A Comparative Study of Sperm Morphology and Morphometrics of Wild and Domestic Male Rabbits in Saudi Arabia

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Abstract: The purpose of this study was to investigate spermatozoa morphometrics in two strains of male rabbits (*Sylvilagus species* and *Oryctolausspecies*) in Saudi Arabia.Semen samples were collected from the caudal epididymis of the adult male rabbits by conventional methods and smeared on the slides then stain, examine under the microscope and photographed. Spermatozoa morphology in both strains was assessed from a total count of 100 spermatozoa. The parameters assessed werehead length,head width,midpiece length and tail length. Abnormalities in spermatozoa such as tail coiled around head, around mid piece, tail coiled below stumpy tail, bent tail and decapitated or detached head were also detected. Results showed a significant difference (p<0.05) in tail length and midpiece length of spermatozoa between the two strains. There was significant difference between the two strainswith regard to abnormal head,tail coiledaround head, tail coiled around mid piece, detached head and condensed acrosomes.The study revealed a negative influence of spermatozoa abnormalities on its quality and fertilization.

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1. Introduction

Male reproductive function include the production of semen containing normal spermatozoa in adequate number together with ability to perform intercourse (Oyeyemi *et. al.*,2008; Oyeyemi and Ubiogoro,2005).Studying sperm morphologyis a useful tool for detection of sperm fertility and estimation of sperm quality in mammals (Irvine and Aitken, 1994). Rabbits are farmed primarily for their high quality meat.Baladi or domestic rabbits (*Sylvilagus sp.*) are of economic importance. Thisstrain of rabbits has been imported into a number of countries including the Kingdom of Saudi Arabia, mainly for the production of meat(Lebas*etal.*,1997). The Bari or Wild strain (Oryctolaus sp.) is a wild strain of rabbits found in certain areas of the Kingdom such as southern Region or wadyalDawaser that have fluctuating environments.

The extensive production of meat in both Domestic and Wild strains has been limited by their fertility rates(Hafez, 1987; Skinner,1975).

Sperm morphology is considered as a predictor of success in fertilizing oocytes during *in vitro* fertilization.The sperm head morphology has been used earlier to determine fertility potential in rabbits (Lavara *et al.*,2008, Cooper *et al.*,1999;Ombelet *et al.*,1995). However, in some strains of rabbits, the problem of quantifying a normal morphology remains a challenge (Gago *et. al.*, 1998;Sancho *et. al.*,1998; Bedford, 1963).This was clearly justified by an earlier study in male rabbits of Wild species, which have several morphological forms in the mid piece and tailof spermatozoa (Sanchez *et al.*, 1997). Consequently, this study aimed to investigate morphometrics in the sperms of male rabbit of Domestic and Wild strainsas well as to determine different morphological traits of the spermatozoa in these strains.

2. Material and Methods

1- Animals:

Adult male rabbits of Domestic strain (*Sylvilagus sp.*) werepurchasedduring periods of maximum gonad activity (spring and winter) from a local bird market in Riyadh city, and their color was white with wool and fur(Fig. 1). TheWild strainrabbits (*Oryctolaus sp.*) were obtained from fishing enthusiasts and were difficult and expensive to obtain. The color of the Wild rabbits was gray having wool and fur (Fig. 2). The number of male rabbits used was four for each strain weighing 3-4 kg.Animals were fed regularlywithbalanced diet, and sexually mature at the time of collection of spermatozoa. The current study was carried out at the Department of Zoology Faculty of Science, King Saud University, KSA.

2- Methods:

a- Isolation of spermatozoa:

The animals were then killed by ether anesthesia. The epididymis was excised and samples of spermatozoa were taken from the caudal part of the epididymis.To release the spermatozoa, tissues from

the caudal epididymis were cut with sterilized scissors into small pieces and were pressed, thus forcing the spermatozoa from the ducts into medium containing phosphate buffer for 5-10 minutes followed by staining with1 % Eosin stain (Wyrobek and Bruce, 1975; Mori *et al.*, 1991; Chandra*et. al.*, 2007).

