Histomorphology and biochemical composition of epididymis and spermatozoa of the house rat, *Rattus rattus* L.

***Charanjit K Dhanju and Navdeep Kaur**
Department of Zoology, Punjab Agricultural University, Ludhiana – 141 004, Punjab, India.

Abstract

Keeping in view the significance of role of epididymis in sperm maturation in mammals, the histomorphology and biochemical composition of its three regions; proximal caput, middle corpus and distal cauda were studied in the house rat, *Rattus rattus*, a species of great economic importance being a commensal rodent pest. Histomorphological observations revealed the presence of rounded and oblong tubules filled with spermatozoa in all the regions of epididymis. The tubular as well as luminal diameter and the thickness of epithelial lining was highest in caput as compared to that in corpus and cauda indicating the more secretory activity in this region. The sperm motility and concentration were significantly higher in cauda as compared to that in caput and corpus. The level of biochemical constituents including proteins, lipids, phospholipids, cholesterol, free fatty acids and sialic acids estimated in tissues of three epididymal regions, their fluids (only proteins) and spermatozoa progressively decreased from caput to corpus and to cauda epididymal tissues while their concentration successively increased in the spermatozoa from caput to corpus and to cauda epididymii. In epididymal fluid, protein content significantly increased from caput to corpus and to cauda. It reveals that maximum secretory activity was in the caput region of epididymis and content of various biomolecules was highest in the spermatozoa collected from cauda epididymis perhaps of its being their storage organ.

Key words: Epididymis*, Rattus rattus*, histomorphology, biochemical composition.

INTRODUCTION

Epididymis is a highly convoluted tubule that links the testis to the vas deferens in mammals and is a site of maturation and storage of spermatozoa. The physiological maturation of the mammalian spermatozoa is completed during their transit from caput; the upper proximal part of epididymis through middle corpus and then to lower cauda epididymis. The spermatozoa undergo significant morphological and biochemical modifications during epididymal maturation which enable them to recognize and fertilize oocyte[1]. The plasma membrane of spermatozoa undergoes substantial remodeling during this process principally because of changes in phospholipids composition and alteration in nature of proteins at specific domains due to exchange of materials with the luminal fluid. However, the accurate identification and quantification of these changes and information on the organization of proteins in membrane elements is lacking in many species especially those of economic importance like the house rat, *Rattus rattus* which is a widely distributed, cosmopolitan commensal rodent pest of household, agricultural and public health importance. The present day rodent management technology is mainly dependent on rodenticides[2] which is a short-lived method. The present study was undertaken to explore the histomorphology of epididymis and the fertility related sperm membrane biochemical alterations occurring during epididymal maturation in *Rattus rattus* to be of value for development of new and more reliable methods of control like immunocontraception for this rodent pest as this kind of information is totally lacking in this species.

***Corresponding author:**
Email: hfhckdhanju@gmail.com

MATERIALS AND METHODS:

Collection, Maintenance and Dissection of Rats

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 anaesthetized and dissected to collect epididymii of both sides which were separated into three regions i.e. caput, corpus and cauda. These were dipped in 0.5 ml of 0.9% saline solution to collect epididymal fluid containing spermatozoa for assessing their motility, concentration and abnormalities. The epididymal tissues were fixed for histological and frozen for biochemical studies.

Sperm Motility, Concentration and Histomorphology

Percent sperm motility and concentration in epididymal fluid of caput, corpus and cauda regions of 45 rats were observed[3] and sperm abnormalities studied microscopically in Giemsa stained sperm smears. The caput, corpus and cauda tissues from 5 rats each were fixed in Bouin's fluid and processed for histological preparations. Eosin-Haematoxylin stained sections of three epididymal regions were microscopically studied for measurement of the diameter of the tubules and lumen thickness of the luminal cells in caput, corpus and cauda epididymii.

