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STEROID HORMONE IN SERUM OF MALE *MUGIL CEPHALUS* FROM LAKE QUARON IN RELATION TO ULTRASTRUCTURE OF STEROIDOGENIC SECRETING TISSUE

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ABSTRACT

The grey mullet *(Mugil cephalus)* is one of the most common fish species in the Mediterranean Sea. Morphological and histological studies on testes of this species indicate six successive maturity stages. The specific changes in serum sex hormone levels were found to occur during the different phases of gonadal development. Concentration of serum steroid hormone in male *Mugil cephalus* revealed a marked drop in serum steroid (estradiol and testosterone) hormone levels in nearly ripe stage when compared with ripe stage, while the concentration of estradiol, testosterone and progesterone at late stage (ripe stage) of sexual maturity was increased. Histological examination and cyclic change in the testes were revealed of active spermatogenesis with all generations in the early stages of testicular development. Nearly ripe testes showed more active spermatogenesis, nests of spermatocytes and small number of spermatids. While at ripe stage, the seminiferous lobules contained a fair quantity of spermatozoa with little spermatids. Ultra-structural examination showed well differentiated interlobular connective tissues which noticed at early stages. In nearly ripe stage, the fine structure revealed that early spermatids have round shaped vacuolized cells, a spherical nucleus with diffuse chromatin and without mitochondria. At this stage the interlobular connective tissue were hypertrophied, increase in thickness and filled with abnormal fibers. The abnormal shape of interlobular tissue was observed. Ultra-structurally, all the spermatozoa cells were deformed in shape, characterized by small ovoid or spherical nucleus with highly compact chromatin, surrounded by thick tissue appeared with two deformed mitochondria. In the present result, the concentration of steroid hormone in *Mugil cephalus* in Lake Quaron was highly correlated with the dysfunction of gonadal hormone, maturation of gametes and considered as good sign of infertility and shortage in secretion function of these tissues may be due to unsuitable environmental condition in Lake Quaron needed for spawning of male.

1. INTRODUCTION

Aquaculture is the most solution to the demand of fishery products but, of the major problems in aquaculture of most marine fish is lack of spontaneous spawning in captivity due primarily to the absence of appropriate environmental cues resulting in alteration of normal neuro-endocrine process, Chen (2001).

Farouk (1995) postulated that mullet species in Lake Quaron fail to undergo final oocyte maturation and ovulation or spawning when reared in captivity in Lake Quaron.

Abd- Ellah (1999) indicated that Lake Quaron doesn't receive direct Nile water, its main water source is agriculture drainage water that is loaded with sewage, fertilizers and pesticides and the balance production of sex steroid hormones and their biological activities are critical to the regulation of puberty development. One of the most locally common and highly economic fish species is grey mullet *Mugil cephalus* Linnaeus. This is

an attractive species for farming in marine, brackish and fresh water. However, the culture of this fish is mostly dependent on the availability of wild fry.

Consten *et al. (*2002) and Fahmy (2006) reported that the endocrine control can not continue without appropriate environmental cues required stimulating reproduction.

Changes in the environmental cues may cause the change in the annual percentage maturation corresponding with shifts in sex ratio and age structure in the spawning run of trout, Tilzey (1999).

Cleary *et al.* (2000) reported that any stress in environment of teleost fish may affects the endocrine and gonadal response associated with elevation in plasma cortisol levels results in decrease in plasma levels of gonadal steroid.

Steroid hormones are just one type of the many hormones that influence the reproductive process. The synthesis of male hormones occurs in lydig cell and the major androgens produced by testicular tissue varies from species to another and developmental stages but may include testosterone, 11-Ketotestosterone and androstenedione (Bourne, 1991 and Mylonas *et al.* (1998).

Plasma levels of steroid hormones (estradiol – testosterone) which were studied varied considerably from month to month especially at certain stages of both sexes as reported by Zaki *et al*. (1994) in *Liza ramada;* Assem (1995); Cornish (1998) and Sulistyo *et al.* (2000).

Seasonal changes in the concentrations of circulating sex hormones and their importance for reproduction has been reported for several species of teleosts, (Barannikova *et al.*, 2002 and Consten *et al.*, 2002).

'Mac-Kenzie and Burton (1998) discussed the influence of hypothalamus-pituitary-gland axis as feed back mechanism for controlling further follicular development. In addition Lee and Yang (2002) studied the failure of fish to produce gonadotropin releasing hormone and concluded that these may be responsible for the lack of final oocyte maturation, ovulation and spawning which also affects directly or indirectly on maturation and spawning of male fish.

Histological examination and cyclic change in the testes have been studied in many teleost fishes by Mousa *et al*. (1998); Assem (1999)*;* Moulton and Burton (1999); Santos *et al.* (2001); Consten *et al.*(2002) and Balubid (2003)

The ultra-structure of testes during spermatogenesis has been investigated in many teleost fishes by various authors such as Assem (1999) in *Caranx crysos* and Fahmy (2006) for *Chrysicthys ruppelli*. While the ultra-structure of spermatogenesis and spermatozoa were studied by many investigators as Romagosa *et al.* (1999) in *Brycon cephalus*; Quagio Grassiotto and Carvalho (2000) in *Sarubim lima* and Assem (2003) in *Pagellus erythrinus.*

In the present study, the plasma concentration of estradiol, testosterone, and progesterone were determined in relation to different maturity stages and gonadosomatic indices throughout the year in male of *Mugil cephalus*. These determinations were correlated with histological and ultra-structure examination of testes and aims to identify and clarify the distribution of the steroidogenic endocrine tissue in the tests of male.

2. MATERIAL AND METHODS

2.1. Fish sampling

Mugil cephalus males were collected throughout a year from Lake Quaron «EL-Fayum Governorate». The collected specimens ranged in total length from 23 to 42 cm and body weight varied between 430 and 970 gm. Sex and maturity stages were identified by three methods as follows:

1- Gonadosomatic index (GSI).
2- Percentage of spermatogenesis.
3- Histological and ultra-structure studies of testes.

2.2. Hormonal assay

Blood sampling were collected from caudal vein. The analysis was based on the (RIA) procedure. Testo-CT2 and Pantex progesterone I 125 Kits number 335 and 337 were used to measure the level of testosterone and progesterone hormones respectively.

Coat A- count estradiol catalog number TKE21 was also used for the quantitative essay of estradiol in serum.

