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OBSERVATIONS ON REPRODUCTIVE ORGANS AND TISSUES OF TWO FRESHWATER CYPRINID FISHES

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ABSTRACT
Two freshwater fish models were selected caught in river Tigris passing through Neinava province, to investigate their reproductive systems. A total of 45 of Hemri *Barbus luteus* and 32 of Ethri, *Varicorhinuus trutta* were examined during the period between January 2008 and November 2009 to study their female and male reproductive systems, from gross morphological and histological aspects .Females shown to possess a pair of elongated ovaries situated at the ventral side of the swim bladder, connected with it and other viscera by thin mesenteries. It revealed from histological studies that ovaries are coated with tunica albuginea, from which folds are protruded towards the cavity of the ovary known as oogerous lamella .Six main stages were distinguished in oogenesis, these are: 1. oogonia. 2. chromatin-nucleolus. 3. peri- nucleolar stage which is further divided into: pre-perinuclear stage, early peri-nuclear stage, later peri- nuclear stage.4. Yolk vesicles stage. 5. Yolk granules stage. 6. Ripe egg stage.The diameter of the mature ovum is: 1161.8 µm in Hemri, while in Ethri is (1215.9) µm. In these ova, yolk vesicles appeared with blue color in Mallary triple stain while yolk granules are red in color with the same stain. In addition atretic oocytes were observed in the final stage of atresia as the vesicular cells absorb the yolk, and the cytoplasm of oocytes and remaining tissue converted into corpus luteum. Males of Hemri and Ethri posses one pair of testes, which are white in color, elongated and their ventral side in Hemri possesses a ventral groove, these testes are situated in ventral side of the swim bladder and are connected with and other viscera by thin mesenteries. The testes are coated by a layer of dense connected tissue which possesses elastic fibers and blood vessels, the thickness of this layer differ by different maturation stages, inner to this layer is a testes stroma which consists of interstitial connective tissue, seminiverous tubules which are separated by fibrous partitions extended from tunica albuginea. Six stages were distinguished in the spermatogensis, these are: 1. Primary spermatogonia. 2. Secondary spermatogonia. 3. Primary spermatocytes. 4. Secondary spermatocytes. 5. Spermatids. 6. Spermatozoa.

KEY WORDS: Barbus luteus, Cyprinid fishes, Reproductive organ, Varicorhinuus trutta

INTRODUCTION

The gonad of fishes differs largely intraspecifically and interspecifically depending on many factors including morphology, anatomy and environmental conditions. Freshwater fishes in Iraq are belonging to different taxonomic groups due to their differences in their morphology and anatomy (Code, 2010). One of the pioneer studies on fish gonads was carried out by Dawood (1976) in his investigation on the freshwater fish, *Varicorhinus trutta* caught from river Tigris passing through Mosul city. He was able to distinguish 6 stages in gonad formation, trace yolk formation, and embryonic membranes surrounding the eggs. He also found that males have more weight than females. Bhatt and Al-Daham (1978) studied the male sexual cycle of *B. luteus* and they distinguished five stages in sexual maturity and they concluded that the spermation occurs in May-July. Furthermore, Al-Daham and Bahatti (1979) studied the sexual cycle of females of *B. luteus* and they distinguished five stages of the ovary development depending on size, color, and gonadosomatic index in addition to egg diameter measurement and spawining period. Study on *B. luteus* was continued by Yousif (1983) who estimated the gonad index and found the high gonad index was in March. Al-Hazza (2005) during his investigation on *B. luteus* in Euphrate river originated from Turkey found that 70% of males and 75 % of females reaches maturity in the 2nd year and all reaches maturity at the third year, and the sex ratio was (1: 1).

In Turkey, an extensive investigation was carried out in Karakaya Dam lake by Kalkam (2008), he found that *V. trutta* is the most abundant fish among cyprinids and the maximum gonad index was in May depending on morphology, size, weight of fishes examined. Furthermore, sexual maturity occurs in 2 -3 years and the reproductive period between March-July and the highest weight of gonad was in May and the highest egg diameter reached 1.04 mm and the sex ratio (1: 0.98). As it appear from above that there is no extensive gross morphological and histological on the gonad study on two selected cyprinid fishes namely *B. luteus* and *V. trutta* caught from River Tigris passing through Mosul city as such investigation was designed.

