

Research Article

The profiles of sex Steroid Hormones in females of grass carp (*Ctenopharyngodon idella*) and their correlation with ovarian growth

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Abstract: The sex steroid hormones includes 17 β -estradiol (E2), testosterone (T) and progesterone (P) were studied during the annual cycle of gonadal maturation. The pituitary gland secretes gonadotropin hormone which is used as monitor and control on the secretion steroid hormones from the theca and granulosa cells of follicular oocyte. The annual fluctuated in 17 β -estradiol (E2) is correlated with the degree of maturation state in the ovary of grass carp as vitellogenesis ceased and the ova reached to the final maturation state, the concentration of E2 decreased to the minimum level 109ng/ml. The testosterone (T) level among the other sex steroid hormones plays an essential role in the sexual maturation. Testosterone (T) fluctuated through the maturation cycle and reached the lowest value at immature state of gonad and measured 1.5ng/ml, however this value gradually increased and peaked in ripe stage to 2.2ng/ml. The value of T decreased to 1.5ng/ml in atretic stage. The fluctuated in the total T level in the serum of grass carp seem to be inconsistent with fluctuation of E2. Progesterone (P) peaked in maturing ovary and measured 12.9 ng/ml and then decreased to 9.2 in ripe stage. Both Follicle stimulating hormone (FSH) and luteinizing hormone (LH) play an important role during the annual cycle for growth oocyte and is necessary for maturation and successful in propagation process.

Keywords: 17 β estradiol (E2), testosterone (T), progesterone (P), Follicle stimulating hormone (FSH) and luteinizing hormone (LH).

INTRODUCTION

The grass carp, *Ctenopharyngodon idella* has economic value and introduced to Egypt to be reared in freshwater ponds and River Nile to control the aquatic plants. The fish are typically not spawned naturally in nature and the final maturation has not been completed. The failure in this process was either due to lack of synthesis of sex steroid hormone from follicular oocytes or pituitary gland gonadotropin deficiency in controlling and regulating this process.

To understand this concept, further research on secreted hormone from the hypophyseal gland and the other hormones secreted from the oocyte follicular must be involved testosterone, 11 keto testosterone, 17 β -estradiol and 17 α ,20 β -Dihydroxy-4-pregnen-3-one (17 α ,20 β -DP) in gonad maturation (Lubzen *et al.*, 2010, Schulz *et al.*, 2010) However, testosterone and 11 keto testosterone are male androgens; they are also present in

female fish Rinchard *et al.*, (1993) and Slanter *et al.*, 1994. There is a relationship between testosterone and 17 β -estradiol in female fish, and testosterone in turn leads to 17 β -estradiol production leading to vitellogenic development Kagawa *et al.*, 1982. Other sex steroid hormones, that played major roles in final oocyte maturation are (17 α , 20 β -DP) and 17 α , 20 β -21-trihydroxy-4-pregnen-3-one (17 α , 20 β -TP) (king *et al.*, 1994 and Maylonas *et al.*, 1997). Injection of luteinizing hormone (LH) initiated the spawning process (Zohar and Mylonas, 2001). In order to illustrate the successful propagation process, the present study was planned to study the changes of steroid hormones at different stages of sexual maturation coinciding with oogenesis.

MATERIAL AND METHOD

The female fish specimens were collected from floating cages in River Nile during annual cycle

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extended from January to December 2016. The body weights were ranged from 2350 to 4754gm, while the total length arranged from 52cm to 53.2cm. The age of collected fish ranged from 3-4 years. The specimens were sacrificed, dissected and the ovaries were separated. The ovaries were weighed and gonadosomatic Index (GSI) was also calculated according to the equation:

$$\text{GSI} = \frac{\text{Gonad weight (g)}}{\text{Gutted weight (g)}} \times 100$$

Histological Investigation:

The gonad were fixed in the bouin's fluid for 24 hours, then dehydrated in ascending degrees of ethyl alcohol, cleared in xylene and embedded in molten paraplast wax (paraplast, the melting point 56°-58°). Six µm thick histological sections were cut and stained with either haematoxylin and eosin or with triple mallory stain

Hormonal Analysis:

The blood samples were collected from the heart and allowed to clot and centrifuged at 4000 RPM. The serum was separated and kept at -20°C in deep freezer. The progesterone, testosterone and estradiol were determined by ELISA Kits according to Bayunova *et al.*, 2002 their values were measured according to Roche Hetachi cobas C311.

