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SPERMATOGENESIS IN THE TESTIS OF OREOCHROMIS SPILURUS (GÜNTHER) REARED IN JEDDAH FISH FARMS SAUDI ARABIAN

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ABSTRACT

The spermatogenises in the testis of Oreochromis Spilurus acclimatized and reared in Saudi Arabian fish farms was investigated. Histological studies indicated that testis maturation is accomplished in five stages as following:

1- Immature stage having spermatogonial cells (about 12μ).

2- Mature or recovery stage display active spermatogenesis and the cells of all stages can be seen (spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids) .

3- Nearly ripe stage characterized by the active spermiogenesis process forming spermatid and spermatozoa, beside the presence of few spermatogonia and spermatocytes.

4- Ripe and spawning stage showing a marked dilatation of semineferous lobules containing a lot of sperms in ripe stage, while the spawning stage as well as the ripe show a decrease in size of the lobules due to the discharge of considerable amount of spermatozoa. This stage extends through the period from March to December.

5- Spent stage, takes place in January have lobules desentegrate and no milt is found.

The ultrastructure studies during spermatogenesis show that the arrangement of mitochondria which surround the base of nucleus. Also the condensation of chromatin in the nuclei as well as secondary spermatocyte and spermatids are present in specific pattern as maturation occurs. Also there is a great decrease in the size of nucleus of spermatids.

The ultrastructural appearance of spermatozoa show that they are of the primitive type and lack an acrosome. The nucleus is basically cylindrical, domed at the anterior pole and flattened at the caudal pole the articular fossa at the caudal pole is key hole shape which contains the proximal and distal centriales.

The spermatogenesis process of O. Spilurus belong to semi-cystic type where spermatogenesis occurs partly out side cysts, in the lumen of the

lobules. The complication of the process of spermatogenesis and the character of the discharge of the sexual products are relative to the asynchronous type.

INTRODUCTION

In most fishes, the testes are elongated paired organs attached to the dorsal body wall by a membrane called mesorchium. Testicular structure in teleosts varies from one species to another according to the differentiation of the germinal tissue (Nagahama, 1983, Zaki *et al* 1986; El-ghamdi 2001). The typical testis of most teleosts is lobular type, this is composed of numerous separated lobules jointed by a thin layer of connective tissue, the arrangement of the lobules varies considerably (Billard *et al*, 1982).

Various authors have described and illuslrated the teleostean testes as Latif and Saady (1973) for *Tilapia nilotica* and Zaki *et al* (1986) for *Clarias garipenis*.

O. Spilurus is successfully aclimatized and reared in Jeddah fish farms Saudi Arabian due to the suitability of environmental factors that include Temperatune, salinity and behavior associated with feeding and reproductive strategy.

As in most teleosts, the testis consists internally of intricotely divided lobules which are separated from each other by mean of interlobular connective tissue. The lobules contain numerous nests composed of maturing germ cells varying in the appearance with the season and the state of maturity (Billar *et al.*, 1982).

The aim of this study is to describe the histological characteristics of the testis to give back grounds for the seasonal changes in the male sexual gland and the reproductive cycle of *O. Spilurus*

MATERIALS AND METHODS

O. Spilurus were cellected during the period from June 2001 to May2002 from Jeddah fish farm. The fish was dissected to determine sex and maturity stage. The testes were removed and weighed to the nearest milligram. The shape, size and colour of each

gonad was recorded and the gonado somatic index was calculated and expressed as the percentage weight of the gonad to the gutted weight of the fish.

For the study of the histological changes in the testes during different developmental stages, pieces of testis were fixed in Bouin's fluid. After fixation the specimens passed into dehydrating, clearing and embedding in paraffin wax. Section at thickness of 5-7 μ. were stained with Eosin and Haematoxylin.

For electron microscope a piece of testes was embedded in 4% gluteraldehyde in 0.1M cacodylate buffer (PH 7.2 – 7.4) at 4°c for 3 h.. Washing was done in 5% sucrose in 0.05 M cacodylate Buffer overnight. Post fixation in 1% Osmium tetroxide in 0.2 M cacodylate buffer. Rinsing and washing in buffer at PH 7.2 – 7.4. Dehydration, embedding , sectioning and staining with uranel acetate (saturated in 70% alcohol). Then examination was done by a transmission electron microscope at 80 KV.

RESULTS

In *o. spilurus* as most teleosts fish the testes morphologically changes in shape, colour and increasing magnitude towards spawning. In the persent study the fish less than 10 cm were immature and were present throughout the year. The male larger than 14 cm were sexually mature.