Processing of Samples and Estimation of Proteins, Lipids, Phospholipids, Cholesterol, Free Fatty Acids and Sialic Acids

The caput, corpus and cauda epididymii were dipped in liquid N_2 and immediately homogenized in pestle and mortar and the powder so formed was suspended in PBS (pH 7.4). The caput, corpus and cauda epididymal fluids and tissue homogenates of 15 rats each were pooled to form three samples for biochemical estimations. Quantitative estimation of proteins was carried out in the tissue homogenates, epididymal luminal fluids and spermatozoa of three regions of epididymis using BSA as standard[4]. Total lipids of tissue homogenates and spermatozoa of caput, corpus and cauda regions were extracted[5] and phospholipids, cholesterol, free fatty acids and sialic acids estimated using KH_2PO_4[6], using cholesterol[7], using palmitic acid[8] and N-acetyl neuraminic acid (NANA) as standard[9] respectively.

Statistical Analysis

The results are expressed as mean ± S.E. and different parameters were compared in caput, corpus and cauda epididymis using ANOVA.

RESULTS

Sperm Parameters and Histomorphological Observations

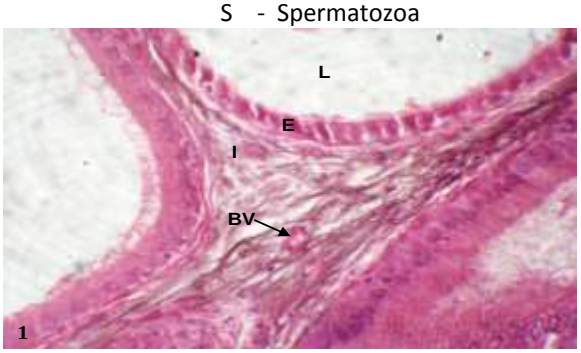
Sperm motility and concentration were highest in cauda followed by caput and corpus and sperm abnormalities were minimal i.e. 1.5% in all three regions of epididymis. Round and oblong tubules in caput, corpus and cauda were observed (Plate1). The tubular and luminal diameter and thickness of epithelial lining were higher in caput as compared to that in corpus and cauda epididymis (Plate 1, Table1). The lumen of all regions of epididymis contained spermatozoa but these were more tightly packed in cauda epididymis (Plate 1).

Biochemical Observations

The concentration of proteins, lipids, phospholipids, free fatty acids, cholesterol and sialic acids was highest in the tissues of caput region as compared to corpus and cauda regions while in spermatozoa it was lowest in those from caput and it successively increased from corpus to cauda spermatozoa. However, the protein content in epididymal fluid was also highest in that from caput region and gradually decreased in corpus and cauda fluids (Table 2).

Abbreviations used:

BV - Blood vessel
E - Epithelial height
L - Lumen
I - Interstitium
S - Spermatozoa



Showing epithelial height of caput epididymis tubule. Magnification X400.

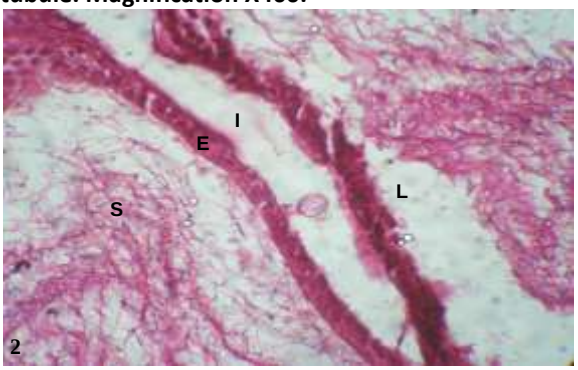


Fig.2 Showing epithelial height of corpus epididymis tubule. Magnification X400.

PLATE 1

Fig.3 Showing epithelial height of cauda epididymis tubule. Magnification X400.

Table 1 : Histomorphological observations and sperm parameters of three regions of epididymis in, *Rattus rattus*

Region of epididymis	Tubular* diameter (mm)	Epithelial* height (mm)	Lumen* diameter (mm)	Sperm motility** (%)	Sperm conc.** (10^9/ml)
Caput	205.10±1.02	22.35±0.39	160.4±0.45	64.20±2.09	82.40±7.09
Corpus	146.37±0.41[a]	11.37±0.13[a]	123.62±0.35[a]	71.60±1.74[a]	51.20±6.00[a]
Cauda	159.21±0.86[a]	15.85±0.26[ab]	127.51±0.36[a]	79.60±1.26[ab]	116.80±7.65[ab]

The data is expressed as mean ± S.E.

