UNIVERSITI PUTRA MALAYSIA

COMPARATIVE STUDY OF THE HISTOLOGICAL STRUCTURE OF THE KEDAH-KELANTAN BULL EPIDIDYMIS AT PREPUBERTY, PUBERTY AND POSTPUBERTY

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BY

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To my mom, ed and ena

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Abstract

Histological structure of the Kedah-Kelantan bull epididymis was investigated during prepuberty (7 months), puberty (18 months) and postpuberty periods (3 years). Two samples of each age group were used for the study. Each sample was designated as head, body and tail.

Under light microscope, the epithel 1 lining of the ductus epididymis represents a pseudostratified ciliated columnar epithelial cell which was made up of 3 cell types i.e. principal cell, basal cell and apical cell.

Tubular diameter increased along the ductus from head to tail and measured at range of 206.2 ± 73 um to 273.7 ± 52 um, 220.2 ± 48 um to 409.4 ± 42 um and 401.07 ± 47 um to 472.0 ± 54 um of the different age group.

Epithelial height also varies from 35.6 ± 10.6 um to 50.0 ± 2.0 um in prepuberty, 36.9 ± 11.0 um to 69.1 ± 9.3 um in puberty and 77.0 ± 11.9 um to 75.0 ± 13.2 um as in postpuberty.

Stereocilia height seem to be varies along the ductus with advancement of age.

CONTENTS

1. INTRODUCTION

It is well established that spermatozoa of mammals undergo important changes during their transit in the ductus epididymis before being able to fertilise. The process of maturation, storage of the spermatozoa and ability to swim depand on the special environment created by androgen-dependent activities of the epithelium of the epididymis. The epithelium of the epididymis of the adult animal plays a major role in modifying the component of the epididymal plasma in which the spermatozoa is bathed and matured.

Studies made by various workers have shown that the structure of the peididymis varies according the species and region of the epididymis. However, until presently there has been no studies made on the epididymis of Kedah-Kelantan bulls. This study was conducted mainly to provide a baseline morphological information on the different regions of the epididymis of postpuberty, at puberty and prepuberty of Kedah-Kelantan bulls.

2. LITERATURE REVIEW

The epididymis is considered as an important segment of the reproductive system and has been assigned with various functions storage and maturation of spermatozoa. In adult animal, the epithelium plays a major role in the modification of the epididumal plasma creating an environment suitable for the nutrition and maturation of the spermatozoa.

In all species of animal so far investigated there are two cell types present in the epithelium of the epididymis, the principal cell and the basal cell and in many species such as rat and hamster additional cell types can be found (4,9). They

reported that principal cell are found along the whole length of the epididymis which extending from basal layer of the duct epithelium, thus reflected in the thickness of the epithelium. The apical surface of the principal cells bear a long and sometimes brancing microvilli (stereocilia) whose length deacrease toward the tail of the epididymis. Cells of the second type, basal cells are small round or pyramidal elements lodged between the bases of the principal cells.

Presence of stereocilia in the epididymis has been studied by various workers. Macmillan & Hafs (1969) and Wislocki (1949) found that the head region of ductus epididymis possesses taller cells with delicate stereocilia as in bull, while in the tail region the cells were smaller and the stereocilia were shorter. Macmillan & Hafs (1969) reported that stereocilia in the head of epididymis of bull was already present at birth and in the body region at 2 months. Stereocilia have been observed in the head and tail of epididymis about 70 days after birth in goat (18). In the head epididymis of rabbits the stereocilia first appear by 63 days of age.

The histological structure of epididymis varies at different ages and also at different regions of the same age. This variations and changes are mainly found within the epithelium of the ductus epididymis. The epithelium of the ductus epididymis changes from simple columnar to pseudostratified columnar with increase in age. Also the completion of pseudostratification occurs earlier in head than the rest of the epididymis. It was shown in buffalo epididymis that at first 7½ months of age, the epithelium of the ductus of the head was simple columnar and in the ductus of the tail the

epithelium was pseudostratified low columnar (6). In Bos taurus bulls the process of completion of pseudostratification of the epithelium of the ductus epididymis was earlier (13). They observed that the completion of pseudostratification in the head body and tail was completed at 4,5 and 6 months respectively. In goat and sheep the pseudostratification of the epithelial lining was completed in the head, body and tail at 2½, 4½ and 5 months respectively (2,8,12,18).

The height of epithelium of the ductus epididymis varies between ages and region of epididymis. In Bos taurus, the height of epithelium was tallest in the tail region at birth and at 12 months of age the epithelium was tallest in the body (11,13). Wildeus & Entwistle (1983) reported that there is a high correlation between epithelial height and ductal diameter.

3. MATERIALS AND METHODS

3.i. ANIMALS.

A total of 6 Kedah-Kelantan bulls were used in this study; 2 of which were at 7 months of age, 2 were yearling at 18 months of age and the remaining 2 were mature bull at 3 years of age. Their testes were free from abnormalities when examine prior to the commencement of the studies.

3.ii. EXPERIMENTAL PROCEDURES.

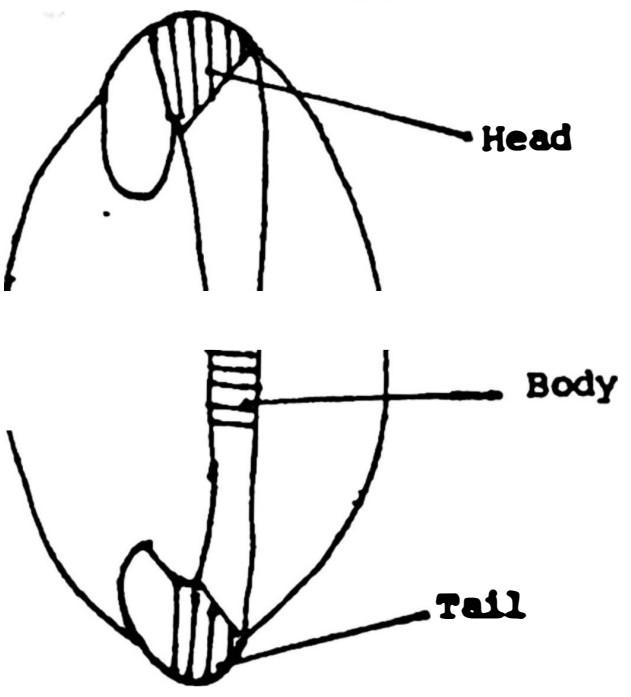
The samples were collected after doing unilateral surgical castration of the right testes. Castration were done in a standing position in the crust. The animals were first sedated with xylazine (Rompun^{R}) with a dosage of 0.5 mg/kg body weight administered intravenously. The site of incision that is

anterior-posterior aspect of the ventral extremities of the testes were throughly cleaned with antiseptic. Then 10 mls. of 2% xylocaine was infiltrated subcutaneously along the incision site and the neck of the scrotum. A clean incision about 1½ inches was made through the skin and tunica vaginalis to expose the testes. The spermatic cord was located and a tranfixing ligatures were a applied to it. The testes was then removed by servring the spertamic cord below the ligatures.

3.iii. FIXATION AND SAMPLING OF EPIDIDYNMS FOR LIGHT MICROSCOPY

The epididymis were separated from the testes and divided into 3 parts; head, body and tail (Fig.1). Each part was divided into two equal blocks, block 1 and 2. Block 1 was used for preparing epon section. Block 2 was further divided into 2 parts, one for frozen section and another for paraffin section.