2.3. Histological and fine structure examination

The fixed testes were washed, dehydrated cleared and embedded in paraffin wax. Four small blocks (2mm x 2mm) of testes specimens were fixed overnight at 4°C in 4% buffered glutaraldihyde and then in 1 % osmium tetraoxide for one hour at room temperature, rinsed twice in cacodylate buffer, dehydrated through a graded ethanol series, cleared in propylene oxide and embedded in polarbed 812 (polaron) epoxy resin. Ultra-thin sections of one micron thick were prepared using glass and diamond knives, and stained, with uranyl acetate and lead citrate. Sections were examined using a transmission electron microscope.

2.4. Statistical analysis

All data were statistically analyzed by Microsoft window (2000) Excel program. The correlation coefficient and significance of data were calculated.

3. RESULTS AND DISCUSSION

In the present study, the determinations of the plasma sex steroid levels, GSI and histological analysis of the testes were employed to describe the sexual cycle and spawning season of males of the *Mugil cephalus* in Lake Quaron. A scale of maturity stages was taken in consideration into account the scales of both (Zaki *et a1.,* 1995) and (EL-Gharabawy, 1996) as indicated in table (1).

3.1. Determination of sex steroid hormones in serum of male *Mugil cephalus*

3.1.1. Estradiol concentration in serum of male Mugil cephalus

The results revealed that the concentration of serum estradiol level in immature stage ranged between 1700 to 1250 with high average level at (1550 ±180 pg/ml) for immature male at $P < 0.01$, when the minimum GSI value was recorded in immature male (0.036). Slight decrease was noticed in average serum estradiol level for maturing male to reach (1200 ± 178 pg/ml) until the average GSI value was reached (0.209).

Although, the average GSI value was increased to reach (0.381), a sharp decrease in the average circulating estradiol value was recorded in serum of the nearly ripe male reached (6.9 ± 3.9 pg/ml). Schulz and Miura (2002) explained that estradiol hormone was associated with testicular development. The maximum average GSI value was recorded as a high average value in the ripe male (1.954), when a sharp increase was noticed in the circulating estradiol value at (918 ±142 pg/ml), which varied between 1260 pg/ml and 730 pg/ml as a maximum and minimum estradiol values, respectively.

Table (1): The spermatogenic cycle of male *Mugil cephalus* in Lake Quaron according to gonadosmatic indices (GSI) and spermatogenic distribution through the period from January to December 2003:- (Scales of Zaki *et al.*, 1995 and El-Gharabawy,1996).

Stages of maturity	Morphology and Duration	Average (GSI)	spermatogenic distribution
1- Immature	Small in size, Pinkish in color, detected throughout the year.	0.063 ± 0.032	Spermatogonia dominate.
2- Mature	Testes increase in size, Pinkish in color, detected throughout the year.	0.209 ± 0.145	Spermatogonia and Spermatocytes were detected.
3- Nearly- ripe	Testes are whitish in color, detected from March to May.	0.381 ± 0.005	Large no. of Spermatids, spermatozoa were detected.
4- Ripe	Testes reach their maximum development, white in color, detected from late June to September.	1.954 ± 0.301	Small no. of Spermatids, & large no. of spermatozoa were detected.
5- Resorbed	Testes began to stretch & shrunken with decrease in size & weight detected from August to November.	0.237 ± 0.073	New generation of spermatogonia & traped spermatozoa.
6- Spent	Testes reduced in size, detected from December 2003 to March.	0.094 ± 0.032	New generation of spermatogonia and empty lobules.

In the present study, there is no role of estradiol hormone in the ripe male, so this elevation may be disrupts a spawning events and showed a degenerative changes in the structure of the testes and may affects negatively of its function. Estradiol profiles do not play any role in the spermatogenesis and the main function of estradiol hormone in immature male only as postulated by Cavaco *et al.* (2001) who concluded that aromatizable androgens (estradiol) play a key role in stimulating gonadotroph activity in immature male fish and in inhibiting the stimulatory effect of 11- Ketotestosterone (KT) on fish spermatogenesis.

Sharp decrease was recorded in the average concentration of estradiol for male *Mugil cephalus* at resorbed and spent stage to reach 11.8 ± 6.2 pg/ml and 2.7 ± 1.6 pg/ml respectively as indicated in table (2) and Figure (1).

3.1.2. Testosterone hormone in serum of male Mugil cephalus:

The present work revealed that level of average circulating serum testosterone hormone was increased gradually during the early stage.

In the immature male *Mugil cephalus* the average circulating serum testosterone was about 5.5 ± 0.9 ng/ml. At this stage the gonadosomatic index was recorded the lowest value 0.063. The circulating testosterone hormone in maturing male *Mugil cephalus* was varied between l7.4 ng/ml maximally and 10.3 ng/ml minimally, with an average value about 15.5 ±1.3 ng/ml and the GSI increased to 0.209, as indicated in table (3) and figure (2).

These results were agree with some authors as Prat *et al*. (1999) and Barannikova *et al.* (2002).Those authors were postulated that the level of testosterone hormone plays an important role during the initial stages of testicular development.

Although, the average GSI value was increased to reach (0.381) at nearly ripe male the average circulating testosterone value decreased to minimum level (0.8 ±0.6 ng/ml).

This sharp decrease in testosterone level recorded during nearly ripe stage may reflect corresponding decline in the reproductive activity and deformation of the spermiation process, may due to unsuitable of environmental condition in Lake Quaron needed for spawning of male. Wen and Lin (2001) and Tollefsen *et al*. (2002) explained that the reproduction is closely related to environment conditions which directly act on gametogenesis and spawning. Any disrupt in testosterone levels affects male sperm producton and fertility, Brinster (2002).

El-Boray (1997) reported that the plasma testosterone in *Rhabdosargus haffara* is increased through immature and developing maturing stages and decreased again during mature, ripe, spawning and spent stages. While Matsuyama *et al*. (1991) observed that the serum testosterone level was relatively low during spermatogenesis rose markedly around the time of spermiation and become low after spawning and during immature period.

Agree with the present result some authors as (Zaki *et al*., 1995 and Cornish, 1998) revealed that the concentration of estradiol, and testosterone hormones were decreased in post-mature fish.

At ripe male the maximum average GSI value was recorded (1.954) and highly significant increase (at $P < 0.01$) in the average serum testosterone hormone was observed (260 ± 39 ng/ml) as indicated in table (3) and figure (2).

