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HISTOMORPHOLOGICAL CHANGES IN THE OVARIES OF *OREOCHROMIS NILOTICUS* DURING BREEDING AND NON BREEDING SEASONS

(With 4 Tables, One Histogram and 13 Figures)

By

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التغيرات الهستومورفولوجية لمبايض البلطي النيلي أثناء موسم التكاثر وموسم عدم التكاثر

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أجريت هذه الدراسة على ٥٨ عينة من إناث البلطي النيلي، وزنت هذه الأسماك بالجرام وقيست الأطوال بالمليميتر. وأعدت مبايض الأسماك للدراسة الهستومورفولوجية بالإضافة لبعض القياسات. يتضح من الدراسة المورفولوجية أن الأسماك لم تظهر إختلافات في الشكل الظاهري أثناء موسم التكاثر (من ابريل إلى سبتمبر) وموسم عدم التكاثر (من أكتوبر إلى مارس). وقد وجد أن لهذه السمكة مبيضان طوليان، أسطوانيان في نفس الحجم تقريبا، يقعان في الجزء الخلفي من التجويف البطنى أسفل المثانة الهوائية ويرتبطان بجدار الجسم العلوي بمساريقا المبيض. أثناء موسم عدم التكاثر، ظهرت المبايض صغيرة بلون أصفر محمر وشغلت جزء ضئيل من التجويف البطنى حيث كان متوسط طول المبيض ٦٣ ، ٣± ١١ ، سم، وقطره ٦١ ، ٣± ٣١ ، مم، ومتوسط وزنه ٨,٢٨±٥١، جم ولم نستطع رؤية البويضات. أثناء موسم التكاثر ظهرت المبايض طويلة وعريضة بلون أصفر ومليئة بالأوعية الدموية وشغلت أغلب التجويف البطنى. كان متوسط طول المبيض٠١ ،٤ ±٢ ، سم، وقطره ٣٦±٨,٢ ، مم، ومتوسط وزنه ٥٨ ، ١٢±٦، جم وشوهدت أعداد كبيرة من البويضات المستديرة بلون برتقالي لامع. تم تصنيف نمو البويضات إلى ستة مراحل هم: أمهات البيض، مرحلة النويات الكروماتينية، مرحلة النويات المحيطية، مرحلة حويصلات المح، مرحلة كريات المح ومرحلة النضج. ظهرت نواة المح في مرحلة النويات المحيطية، بينما ظهرت حويصلات المح محيطيا في مرحلة حويصلات المح. تميزت البويضات الناضجة بقطرها الكبير وتراكم حبيبات المح لتكوين كريات مح كبيرة ووجود حويصلات المح محيطيا في السيتوبلازم. هاجرت النواة إلى قطب الخلية ثم تكسرت عند نهاية تكوين المح. تكون جدار البويضة الناضجة من طبقة شعاعيه سميكة محاطة بالخلايا المحبية وطبقة الخلايا الغمدية. الأخيرة انقسمت إلى طبقة خارجية وداخلية. وتكرر ظهور الحويصلات المرتوقة بعد نهاية

موسم التكاثر. لوحظ من خلال الدراسة النسيجية، أن مبيض البلطي النيلي يحاط بغشاء بريتونى يليه إلى الداخل المحفظة التي كانت سميكة أثناء موسم عدم التكاثر والتي أصبحت رفيعة ومليئة بالأوعية الدموية أثناء موسم التكاثر. أثناء موسم التكاثر، وجد المبيض في مرحلة تكوين المح وزاد كلا من عدد وقطر البويضات الناضجة. بينما في موسم عدم التكاثر، أمتلئت الصفائح المبيضية بالبويضات ما قبل تكوين المح في مرحلة النويات المحيطية وايضا أمهات البيض ومرحلة النويات الكروماتينية.

SUMMARY

The present study was conducted to highlight the relationship between the external morphology of female O.niloticus and the histomorphological changes of the ovaries during different seasons of the year. A total of 58 female fishes of O.niloticus were used in this investigation. The external features of females didn't show any seasonal variations. Ovaries were paired elongated, cylindrical structure of approximate equal size, located in the posterior body cavity, ventral to the swim bladder and attached to the dorsal body wall by mesovarium. During the non-breeding season, ovaries were small, yellowish red in colour and occupied small portion of the body cavity. While during the breeding season, ovaries were extremely long and wide, yellowish in colour and more vascularized. They occupied almost the entire body cavity. The ovary of O. niloticuis was covered by a thick capsule during the non-breeding season, but became thin and vascular during the breeding period. Six arbitrary stages of oogenesis process had been established among the ovarian follicles; oogonia (stagel), chromatine nucleolus stage (stage 2), perinucleolar stage (stage 3), yolk vesicle stage (stage 4), yolk globule stage (stage 5) and mature stage (stage 6). During the non-breeding season, the ovaries were filled with previtellogenic oocytes in perinucleolus stage. Oocytes in the oogonium and chromatin nucleolus stages were abundant; the oogonia reached 10±1.5/ UA and the chromatin nucleolus reached 9.8±1.4 / UA during the non-breeding season. While during the breeding season, the ovaries were in a condition of active vitellogenesis and mature oocytes increased both in number (4.0±0.5 / UA) and in diameter (806.0±11.0 μm). Morphometric studies revealed significant differences in the length, diameter and weight of the ovaries of O.niloticus during breeding and non-breeding seasons.

Key words: *O.niloticus, breeding, ovary*

INTRODUCTION

Nile tilapia belongs to genus Oreochromis. This species is naturally distributed in Palestine, the Nile River as well as most parts of African Rivers & lakes (Trewawas, 1982 and Beamish; Booth and Deacon, 2005). *O. niloticus* is gonochoristic, which each individual possessing a single sexual phenotype. Nile tilapia is characterized by extended spawning seasons, maturity at small size and a fast growth rate. It has been termed the aquatic chicken for its extraordinary production capabilities (Peterson; Slack; Brown- Peterson and McDonald, 2004). Tilapia have one pair of bilateral gonads locating in the posterior part of the body cavity immediately ventral to the swim bladder and attached by mesentries to the parietal peritoneum. Short ducts extend from the posterior end of the gonads to the genital pore. In addition to production of gametes, the gonads also produce hormones from endocrine tissue (Bond, 1979). The aim of the present investigation is to highlight the relationship between the external morphology of the female fish and the histomorphological changes of the ovarian tissue during different seasons of the year. Also, to detect the relationship between some environmental conditions such as water temperature and spawning activity.

MATERIALS and METHODS

The materials employed in this study consisted of randomly obtained 58 female specimens of femle *Oreochromis niloticus*. The materials were collected every month from the Nile River at Elkhazan bridge in Assuit city during the year. The specimens ranging from 14.46 ± 0.22 &13.92 ± 0.16 cm in standard length and from 96.39 ± 2.19 & 95.52 ± 2.00 g. in body weight. A regular record of water temperature for every month was recorded for three times using water thermometer and the mean values were taken in order to study the possible correlation between the temperature and the spawning activity of *O. niloticus* (Table 1).

Ovarian measurements: The ovarian length (cm) was measured individually (right&left) using a ruler from the anterior to the posterior end. In addition the ovarian diameter (mm) was measured individually (right&left) using a caliber. Ovaries (right&left) were weighed individually using Berekel balance.

Gonadosomatic index (GSI): Monthly variation of gonadosomatic index provides good indication of the extent of development of gonad with respect to the time of year (Hatikakoty and Biswas, 2004) (Table 2).

