Anim. Reprod, v.7, n.4, p.373-381, Oct./Dec. 2010.

Changes in plasma levels of steroid hormones during oocyte development of Caspian Kutum (*Rutilus frisii kutum,* Kamensky, 1901)

B. Heidari[1], S.A. Roozati, L. Yavari

Department of Biology, Faculty of Science, the University of Guilan, Rasht, Iran.

Abstract

Kutum (*Rutilus frisii kutum*; Kamensky, 1901) is an economically important Cyprinid species endemic to the Caspian Sea. In this study, the plasma levels of estradiol-17β (E2), 17α-hydroxyprogesterone (17-OHP), testosterone (T), and progesterone (P4) and oocyte developmental stages were studied during the reproductive cycle of *Rutilus frisii kutum*. These hormones were assayed by radioimmunoassay (RIA), and histological features of developmental stages of oocytes were described in detail using light microscopy. The results showed that plasma levels of E2 and T began to increase during the cortical alveolus stage and this trend continued during the vitellogenesis process. The highest plasma levels of E2 and T were measured at the end of the vitellogenic stage (133.4 ± 19.7 and 7.0 ± 1.4 ng/ml respectively) in March. Once oocytes entered the maturing phase in April, E2 and T levels both declined sharply. By contrast, plasma 17-OHP levels started to increase in early April and reached their maximum value in the final maturation stage in mid April (4.0 ± 2.2 ng/ml). Plasma levels of P4 were very low before initiation of the maturational stage but increased notably during maturation by early April (2.6 ± 0.4 ng/ml) and declined again later. These results indicate that in Kutum, the two hormones E2 and T were functionally important during the vitellogenic phase while progestogens were probably associated with the maturational phase of ovarian growth.

Keywords: Caspian Sea, Kutum, oocyte, steroids.

Introduction

The Caspian Sea is the world's largest isolated inland water body. Caspian kutum (Cypriniformes; Cyprinidae; *Rutilus frisii kutum;* Kamenskii, 1901) populations are generally distributed along the coastal regions of the south Caspian Sea. The fish has significant economical importance due to its good taste and culinary customs of local people and is consumed all year round.

The life history of Kutum is recognized as anadromous and sexually maturing adults migrate from the Caspian Sea to freshwater inlets (Anzali lagoon and rivers of southern Caspian Sea) in March for spawning. The fish has a group-synchronous ovary and spawns on aquatic plants or on pebbly substratum in the rivers at the end of April at a water temperature of 9-12ºC (Paykan Heyrati *et al.*, 2007). The association of changes in gonadal development with plasma levels of sex steroids has proven to be a valuable tool for understanding the endocrine control of reproduction and for purposes of restocking valuable teleosts such as Kutum.

It is well known that, in teleosts, vitellogenesis and final oocyte maturation are regulated by gonadotropic hormones that mediate their actions via steroids secreted by the follicular cells surrounding the oocyte (Nagahama, 1994). In many teleosts it has been reported that plasma estradiol-17β (E2) levels increased during the vitellogenic stage but decreased during the maturational stage (Bromage *et al.*, 1982; Kagawa *et al.*, 1983; Shimizu *et al.*, 1985; Sakai *et al.*, 1988). E2 is known to induce the synthesis and release of vitellogenic protein by the liver (Kagawa *et al.*, 1981; Sundararaj and Nath, 1981).

In many if not all teleosts, progestogens can induce oocyte maturation (Kagawa *et al.*, 1984; Richter *et al.*, 1985; Nagahama, 1994; Kobayashi *et al.*, 1996). Correlations between changes in plasma levels of gonadal steroids and oocyte development have been well-documented in a number of species including salmoniforms (Truscott *et al.*, 1986), cyprinids (Kobayashi *et al.*, 1986), catfish (*Heteropneustes fossilis;* Lamba *et al.*, 1983), goldeye (*Hiodon alosoides;* Pankhurst *et al.*, 1986), *Chalcalburnus tarichi* (Unal *et al.*, 2005), Japanese sardine (*Sardinops melanostictus*; Matsuyama *et al.*, 1994), and Korean spotted sea bass (*Lateolabrax maculates*; Lee and Yang, 2002).

In the present study, plasma levels of E2, testosterone (T), 17α -hydroxyprogesterone (17-OHP) and progesterone (P4) were measured in the cyprinid fish, Kutum, from the southern Caspian Sea and adjacent rivers at different stages of oocyte development.

Materials and Methods

Fish capture and blood sampling

Adult Kutum were captured approximately monthly (from October 2007 to May 2008) from the Anzali shore of the Southern Caspian Sea (37º 27′ N, 49º 33′ E ; Guilan Province, Iran) using beach seines.

