# Histological Studies on the Gonads of the Catfish during Different Seasons

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## ABSTRACT

Eighty mature catfish of both sexes were used to study the effect of different seasons on the histological and histochemical structure of the gonads. The results showed that winter season was considered as a resting season of the gonad of the catfish where the testes showed degeneration except intact spermatogonia and the ovaries showed predominance of previtellogenic stage. Both of spring and summer seasons were considered as the spawning (breeding) season of the catfish where the testicular lobules became distended with the different developmental stages with the appearance of spermatozoa while the ovaries showed predominance of vitellogenic and postvitellogenic stage with the appearance of mature follicles. Autumn season was considered as the postspawning season where the testes showed some empty or spent testicular lobules while the ovaries had predominance of the atretic follicles which indicates the spawned fish.

### INTRODUCTION

Fish is not only used for human consumption, but also used as a good source of animal meal; provide it with cheap and high quality protein **Anderson and Mitchum (1974**). Catfish is considered as the cheapest source of high quality protein and rich in calcium, phoshpate, iodine and vitamins **Dadzie and Wangila (1980**).

The gonads are the greater functional significance than their name implies and they were investigated by several workers in the different fish species. Yoakim (1971) in Syndontus sachall, El-Saadny et al, (1991), Khallaf et al, (1991) and Gaber (2000) in Bagrus bayad and Bagrus domac, Dougbag et al., (1988a), Mousa (1998), El-Gohary (2001) and Abd El-Hafez et al., (2007) in Nile tilapia, Oreochromis niloticus and Mousa and Mousa (1998) in mullet, Mugil cephalus. In catfish (Clarias), Zaki et al. (1986a) and Ismail (1992) gave concise information about the gonads. Therefore, it is thought that a detailed study of the gonads of the catfish and their seasonal changes would be very useful for both scientist and who concerning with aquaculture development.

### MATERIALS AND METHODS

A total of 80 sexually mature catfish of both sex with standard length over 35 cm according to **Dowidar et al.**, (1985) were collected alive from El-Sahel fish market in Cairo Province, at the different seasons of the year during the period from January 2007 till October 2008. The catfishes were transported alive to the Histology and Cytology Department. The males were distinguished from the females by the examination of the urogenital area; the male fish had well developed urogenital papilla. For each catfish, body weight to nearest gram was recorded, then immediately dissected and the fish belly was opened to obtain the gonads. The gonads were removed rapidly, weighted to the nearest gram and the middle part of the gonads was fixed in Bouin's fluid, Helly's fluid, Carnoy's fluid. Transverse sections were made at 5  $\mu$  thick. The sections were stained with Haematoxylin and Eosin, Crossman's trichrome stain, Gomeri's reticulin method, Orcein technique, Toluidine blue and Periodic acid Schiff (PAS). The fixatives and staining methods were used as outlined by Crossmon (1937) and Bancroft, Cook, Stirling and Turner (1994).

### The gonado-somatic index (GSI):-

The GSI was used for following up the seasonal variations in the gonads weight as related to the body weight of the fish (to nearest gram) by the formula:

Gonad weight GSI = \_\_\_\_\_ X 100 Han (1978)

Fish body weight

#### RESULTS I- THE TESTES

The testis of the catfish was surrounded by tunica albuginea which consisted of dense collagenous connective tissue (Fig. 1) with few fine elastic fibers (Fig. 2) and also contained smooth muscle cells (Fig. 3). This testicular capsule gave connective tissue septa which divided the testis into large numbers of testicular lobules. These lobules were varying in their shapes and sizes and they were surrounded by interstitium (Fig. 1).

A- The Testicular Lobules

The testicular lobules were surrounded by reticular fibers (Fig.4), collagen fibers and myoid cells appeared as elongated fusiform cells around the lobules (Fig.5). The spermatogenic cells were arranged within these lobules in form of germinal (spermatogenic) cysts. The same lobule contained many different germinal cysts but, each cyst had the same spermatogenic stage (Fig.1).