It is known that the role of sex steroids in controlling the maturation cycle in teleosts especially during spawning times is altered by environmental or hormonal manipulation, and this has both theoretical and practical relevance (Flammarion, 2000).

In contrast with the present results El-Halfawy *et al.* (2007) reported that plasma testosterone content in grey mullet, *Liza ramada* was accompanied by a marked

increase with GSI during spermatogenic period.

Scott *et al.* (1980) and Fostier *et al.* (1983) concluded that in male teleosts growth and development of the testis was always associated with rising plasma levels of testosterone and 11- Ketotestosterone.

Amiri *et al*. (1995) pointed that as spermatogenesis progressed, during rapid spermatogenesis (stage III), the levels of both gonadotropin and testosterone were increased and a high level of testosterone was recorded during ripe stage while the level of serum gonadotropin was low due to the action of feed back mechanism control.

While Rodriguez *et al.* (2001) stated that testosterone and 11- ketotestosterone levels displayed the highest values during the spermiation period (Ripe & spawning). In agreement with the present results Guerriero *et al*. (2005) reported that the peaks of the plasma androgen levels presented in the early stage and during the spawning phase.

At resorbed and spent stage the average GSI value was about 0.237 and 0.094 respectively and the average concentration of testosterone value were about 2.5 $\pm$ 1.2 ng/ml and 17.5±1.7 ng/ml respectively.

In addition to being used as a precursor, testosterone may be important in regulating gonadotropin secretion through the feedback mechanism and as reported in the present study, levels of testosterone and estradiol dropped during resorbed stage may be due to the influence of hypothalamus-pituitary-gonad axis (pituitary control by feed back mechanism), for controlling further gonadal development, Cavaco *et al.* (2001) and Robberts *et al.* (2001).

Many authors detected minimum testosterone value at spent stage in normal conditions as Zaki *et al.* (1994) for *Liza ramada*; Zaki *et al.* (1995) for *Mugil cephalus* and Cornish *et al.* (1998) for *Oreochromis mossambicus.* These results are in disagreement with the testosterone level serum of spent male in Lake Quaron that may be due to unsuitable of environmental condition that needed for spawning of male.

Abd- Ellah (1999) indicated that Lake Quaron doesn't receive direct Nile water, its main water source is agriculture drainage water that is loaded with sewage, fertilizers and pesticides and the balance production of sex steroid hormones and their biological activities are critical to the regulation of puberty development.

3.1.3. Progesterone hormone in male Mugil cephalus

The maximum average GSI value was recorded in the ripe male (1.954), while the minimum value was noticed in immature male (0.063). The average circulating progesterone hormone varied between 0.035 ± 0.07 ng/ml for immature male and 0.013 ± 0.03 ng/ml, for maturing male. In nearly ripe male the level of progesterone hormone was increase in the average concentration until reached 0.15 ± 0.08 ng/ml. Highly significant increase in the average concentration of progesterone hormone reached to 1.81 ±0.13 ng/ml at ripening period ($p<0.01$), when the GSI value also reached the maximal at 1.9. Sharp decline in progesterone hormone was recorded at resorbed (0.021 ± 0.03 ng/ml) and spent (0.019 ± 0.06 ng/ml) male *Mugil cephalus* as indicated in table (4) & Figure (3), that correlated with the decrease in GSI value gradually until reached the minimum value (0.094) at spent.

In male *Mugil cephalus,* an increase in level of progesterone hormone was recorded at nearly ripe stage and ripe stage as the reported results by Abdo (1996); this increase may indicate the dysfunction of testes.

This result was disagreed with the progesterone level in EL-Boray (1997), who postulated that a gradual increase in the concentration of plasma progesterone was observed during the advanced maturing stage but it decreased in the ripe stage.

EL-Gharabawy *et al.* (1994) in *Mugil seheli* observed that plasma progesterone level was at maximum value in the ripe stage.

In the present study *Mugil cephalus* in Lake Quaron, fail to undergo final maturation

and spawning may be due to the impairment of environmental conditions.

Cleary *et al.* (2000) reported that any stress in environment of teleost fish may affect the endocrine and gonadal response associated with elevation in plasma cortisol levels results in decrease in plasma levels of gonadal steroid.

In addition Lee and Yang (2002) indicated that failure of fish to release gonadotropin releasing hormone may be responsible for the lack of final testicular maturation, and spawning.

Seasonal cycle of plasma testosteron have been correlated with testicular development (Mac-Kenzie and Burton,1998) in *Ictalurus punctatus* and act as the levels of the pituitary to regulate gonadotropin hormone secretion, Yeu *et al*. (2002) and Rodolfo *et al*. (2003)

The present analysis showed a marked drop in serum steroid hormone levels in nearly ripe stage when compared with ripe stage. These declines in the steroid secretion may be decrease the negative feedback in the pituitary and allow the elevation of the gonadotropin level in plasma, thus preventing the synthesis and accumulation of gonadotropin in the pituitary which is necessary of completion of fully development gonad, (Khan, *et al*. (1999) and Rodolfo *et al*. (2003).

It appears that the balance production of sex steroid hormones and the control of their biological activities are critical to the regulation of puberty development of fish.

Table (2): Relationship between concentration of Estradiol (pg/ml) and GSI (%) for male *M. cephalus* in Lake Quaron at all stages of maturity.

Stages of maturity	No. of fish	Average GSI (%)	Concentration of serum estradiol (pg/ml)		
			Max.	Min.	Average ± S.D.
Immuture	3	0.063	1700	1250	1550±180**
Mature	4	0.209	1445	1013	1200±178
Nearly Ripe	4	0.381	10.3	2.5	6.9±3.9
Ripe	3	1.954	1260	730	918±142
Resorped	5	0.237	19.4	8.7	11.8±6.2
Spent	6	0.094	5.3	1.8	2.7±1.6

