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Histological Studies of Common Carp Ovarian Development During Breeding Season in Khouzestan Province, Iran

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Abstract: Ovaries of 49 common carp brooders were collected during April to October 2010. Tissue samples were taken from anterior, median and posterior portions of the ovaries. Thin sections were prepared and stained by H&E and PAS. Histological examination showed that breeding seasons in common carp in this area take long at least 7 months and spawning were continued since April to October. It seems that previtellogenesis and vitellogenesis stages are short in Khouzestan climate condition and this species enter in maturation phase rapidly. Histological and histometrical results showed that the minimum and maximum diameters of follicles were observed in chromatin nucleolus and secondary vitellogenic follicles respectively. Zona radiata reached to maximum thickness in secondary vitellogenic follicles. Nucleus reached to maximum diameter in primary vitellogenic follicles. There was significant difference in parenchyma percentage between three stages of reproductive cycle. Also, the gonadosomatic index (GSI) from previtellogenesis to vitellogenesis and maturation stages was increased significantly ($p<0.05$).

Key words: Ovarian development % Histological studies % Spawning % Common carp

INTRODUCTION

Common carp is one of the most important fish species in aquaculture [1-3]. Ovarian structure of cyprinidae family has changes in the annual reproductive cycle [4, 5]. The most suitable method of determining the reproductive cycle in female fishes is to observe seasonal developmental changes in the gonads [5]. It is well known that photoperiod and temperature has profound effects on reproductive performance of fishes [1, 6, 7]. Quintana *et al.* [8] showed that high environmental temperature is enough to trigger sexual maturity in fish from temperate climate. Bapary and Fainuulelei [9] showed that photoperiod and temperature are involved in the regulation of gonadal development and a long photoperiod within a suitable range of water temperatures is required for continuity of reproductive activities. Miranda *et al.* [10] reported that female fishes that kept under short photoperiod had low GSIs and their ovaries contained only previtellogenic oocytes, in contrast, females exposed to the long photoperiod had high GSIs and ovaries with vitellogenic oocytes. Khouzestan province located in the southwest of Iran with long photoperiod and high temperature and is a major centre for the culture and export of freshwater fish [11], but only limited information is available on reproductive biology of common carp in Khouzestan province. The aim of this survey was determination of common carp ovarian status and structure in breeding season and in climate condition of Khouzestan province, Iran.

MATERIALS AND METHODS

Seven mature female common carp were obtained in each month (April to October), the mean length was (68.16±1.13cm) and mean weight was (5.57±0.29kg), from

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Table 1: The average of temperature and photoperiod in Khouzestan province from April to October

Months	April	May	June	July	Aug.	Sep.	Oct.
Temp.°C	26	31	34	36	36	32	27
L/D	13/11	14/10	14/10	14/10	14/10	13/11	12/12

culture ponds of Shush, Khouzestan, Iran. The average temperature and photoperiod of this area are showed in Table1.

This fishes were anesthetized by MS222 at concentration of 250 ppm. The ovaries were collected and weighed and tissue samples were taken from anterior, median and posterior portions. These samples were fixed in Bouin's solution and dehydrated in ethanol and were passaged in autotechnicon. Sections of 6 micron thickness were prepared and stained with H&E. Also, for study of zona radiata and yolk granules, PAS staining was used. The oocytes growth and histometrical details were examined. The numbers of previtellogenic and vitellogenic follicles were counted in three portions of ovaries in each stage. Zona radiata formation and thickness, oocytes diameter were measured during the oocyte growth by digital microscope and Dino-lite capture 1. The status of nucleolus was studied as well as the parenchyma- stroma ratio also was measured by calibrated ocular lens in three portions of ovaries. Gonadosomatic index (GSI) was calculated (GSI=Weight of gonad/ total weight of fish × 100). The results were analyzed by one- way ANOVA, two ways ANOVA and least significantly difference (LSD) multiple comparison test.

RESULTS

Histological and histometrical studies of dissected ovaries indicated that 7 different follicles were present in each stages of reproductive cycle as follows:

Chromatin-Nucleolus Follicles: Multiple nucleoli were present in the nucleus that arranged irregular, then, they lied at peri nuclear position. Ooplasm was thin and basophilic (Fig. 1). The diameter of these follicles was 35.96 ±4.52µ (Table 2).

