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Structure and Seasonal Changes in the Testes of a Hill Stream Fish *Glyptosternum pectinopterum*

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Introduction

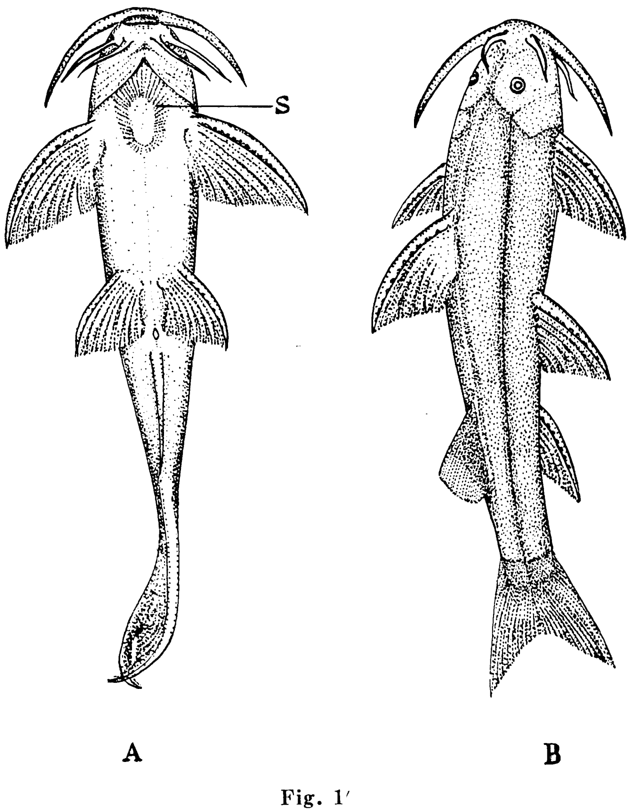
Early studies on the gonads of fishes were mainly concerned with the general structural characteristics of the testes. During the recent years, however, much attention has been paid to the seasonal histological changes in the testes. Important contributions on the testicular cycle have been made by TURNER (1919), VAN OORDT (1925), CRAIG BENNET (1931), MATTHEWS (1938), JONES (1940), WIESEL (1943), JAMES (1946), GHOSE and KAR (1952), GOKHALE (1957), SATHYANESAN (1959), HONMA and TAMURA (1962, 1963), and NAIR (1965) in different species of fishes. The accessory glands associated with the testes in some fishes have been described by WEISEL (1949) in *Gillichthys*, SUNDARARAJ (1958) in *Heteropneustes fossilis*, NAWAR (1960) in *Clarias lazera*, SNEED and CLEMENS (1963) in some cat-fishes and NAIR (1965) in *Heteropneustes fossilis* and *Clarias batrachus*. The present paper deals with the cyclical changes in the testes of a hillstream teleostean siluroid fish, *Glyptosternum pectinopterum* (McCLEILAND), and has been taken with a view to elucidate its reproductive physiology in relation to the pituitary gland.

Material and method

Specimens of *G. pectinopterum* were collected each month during the period 1964-65 at Chaukhutia from the West Ram Ganga at an altitude of about 3,500 feet. The total length and weight of the specimens and their testes were recorded. The anterior, middle and posterior parts of the testes were then separately fixed in bouin's fluid, alcoholic bouin and ZENKER's formol. Sections were cut at 5 or 6 micron thickness and stained with DELAFIELD's haematoxylin, MAYER's haemalum and iron-haematoxylin. In all the cases eosin was used as a counter stain. HEIDENHAIN's azan stain was used to identify the presence of connective tissue. The gonosomatic index was calculated as below:

GSI=weight of the gonad$\times$100$\div$weight of the body.

Fig. 1



A B

Fig. 1′

Fig. 1, 1′ (A, B). *G. pectinopterum*, natural size.
A=Ventral view, B=Dorsal view, S=Sucker.

