal Piece (µm)	Total Flagellar (µm)	Flagellum: head ratio
Length	7.821 ± 0.844	67.695 ± 8.964	100.854 ± 11.167	168.549 ± 9.19	21.550
Width	1.79 ± 0.0672	1.238 ± 0.213	0.817 ± 0.235	--	--

The length of sperm grows as body size decreases in an inverse relation to body size, according to prior research. Structurally intact sperm flagella plays a crucial role in the movement of sperm and the processes of fertilization. Gomendio & Roldan, (1991) discovered variations in the sperm of some rats during competitive situations. The researchers found that among monkeys, the sperm of men in polygamous species is longer than that of males in monogamous ones. Prior research has indicated that the length of the flagellum, particularly the midpiece, is a reliable indication of the speed at which sperm can travel (Firman & Simmons, 2010; Tourmente et al., 2011). According to Gu et al., (2019), there was a positive correlation between the size of the mitochondria in the flagella and the speed at which they move. The energy needed for this process is supplied by the metabolism of cyclic AMP within the compact core of the mitochondria (Gage, 1998). It is believed that longer spermatozoa have a higher swimming speed but a shorter lifespan. According to Gomendio & Roldan, (1991) this is expected to necessitate a greater amount of metabolic energy that is utilized at a faster rate. In a study conducted by Cummins,

& Woodall, in 1985, They reported the middle part and total flagella lengths as 55 and 172 μm, respectively, and the flagellum head ratio as 13.76. In the same study, in Long-Tailed Giant Rats They reported the middle part and total flagella lengths respectively as 67 and 170 μm, and the flagellum head ratio as 14.78. In the same study, the middle part length of sperm in Common White and Brown rats 67 μm, and total flagella lengths was reported to respectively as 188.7 and 190.1 μm. In their morphological study in 2019, Gu et al. reported that the middle part and total flagella lengths were 67.42 and 177.24 μm, respectively, and the flagellum head ratio was 24.87. The data we obtained were consistent with those reported in prior morphometric research.

In conclusion, when we compared the previous measurements with our study, it was observed that sperm head length and width were close to the *Rattus Norvegicus* but different from other rats. However, flagellar length was close to each other among rat species, which is consistent with our study. These differences and similarities are thought to reflect species characteristics. Research has demonstrated that there are variations in the length of sperm, even between different species of rats. There is a requirement for thorough investigations on the morphological measurements of sperm, particularly focusing on rat species.

CONCLUSION

In conclusion, detailed visualization of Wistar rat sperm cells through scanning electron microscope images commonly used in scientific research, along with precise micro morphometric measurements from these high-resolution images, have enabled the determination of the details and measurements of sperm cells. Current findings serve as a reference for future research and provide preliminary data for comparing the male reproductive cells of Wistar rats and other experimental animals.

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Conflict of interest

There is no conflict of interest.

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