[1]Corresponding author: bheidari@guilan.ac.ir
Tel: +98(131)324-3630; Fax +98(131)322-0066
Received: August 8, 2010
Accepted: January 11, 2011

Maturing and migratory fish were captured from the Sefid Rood River inlets to the Caspian Sea during the spawning migration in April-May 2008. The data about photoperiod and sea surface temperature of sampling site is given in Table 1.

All samples with body weight range of 685.5 to 1093.57 g were collected during October 2007 to May 2008, which corresponds to the period of gonadal recrudescence and spawning. Fish (n = 75) were anesthetized with 100 ppm 3-aminobenzoic acid ethyl ester (MS222, Sigma) and blood samples (about 5 ml) were collected from the caudal vein using heparinized syringes with 21-gauge needles. Samples were then centrifuged at 3000 rpm for 20 min, and plasma was separated and stored at -25ºC until used for steroid assay.

Radioimmunoassay (RIA)

Plasma levels of hormones were determined using radioimmunoassay (RIA) after extraction (Kagawa *et al*., 1982). Briefly, 50-100 µl of standards, controls or sample plasma was added into tubes coated by antibody (polyclonal rabbit antibodies were used). Thereafter, 500 µl of 125I-labelled E2 (radioactivity 170 kBq, Orion Diagnostica, Finland), 125I-labelled T (Radioactivity 200 kBq, Orion Diagnostica, Finland), 125I-labelled P4 (Radioactivity 185 kBq, Immunotech, France) or 1 ml of 125I-labelled 17-OHP (Radioactivity 185 kBq, Immunotech, France) tracer was added to all tubes and incubated in a water bath (incubation times varied between steroid assays). Following washes in phosphate buffer, radioactivity was counted using a gamma counter (Wallac/LKB gamma counter). The standard concentrations ranged from 0-300 ng/ml for E2, 0-14.4 ng/ml for T, 0–60 ng/ml for P4 and 0-50 ng/ml for 17-OHP. The E2, T, P4 and 17-OHP antisera had very low cross reactivity with other sex steroids examined. For example, E2 and T antisera showed 0.008 and 0.45% cross-reactivity, with 17α-estradiol and methyltestosterone respectively.

Inter- and intra-assay coefficients of variation (CV) for E2 were 8.3 and 2.9%, respectively. The CVs for T, 17-OHP and P4 were 7 and 7.5%, 5 and 7.1%, 5.1 and 3.5%, respectively. In addition, minimum levels of detection for E2, T, P4 and 17-OHP were 0.006, 0.025, 0.05, and 0.046 ng/ml, respectively.

Histology

After blood sampling, fish were killed by decapitation and the ovaries were dissected. Samples of ovary were fixed in Bouin's solution, embedded in paraffin after dehydration and infiltration, sectioned at 5-6 µm thickness and stained with hematoxylin-eosin for histological examination using binocular light microscopy. The diameters of 30 oocytes per female were determined using a calibrated ocular micrometer.

Statiscal analyses

All data were expressed as means ± standard deviation (± SD). Changes in plasma levels of E, T, P4 and 17-OHP were assessed by one-way ANOVA, Duncan's multiple range tests, and relationships between E2 and T levels were examined by non-linear regression (power type). The minimum significance was set at $P < 0.05$. All analyses were conducted using the SPSS 17.0 for Windows computer package.

Results

Kutum oocytes progressed through different stages of development over the winter and spring seasons. Previtellogenic growth commenced in October, as evidenced by increasing abundance of oocytes in the perinucleolus stage (Fig. 1a, b). The cortical alveoli stage and the accumulation of yolk granules (Fig. 1c, d, e) associated with vitellogenesis occurred between December and March, while final oocyte maturation resulting in spawning took place at the end of April (Fig. 1f). In spent stage in May, freshly spawned ovary possessed a number of empty follicles and unovulated oocytes were found to be at the primary developmental stages (Fig. 1g). Fish sampled in this study were all in the 3+ and 4+ year classes.

Steroid hormone levels during previtellogenic growth

During the previtellogenic growth phase, only plasma E2 levels were elevated (93.5 ± 29.4 ng/ml) whereas those of other steroid hormones (T, P, 17-OHP) were low. At these stages, no significant variations were observed ($P > 0.05$; Table 1).

Steroid hormone levels during vitellogenic growth

During vitellogenic growth, comprising the cortical alveoli and vitellogenic stages, significant elevations in E2 and T plasma levels were observed ($P < 0.05$; Fig. 2, 3) whereas the progestogens did not undergo significant changes ($P > 0.05$) until the oocyte maturation stage.