Statistical Analysis

All data were analyzed by Tukey test. Tukey test was applied to identify which means were significant at $P < 0.05$.

RESULTS:

According to the morphological changes of ovary, the developmental stages could be classified into four stages.

Immature Stage

Morphologically in this stage, the ovary was a colourless compressed strand, adhering to the upper wall of the abdominal cavity and above the kidney. The blood supply cannot detect with naked eye during this stage as in Fig.1 (a). Histological observation of immature ovary showed different stages of oocytes: chromatin nucleus stage, early & late perinucleolus stage, as shown in Fig. (1b).

Maturing Stage

Morphologically, maturing ovary appeared opaque, red in colour. The eggs were visible with naked eyes and the blood capillaries were distributed along the ovarian wall. Histologically, the maturing ovary contains different stages of late perinucleolus stage early and mid-yolk vesicle as shown in Figs.1 (c&d).

Ripe Stage

Morphological observation of ripe ovary was large opaque organ and appeared yellow in color and loaded with yolk and the eggs were clearly visible with naked eyes. The blood supply of the ovary can be observed through a developing of blood capillary and distributed as a network on the outer surface of the ovarian wall. Histologically, ripe ovary characterized with loaded of yolk granule stages (YG) as shown in Figs.1 (e&f).

Atretic Stage

Morphologically the ovary size decreased and was flaccid, flabby and yellowish in colour. Some translucent and opaque residual eggs were visible with the naked eyes. The ovary during this stage was richened with blood supply as in ripe stage. Histologically atretic stage showed presence active invading hypertrophied granulose cell and atretic oocytes remnants of liquefied and yolk granules as shown in Figs.1 (g&h).