**Highly significant (P< 0.01)

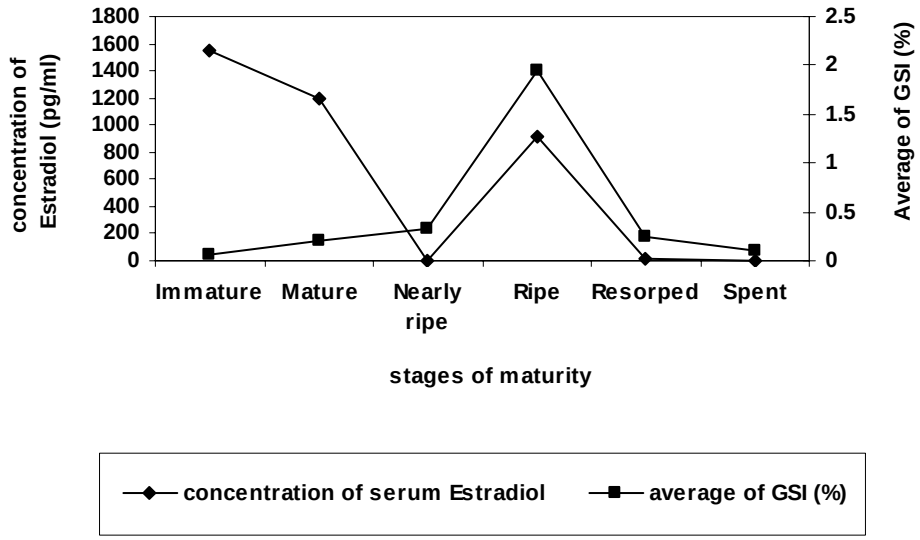

Fig. (1): Relationship between concentration of serum Estradiol (pg/ml) and GSI (%) for male *M. cephalus* in Lake Quaron at all stages of maturity.

Table (3): Relationship between concentration of serum Testosterone (ng/ml) and gonadosamatic index (%) for male *M. cephalus* in Lake Quaron at all stages of maturity.

Stages of maturity	No. of fish	Average GSI (%)	Concentration of serum testosterone (ng/ml)		
			Max.	Min.	Average ± SD
Immature	3	0.063	6.7	3.2	5.52±0.9
Maturing	4	0.209	17.4	10.3	15.52±1.3
Nearly ripe	4	0.381	1.5	0.2	0.82±0.6
Ripe	3	1.954	318	210	260±39**
Resorbed	5	0.237	3.9	1.1	2.52±1.2
Spent	6	0.094	19.3	11.4	17.52±1.7

** Highly significant ($p < 0.01$).

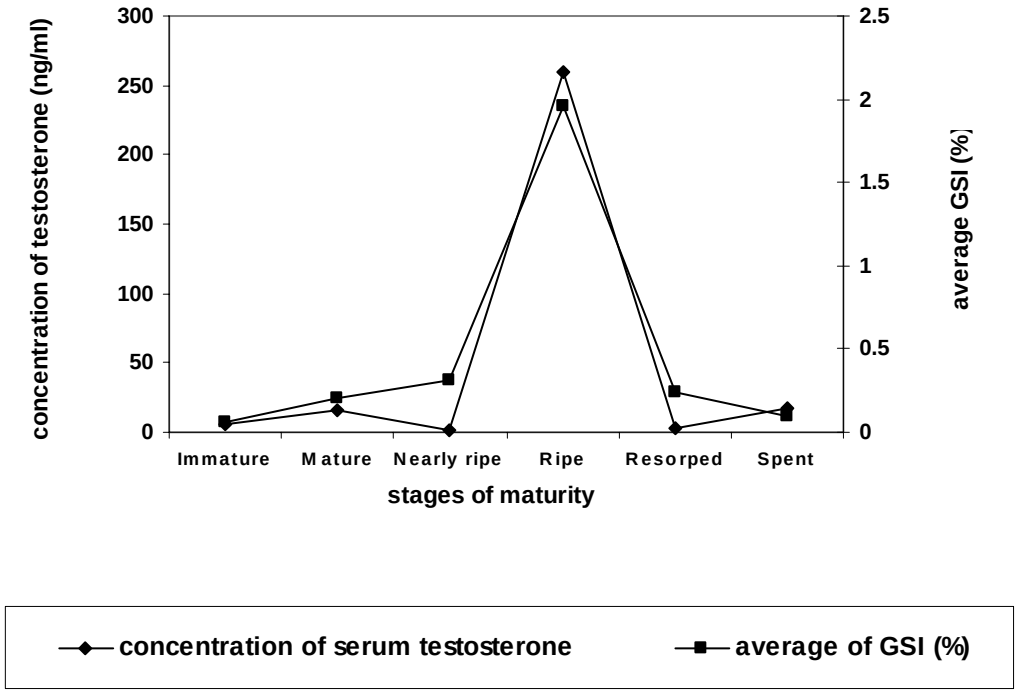

Fig. (2): Relationship between concentration of serum Testosterone (ng/ml) and GSI (%) for male *M. cephalus* in Lake Quaron at all stages of maturity

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Table (4): Relationship between concentration of progesterone (ng/ml) and GSI (%) for male *M. cephalus* in Lake Quaron at all stages of maturity.

Stages of maturity	No. of fish	Average GSI (%)	Concentration of serum progesterone (ng/ml)		
			Max.	Min.	average + SD
Immature	3	0.063	0.125	0.003	0.0352=0.07
Maturing	4	0.209	0.111	0.002	0.0132=0.03
N. Ripe	4	0.381	0.23	0.07	0.152=0.08
Ripe	3	1.954	2.21	1.21	1.812=0.13**
Resorbed	5	0.237	0.121	0.005	0.0212=0.03
Spent	6	0.094	0.113	0.008	0.0192=0.06