E2 levels continued their increasing trend that started at perivitellogenic growth, reaching their highest value (133.43 ± 19.69 ng/ml) at the end of the vitellogenic stage before declining sharply ($P < 0.05$; Fig. 2).

T plasma levels averaged 4.03 ± 1.21 ng/ml at the cortical alveoli stage and peaked (6.99 ± 1.44 ng/ml) in March, which coincided with the end of the vitellogenic stage ($P < 0.05$; Fig. 3). This trend was partly comparable to that for E2 and yielded a strong t non-linear regression relationship ($R^2 = 0.89$; Fig. 4). Following vitellogenesis and initiation of the anadromous migration of the fish, plasma levels of E2 and T both decreased as shown in Table 1 ($P < 0.05$).

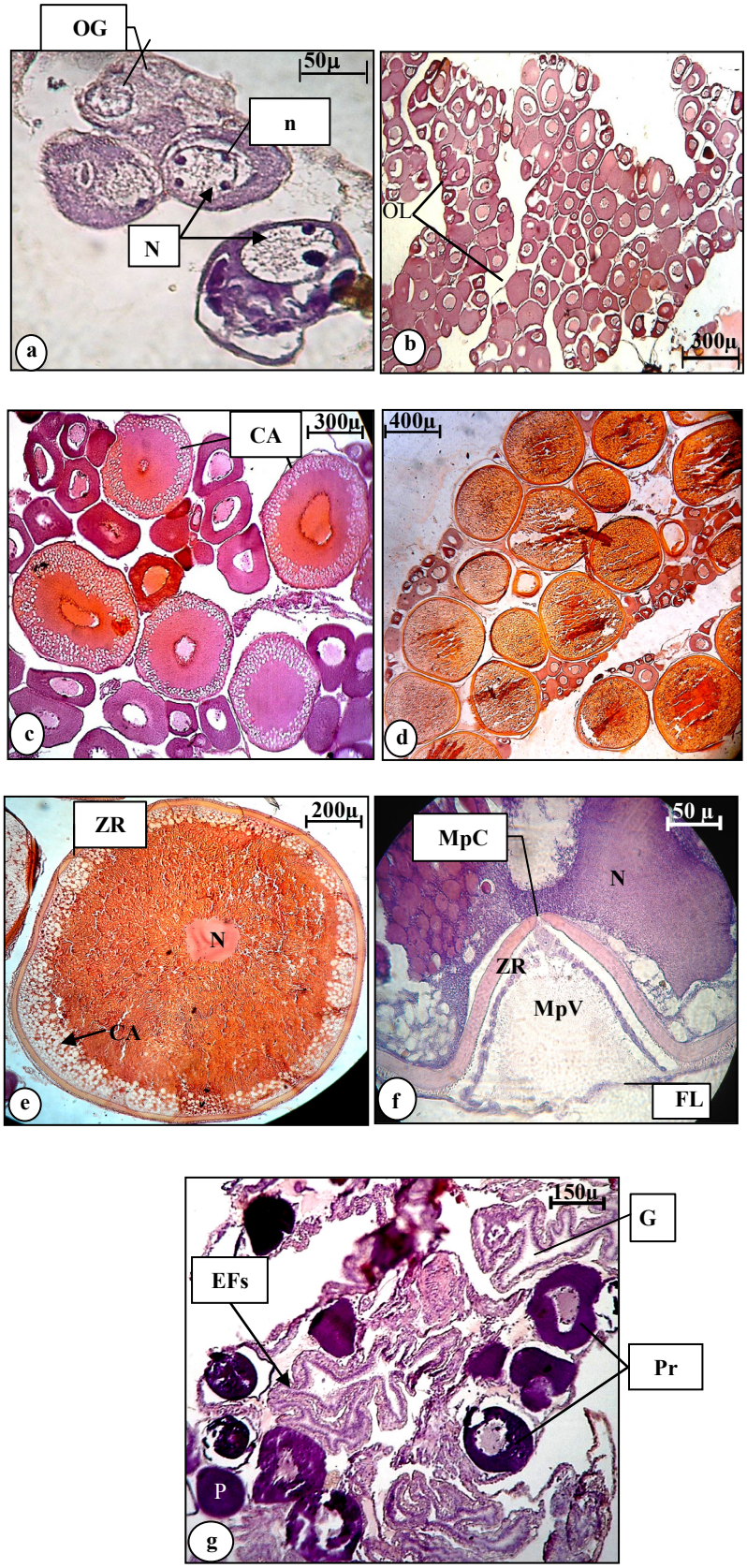

Figure 1. Photomicrographs of Kutum oocytes in the different developmental stages. **a** Primary oocyte stage by October that involved small oocytes (150.9 ± 56 µ) with intense basophilic ooplasm, a high nucleus-cytoplasm ratio, and a few large peripheral nucleoli (*n*). **b** Perinucleolus stage by November, the ovarian lamellae (*OL*) and lower basophilic tendency in H&E markedly were observed. **c** Cortical alveoli stage in December, cortical alveoli (*CA*) were appeared firstly at the peripheral zone of the ooplasm (diameter 475.5 ± 38.3 µ). **d, e** Vitellogenic stage from January to the March, yolk globules characterized the oocytes (diameter 775.5 ± 38.3 µ) at the beginning of Vitellogenesis (**d**) but in advanced stage, they displayed a remarkable increase in both size (1314.3 ± 87.3 µ) and accumulation of yolk bodies (**e**). **f** Maturation stage by April, the stage characterized by completion of germinal vesicle movement to the animal pole, its breakdown (*GVBD*) and coalescence of yolk globules. The highest oocyte diameter was observed at this stage (1435.9 ± 40.3 µ). **g** Spent stage by May, a freshly spawned ovary possessed a number of empty follicles (*EF*) and unovulated oocytes found to be at the primary (*P*) and perinucleolus (*Pr*) stages. *FL*: Follicular Layer; *G*: gap between ovigerous lamellae; *MpC*: Micropylar Canal; *MpV*: Micropylar Vestibule; *N*: Nucleus; *OG*: oogonia; *ZR*: Zona radiata.

Table 1. Mean (+SD) values of parameters measured at the different developmental stages in the sampling period.

Sampling time	Number of samples	GSI (%)	Oocyte diameter (µm)	Photoperiod L/D	Sea surface temperature (°C)	T (ng/ml)	E2 (ng/ml)	P (ng/ml)	17-OHP (ng/ml)	Stages of ovarian growth
04-Oct	8	2 ± 0.6	150.9 ± 56.0	12/12	20.5	0.002 ± 0.001	93.5 ± 29.4	0.02 ± 0.01	0.45 ± 0.15	Primary oocyte
03-Nov	8	2 ± 0.9	262.3 ± 18.0	11/13	19.2	0.003 ± 0.001	9.13 ± 31.2	0.03 ± 0.01	0.53 ± 0.12	Perinucleolus
02-Dec	8	7 ± 2.8	475.5 ± 38.3	10/14	16.1	4.03 ± 1.21	116.43 ± 28.13	0.18 ± 0.04	0.66 ± 0.13	Cortical Alveoli
02-Jan	9	10 ± 0.3	775.5 ± 38.3	10.15'/13.45'	13.2	4.78 ± 0.88	120.80 ± 16.85	0.21 ± 0.08	0.73 ± 0.19	Vitellogenesis
04-Feb	8	14 ± 0.6	1046.7 ± 44.2	11/13	11.4	6.76 ± 0.83	128.86 ± 10.34	0.25 ± 0.06	0.78 ± 0.17	Vitellogenesis
02-Mar	8	18 ± 0.9	1314.3 ± 87.3	12/12	12.6	6.99 ± 1.44	133.43 ± 19.69	0.28 ± 0.05	0.84 ± 0.49	Vitellogenesis
03-Apr	8	18 ± 1.9	1321.7 ± 34.3	13/11	13.8	4.36 ± 1.17	77.5 ± 10.31	2.60 ± 0.37	1.13 ± 0.20	Maturation
15-Apr	10	20 ± 0.9	1435.9 ± 40.3	13.30'/10.30'	14.5	0.26 ± 0.07	47.2 ± 8.57	2.02 ± 0.57	4.02 ± 2.25	Maturation
01-May	8	-------	-------	14/10	15.3	0.02 ± 0.01	16.75 ± 3.86	0.77 ± 0.14	2.54 ± 2.08	Spent