The sexually mature testes was identified morphologicaly into five stages as postulated by zaki *et al.*,(1986) in *Clarias gariepenus* following: Maturing, nearly ripe, ripe, spawning and spent.

1- **Mature stage:** Testes increased in length and width and occupied two fifth of the body cavity and characterized by fleshy colour Gonado somatic index reached 0.6%. This stage can be identified for fish more than 14 cm.

2- **Nearly ripe stage:** At this stage testes increased in length and width and occupied about two thirds of the body cavity and become whitish in color. Gonado somatic index increased to 0.85%. This stages was detected through February and March.

3- **Ripe stage:** At this stage, testes show maximum growth and occupied the entire body cavity. Its colour become white and the melt discharged by squeezing on the abdomen. At this stage the gonado somatic index reached to maximum value ranged

from 1.38 to 1.49. This stage was detected through March and April months when temperature reached 22 - 25 ^{0}c.

4- Spawning stage: Spawning takes place throughout the year at intervals. A considerable amount of sexual products were discharged during April, May, June, July, August, September, October and November. This causes a decrease in gonado somatic index to a value ranging between 0.67 and 0.89.

5- **Spent stage:** At this stage the testes were reduced in size to minimum size and become pinkish in colour and the gonado somatic index reduced to 0.37. This stage was detected through January and February.

The structure and spermatogenesis :

The testes of *o. spilurus* is surrounded by testicular wall composed of a thin membrane of connective tissue. The testes are characterized by the presence of long, branched seminiferous lobules held with each other by interstitial connective tissue Fig.(6).

The seminiferous lobules at immature stage, composed of the germ cells which are inactive. They designate spermotogonium at different stages as show Fig. (1).The seminiferous lobules at mature stage have various types of cells at different developmental stages i.e spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa as shown in Fig. (2,3).

1- Spermatogonia:

There are the primary germ cells. They under go repeated mitotic divisions to form a large number of spermatogonia. It is the first spermatogonial stage in the testes. The formed spermatogonium is nearly round the nucleus of which have a much greater affinity for heamatoxlin its diameter ranged from 12 to 16 μ with nucleus ranging from 8 to 12 μ. There is one nucleolus that lies in the center of some cells but in others located in periphery of the nucleus Fig. (1). The chromatin material is arranged on the peripheral part of nuclear membrane. The cytoplasm appeared lightly eosinophilic, while the nucleoplasm was slightly basophilic Fig. (1).

2- Primary spermatocytes:

As spermatogenesis proceeded, spermatogonia divided and multiplied to give the primary spermatocytes. Spermatogenesis occurrs synchronously Fig. (2, 3). The lobule exhibited the presence of spermatogonia and primary spermatocytes. The latter

appeared smaller than spermatogonia. The cell out-line are not well defined. The nucleus is spherical and measured 8 μ in diameter. This stage is characterized by condensed chromatin material of their nuclei Fig. (2,3).

Ultrastructure studies, show that the primary spermatocytes are characterized by the presence of excentric nucleus which contains a prominent nucleolus in addition to the chromatin threads and granules. The juxta-nuclear mitochondria grow in size from the dust-like granules to appreciably bigger granules. The Golgi bodies are big darkly-stained granules scattered either among the mitochondrial mass or outside it. They are however fewer in number as compared to the mitochondria Fig. (11).

3- Secondary spermatocytes:

The primary spermatocytes multiplied and divided to produce the secondary spermatocytes. These cells were formed by first meiotic division of the primary spermatocyte. The secondary spermatocyte has a short duration and rapidly divided into spermatid. The cell wall of second spermatocytes become indistinct. The nucleus is spherical and measures 5μ in diameter. The chromatin material become either polarized to one pole of the nucleus or deeply condensed with transluescent center Fig. (2, 3).

Ultrastructural study of the secondary spermatocytes showed that they are oval in shape and their nuclei are characterized by thick clump of chromatin. The cytoplasm appeared as thin layer having a few number of mitochondria Fig. (13).

4- Spermatids:

These cells were formed by second meiotic division of secondary spermatocyte as shown in Fig. (12). The spermatid appeared smaller than the primary and secondary spermatocytes are recognized by indistinct cell outlines. The nucleus appears more condensed with chromatine material and becomes homogenous. Each nucleus is spherical and measures 3μ in diameter. According to the degree of maturation the spermatid are either grouped inside a cyst or distributed throughout the central lumen of the lobules (Fig.4, 8).