Fig. 1: Domestic strain (*Sylvilagus sp.*)

Fig. 2: The Wild strain rabbits (*Oryctolaus sp.*)

b- Examination of spermatozoa:

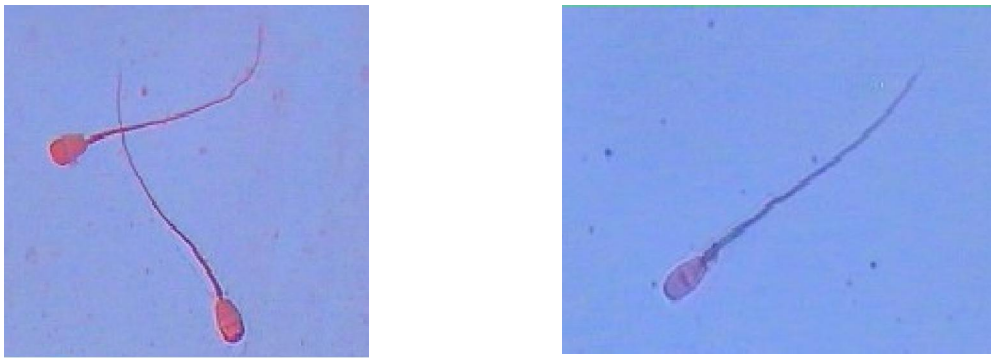
Examination of spermatozoa was performed according to the method described by Bedford (1963). In accordance with this method,the spermatozoa were smeared on the slide, drying in air, fixed with 10% formalin and stained with Eosin. For sperm morphology and morphometrics, prepared slides were examined by research microscope with digital camera and photographed. In each of the four animals,measurements were made in µm of the following traits and parameters: head length, head width,midpiece length and tail length in both Domestic and Wild strains for comparison. Theparameters were studied in 100 spermatozoa.In another set of experiments, spermatozoa samples were divided into two categories normal and abnormal according to strict sperm morphology criteria and all of the morphological abnormalities in both rabbit strains.

3- Statistical analysis

Data obtained were pooled and statistically analyzed with help of statistical package SAS 9.1(SAS Institute Inc.,U.S.A) usingunpaired student`s t-test.

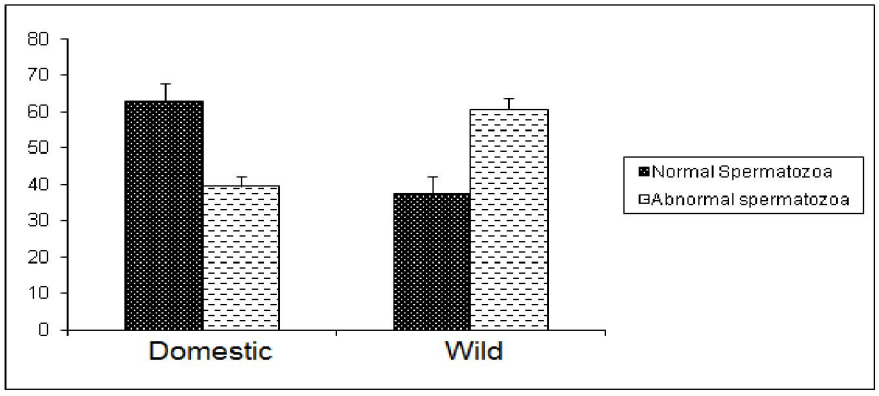
3. Results and Discussion

In Saudi Arabia, the Domestic strain is affordable and easily available when compared to Wild strain, which is more expensive, very limited in numbers and scarce in its natural habitats.The present study was aimed to determine morphological differences in the spermatozoa of two rabbit strains:Domestic and Wild in Saudi Arabia(Fig.3a,b). The study revealed prominent differencesin male spermatozoa morphometrics between the tested strains (Fig.4).



(a) (b)

Fig. 3: A Photomicrograph showing Normal morphology of spermatozoa in male rabbit of a) Wild and b) Domestic stained with Eosin (X 1000)



Values are mean±SEM, n=5, $^{**}P<0.01$ compared to Domestic rabbit spermatozoa
Fig. 4: Abnormal vs. normal morphology spermatozoa in Domestic and Wild rabbits

The head width, mid piece length and tail length of spermatozoa from Wild strain was significantly different from that of Domestic rabbits (Table 1). However, no significant difference was observed in head length of spermatozoa between tested strains.