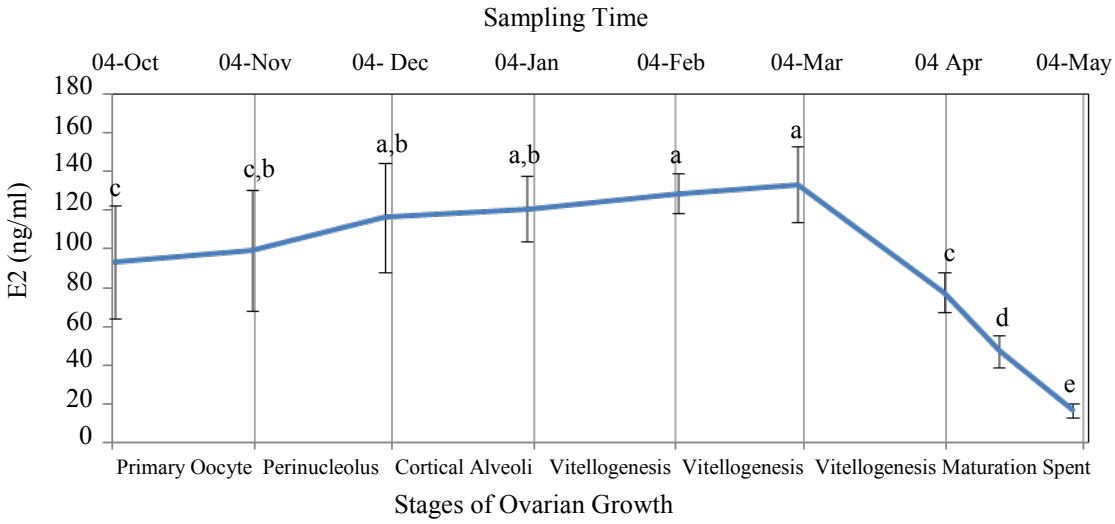

Figure 2. The variations of plasma E2 levels at different developmental stages in the sampling period.

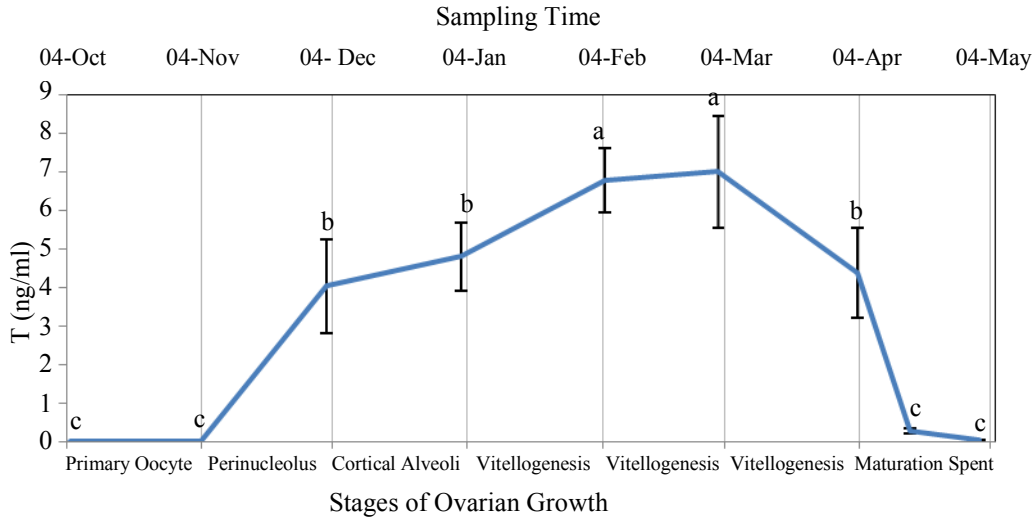

Figure 3. The variations of plasma T levels at different developmental stages in the sampling period.

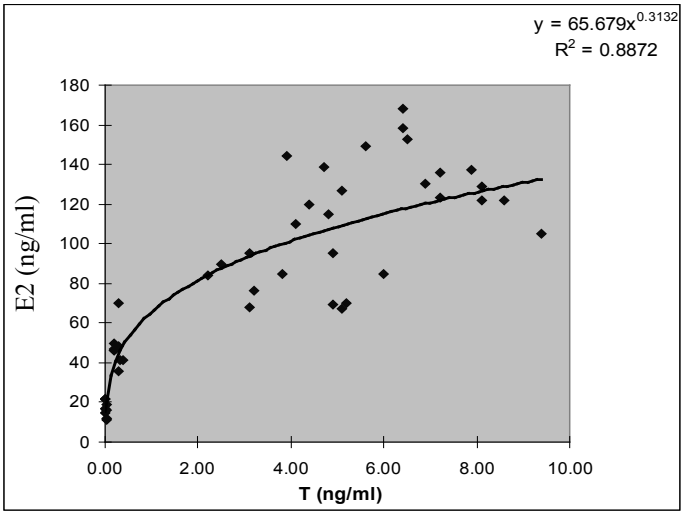

Figure 4. Non-linear regression between T and E2.

Steroid hormone levels during oocyte maturation

The plasma levels of P4 and 17-OHP gradually increased (P > 0.05) from the previtellogenic until the maturational phase (P > 0.05; Table 1). During the maturation stage (early-mid April; Fig. 1f), which corresponded to entry of fish into the rivers, increases in progestogen levels were particularly marked (P < 0.05; Fig. 5, 6).