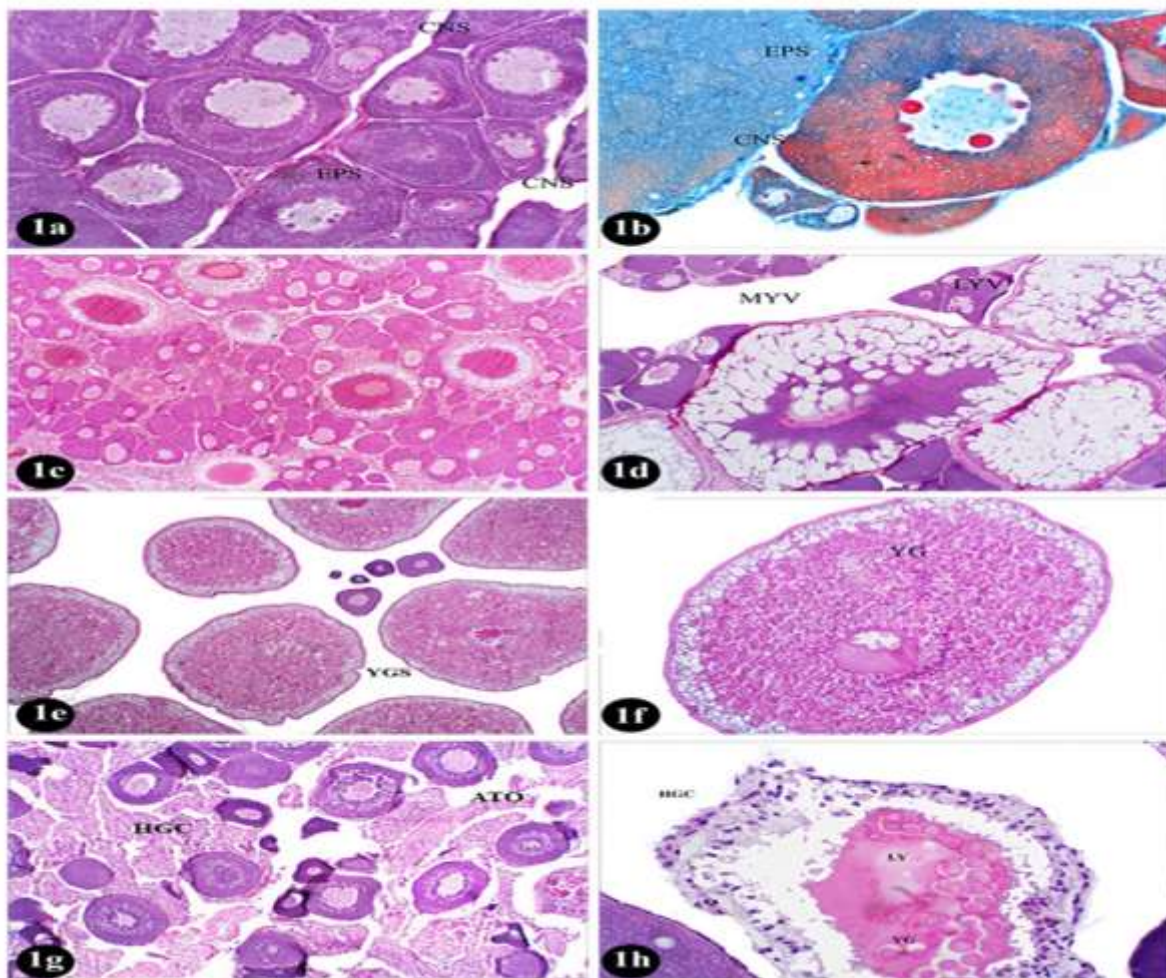


Figure (1). Photomicrograph of histological sections of grass carp, *Ctenopharyngodon idella* ovaries, in different stages of gonadal maturation. Fig. (1a) Immature ovary contain chromatin nucleus stage (CNS), early perinucleolus stage (EPS) as shown in Fig. H&E, X100 & Fig. (1b) Mallory triple stain X100. Figs. (1c&d) Maturing stage showing different stages of late perinucleolus stage (LPN) early and midyolk vesicle (EYV) (MYV) H&E, X400. Figs. (1e&f) Ripe stage characterized with the presence of yolk granule stages (YG) H&E, X200. Figs. (1g&h) Atretic stage showed presence hypertrophied granulose cell (HGC) and atretic oocytes (ATO) remnants of liquefied and yolk granules (LY & YG) H&E, X400

Profile of Sex Steroid Hormones:

In the present study, concentrations of 17β-estradiol (E2) in serum of females grass carp, *Ctenopharyngodon idella* was exhibited a significant variation among the maturation stages of ovarian development. After the end of resting stage and during immature stage, E2 appeared in a peaking value 524 pg/ml.

In the maturing ovaries E2 in serum decreased to reach an average concentration of 381pg/mL. With progress of exogenous accumulation of protein and lipid in to the ovary toward ripe stage, the E2 level in serum was recorded 471pg/mL. As the vitellogenesis

ceased and ova reached to maturation state the concentration of E2 decreased and reached to the minimum level in serum 108pg/mL as showed in Fig. (2) and Table (1).

The trend of gonadosomatic index (GSI) fluctuation was peaked with14.79% in ripe stage, while the E2 recorded to 471pg/mL in serum. Sharply decrease of GSI (3.09%) of female was coincided with decline of E2 (108pg/mL) during atretic ovary events. The value of E2 decreased to 108pg/ml, during atretic stage, while GSI recorded the lowest value 3.097% as showed in Fig. (2) and Table (1).

Total testosterone (T) level fluctuated through the maturation cycle of female grass carp, *Ctenopharyngodon idella*. It seems to be in consistent with fluctuation of E2, since at immature stage T recorded 1.5ng/ml. This value gradually increases from 1.67ng/ml in maturing stage until reached to peak in ripe stage (2.2ng/ml). The value of T decreased to1.5ng/ml again in atretic stage as shown in Fig. (3) and Table (2).

Progesterone (P) level in serum was measured 12.9ng/ml during immature stage then the value peaked in maturing ovary stage and reached 20.2ng/ml. This value sharply decreased and attained in ripe stage

9.2ng/ml, then increase to 12.3ng/ml in atretic stage.as shown in Fig. (4) and Table (3).

Follicle stimulating hormone (FSH) was measured in serum of female grass carp, *Ctenopharyngodon idella* during annual maturation cycle of ovary and the results showed the variation in the level value from immature stage to atretic one.

On the other hands, FSH level in serum was recorded during immature stage 0.13mIU/ml and peaked in maturing stage with 0.15mIU/ml. The minimum value was recorded in ripe stage 0.1m IU/ml then slight increase was attained in atretic stage with 0.12mIU/ml as shown in Fig. (5) and Table (4).

The inverse relationship between fluctuations of FSH and luteinizing hormone (LH) referred as gonadotropin was observed, since lowest value of LH was 0.64 mIU/ml measured in maturing stage, while the FSH recorded the peaked value and when LH peaked in

ripe stage with value 0.8mIU/ml, FSH recorded lowest value. LH was recorded 0.7 &0.67mIU/ml in immature and atretic stage respectively as shown in Fig. (5) and Table (4).

