

Age Related Seasonal Changes in the Gonadal Activity of the Fish *Notopterus notopterus* (Pallas)

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Abstract: Oogenetic and testicular activity of *N. notopterus* was studied in relation to age and different the seasons in a year. The age was determined based on the annuli on the scale (Scalimetry) per year. Age at first maturity was determined based on the gonadal changes in a seasonal cycle. One year age group of fish during March shows maturing ovary with tertiary nucleolus staged ova 2.3% (2.0 ± 0.21) and migratory nucleus staged ova 1.26% (1.1 ± 0.1) with highest number of oogenetic elements in total it is 86.9 ± 2.53 . In August it is 7.1% of (5.3 ± 0.15) tertiary nucleolus stage and 2.13% (1.6 ± 0.16) of migratory nucleus. Similarly, in March the testis shows spermatids 26.13% (117.8 ± 1.1) and spermatozoa 25.86% (31.5 ± 0.32). In August it is 19.26% (61.5 ± 0.47) of spermatids and 25.86% (117.7 ± 0.42) of spermatozoa. This indicates that the fish attains first maturity in one year age of its life, with moderate gonadal activity. Histological structure of two year matured fish shows all nine stages of oogenetic and spermatogenic elements during spawning period. The ovary shows different stages of oogenetic elements (total 118.3 ± 1.35) bearing 45.98% of tertiary nucleolus staged ovum and 1.68% of migratory nucleus ovum. The lobules of testis shows different stages of spermatogenic elements (total 1077.3 ± 1.57) with abundant spermatozoa i.e., 843.2 ± 0.48 (78.26%) in March and 728.4 ± 0.16 (74.53%) in August. Thus 2-year aged fish showing high degree of spermatogenesis. The degree of gonadal activity is 0 age <1 age <2 age.

Keywords: *Notopterus notopterus*, oogenesis, maturity, age.

1. Introduction

Determination of age and growth in fish by scalimetry has been reported by many workers (Kutty, 1961; Sheshappa and Chakrapani, 1982; Sheshappa Bhirnachar, 1991; Snovsky and Shapiro, 2000). As per Avery (1985) knowledge of size and age at first maturity and potential contribution of each size and age group of fish to recruitment process is particularly relevant to proper choice of angling regulations that will sustain balanced population for present and future generation. Knowledge of age at maturity, maximum age and growth rates is a prerequisite for age-based methods of stock assessment which intern can potentially be used for the management of this species. There are studies aimed to determine (i) Age and growth in fish (ii) length weight relationship (Sheshappa and Bhimachar, 1951, 1955, Sheshappa and Chakrapani 1982; Herrera and Fernandez, 1994, Sulikowski et al. 2009), Seasonal variations in somatic condition, hepatic, gonadal activity and nature of gonads was studied in teleosts (Asuncion et al.2006, Piotr et al.2011). The present study was aimed to determine the relationship between the age, gonadal activity in relation to seasonal cycle.

2. Materials and Methods

The fishes for the study were collected from Farahathabad nala winch receives water from the Bheema River, 10 km away from Gulbarga 300 fishes of different sizes ranging from 12-26 cm were used for the study. Fishes were collected every first week of the month for one year (1999-2000). Soon after the fish were brought to the laboratory they were divided into 3 groups viz.,

Group-I Fishes of length 12-16 cms.

Group-II Fishes of length 17-21 cms.

Group-III Fishes of length 22-26 cms.

All fishes were autopsied on the day of arrival for total length, body weight were measured. Since the fish does not exhibits sexual dimorphism. sex was identified only after cutting open the body cavity Testis and ovary were weighed with an electronic balance and fixed in Bouin's fluid.

At the same time 10 scales were taken from the area behind pectoral fin where largest scales were located. Since the scales are of small in size, they were mounted between two slides and studied with the help of a compound microscope. Transverse sections of less than 5 μ , thickness of the ovary and testes of each fish were taken and stained with Ehrlich's haematoxylin and Eosin. Histological stages in the development of gonadal germ cells was identified in accordance with the description by Yamazaki (1965), Davis (1977), Wallace and Selman (1981) and Nagahama (1983). Differential counts of the cells were made in the entire section.