Observations

Morphology of the testes:

The testes of *G. pectinopterum* are paired and elongated structures lying on either side in the posterior half of the abdominal cavity ventral to the kidneys. Each has a spermatic duct running throughout its length and the two ducts unite posteriorly to open by a common aperture. The testes are fused with one another along their whole length. Numerous lobules arise from the centrally placed spermatic duct and are not bound together to form a compact organ. The lobules extend on all sides of the testes and during the breeding season are full of sperms, looking as finger-like projections (Fig. 2.) The pigments are absent from the surface of the testes, which vary in colour from dull brown to brownish pink. The important morphological features are summarized below:

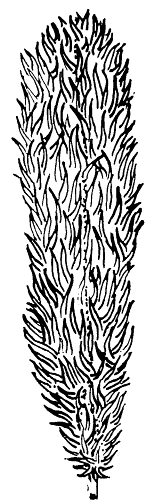


Fig. 2. Tsetes of *G. pectinopterum* in June.

(i) The weight of the testes increases very slowly and attains its maximum in the month of June. The weight then decreases sharply and reaches the minimum in the month of November (Fig. 3).

(ii) There is no change in the length of the testes and therefore, this does not serve as an indication of sexual maturity.

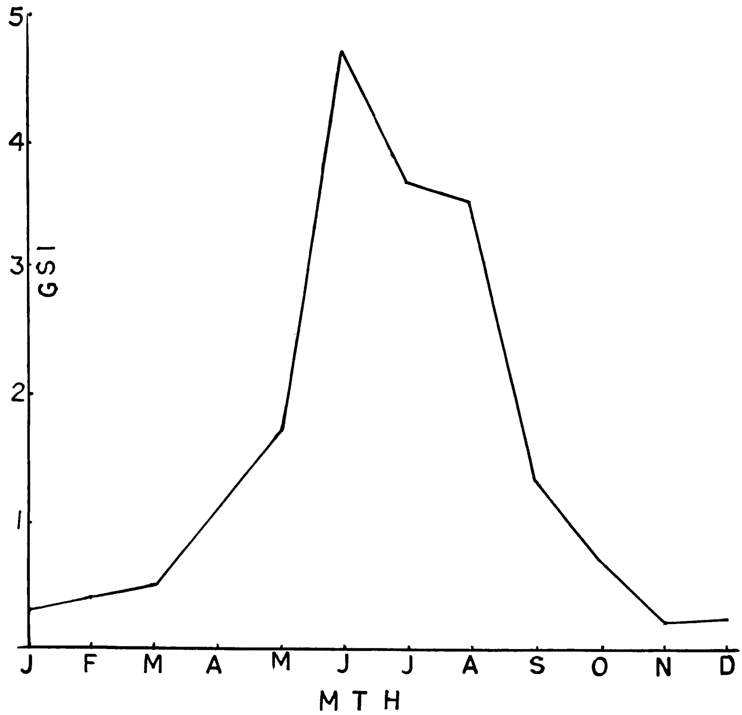
(iii) The testes which are thin, thread-like, flaccid and translucent during the resting period, become swollen, bulging, turgid and opaque on the approach of the breeding season. This change is accompanied with an increase in the weight and volume of the testes.

(iv) The milt oozes out in the form of a colourless viscous fluid on applying pressure on the abdomen during the months of June, July and August.

(v) There is no sexual dimorphism.

Histology of the testes:

The testes of *G. pectinopterum* are simple in structure with lobules radiating out individually from the sperm duct into which each opens by its own aperture (plate 6., fig. 1). The walls of the lobules are lined by the germinal epithelium whose cells divide during the growth period. This is covered over by a layer of elastic tissue and a layer of connective tissue, surrounded by the peritoneum. Each lobule is further divided into a few cysts by means of trabeculae. The two spermatic ducts which are free in the anterior region unite posteriorly inside the testes and each is lined with columnar epithelial cells. These cells are elongated and contain large



Text-fig. 2. Graph showing the testicular cycle in *G. pectinopterum*.
GSI=Gonosomatic Index. MTH=Months.

oval nuclei placed eccentrically. The nucleolus lies in the centre of the nucleus. With the azan technique, the cytoplasm of the cells stains pink, while the thick connective tissue takes a dark blue colour.

The lobule boundary cells are present throughout the year. They resemble more or less with the connective tissue cells and are arranged in a ring-like fashion all round the lobule. These cells are of various shapes—spherical, oval, spindle or oblong. The nucleus of the cell takes a dark colour with haematoxylin while the cytoplasm is faintly stained (Plate 6., fig. 2). The spaces in between the lobules are filled with connective tissue, blood capillaries and a few interstitial cells (Plate 6., fig. 5).