Fig.1 Schematic drawing of epididymis & Testes showing regions examined histologically (10).



3.iii.a. EPON SECTION.

The samples were fixed in 4% Gluteraldehyde in 0.1M cacodylate buffer. The samples were diced into 1 to 2 mm cubes and fixed for another hour. The samples were then rinsed in buffer and post-fixed in 1% Osmium oxide in 0.1M cacodylate buffer for 1 hour. The samples were subsequently rinsed in buffer dehydrated through graded concentration of ethanol followed by propylene oxide and embedded with epon araldite. Semi-thin section of light microscopy was cut by using ultramicrotome MODEL REICHERT - OMU 3 and stained with Toluidine blue.

3.iii.b. PARAFFIN SECTION.

The samples were fixed in Bouin's solution for 24 to 36 hours. After the samples were trimmed into blocks, they were processed through graded concentration of ethanol. The tissue were embedded in the paraffin and sectioned at 5 to 7 um thick using Leitz Rotory microtome. The section were stained with Harris haematoxylin and eosin and periodic acid-schiff method (PAS).

3.iii.c. FROZEN SECTION.

For the frozen section, small blocks of tissue were fixed in 10% buffered formalin. Frozen sections were cut at 4 to 5 um thick using a cryostat. The section was stained with Sudan Black for the demonstration of fats and other lipids.

4. RESULTS

4.i. PREPUBERTY.

At this stage of development, the size of the testes and epididymis were still small. Histological examination of epididymis

revealed a wide range of ductal development. In the head region, the ducts were still small and undeveloped. They were separated by a thick band of interductal connective tissue (Fig.1). The connective tissue was made of thick collagen fibres which were oriented around the duct (Fig.2). The cellular content of the connective tissue was mainly made up of fibrocytes arranged in between the collagen fibres (Fig.3). Fibroblast with large nucleus and basophilic cytopalm were comparatively fewer in number. Large blood vessels and lymphatics were also observed.

The size of tubules at this region measured at 206.2 ± 73 um (Table.1). Within their lumina, traces of secretion was present. The height of epithelium at this stage was low measuring 35.6 ± 10.6 um (Table.1) and made up mainly of pseudostratified columnar and ciliated columnar epithelium. Epithelial cells were mainly classified into 3 types based on their morphology and stained affinity. The most predominant cell had darkly stained cytoplasm with an elongated shape nucleus. Within the nucleus were present 1 to 3 nucleoli which were mostly attached to the membrane. It's nucleus was located at the basal part of the epithelium. This cell was known as principal cell (Fig.4). Second type of cell was identified as flaskshape cell with large oval nucleus. This cell known as apical cell (Fig.4). The third type of cells which present on the basement membrane and beneath the principal cells were known as basal cell. These cells were small in size with a rounded or triangular in shape (Fig.4).

In the body region, the amount of connective tissue between the duct was comparatively thinner than that of the head. Here, the ductal diameter was bigger than in the head i.e, 213.7 ± 45 um

(Table.1). Within the ductal, lumina was present homogenous like secretion. Here the amount of secretion was in larger amount. The epithelium found here was similar to the head except that there was an increase in the height of epithelium to 36.9 ± 11 um (Table.1).

In the tail region, the ductal system was comparatively well developed than the rest of the epididymis. The ductal diameter in this region was bigger than in other part of the epididymis i.e. 273.7 ± 52 um (Table.1). The type of epithelium cell of the ducts were similar to the other region of the epididymis except that its height has increased to 50.0 ± 2.0 um (Table.1). It is interesting to note that in this region, there were the presence of a few large cells in the epithelium (Fig.5).

TABLE. 1. HISTOMETRIC DETAILS OF THE CAPUT, CORPUS AND CAUDA OF EPIDIDYMIS.

A. EPITHELIAL HEIGHT (um)

Age group	Caput	Corpus	Cauda
Prepuberty	35.6 ± 10.6	37.5 ± 6.8	50.0 ± 2.0
Puberty	36.9 ± 11.0	46.6 ± 5.5	69.1 ± 9.3
Postpuberty	77.0 ± 11.9	102.5 ± 15.9	75.0 ± 13.2

B. TUBULAR DIAMETER (um)

Age group	Caput	Corpus	Cauda
Prepuberty	206.2 ± 73	213.7 ± 45	273.7 ± 52
Puberty	220.0 + 48	362.0 + 39	409.4 ± 42
Postpuberty	401.0 ± 47	420.0 ± 49	472.0 + 54

4.ii. PUBERTY.

As the animal reached puberty, the histological changes within the ducts was quite significant. In the head region, there was a great increase in the diameter from 206.2 ± 73 to 220 ± 48 um (Table. 1). The epithelial cells were much taller than during prebuberty stage (Fig.6). Their height measured 36.6 + 11 um (Table.1). The number of basal cells were also numerous. The epithelial stereocilia were fully developed at this stage. They were seen swept in one direction of the duct (Fig.7). In some ducts the stereocilia might not be apparent due to the excessive deposition of secretion on the surface (Fig. 8,9). Within the lumina of the ducts secretion were quite abundant. Secretion was mainly a merocrine type as it was in a globular form. Occasionally spermatozoa were seen interspersed amongst the secretion.

In the body of epididymis, the ducts and its epithelium were quite well developed. There was a further increase in the ductal diameter to 362.0 ± 39 um (Table.1). The height of the ductal epithelial in this part of epididymis has reach 46.6 ± 5.5 um (Table.1). The basal cells in this region were found in large numbers.

Within the lumina of the duct of epididymis, there was a copious amount of epithelial secretion. Some of the secretion were seen extruding from the apical surface of epithelium (Fig. 10). Also present in the lumina of the ducts were large numbers of spermatozoa and some round spermatids which were bathed in the secretion (Fig.11).

The ductal connective tissue was reduced mainly due to the expansion of the ducts. Within in were found large number of blood vessels (Fig.12).

In the tail epididymal region, the ducts were very large with a diameter of 409.4 + 41 um (Table.1). Their lumina were filled with clumps of spermatozoa (Fig.13). The globular secretion was present in abundance in the lumina.

The epithelial showed a further increased in height to 89.0 ± 9.3 um (Table.1). The srereocilia on it's apical surface were very high. At this stage there was a further increase in the number of epithelial cells.

4.iii. POSTPUBERTY.

At postpuberty stage, the ducts were very well developed. Their lumina were big and mainly filled up with large amount of secretion and spermatozoa (Fig.14). The diameter of the ducts had increased further to 410.9 ± 47 um (Table.1). The basement membrane surrounding the ducts appeared to increase in thickness. During this stage, epithelial cells of the ducts were very tall measuring 77.0 ± 12 um (Table.1). The cell were slender with an elongated nuclei (Fig.15). Their nuclei were mainly situated at the base of the cells.

In the body and tail epididymis, the histological picture were quite similar to the head region. Their ductal diameter has increase to 420.0 ± 49 um and 472.0 ± 54 um (Table.1). In the body epididymis, it is interesting to note that the height of epithelium was higher than in the tail.

5. DISCUSSION

Basically, the histology structure of the epididymis of Kedah-Kelantan bulls appear to be similar to that in the epididymis of the buffalo (6), boar (3,16), ram (12) and goat (8). Similar histological picture was observed in the other region of the epididymis. During period of differentiation, the different region of the epididymis become distinct and each region could be identified by the height of the epithelium and the different cell type comprising it (5). The epithelial height in the head was lower than in other part of ductus epididymis, however this region gain height over the tail at postpuberty.