GSI was calculated monthly from each fish using the following formula:
GSI % = Gonads (ovaries) weight (g.) / Body weight (g.) x 100

Histological preparations: Samples for histological examination were dissected as soon as possible from the anterior, middle and posterior parts of each ovary (1x1x.05 cm) and were immediately fixed in Bouin's fluid for 24 hours. The fixed materials were dehydrated in an ascending series of ethanol, cleared in methyl benzoate and then embedded in paraffin wax. Transverse and longitudinal paraffin sections at 5-8 μm in thickness were cut and stained with the following histological stains; Harris haematoxylin and Eosin (Harri's, 1900), Grossmon's Trichrome (Grossmon, 1937), Periodic Acid –Schiff (PAS) (McManus, 1946).

Morphometrical measurements: were applied including thickness of the tunica albuginea., oocytes diameter and number/ unit area and diameter of mature ovarian follicles. By using Image analysis system (Leica Q500 MC) (Tables 3&4).

RESULTS

Morphological study:

During the present study, the mature fish exhibited silver colouration and there was no variation in the external appearance of the fish during the breeding (from April to September) and the non-breeding seasons (from October to March). The ovaries of female *O. niloticus* were paired elongated, cylindrical structure and approximatly of equal size. They were located in the posterior part of the body cavity, ventral to the swim bladder. During the breeding season, ovaries were extremely long, wide and occupied almost entire of the body cavity. Large numbers of spherical bright orange oocytes were easily visible, which gave it granular texture. In addition, the ovaries were yellowish in colour and vascularized. Ripped oocytes were visible through thin transparent ovarian wall (Fig. 1). During the non breeding season, ovaries were small, ribbon- like, yellowish red in colour, occupied less than one third of the body cavity and the ova could not be seen (Fig. 2). From the present investigation, it was observed that the ovaries showed great variation in oocytes during different months of the year (Table 3) and (Histogram 1).

There was non-significant increase in mean total and standard length of female *O. niloticus* during breeding and non-breeding seasons, while there was highly significant increase in mean body weight of female *O. niloticus* during breeding and non-breeding seasons.

Histological study:

The histological investigation of the ovaries of *O. niloticus* showed that the ovary was covered with the peritoneal membrane, which overlied the tunica albuginea that consisted of vascular collagenous connective tissue (Fig. 3) elastic fibers and smooth muscle fibers. The ovarian cavity (ovocoel) remained

in the center of the ovary as irregular space lined with germinal epithelium (simple squamous epithelium), which divided to give oogonia. The ovocoel continued posteriorly as the lumen of the oviduct (Fig. 4 a,b). The developing ova were held together by the stroma (vascular collagenous connective tissue and few strands of smooth muscle fibers). The stroma consisted of finger- like ovarian (ovigerous) lamellae which contained ovarian follicles at different stages of oogenesis in addition to atretic follicles (Figs. 4 a&b). Ovigerous lamellae protruded into the ovocoel from the ovarian wall and oogenesis occurred in these lamellae.

a)- *Oogenesis:* Oogenesis involved the proliferation of oogonia by mitosis and the development of oocytes. According to changes in size, nucleus, ooplasm and egg membranes of the developing ova, six stages were observed in *O. niloticus:* Stage 1- Oogonia: They were found in groups or nests in the ovigerous lamellae, associated with the germinal epithelium and they were the smallest cells of the germinative lineage. They were small spherical cells with large light basophilic nucleus with a single nucleolus. Thin film of faintly stained ooplasm surrounds the nucleus (Fig. 5a&b). All ovaries of developing and mature females had several patches of oogonia Oogonia divided by mitosis to give primary oocytes.

All ovaries of developing and mature females had several patches of oogonia. Oogonia divided by mitosis to give primary oocytes. During the breeding seasons, they reached 6.4 ± 2.1 in number / unit area and 28 ± 0.8 µm in diameter while during the non-breeding seasons, they reached 10 ± 1.5 in number / unit area and 24.8 ± 2.0 µm in diameter (Table 3). Stage 2- Early oocyte (chromatin nucleolus stage): Transformation of oogonia to oocyte includes increase in the size of the cell and nucleus (Table 3). Chromatin of the nucleus appeared thread like and distributed throughout the nucleus. The number of nucleoli increased which distributed throughout the nucleoplasm. The ooplasm was reduced and deeply basophilic. These oocytes had little or no affinity with dyes used (Fig. 6). During the breeding seasons, they reached 3.2 ± 0.50 in number / unit area and 44.0 ± 2.4 µm in diameter while during the non-breeding seasons; they reached 9.8 ± 1.4 in number / unit area and 40.0 ± 2.4 µm in diameter (Table 3). Early oocytes showed highly significant decrease in number / unit area but non-significant increase were found in diameter in the breeding seasons. Stage 3- Late oocyte (perinucleolar stage):

The nucleus stained lightly and the number of nucleoli increased, arranged themselvs in the peripheral part of the nucleus. Chromosomes were assembled at one side of the nucleus. Ooplasm contained yolk nucleus (Balbiani bodies), which appeared as basophilic round mass. Oocyte surrounded by a single layer of squamous follicular epithelium (Figs.5c&7). Oocyte increased progressively in number and diameter (Table 3). During the breeding seasons, they reached 4.0 ± 1.1 in number / unit area and 100.0 ± 6.1 µm in diameter, while during the non-breeding season, they reached 9.0 ± 2.4

in number / unit area and 98.0 ± 3.8 µm in diameter. Late oocytes showed significant decrease in number but non-significant increase were found in diameter in the breeding season (Table 3). Stage 4- Vacuolated follicles (yolk vesicle or cortical alveolar stage): Nucleus increased in size to become the germinal vesicle. Nucleoplasm attained acidophilic reaction and the nuclear membrane became irregular. Ooplasm became faintly basophilic and yolk vesicles were peripherally arranged (PAS positive), late become cortical alveoli and take part in the formation of perivitelline space. Yolk granules appeared in the ooplasm around the nucleus. Zona radiata (oolemma) begin to appear which was acellular thin hyaline acidophilic membrane. The follicular layer was formed of cuboidal cells. The stroma of the ovary was formed of flat thecal cells (thin layer of fibroblasts) that surrounded the follicular layer (Figs. 8). Oocytes increased both in number and diameter (Table 3). During the breeding season, they reached 5.4 ± 2.2 in number / unit area and 242.0 ± 7.0 µm in diameter while during the non-breeding season, they reached 5.0 ± 0.4 in number / unit area and 204.0 ± 7.3 µm in diameter. Late oocytes showed non-significant decrease in number but highly significant increase were found in diameter in the breeding season (Table 3). Stage 5- Yolk globule stage (vitellogenesis): Yolk vesicles increased in size. Yolk granules accumulated into large yolk globules. Several rounded yolk globules (platelets) appeared near the center of the oocyte and extend centrifugally until only a thin peripheral shell of cytoplasm remains. These globules gave PAS positive reaction. Zona radiata appeared thick and the follicular epithelium (granulosa) made up of cuboidal cells. Theca folliculi divided into outer vascular, collagenous connective tissue thecal layer and inner cellular theca cells (Fig. 9). Basal lamina (thin fibrous layer, PAS positive) was found between follicular epithelium and theca layer. When vitellogenesis begins, the egg membranes became clear and PAS positive. Oocyte increased in size (Table 3). During the breeding seasons, they reached 4.0 ± 0.31 in number / unit area and 492.0 ± 2.7 µm in diameter while during the non-breeding season they reached 2.0 ± 0.6 in number / unit area and 313.0 ± 6.0 µm in diameter. Yolk globule oocytes showed highly significant increase in number and significant increase were found in diameter in the breeding season. Stage 6- Mature follicles: Many empty large vacuoles found towards oocyte periphery and the yolk globules increase in size. The nucleus gradually disappeared and begin to migrate to the animal pole (germinal vesicle migration stage) which usually occur at the end of vitellogenesis (Figs. 11 a,b,c,d). At the end of this stage, Zona radiata attained its maximal thickness surrounded by granulosa and theca cells. Strong PAS positive reaction was observed in the cytoplasm of mature follicles between yolk globules (Figs. 10 a,b,c &11 d). Oocyte was large in diameter, they reached 1049.5 µm in May (Table 3). During the breeding season, they reached 4.0 ± 0.5 in number / unit area and its mean 806.0 ± 15.0 µm in diameter while during the non-breeding season, they reached 1.2 ± 0.2 in

number / unit area and 509.0 ± 4.0 µm in diameter. Mature oocytes showed significant increase in number and highly significant increase were found in diameter in the breeding season (Table 3).

