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Spermatozoan Maturation in the Epididymis of Sheep *(Ovis aries)*

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Abstract

The study was performed to study spermatozoan maturation in different parts of ram epididymis. Spermatozon suspensions were prepared from caput, corpus and cauda epididymis of 40 rams and thereafter tested for motility, livability, membrane integrity and percentage of normal acrosome. Motility of epididymal spermatozoa was examined under low power and high power microscope and scored from 0 to 100% by eye estimation. Livability, membrane integrity and percentage of normal acrosome in the three regions of epididymis were tested by staining with Eosin-Negrosin, Giemsa's stain and hypo-osmotic swelling test (HOST) respectively. Significant difference in motility, livability, membrane integrity (percentage of HOST positive sperm) and percentage of normal acrosome was observed among the three regions of epididymis. Motility was highest in the cauda (70.65 ± 0.41) and nil in caput. The corpus however showed some oscillatory motion. Dead spermatozoa stained red by Eosin-Nigrosin whereas live spermatozoa stained white. Maximum livability was observed in cauda (81.09 ± 0.47%) that had significant difference with corpus but the difference with caput was non-significant. HOST positive spermatozoa exhibited various types of tail coiling being maximum in cauda (68.59 ± 0.25%) and minimum in caput (53.89 ± 0.33%). Maximum percent of normal acrosome was found in cauda (86.40 ± 0.35%) and minimum in caput (84.92 ± 0.38%). The results indicated that there is progressive increase in motility, livability, membrane integrity and percentage of normal acrosome as spermatozoa pass through the epididymis indicating that important steps of maturation of spermatozoa take place in the epididymis that is vital for fertilization.

Key words : Ram, Epididymis, Spermatozoa, Maturation.

The study of spermatozoon maturation in the excurrent duct, the histological nature of the duct, which plays an essential role for maintaining the activities of spermatozoa have been conducted in rams. Spermatozoa have been evaluated for motility, livability, membrane integrity, and normal acrosome in caput, corpus and cauda epididymis. Motility of the spermatozoa is the main index for normal metabolism. Motility has long been considered necessary for spermatozoan transport through the female reproductive tract (1) and is essential in the oviducts for fertilization (2). Motility and livability is one of the most important semen characteristics to predict fertility in a male (3). The functional integrity of the sperm's plasma membrane plays a vital role during capacitation, acrosome reaction, and sperm binding on the oocyte and penetration. Normal, intact and fully mature acrosome is vital for capacitation and subsequent acrosome reaction, a process essential for successful penetration of sperm through the cumulus oophorus and fertilization of ovum. The study is, therefore made to evaluate the changes in these parameters, vital for fertility, in ram epididymal spermatozoa, during their transit through the epididymis regions. The roles of epididymis, apart from being a storehouse of spermatozoa, have been emphasized by the results of these studies.

Methods

Collection of Fluid From Caput, Corpus and Cauda Epididymis

The testes of adult healthy sheep averaging 3-3 ½ years of age and body weight (10.5 to 12.5 kg) were collected from local abattoir immediately after slaughter. Both the testes were brought to the laboratory in a thermoflask containing normal saline solution. Testes were washed thoroughly with the normal saline solution. Tunica albugenia were removed from both testes. Ligatures were placed unilaterally at the proxi-

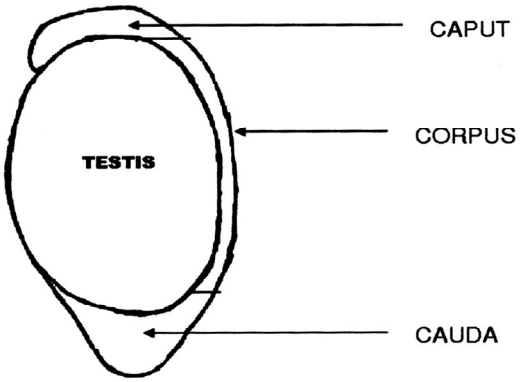

Figure 1. Caput, corpus and cauda epididymis in relation to the testes of ram.

mal end of the vas deferens, ampullae and cauda epididymis separately and distal to the caput epididymis and vas efferentia. After making ligations, whole epididymis (Fig. 1) was dissected out from the testes. Each individual ligated region (caput, corpus and cauda) was cut again and minced separately into 2 ml of Ringer's solution, Ph 7.4, at 37 C into different watch glasses. Luminal content from each portion was collected separately by gentle pressure on the excised tissues into the medium with individual clean glass rods. The resultant suspensions were filtered through double glass wool column to free the cellular debris. Each filtrate was collected into individual glass test tubes, centrifuged at 500g for 10 minutes and the supernatants were discarded. Finally, 200 µl of Ringer's solution was added to each sperm pellet separately and kept at 37 C into an incubator prior to experimentation.