MATERIALS AND METHODS

A total 0f 45 specimens of *Barbus luteus* and 32 of *Varicorhinus trutta* were brought to the laboratory after have been caught from River Tigris passing through Mosul city. Total weight, total lengths were measured in addition to the removal of scales for age determination. After dissection testes and ovary were removed, examined gross morphologically, thin films were prepared from gonad to determine maturity. Gonads were fixed in Duboscq-Brasil or alcoholic Bouin were (see Gurr, 1962). Specimens were dehydrated, cleared in xylene, embedded in paraffin wax , sectioned at 8 – 10 microns then stained in Harris-haematoxyline-eosin(Luna, 1968), Aldehyde-fuchsin(Ewen. 1962),

Mallory triple stain(Culling *et al.* 1985), ammonical silver nitrate(Culling *et al.* 1985). Stained sections were examined and photographed using Olympus Microscope. Measurements were done using ocular micrometer.

RESULTS AND DISCUSSION

Female Reproductive System

In both *B. luteus* and *V. trutta* a pair of elongated ovaries was observed in the coelomic cavity, each ovary is connected by other viscera by a mesovarian.its length from 12 – 36.5 cm in *B luteus* and 16.5 – 34 cm in *V trutta* and the mean weight 43.17 – 704.16 g and 79.90 – 608.73 g respectively . From each ovary a short oviduct emerge, both oviducts united to open to the outside by urinogenital opening. Ovaries are yellow with a granular appearance, the ovaries occupy 2/3 of the body. The above observation is similar to the description given in the studies on cyprinid fishes (Al_Daham and Bahatti, 1979; Al-Nouri 1996; Bardakci *et al.* 2000).

The ovary *of B. luteus* is surrounded by thick germinal cuboidal epithelium while that of *V. trutta* by a thin peritoneum. Underneath this epithelium there is a connective tissue called tunica albugina which in turn surrounded by ovarian stroma at which female sex cells are embedded. (Figure 1).Similar observations were seen by Al-Daham and Bahatti (1979) and Dawood (1976). It is revealed from the sections that tunica has growth inside the ovary to form what is known as ovigerous lamellae which extend to the ovarian lumen (Fig 40), six stages of oogenesis can be distinguished, these are": 1-oogonia; 2-chromatin-nucleolus stage;3-peri-nuclear stage;4-yolk vesicle stage 5-yolk granules stage;6-ripe egg stage.

Oogonia are arranged in a single or cluster stages (Fig.2), attended to ovigerous lamella, rounded nucleus or spherical occupy most of the cell space. Oogonia were easily distinguished from primary oocytes in their shape of nucleus as oogonia have oval nucleus, single nucleolus, and chromophobic cells (Figs. 3-5). These observations coincide with those of Figueiredo *et al.* 2008. In these fishes the maturing peri-nuclear oocytes contains numerous nucleoli scattered irregularly in the nucleoplasm as growth continue and increasing of the cells gradually and after that starting the migration of the peripheral nucleoli, fusion may happen between nucleoli before formation of yolk(Figs. 6,7,8). Such migration and formation of yolk is similar to the conclusion of Al-Hamdani (1999) for the ovary of mosquito fish and those of Cakici and Uncuncu (2007) for the zebra fish, *Danio s rerio.* Furthermore, number of nucleoli in both fishes studied differs, similar differences were observed by the study of Fishelson *et al.*2003 (see Koc *et al.* 2008) who concluded that increasing of peripheral nucleoli is indication of starting of yolk formation, this means nucleoli have special role in the formation of rRNA (Al-Mokhtar *et al.* 1981). Oocytes contain a peculiar structure known as yolk nucleus (Fig.6) in both *B. luteus* and *V. trutta* when the cell passes in the peri-nuclear stage. Some scientist believes it is from nuclear or cytoplasmic origin (see Malhorata *et al.*1978). The peri-nuclear follicular cells increase in their thickness and differentiations to be arranged into two rows of follicular cells which were very close to each other forming an external layer, the theca and the inner granular layer, the granulose (Fig 9). These observations coincide with similar results obtained by Al-Hamddani (1999) in the ovary of mosquito fish.

