MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES IN SPERMATOZOA OF HOUSE RAT DURING PASSAGE THROUGH THE EPIDIDYMIS

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ABSTRACT

Morphological changes in sperm are one aspect of a maturation process during epididymal transit in mammals. Taking into account the importance of the sperm epididymal maturation process and the consequential changes in the spermatozoa, we studied different sperm malformations in the caput, corpus and cauda regions of the epididymis of healthy and sexually mature house rats in order to determine the origin of these sperm abnormalities. Migration of the cytoplasmic droplet and induction of motility originate in caput and increases progressively through corpus to cauda regions. During epididymal transit the percentage of immature and unviable spermatozoa decreases, indicating the existence of a mechanism that removes abnormal spermatozoa. The primary abnormalities like sperm head/acrosomal defects, acrosomal abnormalities and midpiece abnormalities were observed to be constant. In contrast, there was a small but significant increase in the proportion of spermatozoa with secondary abnormalities during epididymal transit. The results of this study demonstrate that the proportions of sperm abnormalities originating in the testes decrease during epididymal transport, while some sperm tail abnormalities may actually originate in the epididymis. Furthermore, the morphometric studies showed a significant decrease in sperm head dimensions during passage through epididymis, probably due to acrosomal condensation. The change in acrosome size during epididymal passage is discussed in relation to the development of fertilizing ability of rat spermatozoa. The complex epididymal maturation process of the sperm results in quantitative and qualitative changes that can be characterized in each of the three epididymal regions.

KEYWORDS: Epididymal Maturation, House Rat, Spermatozoa, Abnormalities, Morphometry, Cytoplasmic Droplet

INTRODUCTION

Mammalian sperm differentiation is completed during their transit from the caput to the cauda epididymis and they undergo significant morphological and biochemical modifications of their plasma membrane which enable them to recognize and fertilize the oocyte (Cornwall, 2009). Under normal circumstances the morphological changes in spermatozoa within the epididymis primarily involve modifications to the sperm head, with final adjustment of the shape of the acrosome. One of the most striking changes in spermatozoa during their maturation phases are migration and alteration in the shape and size of the cytoplasmic droplet, the remnants of spermatid cytoplasm which appears on the sperm cells during the process of spermatogenesis and ultimately excludes from the lumen by epithelial phagocytosis (Cooper, 2005). Displacement of the cytoplasmic droplet from a proximal to a distal position is one obvious morphological change that occurs during the epididymal passage. Additionally, a small number of abnormally-shaped spermatozoa originates during the metamorphic stage of spermatogenesis or during the process of maturation itself (Bonet et al., 1992).
Other important changes include condensation of the chromatin (Hingst et al., 1995), change in size of the acrosome (Bedford, 1963), initiation of motility, and ability to recognize and bind to the oocytes.

Permissible numbers of abnormal spermatozoa in ejaculates depend largely on the types of abnormalities present (Jalkanen, 1993). Primary defects originate during the metamorphic stage of spermatogenesis while secondary defects are thought to occur after the sperm cells have left the testis. Defects such as deformed heads, segmentary hypoplasia of the mitochondrial sheath or short tails are certainly initiated during spermatogenesis in the testis and appear to have a genetic origin (Baccetti et al., 1993). Other signs, like detached heads, swelling of the acrosome and even serious structural defects such as tightly coiled and/or folded tails may, however, arise at the time of maturation and the storing of spermatozoa in the epididymis (Bonet et al., 1992). The study of sperm morphology in the different regions of the male reproductive tract is, therefore, of utmost importance for the determination of the site of formation of a definite type of morphological abnormality.

In this study, the microscopical quality of spermatozoa coming from the caput, corpus and cauda of the epididymis has been examined in house rats to accurately characterize the spermatozoa in each epididymal region in order to obtain new data on the extremely complex process of epididymal maturation.