b) - Atretic follicles: Its diameter was too variable to be measured, since it was irregular structure that could be seen at any time during oocyte maturation, but it always formed at the period of post-spawning in oocytes at any stage. These follicles characterized by breakdown of oolemma, yolk liquefaction, presence of many empty spaces, the granulosa hypertrophied and their cells increased in number and the connective tissue surrounding the follicles thickened and become more vascular and appearance of small acidophilic mass of unclear structure in the cytoplasm of these follicles to give structure resemble corpora atretica or yellow brown bodies (Fig. 12). First oocytes to be reabsorbed were those containing yolk.

Histological variation of the ovarian tissue during the breeding and non- breeding seasons:

a) - During the non-breeding seasons:

The tunica albuginea was thick during the non- breeding seasons and contained much amount of smooth muscle fibers (Fig. 13), its mean thickness reached 48.01 ± 4.56 µm (Table 4) and the maximum mean value was 72.18 ± 0.79 µm in February.

The ovarian lamellae were thin in non-breeding seasons. The lamellae were filled with previtellogenic oocytes in perinucleolus stage. Oocytes in the oogonium and chromatin nucleolus stage were abundant, while mature ovarian follicles were absent in January (Table 3). The lamellae were disrupted and disorganized with several empty spaces and extensive vascularization. Remnants of atretic follicles were present throughout the ovary (Fig.12).

B)- During the breeding seasons:

The tunica albuginea was thin and vascular, its mean thickness reached 29.18 ± 0.67 µm (Table 6).

The ovarian lamellae were thick and completely obliterated ovaries. Mature ovary was in active vitellogenesis. Oocytes in all stages of development, from perinucleolus to ripe stages could be identified, but late stages of vitellogenesis were dominant (Figs. 4 a&b).

Mature oocytes increased both in number to reach about 5 / unit area in May and July and also in diameter to reach about 1049.5 µm in May, while perinucleolar oocytes were absent in June and July (Table 3). Stroma of mature breeding ovary was pressed due to enlargement of ocytes (Figs. 4 a&b).

Gonadosomatic index:

GSI= gonads weight \ body weight x 100

GSI can be used as indicator for gonadal development. When gonadosomatic index reach a maximum value, this gives a perfect indication to the time of spawning.

Gonadosomatic indix were highest between May to July. GSI peaked in June (Table 1).

In our study we found that the mean GSI during the breeding seasons was 12.58 ± 0.59 %. While the mean GSI during the non- breeding seasons was 9.17 ± 0.36 % (Table 1). From the present investigation, the water temperatures were highly correlated with GSI and the optimum temperature for spawning was between 21 °C to 24 °C (from May to July) (Table 2) that was the peak period for the GSI.

Table 1: Average monthly fluctuations in GSI.

	Number of specimens	Mean body weight (g.)	Mean ovary weight (g.)	GSI
Non-breeding season				
October	5	95.0	9.3	9.78
November	5	92.6	8.9	9.61
December	5	86.4	7.8	9.02
January	5	83.0	7.0	8.43
February	5	85.8	6.8	7.92
March	4	96.25	9.9	10.28
M±SE			8.28	9.17
breeding season	--------	--------	--------	-------
April				
May	5	105.2	12.1	11.5
June	5	101.0	13.7	13.56
July	5	102.6	14.5	14.13
August	5	103.5	14.5	14.0
September	4	100.6	11.3	11.23
M±SE	5	98.4	11.0	11.1
			12.58	12.58

Table 2: The mean values of water temperature during different months of the year.

Month	Temperature
October	18 °C
November	16 °C
December	11 °C
January	11 °C
February	13 °C
March	15 °C
April	19 °C
May	21 °C
June	22 °C
July	24 °C
August	25 °C
September	26 °C

Table 3: Number and diameter (μm) of all stages of oocyte development during different months of the year / unit area.

Stages	Stage 1		Stage 2		Stage 3		Stage 4		Stage 5		Stage 6	
Months	Number	diameter	Number	diameter	Number	diameter	Number	diameter	Number	diameter	Number	diameter
non breeding season October	10.0±0.1	24.8	9.8±0.21	40.0	9.0±0.6	98.0	5.0±0.2	204.0	2.0±0.1	313.0	1.2±0.5	509.0
November	14±0.36	28.1	12±0.36	42.78	10±0.21	108.86	4±0.47	224.01	1±0.61	297.19	2±0.11	521.63
December	10±0.26	20.53	11±0.25	35.68	7±0.11	84.92	6±0.16	220.01	1±0.33	300.99	1±1.0	505.41
January	12±0.52	19.77	13±0.84	33.87	18±0.1	100.82	4±0.15	189.39	3±0.58	318.36	--	--
February	9±0.54	30.4	8±0.54	45.55	6±0.41	95.71	6±0.46	194.5	4±0.91	330.5	1±0.4	496.39
March	5±.0.33	25.41	5±0.9	44.17	4±0.13	100.38	5±0.54	193.33	1±0.28	320.6	1±0.91	514.37
M±SE	10±1.5	24.8±2	9.8±1.4	40±2.4	9±2.4	98±3.8	5±0.4	204±7.3	2±0.6	313± 6	1.2±0.2	509±4
Breeding Season April	15±0.11	27.24	5±0.33	45.12	4±0.25	97.44	14±0.33	234.73	5±0.93	488.85	4±0.16	671.91
May	5±0.22	28.95	4±0.14	36.62	2±0.41	91.22	2±0.51	227.48	3±1.0	484.01	5±0.12	1049.5
June	4±0.31	29.52	2±0.43	42.01	--	--	2±0.32	267.8	4±0.28	495.1	4±0.45	874.18
July	3±0.51	30.23	2±0.41	51.33	--	--	3±0.41	246.3	4±0.54	492.6	5±0.56	742.06
August	5±0.24	25.3	3±0.25	46.52	6±0.25	111.99	6±0.25	236.7	4±0.28	500.24	2±0.47	692.08
September	6.0±0.61	28.0	4.0±0.41	44.0	4.0±0.2	100±6.	5.4±2.2	242±7	4±0.31	492±2.7	4±0.5	806± 4
M±SE	6.4±2.1 *	28±0.8 n.s	3.2±0.5 **	44±2.4 n.s	4±1.1 **	100±6 n.s	5.4±2.2 n.s	242±7 **	4±0.31 **	492±2.7 *	4±0.5 *	806±11 **

n.s means the values are non- significant.

* means the values are significant. ** means the values are highly significant.

Histogram 1: The mean number of all stages of oocytes development /unit area during different months of the year.