In some ovary sections in *B. luteus* and *V. trutta* a yolk deposition rings which indicates starting of vitellogenesis and storage of yolk in oviparous fish during oogenesis. Some authors called these as yolk vesicles or lipid droplets or cortical alveoli (see Ravaglia and Maggese, 2002). It is worthy to note that the yolk vesicles were observed for the first time in cytoplasm of oocytes as vacuoles and in small number, small size in the peripheral part of cell, while yolk droplets formed inside these vacuoles leading to the formation of yolk vesicles which appeared in two fishes in 2-year old fishes, or more. These yolk vesicles increased in number and volume running parallel to the increase of oocytes (270 microns) in diameter in *B. luteus* starting yolk formation to reach 540 microns, and from 243 microns to 675 microns in *V. trutta*, then a new zone was found which granular in nature which is known as zona radiata.
The above results agree with those of Al-Daham and Bahatti (1979) in *B. luteus* and Al-Nouri (1996) in *A. marmid* and by Figueiredo *et al.*(2008) in *Thunnus obescus*. Inner to the zona radiata the yolk spheres are present which appear as red corpuscles after staining with Mallory triple. These spheres duplicates and increase in size, appear in cytoplasm between yolk follicles which appeared blue in color with same stain which accumulate in the center of oocyte and occupy ¾ of the cell. Such arrangement is due to accumulation of yolk which masks the observation of the nucleus. Also inner to zona radiata a new layer is formed known as vitelline membrane such finding can be confirmed by the previous observation of some authors (Koc *et al.* 2008). The formation of yolk sphere indicates the yolk formation is about to finish, along with disappearance of nuclear membrane and the nucleoli and primary oocytes converted to mature or ripe eggs with a diameter of 1611 microns in *B. luteus* and 1215 microns in *V. trutta* as such are ready to lay out their eggs starting from March to July. Similar finding was found by Dawood (1976) and Al-Daham and Bahatti (1979). Furthermore increase in thickness of zona radiata from 10.8 micron to 18.9 microns in both *B. luteus* and *V. trutta* respectively was observed. This is in agree with the description given by Al-Daham and Bahatti(1979)in *B. luteus* and Cakici and Ucuncu(2007) in zebra fish, and those of Kayaba *et al.* 2001(see Cakici and Ucuncu, 2007) in japanees eel.