** Highly significant (P< 0.01).

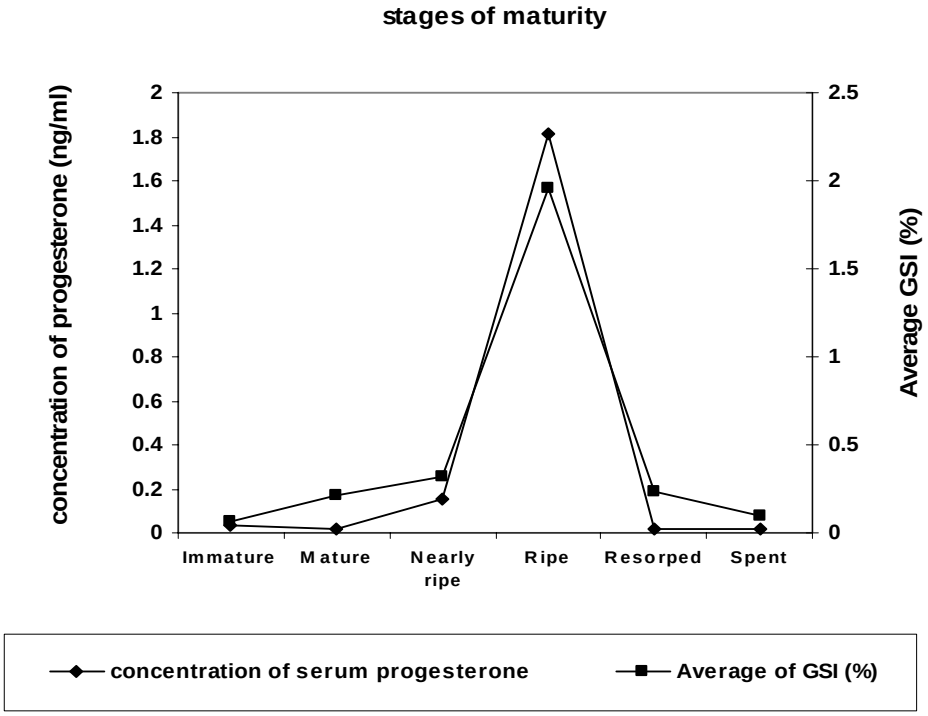


Fig. (3): Relationship between concentration of serum Progesterone (ng/ml) and GSI (%) for male *M. cephalus* in Lake Quaron at all stages of maturity.

3.2. Histological and ultra-structural studies in testes of male *Mugil cephalus*

Immature testes consisted mainly of nests of spermatogonia (Fig. 4) ranged in diameter from 8µm to 12 µm, and has spherical nucleus varying from 4µm to 5µm in diameter.

While ultra-structurally revealed the abnormal small spermatogonia (Fig. 5) can be distinguished by their conspicuous boundary and nuclear outline. A darkly stained nucleolus was shown in the central zone of the nucleus. The chromatin material is arranged on the periphery of the nuclear membrane. Well differentiated interlobular connective tissues were noticed between the lobules. Fine structure of immature testes revealed presence of large number of sertoli and lydig cells (Fig. 6) immersed between mitochondria. In the present study, the main functions of interstitial cells are synthesis of steroid hormone which carried out in steps as postulated by Hines *et al.* (1999).

Maturing testes marked the beginning of active spermatogenesis with all generations. In spermatocytes neither nucleoli nor the cell membrane could be clearly differentiated. These cells were distinguished by their diameters and their comparatively little cytoplasm, the primary spermatocytes measured 6µm in diameter and the secondary spermatocytes were about 3µm in diameter, (Fig. 7).

Ultra-structurally, spermatocytes are characterized by complex nuclear structure. These cells were oval and have voluminous nucleus with thick clumps of centrally located chromatin (as irregular strands) and little chromatin at the nuclear periphery. The cytoplasm was densely occupied by mitochondria.(Fig. 8).The investigations for spermatogonia and spermatocytes are similar as reported by other fishes *Parasilurus aristototelis* (Liadou and Fishelsen,1995) *Mugil cephalus* (Mousa *et al*.,1998), *Pagellus erythrirus* (Assem, 2003) and *Chrysicthys ruppelli* (Fahmy, 2006).

The nearly ripe testes showed more active spermatogenesis, nest of spermatocytes, spermatids and few number of spermatozoa (Fig. 9a).The interlobular connective tissue were hypertrophied, increase in thickness and filled with abnormal fibers. Magnification of interlobular tissue by ultra structure revealed the presence of elongated cells rich in chromatin material intermingled with vacuoles and collagenous tissue in abnormal shape, (Fig. 9b).

In the present study the abnormal shape of interlobular tissue in *Mugil cephalus* was considered as good sign of infertility and shortage in function of these tissue as reported by Hines *et al.* (1999) and El-Zaeem and Assem (2004).These authors postulated that the interstitial cells of teleost fish are involved in steroid synthesis which are carried out in steps. The hypertrophied of interlobular connective tissue was considered as an evidence of shortage in secretion of steroid hormones as reported by Sulistyo *et al.* (2000). In the present study the early spermatids have round shaped vacuolized cells, a spherical nucleus with diffuse chromatin and without mitochondria.

At nearly ripe stage the results showed all generations of spermatogenesis,(Fig. 9c). Spermatocytes are characterized by voluminous nucleus and have complex thick clumps of centrally located chromatin material, (Fig. 9d).

During spermiogenesis, the meiotic products round spermatids are converted into sperm through drastic morphological changes including the package of chromosomes into a condensed sperm head, formation of a flagellar tail, and extrusion of most cytoplasm as the residual body, Hong, *et al.*(2004)

At ripe stage, the seminiferous lobules contained a fair quantity of spermatozoa with little spermatids, (Fig. 10a).The fine structure of spermatids were characterized by nucleus with dense and irregular strands of chromatin, attached to the inner nuclear membrane, (Fig. 10b) more or less migrated to the one side of the nucleus. The chromatin material migrates and become eccentrally placed in an area that

corresponds to the anterior region of spermatid. The basal part of the nucleus forms a depression to which the proximal centrals migrate with deformed dark irregular strands of chromatin and mitochondria in large number are present in the cytoplasm, (Fig. 10c).

The spermiogenesis process was not completed and the metamorphosis of spermatid did not occur. All the spermatozoa cells were deformed in shape, characterized by small ovoid or spherical nucleus. The sperm was surrounded by thick tissue appeared with two deformed mitochondria, (Fig. 11a). The majority of sperms were free from mitochondria, which appeared as vacuolated bullet shape of chromatin material (Fig. 11b). In the present study the abnormal function of spermatozoa was considered as a result of abnormal shape of interlobular connective tissue, which causes shortage in secretion of gonadal hormones as reported by Cochran (1992) who postulated that the primary function of interlobular cells is to produce steroids needed for gametogenesis and expression of secondary sex characteristics. In the present result the concentration of steroid hormone in *Mugil cephalus* in Lake Quaron was highly correlated with the dysfunction of gonadal hormone and maturation of gametes. Brinster (2002) explained that the infertility of fish may be depending on dysfunction of sperm production.

All these results indicate that, the male of *Mugil cephalus* in Lake Quaron is infertile.