Perinucleolus Follicles: Numerous large nucleoli were at the periphery of the nucleus. The ooplasm was contained the juxta nuclear complex of organelles (Balbiani body) (Fig. 2). The mean diameter of these follicles was 111.22 ±17.67µ (Table 2).

Cortical Alveolus Follicles: Cortical alveoli appeared at various depths in the ooplasm. Also, there were small lipid droplets around the nucleus. Zona radiata appeared as a thin band. Balbiani body became disappear (Fig. 3). The diameter of these follicles was 200.46 ±22.57µ (Table 2)

We considered chromatin-nucleolus, perinucleolus and cortical alveolus follicles as pre vitellogenic follicles.

Primary Vitellogenic Follicles: Yolk spheres lied between the cortical alveoli. The ooplasm was less basophilic (Fig. 4). The diameter of the nucleus reached to maximum diameter in this stage. The diameter of these follicles was 413.10± 27.53µ (Table 2).

Table 2: The mean and SEM of zona pellucida thickness, nucleus diameter and follicle diameter in different stages of growing follicles (µ).

Structure Type of follicle		Zona radiate thickness (µ)	Nucleus diameter (µ)	Follicle diameter (µ)
Chromatin-nucleolus	(a)	-	20.98±1.69 (bcd)	35.96±4.52 (cde)
Peri nucleolus	(b)	-	69.26±8.20 (acd)	111.22±17.67 (cde)
Cortical alveolus	(c)	1.78±0.29 (de)	101.23±5.79 (abd)	200.46±22.57 (abde)
Primary vitellogenesis	(d)	7.31±0.71 (c)	132.53±4.09 (abc)	413.10±27.53 (abce)
Secondary vitellogenesis	(e)	11.64±0.7 (c)	irregular	769.50±44.41 (abcd)

*SEM: standard error of mean

a, b. c, d and e, indicated significantly difference between these follicles($p<0.05$).

Table 3: The mean and SEM of vitellogenic follicles percentage in different portions of ovary in each stages

Stage Portion		Previtellogenesis	Vitellogenesis	Maturation
Anterior	(a)	0.049±0.001 (bc)	0.21±0.008 (bc)	0.27±0.006 (bc)
Median	(b)	0.080±0.001(ac)	0.29±0.015 (ac)	0.37±0.003
Posterior	(c)	0.12±0.003 (ab)	0.36±0.013 (ab)	0.37±0.004

Table 4: The mean and SEM of parenchyma percentage in three stages of reproductive cycle

Portion Stage		Anterior	Median	Posterior
Maturation	(a)	73.60±1.10 (bc)	77.72±1.63 (bc)	75.56±0.35 (bc)
Previtellogenesis	(b)	47.08±0.14 (ac)	48.80±0.3 (ac)	53.24±0.12 (ac)
Vitellogenesis	(c)	70.92±0.59 (ab)	73.16±0.50 (ab)	71.80±0.44 (ab)

Table 5: The mean and SEM of GSI in three stages of reproductive cycle

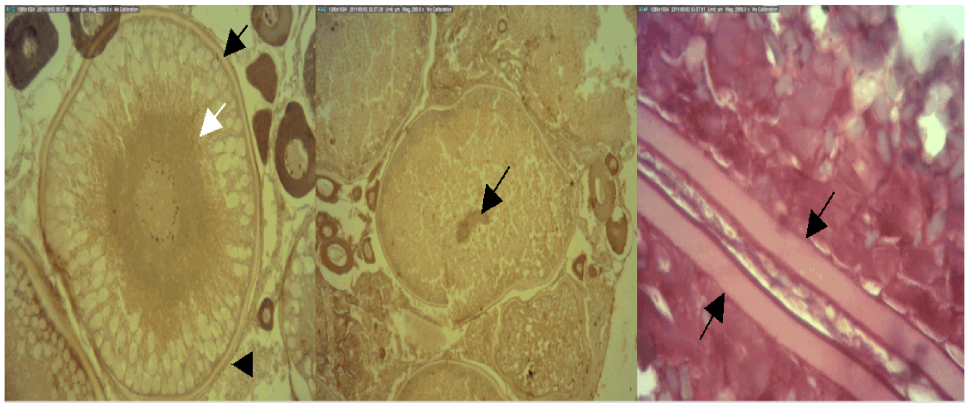
Stage		Average± SEM
Maturation	(a)	8.4 ± 0.1 (bc)
Previtellogenesis	(b)	1.9 6± 0.18 (ac)
Vitellogenesis	(c)	7 ± 0.18 (ab)

(1) (2) (3)

Fig. 1: Chromatin nucleolus follicle of common carp ovary. Peripheral nucleoli (arrows), nucleus (N) and basophilic cytoplasm (C), (H&E ×40).