Ultrastructurally the spermatid had nuclei with thick clumped homogenous chromatin material Fig. (12). The nuclear fossa was developed as a depression in the nucleus Fig. (14). At this stage, the cytoplasm pushed towared the nuclear fossa as two rows Fig. (14).

5 - Spermatozoa:

Spermatozoa were distinguished by small round heads and slender elongated tails. The chromatin material was condensed in the head of spermatozoa and measured about 2μ in diameter. The active spermatozoa migrated towards the center of the lumen of the lobule (Fig. 5, 6).

Ultrastructurally the mature spermatozoa is relatively elongated cell divisible into a head devoid of acrosome, a short midpiece and tail. The midpiece is rudimentary with mitochondrial ring around an articular fossa which contains proximal and distal centrioles.

The nucleus is basically cylindrical, domed at the anterior pole and flattened at the caudal pole Fig.(15). The articular fossa at the caudal pole is keyhole shaped in longitudinal section. The inner and outer membrane of the nuclear envelope maintain a close association over the entire nuclear surface with the exception of the posterior exteremity of the articular fossa.

Seasonal changes in the testes during the annual reproductive cycle:

The immature stage is detected throughout the year for fish less than 12 cm in length. A cross section of testes is mainly built up of nests spermatogonia Fig. (1). A spermatogonium can be distinguishd by its conspicuous all boundary and its special nucleus with well differentiated nuclear membrane, The inter lobular connective tissue appears in moderate thickness. The GSI at this stage ranged between 0.25% and 0.32%.

The mature or recovery stages reveals the beginning of active spermatogenesis, also this stage is detected throughout the year except February, March and April. At this stage primary and secondary spermatocyte are formed with few spermatids. The central lobules of the testis shows more spermatogenec activity Fig. (2, 3). The GSI at this stage ranged between 0.5% and 0.6%.

Nearly ripe stage revealed more active spermatogenesis and the lobules are distended by spermatid and spermatozoa while the primary and secondary spermatocyte are moderetly detected (Fig. 3). This stage is detected throughout February and march. In this period the testes started to increase in weight and GSI increased gradually with average 0.75% during February to 0.85% in March.

Ripe and spawning stage The ripe testes appears full with spermatozoa and the semineforous lobules distended with a fair quantity of spermatozoa as showing Fig. (4, 5) . Where the inter lobular connective tissue break down and few spermatocytes and the spermatogonia rarely detected Fig.(4). At this stages the GSI value reached peak value 1.49% in March. The spawning period continued from March to December. Where the GSI varied between 1.37% and 0.85%. This stage characterized by discharge of considerable quantity of sperm during the spawning season. At this stage the semineforous lobules accompanied by a decrease in the width. Fig. (6, 7, 8) Despite this fact there was still a considerable quantity of spermatozoa.

Spent and recovery stage the spent testis in this stage often contain residual sperm in lumen of the semineforous lobules Fig. (9) , This stage present from December to February. Spermatogonia appear on the periphery of some lobules. These spermatogonia are responsible for the spermatogenesis of the next spawning season. While in Recovery stage a remarkable thick wall is obvious of the lobules which filled with spermatogonia and blood supply Fig.(10). This period extend through January and February.

DISCUSSION

O. spilurus considered as one of the economical important fish species, beside its ease adaptability for culture, an increasing interest has been given to its propagation and productivity. However, little attention has been paid to the spermatogenesis after transplantation and acclimation in Saudi Arabian fish farms. In the present study the general pattern of developmental stages of *Oreochromis Spilurus* were Immature, Mature, Nearly Ripe, Ripe, Spawning, Spent and recovery.

As in most teleosts fish the testes of *Oreochromis Spilurus* are composed internally of intricately divided lobules separated from each other by septa and interlobulas connective tissue. The straight, simple spermatic duct open posteriorly into a seminal vesicle (Zaki *et al*, 1986; Gaber,2000).

The present results revealed that the spermatogenesis process which involves a proliferation of spermatogonia through repeated mitotic division and growth to form primary spermatocytes which then undergo reduction division to form secondary spermatocytes that divided to produce spermatids which then metamorphose to motile and potentially functions gametes (spermatozoa). Within these sequences, the cells are seens decreasing in size but then increasing in numbers. This results conforms to that shown for most teleosts as reported (Hoar 1983; Zaki *et al*., 1986; Lahnsteiner *et al*., 1995; Assem 1999; Gaber 2000 and El-gamdy 2001).