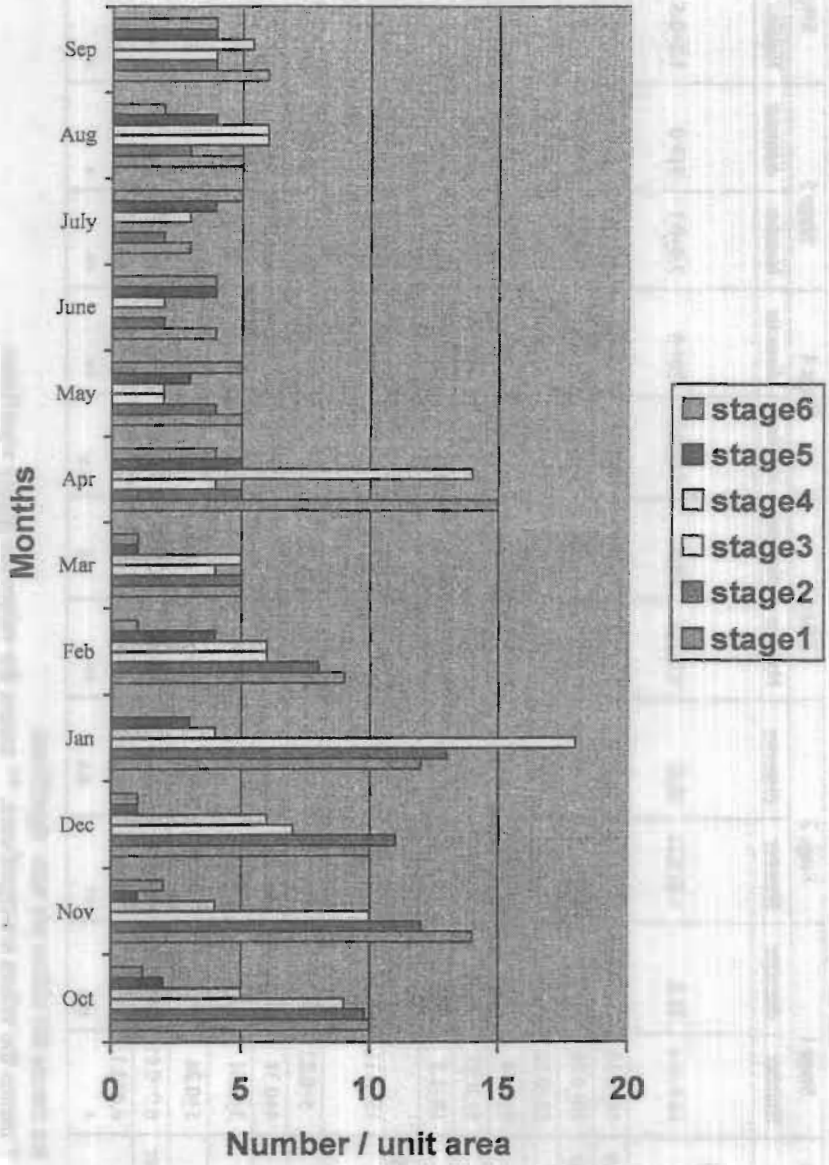
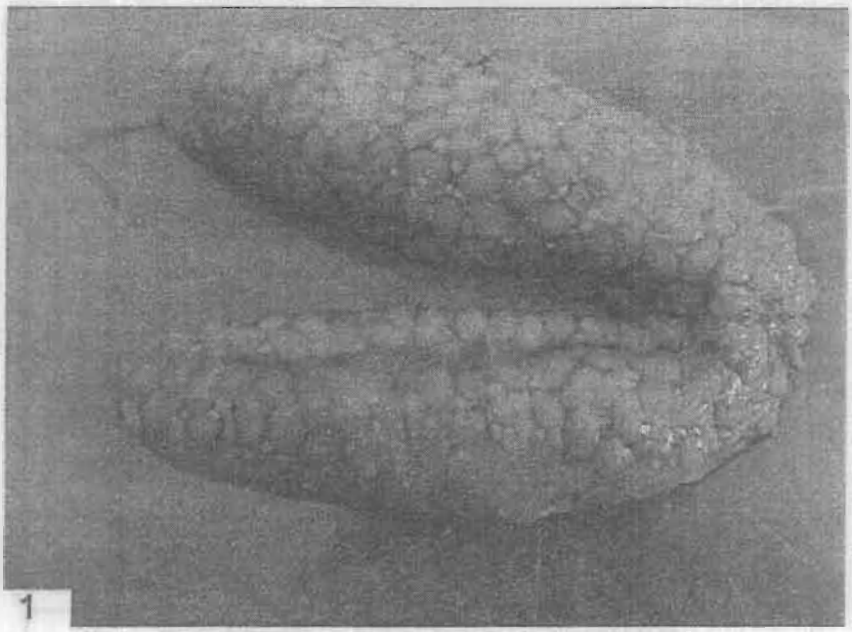


Table 4: The thickness of the tunica albuginea (μm) during different months of the year.

Month	Thickness of tunica albuginea (μm)
Non-breeding season	
October	35.01 ± 0.98
November	31.99 ± 0.78
December	33.79± 0.81
January	34.96 ± 1.03
February	72.18 ± 0.79
March	66.11 ± 0.54
M+SE	48.01 ± 4.56
Breeding season	
April	28.56 ± 1.30
May	29.31 ± 0.46
June	29.17 ± 0.53
July	27.36 ± 0.79
August	31.51 ± 1.21
September	30.18 ± 0.77
M+SE	29.18 ± 0.67 **

Values were represented by mean (M) ± standard error (SE).
** means the values were highly significant.



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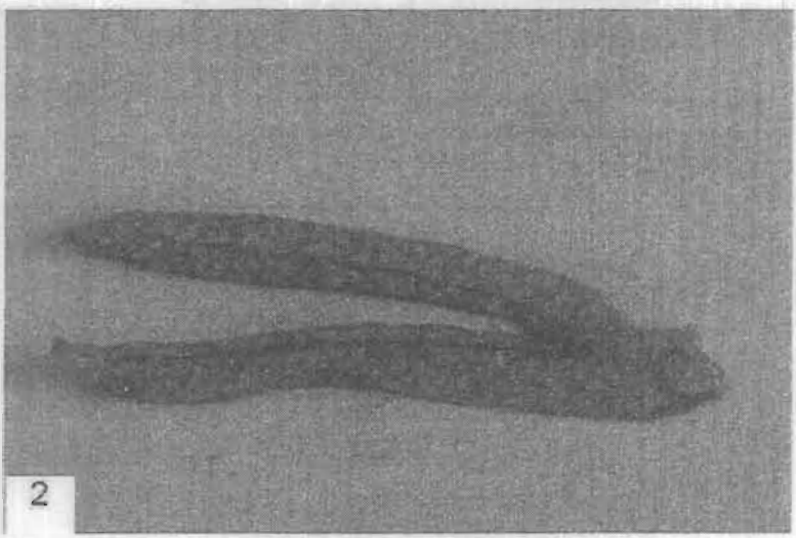

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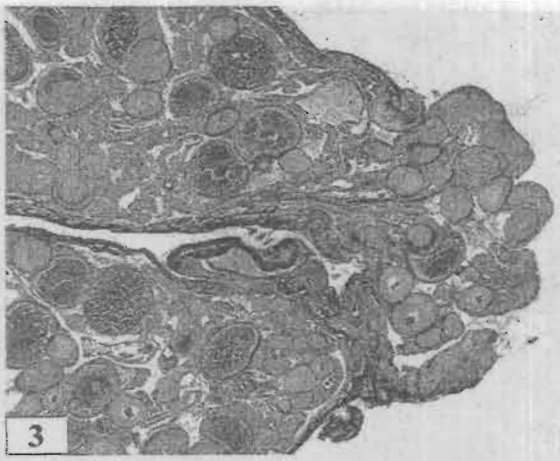