#### B- The Interstitium

The interstitium filled the interlobular spaces and composed mainly of fine collagnous connective tissue which contained Leydig (interstitial) cells and blood capillaries (Figs.5). The interstitial cells (Leydig cells) were polygonal in shape, with centrally located spherical nuclei and were present either singly or in groups in the interlobular space (Fig.6).

# Seasonal changes in testes of the catfish

### During winter season:

The testes showed a great increase in the connective tissue of both tunica albuginea and interstitium. Most of the spermatogenic cells were degenerated except the spermatogonia which appeared intact and predominant (Fig.7). Both the interstitial connective tissue and the degenerated spermatogenic cells gave strong positive PAS reaction (Fig.8). The mean gonadosomatic index (GSI) of the male catfish during this season was 0.25 which considered the lowest value throughout the year (table 1).

### During spring and summer seasons:

Both tunica albuginea and interstitial connective tissue were very thin and reduced due to the pressure exerted on them by the distended testicular lobules with different spermatogenic stages with appearance of the spermatozoa in the lumen of some lobules (Fig.9). The testicular lobules showed different activity where most of lobules were distended with spermatozoa and some lobules appeared empty (spent lobules) due to that they discharged their spermatozoa (Fig.10). Leydig cells increased in field occupation than those of the winter (Figs.9&11). The Leydig cells were abundant and interstitial connective tissue gave faint positive PAS reaction (Fig.12). The mean gonadosomatic index (GSI) of the male catfishes was greatly increased during spring to reach 0.7, while during summer reached 1.1 which was the maximum value throughout the year (table 1).

## During autumn season:

Both tunica albuginea and interstitial connective tissue were still relatively thicker than those of spring and summer but, still thinner than those of winter (Fig.13). Some testicular lobules contained residues of undischarged spermatozoa, while other lobules appeared partially empty or completely empty (Fig.14). The different spermatogenic stages were present but, the most predominant spermatogenic stages were spermatocytes (Figs.13&14). The mean gonadosomatic index (GSI) begun to decrease during this season to reach 0.5 (table 1).

The season	Fish weight	Gonad weight	GSI
Winter	600-700 gm Average 650 gm	1.5 – 1.7gm Average 1.6 gm	0.25
Spring	600-750 gm Average 675 gm	4.2 - 5.3gm Average 4.75gm	0.7
Summer	650-850 gm Average 750 gm	8.5 –8.7 gm Average 8.1 gm	1.1
Autumn	700-800 gm Average 750 gm	3.73 - 4.7 gm Average 3.9 gm	0.5

Table (1)	: Seasonal	variation in t	the gona	dosomatic	(GST	) value of	sexually	v mature	males'	catfish.
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# II- THE OVARY

The ovary of the catfish was surrounded by tunica albuginea which consisted of a vascular collagenous connective tissue contained smooth muscle cells which differentiated into outer circular and inner longitudinal smooth muscle cells (Fig.15), elastic fibers (Fig.16) and network of reticular fibers (Fig.17) which increased in

the stroma. The ovarian follicles passed three developmental stages; previtellogenic stage which had basophilic cytoplasm as the oocytes did not undergo vitellogenesis yet, vitellogenic in which their cytoplasm became acidophilic due to the deposition of vitellogenin into the oocytes (vitellogenesis) and postvitellogenic stages (Fig.18). Previtellogenic stage, vitellogenic stage while postvitellogenic stage in which their cytoplasm was characterized by being full of large yolk globules.

### Seasonal changes in ovary of the catfish

## During winter season:

The tunica albuginea surrounding the ovary reached a great thickness and also, the stromal connective tissue were increased (Fig.19). The ovarian structure showed some degenerated follicles (Fig.19) but, the most predominent stage was the previtellogenic stage where the early and late oocytes were common and abundant and some follicles were in atretic stage (Fig.20). The mean gonadosomatic index (GSI) was 2.4 which considered the lowest value throughout the year (table 2).