Transverse section of less than 5 μ thickness of the testis of each fish were taken and stained group Ehrlich's haematoxylin and Eosin. Identification of the male elements was made by employing the criteria of Gokhale (1957), Rai (1965) and Davis (1977). Meopta optical light microscope (Czechoslovakia) offering upto 1500 \times magnification was used for visualizing the testicular cells. The spermatogenic populations were enumerated by using Meopta at 450 \times with a ocular micrometer having a 5 mm² area divided into 25 identical squares. The entire sample of 10 testis / month was thus considered for calculating the mean number and percentage of the germ cells / 5 mm² area.

The data from histological examination of the ovaries and testes were averaged since the tissue was obtained from 10 fishes. The monthly ($X \pm SD$) values were also based on 10 individuals in each group (i.e., $n=10$).

3. Results

The morphological observations and classification of maturity stages of gonads spread over a period of 12 months have been categorised. Since the fish *N. notopterus* is a fractional spawner, the seasonal cycle can be divided into three phases.

- 1) Prespawning phase (February and July).
- 2) Spawning phase (March and August)
- 3) Resting phase (September to January).

Oogenetic activity in '0' year age group of fish:

The transverse sections of ovaries of fish of '0' year age group with one annuli (Fig. 1), showed the ova in immature stage. At this stage it is difficult to differentiate different phases of germinal cells in the ovary and testis as then were packed with early stages of germinal elements

Gonadal activity in '1' year age group of fish:

One year age group fishes with one annuli (Fig. 2 and 4) measuring 17-21 cm in length were in maturing stage. The histological structure of this stage shows the nine stages of oogenetic activity, namely (i) Oogonium (ii) Chromatin nucleolus (iii) Early perinucleolus (iv) Late perinucleolus (v) Cortical alveoli (vi) Primary yolk globule stage (vii) Secondary yolk globule stage (viii) Tertiary nucleolus stage (ix) Migratory nucleus (Table. 1). During March (spawning phase) increased number of tertiary nucleolus stage ova i.e., 2.3% observed (March and August) (Table-1). The histological structure of this stage shows that, the testicular lobules filled with all stages of spermatogenic activity during spawning phase (Table-3).

During August also, there was an increase of tertiary nucleolus staged ova (7.1%), while the migratory nucleolus staged ova were 1.26% in March and 2.1% in August. During March (spawning) quantitative increase in the number of spermatids i.e., 26.13% (117.8 ± 1.1) and spermatozoa i.e., 25.86% (116.6 ± 0.47) was observed. During August also there increase in upto 19.26% (61.5 ± 0.47) of spermatids and 35.29% (112.7 ± 0.42) suggesting that fish matures in its one year age of life (Table-1).

Gonadal activity in '2' year aged fish:

Two-year age group of fish (Fig.3) measuring 22-26 cm were in completely matured stage, and show comparatively high degree of oogenetic activity. The ovary shows all the stages of oogenesis. During spawning phase high count of tertiary nucleoli and migratory nucleus stage ova were seen. In March 45.98% of tertiary nucleolus staged ova and only 1.68% of migratory nucleus were seen. Again in August, there is an increase up to 53.65% of tertiary nucleolus staged ova and 2.76% of migratory nucleus staged ova.

Comparatively high numbers of oogenetic elements were observed in the ovaries of two year age group fish (Table-2).

The fish of 2-year age showing two annuli on its scale (Fig. 3) shows comparatively high spermatogenic activity. The lobules of testis show the cysts containing different stages of spermatogenesis, such as primary and secondary spermatogonia, spermatocytes and spermatids, spermatozoa. During spawning phase high count of spermatids (68.1 ± 0.03 i.e., 6.32%) and spermatozoa (843 ± 0.48 i.e., 78.26%) were observed in the month of March. During August spermatids are (69.1 ± 0.27) i.e., 7.07% and spermatozoa were 74.53% (28.4 ± 0.16) (Table-4). qualitatively more spermatogenic elements were seen in 2-year age group fish than 0 and one year aged fishes.

4. Discussion

The reproductive cycle of the teleosts is a seasonal phenomenon and the gonads vary in their appearance. It has been excellently reviewed by several workers like Nagahama (1983), Munro (1990), Malhotra et al. (1989), Qasim (1973), Munnar et al. (2010), P. Hliwa et al. (2011) etc. It is stated that most of the tropical fishes have a short life span of two to three years and mature when they are 1-2 years old except for a few species.

