

## Age-Related Changes in Ovarian Gross and Histological Characteristics During Pubertal Development in Captive *Catla catla* (Hamilton, 1822) of Age 18-29 Months

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**Abstract.** Annual reproductive cycle of female *Catla catla* was investigated during the age of 18-29 months through gross and histological studies. *Catla* is a warm water gonochoristic summer spawner. Ovarian development is quite fast with the increase in the temperature (above 29°C) and photoperiod (above 13.50 H) in April. Peak GSI (13.00±9.30 %) was observed in June concomitant with the maximum water temperature and photoperiod. On the basis of gross and histological studies, seven ovarian stages namely, immature/resting, regenerating, developing, maturing, mature/gravid, regressing and regressed were distinguished. Based on GSI studies, spawning seem to last for a very short period (mid June-mid July). Histological studies revealed six stages of oocyte development namely, chromatin nucleolar, perinucleolar, cortical alveolar, early vitellogenic, late vitellogenic and early germinal vesicle movement. Final cue for spawning seems to be the heavy monsoon rains. However, spawning in captivity was not observed in the present study as no final oocytes maturation or post-ovulatory follicles were seen. Total fecundity was measured in May and June separately. Relative fecundity was noted as 2537.80±857.21 oocytes/g of ovary amounting to 41.33±8.66 oocytes/g body weight in May of fish with 1861.40±138.70 g body weight and 49.83±2.05 cm total length. These values for the June fish were 1183.61±681.72/g ovary and 121.73±81.49/g body weight for fish of 1935.00±225.70 gram average body weight and 49.67±1.69 cm total length. Atretic follicles were quite prevalent in regressing ovaries. Since this is the first endeavor from Pakistan, results are discussed in the light of available literature and in comparison with the published work on this species elsewhere.

**Key words:** Age-at-maturity, Annual cycle, GSI, hepatosomatic index, oogenesis, photoperiod, reproductive biology, temperature.

### INTRODUCTION

There is very little awareness about the role of animal proteins in general and fish based proteins, in particular, in health and well being in the Pakistani population. However, some knowledge in this regard and importance of fish in human nutrition is trickling down to the local population. This trend has made fish culture a very attractive and profitable business in Pakistan.

Reproduction in the most free-living vertebrates, including fish, is periodic. It ensures an optimal timing for the young ones produced and, as

a consequence, maximizes the chances of survival of species. Reproductive biology of fish has long been a widely investigated field (Murua and Saborido-Rey, 2003; Murua *et al.*, 2003; Orlando *et al.*, 2003; Sisneros *et al.*, 2004).

Fish living in the temperate zone exhibit an annual reproductive cycle (Nash, 1999). Therefore, reproduction is closely related to the environment which directly influences the gametogenesis and spawning (Wen and Lin, 2001; Tollefsen *et al.*, 2002; Lubzens *et al.*, 2010; Schulz *et al.*, 2010). Seasonal changes in reproductive cycles have been reported for several species of teleosts (Fostier *et al.*, 1983; Nagahama, 1994; Bromage *et al.*, 2001; Lone and Hussain *et al.*, 2009; Lone *et al.*, 2001, 2008a, b; 2009).

Puberty is a state of development in the life of a creature when it becomes capable of reproducing, for the first time, through sexual maturation by transforming from immature to mature individual. This definition has been used invariably by the fish biologists also and points to the functional competence of the brain-pituitary-gonad axis

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(Okuzawa, 2002; Jalabert, 2005; Dufour and Rousseau, 2007). Age and size at puberty varies between species and even within the same species and is dependent upon genetic differences, nutrition, and environmental parameters. Puberty is initiated by the activation of the brain-pituitary-gonad axis and is very dependent on the somatic growth of the individuals in a population (Taranger *et al.*, 2010). Once the puberty sets in, the growth of the fish is retarded due to the repartitioning of the dietary energy between soma and gonads. Therefore, early puberty can be detrimental to the commercial culture of a species while delayed puberty can hinder reproductive control and hatchery operations. This has been seen many times in studies with the culture of temperate species (Longalong *et al.*, 1999; Gines *et al.*, 2003, 2004; McClure *et al.*, 2007; Felip *et al.*, 2008).

The major carp, *Catla catla*, is one of the favorite and economically important fish in Pakistan. It is found in Pakistan, India, Bangladesh, Sri Lanka, and Burma (Costa-Pierce, 2005). It is an annual breeder and in nature attains maturity in the second year of life. Wild catla breeds during monsoon (June-September) in shallow pockets and in marginal areas adjacent to the rivers which are flooded after heavy showers (Khan, 1924; Mookerjee, 1945; Ahmed, 1955; Duby and Tuli, 1961).

There is no detailed information available regarding gross changes and histology of gonads during the first maturational cycle in *Catla catla*. Recently, some publications have appeared from Indian south, with tropical to subtropical environment, on the reproduction of this fish in the wild (Dey *et al.*, 2004, 2005; Bhattacharya *et al.*, 2005; Nandi *et al.*, 2007). Present detailed studies are first of its kind from Pakistan and were done on fish cultured in ponds for commercial purpose. We hope that the results obtained will be directly beneficial and applicable to the fish farm practices in Pakistan.

## MATERIALS AND METHODS

### *Collection of fish*

Fish were reared at and collected from a commercial fish farm 40 Km north of Lahore

(Latitude 31° 37' N, Longitude 74° 13' E). At the start of the studies, *i.e.*, November, 2006, the fish were 18 months of age. The study ended in October, 2007 when the fish were 29 months of age. At least, 24 hours before the sampling, the fish were collected from the farm and brought to the laboratory and kept in cemented fish tanks (4.12 m (L) × 1.7 m (W) × 1.0 m (D)), to be relieved from the stress of capture and haulage. Every month, ten (10) female fish were randomly sampled. Fish could not be distinguished into male and female due to lack of sexual dimorphism, therefore, this number was some times higher in order to get the required number of fish.

### *Physical parameters*

Water and atmospheric temperature were measured at the time of sampling. pH of water and dissolved oxygen were also noted. Other environmental data were obtained from the Meteorological Department, Govt. of Pakistan's Lahore office.

### *Sampling and anesthetization process*

At the time of sampling, a single fish was caught by scoop net and anesthetized immediately. Clove oil was used as anesthesia (Berka, 1986; Kaiser *et al.*, 2006). Anesthesia was always prepared fresh by dissolving the clove oil into absolute alcohol (Merck, Germany) in a ratio of 1:2. This solution was mixed with water and fish was dipped into it till sedated completely. Concentration and time period of anesthesia was changed according to the age, size and resistance of the fish.

### *Morphological parameters*

Total body weight (g), total body length (cm), standard length and body depth (cm) were measured before dissection.

### *Dissection and removal of organs*

Lower abdomen of fish was cut from its posterior to anterior end to remove ovaries. Morphological features of ovaries like its color, thickness, blood vessels, position and color of the opening of oviduct and any visible abnormality in ovaries shape was noted and photographed *in situ*. After removal, gonads were immediately weighed and preserved in 10% buffered formalin following

Troyer (1980). Similarly, liver was also removed, weighed and frozen at  $-20^{\circ}\text{C}$ .

The gonadosomatic index (GSI) and hepatosomatic index was calculated as Tissue Index =  $100 W_T \cdot W^{-1}$  where  $W_T$  is weight of tissue and  $W^{-1}$  is total body weight of fish (Lone and Al-Marzouk, 2000).

### Histological studies

Tissues were processed for routine haematoxyline-eosine staining. Stained slides were microphotographed by a high resolution microscope (Leica, Japan) fitted with a digital camera. Thickness of tunica, diameters of germ cells, nuclei, nucleoli etc. were measured by ocular micrometry.

### Fecundity

Total fecundity was estimated as standing stock of advanced yolked oocytes in the ovary following Almatar *et al.* (2004). Pieces of 100 mg were taken from right and left ovary separately. Eggs were separated with help of brush and then counted in left and right piece of ovary. Mean of these two values was computed to calculate the total number of ova in both ovaries. Relative fecundity was calculated following Murua *et al.* (2003) and Narimatsu *et al.* (2007). The fecundity studies were performed on fish from May and June separately.

## RESULTS

### Environmental and somatic parameters

The details of the environmental parameters are given in Figure 1. Total body weight, length, ovarian weight, liver weight and their indices are given in Table I and Figure 1.