MATERIALS AND METHODS

Animals and Samples

The present study was conducted from 2004 to 2009 in the Department of Zoology, Punjab Agricultural University and Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The research complied with the institutional guidelines of animal ethics and adhered to the local legal requirements (Regd. No. 497/01/a/CPCSEA). Adult *R. rattus* trapped live from poultry farms, residential premises and godowns of Ludhiana were used on the day or next of their collection by maintaining them in the laboratory cages on plain food consisting of a mixture of crushed wheat, sugar powder and groundnut oil (96:2:2) and water *ad libitum*. The rats were weighed, anaesthetized and dissected to collect testis and epididymides of both sides. The epididymis was divided into three regions i.e. caput, corpus and cauda and were suspended in 0.5 ml of PBS to obtain epididymal fluid containing spermatozoa. Epididymal spermatozoa were analyzed for various morphological parameters.

Sperm Motility (Salisbury Et Al., 1978)

A drop of the caput, corpus and cauda epididymal fluid was placed on a pre-warmed glass slide immediately after dissection and observed under the microscope. About 100 motile and immotile spermatozoa were observed in different fields at 400X. The percentage of motility was calculated by using the following formula:

\[
\text{No. of motile spermatozoa} \times 100 \\
\text{Total Spermatozoa}
\]

Sperm Viability (Campbell Et Al., 1956)

A drop of epididymal fluid containing spermatozoa was mixed with a drop of eosin/nigrosin, kept at 37°C for 2 minutes, smears were prepared on clean glass slides. Air dried slides were examined at 1000X for live (unstained) and dead (pink) spermatozoa. About 100 live and dead spermatozoa were observed in different fields and percentage of live
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spermatozoa was calculated.

**Sperm Abnormalities**

A drop of epididymal fluid containing spermatozoa was mixed with a drop of eosin/ nigrosin stain, mixed and incubated at 37°C and smears were prepared on clean glass slides. About 100 spermatozoa with normal morphology, abnormal head (AH), coiled tail (CT), bent tail (BT), detached heads (DH) and midpiece thickening (MPT) were counted in different fields at 1000X using Olympus microscope (CH-21) and percentage of each was calculated.

**Sperm Morphometry**

About 50 spermatozoa in eosin/ nigrosin stained smears of each rat were measured for head length (HL), head area (HA), head perimeter (HP), midpiece length (MPL) and tail length (TL) by using Magnus pro analytic software.

**RESULTS**

**Sperm Motility**

A forward motility pattern was observed in caput, corpus and cauda spermatozoa with an average percentage of 68.13 ± 2.1, 72.5 ± 1.86 and 80.6 ± 2.27 respectively. Sperm motility increased progressively from caput to cauda epididymis (Fig. 1). The percent sperm motility differ significantly (p≤0.05) in the three regions of epididymis.

**Sperm Viability**

Live (colourless) and dead (pink coloured) spermatozoa were observed in eosin-nigrosin stained smears of epididymal fluid containing spermatozoa. The percentage of live spermatozoa was 88.22 ± 0.6, 85.45 ± 1.26 and 83.1 ± 1.98% in caput, corpus and cauda epididymides respectively. A progressive decline from caput to cauda epididymis was observed in sperm viability (Fig. 1). Sperm viability was significantly different in case of cauda spermatozoa from that of caput and corpus (p≤0.05).

**Sperm Morphology and Abnormalities**

Sperm abnormalities like, abnormal head (AH), mid-piece thickening (MPT), coiled tail (CT), bent tail (BT) and detached heads (DH) were observed in caput, corpus and cauda spermatozoa. The average percentage of abnormalities of sperm head i.e. AH was 0.83 ± 0.12, 0.85 ± 0.12 and 0.88 ± 0.14% in caput, corpus and cauda spermatozoa respectively. The average percentage of MPT was 0.88 ± 0.12, 0.84 ± 0.14 and 0.84 ± 0.16% in caput, corpus and cauda spermatozoa.