The present ultrastuctural study during spermatogenesis in *O. Spilurus* show that the arrangement of mitochondria is similar to that described in other species, were these organelles surround the base of nucleus in *Platichthys flesus* (Jones and Butler, 1988) and a number of species of bleunidae (Lahnsteiner and Patzner, 1990). In addition Fishelson *et al.*, (1990) reported that in several species of Gobies, the mitochondria present around the spermatids disappeared to be replaced by two large mitochondria.

Also in the present study on *O. Spilurus* as reported in several teleost species, the condensation of chromatin in the nuclei of secondary spermatocytes and spermatids as they reach maturation occurs in specific pattern that is often in the region adjacent to the developing flagellum (Billard, 1984; Sperando *et al.* , 1988).

As the chromatin condenses there is obviously a great decrease in the size of the nucleus. This result is in agreement with that described in several teleosts species (Mousa *et al.*,1998; Quagio-Grassiotto and Carvalho 2000; Quagio-Grassiotto *et al.*, 2001).

From the histological characteristics of immature testes in which the lobules containing only the spermatogonial cells in different size and the interlobular connective tissue thick relatively as represented by Latif and Saady (1973) for *Tilapia nilotice* and Ghabrial (1990) for *Oreochromis nilotica* .

The mature testes of *Oreochromis Spilurus* display active spermatogenesis and the cells of all stages can be seen, spermatogenia, spermatocytes (Primary and Secondary) and spermatides . This results are in agreement with Zaki *et al.*, 1986) for *clasias lazera*; Mousa *et al.*, 1998 for *Mugil cephalus*; El-ghamdy (2001) for *Acanthopagrus bifasciatus.*

For the nearly ripe stage the present results revealed the presence of few spermatogonia and spermatocytes showing moderate quantity of spermatozoa. These results confirm with most teleosts as reported by Ghabrial 1990 and El-Gohary (2001) in *Oreochromis nilotica.*

Ripe stages of the *O. Spilurus* , show a marked dilation of semineferous lobules containing a lot of sperms. Also the present study revealed that the spawning stage similar to the ripe stage show a decreas in the size of lobules due to discharge of considerable amounts of spermatozoa. This stage extended through the period from March to December.