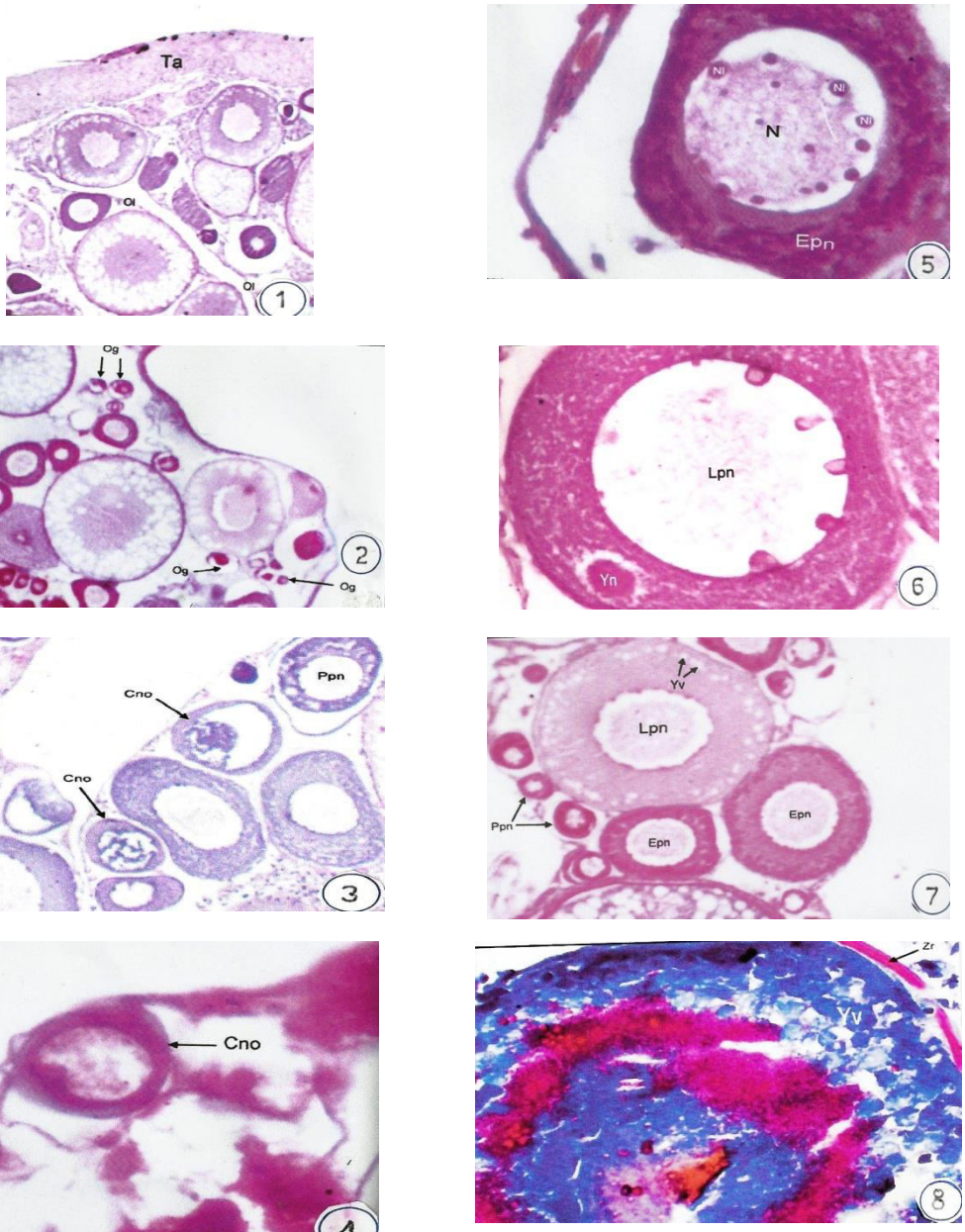
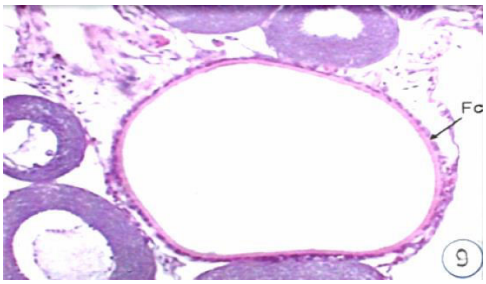
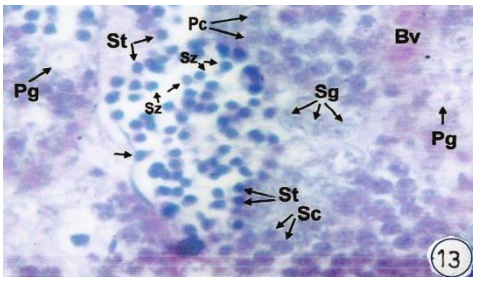
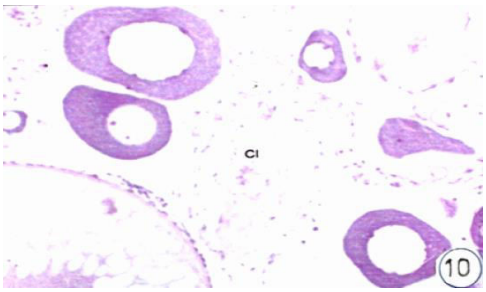
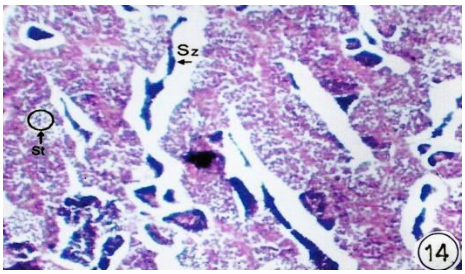


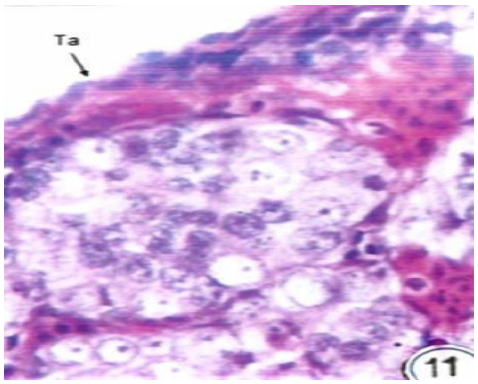
Figure -1. micrograph of a section in the ovary of *Barbus luteus* showing oogerous lamellae (OI) arises from Tunica albuginea. Heamatoxylin-eosin (H-E), X100.
Figure -2. micrograph of a section in the ovary of *B.luteus* illustrating the oogonia (Og) as single or cluster. H-E, X100.
Figure -3. micrograph of a section in the ovary of *B. luteus* showing oocyte in the chromatin-nucleolus stage (Cno) and the pre-peri-nucleolar oocyte(Ppn).H-E. X100.
Figure -4. micrograph of enlarged oocyte of *B. luteus* in the peri-nuclear stage (Cno). H-E. X 400.
Figure -5. micrograph of a section on the ovary of *Varicorhinus trutta* showing oocyte in early peri-nucleolar stage(Epn), nucleus(N), nucleoli(NI). H-E X400.
Figure -6. micrograph of a section in the ovary of *B. luteus* showing late peri-nucleolar stage (Lpn) and the yolk nucleus(Yn). H-E. X400.
Figure -7. micrograph of a section in the ovary of *B. luteus* illustrating the oocyte in pre-perinucleolar stage(Ppn) and early peri-nucleolar stage (Epn) and the late peri-nucleolar stage possessing the yolk vesicles(Yv), small cells are intensively stained while the large are faintly stained.H-E., X100.
Figure -8. micrograph of a section in the ovary of *V. trutta* showing the oocyte with yolk vesicles(Yv) which stained with blue color and zona radiate(Zr) with red color. Mallory triple stain X40.