GSI was peaked with maximum value 14.79% during ripe stage inconsistent with maximum level of serum LH during same stage, while FSH recoded the lowest value 0.1mIU/ml. During mature stage, the value of GSI was recorded 4.68%, while LH measured the lowest value 0.64mIU/ml and at the same time FSH attained the maximum level 0.15mIU/ml.

The inverse relationship between the fluctuations of progesterone (P) and E2 was observed as shown in Fig. (5), since the value of E2 was 381pg/ml in maturing stage, while the P recorded the peaked value 202ng/ml and when E2 peaked in ripe stage with value 471pg/ml P decreased to 92ng/ml.

Table (1): Relationship between concentration of serum estradiol (pg/ml) and gonadosomatic index (%) of females grass carp, *Ctenopharyngodon idella*.

maturity Stage	No. of fish	Average GSI (%) ± SD	Concentration of serum estradiol (pg/ml)		
			Max.	Min.	Average ± SD
Immature	5	3.5±0.057	528	520	524±3.265
Mature	5	4.68±0.1053	384	379	381±1.885
Ripe	5	14.798±0.098	475	468	471±3.091
Atretic	5	3.097±0.1250	110	106	108±1.699

* $P < 0.05$ as determined by one way ANOVA test and Tuky test and multiple comparison test.

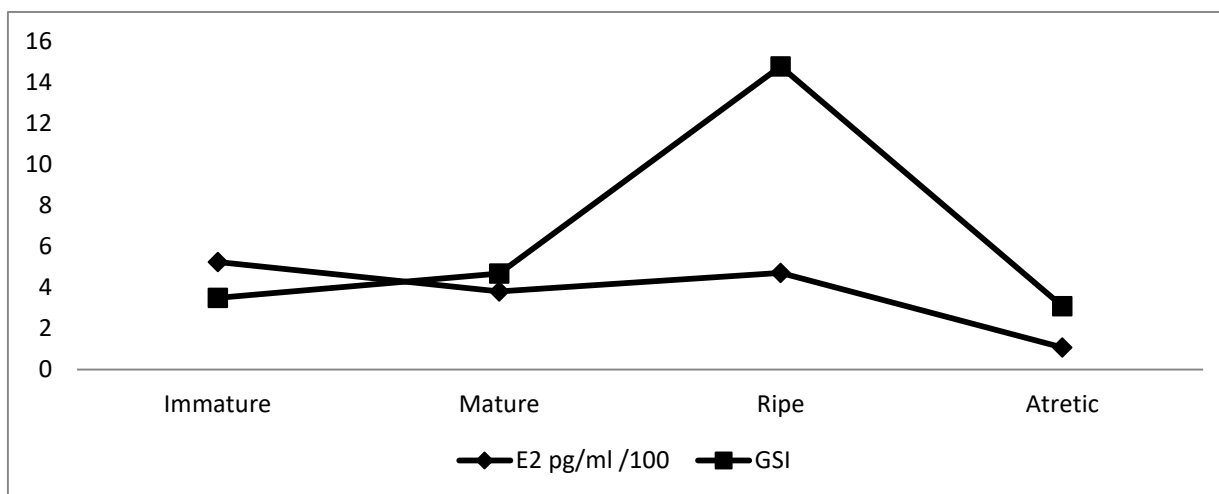


Fig. (2): Relationship between concentration of serum estradiol (pg/ml) and gonadosomatic index (%) of females grass carp, *Ctenopharyngodon idella*.

Table (2): Relationship between concentration of serum Testosterone T (ng/ml) and gonadosomatic index (%) of females grass carp, *Ctenopharyngodon idella*

maturity Stage	No. of fish	Average GSI (%) ± SD	Concentration of serum testosterone (ng/ml)		
			Max.	Min.	Average ± SD
Immature	5	3.50±0.057	1.62	1.44	1.50±0.032*
Mature	5	4.68±0.105	1.75	1.6	1.67±0.068*
Ripe	5	14.79±0.098	2.36	2.1	2.20±0.085*
Atretic	5	3.09±0.125	1.63	1.43	1.50±0.046*

* $P < 0.05$ as determined by ANOVA data and Tuky test and multiple comparison test.