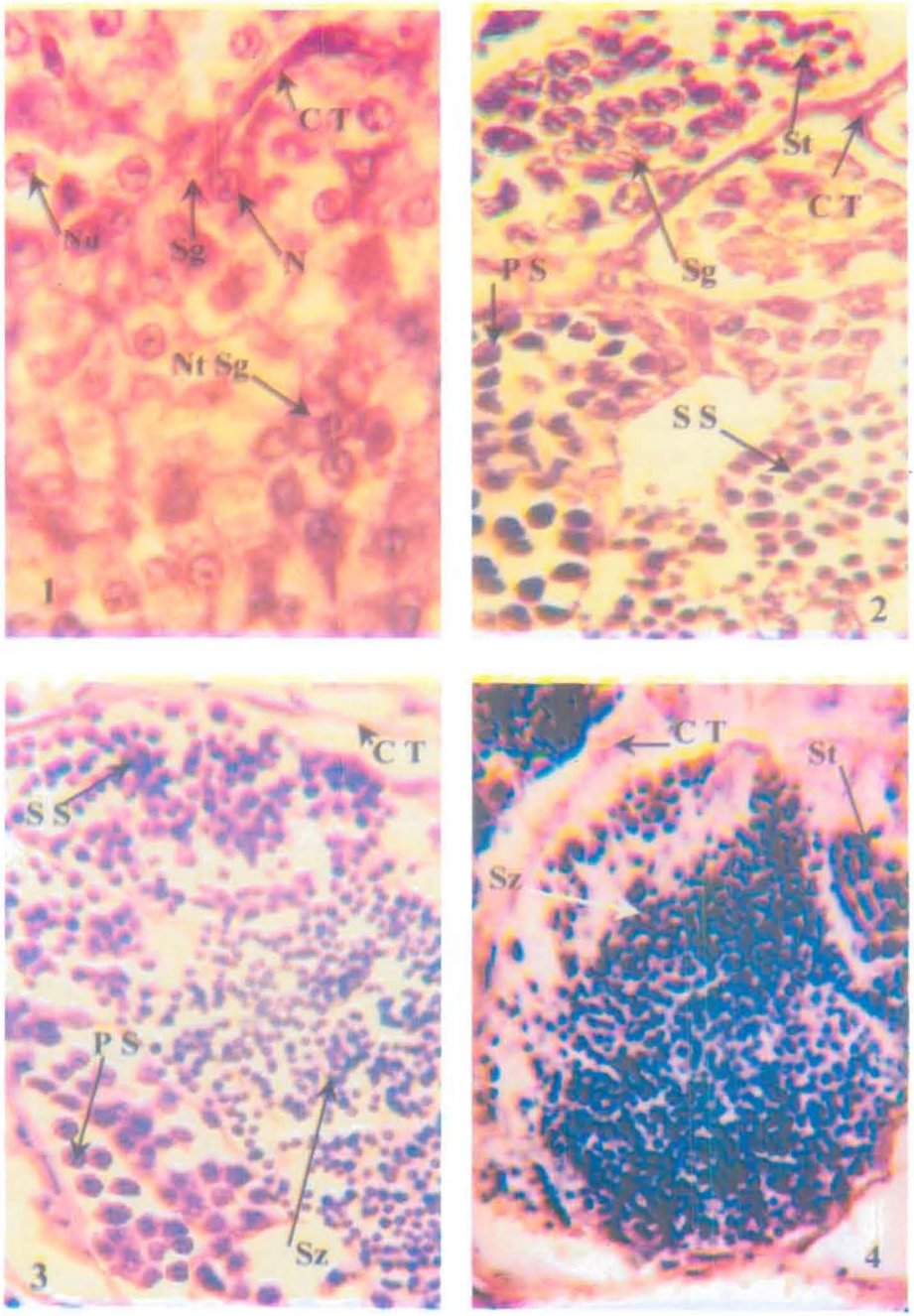
Mattei *et al.*,(1993) recognized two types of spermatogenesis: A cystic where spermatogenesis is completed with in cysts which lead to synchronous development of germ cells; and semi-cystic type where spermatogenesis occur partly outside cysts. This may produce asynchronous spermatogenesis. In *O. Spilurus* spermatid are released into the lumen of the lobules where spermatogenises occurs as the semi-cystic type.

The spent stage appear only in January, in which the semineferous lobules desentegrate and no milt are left in it. The function of the seminal vesicle in teleosten fishes varies with the type of fish. According to Zaki *et al.*, (1986) reported that with the exact function of the seminal vesicle in *Clarias gariepinus* was not determined but a change in its size was observed during the annual reproductive cycle of sexually mature fish. The change may be due to storage and discharge of sperm cells, which is turn may be an adaptation of the long and continuous spawning period of *Clarias gariepinus* .

For male *O. Spilurus* spawning season is characterised by the presence of different individuals at different levels of maturity during the spawning period which extend from March to December. Besides additional amount of sperms are always formed during the spawning period. This results support the finding of Latif and Saady (1973) in *Tilapia nilotica*; Zaki *et al.*, (1986) in *Claries gariepinus* ; Assem (1992) in Oblada melanura; Zaki *et al.*, (1994) in *Mugil seheli.*

The complication of the process of spermatogenesis and the character of the discharge of the sexual products are relative to the asynchronism in the reproduction of the primary spermatocyte as reported by Koppel (1955). Also this asynchronism may be due to the progress of spermatogenesis wave along the different parts of the testes (Butskaya – 1955).