Plasma levels of P4 reached a maximum (2.6 ± 0.37 ng/ml; P < 0.05; early oocyte maturation) by early April and subsequently declined rapidly (P < 0.05; Fig. 5). In contrast, the final maturation-related increase in the 17-OHP plasma level peaked slightly later, by mid April (4.02 ± 2.25 ng/ml; P < 0.05; Fig. 6), prior to a rapid decrease. In the spent stage (Fig. 1g), levels of all sexual steroids declined sharply (P < 0.05).

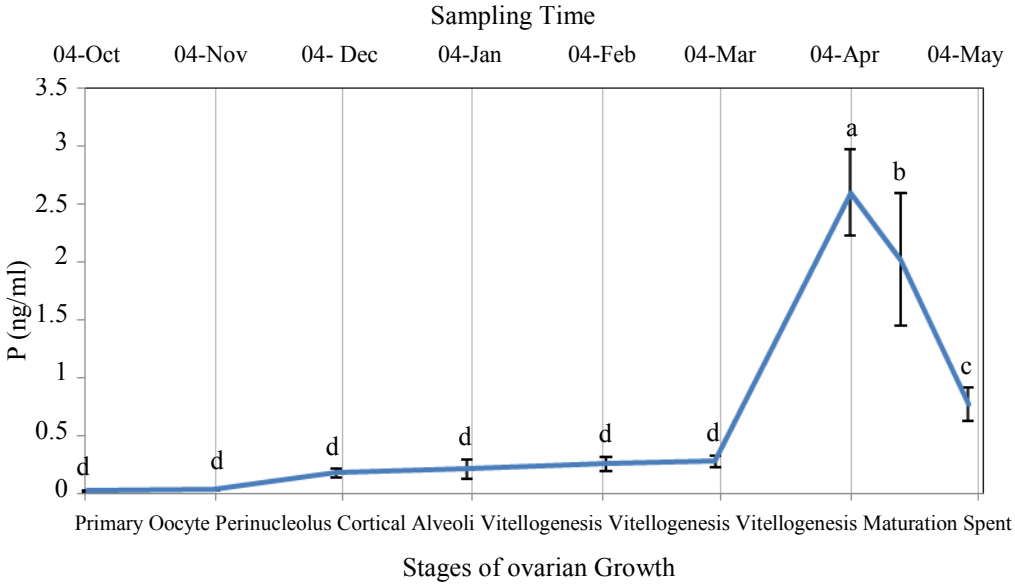

Figure 5. The variations of plasma P4 level at different developmental stages in the sampling period.

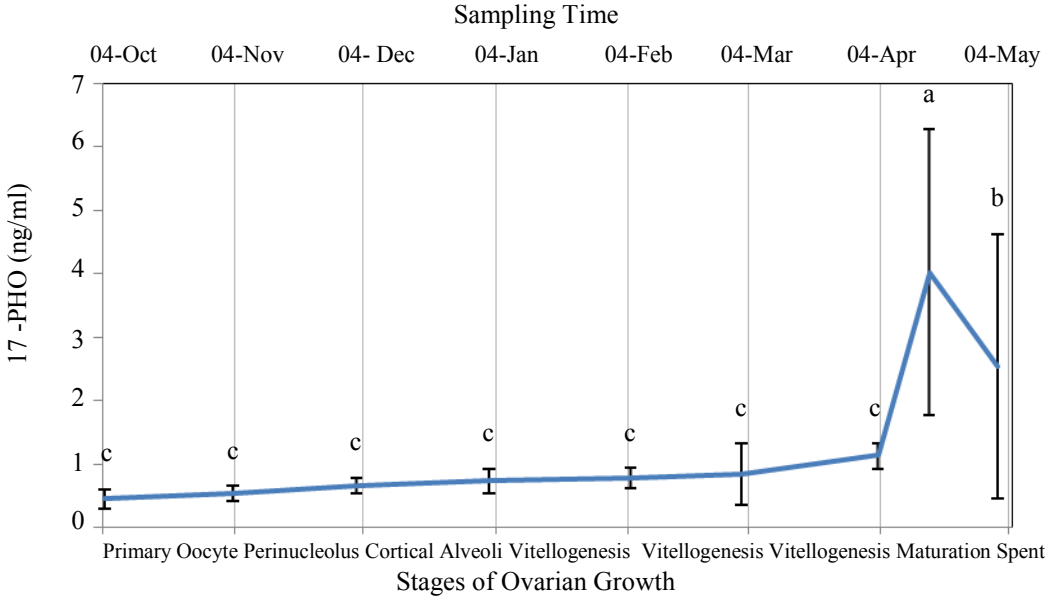

Figure 6. The variations of plasma 17-OHP level at different developmental stages in the sampling period.