Figure 1: Percentage of Sperm Motility and Viability in Three Regions of Epididymis
respectively. The percentage of CT was observed to be 1.42 ± 0.21, 1.59 ± 0.18 and 1.96 ± 0.22% in caput, corpus and cauda spermatozoa respectively. The average percentage of BT was 2.13 ± 0.24, 2.39 ± 0.22 and 2.89 ± 0.24% in caput, corpus and cauda spermatozoa respectively. The average percentage of DH was 1.79 ± 0.30, 2.25 ± 0.29 and 3.73 ± 0.52% in caput, corpus and cauda spermatozoa respectively (Table 1). A continuous increase in secondary abnormalities was observed as the spermatozoa passes through epididymis, whereas no such increase was observed in case of primary abnormalities. The total sperm abnormalities in cauda epididymis were significantly different from that of caput and corpus (p≤0.05). In present studies, the position of cytoplasmic droplet in caput, corpus and cauda epididymal spermatozoa was proximal, middle and distal (Fig. 2).

![Figure 2: Eosin-Nigrosin Stained Epididymal Spermatozoa of Rattus Rattus L Showing](image)

**Sperm Morphometry**

The HL of caput, corpus and cauda spermatozoa was 28.13 ± 0.19, 26.94 ± 0.34 and 25.98 ± 0.32 µm respectively. The MPL was observed to be 100.52 ± 0.58, 100.97 ± 0.76 and 101.52 ± 1.06 µm in caput, corpus and cauda spermatozoa respectively. The TL of caput, corpus and cauda spermatozoa was 119.52 ± 0.76, 119.86 ± 0.95 and 120.21 ± 1.41 µm respectively. The HA of spermatozoa showed a significant decrease (p≤0.05) from caput to corpus to cauda with the values 927.40 ± 0.94, 864.31 ± 0.54 and 824.94 ± 0.96 µm² respectively. In caput, corpus and cauda spermatozoa HP was measured to be 46.43 ± 0.95, 44.51 ± 1.18 and 43.51 ± 1.10 µm respectively (Table 2).

**DISCUSSIONS**

Sperm maturation in the epididymis is a gradual process and results from a number of different and successive changes that a normal spermatozoon must undergo before being able to fertilize an egg. These changes affect the morphology and function of the spermatozoon, so that their properties are modified while they move along the epididymal duct. This study demonstrated morphological and physiological differences among rat sperm isolated from three segments of the epididymis (caput, corpus, and cauda). A progressive increase in sperm motility of rat spermatozoa was observed from caput to cauda epididymis. Sperm motility improves along the length of the epididymis in parallel with the development of fertilizing capacity. The epididymal epithelium through its secretory, synthetic and metabolic activities creates an appropriate luminal environment for the acquisition of fertilizing ability and motility of the spermatozoa (Cooper, 2011). The acquisition of motility by the spermatozoa perhaps initiates in caput, reaches the higher levels after passing through the consecutive regions of the epididymis having more and more appropriate luminal environment created through the region specific secretory molecules. The increase in percent motility of spermatozoa from caput to cauda in present studies is thus indicative of the acquisition of functionality by house rat spermatozoa during epididymal transit. Several different factors involved in the development of motility during epididymal transit have been described, such as intracellular Ca²⁺ (Vijayaraghavan and Hoskins, 1990), cyclic adenosine monophosphate (cAMP; Tash, 1990) and intracellular pH (Hamamh and Gatti, 1998) as components of luminal fluid in different regions. Syntin and Robaire (2001)
observed that the percentage of motile spermatozoa in Brown Norway young adult rat (3-4 months) increased from 16.9 ± 1.8% (mean ± S.E.) in the distal caput epididymis to a maximum of 76.8 ± 1.3% in the distal cauda epididymis. Betzalel et al. (1986) reported that in rat, spermatozoa acquire motility and fertilizing ability upon passage from corpus to cauda epididymidis, approximately 2 wk after entering the epididymis.

Sperm vitality decreases progressively as the sperm moves along the epididymal duct which may perhaps be because of the fact that cauda epididymis acts as a quality control organ and the immature spermatozoa cannot survive for long duration. Sperm viability was significantly different in case of cauda spermatozoa from that of caput and corpus ($p<0.05$). The results are further supported by the observations of Briz et al. (1995) who expressed the sperm viability as the percent of live spermatozoa and observed a significant decrease as the sperm moved along the three epididymal regions ($p=0.0001$) in boar.