Determination of age based on the annuli or growth rings on scales was followed by many workers like Le Cren (1977), Sheshappa and Bhimachar (1951, 1955), Hora and Pillay (1962), Alikunhi (1957) etc., but the age related changes of the gonadal activity in the seasonal cycle of this fish *N. notopterus* was not reported. The ovary of *N. notopterus* studied during prespawning phase exhibits nine oogenetic stages of oocytes which are in agreement with the classification of oogenetic stages by Yamamoto et al. (1965). Oocyte growth followed similar pattern in most teleosts (Arockiaraj et al. 2004). In *N. notopterus* oogenesis begins during January and continues up to March and again it starts from June and reaches the maximum during August in both the one and two year age group of fishes. The percentage of tertiary nucleolus stages in both age group of fishes was less in the spawning phases during March and August Table-1 and 2. The reduction is due to their conversion to more mature stages.

Such reduction of immature stages and development of more and more mature ova during prespawning period was observed in many other Indian teleosts (Lehri, 1967, Sundarabarathy et al. 2004), in *C. balrachus* Biswas et al. (1984), in labeo species and also Malhotra et al. (1989). It was evident that each individual of *N. notopterus* spawns twice in a breeding season as found in *Puntius sophore* (Dixit and Agarwal, 1974), *Peropthalmus barbarus* (King and Udo, 2001) and in the fishes of Caribbean sea (Arocha and Marciano, 2008).

In conclusion, the results of the present study clearly suggests that, in the fish *N. notopterus* growth rings are formed annually or age can be determined by counting the rings on the scales. Both female and male fish attains sexual maturity after completion of one year of age, the breeding activity including growth of gonads, active vitellogenesis

and spermatogenesis along with spawning activity begins in the age of fish belonging to 1-2 years.

Seasonal cyclical changes in the gonads of *N. notopterus* shows all oogenetic and spermatogenic elements during pre-spawning and spawning periods.

5. Future Prospects of our Study

Knowledge of size and age at first maturity and potential contribution of each size and age group of fish to recruitment process is particularly relevant to proper choice of angling regulations that will sustain balanced population for present and future generation. Knowledge of age at maturity, maximum age and growth rates is a prerequisite for age-based methods of stock assessment which intern can potentially be used for the management of fish species

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Figure 1: Showing cycloid scale of '0' year aged fish without concentric rings $\times 128$.

Figure 2 & 4: Showing the cycloid scale of '1' year aged fish with 1 annuli (1 arrow) $\times 128$.

Figure 3: Showing the cycloid scale of '2' year aged fish with 2 annuli (2 arrows) $\times 128$.

Table 1: Seasonal variations in the Oogenetic activity in the female fish *Notopterus notopterus* (One year age group).