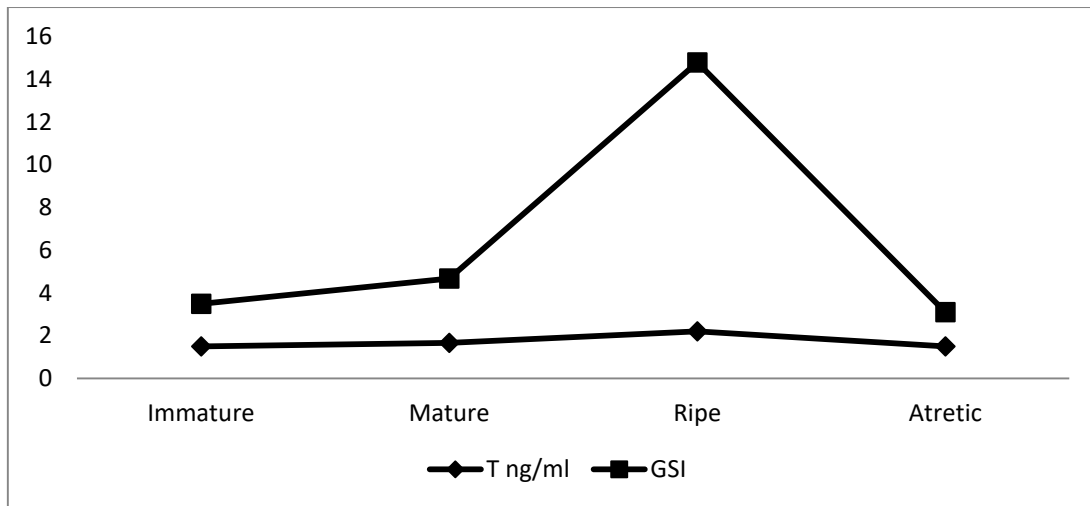


Fig. (3): Relationship between concentration of serum Testosterone (ng/ml) and gonadosomatic index (%) of females grass carp, *Ctenopharyngodon idella*.

Table (3): Relationship between concentration of serum progesterone (ng/ml) and gonadosomatic index (%) of females grass carp, *Ctenopharyngodon idella*

maturity Stage	No. of fish	Average GSI (%) ± SD	Concentration of serum progesterone (ng/ml)		
			Max.	Min.	Average ± SD
Immature	5	3.50±0.057	131	126	129±0.942
Mature	5	4.68±0.105	236	207	202±4.109
Ripe	5	14.79±0.098	95	90	92±0.942
Atretic	5	3.09±0.125	126	120	123±1.414

* $P < 0.05$ as determined by ANOVA data and Tuky test and multiple comparison test.

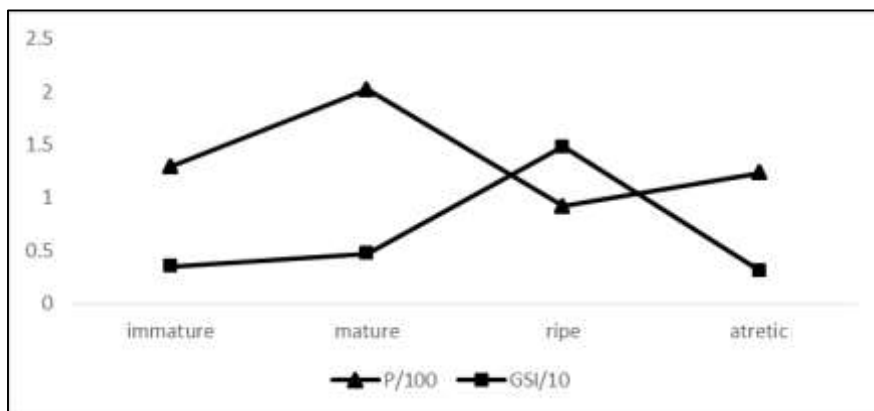


Fig. (4): Relationship between concentration of serum progesterone (ng/ml) and gonadosomatic index (%) of females grass carp, *Ctenopharyngodon idella*.

Table (4): Relationship between concentration of serum FSH and LH ((mIU/ml) for of females grass carp, *Ctenopharyngodon idella*.

	No. of fish	Immature	Mature	Ripe	Atretic
		Average* ± SD (Min., Max.)	Average* ± SD (Min., Max.)	Average* ± SD (Min., Max.)	Average* ± SD (Min., Max.)
FSH mIU/ml ± STD	6	0.13±0.005 (0.122, 138)	0.15±0.003 (0.091, 0.11)	0.1±0.004 (0.14, 0.16)	0.12±0.004 (0.118, 0.126)
LH mIU/ml± STD	6	0.7±0.031 (0.66, 0.74)	0.64±0.008 (0.62, 0.66)	0.8±0.020 (0.77, 0.84)	0.67±0.012 (0.65, 0.71)

* $P < 0.05$ as determined by ANOVA data and Tuky test and multiple comparison test.