Table 1: Spermatozoa morphological parameters in male rabbits of Domestic and Wild varieties

Parameter(μ)	Domestic	Wild
Head length	8.0±0.07	9.6.0±0.71
Head width	4.0±0.03	5.0±0.10***
Midpiece length	10.0±0.06	8.3.0±0.02***
Tail length	37.5±0.05	32.0±0.08***

Values are mean ± SEM, n=5, *** $P<0.001$ compared to Domestic rabbit spermatozoa

The sperm swimming velocity is an important factor for male fertilization success. The sperm swimming capacity depends on sperm morphometrics, that includes the head length, head width, mid piece length and tail length (Firman and Simmons, 2010;Malo *et. al.*,2006) .Spermatozoa with longer mid piece swim more slowly, while those with elongated heads and longer tails swim faster. In the present study, the head width of the spermatozoa from the Wild strain was wider and the mid piece was shorter indicating that the sperm swimming capacity of the Wild strain may be more slowly compared to the Domestic strain. On the contrary, the tail length of the Wild strain was shorter compared to the Domestic strain, which suggests slower swimming of the spermatozoa in the Wild strains. The relative contribution of each of these sperm morphometrics on the sperm swimming capacity is notclearly known, though the mid piece length is considered as the most important factor. Hence, it can be concluded that sperm swimming capacity of the Wild strain may be higherthan that of Domestic strain.

In the ejaculates of Bouscat White and New Zealand White males the incidence ofsperm abnormalities was higher in summer compared with other seasons, but therewas no difference according to breed (Amin *et al.*, 1987). In contrast to the results of (Finzi *et al.*, 1994), unexpectedly, at least some parameters can even improve duringacute and chronic heat stress.

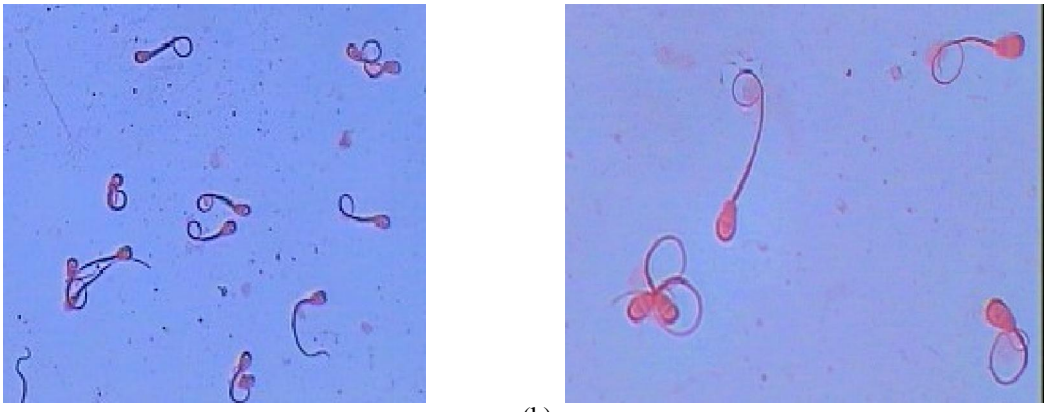
In the other set of experiments abnormalities in spermatozoa of the two varieties Domestic and Wild was examined critically (Table2).

Table 2: Abnormal morphological traits in Spermatozoa of Domestic and Wild rabbits

Abnormalities	Domestic variety	Wild variety
Twin head	0.8±0.490	0. 6 ±0.400
Dag defect	3.0±0.447	3.8±0.663
Abnormal head	6.6±0.812	9.6±0.812*
Proximal cytoplasmic droplet	4.4±0.748	4.0±1.225
Distal cytoplasmic droplet	1.6±0.509	3.6±1.07
Tail coiled around head	6.2±0.860	10.8±1.393*
Tail coiled around midpiece	4.0±0.707	8.4±0.927**
Tail coiled below stumpy tail	1.8±0.800	1.8±0.583
Detached head or separate flagellum(decapitated)	3.4±1.030	6.6±1.208
Simple bent tail	1.4±0.510	5.0±1.304
Simple coiled tail	4.0±1.761	6.4±1.634

Values are mean±SEM, n=5, $^{*}P<0.05$, $^{**}P<0.01$ compared to Domestic variety spermatozoa

The abnormalities include twin head,dag defect,abnormal head, proximal cytoplasmic droplet, distal cytoplasmic droplet,tail coiled around midpiece, tail coiled below stumpy tail,detached head or separate flagellum and simple coiled tail (Fig.5 and Fig.6).