Adaptation of prolonged and continuous spawning is characterized by fractional dischasge of the sperm cells. The prolonged spawning is enhanced by the presence of different individual caught at the same period exhibite different spermatogenic activites and the spermatozoa are discharged gradually from the semineferous lobules and the reduced size of testes, so the specific characteristic of spermatogenesis is related to the type of spawning depending on the character of spawning in female (Zaki *et al.*, 1986; Assem, 1999 and El-ghamaly, 2001).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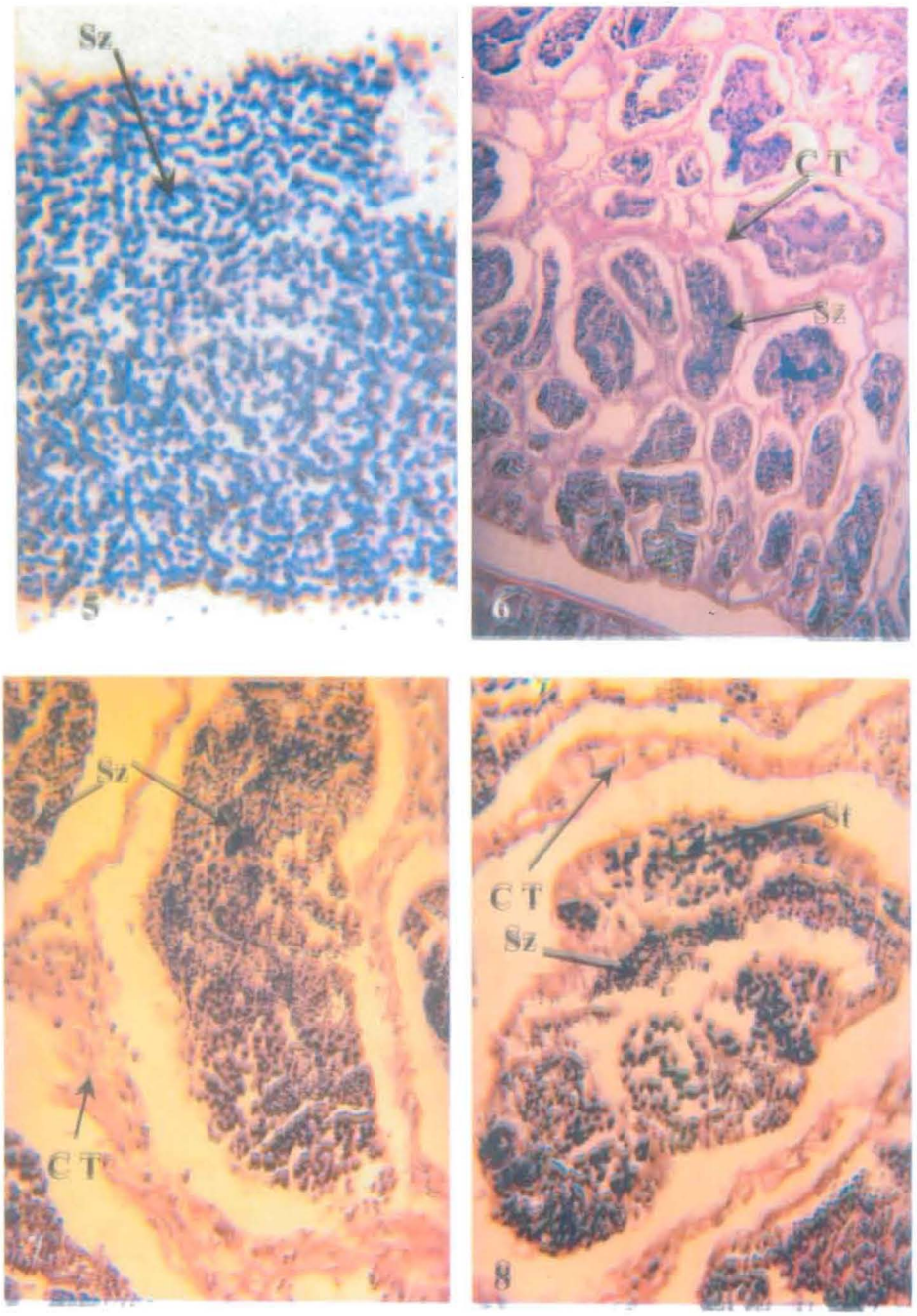