Fig. 2: Balbiani body (arrow) in the ooplasm of a perinucleolus follicle (H&E ×40).

Fig. 3: Cortical alveolus follicle. Lipid droplets (LD) and thin zona radiata (arrow), (H&E ×10).



(4) (5) (6)

Fig. 4: Primary vitellogenic follicle, thicker zona radiata (black arrow), yolk spheres (white arrow), (H&E ×10).

Fig. 5: Secondary vitellogenic follicle, irregular nucleus membrane (arrow), the yolk spheres were moved toward the center and cortical alveoli and lipid droplets moved periphery of the ooplasm, (H&E ×10).

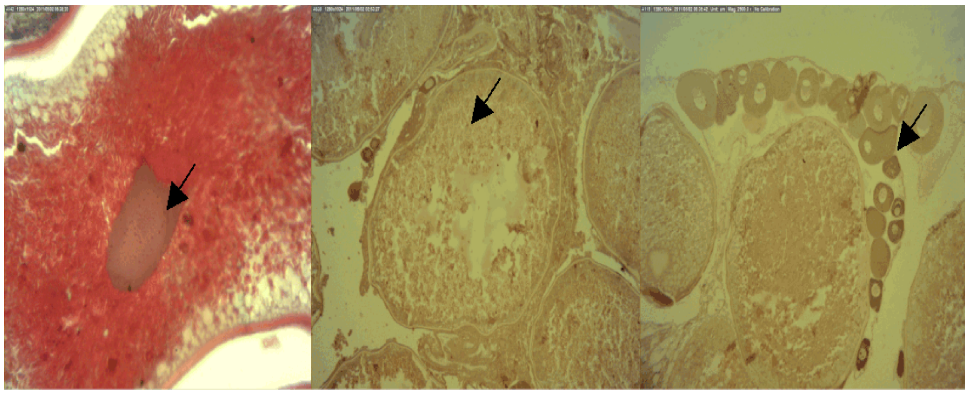
Fig. 6: Zona radiata in two secondary vitellogenic follicles, thick zona pellucida (arrows) is considerable (PAS ×40).

Secondary Vitellogenic Follicles: The yolk spheres were moved toward the center and cortical alveoli and lipid droplets moved periphery. The nucleus membrane became irregular (Fig. 5). The zona radiata thickness and follicles diameter were reached to maximum in this stage (Fig. 6) (Table 2).

Tertiary Vitellogenic Follicles: The yolk spheres were increased and combined together and filled the ooplasm totally. The nucleoli gradually moved toward the center of the nucleus (Fig. 7).

Maturation: The nucleus gradually was displaced toward the animal pole. Later, nuclear membrane was disappeared (Fig. 8). We considered primary, secondary, tertiary vitellogenic and mature follicles as vitellogenic follicles. Results showed also the reproductive cycle of common carp can divided to 3 stages according to percentage of different types of ovarian follicles including: previtellogenesis, vitellogenesis and maturation stages.

In previtellogenesis and vitellogenesis stages, the percentage of vitellogenic follicles from anterior to posterior portion increased significantly ($p<0.05$).



(7) (8) (9)

Fig. 7: Migration of nucleoli to the center of nucleus in tertiary vitellogenic follicle (arrow) is considerable (PAS ×10).
Fig. 8: The mature follicle; the ooplasm filled completely by yolk spheres (arrow) (H&E ×10).
Fig. 9: Ovigenous lamellae (arrow) (H&E ×10).

In maturation stage, the percentage of vitellogenic follicles in median portion was more than anterior portion ($p<0.05$), but there was not any difference between median and posterior portion significantly (Table 3). Also, from previtellogenesis stage to vitellogenesis and maturation stage the parenchyma increased significantly ($p<0.05$) (Table 4).