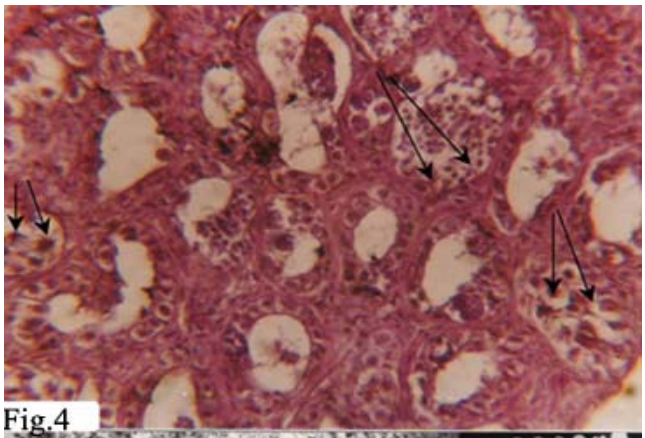


Fig.4

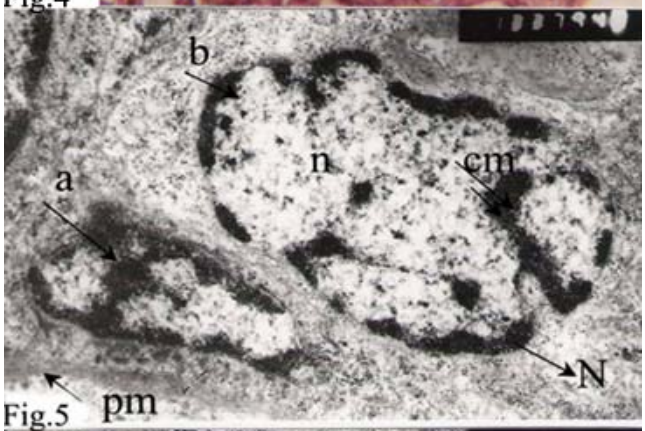


Fig.5

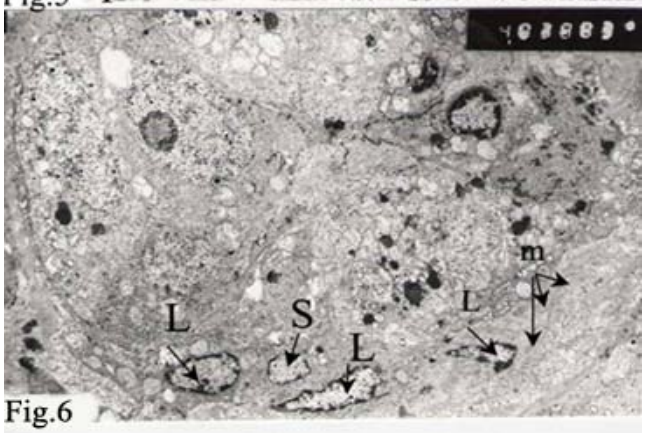
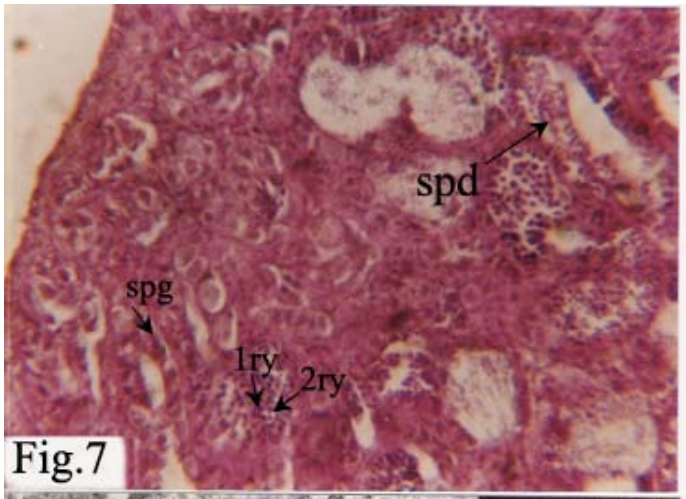


Fig.6



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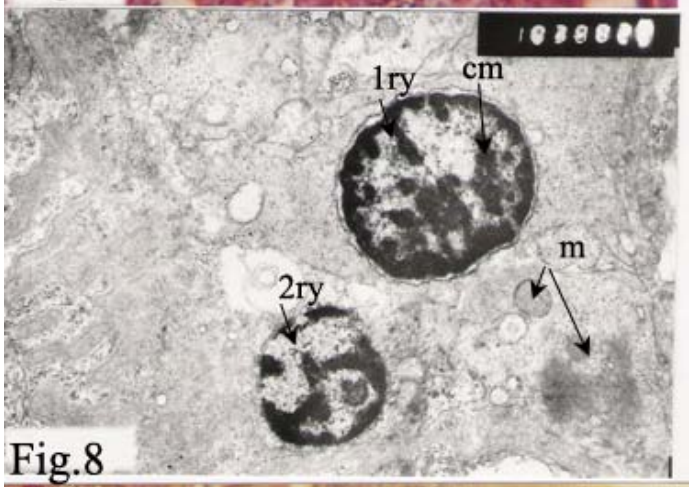