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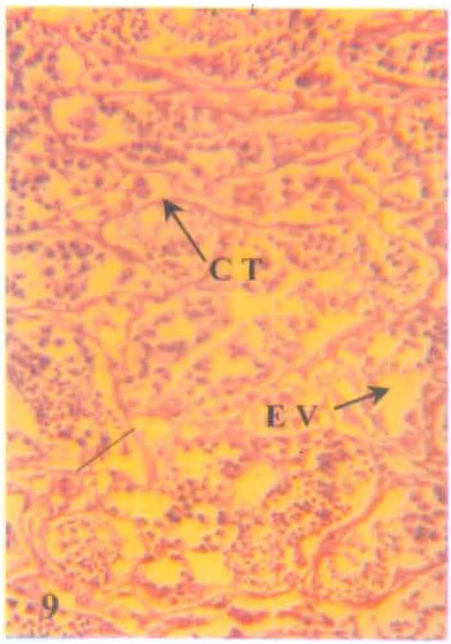
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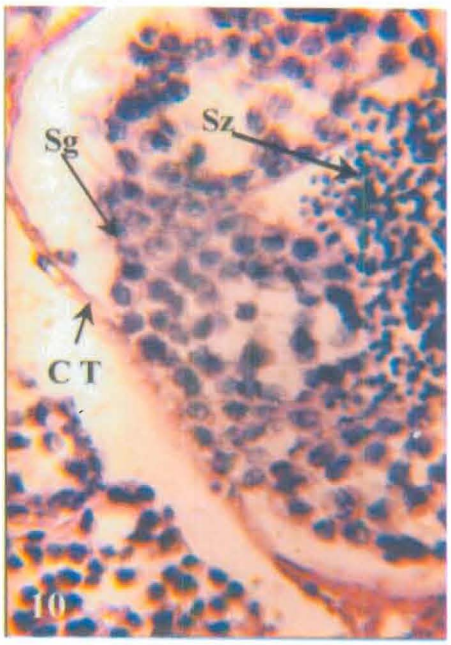
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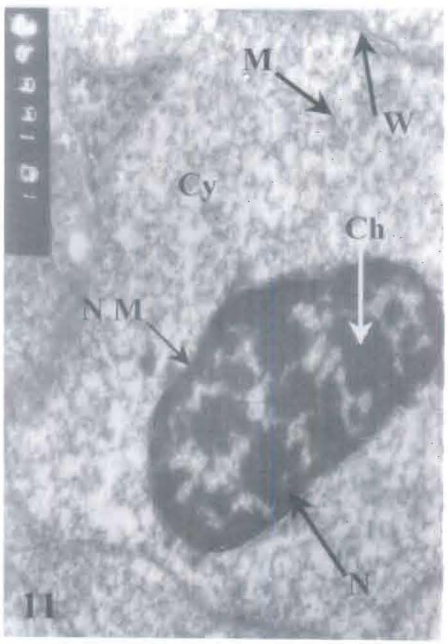
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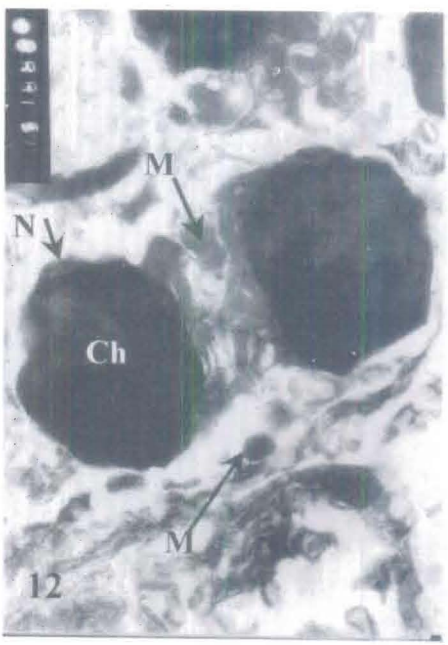
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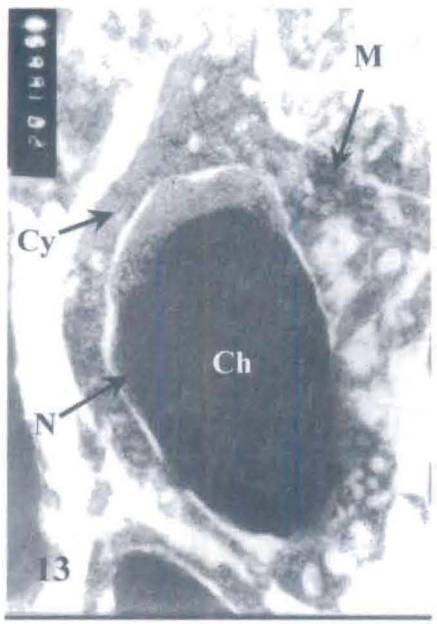
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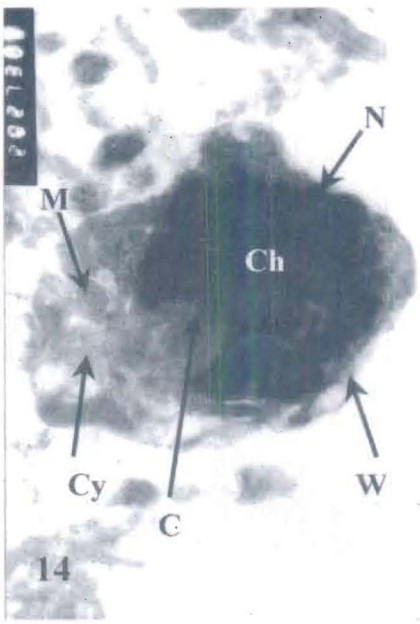
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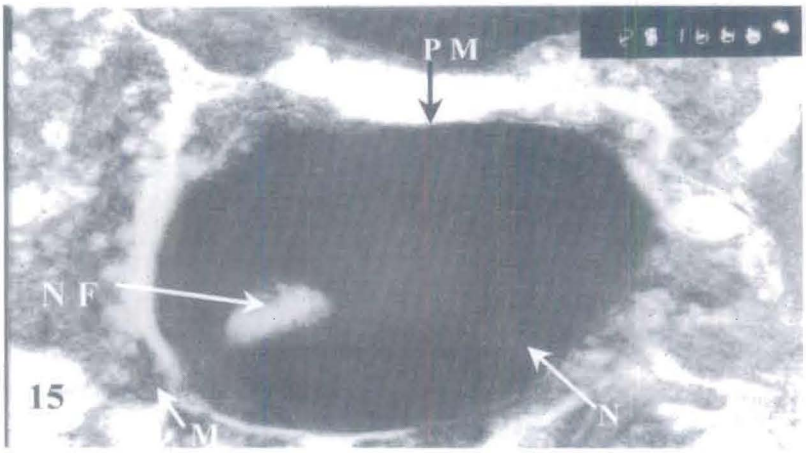
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EXPLANATION OF FIGRES