### Gross structure of the ovary

*Catla catla* is gonochoristic species with no sexual dimorphism between male and female fish. Externally, sex could be recognized only during breeding season when female shows a fully bulged, soft abdomen and red bulging cloacal region. The female reproductive system consisted of a pair of ovaries, suspended antero-posteriorly in the peritoneal cavity and attached to the dorsal air bladder by thin mesenteries. Ovaries joined together posteriorly, sharing a single oviduct which opened into cloaca through genital pore along with the

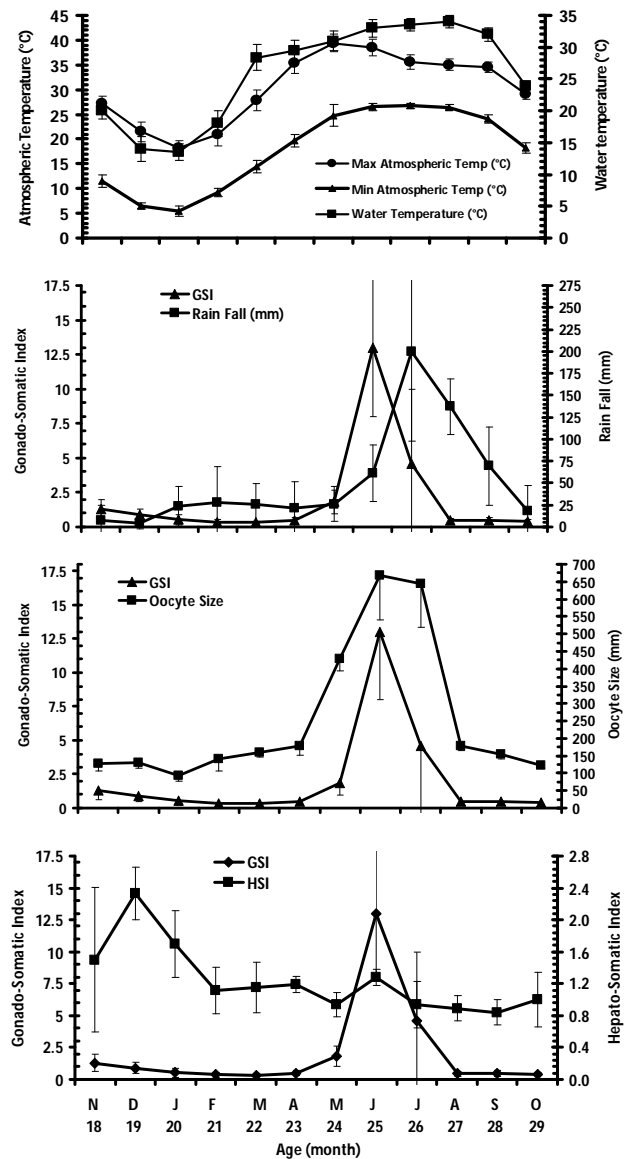


Fig. 1. Environmental parameters and age-related variations in gonado-somatic index (GSI), oocyte size (mm) and hepato-somatic index (HSI) of *Catla catla* reared in ponds. Values given are mean  $\pm$  SD. For more details see Materials and Methods.

openings of gut and ureters. Oviduct was very thin and narrow white tube, which varied in its length and prominence seasonally. The length and girth of both ovaries were not always equal. Their cephalic end was thicker when compared with the caudal end. Externally, each ovary was covered by thin peritoneal membrane, beneath the peritoneal layer

lies tunica albugenia. Tunica albugenia becomes thinner as fish reaches full maturity (Fig. 2A; 2B).

*Annual ovarian cycle*

*November (18 months)*

Ovaries in November were very thin and white, with very little blood supply. The GSI was  $1.28 \pm 0.67\%$ . Histologically, ovaries were at rest, containing only primary oocytes (POC) at chromatin nucleolar stage, having diameter of  $129.04 \pm 22.30 \mu\text{m}$ . Tunic was thick ( $178.56 \mu\text{m}$ ) (Table I; Fig. 2A)

*December (19 months)*

Gross and histological structure of gonads in December did not differ from that in November. GSI was  $0.90 \pm 0.42\%$ . POCs ( $131.00 \pm 15.23 \mu\text{m}$ ) were at chromatin nucleolar stage. Tunic was thick enough ( $178.81 \pm 35.24 \mu\text{m}$ ). There was no mitotic activity in ovaries (Table I; Fig. 2A).

*January (20 months)*

January was the month when the recruitment started beginning with initiation of mitosis. Apparently, ovaries were immature, thin and with little blood supply. GSI was  $0.77 \pm 0.40\%$ . Tunic became thinner ( $46.72 \pm 6.23 \mu\text{m}$ ) because of mitotic activity and increase in POC size. POCs were at chromatin nucleolar and perinucleolar stages, having diameter of  $93.00 \pm 0.40 \mu\text{m}$ . Temperature and photoperiod were still low (Table I; Fig. 2A).

*February (21 months)*

February, showed similar gross features as in January. GSI decreased slightly and was  $0.31 \pm 0.41\%$ . Most of the POCs were at perinucleolar stage ( $141.18 \pm 34.00 \mu\text{m}$ ) with a single large nucleus ( $60.00 \pm 16.19 \mu\text{m}$ ). The size of the nucleoli ( $22.00 \pm 12.35 \mu\text{m}$ ) increased as compared to the previous months showing active nuclear activity.

*March (22 months)*

Water temperature increased from the minimum  $13.50 \pm 1.25 \text{ }^\circ\text{C}$  in January to  $28.40 \pm 1.99 \text{ }^\circ\text{C}$ . Photoperiod while reached  $13.03 \pm 0.03$  hours during this month. These changes reflected positively on the ovarian growth (Fig. 1). GSI ( $0.35 \pm 0.09\%$ ) was not higher than that in February because of faster increase in body weight than

**Table I.- Monthly variations (Mean  $\pm$  SD) in body and ovarian parameters of female *Cailla catla* during the study period (2006-2007).**

Months (Age)	Body weight (g)	Total length (cm)	Condition factor (k)	Body width (cm)	Ovarian weight (g)	Ovarian length (cm)	Ovarian girth (cm)	Oocytes size ( $\mu\text{m}$ )	GSI	Liver weight (g)	HSI
Nov. (18)	762.00 $\pm$ 37.06	48.35 $\pm$ 2.14	0.56 $\pm$ 0.21	16.50 $\pm$ 1.90	10.89 $\pm$ 0.85	12.94 $\pm$ 0.75	04.00 $\pm$ 0.48	129.04 $\pm$ 22.30	1.28 $\pm$ 0.67	16.80 $\pm$ 1.60	1.50 $\pm$ 0.91
Dec. (19)	690.12 $\pm$ 52.14	46.46 $\pm$ 1.85	0.68 $\pm$ 0.05	14.87 $\pm$ 1.21	06.16 $\pm$ 2.60	12.85 $\pm$ 0.92	03.84 $\pm$ 0.71	131.00 $\pm$ 15.23	0.90 $\pm$ 0.42	16.12 $\pm$ 2.61	2.33 $\pm$ 0.33
Jan. (20)	1088.64 $\pm$ 226.80	48.83 $\pm$ 2.84	0.93 $\pm$ 0.15	16.08 $\pm$ 1.94	08.39 $\pm$ 5.81	13.67 $\pm$ 1.15	04.40 $\pm$ 0.72	93.00 $\pm$ 15.00	0.77 $\pm$ 0.40	18.68 $\pm$ 5.94	1.70 $\pm$ 0.42
Feb. (21)	1520.00 $\pm$ 482.40	48.25 $\pm$ 2.87	1.32 $\pm$ 0.18	15.87 $\pm$ 0.73	04.79 $\pm$ 2.28	12.75 $\pm$ 0.80	03.75 $\pm$ 0.60	141.18 $\pm$ 34.00	0.31 $\pm$ 0.41	16.07 $\pm$ 2.77	1.11 $\pm$ 0.29
March (22)	1407.00 $\pm$ 179.74	45.26 $\pm$ 2.02	1.52 $\pm$ 0.19	14.23 $\pm$ 1.06	05.06 $\pm$ 1.61	11.40 $\pm$ 1.51	03.42 $\pm$ 1.09	161.00 $\pm$ 14.00	0.36 $\pm$ 0.09	15.75 $\pm$ 2.24	1.15 $\pm$ 0.32
April (23)	1617.85 $\pm$ 336.08	48.07 $\pm$ 3.58	1.44 $\pm$ 0.04	17.36 $\pm$ 1.47	09.92 $\pm$ 4.96	13.00 $\pm$ 2.00	05.00 $\pm$ 0.26	179.32 $\pm$ 28.27	0.60 $\pm$ 0.21	17.41 $\pm$ 5.50	1.19 $\pm$ 0.10
May (24)	1979.00 $\pm$ 247.84	51.00 $\pm$ 3.00	1.50 $\pm$ 0.07	15.75 $\pm$ 1.46	30.32 $\pm$ 18.80	13.32 $\pm$ 1.00	16.25 $\pm$ 3.18	429.0 $\pm$ 35.07	1.57 $\pm$ 0.92	18.31 $\pm$ 2.89	0.94 $\pm$ 0.15
June (25)	2192.00 $\pm$ 287.14	50.75 $\pm$ 3.18	1.68 $\pm$ 0.12	23.50 $\pm$ 0.90	199.00 $\pm$ 23.13	19.33 $\pm$ 1.87	19.33 $\pm$ 1.87	667.43 $\pm$ 128.40	13.00 $\pm$ 9.30	28.08 $\pm$ 5.72	1.28 $\pm$ 0.10
July (26)	2144.89 $\pm$ 119.17	50.97 $\pm$ 1.31	1.62 $\pm$ 0.10	22.14 $\pm$ 1.00	98.16 $\pm$ 47.43	14.44 $\pm$ 3.07	08.11 $\pm$ 4.25	645.00 $\pm$ 126.00	4.57 $\pm$ 2.21	20.06 $\pm$ 5.56	0.94 $\pm$ 0.29
Aug. (27)	1820.84 $\pm$ 244.87	48.72 $\pm$ 1.10	1.59 $\pm$ 0.25	20.13 $\pm$ 0.48	10.18 $\pm$ 6.38	11.83 $\pm$ 2.62	04.64 $\pm$ 1.18	177.46 $\pm$ 13.00	0.45 $\pm$ 0.18	15.85 $\pm$ 1.47	0.89 $\pm$ 0.16
Sept. (28)	1232.26 $\pm$ 152.98	48.78 $\pm$ 2.73	1.06 $\pm$ 0.14	17.14 $\pm$ 2.37	04.50 $\pm$ 3.03	12.66 $\pm$ 1.12	03.53 $\pm$ 0.48	155.60 $\pm$ 15.65	0.46 $\pm$ 0.24	10.56 $\pm$ 3.15	0.84 $\pm$ 0.16
Oct. (29)	1965.57 $\pm$ 309.86	51.03 $\pm$ 1.49	1.47 $\pm$ 0.13	16.98 $\pm$ 0.63	12.68 $\pm$ 7.82	12.58 $\pm$ 2.17	04.76 $\pm$ 1.12	123.16 $\pm$ 8.00	0.41 $\pm$ 0.11	18.80 $\pm$ 3.56	1.00 $\pm$ 0.34