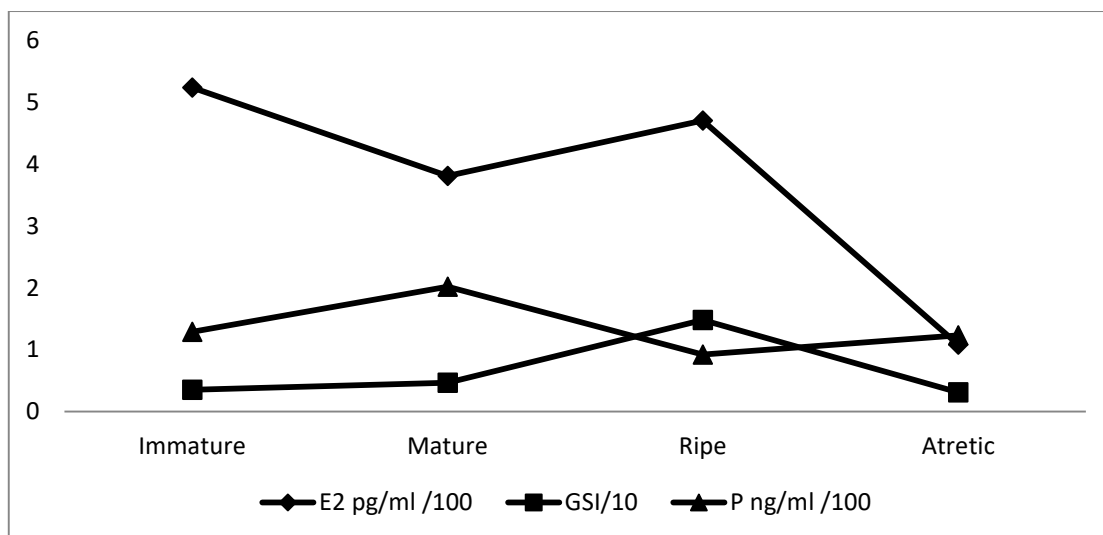


Fig. (5): Relationship between concentration of serum FSH and LH (mIU/ml) and GSI (%) for of females grass carp, *Ctenopharyngodon idella*.

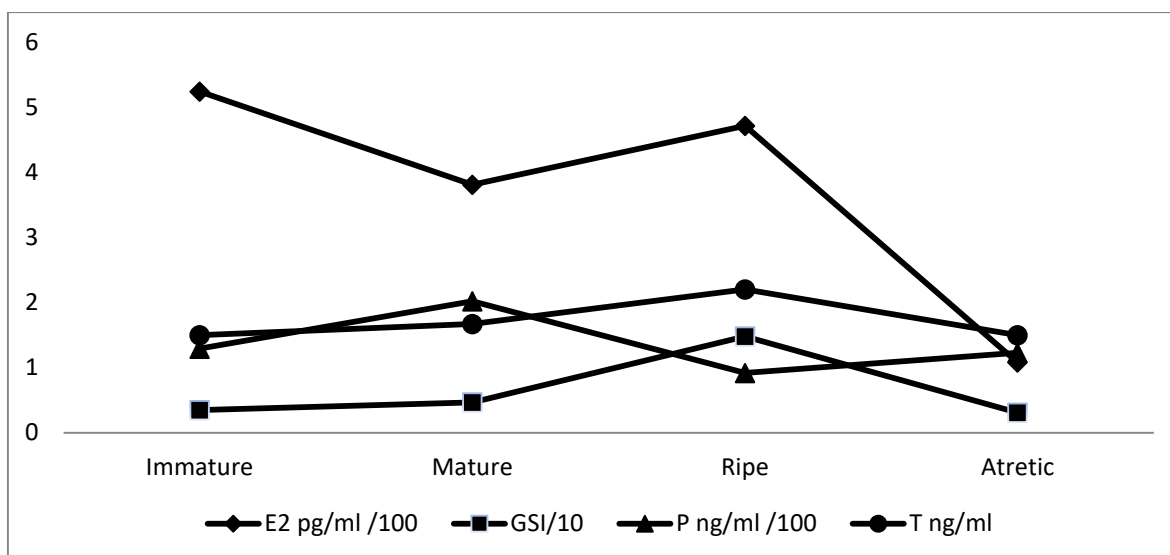


Fig. (6): Relationship between concentration of serum E2 (pg/ml), P (ng/ml) and T (ng/ml) with GSI (%) in the ovary of females grass carp, *Ctenopharyngodon idella*.