(a) (b)

Fig. 5: Aphotomicrograph showing abnormal morphology of spermatozoa in male rabbit of a) Wild and b) Domestic stained with Eosin. Note: spiral tail or coiled tail (X 1000)

(a) (b)

Fig. 6: A Photomicrograph showing abnormal morphology of spermatozoa in male rabbit of a)Wild and b) Domestic stained with Eosin . Note: bent tail or spiral tail (X 1000)

It was found that the percentage of spermatozoa withabnormal heads, tail coiled around and tail coiled mid piece was significantly more in Wild strain compared to the Domestic strain(Table2).The overall percentage of abnormal spermatozoa in Wild strain was significantly more than Domestic strain (Fig.4).

The increased percentage of abnormal spermatozoa in the Wild strain probably explains the scarce number of these rabbits in the kingdom. The percentage of speramtozoa with normal acrosome in Wild strain was significantly less compared to the Domestic strain (Table3). Thus, the Domestic strain spermatozoa have less morphological abnormalities compared to that observed in Wild strain.

These findings are in line with what observed by(Kuzminsky,G.et.al.1996) who explained that ,in New Zealand White rabbits , head abnormalities and broken spermatozoa represented a low proportion of the total number of spermatozoa viewed .Tail abnormalities (coiled tails, cytoplasmic droplets , bent tails and swollen tails) represented 13.6% of the observed spermatozoa and 74.7% of the total abnormalities .

There have been, in fact, very few studies in the literature relative to abnormalities in rabbit spermatozoa, other than tail abnormalities, and the studies that do exist do not agree among themselves (Kasa and Thwaites, 1992; Virag *et Al.,* 1992; Roca, 1993) or with the results reported here. This is no doubt due to the fact that each study had an independent analysis protocol and different abnormality criterion was considered. Seasonal and genetic differences could be involved as well (Virag*etal.,* 1992).

Table 3: Abnormal vs. normal morphology (acrosome) of male rabbit Spermatozoa during the periods of activity*

Morphological trait	Domestic variety	Wild variety
Normal acrosome (covered ¼ of the head area)	37±0.06	28±0.08***
Condensed acrosome (covered 2/3 of the head area)	13±0.02	22±0.06***

Values are mean±SEM, n=5, *** $P<0.001$ compared to Domestic rabbit spermatozoa

The sperm morphological abnormalities are closely related to fertility. Wild strain seems to respond to seasonal fluctuations in environmental factors, which result in more abnormalities of the sperms or behavioral changes such as psychological disturbance in head and tail of the sperm morphology. However, thereis a positive correlation betweennormal shape of the sperms and fertility which increase the probability of ovulation and zygote formation. The more marked abnormalities of sperms in Wild species justified its scarcity in markets as compared to Domestic species. Further research is needed to correlate seasonal fluctuations in environmental factors, spermcount, motility, hypospermia (low semen volume) or oligospermia (low sperm count) with sperm morphology and quality. Also computer assisted semen analysis (CASA) which is a catch-all phrase for automatic semen analysis as modern techniques should also be included for future research plans.

Bodnar,*etal*., (2000) observed that in the case of New Zealand White lower rate of tail abnormalities were found. (Kasaand Thwaites, 1992) observed significant increases in the ratio of dead and piriform sperm after an increase in thelevel of heat stress.The Pannon White and New Zealand White bucks showed a significantly higher (by 6-10 %) ratio of intact spermatozoa in spring and winter. (Kadlecik,1983) found thesame tendency in the Russian breed, but in his opinion the percentages of abnormalspermatozoa also depend on the level of inbreeding in the population. Bodnar,*etal.,*(2000) observed that Pannon White and New Zealand White males produce lessabnormal spermatozoa (about 16 %) throughout the year . The mean values of abnormal cells in the Pannon White and the New Zealand Whitesemen were the same, but the distribution of the main types of spermatozoa abnormalities were slightly different. Acrosome, head and tail abnormalities showed the highest incidental rate in every group; however, every kind of deformation type wasfound in the samples. (Finzi *et al.,* 1995) developed a method to verify the presence ofheat stress in males, based on ratios among different sperm deformations.They also demonstrated that both the Pannon white and the New Zealand White samplescontained about 5-10 % more abnormal sperm cells in summer and autumn thanspring, and winter.

The present study is in agreement with the results of (Martyna*etal*.,2013) who explained that, spermatozoa morphology (the length and the width of head and tail; presence of abnormal spermatozoa) were done using Quick Photo Micro system. Received data were statistically analyzed and showed decrease of semen parameters value after one hour storage in 37°C. From among 3000 analyzed spermatozoa 14.2% posed abnormal forms.

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