During spring and summer seasons:

The tunica albuginea became less in thickness and thinner than winter (Fig.21). The most predominant follicles were in vitellogenic stages (Fig.22). Many follicles in the postvitellogenic stage were firstly observed and were ready to ovulate and spawn (Fig.21). The mean (GSI) were greatly increased in comparison to winter season where it was 7.2 during spring season, while reached 10.2 as a maximum value during summer (table 2).

# During autumn season:

The tunica albuginea was relatively thicker than those of spring and summer (Figs.23). The most predominent stages were atretic follicles (Fig.23), empty and previtellogenic follicles (Fig.24). Unspawned mature follicles were still present (Fig.23). The mean (GSI) of the females begun to decrease during this season till reached 5.4 (table 2).

The season	Fish weight	Gonad weight	GSI
Winter	500-600 gm Av. 550 gm	12.2-14.4 gm Av. 13.8gm	2.4
Spring	490-540 gm Av. 515gm	36.2-38.3 gm Av. 37.3gm	7.2
Summer	480-600 gm Av. 540gm	48.1-60.1 gm Av. 54.1gm	10.2
Autumn	650-800 gm Av. 725 gm	38.4-40.9 gm Av. 39.7gm	5.4

#### Table (2): Seasonal variation in the gonadosomatic (GSI) value of sexually mature females catfish.



# Histogram of GSI values of females' catfish revealed the changes during different seasons

# List of figures:-

Fig. (1): Transverse section of testis of catfish, showing tunica albuginea (TA), seminiferous lobules (SL), different germinal cysts (GC) and the interstitium (I). Crossman's trichrome (X400).

Fig. (2): T.S of testis of catfish, showing fine elastic fibers (E) within the tunica albuginea that is covered by mesothelium (Me). Orcien (X400).

Fig. (3): T.S of testis of catfish, showing smooth muscle cells (arrow) within dense collagenous tunica albuginea. Crossman's trichrome (X400).

Fig. (4): T.S of testis of catfish, showing reticular fibers (R) surrounding seminiferous lobules and within interstitium. Gomeri's reticulin (X100).

Fig. (5): T.S of testis of catfish, showing elongated myoid cells around the lobules. Leydig cells (black arrow), blood capillary (white arrow) and collagen fiber within the interstitium. Crossman's trichrome (X400).

Fig. (6): T.S of testis of catfish, showing blood capillary (B.C) and Leydig cells (L) within the interstitium. Toluidine blue (X1000).

Fig. (7): T.S of testis of catfish during winter season, showing thick tunica albuginea (TA) and interstitium (I), degenerated spermatogenic cells with intact spermatogonia (arrow). Crossman's trichrome (X400).

Fig. (8): T.S of testis of catfish during winter season, showing strong positive reaction. PAS – Haematoxylin counter stain (X400).

Fig. (9): T.S of testis of catfish during spring season, showing thin tunica albuginea and interstitium, seminiferous lobules have different stages with appearance of spermatozoa within some lobules. H&E (X400).

Fig. (10): T.S of testis of catfish during summer season, showing thin tunica albuginea (TA) and interstitium, seminiferous lobules filled with spermatozoa (S).

Crossman's trichrome (X100).

Fig. (11): T.S of testis of catfish during spring season, showing increasing number of Leydig cells (arrow). H & E (X400).

Fig. (12): T.S of testis of catfish during summer season, showing faint positive interstitium. Note abundant Leydig cells. PAS (X400).

Fig. (13): T.S of testis of catfish during autumn season, showing relatively thick tunica albuginea with predominance of spermatocytes cysts (SC). H & E (X400).

Fig. (14): T.S of testis of catfish during autumn season, showing either fully distended partially or nearly empty lobules by spermatozoa. Note predominance of spermatocytes cysts (SC). H&E (X400).