Motility

To determine the spermatozoa motility in the caput, corpus and cauda of epididymis one drop of suspension from each respective region were taken using 1 ml pipette separately on a clean, dry and grease free microscopic glass slide. A cover slip was then placed gently over it. The motility of epididymal spermatozoa was examined under low power and high power of microscope. Motility was scored from 0-100% by eye estimation.

Livability

The spermatozoa were stained by Eosin-Nigrosin following standard procedure (4). The percentage of live spermatozoa was counted at magnification of X100 and X450.

Membrane Integrity

Hypo-osmotic swelling test was performed to assess the spermatozoa plasma membrane integrity, following the standard method (5). The spermatozoa were observed under phase contrast microscope at a magnification of X450. The percentage of spermatozoa exhibiting tail coiling was counted.

Percentage of Normal Acrosome

Standard Giemsa staining technique was followed (6). The spermatozoa were counted for normal acrosome at X1000 magnification.

Statistical Analysis

The difference in motility, livability, membrane integrity and percentage of normal acrosome among the three regions of epididymis were compared by analysis of variance and between two regions by critical difference test. The significance of correlation among motility, livability, membrane integrity and percentage of normal acrosome in the three different regions of epididymis was estimated by *t*-test.

Results and Discussion

Higher motility was observed in cauda epididymis averaging about 70.65 ± 0.41% followed by 14.85 ± 0.36% in corpus and was totally absent in caput epididymis (Table 1) and the difference was highly significant ($P < 0.01$). In corpus epididymis, very few sperms were motile, with only a few sperms exhibiting an oscillatory motion. The results of the study, indicates that during sojourn of spermatozoa through the reproductive tract the motion was strongest in the cauda and was absent in the caput. Similar results were also recorded in bulls (7), goats (8) and human (9).

The reasons for the effect of increase in motility of the spermatozoa during their transit through the epididymis in spite of the presence of different inhibitory factors, metabolic regulators are not clearly

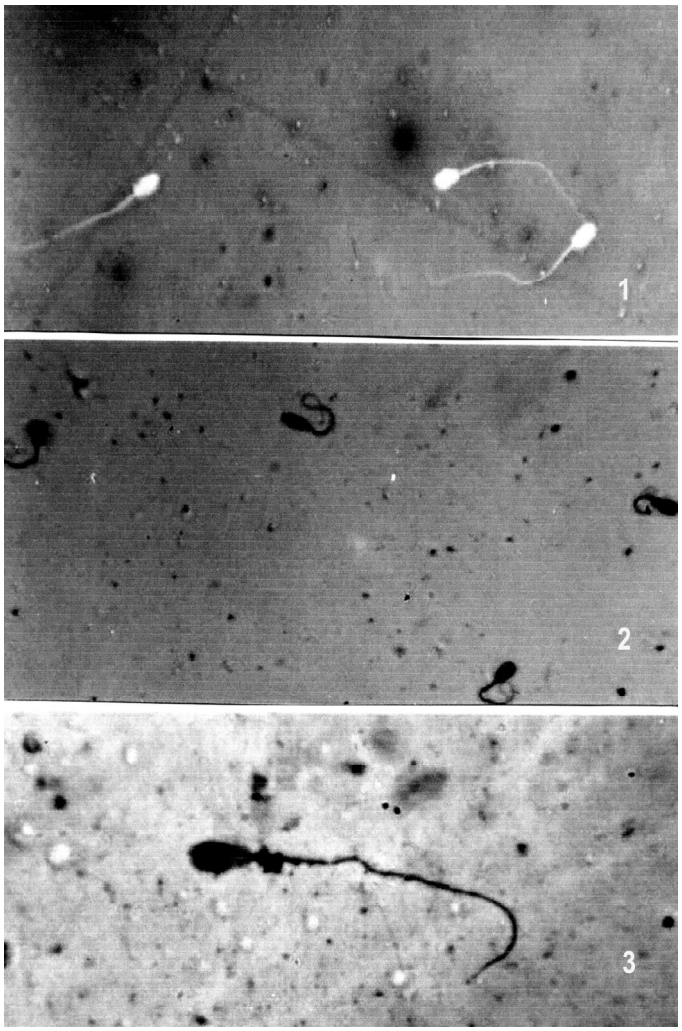

Figure 2. Photomicrograph showing the live spermatozoa from cauda epididymis of ram, (Eosin-Nigrosin, X450). **Figure 3**. Photomicrograph showing coiled tail spermatozoa after HOST from caput epididymis, (Rose Bengal, X450). **Figure 4.** Photomicrograph showing spermatozoon with normal acrosome from corpus epididymis, (Giemsa's stain, X1000).

known (10). However, it was suggested that the increase in motility might be due to the variation in chemical composition and local steroid hormone concentration in different parts of the epididymis and with the age of maturing sperm (11). Similarly, the significant increase in motility in the vas deferens may be due to the reason that carbon dioxide tension, which inhibits the motility, is lesser in the vas deferns than that of the cauda (12).