The germinal epithelial cells become active after spawning, and are firmly lodged inside the cysts. During growth period, these cells start dividing and are transformed into sperm mother cells or spermatogonia. The germinal cells are faintly stained in comparison to the spermatogonia which take a darker colour with haematoxylin.

Each spermatogonium is a spherical structure containing a large, round, deeply stained centrally placed nucleus. The cytoplasm does not take much stain. The nucleolus is situated rather eccentrically and is also deeply stained (Plate 6., Fig. 2). These cells divide mitotically and give rise to a large number of cells.

The primary spermatocyte has a much darker nucleus but is smaller in size than the spermatogonium. It undergoes various stages of division. The chromatin matter is however, unevenly dispersed in the primary stages but is gradually transformed into a fine reticulum, and later gets thickened. The chromatin threads gather on one side of the nucleus, leaving a clear space but no nucleolus is seen. This stage is known as synizesis knot (plate 6., fig. 7).

The secondary spermatocytes are smaller than the primary ones. The chromatin material is seen in the form of thick clump. They last for a short duration only and later give rise to spermatids (Plate 1., Fig. 3). The spermatids are further reduced in size and their chromatin matter is highly chromatic in nature and is deeply stained with iron haematoxylin. A spermatid has a somewhat elliptical nucleus (Plate 6., fig. 3). The sperms are the final products of spermatogenesis. They are similar to spermatids but are further reduced in size. The nucleus of the sperm has a strong affinity for the basic dyes.

The posterior region of the testes in *G. pectinopterum* is not sterile as reported by SATHYANESAN (1959) in *Mystus seenghala* and NAIR (1965) in *Clarias batrachus*. All the regions of the testes in this species are structurally and functionally identical. The sperm ducts are occasionally seen full of sperms during the breeding season and probably serve for the temporary storage of sperms which are finally expelled out by the common aperture.

The testes under study, can be conveniently divided into the following stages on the basis of their morphological and histological structure (see fig. 2): *Stage A:* (Resting condition). November to January.

The testes possess thick walled germinal epithelium whose cells become very active and start dividing. They soon form a large number of cells called the spermatogonia. The spermatogonia are the only germ cells of this stage and are arranged along the wall of the lobule. Often these germ cells completely fill the lumen of the lobule which, therefore looks like a cord of cells (plate 6., fig. 2). The lobule boundary cells are present abundantly.

Stage B: (Slightly enlarged condition). January to April.

The spermatogenesis is in progress during this period. The cysts are thick walled and contain a large number of spermatogonia which undergo division and a few stages of synizesis knot are also present (plate 6., fig. 7). During the months of January and February, primary spermatocytes and spermatogonia are the only cells of the cyst, but in later months the secondary spermatocytes and spermatids are also present in considerable numbers (plate 6., Fig. 3). It is interesting to note that spermatids are formed in the month of April only. Sperms are not yet formed. Every successive stage of germ cells appears to be smaller and smaller. Numerous lobule boundary cells are also visible.

Stage C: (Greatly enlarged condition). April to June.

The germinal epithelium of the testes has become considerably thin and the spermatogonia are reduced in number. A few resting sperm mother cells or spermatogonia lying at the periphery of the cysts are also visible along with the spermatocytes, spermatids and sperms (plate 6., fig. 3). The sperms appear for the first time in the month of May. The cysts show all the stages of spermatogenesis in this month. In the month of June, all the lobules are full of sperms (plate 6., fig. 5). The spermatocytes are reduced considerably. Only a few interstitial cells are seen in between the lobules. The lobule boundary cells do not show any change in their structure.

Stage D and E: (Spawning and partly spent condition). July to September.

The germinal epithelium is very thin and the spermatogonia are further reduced in number. The cysts are full of spermatids and sperms, during the months of July and August. Spermatocytes are further reduced in number. Although the lobules are full of sperms in the month of August, a slight increase in the number of spermatogonia is noticeable. In the begining of September, the germinal epithelium becomes slightly thicker and the spermatogonia increase relatively. The cysts are packed with sperms in the centre and the spermatogonia are arranged towards the periphery (plate 6., fig. 4). The spermatocytes are rare. The interstitial cells are found in between the lobules and their cytoplasm takes a light stain. The nucleus stains deeply with haematoxylin. Besides the interstitial cells, lobule boundary cells are also present. The cavity of the spermduct is also full of sperms.