At the prepuberty stage i.e. 7 months, the epithelium lining of the epididymis has already completed, its pseudostratification progress. However the growth and development of sterecilia of the epithelium was still incomplete. It is now assumed that the pseudotratification progress finishes later than the Bos taurus (13). The trend of develpoment of the duct of Kedah-Kelantan epididymis is quite similar to that of the buffalo (6).

In Bos taurus, 2 cell types were only present in the epididymis i.e. principal cell and basal cell. However it is interesting to note that in the Bos indicus breed there were 4 main type cell types present i.e. principal cell, basal cell, apical cell and halo cell. Principal cells are found along the whole length of the epididymis and extend from the basal lumina of the ductal epithelium to the lumen. It's height reflected in the thickness of the epithelium. On the basis of the number of cells present, it suggested that principal cells involved in the synthetic activity of the organ and resorption of epididymis plasma (9). The basal

cells are fewer in number particularly in the head and body as compared to the tail of the epididymis. However in the tail region the population of these cells were increased. This is probably due to the presence of larger number of sperm in the region as it is the main storage region, and these cells are responsible for the spermiogenesis (6). The population of the apical cells were also found to increase from prepuberty to postpuberty. This is probably due to increase in the population of the epithelium cells proper or principal cell as Reid & Cleland (1957) claimed that these cells are precursor of the epithelial cells principal. The population of the halo cells seem to be correlate with age. It was believed that their number increased significantly as the animals reached postpuberty stage.

The development of stereocilia was completed later than the development of the epithelium. At prepuberty stage in the head and body region the stereocilia was still developed. However the tail region already had comparatively well developed stereocilia. During puberty stage, all the 3 region of the epididymis had well developed stereocilia. Similar pattern of stereocilia was reported in Bos taurus bull (1,11). In present study, stereocilia seem to be swept into one direction, probably due to movement of spermatozoa along the ductus. In the body of the epididymis during puberty stage, stereocilia was not observed mainly due to excessive deposition of secretion on the apical surface of the epithelium. Secretion produced by epithelial cells mainly in the head and body of the epididymis. The secretion composed of

various substances such as glycerylphosphorycholine (GPC), carnitine, sialic acid and glycoprotein (4,9,14).

The vacoule in the cytoplasm of the epithelium appeared to ancrease in number with age. This is mainly because of the increase in the secretion and absorption activity of the epithelium as the animal increase in age. The increase in number of vacoule is related to the increase in volume of secretion. The vacoules were unreactive to sudan black suggest that its component is not made up of lipid.

6. CONCLUSION

From the study, it is showed that the epididymis is a dynamic organ comprising epithelial cells with morphological feature suggestive a very high degree of activity.

Structural abnormalities of epithelial cells were also influences morphological structure of the spermatozoa. This indirectly affect the performance of spermatozoa which kill kept in the cauda epididymis.

As the age advances, the ductal system will further developed from the head to the tail of epididymis. There is also a correlation between tubular diameter and age of animal.

Tubular diameter, epithelial height and stereocilia height increased along the ductus from head to tail. Basal cell increased in number from head to tail of ductus epididymis.

Homogenous secretion first appeared in traces during prepuberty. When the animals reach puberty, there was copious amount of secretion present in the lumina of ductus epididymis. Spermatozoa were evident during the puberty stage and were stored in the tail

region.

Generally, the head and body are involved in the maturation process of spermatozoa, whereas the cauda epididymis serve as their main storage place.

It's hoped that further study of the structure and function of epididymis may contribute to an understanding of the control of male fertility.

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APPENDIX

Fig. 1. Cross-section of head of epididymis from prepuberty animals. The microphotograph shows ducts were small and underveloped separated by thick interductal connective tissue. Toluidine blue x 10.

Fig. 2. Cross-section of head of epididymis shows fibrocytes in between collagen fibres. Note the pseudoatratified ciliated columnar epithelium on the surface of the epithelium. Toluidine blue x 20.

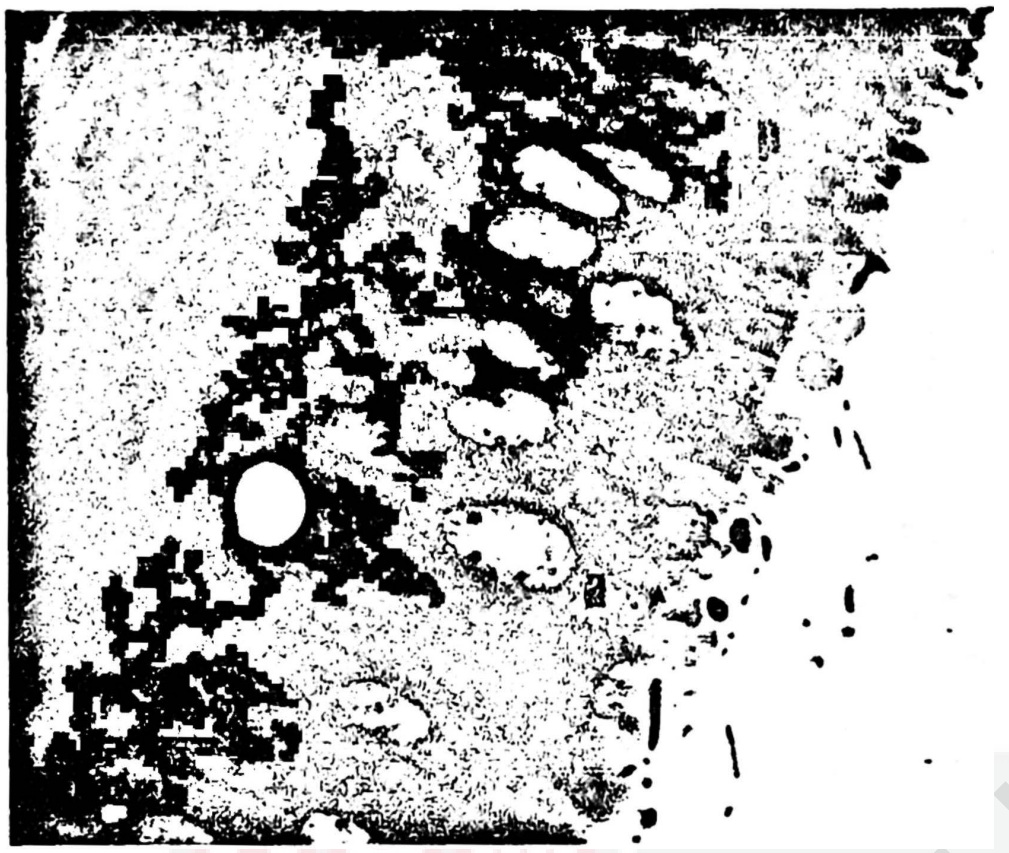
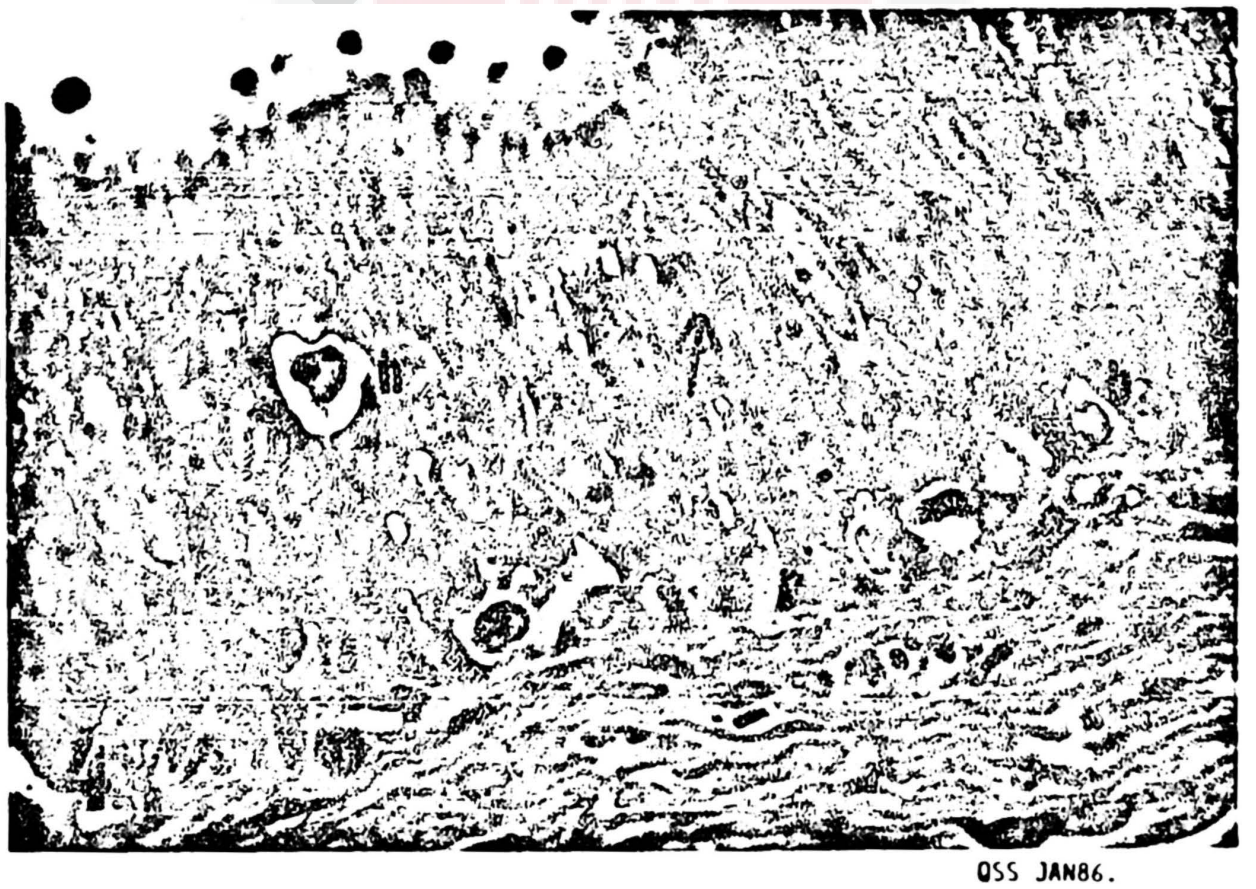