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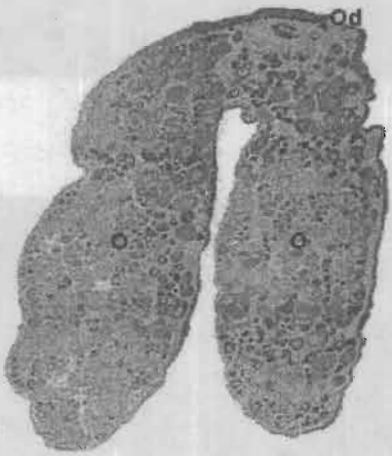
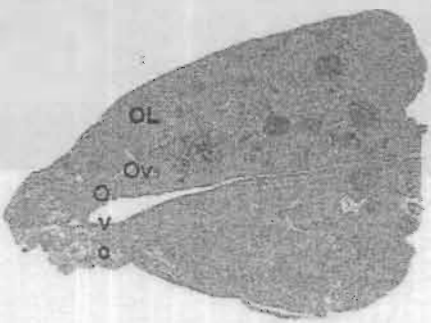
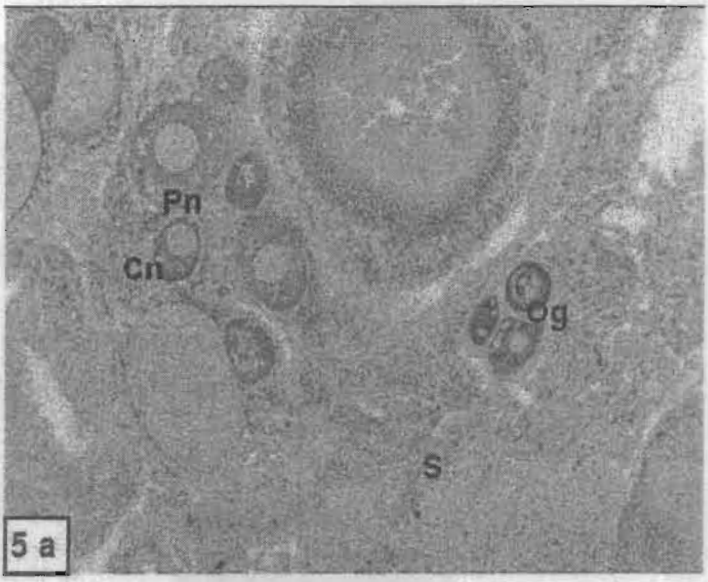


4a



4b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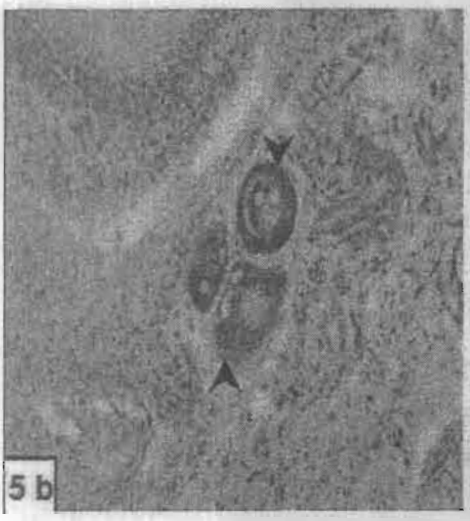
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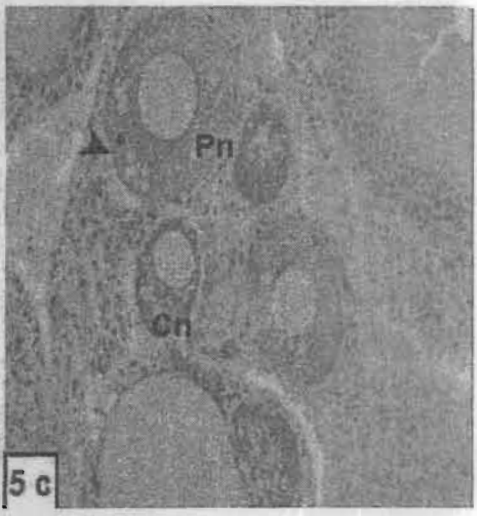
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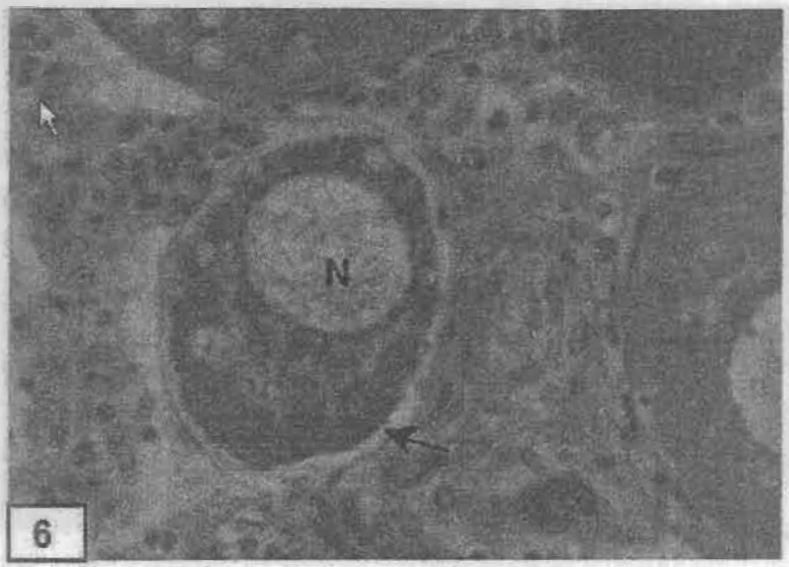

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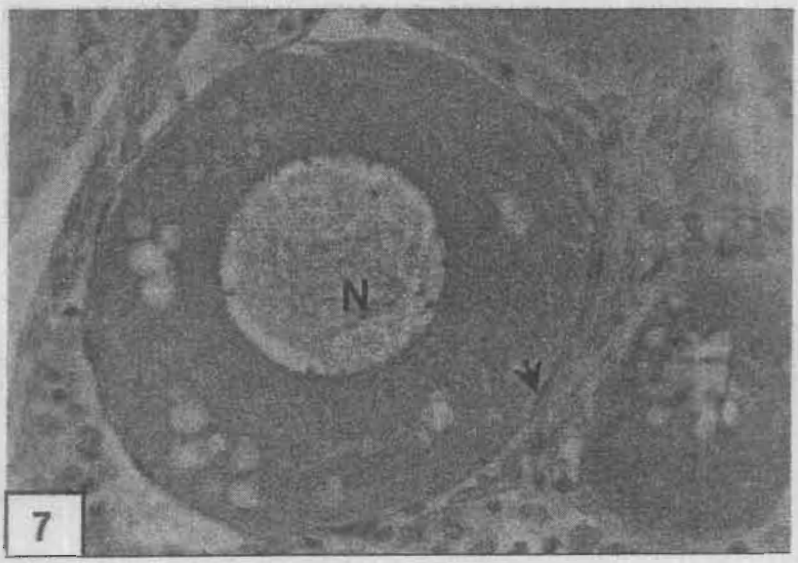