Fig.8

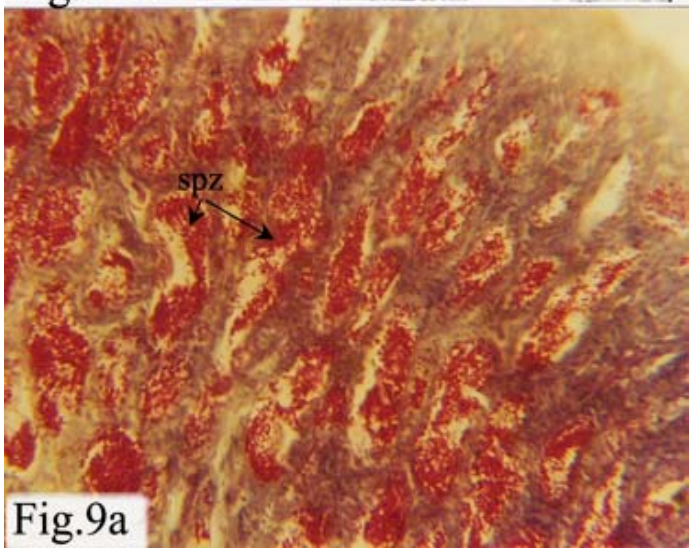

Fig.9a

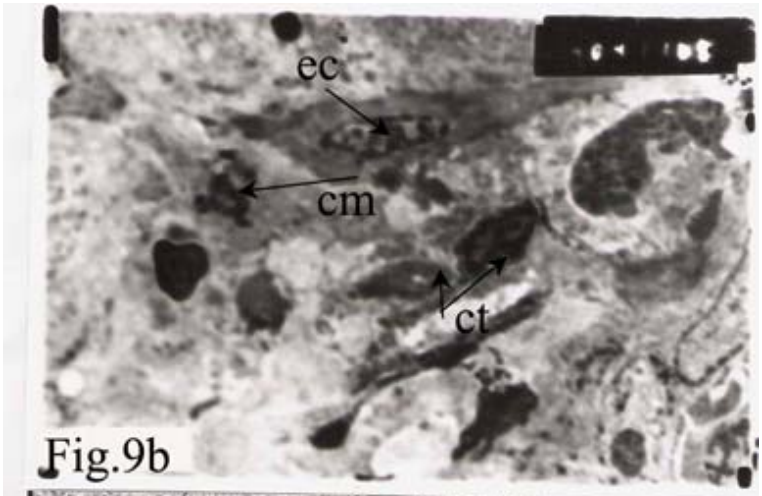

Fig.9b

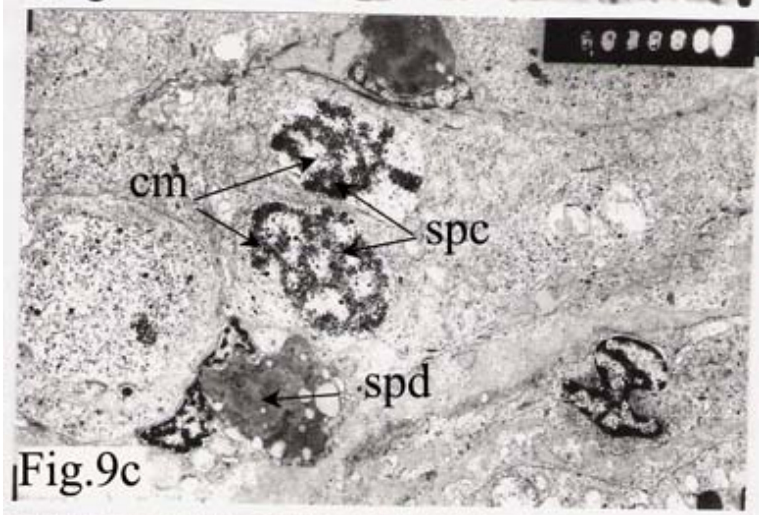

Fig.9c

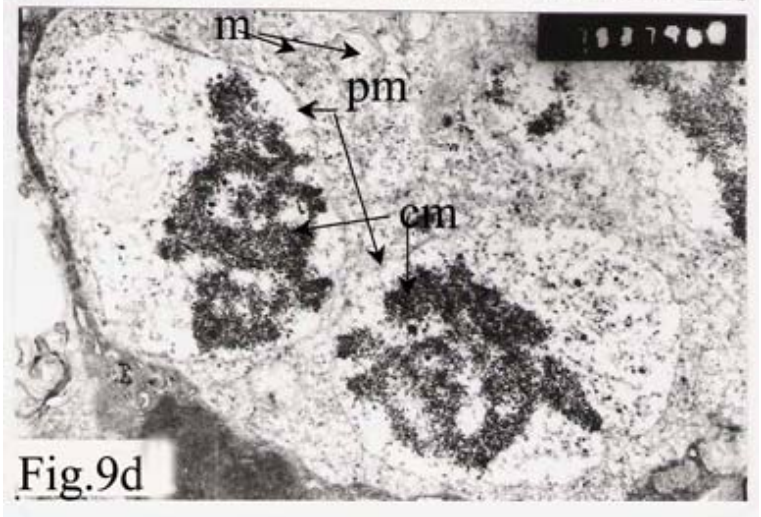

Fig.9d

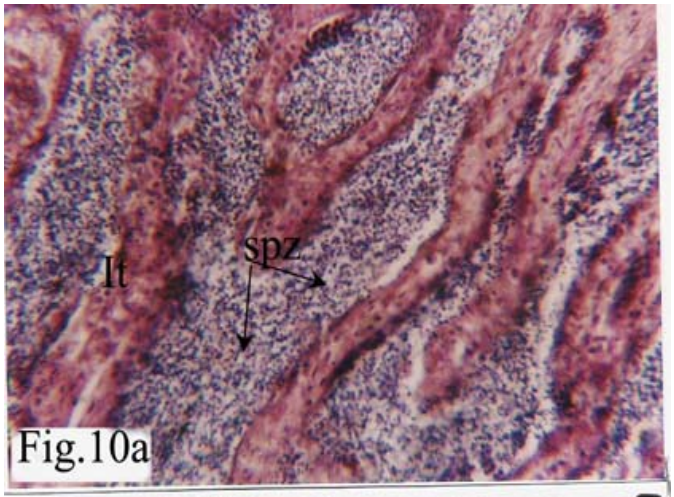

Fig.10a

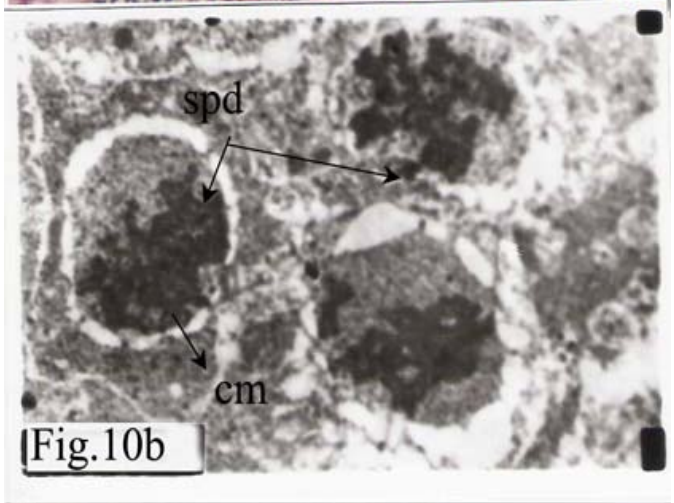

Fig.10b

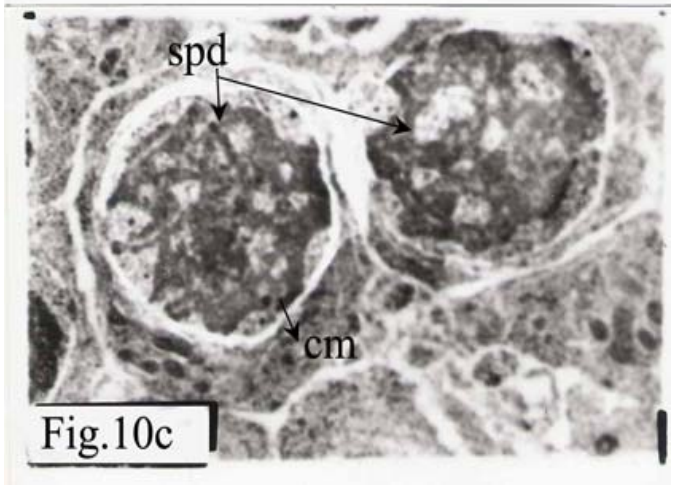

Fig.10c

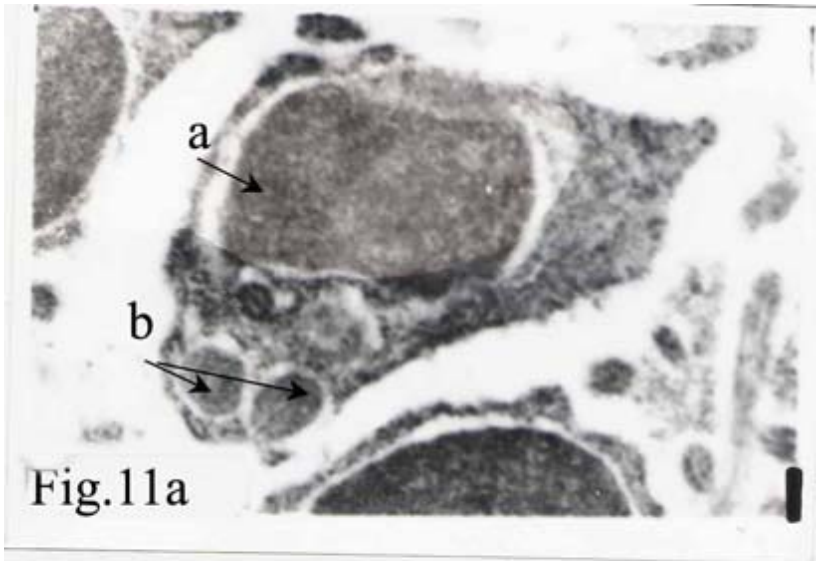

Fig.11a

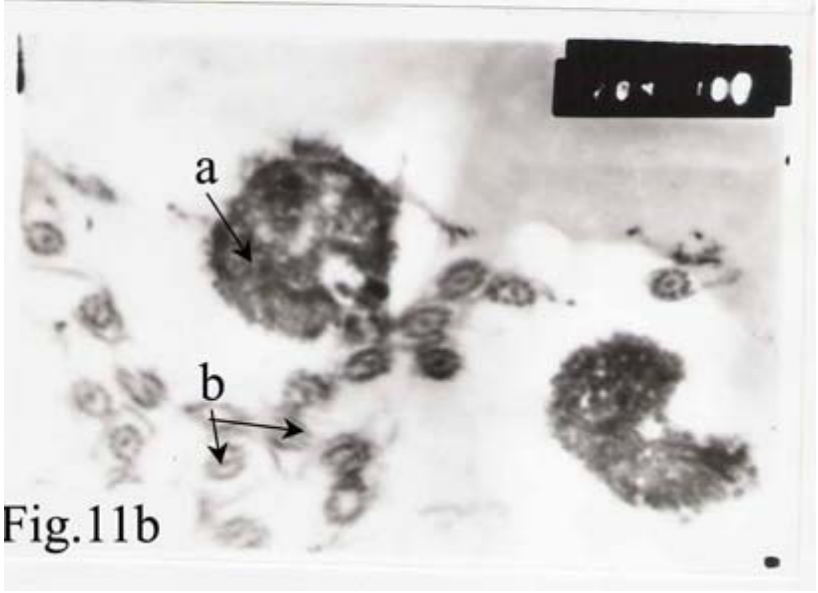

Fig.11b

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EXPLANATION OF FIGURES

Fig. (1-3): Relation between gonadosomatic indexes (GSI) and concentration of serum 1- Estradiol (pg/ml); 2- Testostrone (ng/ml) and Progestrone (ng/ml) for male *Mugil cephalus* in Lake Quaron at all stages of maturity.
Fig. (4): Photomicrograph of a cross section (CS) in testes of *Mugil cephalus* at immature testes showing, spermatogonia (arrows). (H & E) x400.
Fig. (5): Electron micrograph of (CS) in spermatogonia cells showing a-small and b- large spermatogonia, chromatin material (cm), plasma membrane (pm),nucleus(N) and nucleolus (n). x 10000.
Fig. (6): Electron micrograph of (CS) in immature testes showing large number of Sertoli (S) and lydig cells (L) immersed between mitochondria (m).x 4000.
Fig. (7): Photomicrograph of a cross section (CS) in maturing testes showing: spermatogonia (Spg),primary (1ry) and secondary (2ry) spermatocyte, few nests of spermatids (spd). (H & E) x400.