Fig. 3. Principal cell with darky stained cytoplasm (p). Apical cell (a) with large and oval nucleus with fine chromatine. Few basal cell (b) on the basement membrane which rounded in shape. Toludine blue x 40.

Fig. 4. Tall epithelial cell with nucleus in the middle part of the cell. Several vacoules were present in the cytoplasm of principal cell (arrow). Few halo cell (h) and basal cell found in large number (b). Toludine blue x 40.



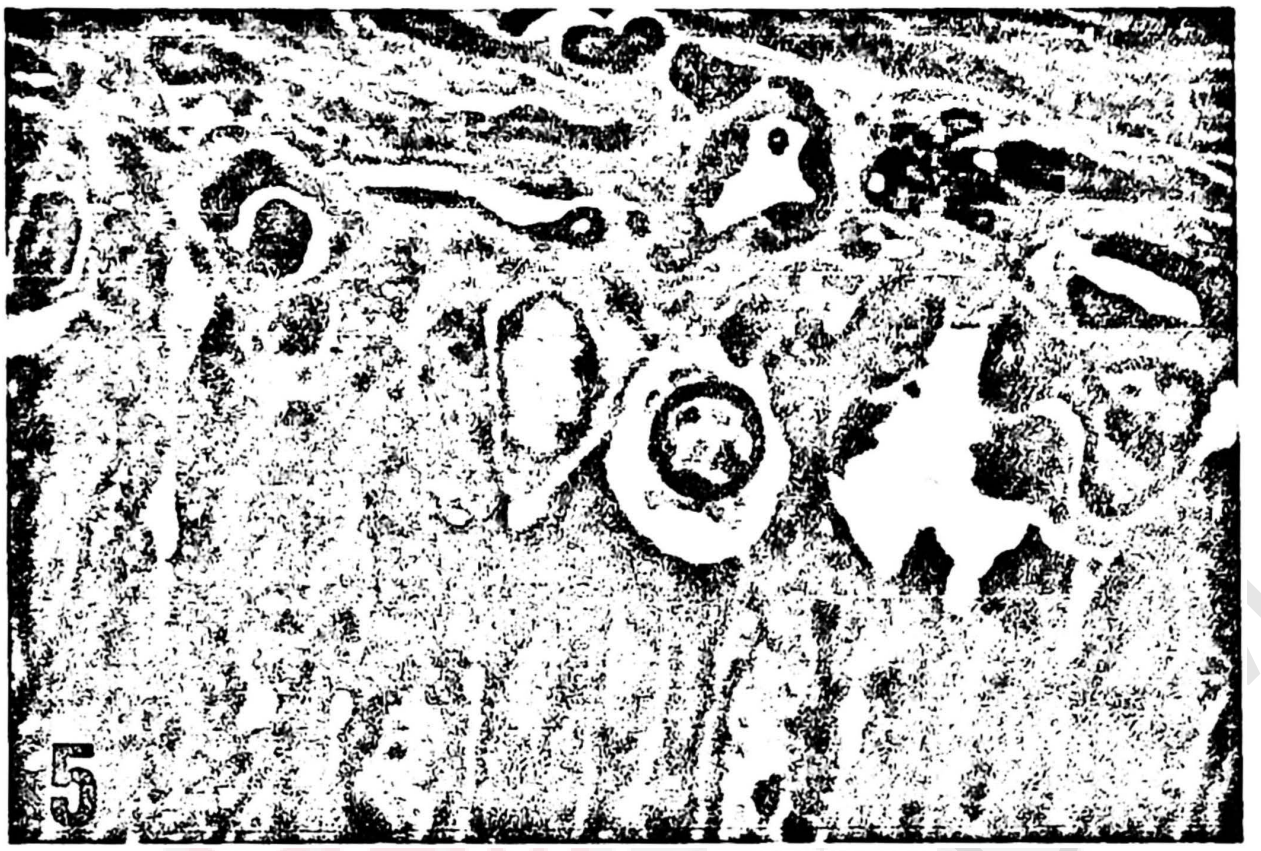
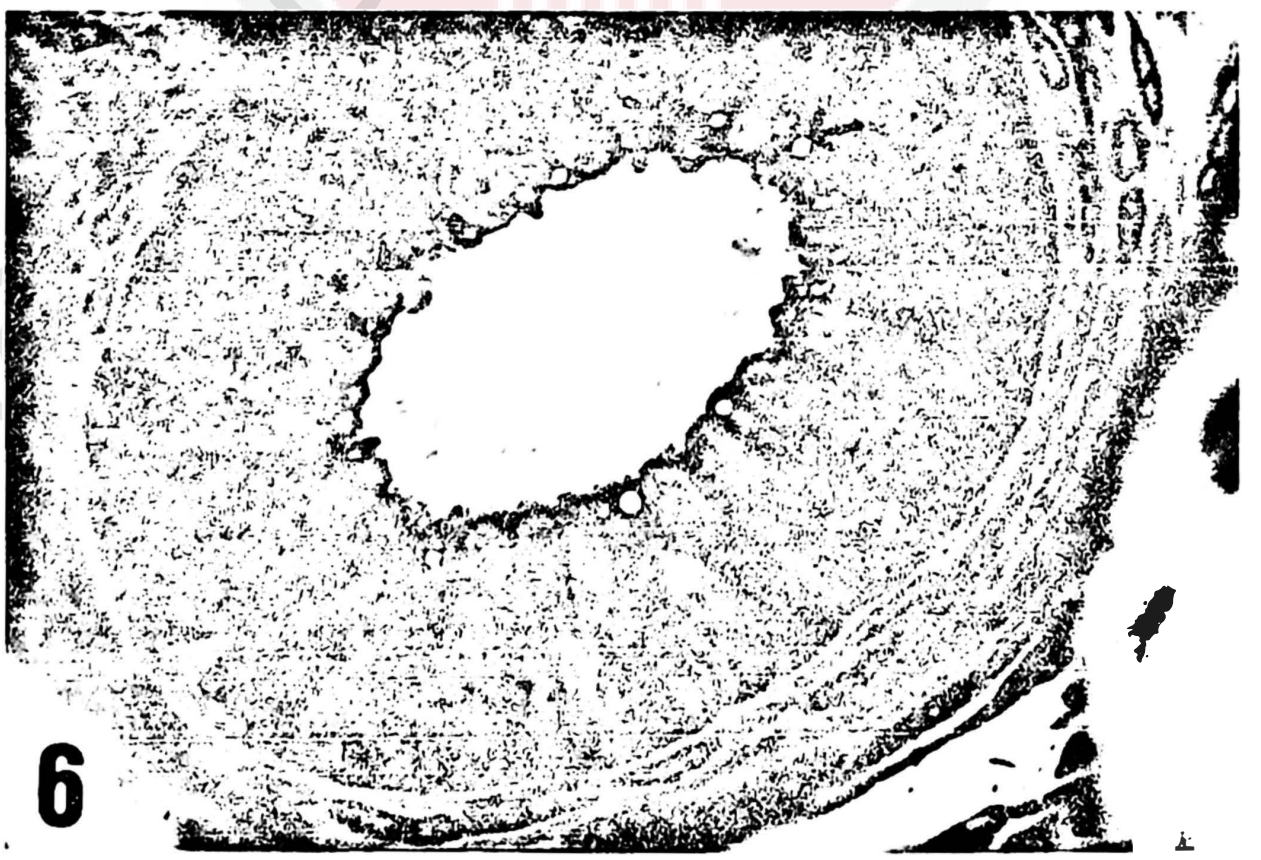