Months	Oogonium	Chromatin nucleolus	Early peri nucleolus	Late peri nucleolus	Cortical alveoli	Primary yolk globule	Secondary yolk globule	Tertiary nucleolus	Migratory nucleus	Total
January	13.2 ± 0.51 (20.61)	10.9 ± 0.34 (17.08)	23.0 ± 0.55 (36.06)	10.5 ± 0.54 (16.45)	3.0 ± 0.21 (4.70)	3.2 ± 0.35 (5.01)	--	--	--	63.8 ± 2.5
February	4.9 ± 0.27 (10.1)	21.5 ± 0.34 (44.33)	7.9 ± 0.27 (16.29)	2.0 ± 0.29 (4.12)	--	1.2 ± 0.13 (2.47)	± 0.33 (22.68)	--	--	48.5 ± 1.63
March	13.6 ± 0.37 (15.65)	27.6 ± 0.49 (31.76)	12.5 ± 0.52 (14.38)	14.3 ± 0.36 (16.46)	14.4 ± 0.37 (16.57)	--	1.4 ± 0.51 (1.61)	2.0 ± 0.21 (2.3)	1.1 ± 0.1 (1.26)	86.9 ± 2.83
April	10.4 ± 0.3 (16.99)	4.1 ± 0.27 (6.7)	34.5 ± 0.45 (56.37)	12.2 ± 0.24 (19.9)	--	--	---	--	--	61.2 ± 1.26
May	14.0 ± 0.36 (31.25)	9.9 ± 0.23 (22.1)	20.9 ± 0.48 (46.65)	---	---	---	-----	----	----	44.8 ± 1.07
June	5.5 ± 0.16 (7.1)	11.9 ± 0.23 (15.37)	23.8 ± 0.29 (30.75)	17.2 ± 0.2 (22.22)	17.7 ± 0.42 (22.86)	1.3 ± 0.15 (1.68)	---	---	---	77.4 ± 1.45
July	13.8 ± 0.24 (16.83)	25.8 ± 0.32 (31.46)	11.4 ± 0.26 (13.9)	12.8 ± 0.2 (15.61)	± 0.25 (2.44)	± 0.1 (1.34)	1.2 ± 0.22 (1.46)	13.0 ± 0.25 (15.85)	0.9 ± 0.17 (1.1)	82.0 ± 1.84
August	2.4 ± 0.16 (3.2)	14.9 ± 0.23 (19.84)	20.5 ± 0.16 (27.3)	23.7 ± 0.21 (31.56)	5.2 ± 0.02 (6.92)	1.5 ± 0.16 (1.99)	--	5.3 ± 0.15 (7.1)	1.6 ± 0.16 (2.13)	75.1 ± 1.27
September	12.8 ± 0.2 (23.49)	8.0 ± 0.25 (14.68)	22.8 ± 0.32 (41.83)	10.9 ± 0.73 (2.0)	--	----	----	----	---	54.5 ± 1.0
October	6.0 ± 0.25 (10.23)	24.6 ± 0.26 (41.8)	22.5 ± 0.16 (38.39)	5.5 ± 0.16 (9.4)	--	--	--	--	--	58.6 ± 0.83
November	21.6 ± 0.22 (36.73)	15.0 ± 0.14 (25.5)	22.2 ± 0.2 (37.76)	---	---	---	---	---	--	58.8 ± 0.56
December	8.3 ± 0.15 (18.65)	18.0 ± 0.25 (40.45)	12.1 ± 0.27 (27.19)	6.1 ± 0.17 (13.71)	---	---	---	---	---	44.5 ± 0.84

Values are means ± S E for each Month. Values in parentheses are percentage of cells. Each value denotes the number of percentage of cells in an average transverse section of the ovary. Monthly values are calculated separately for each of 10 ovaries (n = 10). Ten sections / ovary were scored to obtain the average value for each cell type.

Table 2: Seasonal variations in the Oogenetic activity in the female fish *Notopterus notopterus* (Two year age group).

Months	Oogonium	Chromatin nucleolus	Early peri nucleolus	Late peri nucleolus	Cortical alveoli	Primary yolk globule	Secondary yolk globule	Tertiary nucleolus	Migratory nucleus	Total
January	5.8 ± 0.13 (07.16)	12.3 ± 0.21 (15.18)	23.4 ± 0.22 (28.9)	10.4 ± 0.22 (12.84)	15.2 ± 0.2 (18.76)	5.3 ± 0.15 (06.54)	8.6 ± 0.16 (10.62)	-	-	81.0 ± 1.29
February	3.11 ± 0.2 (03.6)	10.66 ± 0.44 (12.25)	12.22 ± 0.14 (14.12)	10.66 ± 0.33 (12.32)	4.33 ± 0.23 (5.0)	1.22 ± 0.14 (1.4)	3.33 ± 0.16 (3.85)	37.33 ± 0.33 (43.15)	3.66 ± 0.16 (04.23)	86.5 ± 3.47
March	5.7 ± 0.15 (4.84)	4.6 ± 0.16 (3.89)	10.6 ± 0.26 (8.96)	31.5 ± 0.22 (26.6)	8.3 ± 0.21 (7.0)	--	1.2 ± 0.13 (1.01)	54.4 ± 0.22 (45.98)	2.0 ± 0 (1.68)	118.3 ± 1.35
April	12.4 ± 0.221 (16.12)	11.0 ± 0.298 (14.3)	40.9 ± 0.23 (53.18)	12.6 ± 0.22 (16.38)	--	--	--	--	--	76.9 ± 0.96
May	5.72 ± 0.41 (13.48)	13.0 ± 0.23 (30.65)	23.7 ± 0.19 (55.87)	--	--	--	--	--	--	42.42 ± 0.83
June	7.3 ± 0.15 (9.24)	14.9 ± 0.23 (18.85)	21.3 ± 0.3 (26.95)	12.5 ± 0.22 (15.82)	21.33 ± 0.55 (26.99)	1.7 ± 0.15 (2.15)	--	--	--	79.03 ± 1.95
July	7.8 ± 0.24 (7.7)	6.8 ± 0.24 (6.72)	15 ± 0.25 (14.82)	17.2 ± 0.24 (16.99)	4.6 ± 0.26 (4.55)	1.2 ± 0.2 (1.19)	3.1 ± 0.2 (3.06)	43.2 ± 0.32 (42.69)	2.3 ± 2.3 (2.27)	101.2 ± 2.25
August	2.8 ± 0.13 (1.84)	8.1 ± 0.23 (5.32)	12.6 ± 0.26 (8.28)	32.8 ± 0.24 (21.56)	7.5 ± 0.16 (4.93)	--	2.5 ± 0.16 (1.64)	81.6 ± 0.16 (53.65)	4.2 ± 0.2 (2.76)	152.1 ± 1.54
September	15.6 ± 0.15 (15.24)	17.3 ± 0.24 (16.9)	57 ± 0.47 (55.69)	12.45 ± 0.2 (12.16)	--	--	--	--	--	102.35 ± 1.06
October	7.8 ± 0.2 (10.29)	14.9 ± 0.23 (19.66)	47.6 ± 0.26 (62.8)	5.5 ± 0.16 (7.23)	--	--	--	--	--	75.8 ± 0.85
November	18.2 ± 0.2 (23.33)	13.6 ± 0.3 (17.44)	46.2 ± 0.32 (59.23)	--	--	--	--	--	--	78.0 ± 0.83
December	18.2 ± 0.22 (27.33)	15 ± 0.21 (22.52)	22.5 ± 0.22 (33.78)	10.9 ± 0.34 (16.34)	--	--	--	--	--	66.6 ± 0.89