Fig. (15): T.S of ovary of catfish, showing circular (C) and longitudinal (L) smooth muscle cells within tunica albuginea and ovigerous lamella (OL). H & E(X400).

Fig. (16): T.S of ovary of catfish, showing fine elastic fibers (E) within tunica albuginea that is covered by mesothelium (Me). Orcien (X400).

Fig. (17): T.S of ovary of catfish, showing network of reticular fibers (R) within the stroma. Gomeri's reticulin (X400).

Fig. (18): T.S of ovary of catfish showing previtellogenic stages (PV), vitellogenic (V) and postvitellogenic stages (PO). H & E (X100).

Fig. (19): T.S of ovary of catfish during winter season, showing degenerated follicles with thick tunica albuginea (TA) and stroma (ST).H & E (X100).

Fig. (20): T.S of ovary of catfish during winter season, showing predominance of previtellogenic stages (PV) and atretic follicles (AT).H & E (X100).

Fig. (21): T.S of ovary of catfish during spring (spawning) season, showing thin tunica albuginea (TA) and mature follicle (MF). H&E (X100).

Fig. (22): T.S of ovary of catfish during summer (spawning) season showing vitellogenic (v) and post-vitellogenic stages (PO). PAS (X400).

Fig. (23): T.S of ovary of catfish during autumn season showing attract follicle (AT) and mature follicle (MF). H & E(X100).

Fig. (24): T.S of ovary of catfish during autumn season showing, previtellogenic follicle (PV) and empty follicle (E). H & E (X100).

## DISCUSSION

The testis of the catfish was covered by collagenous connective tissue capsule which gave many septa dividing the testis into several lobules. The testicular lobules decreased in size during winter and begun to increase during spring and reached a maximum size during summer and they begun to decrease again during autumn. This finding was supported by Yoakim (1971) in S.schall, Latif and Salem (1983) in L. nebulosus and Gaber (2000) in Bagrus species. The interstitial cells were present in the interlobular spaces with collagen fibers and blood capillaries. This result was supported by This result was similar to those of Bhatti and Al-Daham (1978) in B. luteus, Rosenblum et al., (1987) in I. nebulosus, and Smita et al., (2005) in I.tricolor. The testicular lobules contained many germinal cysts and each cyst had the same spermatogenic stage. This resembled the results of Abraham et al., (1980) in A. dispar, Saad and Billard (1987) in C. carpio and Arenas et al., (1995) in G. affinis. Spermatogonia were abundant during the winter season to replenish the testes after this resting season giving new generations of spermatogenic cells for the next spawning season. This finding was similar to those of Zaki et al. (1986b) in Clarias gariepinus, Dziewulska and Domaga (2003) in salmonid and Guerriero et al., (2005) in L. cephalus. Spermatocytes were abundant during the spring, summer and autumn seasons i.e increased toward the spawning seasons that agreed with results of Rosenblum et al., (1987) in C.gariepinus and Gaber (2000) in Bagrus. The testicular lobules showed different activity where some lobules were filled with spermatozoa and others were empty that similar to result of Latif and Salem (1983) in L. nebulosus, Resink et al., (1987) in C. gariepinus, Gaber (2000) in Bagrus and Guerriero et al., (2005) in L. cephalus.