Fig. 5. Halo cell and basal cell on basal layer. Toluidine blue x 100.

Fig. 6. Cross-section of head of epididymis from puberty animals. Tall epithelium cell with empty vacoules (arrow) within apical cytoplasm. Few basal cells and presence of tall stereocilia. Toluidine blue x 20.



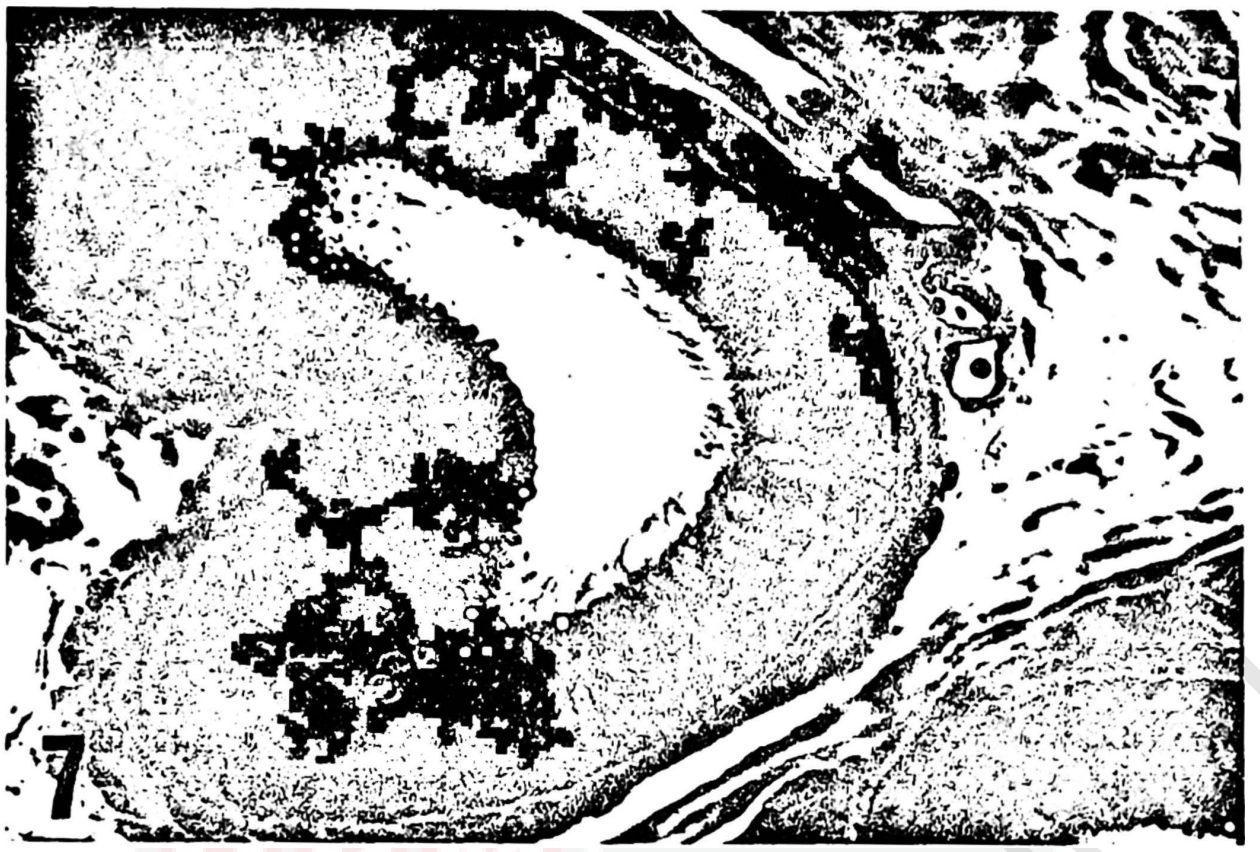
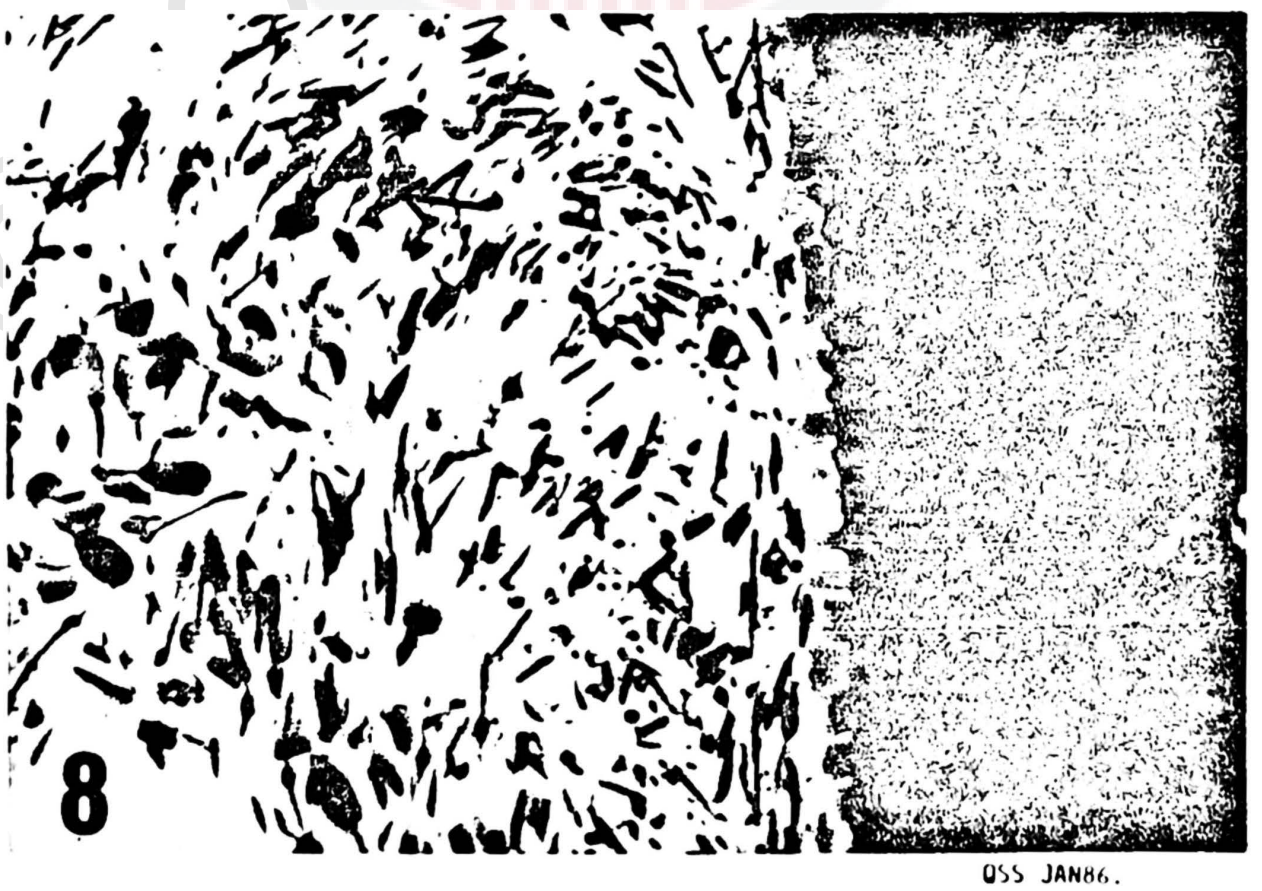