Values are means ± S E for each Month. Values in parentheses are percentage of cells. Each value denotes the number of percentage of cells in an average transverse section of the ovary. Monthly values are calculated separately for each of 10 ovaries (n = 10). Ten sections / ovary were scored to obtain the average value for each cell type.

Table 3: Seasonal variations in the spermatogenic activity in the male fish *Notopterus notopterus* (one year age group)

Months	Primary spermatogonia	Secondary spermatogonia	Primary spermatocytes	Secondary spermatocytes	Spermatids	Spermatozoa	Total Number
January	12.66 ± 0.22 (4.76)	14.5 ± 0.22 (5.45)	116.2 ± 1.24 (43.66)	122.8 ± 0.77 (46.14)	--	--	266.16 ± 2.45
February	22.2 ± 0.78 (6.36)	35.2 ± 0.24 (10.8)	42.0 ± 0.39 (12.03)	153.5 ± 0.8 (43.98)	62.6 ± 0.61 (17.93)	33.5 ± 0.52 (9.52)	349.0 ± 3.34
March	14.7 ± 0.21 (3.26)	15.2 ± 0.2 (3.37)	32.7 ± 0.3 (7.25)	153.8 ± 0.8 (34.11)	117.8 ± 1.1 (26.13)	116.6 ± 0.47 (25.86)	450.8 ± 2.81
April	51.7 ± 0.3 (21.75)	58.7 ± 0.39 (24.69)	12.6 ± 0.22 (5.30)	114.7 ± 0.47 (48.25)	--	--	237.7 ± 1.38
May	41.81 ± 0.37 (21.75)	114.4 ± 0.43 (57.63)	42.27 ± 0.33 (21.29)	--	--	--	198.48 ± 1.13
June	123.1 ± 0.43 (41.60)	14.4 ± 0.26 (4.86)	35 ± 0.25 (11.82)	63.4 ± 0.47 (21.42)	42.5 ± 0.3 (14.36)	17.5 ± 0.52 (5.91)	295.9 ± 2.23
July	12.0 ± 0.5 (2.91)	12.5 ± 0.16 (3.02)	133.7 ± 0.49 (32.31)	92.1 ± 0.69 (22.34)	115.3 ± 0.3 (27.97)	46.6 ± 0.47 (11.30)	412.2 ± 2.61
August	22.2 ± 0.44 (6.95)	24.1 ± 0.4 (7.54)	16.9 ± 0.34 (5.29)	81.9 ± 0.27 (25.52)	61.5 ± 0.47 (19.26)	112.7 ± 0.42 (35.29)	319.3 ± 2.34
September	58.4 ± 0.22 (22.78)	183.2 ± 0.41 (71.47)	14.7 ± 0.22 (5.37)	--	--	--	256.3 ± 0.84
October	41.6 ± 0.266 (23.03)	126.7 ± 0.44 (70.11)	12.4 ± 0.22 (6.86)	--	--	--	180.7 ± 0.926
November	25.6 ± 0.16 (12.92)	131.8 ± 0.38 (66.53)	40.7 ± 0.36 (20.54)	--	--	--	198.1 ± 0.9
December	49.1 ± 0.31 (30.27)	113.1 ± 0.3 (80.82)	--	--	--	--	162.2 ± 0.61