The ovary of catfish was covered by tunica albuginea which consisted of dense collagenous connective tissue, elastic fibers and network of reticular fibers. The ovarioan wall was supported with smooth muscle cells which differentiated into an outer circular and inner longitudinal cells and this agreed with the result of Rizkalla (1970) in C.lazera, Yoakim (1971) in S.shall, Khallaf et al. (1991) and Gaber (2000) in B.bayad. This tunica albuginea of the catfish ovary had no uniform thickness around the year that was similar to those obtained by Gaber (2000) in B.domac and Abel El Hafez et al., (2007) reported that the ovary of O.niloticus. This tunica albuginea greatly increased during winter and decreased during the following breeding seasons. This finding was similar to the result of Gaber (2000) in B.domac and Abd El-Hafez et al., (2007) reported that the ovary of O.niloticus. The ovigerous lamellae contained oogonia and various developmental stages of follicles without any arrangement. This finding was similar to Dougbag et al., (1988c) in T.niloticus and Ismail (1992) in Clarias lazer, Mousa (1998) and El-Gohary (2001) in O.niloticus. The previtellogenic stages which had basophilic cytoplasm were common in autumn and winter. This result was supported by Dougbag et al., (1988d) and Abd El-Hafez et al., (2007) in O.niloticus. Since the ovary during winter, would stay several months in previtellogenetic stage till enter vitellogenesis, it can be known as (resting season). The vitellogenic follicles which characterized by the appearance of large number of yolk vesicles were abundant during spring and summer. This finding was similar to those obtained by Van Oordt et al., (1987) in C.gariepinus and Gaber (2000) in Bagrus. The postvitellogenic follicles were abundant during spring and summer seasons but, rarely observed winter which was the resting season of the ovarian activity. This result agreed with Dougbag et al., (1988d) in T.noliticus and Gaber (2000) in Bagrus. These attretic follicles were were abundant during autumn and winter where the resting season and these atretic follicles indicated the spawned individuals. This finding similar to those obtained by Yoakim (1971) in S.schall, Gaber (2000) in Bagrus and Merson et al., (2000) in summer flounder.

Another method of studying the seasonal variations of the gonads was the values of gonadosomatic index (GSI) for both male and female catfish which used as an indicator of gonadal development as when the GSI reached a maximum value, this gave a perfect indication to the time of spawning. This was supported by **Moustafa (1984)** and **Ismail (1992)** in C.lazera, **Resink et al., (1987)** in C.garipienus, **Dougbag et al., (1988b&d)** in T.niloticus, **Khallaf et al., (1991)** and **Gaber (2000)** in Bagrus species. On other hand, the GSI dropped during the spawning season of I.nebulosus **as** mentioned by **Rosenblum et al., (1987)**. The results about GSI were coincided with the obtained results where the GSI of both sex reached their minimum values during winter season where the gonads were in resting season, began to increase greatly during spring season, reached their maximum values during summer where the gonads were in the spawning season and began to decrease again during autumn season. This indicated long spawning season of the catfish extending from spring to summer, but its peak was reached during summer season.

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دراسات هستولوجية على مناسل سمك القرموط في المواسم

# المختلفة

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- أجريت هذه الدراسة على عدد 80 من سمك القراميط من كلا الجنسين لتوضيح التركيب الهستولوجى و الهستوكيميائى للمناسل في المواسم المختلفة.
- أوضحت النتائج أن موسم الشتاء هو موسم السكون والراحة لمناسل سمك القرموط حيث كانت كل مراحل تكوين الحيوانات المنوية بالخصية مدمرة ما عدا أمهات المنى وأوضحت المبايض سيادة مرحلة ما قبل تكوين المح.
- > وكان كل من الربيع و الصيف بمثابة مواسم التزاوج لسمك القرموط حيث كانت فصيصات الخصية ممتلئة بكل مراحل تكوين الحيوانات المنوية وقد ظهرت بعض الفصيصات ممتلئة بالحيوانات المنوية و أوضحت المبايض سيادة مرحلة تكوين المح و مرحلة ما بعد تكوين المح مع ظهور الحويصلات الناضجة.
- وكان فصل الخريف بمثابة موسم ما بعد التزاوج حيث قل نشاط الخصية عما كانت عليه في مواسم التزاوج السابقة فظهرت بعض الفصيصات فارغة تماما وأوضحت المبايض سيادة الحويصلات المرتوقة التي تدل على إتمام التزاوج.