Fig. 7. Section of head of epididymis from puberty animals shows stereocilia were fully developed abd directed toward one direction. Empty vacoules also present. Large blood vessels in the interductal connective tissue. Toluidine blue x 10.

Fig. 8. Section of head of epididymis from puberty animals. Note the deposition of secretion on the luminal surface with spermatozoa within lumen (arrow and also spermatid. Toluidine blue x 20.



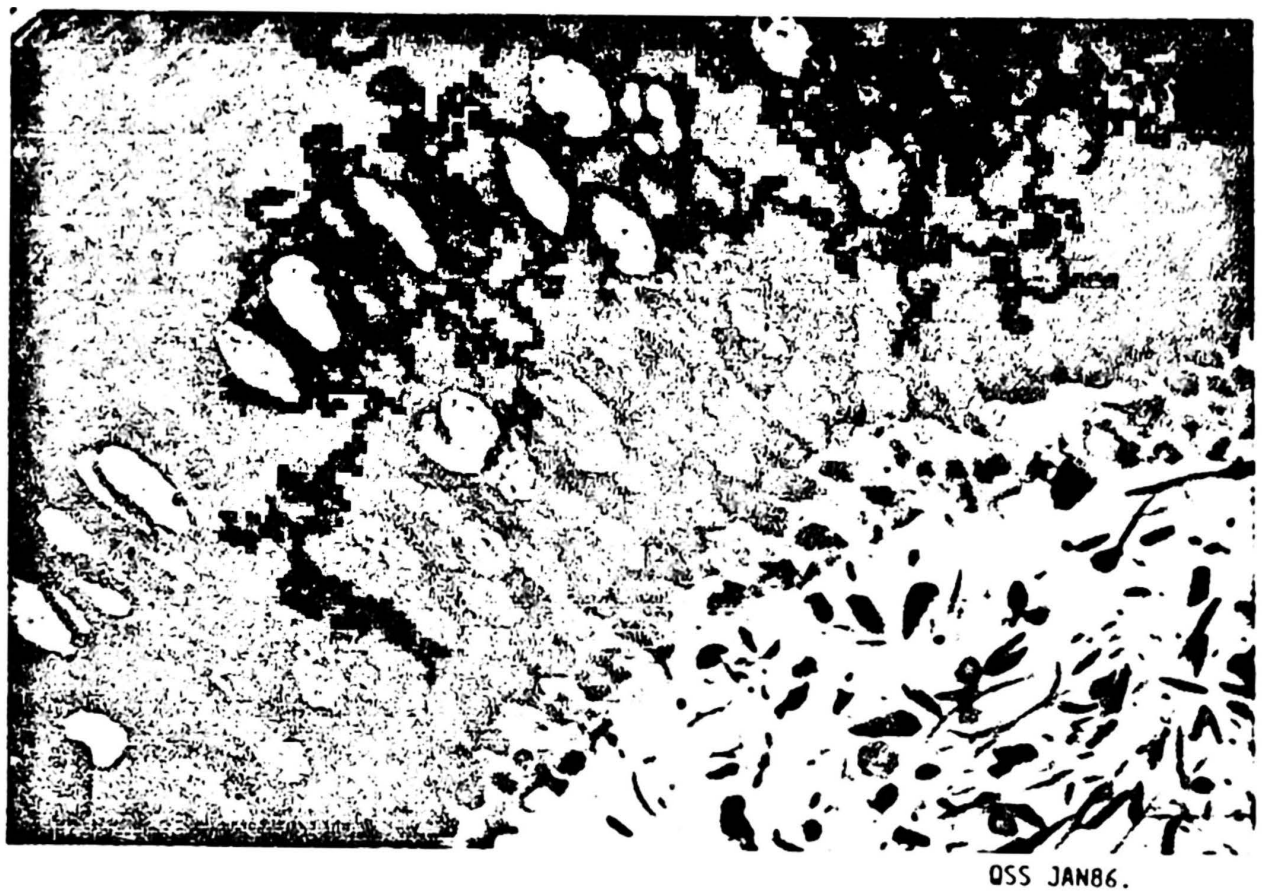


Fig. 9. Section from head of epididymis of puberty animals. On the epithelium surface were present a lot of secretion. some of secretory globules were seen extruding from the apical surface. Toluidine blue x 20.

Fig. 10. Section of the body epididymis of animals at puberty stage. The secretion were seen to be extruded from the apical surface (arrow). Within the epithelial were numerous vacoule (small arrow). Toluidine blue x 40.