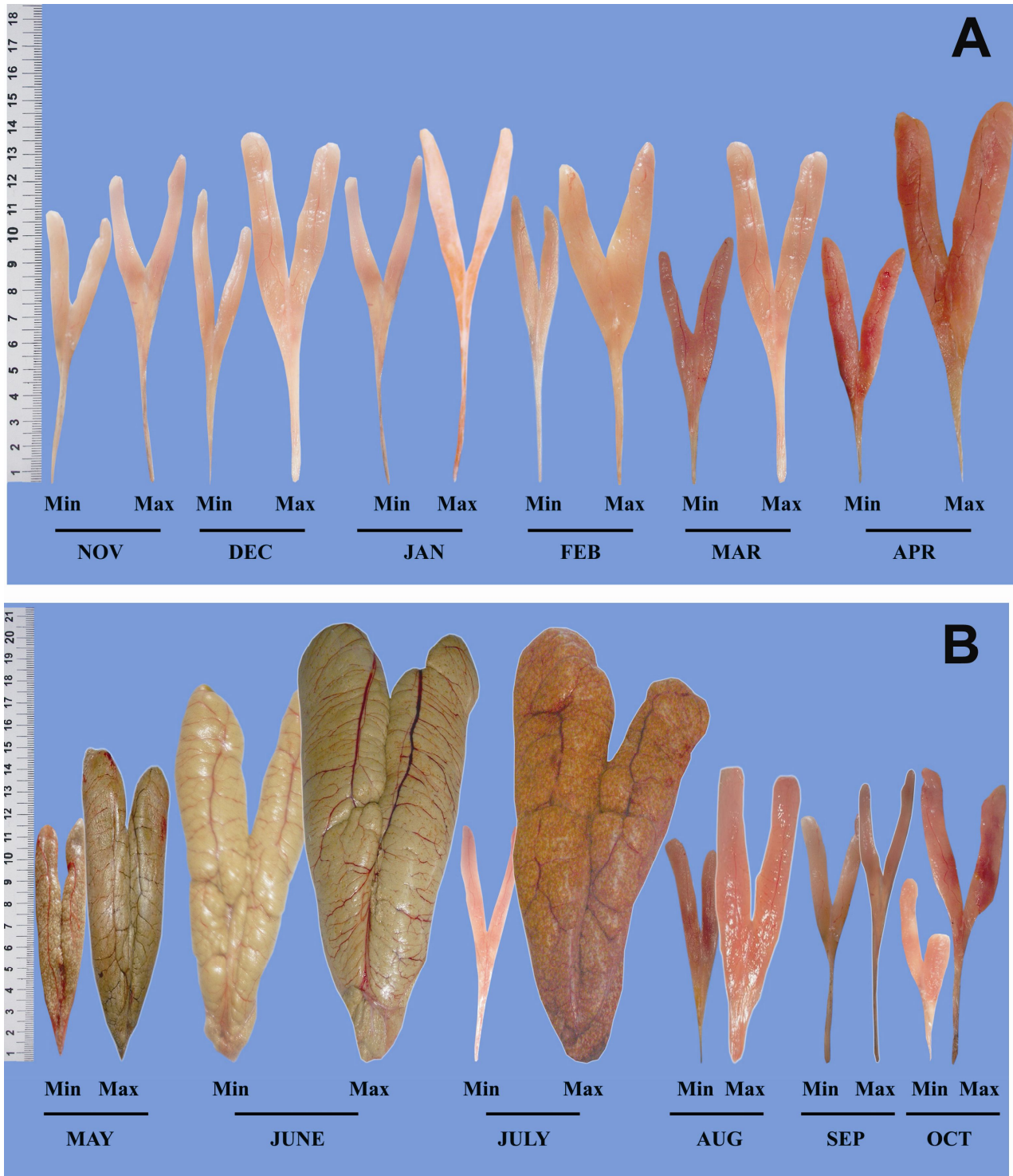


Fig. 2A,B. Size, Gross structure and developmental stages of *Catla catla* ovary from November 2006 (age 18 months) to October 2007 (age 29 months). The photographs were taken from the fresh dissected samples. For other details see Table I and II.

ovarian weight. Diameter of POCs was  $98.30 \pm 3.56 \mu\text{m}$ , having large nuclei ( $44.00 \pm 2.00 \mu\text{m}$ ) but fewer nucleoli. Tunic was thick ( $36.00 \pm 5.20 \mu\text{m}$ ).

#### *April (23 months)*

GSI increased in April ( $0.60 \pm 0.21 \%$ ). Ovarian blood supply increased and Tunica was  $48.00 \pm 11.15 \mu\text{m}$ . Ovaries showed initiation of meiotic division and vitellogenesis as cortical alveolar oocytes (CAO) ( $161.00 \pm 14.00 - 179.32 \pm 28.27 \mu\text{m}$ ) were observed for the first time. POCs were still more prevalent than CAOs (Table II; Fig. 2A and Fig. 3).

#### *May (24 months)*

A rapid increase in GSI and advancement in histological picture of ovaries was observed. Oocytes could be seen through the thin tunic ( $25.04 \pm 9.10 \mu\text{m}$ ) of large baggy ovaries with prominent blood vessels. All stages of germ cells were observed. POCs were few while secondary yolk oocytes were also noted at early (SOE) and late yolk stages (SOL). The largest germ cells were tertiary yolk oocytes (TYO). Very early stages of GVM could be seen at few places (Table II; Fig. 2B and Fig. 3).

#### *June (25 Months)*

June was the month when the maximum ovarian development was observed. The water temperature ( $33.00 \pm 1.40^\circ\text{C}$ ) and photoperiod ( $15.11 \pm 0.05 \text{H}$ ) was also the maximum (Fig. 1). GSI ( $13.00 \pm 9.30 \%$ ) showed its peak during this month. Females could be differentiated from males by their soft and bulging abdomens. Genital pore was very red and bulged out. Ovaries had very prominent blood vessels and near transparent with thin tunic ( $21.53 \pm 0.25 \mu\text{m}$ ). No egg was released even on tightly pressing the abdomen. Histologically, no finally mature oocyte or post ovulatory follicle (POF) was observed in any ovary (Table II; Fig. 2B and Fig. 3).