DISCUSSION

The annual fluctuation of steroids hormones concentrations in related to maturation state in the gonads of several teleosts species has been studied. These studies conducted that the annual variation of steroids hormones concentrations closely related to factors such as temperature, environment, species of fish, length of day and gonadal sex Pavlidis *et al.*, 2000. While Abbasi *et al.*, 2008 added that annual fluctuations of steroids related to reproductive, feeding and growth cycles in fishes. 17β-estradiol (E2) is the main ovarian steroid in teleost fish, which is produced by a hepatic yolk precursor as stated by Jerez *et al.*, 2006. Erdođan *et al.*, 2002 found that in case of in *Capoeta capoeta umbla*, the seasonal changes in both serum lipids and steroid hormones were associated with reproductive activity.

The result of present finding showed that E2 concentrations in serum of females grass carp; at the beginning of maturation E2 maintains high level then

decrease in mature stage. This decrease may be to the available concentration of E2 not renewed and these amounts utilized in activate the endogenous growth. This finding was also similar to the results obtained by Heidari *et al.*, 2010, who reported that the high level of plasma E2 during the primary growth phase of the oocyte may be related to recruitment (proliferation) of ovarian germ cells.

In the proliferation stage, (maturing ovaries), E2 in serum decreased to 381pg/ml. This decrease may be due to utilized in activation of vitellogenesis as mentioned by Cornish, 1998, Who suggested that during the mid-cycle of *Oreochromis mossambicus*, the decline in Estradiol levels could be due to a rapid utilization of the hormone in stimulating vitellogenesis. With the exogenous accumulation of protein and lipid (during proliferation period) commenced and with acceleration of vitellogenesis E2 and returned to increase again to form peak during ripe stage, this result

confirmed with other authors, De Vlaming *et al.*, 1980 Smith and Haley, 1988, Galas *et al.*, 1999 and Alvarado *et al.* 2015. As in the present study the decline of E2 level during final maturation and atretic period in case of female grass carp was also reported as in case of Major Carp, *Catla Catla* Sehafii *et al.*, 2014. Cornish, 1998 confirm the same result of decline of E2 during final maturation stage in case of *Oreochromis mossambicus*. In contrast to decline of E2 in period of oocyte final maturation in grass carp as reported in present study Rinchar *et al.*, 1993 mentioned that, in other teleosts such as gudgeon (*Gobiogobio*), there was no decrease of E2 level during oocyte maturation.

Metwally and Fouad. (2008) found that during artificial spawning in female grass carp, the induced spawning with injection by Ovaprim and Pregnyl, high Estradiol concentration recorded 855.54pg and after injection with Pregnyl 894.42pg ml and 855.54pg ml after injection of Ovaprim .

The result of present investigation on grass carp showed that the level of E2 increase with progress of exogenous accumulation of protein and lipid in the ovary toward ripe stage which peaked with value 471pg/mL, then declined to reach to the minimum level in serum (108pg/mL) during atretic stage.

The pervious results was inconsistent with the results of other authors (Mayer *et al.*, 1990, Schulz *et al.*, 2010 and Alvarado *et al.*, 2015) who reported that, in female teleosts, the level of the E2 tended to increase gradually during cortical alveoli phase and peaked in the vitellogenesis phase and then declining prior to the ovulation phase. Also Matsuyama *et al.*, 1991 found that E2 begins to increase in accordance with the appearance of active vitellogenic oocytes, and peaked in the tertiary yolk stage oocyte and sharply declines in postvitellogenic and atretic ovaries. Also Hainfellner *et al.*, 2012 reported that the increase of gonadal steroids levels with commenced the vitellogenesis in case of *Prochilodus lineatus* and on Common carp, Taghizadeh *et al.*, 2013. In this respect, Hassan zadeh *et al.*, 2013 considered that the decrease in concentration of plasma E2 is indicator of gonad development.