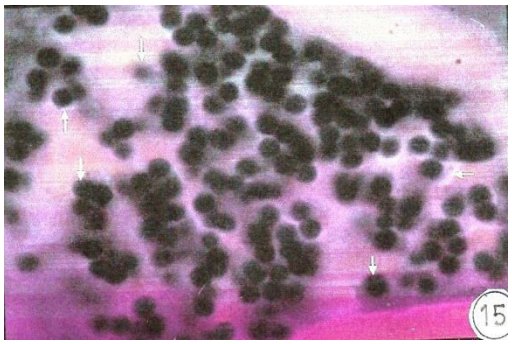












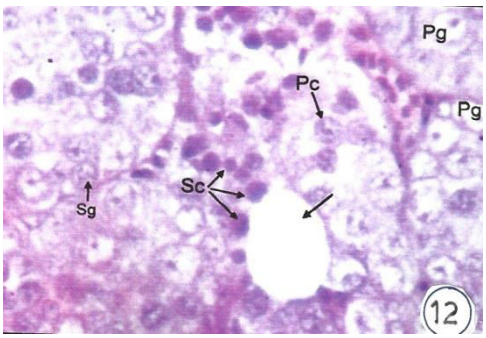


Figure -9. micrograph of a section in the ovary of mature oocyte of *V.trutta* after its expulsion showing the follicle cells (Fc) of granular layer.H-E.X100.
Figure -10. micrograph of a section in the ovary of *B. luteus* showing late stage of atresia containing a damage tissue possessing scattered cells which is corpus luteum(CI). H-E.X 40.
Figure -11. micrograph of a section in the testis of *B. luteum* showing Tunica albunogena(Ta), clear blood vessels with nucleated RBC and eosinic cytoplasm near the seminiferous tube.H-E.X400.
Figure -12. micrograph of a section in the testis of *V. trutta* showing the lumen(arrow) of seminiferous tubule, primary spermatogonias as cluster(Sg) and primary spermatocyte(Pc) and secondary spermatocyte(Sc) close to the lumen. H-E X 400.
Figure -13. micrograph of a section in the testis of *B. luteus* showing primary spermatogonia(Pg) and Secondary permatogonia (Sg) primary spermatocytes(PC) and secondary spermatocytes(Sc), spermatids(St) and the short tails(arrows) of spermatozoa, blood vessels(Bv) between seminiferous tubules. H-E. X400.
Figure -14. micrograph of a section in a testis of *B. luteus* showing elongated seminiferous tubules, permatids(St) as clusters, and the lumen inside the seminiferous tubules full of spermatozoa(Sz)/ H-E. X 100.
Figure -15. micrograph of a section in the testis of *V. trutta* showing spermetazoan and and some of the short tails(arrow). H-E.X1000

In some ovary sections atretic ovary were observed (Fig.10) such phenomena was described by some scientist (Al_Daham and Bahatti, 1979; Ravaglia and Maggese (2002) in the eel, *Synbranchus marmoratus* and Koc *et al.*(2008) in *D. rerio*.In these ova, yolk vesicles appeared with blue color in Mallary triple stain while yolk granules are red in color with the same stain. In addition atretic oocytes were observed in the final stage of atresia as the vesicular cells absorb the yolk, and the cytoplasm of oocytes and remaining tissue converted into *corpus luteum* (Fig. 10). Males of Hemri, *B. luteus* and Ethri , *V. trutta* posses one pair of testes, their lengths from 2 -8.5 and 3 -6 cm respectively , white in color, elongated and their ventral side in Hemri possesses a ventral groove, these testes are situated in ventral side of the swim bladder and are connected with and other viscera by thin mesenteries. From each testis a vas deferns emerge, both from each side unite to form sperm duct which open through urinogenital opening. The testes are slender during resting stage became flattened, occupy two third of the body cavity. The above observations are similar to those found by Bhatti and Al-Daham (1978) during their study on *B. luteus* collected from Shatt Al-Arab, Dawood (1976) when investigated the gonad of *Varicorhinus trutta,* and Suwanjarat *et al.*(2005) when studied the fish sand goby *Oxyeleotris marmoratus*, Dadzie and Abou-Seed (2004) when studied the fish, silvery croaker *Otolithes ruber.*