#### *July (26 months)*

GSI declined in July ( $4.57 \pm 2.21 \%$ ) concomitant with the peak in rainfall ( $199.86 \pm 32.31 \text{mm}$ ) and a small decrease in photoperiod after summer solstice (Fig. 1). Both mature and immature specimens were observed during this month.

Histologically, primary oocytes were quite prevalent both in mature and immature ovaries. Numerous atretic follicles were noted. Stromal tissue became quite prominent. Tunic became thicker again ( $45.00 \pm 8.45 \mu\text{m}$ ). Granulocytes were seen, probably clearing the cellular debris accumulating due to atresia. Ovaries were loose and reddish in color (Table II; Fig. 3).

#### *August (27 months)*

Ovaries were completely regressed and only immature specimens were encountered (GSI  $0.45 \pm 0.18 \%$ ). Only POCs were present along with stromal tissue. Granulocytes could still be seen in stroma.

#### *September (28 months)*

Gross and histological picture of ovaries in September was same (GSI  $0.46 \pm 0.24 \%$ ) as observed in August (Table II; Fig. 2B and 3).

#### *October (29 months)*

Ovaries were at a complete resting stage. Stromal tissue was cleared and granulocytes were absent. GSI was  $0.41 \pm 0.11 \%$ . Thickness of tunic was  $153.06 \pm 22.03 \mu\text{m}$ . In one of the samples, ovary with right lobe half the length of the left lobe was observed. Histologically, only POCs were observed and ovaries were completely regressed (Table II; Fig. 2B and Fig. 3).

#### *Fecundity*

Total fecundity was calculated by simple gravimetric method on six fish each in May and June separately, when the overall GSI of the fish was  $1.57 \pm 0.92$  and  $13.00 \pm 9.30$  respectively. The total fecundity of the fish in May was  $2537.80 \pm 857.21$  per gram of the ovary which amounts to  $41.33 \pm 8.66$  eggs per gram body weight of fish (oocyte size =  $429.0 \pm 35.07 \mu\text{m}$ ). The average body weight of the fish used in these measurements was  $1816.40 \pm 138.70 \text{g}$  and the length  $49.83 \pm 2.05 \text{cm}$ . In June, when the GSI increased some 8 times the values in May, the average fecundity was  $1183.61 \pm 681.72/\text{g}$  ovary weight and  $121.73 \pm 81.49$  per gram body weight (oocyte size =  $667.43 \pm 128.40 \mu\text{m}$ ) of fish with  $1935.00 \pm 225.70$  gram body weight and  $49.67 \pm 1.69 \text{cm}$  average total body length (Table I).

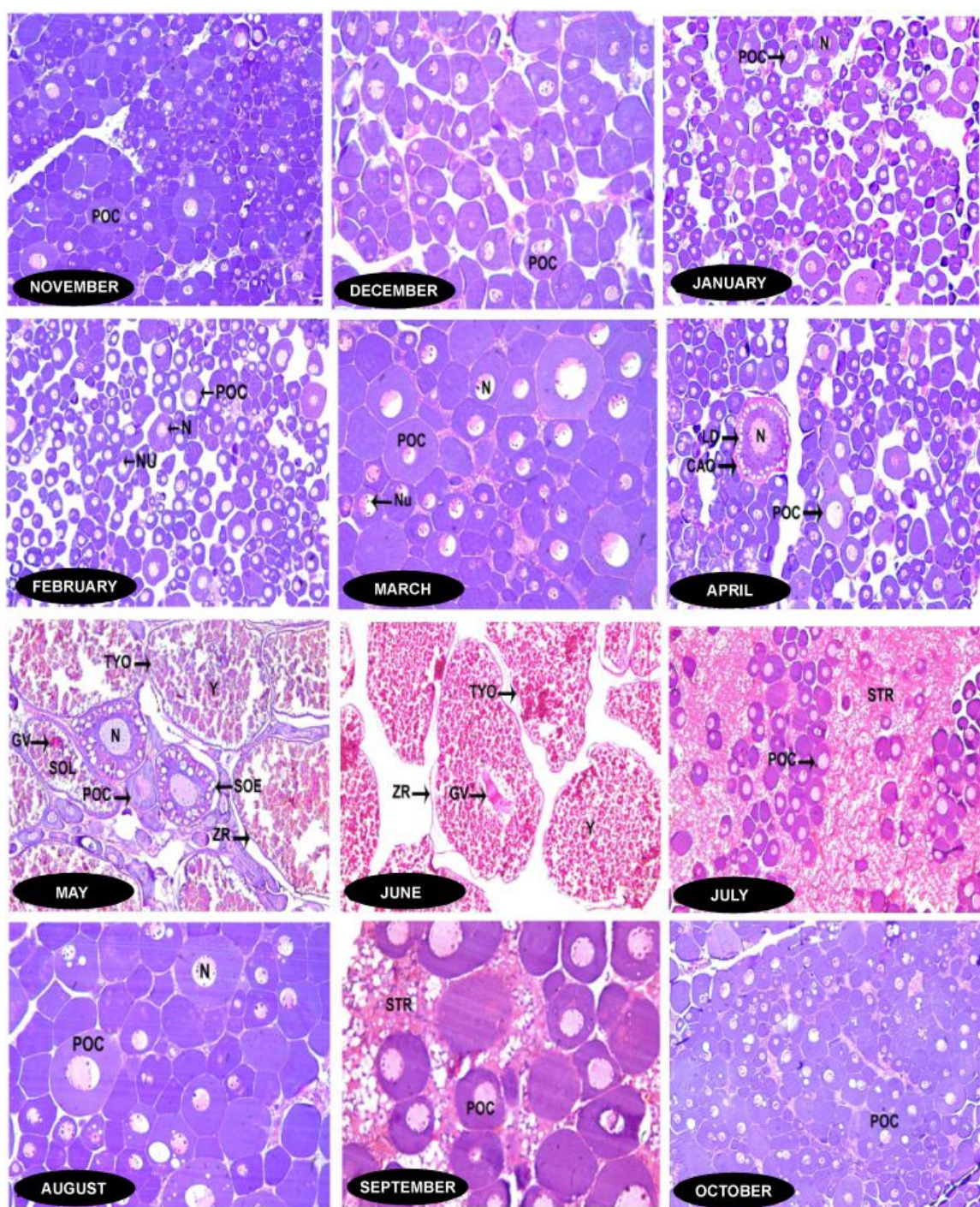


Fig. 3. Formalin fixed and hematoxylin and eosin-stained paraffin sections of *Catla catla* ovary showing different stages of oogenesis and seasonal, age-related changes. X 50. CAO, Cortical Alveoli Oocyte; GV: Germinal Vesicle; LD: Lipid Droplets; N: Nucleus; NU: Nucleolus; POC: Primary Oocyte; SOE: Secondary Oocyte (early); SOL: Secondary Oocyte (late); STR: Stroma; TYO: Tertiary Yolk Oocyte; Y: Yolk; ZR: Zona Radiata.

Table II.- Classification of annual ovarian cycle and maturing stages of female *Catla catla* between the age of 18-29 months. Values given are Mean±SD.