Three different follicles were recognized in the ovigenous lamellae: chromatin nucleolus, pre nucleolus and early cortical alveolus follicles (Fig. 9).

There was a significant difference in gonadosomatic index (GSI) between three stages of reproductive cycle, from previtellogenesis to vitellogenesis and maturation stages which it increased significantly ($p<0.05$) (Table 5). The mature ovaries were seen during April, May and June. In July, 1 fish (14.3%) was in previtellogenesis stage and 6 fishes (85.7%) had mature ovary. In August, 4 fishes (57.1%) and 3 fishes (42.9%) were in previtellogenesis and maturation stages respectively. In September, 4 fishes (57.1%) and 3 fishes (42.9%) were in vitellogenesis and maturation stages respectively. In October, 3 fishes (42.9%) and 4 fishes (57.1%) were in late vitellogenesis and maturation stages respectively.

DISCUSSION

It is well known that environmental factors can have profound effects on the timing of gametogenesis, vitellogenesis and maturation in fishes [7, 10]. Miranda *et al.* [10] showed that all levels of the brain-pituitary-gonadal axis were stimulated by the increase in day length. The spawning period in common carp is typically when water temperature is between 18-28°C [12]. Khouzestan province located in southwestern of Iran with high temperature and long photoperiod since April to October [11]. The histological results showed that common carp in this area had mature ovaries in April and these fishes were ready to spawning in onset of spring season. The histological observation showed that some of fishes are in maturation stage since April to October. It indicated that common carp breeding season in Khouzestan province take long about 7 months and this species were spawned since April to October. The present study confirms that common carp ovarian maturation correlated with increasing of temperature and photoperiod which it mentioned in table 1. Davies *et al.* [13] showed that common carp ovulated one month earlier at long photoperiod. Guha and Mukherjee [14] reported two clear reproductive cycles in one year in common carp in west bengal. In tropical Brazil and Bangladesh, spawning seasons extended for 5-6 months [5]. In our study there was a significant increasing in GSI from previtellogenesis to vitellogenesis and maturation stages. This observation was in corresponds with Sivakumaran *et al.* [5] in Australia and Tempero *et al.* [12] in New Zealand. They reported that female GSI was negatively related to water temperature and this index increased as oocytes matured and release of oocytes caused a decrease in GSI. Tempero *et al.* [12] reported that female common carp GSI was minimum after spawning and rising to a peak in September when the first spawning activity was observed, also only 20% of fishes were in maturation stage in September [12] while in presence study 42.9% of examined fishes were in maturation stage in September. Bapary and Fainuulelei [9] reported that long photoperiod and high water temperature resulted to increasing in GSI and induction of vitellogenic oocytes.

It seems that previtellogenesis and vitellogenesis stages were short in Khouzestan climate condition and this species enter in maturation phase rapidly. It was in agreement with Bieniarz *et al*. [15] in Poland, they reported that soon after spawning, under long photoperiod and warm temperature common carp re-initiate gonadal growth.

A size increasing is the most obvious manifestation of oocytes development, also the status of nucleus can help to determine the type of follicles. The minimum diameter of oocytes observed in chromatin nucleolus stage and it reached to maximum diameter in secondary vitellogenesis stage. The difference between secondary and tertiary vitellogenesis was in combination of yolk spheres and position of nucleoli. These observations are in agreement with Sivakumaran *et al.* [5] and Mabudi *et al.* [4].

In spawning, the ovaries released its mature oocytes and the connective tissue replaced the ovarian structure after ovulation. Because of this, in previtellogenesis stage the parenchyma were decreased significantly ($p<0.05$). In vitellogenesis stage immature oocytes enter in vitellogenesis phase and the number of vitellogenic oocytes increased significantly ($p<0.05$). Because of this, the parenchyma was more than stroma significantly. The results showed that, however the ovary of common carp is asynchronous and different types of follicles can be found in all stages of reproductive cycle, but the percentage of maturating oocytes were increased from anterior to posterior portion of ovaries in three stages of reproductive cycle, It seemed that since in the boney fish spawning has down from posterior of the ovary [16], this may be cause of further migration of mature follicles to the posterior portion.

CONCLUSION

The histological results showed that common carp has mature ovaries from April up to October which it indicated that breeding season of common carp take long about 7 months in this area and it has spawning activity in this period.

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