*From 50 observations i.e. ten from each five rats, **From 45 animals

[a] Significantly differ from caput, p≤0.05 , [b] Significantly differ from corpus, p≤0.05

Table 2: Biochemical composition of three regions of epididymis and their contents in *Rattus rattus*

	CAPUT			CORPUS			CAUDA		
	Tissue (mg/g)	Epididymal fluid (mg/ml)	Spermato-zoa (mg/10^9)	Tissue (mg/g)	Epididymal fluid (mg/ml)	Spermato-zoa (mg/10^9)	Tissue (mg/g)	Epididymal fluid (mg/ml)	Spermato-zoa (mg/10^9)
Proteins	15.81± 0.45	1.76±0.18	15.40±0.61	12.63± 0.34[a]	1.43±0.14a	27.76±1.71[a]	9.02± 0.26[ab]	1.33±0.006[ab]	56.40±1.30[ab]
Lipids	32.74± 2.98	-	9.30±1.50	20.83± 2.96[a]	-	8.67±0.47[a]	14.32± 0.20[ab]	-	7.37±0.45[ab]
Phospho-lipids	1.87± 0.03	-	1.82±0.81	1.16± 0.24[a]	-	1.65±0.98[a]	0.93± 0.05	-	1.03±0.96[ab]
Cholesterol	1.06± 0.0005	-	0.74±0.06	0.91± 0.19	-	1.30±0.05[a]	0.69± 0.01	-	1.60±0.05[ab]
Free fatty acids	9.38± 0.99	-	0.90±0.12	2.55± 0.05[a]	-	0.55±0.08	2.43± 0.02[a]	-	0.47±0.12
Sialic acids	0.265± 0.004	-	0.362± 0.005	0.179± 0.004	-	0.321±0.003	0.102± 0.002	-	0.433±0.001

*The values of above are expressed as mean ± S.E. of 3 observations from pooled samples of 3 groups of 15 rats each

[a] Significantly differ from caput, p≤0.05, [b] Significantly differ from corpus, p≤0.05

DISCUSSION

The maximal luminal cell height and highest concentrations of major biomolecules; proteins, lipids, phospholipids, free fatty acids, cholesterol and sialic acids in the tissues of caput epididymis and its fluid in present studies are indicative of the secretory activities being maximum in this region[10]. The concentration of these biomolecules in spermatozoa being highest in those from cauda suggests their maturation process initiated in caput and completed here as also indicated by their highest motility in this region. The highest sperm concentration in cauda region of epididymis is indicative of this being a storage organ for spermatozoa in *Rattus rattus* as in other mammalian species. However, in the efferent ducts and caput epididymis, almost all the proteins transported by the rete testis are actively reabsorbed by the epithelium[11-15]. Thereafter all the major changes in the proteins are due to epididymal secretions because of the existence of heamato-epididymal barrier that precludes the exchange of proteins with blood and lymph [11-16]. Moreover, the mammalian spermatozoa are highly specialized cells whose DNA is biosynthetically (transcriptionally) inactive but they are sensitive to external signals from their environment and regulate their fertilizing capacity until they are in vicinity of the egg. Therefore, it could appear that the interactions of spermatozoa with surrounding fluid could play a major role in the surface membrane evolution which initiates in caput and completes in cauda as indicated by biochemical composition of epididymal spermatozoa in *Rattus rattus* in the present studies. Thus the post-testicular remodeling leading to the acquisition of functional maturity by spermatozoa during their epididymal transit is mediated mainly by the secretory activities of epididymal epithelium[16, 17]. Epididymis synthesizes and secretes proteins, some of which become associated with the sperm plasma membrane[18, 19]. Alterations in lipid composition of three epididymal regions observed in present studies are perhaps because these in the form of protasome like particles play an important functional role in epididymal sperm maturation by transferring selective proteins from

luminal fluid to the sperm surface[20]. Free fatty acids like arachdonic acid form a significant component of vesicular structures called epididymosomes in mouse[21] and thus are important for epididymal maturation of spermatozoa. Sialic acid groups contribute significantly to surface changes in spermatozoa during epididymal maturation in many species[22] as also indicated by their altered status in spermatozoa from caput to cauda. The importance of all these molecular changes need to be correlated more precisely with the physiological functioning of the spermatozoa so that the sperm surface specific molecules may be used for regulation of their fertility especially in species of economic importance like the rodent pest *Rattus rattus* used in present studies.

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