Maturity Stage	Duration (Months)	GSI	Thickness of tunica (T) and ZR (µm)	Diameter of germ cells (µm)	Diameter of nucleus (µm)	Diameter of nucleolus (µm)	Macroscopic Appearance	Microscopic Appearance
Immature/ Resting	October to	0.41±0.11-	T:	POC:	POC:	POC:	Ovaries were small transparent or off-white in color, joined posteriorly. Oocytes were not visible. A thin blood vessel may be seen on the surface.	Only small primary oocytes were present at chromatin and perinucleolar stage. Tunica were thick.
	December	0.9 ±0.42	153.06±22.03- 178.81±35.24	123.16± 8.00 – 131.00± 15.23	40.00±6.00- 51.25±9.23	13.00±2.03- 18.06±4.00		
Regenerating	January to	0.77±0.40 –	T:	POC:	POC:	POC:	Ovaries were slightly larger than previous months. Compact in structure. Blood vessels were not prominent.	Historically, same as in previous months except the size of cell, nuclei and nucleoli was larger.
	February	0.31±0.41	46.72±6.23- 105.00±19.0	93.00±15.00- 141.18±34.00	47.65± 6.20- 60.00±16.19	18.42±5.62- 20.00±12.35		
Developing	Mar. – April	0.36±0.09- 0.60±0.21	T:	POC:	POC:	POC:	Ovaries pinkish red with opaque oocytes. Blood vessels were visible. Ovary solid and compact. Ovarian ducts were thin and transparent.	Primary oocytes were dominant. Few oocytes were found at cortical alveoli stage with formation of oil droplets on periphery. Atretic follicles were also observed.
			36.00±5.20- 43.00±11.15	98.30± 3.56- 100.10±10.11	44.00± 2.00- 45.19±7.74	15.56±6.90- 21.00±13.56		
Maturing	May	1.57±0.92	T:	POC:	POC:	POC:	Maturing ovaries filling almost 2/3 of body cavity. Yellowish in color. Prominent Blood vessels and yolk oocytes were clearly visible through very thin tunica.	Ovaries contained all stages of oogenesis with secondary oocytes at early and late yolk globule stage. Some ovaries had predominantly tertiary yolk oocytes and few primary oocytes. Early GVM was observed. No POF was seen.
			25.04±9.10 16.00±0.20	161.00±14.00- 179.32±28.27	85.80±22.61- 96.53±7.15	21.45±10.00- 30.25±10.00		
Mature/ gravid	June	13.00±9.30	T:	TYO:	TYO*:	TYO:	Ovaries were still compact and yellowish. No egg was released by tightly pressing the abdomen. Blood vessels were well developed	Only tertiary yolk oocytes at late yolk globule stage were observed. Some oocytes showed GVM. No hydrated oocyte and POF was seen.
			21.53± 0.25 ZR: 18.40±6.5	667.43±128.40	147.00 ±67.00 103.00±21.21 150.25±47.39	32.00±11.21 32.00±13.00 22.00±8.95		
Regressing	July	1.56±2.21	T:	POC:	TYO*:	POC:	Ovaries were slightly loose and smaller than June samples and with prominent blood supply. Few ovaries contained mature (?) follicles. Color was reddish and tunica thin.	Atretic Follicles were very prominent but no POF was seen. Few tertiary oocytes were observed. Stromal tissue was seen within the empty spaces. Granulocyte activity was not prominent.
			45.00± 8.45	146.00±18.64	86.24±11.01	12.52±3.56 Not Observed		
Regressed	Aug-Sep	0.45±0.18- 0.46± 0.24	T:	POC:	POC:	POC:	Some ovaries were still loose but follicles were not seen. Color was reddish or pink. Ovarian wall was thick. Blood vessels may be visible on the surface.	Only primary oocytes were seen at perinucleolar stage. Well developed ovarian stroma was observed. Atresia was very prominent. No POF was observed. Granulocytes were seen.
			60.10±20.45- 118.50±25.45	155.60 ± 15.65- 177.46± 13.00	60.10±6.40- 69.00±16.00	12.66±4.58- 16.10±6.50		

CAO, Cortical alveoli oocyte; POC, Primary oocyte; SOE, Secondary oocyte (Early); SOL, Secondary oocyte (Late); TYO, Tertiary Yolk Oocyte; \*TYO, Mean value of thickness at different points because of crescent shaped GV (Germinal Vesicle).



## DISCUSSION

The reproductive cycle of female *Catla catla* of age 18-29 months was divided into seven stages on the basis of gross ovarian morphology and histological features. These stages are: immature/resting, regenerating, developing, maturing, mature/gravid, regressing and regressed. Histological studies also support this division (Table II). In the oogenetic cycle, six stages of oocytes were observed throughout the histological study. These were: chromatin nucleolar, perinucleolar, cortical alveolar, early vitellogenic, late vitellogenic and early germinal vesicle movement (GVM) oocytes. These stages were defined on the basis of number and position of nucleoli, nuclear diameter, nuclei movement and breakdown, appearance of oil droplets and yolk globules, thickness of tunica and zona radiata.

Based on the size of ovary and its histological picture, an annual (seasonal) reproductive cycle was also determined. This study showed that catla is an annual, group-synchronous and total spawner, probably spawning in high summer like many other teleosts from the temperate regions (Barcellos *et al.*, 2001; Yi Wang *et al.*, 2001; Marraro *et al.*, 2005; Chen *et al.*, 2006; Dadzie, 2007). GSI started increasing in April and reached its peak in June ( $13.00 \pm 9.30$ ), when aged 25 months. As this study is based on first maturational cycle, GSI showed that catla matures at the age of 24-25 months. The same range of age had been reported by Alikunhi (1957) and Dholakia (2004) for the wild *catla catla* from the sub-tropical Indian Bengal.

Gross picture of ovaries and GSI showed no apparent development till April (Fig. 2). However, histologically the recrudescence seem to have been started in February-March when a decrease in average cell to nucleus ratio of oocytes was observed with a concomitant increase in number and size of nucleoli (Table I) in the germinal vesicle pointing to an increase in mRNA synthesis. A marked increase in GSI was noted in May, simultaneous with the ovarian development, showing vitellogenic tertiary oocytes as the most advanced stage. The size of oocytes increased with the advancement in ovarian cycle. The average size of mature follicles was  $667.43 \pm 128.40 \mu\text{m}$  with very

thick zona radiata, showing maximum vitellogenin and estrogen synthesis (Lenhardt and Cakic, 2002). The slow response of female catla to water temperature and photoperiod changes showed that it is less sensitive to environmental changes as compared to its male counterpart (Lone *et al.*, 2009). Ovaries developed later than males (testis), however, once started the maturation cycle was completed within a short period.

The maximum GSI was observed in June, a time slightly later than the male catla that were already running in May (Lone *et al.*, 2009). The GSI declined in July, when the rainfall was maximum, indicating the occurrence of spawning. However, in the present studies, absence of final oocytes maturation (FOM), hydrated oocytes or eggs in oviduct, or post ovulatory follicles (POF) strongly point out that catla did not spawn in captivity. Hydrated oocytes are the indicators of imminent spawning and fresh POFs presence points to the fact that spawning has occurred in the near past (Sivakumaran *et al.*, 2003; Yanagimoto and Humphreys, 2005; Chen *et al.*, 2006; Lone *et al.*, 2001, 2008b). This observation has been seen earlier also by the workers in India and Pakistan for the wild catla kept in ponds for spawning (Chako and Kurian, 1950; Jhingran, 1968; Dholakia, 2004; Bhattacharya *et al.*, 2005; Dey *et al.*, 2004, 2005). Earlier authors attributed the failure in reproduction in captivity due to the lack of ecological and behavioral factors, such as water currents, temperature and suitable spawning substrates etc. These factors are critical for the process of final sexual maturation and spawning under natural conditions and transduce their effect through neuro-endocrine reflexes (Donaldson and Hunter, 1983; Bromage *et al.*, 2001; Lone and Hussain, 2009; Mananos *et al.*, 2009; Mylonas *et al.*, 2010). This failure can also be attributed to the stress of captivity, insufficient food and high stocking density (Billard, 1992). We have also seen this failure of spawning under the same conditions of pond culture in *Labeo rohita* females (Lone and Hussain, 2009).

Based on the data obtained during present studies, it appears that spawning season in catla is probably between mid-June to mid-July. Our observations differ from those reported earlier by

Hora and Pillay (1962), Talwar and Jhingran (1991) and Bhattacharyya and Maitra (2006). These authors reported that in India the spawning of wild catla takes place between July and August. These differences probably are because of variations in latitudes of Lahore (Pakistan) and West Bengal (India), from where the maximum Indian reports originated and greater adaptability of cyprinids to the ambient environments (Sivakumaran *et al.*, 2003; Billard, 1995). Also, the differences between the wild and the domesticated fish should also be kept mind in terms of nutritional supplies, constant and variable conditions of water temperature, photoperiod and genetic factors. The steroid analysis performed on these specimens showed that titers of sex steroids had also regressed by this time since ovaries were inactive and regressive (Unpublished data). The presence of atretic tertiary yolked oocytes in the ovaries in July also point towards middle June-early July spawning in catla females in Lahore. However, further studies on fish aged 3-5 years will help in resolving this matter. The ovaries were cleared of advanced yolked oocytes in the beginning of August and by mid-August, only the primary oocytes were present in the ovaries.