Stage F: (Depleted condition). September and October.

The testes have a slightly thicker germinal epithelium and a large number of spermatogonial cells are present at the periphery of the lobules. Spermatocytes are nearly absent indicating the cessation of spermatogenesis. A few spermatids are present. The interstitial and the lobule boundary cells do not show any change from the previous stage. In the begining of this stage, the sperms are packed inside the cavity of the lobule and sperm duct, but as this comes to an end, the spermatogonia show further increase in their number, the interlobular spaces are filled with connective tissue and the sperm masses become rarefied both in the lobules and the sperm duct. The residual sperms are retained inside the sperm duct for some time but finally disappear completely (plate 6., fig. 6). The entire cavity of the lobule is then filled with spermatogonial cells and the testes come to rest till late January when the primary spermatocytes appear again to continue the cycle.

Thus, it is evident from the above description that the testicular cycle of this fish comprises five main stages viz. 'Growth', 'Maturation', 'Activation', 'Depletion' and 'Rest'. The testes start growing actively during the months of January, February, March and April when the weight increases gradually. The maturation period extends from April to June, during which active meiotic divisions take place and the testes attain the maximum weight. The sperms are seen filling the cavity of the lobule. The period of activation starts from June to August when the lobules are full of sperms and the number of spermatogonia, spermatocytes and spermatids is reduced considerably.

The spermatogenesis ceases by the end of September, when the sperm masses are seen in the lobule and sperm duct, and a depleted condition of testes is attained by the end of October when the germinal epithelium and spermatogonia increase appreciably. Finally, the testes come to rest during November and December.

The histomorphological observations described above in the testicular cycle of *G. pectinopterum* are in conformity with the analysis of the gonosomatic index.

Discussion

The above study reveals many interesting features. There is no sexual dimorphism in *G. pectinopterum* and both the sexes are similar in appearance.

SWARUP (1959) reported the appearance of melanophores in the testis of *Gasterosteus aculeatus* during breeding season and considers it as a sign of the maturation of testes. No pigmentation is seen during maturation of the testes of *G. pectinopterum*. However, the colour changes from dull brown to brownish pink due to a large number of blood capillaries.

TURNER (1919), JAMES (1946) and GHOSE and KAR (1952) reported the fusion of the testes in the posterior region. In *G. pectinopterum*, the testes are fused along their whole length and this may by due to a limited space available to it inside the body cavity of the fish. The testes present a peculiar shape in the breeding season, with hollow finger-like lobules projecting freely into the body cavity, as in *Gillichthys* (WEISEL, 1949). This condition of the testes appears to be a very much simplified feature not usually found in the teleosts.

The posterior region of the testes has been reported to be sterile in several species of teleosts (SATHYANESAN, 1959; NAIR, 1965). Sterile lamellae are present in this region which is reported to serve for the storage of sperms. In *G. pectinopterum*, however, the posterior portion of the testes is equally capable of producing germ cells as any other part. Hence, the testes of this fish are not divisible into three regions as in other species. Accessory glands similar to the seminal vesicles of mammals are present in *Gillichthys* (WEISEL, 1949), *Heteropneustes fossilis* (SUNDARARAJ, 1958), *Clarias lazera* (NAWAR, 1960) and in two species of siluroid fishes (NAIR, 1965). Such structures are absent in *G. pectinopterum*. However, it appears that the sperms are temporarily stored in the spermatic ducts of this fish until they are expelled out into the water.

The lobules are simple sacs with a few trabeculae and do not form a complex network as in the perch (TURNER, 1919) and in other species. There is no radial septation in the testes of this fish as in salmon (WEISEL, 1943). The cortical and medullary zones are also not distinguishable as in *Fundulus* (MATTHEWS, 1938).