The spermatozoa of most murine rodents are characterized by a falciform head with an apical hook at the proximal end of head that varies markedly in size and curvature across species (Breed, 2004). A fraction of spermatozoa from an ejaculate are morphologically abnormal, but when that fraction becomes excessive, fertility may decrease. Morphological abnormalities in the spermatozoa are generally classified as primary and secondary (Ball et al., 1983). The primary abnormalities are believed to originate during spermatogenesis, while secondary abnormalities arise during epididymal maturation, transit or ejaculation (Christiansen, 1984). In rats head defects include reduced hook, banana/flattened head or pinhead and midpiece defects include thickening of midpiece, kinkled or coiled neck at varying degrees from slight to greater than 180° (Brown et al., 1994). Nwanjo et al. (2007) also classified small and pyriform heads (abnormal heads) and mid piece thickening as primary abnormalities and the presence of coiled and bent tails as secondary abnormalities in rat spermatozoa. Orgebin-Crist (1968) reported that the frequency of primary abnormalities beyond the proximal caput of the epididymis remained unchanged. However, the frequency of some secondary abnormalities of the sperm tail (i.e. coiled, duplicated or fused tails in intact animals) increased as spermatozoa traverse the epididymis in different animal species (Bonet et al., 1992). It was found that the various levels of the reproductive tract of the bull did not differ significantly in the proportions of abnormal spermatozoa and that the predominant types of abnormalities were those affecting the heads of the spermatozoa (Branton and Salisbury, 1947). It was, therefore, concluded that the testis is the original source of spermatozoa with morphologically abnormal heads i.e primary abnormalities but secondary abnormalities increases during epididymal transit. A characteristic morphological change to spermatozoa during epididymal transit is the caudal migration of the cytoplasmic droplet away from the neck, behind the head, to the annulus or at the end of the midpiece (Cooper, 2011). Branton and Salisbury (1947) also studied spermatozoa from three regions of epididymis to locate the cytoplasmic droplet and concluded that these were morphological stages in the development of the spermatozoa and that all normal bulls show the same stages. According to Perez-Sanchez et al. (1997) the progressive migration of the cytoplasmic droplet along the midpiece of the spermatozoa and its eventual loss as they reach the more distal regions of the epididymis contributes to their maturation process. It is possible that persistence of the cytoplasmic droplet in a proximal position indicates a general failure of sperm maturation, which also affects the sperm membranes and the receptors that interact with the zona pellucida. The exclusion of defective spermatozoa and the morphological maturation of epididymal sperm cells may be a prerequisite for the acquisition of normal motility and fertilizing capacity which for most species occurs during the passage of spermatozoa through more distal regions of the epididymis (Soler et al., 1994).
The morphometric dimensions of spermatozoa including total sperm length, head length, mid piece length, tail length, head perimeter and head area are rarely described in rats. However, Cummins and Woodall (1985) observed linear sperm dimensions in different species of mammals including rodents and reported the total sperm length to be 164.0, 162-164, 188.7 and 190.1 µm in *Rattus fuscipes* (bush rat), *Rattus lutreolus* (swamp rat), *Rattus norvegicus white* (common white rat) and *Rattus norvegicus brown* (common brown rat) respectively and found the greatest diversity in sperm length in mammalian orders like Chiroptera, Rodentia and Marsupialia. The values for mean HL, HA and HP of cauda spermatozoa, were less than the values for the corresponding caput and corpus spermatozoa, whereas the MPL and TL found to be same in all the regions. The decrease in head morphometric indices may be because the spermatozoa undergo the final adjustment of the acrosome shape and size during epididymal transit (Bedford, 1966). These results were further supported by the observations of Bedford (1963) in rabbit epididymis who observed a decrease in head dimensions from caput to cauda spermatozoa. The epididymal maturation involves changes in several morpho-functional aspects of the spermatozoon like changes in the nuclear chromatin and modification of the acrosomal shape (Olson et al., 2002). It seems reasonable therefore to suppose that reduction in acrosome size during epididymal passage is an essential preliminary step to the acquisition of fertilizing ability by rat epididymal spermatozoa. In the present study, total sperm length was 248.18 ± 0.85, 247.79 ± 0.66 and 245.18 ± 1.01 µm in caput, corpus and cauda respectively. The dimensions of spermatozoa obtained during the present study vary a little from those of previous findings. The small differences may perhaps be because of the different staining method used, which was reported to influence the sperm head dimensions considerably (Root-Kustritz et al., 1998). Cummins (1983) suggested that very large spermatozoa may be selected when circumstances in the female tract favour large, vigorous spermatozoa competing between each other to be first to reach the eggs. It has also been postulated that sperm head area and shape affects the total sperm count.