Live spermatozoa do not take stain and appear white by Eosin-Negrosin staining (Fig. 2). Analysis of variance revealed highly significant difference (P <0.01) in spermatozoan livability from caput, corpus, and cauda epididymis (Table 1). Critical difference test showed highly significant (P < 0.01) difference in spermatozoan livability between corpus and cauda epididymis, whereas the difference in spermatozoan livability of caput with corpus and cauda were non-significant (P >0.05). Epididymal spermatozoan livability had positive and highly significant (P < 0.01) correlation with HOST reactivity, while a negative and non-significant (P > 0.05) correlation was found with normal acrosome (Table 2).

Similar trend in spermatozoan livability was ob-

Table 1. Spermatozoan motility, livability, HOST positivity and percentage of normal acrosome from caput, corpus and cauda epididymis. Values expressed as Mean ± SE, a, b, c : Similar alphabets denote homogenous ($P > 0.05$) means.

Observation	Caput (n = 40)	Corpus (n = 40)	Cauda (n = 40)
Motility	0^{a}	14.85 ± 0.36^{b}	70.65 ± 0.41^{c}
Live (%)	79.71 ± 0.49^{ab}	78.65 ± 0.53^{a}	81.09 ± 0.47^{b}
Host (%)	53.89 ± 0.33^{a}	64.97 ± 0.31^{b}	68.59 ± 0.25^{c}
Normal Acrosome (%)	84.92 ± 0.38^{a}	85.47 ± 0.35^{ab}	86.40 ± 0.35^{b}

served among regions in boar (13) and bull (7). Dead sperms are selectively removed during their transit through the caput them and is possibly responsible for the presence of greater quantity of live spermatozoa in the cauda of present experiment. Percentage of dead spermatozoa increased beyond the cauda epididymis (7, 13). Moreover, apocrine secretion in the adult mouse epididymis and vas deferens have been reported (14) that could play important roles in relation to sperm maturation, protection and viability.

The membrane integrity was measured by hypoosmotic swelling test (HOST). Spermatozoa exhibiting coiling of tail were considered HOST positive. Various types of tail coiling were observed (Fig. 3) such - as hairpin curvature of the tail, g-type coiling, shortened and thickened tail, swollen area that partly or completely enveloped the curved tail of the spermatozoa. Analysis of variance revealed highly significant difference ($P < 0.01$) in HOST reactive spermatozoa from caput, corpus, and cauda epididymis (Table 1). HOST reactivity of epididymal spermatozoa showed highly significant ($P < 0.01$) and positive correlation with motility, livability and percentage of normal acrosome (Table 2).

Thus there was significant difference in membrane integrity between caput, corpus and cauda epididymis, which means that there is a progressive increase in membrane integrity as spermatozoa pass through various regions of epididymis. The structural proteins of mature mammalian spermatozoa possess a high degree of disulfide bonding. Immature spermatozoa taken from the caput epididymis show a relative deficiency of these cross linkages, but significant structural changes involving establishment of the disulfide linkages take place in the

Table 2. Correlation among observations (%) of epididymal spermatozoan motility, livability, normal acrosome and Host positive sperm. Degrees of freedom = 39,$^{*}P > 0.01$.

	Live % dead	Host	Acrosome
Motility	0.29^{*}	0.78^{*}	0.25^{*}
Live % dead		0.28^{*}	-0.08
HOST			0.24^{*}

nucleus and in the tail structures during sperm maturation in the epididymis (15). A very high degree of positive correlation ($r = 0.78$) was found between motility and HOST that is in accordance with the findings of others (16, 17).

Spermatozoa with normal intact acrosome were counted after staining by Giemsa's stain (Fig. 4). Various types of acrosomal abnormalities were observed i.e. knobbed acrosome, ruffled acrosome, incomplete acrosome, and detached galea capitis. Analysis of variance revealed significant difference ($P < 0.05$) in percentage of normal acrosome between the three regions (Table 1), but critical difference test showed highly significant ($P < 0.01$) differences in percentage of normal acrosome between caput and cauda, while the difference between corpus with caput and cauda was found to be non-significant ($P > 0.05$). Percentage of normal acrosome had positive and highly significant ($P < 0.1$) correlation, with HOST reactivity and motility while a non-significant and negative correlation was found with livability (Table 2).

The significant decrease in the percentage of the abnormal acrosome (Table 1) in domestic sheep agrees with the findings in Guineapig (18) and rabbit (19) that during the passage through the excurrent ducts there is significant decrease in the percentage of abnormal sperms in all parts of the epididymis as the acrosome adhere strongly with sperm nucleus. This might be due to dehydration of spermatozoa during passage through the epididymis (20), decrease in permeability and water content of acrosome (10) and adherence of acrosome to the sperm nucleus (21).

Thus it can be concluded that as spermatozoa pass through the epididymis, there is is increase in motility, livability, membrane integrity and percentage of normal acrosome. These four parameters viz. motility, livability, membrane integrity and percentage of normal acrosome are vital for fertility and are

also found to be significantly correlated among themselves as spermatozoa pass through the epididymis.

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