Values are means ± S E for each Month. Values in parentheses are percentage of cells. Each value denotes the number of percentage of cells in a 5 mm area of the testicular section. Monthly values are calculated separately for each of 10 testis (n = 10) . Ten sections / testis were scored to obtain the average value for each cell type.

Table 4: Seasonal variations in the spermatogenic activity in the male fish *Notopterus notopterus* (Two age group).

Months	Primary spermatogonia	Secondary spermatogonia	Primary spermatocytes	Secondary spermatocytes	Spermatids	Spermatozoa	Total Number
January	25.1 ± 0.23 (6.59)	20.7 ± 0.3 (5.43)	192.2 ± 0.24 (50.49)	142.6 ± 0.42 (37.46)	--	--	380.6 ± 1.19
February	23.5 ± 0.16 (2.65)	42.6 ± 0.4 (4.81)	53.5 ± 0.22 (4.96)	68.0 ± 0.2 (7.69)	185.1 ± 0.56 (20.9)	512.6 ± 0.37 (57.90)	885.3 ± 1.91
March	23.5 ± 0.16 (2.18)	25.0 ± 0.25 (2.32)	26.8 ± 0.24 (2.48)	90.7 ± 0.213 (8.41)	68.1 ± 0.23 (6.32)	843.2 ± 0.48 (78.26)	1077.3 ± 1.57
April	116.2 ± 0.41 (41.96)	107.6 ± 0.3 (38.85)	21.8 ± 0.35 (7.87)	31.3 ± 0.15 (11.30)	--	--	276.9 ± 1.21
May	85.9 ± 0.23 (28.7)	131.9 ± 0.4 (44.09)	81.3 ± 0.21 (27.18)	--	--	--	299.1 ± 0.84
June	27.4 ± 0.3 (3.33)	32.4 ± 0.26 (3.94)	67.1 ± 0.45 (8.16)	152.3 ± 0.26 (18.53)	127.8 ± 0.249 (15.54)	414.9 ± 0.34 (50.48)	821.9 ± 1.85
July	17.5 ± 0.16 (1.86)	35.6 ± 0.16 (3.79)	45.4 ± 0.16 (4.83)	88.3 ± 0.36 (9.41)	187.7 ± 0.3 (20.0)	563.7 ± 0.53 (60.08)	938.2 ± 1.67
August	27.7 ± 0.39 (2.83)	20.5 ± 0.16 (2.09)	23.4 ± 0.16 (2.39)	108.2 ± 0.24 (11.07)	69.1 ± 0.27 (7.07)	728.4 ± 0.16 (74.53)	977.3 ± 1.38
September	117.9 ± 0.17 (43.37)	127.4 ± 0.16 (46.87)	26.5 ± 0.26 (9.74)	--	--	--	271.8 ± 0.59
October	86.2 ± 0.2 (22.73)	142.8 ± 0.29 (37.66)	87.6 ± 0.33 (23.10)	62.5 ± 0.22 (16.48)	--	--	379.1 ± 1.04
November	48.7 ± 0.21 (16.34)	162.6 ± 0.3 (54.58)	69.0 ± 0.21 (23.16)	17.6 ± 0.22 (5.90)	--	--	297.9 ± 0.94
December	32.0 ± 0.14 (19.42)	132.7 ± 0.47 (80.57)	--	--	--	--	164.7 ± 0.61

Values are means ± S E for each Month. Values in parentheses are percentage of cells. Each value denotes the number of percentage of cells in a 5 mm area of the testicular section. Monthly values are calculated separately for each of 10 testis (n/ month = 10). Ten sections / testis

were scored to obtain the average value for each cell type.

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