Discussion

In this study, we measured the changes in plasma levels of steroid hormones (E2, T, P4, 17-OHP) during oocyte development in *Rutilus frisii kutum*. Ovarian growth typically is divided into three phases, i.e., primary (previtellogenic) growth, oocyte growth (vitellogenesis) and oocyte maturation (Tyler and Sumpter, 1996). Further details of developmental stages of Kutum have already been published (Heidari *et al*.,

2009). Fluctuations in steroid hormones during vitellogenesis and the maturational phase tend to depend on spawning strategy; 1) the pattern of steroid secretion in species with synchronous gamete development is typified by one or two main peaks of activity, as seen in some salmonids and cyprinids where there is annual or bi-annual spawning and production of a single ovulatory clutch (group synchronous; Tyler *et al.*, 1990; King and Pankhurst, 2003). Under this scenario, plasma levels of sex steroids are low or undetectable prior to vitellogenesis. During vitellogenesis, there is a gradual increase in plasma E2 levels in females with matching patterns of T. Plasma E2 levels peak towards the end of vitellogenesis and they decline rapidly in the maturation phase. Plasma T levels decline as oocyte maturation proceeds, whereas plasma maturation-inducing hormone (17,20β dihydroxy-4-pregnen-3-one) levels rise rapidly (Scott *et al.*, 1980; Pankhurst and Thomas, 1998; King and Pankhurst, 2003); 2) the patterns of ovarian development in species with asynchronous gamete development, are more variable, and there is an extended spawning season with multiple cycles of gamete maturation and spawning.

Therefore, patterns of plasma steroid hormones are dissimilar to those of group synchronous species. The main difference is that there is not necessarily a fall in plasma T and E2 levels at ovulation especially if there are further developing oocyte clutches in the ovary. In some species, the highest T and E2 levels occur in females undergoing oocyte maturation and ovulation (Pankhurst *et al.*, 1999).

Kutum presented group-synchronous germ cells (Fig. 1b) with a single annual-spawning episode (end of April) in accordance with the first described pattern of development. Plasma E2 levels began to increase during primary growth, reached 116.43 ± 28.13 ng/ml at the cortical alveoli stage and peaked at the end of vitellogenesis (March; 133.43 ± 19.69 ng/ml) before sharply declining during the maturational phase ($P < 0.05$; Fig. 2). This is consistent with the recognized role of E2 in stimulating hepatic synthesis of the yolk protein precursor, vitellogenin (Kagawa *et al.* 1982; Mommsen and Walsh, 1988; Venkatesh *et al.* 1990; Tyler and Sumpter, 1996). The high level of plasma E2 during the primary growth phase of the oocyte may be related to recruitment (proliferation) of ovarian germ cells. Unal *et al.* (2005) reported a high value of 208 ± 17.3 ng/ml for E2 in anadromous *Chalcalburnus tarichi* at the primary stage of ovarian growth, although Nagahama (1994) reported that plasma levels of steroid hormones during primary growth were low because of its gonadotropin independence.

A similar trend for T levels and close parallel variations of E2 and T, may suggest that T was precursor for E2 in Kutum, akin to the situation for another cyprinid, *Carassius auratus* (Kagawa *et al.*, 1984), the guppy *Poecilia reticulata* (Venkatesh *et al.*, 1990), the medaka *Oryzias latipes* (Kobayashi *et al.*, 1996) and the Persian sturgeon *Acipencer persicus* (Nazari, 2010).

The plasma levels of P4 and 17-OHP increased significantly during the maturation phase of Kutum ovarian growth (Fig. 5, 6). C21 steroids including 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP), 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S), 20 β-dihydroprogesterone and 11-deoxycorticosterone (DOC) have been show to be potent steroid inducers of germinal vesicle breakdown (GVBD; Nagahama, 1994; Nagahama and Yamashita, 2008). Among them, 17α,20β-DP is the most effective steroid in the induction of GVBD in the majority of teleost species (Nagahama and Yamashita, 2008).

In most teleosts 17-OHP, plays a role as precursor of 17α,20β-DP rather than having a direct effect on final oocyte maturation (FOM). Thus in FOM, 17-OHP is converted to 17α,20β-DP by 20β-hydroxysteroid dehydrogenase (20β-HSD) in follicular cells of oocyte (Nagahama and Yamashita, 2008). Sometimes, the plasma level of 17-OHP (a potential precursor for most other ovarian steroids) rises concurrent with plasma level of 17α,20β-DP in time of FOM and ovulation. This concomitant increment trend has been shown in three cyprinid teleosts, *Carassius auratus* (Kobayashi *et al.*, 1986), *Chalcalburnus auratus* (Scott *et al.*, 1983) and bitterling *Acheilognathus rhombea* (Shimizu *et al.*, 1985).