**CONCLUSIONS**

In conclusion, we found a slight increase in the proportion of spermatozoa with abnormalities of tail, suggesting that these abnormalities may have originated in the epididymis. Movement of the cytoplasmic droplet from a proximal to a distal position and capacity for motility occured simultaneously. Sperm viability and size of sperm head decreased during the epididymal transit. This information gives a better understanding of the mechanisms affecting sperm morphology and maturation in the house rat and suggest that this is a slow and extremely complex process, and sperm quality depends on a complete maturation process.

**REFERENCES**


**Table 1: Sperm Abnormalities in Different Regions of Epididymis in House Rat, Rattus Rattus**

<table>
<thead>
<tr>
<th>Region of Epididymis</th>
<th>Abnormal Head (%)</th>
<th>Mid Piece Abnormalities (%)</th>
<th>Coiled Tail (%)</th>
<th>Bent Tail (%)</th>
<th>Detached Heads (%)</th>
<th>Total Abnormalities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caput</td>
<td>0.83 ± 0.12</td>
<td>0.88 ± 0.12</td>
<td>1.42 ± 0.21</td>
<td>2.13 ± 0.24</td>
<td>1.79 ± 0.30</td>
<td>6.88 ± 0.61</td>
</tr>
<tr>
<td>Corpus</td>
<td>0.85 ± 0.12</td>
<td>0.84 ± 0.14</td>
<td>1.59 ± 0.18</td>
<td>2.39 ± 0.22</td>
<td>2.25 ± 0.29</td>
<td>7.68 ± 0.66</td>
</tr>
<tr>
<td>Cauda</td>
<td>0.88 ± 0.14</td>
<td>0.84 ± 0.16</td>
<td>1.96 ± 0.22</td>
<td>2.89 ± 0.24</td>
<td>3.73 ± 0.52</td>
<td>10.70 ± 0.66</td>
</tr>
</tbody>
</table>

The data is expressed as mean ± S.E. from 30 animals.

a Significantly differ from caput, \( p < 0.05 \).

b Significantly differ from corpus, \( p < 0.05 \).
Table 2: Sperm Morphometry in Different Regions of Epididymis in House Rat, Rattus Rattus

<table>
<thead>
<tr>
<th>Region of Epididymis</th>
<th>Head Length (µm)</th>
<th>Mid Piece Length (µm)</th>
<th>Tail Length (µm)</th>
<th>Head Area (µm²)</th>
<th>Head Perimeter (µm)</th>
<th>Total Sperm Length (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caput</td>
<td>28.13 ± 0.19</td>
<td>100.52 ± 0.58</td>
<td>119.52 ± 0.76</td>
<td>927.40 ± 0.94</td>
<td>46.43 ± 0.95</td>
<td>248.18 ± 0.85</td>
</tr>
<tr>
<td>Corpus</td>
<td>26.94 ± 0.34</td>
<td>100.97 ± 0.76</td>
<td>119.86 ± 0.95</td>
<td>864.31 ± 0.54a</td>
<td>44.51 ± 1.18</td>
<td>247.79 ± 0.66</td>
</tr>
<tr>
<td>Cauda</td>
<td>25.98 ± 0.32</td>
<td>101.52 ± 1.06</td>
<td>120.21 ± 1.41</td>
<td>824.94 ± 0.96ab</td>
<td>43.51 ± 1.10</td>
<td>245.18 ± 1.01</td>
</tr>
</tbody>
</table>

The data is expressed as mean ± S.E. from 30 animals.

a Significantly differ from caput, \( p \leq 0.05 \).
b Significantly differ from corpus, \( p \leq 0.05 \).