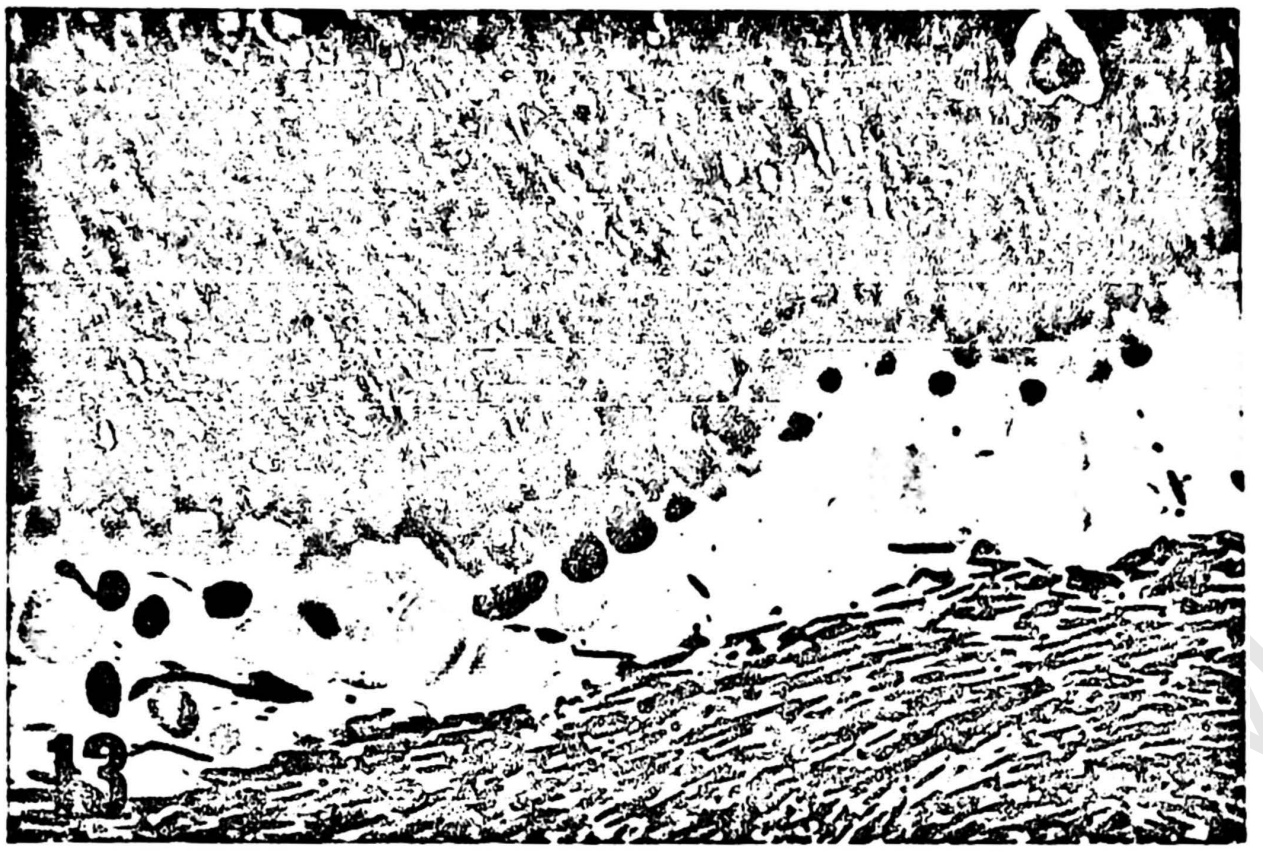
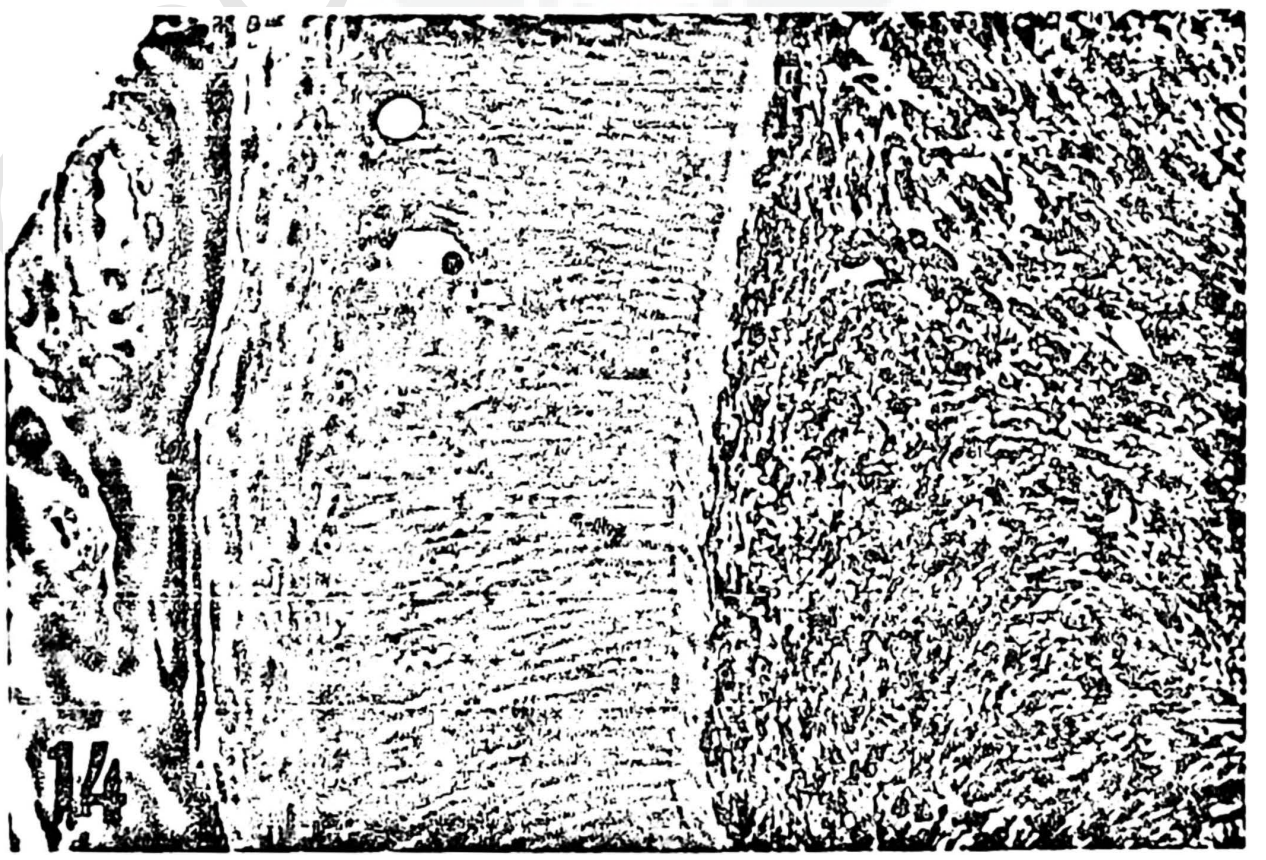


Fig. 13. Section of tail of epididymis at puberty. Note the presence of secretory globules and spermatozoa in the lumen. The epithelial cells were tall with nuclei situated at the middle part. Toluidine blue x 40.

Fig. 14. Section of head of epididymis at postpuberty. The epithelial cell was increased in height with fully developed stereocilia. Note the stereocilia were swept to one side of the duct. The nuclei of peithelial cells were mainly at the basal region. Within the lumina there were clumps of spermatozoa. Toluidine blue x 40.