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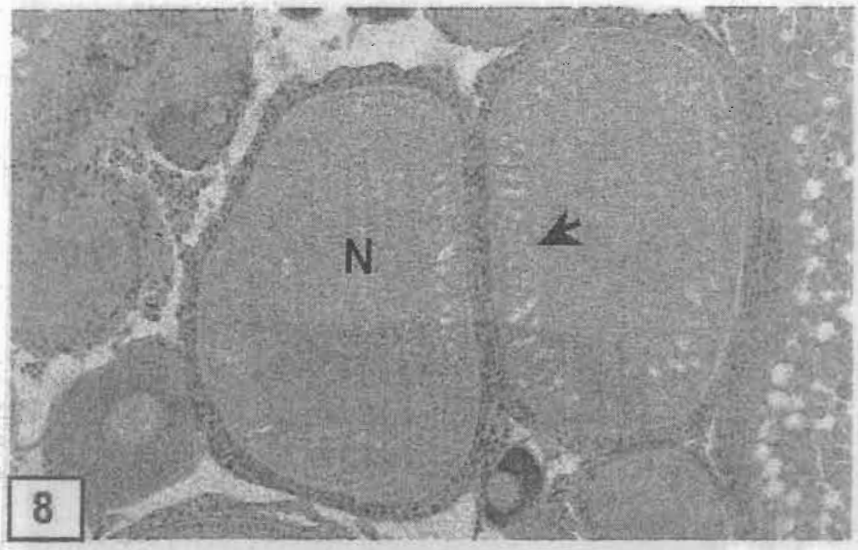

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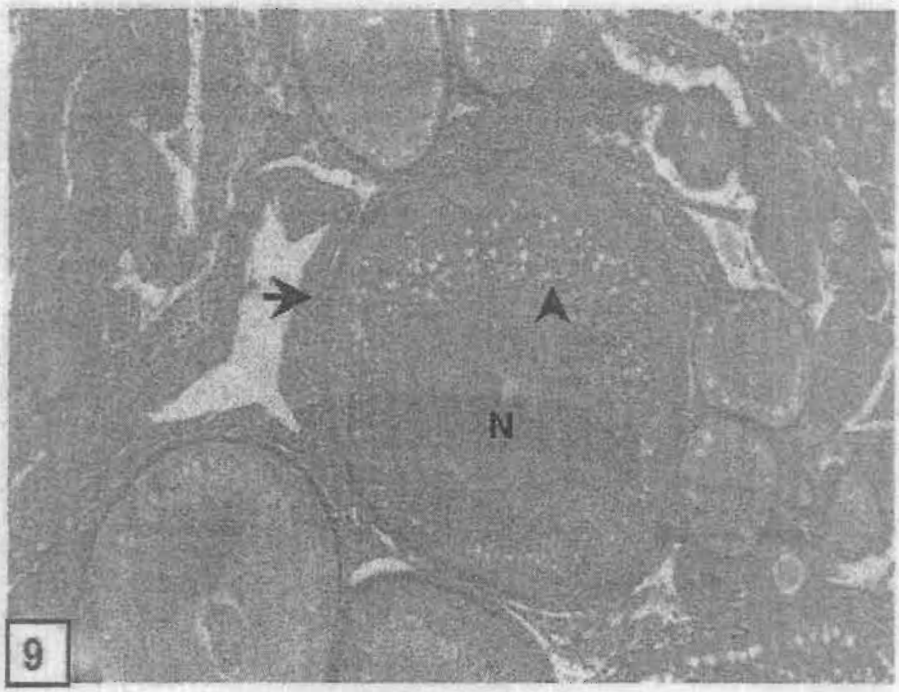

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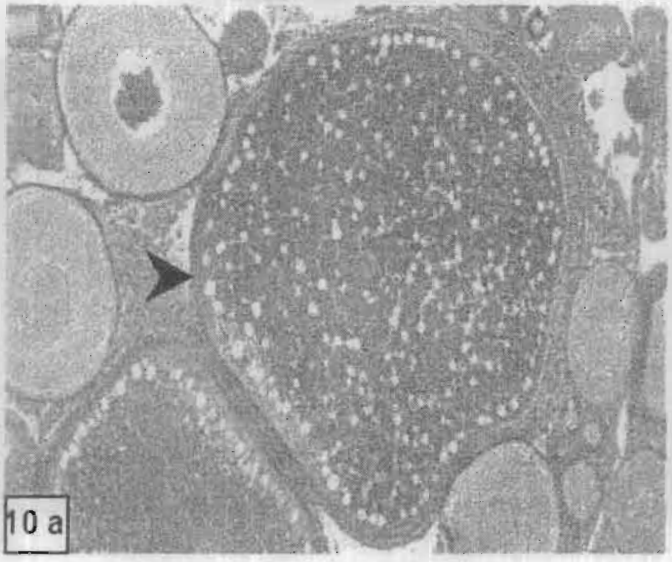
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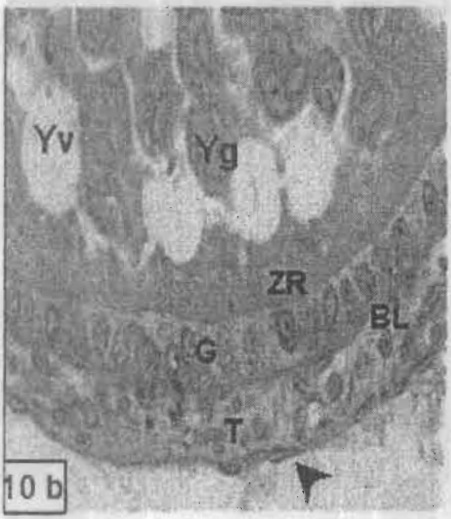
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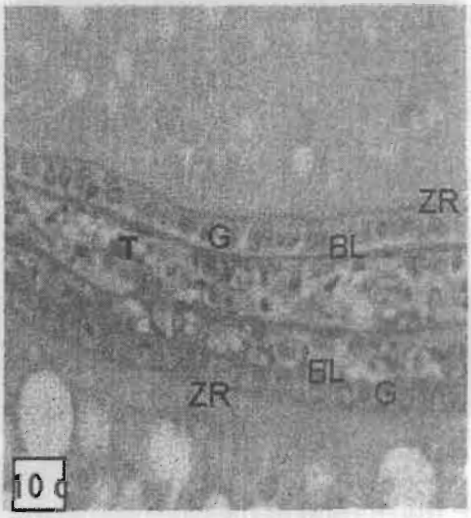
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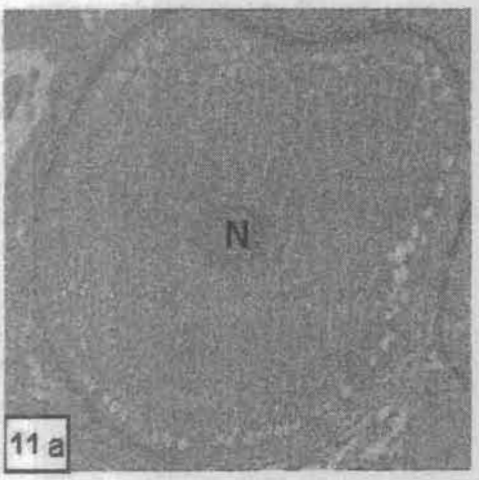