Fig. (8): Electron micrograph of (CS) in primary (1ry) and secondary (2ry) spermatocyte showing chromatin material (cm), and mitochondria (m).13000.
Fig. (9a): Photomicrograph of a cross section (CS) in nearly ripe testes showing, active spermatognesis in all stages of development, spermatozoa (spz) Azan stain , x 250
Fig. (9b): Electron micrograph of (CS) in interlobular tissue showing; elongated cells (Ec), chromatin material (cm), Vacuoles and collagenous tissue (ct) x10000.
Fig. (9c): Electron micrograph of (CS) in nearly ripe testes showing, spermatocyte (spc), chromatin material (cm), spermatids (spd). x 7500
Fig. (9d): Magnification of spermatocyte cells showing chromatin material (cm), plasma membrane (pm), and mitochondria (m). x 13000
Fig. (10a): Photomicrograph of a (CS) in ripe testes showing: a fair quantity of spermatozoa (Spd) and abnormal shape of interlobular connective tissue (It).(H&E) x250.
Fig. (10 b&c): Electron micrograph of (CS) in early (b) and late (c) spermatids (Spd) and chromatin material (cm).
Fig. (11a): Electron micrograph of (CS) in sperm surrounded by thick tissue, showing abnormal shape of spermatozoa (a), with two deformed mitochondria (b). X13000
Fig. (11b): Electron micrograph of (CS) in sperm showing a numbers of vacuolated bullet sperm free from mitochondria (a) and (T.S.) of sperm's tail (b).X10000