Various opinions have been expressed about the interstitial cells in fishes. CRAIG-

BENNET (1931) correlated the seasonal cycle of these cells with that of the secondary sexual characters in *Gasterosteus aculeatus*. In this fish the interstitial cells are reported to be responsible for the secretion of male hormones. GOKHALE (1959) attributed a nutritive function to the interstitial cells in *Gadus*. ESSENBERG (1923), VAN OORDT (1925), MATTHEWS (1938), RASQUIN and HAFTER (1951) and SATHYANESAN (1959) could not find any secretory activity in the interstitial cells of the species studied by them. MARSHALL and LOFTS (1956) found two distinctly different arrangements of endocrine tissue in fish testes. The typical vertebrate interstitial gland (Leydig cell) pattern is present in such species as *Gasterosteus aculeatus*, *Tilapia* sp., *Clupea sparatus*, *Latemeria*, *Scyliorhinus* and *Chimera*. On the other hand, fish such as *Esox lucius*, *Salvelinus willughbii*, *Plecoglossus altivelis* and *Labeo* sp. lack the secretory interstitium but possess 'lobule boundary cells' which are histochemically similar to the Leydig cell. Interstitial cells and lobule boundary cells are morphologically similar and may be presumed to perform the same function (HOAR, 1957). In *G. pectinopterum* both the interstitial and lobule boundary cells are present but nothing can be said about their secretory nature.

The spermatogonia are the only germ cells during the resting phase, but they are also present throughout the year, although their number is considerably reduced during breeding period. Various names have been assigned to these sperm mother cells. HANN (1927) named them 'dormant germ cells', SUZUKI (1939) called them 'reserve germ cells', WEISEL (1943), HONMA and TAMURA (1962) and JONES (1940) reffered to them as 'resting germ cells'.

The testes of *G. pectinopterum* during its seasonal testicular cycle exhibit a period of Growth, Maturation, Activation, Depletion and Rest. TURNER (1919) recorded the maximum size of the testes of *Perca flavescens* in the month of October and minimum in July and August. The testes of *Fundulus* attain maximum weight in the late spring and early summer (MATTHEWS, 1938). RAI (1965) reported that the testes of *B. tor* attain maximum weight and volume in the month of March and April and minimum in September and October. NAIR (1965) found the maximum size and weight of the testes in *Heteropneuste fossilis* in the months of July and August and minimum in January and February. In *G. pectinopterum* the testes reach maximum weight and volume in the month of June and minimum in the months of October and November. During the successive months the weight of the testes increases gradually. The spermatogenic activity starts at different times of the year in various species of fishes studied by TURNER (1919), BULLOUGH (1939), JONES (1940), HONMA and TAMURA (1962, 1963), NAIR (1965) and RAI (1965). These variations are clearly due to differences in the environmental conditions like temperature and light prevailing in the locality. However, SWARUP (1958) found that the testes become sexually mature at any time of the year but its functional maturity is obtained only during the breeding period (April and May).

In *G. pectinopterum* the spermatogenic activity commences in the month of January and progresses rather slowly through February, March and April and reaches a peak in the months of June and July, when the lobules are full of spermatids and sperms. From June onwards, the spermatogenesis is slowed down and comes to an end by October and early November. After the cessation of spermatogenic activity the testes enter a period of rest.

Summary

(1) The sexes of *G. pectinopterum* can not be distinguished externally at any phase of maturity.

(2) Numerous lobules arise from the centrally situated spermatic duct and radiate individually on all sides of the testes. The two sperm ducts are free in the anterior part of the testes and join posteriorly to open out by a common aperture.

(3) The entire testis produces sperms and its posterior part is not sterile.

(4) Cyclic changes in the testes have been described. The spermatogenesis commences in the month of January and progresses slowly during the following months reaching its peak in June and July. It then slows down and ceases by the end of October and November. The testes enter a period of rest during November and December.

(5) The interstitial and the lobule boundary cells are present in the testes but secretory activity or cyclic changes have not been observed in them.