It may also be noted here that the fish used in the present studies were of the age between 18-29 months. This means that the fish were studied during their first maturation season or that the fish were entering the puberty. Puberty in fish, as in mammals, is a transition state in which an individual becomes mature for the first time and that before this time a juvenile stage existed (Okuzawa, 2002; Taranger *et al.*, 2010). Catla used in the present study were of known age since the fish were reared from the spawn of the broodstocks kept in the same farm from which all the fish used in the present study were collected. Based on the results obtained from the gross changes in the ovarian pattern and histological changes, it appears that the fish matures in the captivity, for the first time, more or less at the age of 2 years. This date is similar to the one already reported for the wild catla from India (Jhingran, 1968; Chakraborti, 1998; Jhingran and Pullin, 1988; Bhattacharya *et al.*, 2005; Bhattacharya and Maitra, 2006; Dey *et al.*, 2004, 2005). Moreover, it was also observed that not all the fish entered puberty or

showed maturational changes. There were fish in the samples taken from the same population and kept in the same place which were immature even in May and June when other fish were showing maximum ovarian growth (Fig. 3). Whether these fish will mature in the next season or they do not mature at all is not clear. Studies on fish with known age and beyond the age of 28 months will answer these questions more precisely.

In tropical and temperate cyprinids, gametogenesis is associated with environmental changes. Seasonal rains of monsoon, flooding and critical values of water temperature and photoperiod are ultimate factors regulating ovarian growth and time of spawning in carps (Billard, 1995; Munro *et al.*, 1995). Water level, water quality, nutrients, breeding substrate and vegetation are also important factors which may determine the spawning success in cyprinids as in other fish (Hontela and Stacey, 1995; Mananos *et al.*, 2009; Mylonas *et al.*, 2010). In the present study also, water temperature and photoperiod were of critical importance in the initiation of gonadal recrudescence in *Catla catla*. Gonadal development started in April when the average photoperiod was 14.03 hours and the water temperature  $29 \pm 1.76^\circ\text{C}$ . Maximum ovarian development was noted in May and June when both water temperature and photoperiod were nearly the maximum (Fig.1). It appears that both temperature and photoperiod were responsible for oocyte development and maturation. Dey *et al.* (2005) reported that exposure of catla female to short photoperiod resulted in significant decrease in GSI while male catla showed no influence of photoperiod on maturity or spawning stage (Bhattacharyya *et al.*, 2005). This led to the possibility of a bit different behaviors of male and female catla to environmental factors at different sexual stages, showing that female needs a photoperiod higher than 12 hours and water temperature above  $29^\circ\text{C}$  for ovarian development. In the present study, we observed that female catla had the maximum GSI in June during summer solstice and near highest water temperatures, whereas the catla male became mature earlier (Lone *et al.*, 2009). In the present study, the optimum water temperature for the advancement of oogenesis in female catla was around  $28-29^\circ\text{C}$ , because when

it reached a peak (34°C) in August, the GSI was already regressing. The range for oogenesis reported here is closer to the range reported in previous studies on wild catla; *i.e.* 24-31°C (Khan, 1942), 22-28°C (Chacko and Kuriyan, 1950) and 26-33°C (Dubey and Tuli, 1961). Bhattacharyya and Maitra (2006) reported the temperature range of 33-35°C and the photoperiod range of 11.00-13.30 H for male catla in Bengal, India.

A sudden decline in GSI in July (photoperiod 15.10±0.05 H; water temperature 33.50±1.00 °C) was concomitant with a slight decrease in photoperiod while water temperature was the maximum (Fig. 1) points towards the more significant role of water temperature than photoperiod in regression of the ovary. This may also be because the fish did not spawn, therefore, regression of the ovary was faster than in the wild where spawning normally occurs or extended up to August. However, a detailed study of exposing the fish to different regimes of photoperiod and water temperature combinations is required to reach at a final conclusion. A similar role of photoperiod in initiation of oogenesis and advancement of ovulation was studied in Atlantic salmon, perch and sting rays recently (Taranger *et al.*, 2003; Miguad *et al.*, 2004; Christopher *et al.*, 2008). In a study, *Catla catla* with regressed ovaries were exposed to a long (16H light: 8H dark) photoperiod in August (Dey *et al.*, 2005; Bhattacharyya and Maitra, 2006) in August. The results showed that ovaries did not respond to this treatment although, a similar treatment in the spring caused the precocious maturation of the ovary. We found that the heaviest rains of monsoon in July (199.86±32.31 mm) caused a sudden drop in the GSI (Fig.1), probably pointing to the spawning in nature and the role of rains as the final (?) cue for the spawning. Earlier workers also suggested to the similar role of monsoon rains in catla spawning (Khan, 1942; Ganapati and Chacko, 1954; Chakraborti, 1998; Bhattacharyya and Maitra, 2006). Recently, we observed a similar phenomenon in female *Labeo rohita* of the same age group and kept with the fish used in the present studies (Lone and Hussain, 2009).

Total fecundity was computed on six fish each in May and June separately. The fecundity in

May was 2537.80±857.21 per gram of the ovary (oocyte size = 429.0±35.07 µm) of the fish having 1816.40±138.70 g average body weight. In June, when the GSI increased 8 times the values in May, the average fecundity was 1183.61±681.72/g ovary (oocyte size= 667.43±128.40 µm) of fish with 1935.00±225.70 gram body weight. The decrease in fecundity in June as compared to May can be attributed to the increase in size of the ovarian oocytes. The average increase in size of oocytes from May to June was 55.57% which caused a decrease in relative fecundity of 53.36%, showing that the decrease in fecundity was almost due to the increase in size of the oocytes. This has also been reported earlier from many fish (Sehgal and Toor, 1991; Juchno *et al.*, 2007; Plaza *et al.*, 2007).

Fecundity values calculated in the present study are slightly higher than the values of fecundity given for the wild *Catla catla*. For example, catla of age three plus years had 767-821 ova per gram of ovary (GSI 2.66-20.91) and total body weight of 11.33-14.37 kg (Jhingran and Pullin, 1988). The values given for the wild fish were less than the fish in the present study (Age 24 months) probably because the fish from the present study were taken from a farm where they were fed regularly whereas the wild fish generally have erratic food availability (De Vlaming, 1972; Narimatsu *et al.*, 2007). Jhingran and Pullin (1988) also showed that the fecundity of the wild fish increased up to five years of age and then plateaued. The total body weight of the fish at five year of age reached up to 18.91 kg while the GSI reached up to 25.87. The maximum GSI noted in the present study was 13.00±9.30.

Present study is the first detailed study of annual ovarian cycle of cultured *Catla catla* and its environmental control in temperate region (Pakistan). It can be concluded from the present studies that catla is an iteroparous seasonal breeder which does not spawn in captivity. However, a comprehensive and more advanced study is needed for understanding of environmental control of reproduction in this carp.

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**Table I.- Monthly variations (Mean  $\pm$  SD) in body and ovarian parameters of female *Catla catla* during the study period (2006-2007).**