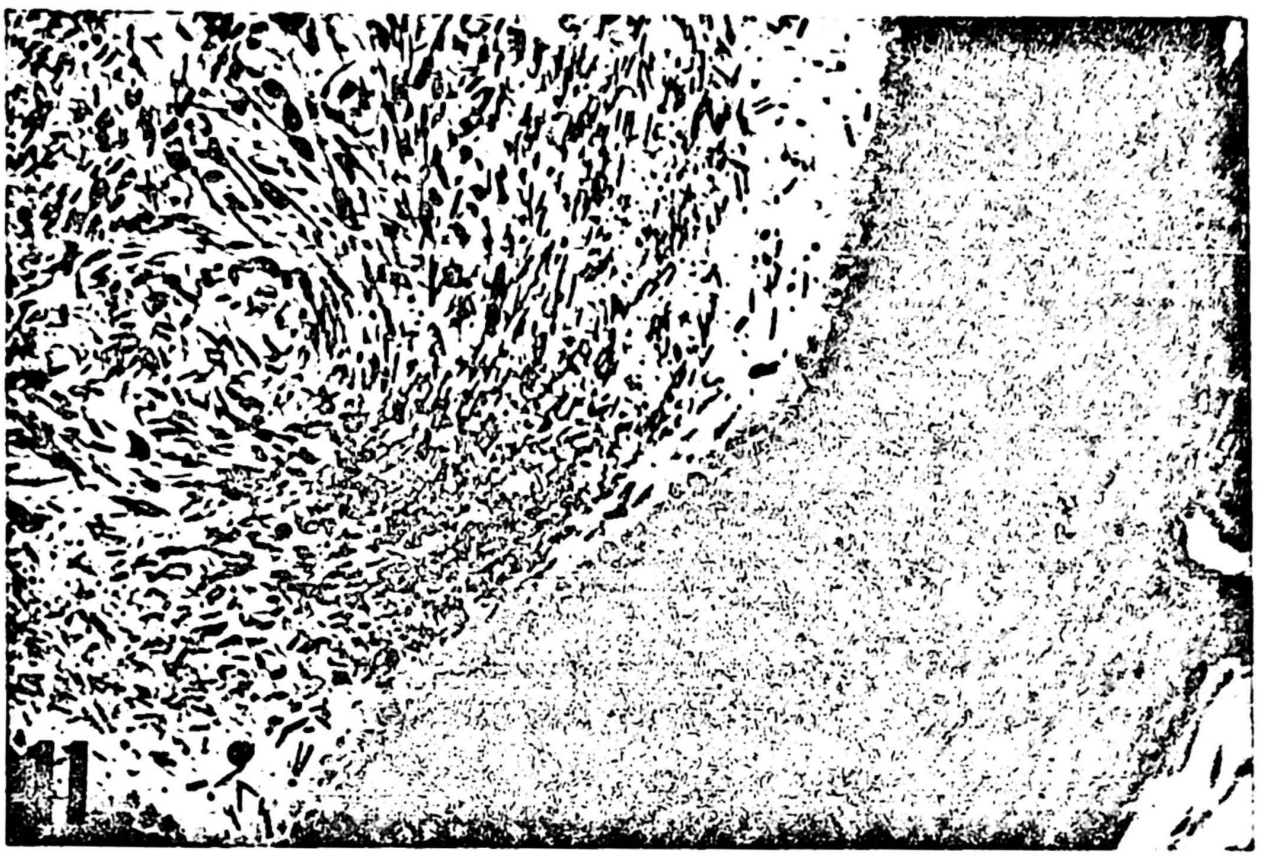


Fig. 11. Section of the body epididymis at puberty stage. Within the lumen were present of large numbers of spermatozoa and spermatid. Toluidine blue x 20.

Fig. 12. Section of body of epididymis during puberty stage. There were present numerous large blood vessels within interductal connective tissue (arrow). Toluidine blue x 20.

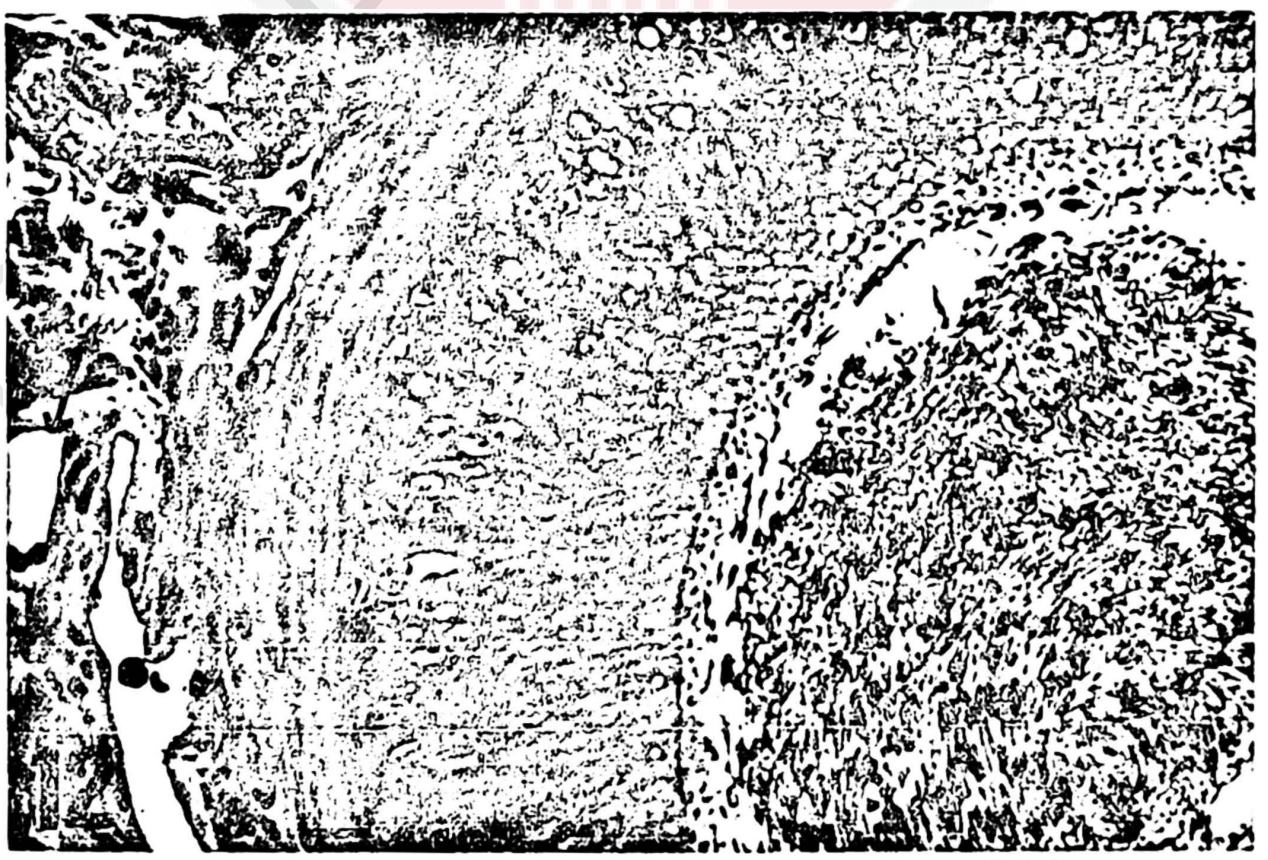


Fig. 15. Section of head of epididymis at postpuberty. Note the tall epithelial cells with elongated nuclei and a large halo cell (h). Toluidine blue x 40.