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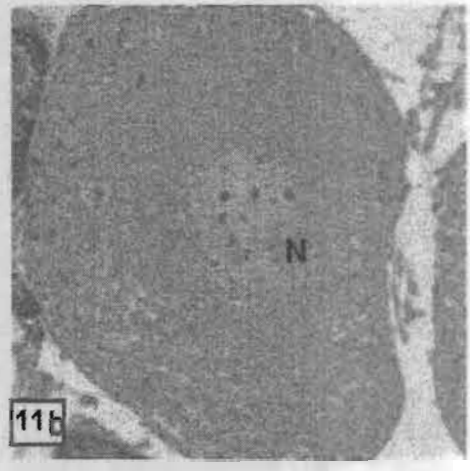

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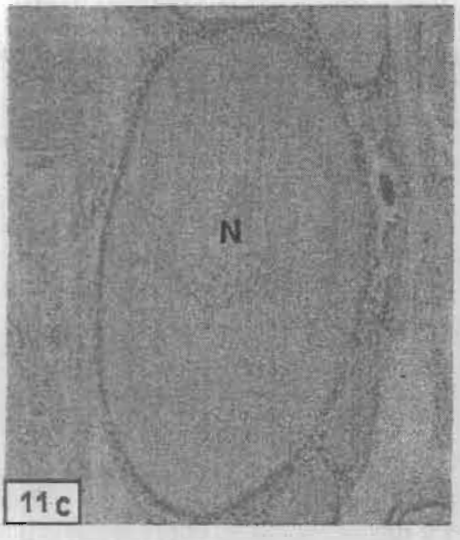

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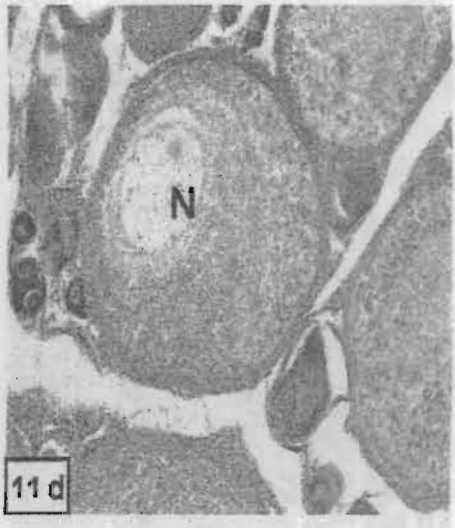

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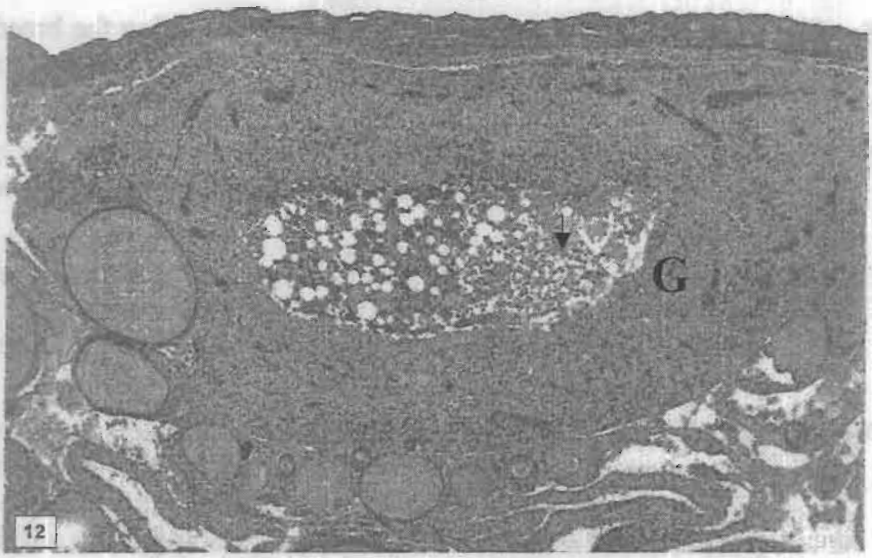
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13

LEGENDS

Fig. (1): Photograph of ovaries of O. niloticus representing the breeding season, they were large yellowish in coloure and vascularized. Notice that the ripped oocytes were visible by naked eye.

Fig. (2): Photograph of the ovaries of O. niloticus representing the non-breeding season, the ovaries were small pale yellowish red in coloure.

Fig. (3): Photomicrograph of the posterior part of the two ovaries showing the tunica albuginea (arrow) stained green and the nucleus of the oocytes (arrow head) stained red. (Grossmon trichrome, X 10)

Figs. (4 a & b): Photomicrograph of the ovaries of O. niloticus showing connection of the ovaries(O) with the oviducts (Od). Notice that the ovigerous lamellae (OL) protruded into the ovocoel (Ov) from the ovarian wall. (Heamatoxylin and Eosin a & b; X 50)

Fig. (5a): Photomicrograph of the ovary showing nests of oogonia (Og), chromatin nucleolus oocyte stage (Cn) and perinucleolus oocyte stage (Pn) that embedded in the stroma (S). Notice the presence of smooth muscle strands in the stroma. (Heamatoxylin and Eosin, X 80)

Fig. (5b): Photomicrograph of the ovary showing groups of deep basophilic oogonia (arrow heads) with light basophilic nucleus. (Heamatoxylin and Eosin, X 80)

Fig. (5c): Photomicrograph of the ovary showing chromatin nucleolus oocyte stage (Cn) and perinucleolus stage (Pn). Notice the presence of Balbiani' body (arrow). (Heamatoxylin and Eosin, X 80)

Fig. (6): Photomicrograph of the ovary showing chromatin nucleolus oocyte stage (arrow) contained nucleus (N) had chromatin threads. (Heamatoxylin and Eosin, X 400)

Fig. (7): Photomicrograph of the ovary showing nucleus (N) of perinucleolar oocyte stage. The oocye surrounded by flat squamous cells (arrow head). (Heamatoxylin and Eosin, X 400)

Fig. (8): Photomicrograph of the ovary showing yolk vesicle stage contained central nucleus (N) that had large number of peripherally arranged nucleoli. Notice the presence of peripherally arranged yolk vesicles (arrow) (Heamatoxylin and Eosin, X 100)

Fig. (9): Photomicrograph of the ovary showing yolk globule oocyte stage (arrow) contained central nucleus (N) which began to migrate to the animal pole. Notice the increase oocyte siz addition the yolk vesicles (arrow head) increased in size and number. (Heamatoxylin and Eosin, X 200)

Fig. (10a): Photomicrograph of the ovary showing the mature oocyte (arrow head). Notice its large size. (Heamatoxylin and Eosin, X 100)

Fig. (10b): Photomicrograph of the ovary showing part of the mature oocyte contained large yolk globules (Yg) and yolk vesicles (Yv). The wall consisted of zona radiata (ZR), granulosa layer (G), basal lamina (BL) and thecal layer (T). Notice the lumen of the ovary lined with flat squamous cells (arrow head). (Heamatoxylin and Eosin, X1000)

Fig. (10c): Photomicrograph of the ovary showing the wall of two mature ovarian follicle, consisted of zona radiata (ZR), granulosa layer (G), basal lamina (BL) and thecal layer (T). (Heamatoxylin and Eosin, X 400)

Figs. (11a, b, c&d): Photomicrograph of the ovary showing stages of migration of nucleus (N) of mature oocytes central position to the animal pole. (Heamatoxylin and Eosin a; X 160) (Heamatoxylin and Eosin b & c; X 100) (Periodic acid Schiff reagent d; X 80).