Fig. 1 : Cross section of immature testis. showing preponderance of the spermatogonia (Sg); nucleus (N); nucleolus (Nu); nest of spermatogonia (Nt Sg) and inter lobular connective tissue (C T).

Fig. 2 : Cross section of mature testis, showing the on set of active spermatogenesis (Sg) spermatogonium; (P S) primary spermatocyte; (S S) secondary spermatocyte; (St) spermatide and (C T) inter lobular connective tissue.

Fig. 3 : Cross section of nearly ripe testis, showing active spermatogenesis throughout the testis. (P S) nest primary spermatocyte; (S S) nest of secondary spermatocyte; (S Z) spermatozoa and (C T) inter lobular connective tissue.

Fig. 4 : Cross section of ripe testis, showing active spermiogenesis throughout the testis. (C T) inter lobular connective tissue; (S Z) spermatozoa and (St) spermatide.

Fig. 5 : Cross section of ripe testis, showng losses (C T) inter lobular connective tissue; A lot amount of free sperms (Sz).

Fig. 6 : Cross section of spawning testis showing reduction in size of lobules leaving space between them, decrease of the intensity of spermatozoa (Sz).

Fig. 7 : Cross section of magnified lobules at spawning showing reduction in its size and thick (C T) inter lobular connective tissue; spermatozoa (Sz).

Fig. 8 : Cross section of magnified lobules at spawning showing reduction in its size; (Sz) spermatozoa; (St) spermatide; thick (C T) inter lobular connective tissue.

Fig. 9 : Cross section of spent testis showng residual sperms and (E V) empty vesicles.

Fig. 10: Cross section of recovering testis, showing preponderance of (Sg) spermatogonium; residual of (Sz) spermatozoa and (C T) inter lobular connective tissue.

Fig. 11: Electronmicrogaph of T.S in testis of *O. spilurus*, showing the structure of primary spermatocyte cell, (N) nucleus with dens (Ch) chromatin material; (N M) nuclear membrane; (Cy) cytoplasm; (M) mitochondria and (W) cell wall.

Fig. 12: Electronmicrogaph of T.S in testis of *O. spilurus*, showing the structure of secondary spermatocyte cell, (N) nucleus; (Ch) chromatin material and (M) mitochondria.

Fig.13: Electronmicrogaph of T.S in testis of *O. spilurus*, showing the structure of secondary spermatocyte cell, (N) nucleus; (Ch) chromatin material; (M) mitochondria and (Cy) cytoplasm.

Fig. 14: Electronmicrogaph of T.S in testis of *O. spilurus*, showing the structure of spermatid cell, (N) nucleus with compact (Ch) chromatin material; (C) centriolar complex; (Cy) cytoplasm; (W) cell wall and (M) mitochondria.

Fig. 15: Electronmicrogaph of T.S in testis of *O. spilurus*, showing the structure of early stage of spermatozoa cell, (N) nucleus; (N F) nuclear fossa; (M) mitochondria and (P M) plasma membrane.

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