Acknowledgements

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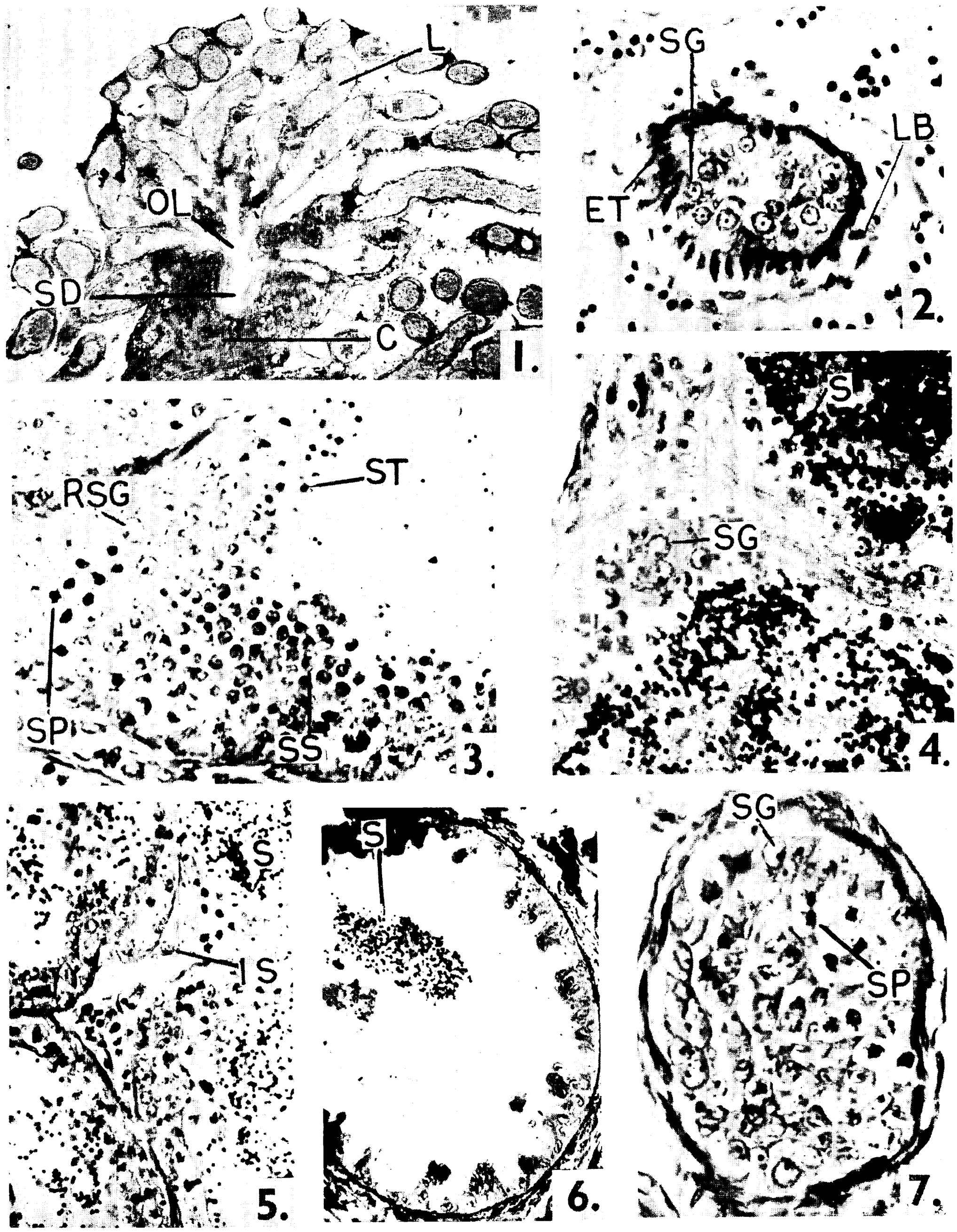
Abbrvlations used

SD=sperm duct.	S=sperm.
SG=spermatogonia.	L=lobule.
RSG=resting spermatogonia.	LB=lobule boundary cell.
SP=primary spermatocyte.	C=connective tissue.
SS=secondary spermatocyte.	ET=elastic tissue.
ST=spermatid.	IS=interstitial cell.

Explanation of the plate 14

Fig. 1. T. S. testis showing radiation of lobules and sperm duct ×110.
Fig. 2. T. S. of a lobule of testis showing spermatogonia and lobule boundary cells. ×240.
Fig. 3. T. S. testis showing primary and secondary spermatocytes, spermatids and resting spermatogonia. ×400.
Fig. 4. T. S. testis showing thick walled cysts containing spermatogonia and sperms. ×320.
Fig. 5. T. S. testis showing thin walled cysts, sperms and interstitial cells. ×400.
Fig. 6. T. S. sperm duct containing sperms. ×90.
Fig. 7. T. S. testis showing spermatogonia and primary spermatocytes. ×320.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