Fig. (12): Photomicrograph in the ovary showing the atretic follicle. Notice the presence of empty spaces (arrow) and granulose (G) hypertrophied. (Heamatoxylin and Eosin, X 100)

Fig. (13): Photomicrograph in the ovary showing the thick tunica albuginea during the non-breeding season. Notice the presence of strands of smooth muscle fibers (arrow). (Heamatoxylin and Eosin, X 400)

DISCUSSION

The present work was carried out on 58 specimens of *O. niloticus* throughout the year, in order to observe the morphological and histological changes in the ovaries and testes during different seasons of the year. The result showed that the breeding season for reproduction was between April and September, while non-breeding season was between October and March, the current findings simulate those of Caputo, V.; Mesa, M.L.; Candi, G. and Cerioni, P.N. (2003) and Cinquetti and Dramis (2003). The ovarian morphology in the present study resembled that described in anglerfish (Afonso- Dias and Hislop, 1996); the catfish (Gomes and Araujo, 2004) and *Oreochromis mossambicus* (Hatikakoty and Biswas, 2004). As ovaries develop, they present accentuated differences in size and form. The mature stage was well evidenced by its largest volume corresponding to increasing size of cells of the germinative lineage. Variation in the ovarian form occurred from the filiform appearance during the non-breeding season and became large and cylindrical during the breeding season. In addition, the ripe ovaries during the breeding season occupied most of the body cavity and were turgid, orange yellow in color, had a granular appearance due to the presence of mature oocytes that could be seen through the distended translucent gonadal wall. Ovaries of tilapia are of cystovarian type, as the ovarian cavity is connected directly with the oviduct (Alka'abi, 1996), subsequently the *O. niloticus* don't release their mature ova into coelemic cavity. The overall pattern of oocyte development in *O. niloticus* was the same as in other teleost species (Coward and Bromage, 1998). In agreement with Alka'abi (1996), the process of oogenesis in *O. niloticus* classified according to changes in size, nucleus, cytoplasm and egg membranes of the developing ova into six stages beginning with oogonia. In addition, the present study indicated the presence of the six stages for proliferation of oogonia and development of mature ova. These stages are oogonia, chromatin nucleolus stage, perinucleolar stage, yolk vesicle stage, yolk globule stage and mature stage. However, Coward and Bromage (1998) recorded nine stages for oogenesis process in *Tilapia zilli.* The present study revealed that the peak period of oogonial proliferation by mitosis occurred during the immediate post- spawning period, since nests of oogonia were present in greatest abundance reaching 15 oogonia / unit area in April. The current findings are in agreement with that revealed by Mayer; Shackley and Ryland (1988). The result showed that immature oocytes were small in

size and then increased gradually where the nucleoli arranged on the nuclear membrane in the perinucleolar stage (stage 3) and the yolk nucleus appeared at this stage with decrease in basophilia of cytoplasm. The current findings simulate those of Coward and Bromage (1998) & Hatikakoty and Biswas (2004). A characteristic Balbiani's body as that seen by Yoakim (1975); Alka'abi (1996); Hamdoon and Zayed (1998) and Bardakci, F.; Ozansoy, U. and Koptagel, E. (2000) A comparison of oogenesis under constant and fluctuating temperatures in Doctor fish, *Garra rufa* Heckel, 1843 (Teleostei: Cyprinidae). J. Biology., 35(5): 193-212. was clearly demonstrated as basophilic round mass in the cytoplasm of perinucleolar oocytes and was found most commonly either close to the nucleus or the oocyte periphery.

Perinucleolar and yolk vesicle oocytes showed numerous nucleoli. The origin and the role of them are subjects of plausible arguments. Yoakim (1975) found numerous of nucleoli in perinucleolar and yolk vesicle oocytes and some of them were extruded into the surrounding ooplasm. The number and the size of nucleoli decrease in the yolk globule and mature oocytes. He suggested that the nucleolar extrusion has something to do with the process of oogenesis and /or the various metabolic activities taking place in the cell. Further work had to be done to determine the precise role of nucleolar extrusion. Concerning the yolk vesicle stage (stage 4), the present study revealed that this stage was characterized by increase in size of oocyte with appearance of large number of small, clear vacuoles called the yolk vesicles appeared in the periphery of the ooplasm and formation of zona radiata around oocyte, followed by granulosa and thecal cells layer. Similar findings were reported by Coward and Bromage (1998) & Hatikakoty and Biswas (2004). However, Maack and Segner (2003) reported that the ovarian stroma is the responsible tissue for formation of the thecal layer. During the latter part of the yolk globule stage (stage 5), large yolk globules were dispersed throughout the entire ooplasm. Numerous empty vacuoles occurred throughout the ooplasm. Their possible functional role was not mentioned in the available literature. It is possible that the small vesicles located close to the oocyte periphery are cortical alveoli, since their size, shape and distribution are very similar to those of the alveoli at yolk vesicle stage. On the other hand, Hibiya (1982) and Alves, M.M.; Leme Dos Santos, H.S.; Lopes, R.A.; Petenusci, S.O. and Haiyashi, C. (1983)stated that yolk vesicles contained glycoprotein and exhibited a strong positive reaction to PAS stain, while yolk globules

consisted mainly of lipoprotein and gave weak positive reaction to PAS stain.

Yolk globule stage is the most important phase of oocyte development; since it is during this phase, vitellogenesis occurs, resulting in an extensive oocyte growth. Coward and Bromage (1998) and Chmilevskii and Kameneva (2003) stated that oocytes were enlarged chiefly by rapid incorporation of large amounts of exogenous hepatically derived vitellogenin. While Patino (1997); Arockiaraj, *et al.* (2004) and Jalabert (2005) reported that growing ovarian follicles produce steroid hormones. This steroid leaves the follicle via blood vessels supplying the theca cell layer and was transported to the liver where it induced the production of vitellogenin. Vitellogenin was transferred to the ovary via circulation, where it taken up by the oocyte and is deposited as yolk protein which serves as building and energy material after fertilization. The oocytes reach their maximal diameter of about 500 µm.

During the maturation stage (stage 6), the follicular layers became extremely well developed and consisted of a cuboidal granulosa and a single thecal layer, this would reflect the increased steroidogenic function of the granulosa and theca during active vitellogenesis. In addition, the results of the present work showed that during the maturation stage, the nucleus (germinal vesicle) migrated to the animal pole just beneath the oocyte surface and then breakdown. With germinal vesicle breakdown, the protein yolk globules start to coalesce and the oocyte rapidly increases in volume, then the oocyte ovulated into the ovarian lumen and became mature ovum. These findings agreed with that mentioned by Scott (1979); Mylonas, C.C.; Woods III, L.C. and Zohar, Y. (1997); Bardakci, F.; Ozansoy, U. and Koptagel, E. (2000) and Francolini, M.; Lora Lamia, C.; Bonsignorio, D. and Cotelli, F. (2003). In agreement with many authors Grizzle and Rogers, 1976; Coward and Bromage, 1998 and Bardakci, *et al.* (2000), the presence of follicular atresia as shown in the present study seem to be a very common phenomenon of the teleost ovary. The atretic follicles are usually seen during the period of post-spawning in oocytes at any stage, where the follicular cells enlarge and phagocytize the oocytes where not spawned and the connective tissue surround the follicle thickened and become more vascular. The follicles contracted and folded probably as a result of the elastic properties of the theca externa. The result showed that the ovarian wall of tilapia was composed of three layers; the outermost is thin layer of mesothelium, the middle layer is the tunica albuginea, which made up of collagenous, elastic fibers and smooth

muscle cells with some blood capillaries. Tunica albuginea was thin and highly vascular during the breeding season, but became thick during the non- breeding season. This may be due to expansion of the ovary by presence of many large mature oocytes in the breeding season that pressed on the wall. The innermost layer was the germinal epithelium, which projected into ovocoel in the form of finger like ovarian lamellae, that contain many oocytes at different stages of development.

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