The relation between estradiol's (E2) and GSI were studied by several authors in the other teleosts. The vitellogenesis and oocyte maturation were regulated by steroids secreted from the follicular cells surrounding the oocyte as reported by Lee and Yang, 2002, so the variation of E2 could be expressed to the state of gonad. The present results showed that a strong correlation between variation of GSI and E2 level in serum of female. At the time of GSI peaked to 14.79% in ripe stage, the E2 reached to 471pg/mL in serum. The sharply decline in GSI value was coincided with decrease of E2 (108pg/mL.) during atretic. These results are similar with the finding of Santos *et al.*, 1986; Matsuyama *et al.*, 1991; Lee, 1998 who reported that in several species. The level of serum E2 begins to

increase with the appearance of active vitellogenic oocytes, and peaked when the dominant stage on the ovary was tertiary yolk stage oocyte. Sharply declines in fish with postvitellogenic and atretic ovaries. Galas *et al.*, 1999 reported that high level of E2 secretion was closely correlated with vitellogenesis in *Cyprinus Carpio*. Also the paralleled increasing of plasma estradiol (E2), GSI and calcium levels in female *Oreochromis mossambicus* was recorded by Cornish 1993.

In case of female, grass carp under the present study indicated that the peak of E2 and GSI are consistent in ripe stage and decline in atretic ovaries. Also Sabet *et al.*, 2009 found E2 and testosterone (T) reached their highest values in month of April, coinciding with peaking of GSI. Erdoúan *et al.*, 2002 reported that the concentrations of testosterone and 17 β estradiol in case of Capoeta, *capoeta umbla* were significantly correlated with the gonadosomatic index (GSI) in the both of male and female.

The result of present investigation showed that the level of serum testosterone (T) fluctuated through the maturation cycle in female of grass carp seem to be inconsistent with fluctuation of E2 level, with progress of maturation T gradually increase during maturing stage until peaked in ripe stage and during vitellogenesis period, and then T decreased in atretic stage.

Galas *et al.*, 1999 related increase T during intense vitellogenesis to this steroid could be used as the substrate for aromatization to E2. In contrast to present investigation Sehafii *et al.*, 2014 reported in case Major Carp *Catla Catla* T of female recorded the lowest value at the time of E2 recorded in the highest level.

Kobayashi *et al.*, (1989) considered the rise T level as a sign of the ovary compacted with fully mature oocyte and became ready to ovulate. So the increase of T level may be due to stop conversion of T to the other steroids through aromatization process. In the present study the beginning of maturation, Progesterone (P) recorded 12.9 ng/ml levels in serum of female grass carp then P peaked in the maturing ovary. The elevation of P attributed to increase in water temperature as in case of *Oreochromis mossambicus* that reported by Cornish, 1998.

The result in the present study investigated that the P level returned to increase again in atretic stage and is considered as indication of the ending of maturation. Similar results were reported with Hobby *et al.*, 2000 in female fish in which the levels of P are elevated during final oocyte maturation and ovulation. Progesterone (P) is an intermediary factor in the formation of some very important estereoids 17 β -estradiol (E2) & testosterone (T) in the gonad of *M. cephalus* (Kobayashi *et al.*, 1989).

Tan, 1985 stated that production of two gonadotropin FSH & LH, by a single cell type in mammalian is

established and the number of gonadotropins secreting cells in teleosts is still unresolved. However, some workers claim to be able to demonstrate only one type of gonadotropin on carp as stated by Nagahama, 1973. In the present study, to demonstrate of the number of gonadotropin in grass carp, the work need antibody prepared against vitellogenin and maturational fraction and must be studied in the future investigation.

Conclusion

It can be concluded that the annual fluctuation of steroid hormone in related to maturation state in the ovary of grass carp has been investigated. The concentration of 17 β -estradiol (E2) in the serum of female was exhibited a significant variation among the maturation of ovarian development. As the vitellogenesis ceased and the ova reached to maturation state and the concentration of E2 decreased. The total testosterone (T) level fluctuated through the maturation cycle and seems to be inconsistent with fluctuation of E2. Progesterone (P) peaked in maturing ovary and sharply decreases in the ripe stage. Both folliculate stimulating hormone (FSH) and luteinizing hormone (LH) play an important role during the annual cycle of ovarian maturation and provide basic information necessary for its successful propagation.

CONCLUSION

It can be concluded that the annual fluctuation of steroid hormone in related to maturation state in the ovary of grass carp has been investigated. The concentration of 17 β -estradiol (E2) in the serum of female was exhibited a significant variation among the maturation of ovarian development. As the vitellogenesis ceased and the ova reached to maturation state and the concentration of E2 decreased. The total testosterone (T) level fluctuated through the maturation cycle and seems to be inconsistent with fluctuation of E2. Progesterone (P) peaked in maturing ovary and sharply decreases in the ripe stage. Both folliculate stimulating hormone (FSH) and luteinizing hormone (LH) play an important role during the annual cycle of ovarian maturation and provide basic information necessary for its successful propagation.

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