Months (Age)	Body weight (g)	Total length (cm)	Condition factor (k)	Body width (cm)	Ovarian weight (g)	Ovarian length (cm)	Ovarian girth (cm)	Oocytes size ( $\mu$ m)	GSI	Liver weight (g)	HSI
Nov. (18)	762.00 $\pm$ 37.06	48.35 $\pm$ 2.14	0.56 $\pm$ 0.21	16.50 $\pm$ 1.90	10.89 $\pm$ 0.85	12.94 $\pm$ 0.75	04.00 $\pm$ 0.48	129.04 $\pm$ 22.30	1.28 $\pm$ 0.67	16.80 $\pm$ 1.60	1.50 $\pm$ 0.91
Dec. (19)	690.12 $\pm$ 52.14	46.46 $\pm$ 1.85	0.68 $\pm$ 0.05	14.87 $\pm$ 1.21	06.16 $\pm$ 2.60	12.85 $\pm$ 0.92	03.84 $\pm$ 0.71	131.00 $\pm$ 15.23	0.90 $\pm$ 0.42	16.12 $\pm$ 2.61	2.33 $\pm$ 0.33
Jan. (20)	1088.64 $\pm$ 226.80	48.83 $\pm$ 2.84	0.93 $\pm$ 0.15	16.08 $\pm$ 1.94	08.39 $\pm$ 5.81	13.67 $\pm$ 1.15	04.40 $\pm$ 0.72	93.00 $\pm$ 15.00	0.77 $\pm$ 0.40	18.684 $\pm$ 5.94	1.70 $\pm$ 0.42
Feb. (21)	1520.00 $\pm$ 482.40	48.25 $\pm$ 2.87	1.32 $\pm$ 0.18	15.87 $\pm$ 0.73	04.79 $\pm$ 2.28	12.75 $\pm$ 0.80	03.75 $\pm$ 0.60	141.18 $\pm$ 34.00	0.31 $\pm$ 0.41	16.07 $\pm$ 2.77	1.11 $\pm$ 0.29
March (22)	1407.00 $\pm$ 179.74	45.26 $\pm$ 2.02	1.52 $\pm$ 0.19	14.23 $\pm$ 1.06	05.06 $\pm$ 1.61	11.40 $\pm$ 1.51	03.42 $\pm$ 1.09	161.00 $\pm$ 14.00	0.36 $\pm$ 0.09	15.75 $\pm$ 2.24	1.15 $\pm$ 0.32
April (23)	1617.85 $\pm$ 336.08	48.07 $\pm$ 3.58	1.44 $\pm$ 0.04	17.36 $\pm$ 1.47	09.92 $\pm$ 4.96	13.00 $\pm$ 2.00	05.00 $\pm$ 0.26	179.32 $\pm$ 28.27	0.60 $\pm$ 0.21	17.41 $\pm$ 5.50	1.19 $\pm$ 0.10
May (24)	1979.00 $\pm$ 247.84	51.00 $\pm$ 3.00	1.50 $\pm$ 0.07	15.75 $\pm$ 1.46	30.32 $\pm$ 18.80	13.32 $\pm$ 1.00	16.25 $\pm$ 3.18	429.0 $\pm$ 35.07	1.57 $\pm$ 0.92	18.31 $\pm$ 2.89	0.94 $\pm$ 0.15
June (25)	2192.00 $\pm$ 287.14	50.75 $\pm$ 3.18	1.68 $\pm$ 0.12	23.50 $\pm$ 0.90	199.00 $\pm$ 23.13	19.33 $\pm$ 1.87	19.33 $\pm$ 1.87	667.43 $\pm$ 128.40	13.00 $\pm$ 9.30	28.08 $\pm$ 5.72	1.28 $\pm$ 0.10
July (26)	2144.89 $\pm$ 119.17	50.97 $\pm$ 1.31	1.62 $\pm$ 0.10	22.14 $\pm$ 1.00	98.16 $\pm$ 47.43	14.44 $\pm$ 3.07	08.11 $\pm$ 4.25	645.00 $\pm$ 126.00	4.57 $\pm$ 2.21	20.06 $\pm$ 5.56	0.94 $\pm$ 0.29
Aug. (27)	1820.84 $\pm$ 244.87	48.72 $\pm$ 1.10	1.59 $\pm$ 0.25	20.13 $\pm$ 0.48	10.18 $\pm$ 6.38	11.83 $\pm$ 2.62	04.64 $\pm$ 1.18	177.46 $\pm$ 13.00	0.45 $\pm$ 0.18	15.85 $\pm$ 1.47	0.89 $\pm$ 0.16
Sept. (28)	1232.26 $\pm$ 152.98	48.78 $\pm$ 2.73	1.06 $\pm$ 0.14	17.14 $\pm$ 2.37	04.50 $\pm$ 3.03	12.66 $\pm$ 1.12	03.53 $\pm$ 0.48	155.60 $\pm$ 15.65	0.46 $\pm$ 0.24	10.56 $\pm$ 3.15	0.84 $\pm$ 0.16
Oct. (29)	1965.57 $\pm$ 309.86	51.03 $\pm$ 1.49	1.47 $\pm$ 0.13	16.98 $\pm$ 0.63	12.68 $\pm$ 7.82	12.58 $\pm$ 2.17	04.76 $\pm$ 1.12	123.16 $\pm$ 8.00	0.41 $\pm$ 0.11	18.80 $\pm$ 3.56	1.00 $\pm$ 0.34

**Table II.- Classification of annual ovarian cycle and maturing stages of female *Catla catla* between the age of 18-29 months. Values are Mean±SD.**

Maturity Stage	Duration (Months)	GSI	Thickness of tunica (T) and ZR (µm)	Diameter of germ cells (µm)	Diameter of nucleus (µm)	Diameter of nucleolus (µm)	Macroscopic Appearance	Microscopic Appearance
Immature/ Resting	October to December	0.41±0.11- 0.9 ±0.42	<b>T:</b> 153.06±22.03- 178.81±35.24	<b>POC:</b> 123.16± 8.00 – 131.00± 15.23	<b>POC:</b> 40.00±6.00- 51.25±9.23	<b>POC:</b> 13.00±2.03- 18.06±4.00	Ovaries were small transparent or off-white in color, joined posteriorly. Oocytes were not visible. A thin blood vessel may be seen on the surface.	Only small primary oocytes were present at chromatin and perinucleolar stage. Tunica were thick.
Regenerating	January to February	0.77±0.40 – 0.31±0.41	<b>T:</b> 46.72±6.23- 105.00±19.0	<b>POC:</b> 93.00±15.00- 141.18±34.00	<b>POC:</b> 47.65± 6.20- 60.00±16.19	<b>POC:</b> 18.42±5.62- 20.00±12.35	Ovaries were slightly larger than previous months. Compact in structure. Blood vessels were not prominent.	Histologically, same as in previous months except the size of cell, nuclei and nucleoli was larger.
Developing	Mar. – April	0.36±0.09- 0.60±0.21	<b>T:</b> 36.00±5.20- 43.00±11.15	<b>POC:</b> 98.30± 3.56- 100.10±10.11 <b>CAO:</b> 161.00±14.00- 179.32±28.27	<b>POC:</b> 44.00± 2.00- 45.19±7.74 <b>CAO:</b> 85.80±22.61- 96.53±7.15	<b>POC:</b> 15.56±6.90- 21.00±13.56 <b>CAO:</b> 21.45±10.00- 30.25±10.00	Ovaries pinkish red with opaque oocytes. Blood vessels were visible. Ovary solid and compact. Ovarian ducts were thin and transparent.	Primary oocytes were dominant. Few oocytes were found at cortical alveoli stage with formation of oil droplets on periphery. Atretic follicles were also observed.
Maturing	May	1.57±0.92	<b>T:</b> 25.04±9.10 <b>ZR:</b> 16.00±0.20	<b>POC:</b> 129.00± 35.03 <b>SOE:</b> 314.61±71.00 <b>SOL:</b> 329.00±48.54 <b>TYO:</b> 429.03±35.07	<b>POC:</b> 66.35±16.60 <b>SOE:</b> 147.00 ±67.00 <b>SOL:</b> 103.00±21.21 <b>TYO*:</b> 150.25±47.39	<b>POC:</b> 35.30±16.25 <b>SOE:</b> 32.00±11.21 <b>SOL:</b> 32.00±13.00 <b>TYO:</b> 22.00±8.95	Maturing ovaries filling almost 2/3 of body cavity. Yellowish in color. Prominent Blood vessels and yolky oocytes were clearly visible through very thin tunica.	Ovaries contained all stages of oogenesis with secondary oocytes at early and late yolk globule stage. Some ovaries had predominantly tertiary yolk oocytes and few primary oocytes. Early GVM was observed. No POF was seen.
Mature/ gravid	June	13.00±9.30	<b>T:</b> 21.53± 0.25 <b>ZR:</b> 18.40±6.5	<b>TYO:</b> 667.43±128.40	<b>TYO*:</b> 93.00±18.44	<b>TYO:</b> 21.50±4.56	Ovaries were still compact and yellowish. No egg was released by tightly pressing the abdomen. Blood vessels were well developed	Only tertiary yolk oocytes at late yolk globule stage were observed. Some oocytes showed GVM. No hydrated oocyte and POF was seen.
Regressing	July	1.56±2.21	<b>T:</b> 45.00± 8.45	<b>POC:</b> 146.00±18.64 <b>TYO:</b> 645.00±126.00	<b>POC:</b> 57.22±10.08 <b>TYO*:</b> 86.24±11.01	<b>POC:</b> 12.52±3.56 <b>TYO:</b> Not Observed	Ovaries were slightly loose and smaller than June samples and with prominent blood supply. Few ovaries contained mature (?) follicles. Color was reddish and tunica thin.	Atretic Follicles were very prominent but no POF was seen. Few tertiary oocytes were observed. Stromal tissue was seen within the empty spaces. Granulocyte activity was not prominent.
Regressed	Aug-Sep	0.45±0.18- 0.46± 0.24	<b>T:</b> 60.10±20.45- 118.50±25.45	<b>POC:</b> 155.60 ± 15.65- 177.46± 13.00	<b>POC:</b> 60.10±6.40- 69.00±16.00	<b>POC:</b> 12.66±4.58- 16.10±6.50	Some ovaries were still loose but follicles were not seen. Color was reddish or pink. Ovarian wall was thick. Blood vessels may be visible on the surface.	Only primary oocytes were seen at perinucleolar stage. Well developed ovarian stroma was observed. Atresia was very prominent. No POF was observed. Granulocytes were seen.