The testis of *B. luteus* are tubular glands surrounded by testicular wall, consists of two layers outside is a peritoneum consist of cuboidal epithelium and the inner by tunica albugenia(Fig. 11) which consist of connective tissue containing elastic fibers, some smooth muscles, fibroblasts and blood vessels. This layer is thick in the beginning of maturation becoming thin as maturation proceeded. The wall of the testis is known as testis stroma which consist of interstitial connective tissue and semineferous tubules which are rounded in shape . Similar results were obtained in *Acanthobrama marmid* by Al-Nouri (1996), and in *Otolithes rubber* by Dadzie and Abou-Seed o(2004) and in *Oligasarcus hepseutus* by Santos *et al.*(2006).Each of the seminiferous tubule is surrounded by basal thin membrane, connective tissue, fibrocytes, smooth muscles and blood vessels, and inside each tubule nest of germinal epithelium connected to the basal membrane of the tubule long cells are supporting cells and Sertoli cells and the central cavity in each tube for sperm passing (Figure 12, 13, 14) .Six stages of spermatogenesis were distinguished in both fishes , these are

1-Primary spermatogonia; 2-Secondary spermatogonia; 3-Primary spermatocytes 4-Secondary spermatocytes; 5-Spermatids;6-Spermatozoa.

The primary sperpatogonia in *B. luteus* are large in size lying in tubules they are arranged in single in *B. luteus and as* clusters in *V. trutta*, this nucleus are spherical in shape occupying most of the cell space and has basic staining ability, it has one or two nucleoli. Some differences are existing between the present fish in the primary spermatogonia and those described by Dadzie and Abou-Seedo 2004, Bhatti and Al-Daham (1978) and Al-Nouri(1996). These differences may run with morphological differences and in turn with taxonomic differences. The secondary spermatogonia are present in cluster in both *B. luteus* and *V. trutta* they are ovoid cells with clear cell membrane, nuclei stained intensely, with single nucleolus. These results are in agree with those found by Bahatti and Al-Daham (1978) for *B. luteus* and with Dawood (1976) for *V. trutta* and Al-Nouri (1996) in *A. marmid.* Primary spermatocytes are oval in shape, small 3.7 -5.4 microns with spherical nucleus, nucleoli which are stained intensively with haematoxylin. These primary spermatocytes transfer into secondary spermatocytes, each with oval irregular nucleus. Spermatids are smaller than secondary spermatocytes present as clusters near the center of tubule, they are spherical in shape and their average diameter 2.7 microns. Spermatozoa are smallest cells, occupy the lumen of seminiferous tubules, about 1.5 microns in diameter, ovoid , nucleus appear to occupy most of the cell. In some sections spermatozoa were with tails. Sertoli cells are pyramid in shape, irregular, branched inside the tubules, large in size, always lie near primary spermatogonia and contain central nuclei, irregular in shape , their diameter is about 13.51 microns with large nucleolus with intense haematoxyline stain(Figure 15). As concern interstitial cells or Leydig cells present usually in interstitial tissue between the tubules sometime in single, 13.5 – 18.9 microns in length. These cells contain large nuclei with 8.1-10.8 micrometer in diameter, they are situated between seminiferous tubule with weakly stained acid cytoplasm , oval nucleus, clear nucleolus, single or cluster near blood vessels .Similar results were obtained by Al-Nouri(1996) in A. marmid , Rutaisire (2003) in ningu, *Labeo victoninus* while Dawood (1976) no such cells were observed. The nuclei of Sertoli cells which appear as ovoid or pyramid in shape and with eosinic cytoplasm which are frequently difficult to distinguish , these cells especially in the resting stage are similar to those observed in testes of the fish tambaqui, *Colossoma macropomum* described by Nakaghi *et al.*(2003). These cells were not recognized by Bahatti and Al-Daham (1978), Dawood (1976) possibly because they examined only mature fishes in which these cells atrophied or transformed into comma shape as concluded by Nakaghi *et al.* (2003). .

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