CAO, Cortical alveoli oocyte; POC, Primary oocyte; SOE, Secondary oocyte (early); SOL, Secondary oocyte (late); TYO, Tertiary yolk oocyte; \*TYO, mean value of thickness at different points because of crescent shaped GV (germinal vesicle).



Maturity Stage	Duration (Months)	GSI	Thickness of tunica (T) and ZR (µm)	Diameter of germ cells (µm)	Diameter of nucleus (µm)	Diameter of nucleolus (µm)	Macroscopic Appearance
Immature/ Resting	October to December	0.41±0.11-0.9 ±0.42	T:153.06±22.03-178.81±35.24	POC: 123.16± 8.00 – 131.00± 15.23	POC: 40.00±6.00-51.25±9.23	POC: 13.00±2.03-18.06±4.00	Ovaries were small transparent or off-white in color, joined posteriorly. Oocytes were not visible. A thin blood vessel may be seen on the surface.
Regenerating	January to February	0.77±0.40 – 0.31±0.41	T: 46.72±6.23-105.00±19.0	POC: 93.00±15.00-141.18±34.00	POC: 47.65± 6.20-60.00±16.19	POC: 18.42±5.62-20.00±12.35	Ovaries were slightly larger than previous months. Compact in structure. Blood vessels were not prominent.
Developing	Mar. – April	0.36±0.09-0.60±0.21	T: 36.00±5.20-43.00±11.15	POC: 98.30± 3.56-100.10±10.11 CAO: 161.00±14.00-179.32±28.27	POC: 44.00± 2.00-45.19±7.74 CAO: 85.80±22.61-96.53±7.15	POC: 15.56±6.90-21.00±13.56 CAO: 21.45±10.00-30.25±10.00	Ovaries pinkish red with opaque oocytes. Blood vessels were visible. Ovary solid and compact. Ovarian ducts were thin and transparent.
Maturing	May	1.57±0.92	T: 25.04±9.10 ZR: 16.00±0.20	POC: 129.00± 35.03 SOE: 314.61±71.00 SOL: 329.00±48.54 TYO: 429.03±35.07	POC: 66.35±16.60 SOE: 147.00 ±67.00 SOL: 103.00±21.21 TYO*: 150.25±47.39	POC: 35.30±16.25 SOE: 32.00±11.21 SOL: 32.00±13.00 TYO: 22.00±8.95	Maturing ovaries filling almost 2/3 of body cavity. Yellowish in color. Prominent Blood vessels and yolky oocytes were clearly visible through very thin tunica.
Mature/ gravid	June	13.00±9.30	T: 21.53± 0.25 ZR: 18.40±6.5	TYO: 667.43±128.40	TYO*: 93.00±18.44	TYO: 21.50±4.56	Ovaries were still compact and yellowish. No egg was released by tightly pressing the abdomen. Blood vessels were well developed

Maturity Stage	Duration (Months)	GSI	Thickness of Tunica (T) and ZR (µm)	Diameter of Germ Cells (µm)	Diameter of Nucleus (µm)	Diameter of Nucleolus (µm)	Macroscopic Appearance	Microscopic Appearance
Immature/ Resting	OCT-DEC	0.41±0.11-0.9 ±0.42	T: 153.06±22.03-178.81±35.24	POC: 123.16± 8.00 - 131.00± 15.23	POC: 40.00± 6.00-51.25±9.23	POC: 13.00±2.03-18.06±4.00	Ovaries were small transparent or off-white in color, joined posteriorly. Oocytes were not visible. A thin blood vessel may be seen on the surface.	Only small primary oocytes present at oocyte perinucleolar stage thick.

<b>Regenera- ting</b>	<b>JAN-FEB</b>	0.77±0.40 – 0.31±0.41	<b>T:</b> 46.72±6.23- 105.00±19.0	<b>POC:</b> 93.00±15.00- 141.18±34.00	<b>POC:</b> 47.65± 6.20- 60.00± 16.19	<b>POC:</b> 18.42±5.62- 20.00±12.35	Ovaries were slightly larger than previous months. Compact in structure. Blood vessels were not prominent.	Histologically, same as previous months except the nuclei and nucleoli
<b>Developing</b>	<b>MAR-APR</b>	0.36±0.09- 0.60± 0.21	<b>T:</b> 36.00±5.20- 43.00±11.15	<b>POC:</b> 98.30± 3.56- 100.10±10.11 <b>CAO:</b> 161.00±14.00- 179.32±28.27	<b>POC:</b> 44.00± 2.00- 45.19±7.74 <b>CAO:</b> 85.80±22.61- 96.53±7.15	<b>POC:</b> 15.56±6.90- 21.00±13.56 <b>CAO:</b> 21.45±10.00- 30.25±10.00	Ovaries pinkish red with opaque oocytes. Blood vessels were visible. Ovary solid and compact. Ovarian ducts were thin and transparent.	Primary oocytes were observed. Few oocytes were in the alveoli stage with yolk droplets on the surface. Some follicles were also observed.
<b>Maturing</b>	<b>MAY</b>	1.57±0.92	<b>T:</b> 25.04±9.10 <b>ZR:</b> 16.00±0.20	<b>POC:</b> 129.00± 35.03 <b>SOE:</b> 314.61±71.00 <b>SOL:</b> 329.00±48.54 <b>TYO:</b> 429.03±35.07	<b>POC:</b> 66.35±16.60 <b>SOE:</b> 147.00 ±67.00 <b>SOL:</b> 103.00±21.21 <b>TYO*:</b> 150.25±47.39	<b>POC:</b> 35.30±16.25 <b>SOE:</b> 32.00±11.21 <b>SOL:</b> 32.00±13.00 <b>TYO:</b> 22.00±8.95	Maturing ovaries filling almost 2/3 of body cavity. Yellowish in color. Prominent Blood vessels and yolk oocytes were clearly visible through very thin tunica.	Ovaries contained oocytes in various stages of oogenesis with secondary and early and late yolk oocytes. Some ovaries had tertiary yolk oocytes. No POF observed.
<b>Mature/ Gravid</b>	<b>JUNE</b>	13.00±9.30	<b>T:</b> 21.53± 0.25 <b>ZR:</b> 18.40±6.5	<b>TYO:</b> 667.43±128.40	<b>TYO*:</b> 93.00±18.44	<b>TYO:</b> 21.50±4.56	Ovaries were still compact and yellowish. No egg was released by tightly pressing the abdomen. Blood vessels were well developed	Only tertiary yolk oocytes and yolk globule stage were observed. Some oocytes showed a hydrated oocyte and
<b>Regressing</b>	<b>JULY</b>	1.56±2.21	<b>T:</b> 45.00± 8.45	<b>POC:</b> 146.00±18.64 <b>TYO:</b> 645.00±126.00	<b>POC:</b> 57.22±10.08 <b>TYO*:</b> 86.24±11.01	<b>POC:</b> 12.52±3.56 <b>TYO:</b> Not Observed	Ovaries were slightly loose and smaller than June samples and with prominent blood supply. Few ovaries contained mature (?) follicles. Color was reddish and tunica thin.	Atretic Follicles were observed. No POF was seen. Follicles were observed. Strongly atretic activity was not prominent within the empty space.
<b>Regressed</b>	<b>AUG-SEP</b>	0.45±0.18- 0.46± 0.24	<b>T:</b> 60.10±20.45- 118.50±25.45	<b>POC:</b> 155.60 ± 15.65- 177.46± 13.00	<b>POC:</b> 60.10±6.40- 69.00±16.00	<b>POC:</b> 12.66±4.58-16.10±6.50	Some ovaries were still loose but follicles were not seen. Color was reddish or pink. Ovarian wall was thick. Blood vessels may be visible on the surface.	Only primary oocytes were observed. Granulocytes were observed. Ovarian stroma was very prominent.

POC= Primary Oocyte; CAO= Cortical Alveoli Oocyte; SOE= Secondary Oocyte (Early); SOL= Secondary Oocyte (Late); TYO= Tertiary Yolk Oocyte; \*TYO: Mean value of thickness at different points because of crescent shaped GV (Germinal Vesicle)