In Kutum, the highest plasma levels of 17-OHP (4.02 ± 2.25 ng/ml) were measured during FOM and this steroid can be considered as one of the most important steroid hormones contributing to oocyte maturation. A study on black gorgy has shown that 2 ng/ml of 17-OHP can cause about 30% of GVBD while 20β-S and 17α,20β-DP in the same concentration caused about 50% of GVBD (Yueh and Chang, 2002). Also in catfish, *Clarias gariepinus*, FOM and ovulation were successfully induced by injection of intramuscular 17-OHP (Richter *et al.*, 1985).

Plasma levels of P4 in Kutum reached their highest value preceding oocyte maturation (early April; 2.6 ± 0.37 ng/ml) and then with increasing of 17-OHP, it decreased in FOM ($P < 0.05$). It is known that P4 is a precursor to other steroids (Scott *et al.*, 1983). In Kutum, P4 may also be a precursor to other steroids.

In conclusion, this study has investigated ovarian growth and accompanying changes in plasma steroid levels in Kutum and demonstrated that plasma levels of E2 and T correlated with the vitellogenic phase and that P4 and 17-OHP were associated with oocyte maturation.

Acknowledgments

The authors thank the staff of the Shahid Ansari Cyprinid Fish Complex (Rasht, Guilan, Iran) for kindly providing the wild freshwater fish samples and Mr. Nahrevar (the member of Inland Aquaculture

Research Center, Anzali, Guilan, Iran) for age determination of samples. In addition, the authors are grateful to Dr. Mark Lokman, University of Otago (Dunedin, New Zealand) for critically reviewing earlier drafts of this manuscript.

References

Bromage NR, Whitehead C, Breton B. 1982. Relationships between serum levels of gonadotropin, oestradiol-17β, and vitellogenin in the control of ovarian development in the rainbow trout. II. The effects of alterations in environmental photoperiod. *Gen Comp Endocrinol*, 47:366-376.

Heidari B, Shabanipour N, Savari A, Yavari V, Hosseini N. 2009. The oocyte development of Kutum, *Rutilus frisii kutum*, Kamensky with special emphasis on the zona radiata structure. *Anim Reprod*, 6:465-472.

Kagawa H, Takano K, Nagahama Y. 1981. Correlation of plasma estradiol-17β and progesterone levels with ultrastructure and histochemistry of ovarian follicles in the white-spotted char, *Salvelinus leucomaenis*. *Cell Tissue Res*, 218:315-329.

Kagawa H, Young G, Adachi S, Nagahama Y. 1982. Estradiol-17 beta production in amago salmon (*Oncorhynchus rhodurus*) ovarian follicles: role of the thecal and granulosa cells. *Gen Comp Endocrinol*, 47:440-448.

Kagawa H, Young G, Nagahama Y. 1983. Relationship between seasonal plasma estradiol-17β and testosterone levels and *in vitro* production by ovarian follicles of Amago salmon (*Oncorhynchus rhodurus*). *Biol Reprod*, 29:301-309.

Kagawa H, Young G, Nagahama, Y. 1984. In vitro estradiol-17b and testosterone production by ovarian follicles of the goldfish, *Carassius auratus*. *Gen Comp Endocrinol*, 54:139-143.

King HR, Pankhurst NW. 2003. Ovarian growth and plasma sex steroid and vitellogenin profiles during vitellogenesis in Tasmanian female Atlantic salmon (*Salmo salar*). *Aquaculture*, 219:797-813.

Kobayashi D, Tanaka M, Fukada S, Nagahama YS. 1996. Steroidogenesis in the ovarian follicles of the medaka (*Oryzias latipes*) during vitellogenesis and oocyte maturation. *Zool Sci*, 13:921-927.

Kobayashi M, Aida K, Hanyu U. 1986. Annual changes in plasma levels of gonadotropin and steroid hormones in goldfish. *Bull Jpn Soc Sci Fish*, 52:1153-1158.

Lamba V, Goswami SV, Sundararaj BI. 1983. Circannual and circadian variations in plasma levels of steroids (cortisol, estradiol-17b, estrone, and testosterone) correlated with the annual gonadal cycle in the catfish, *Heteropneustes fossilis* (Bloch). *Gen Comp Endocrinol*, 50:205-225.

Lee WK, Yang SW. 2002. Relationship between ovarian development and serum levels of gonadal steroid hormones, and induction of oocyte maturation and ovulation in the cultured female Korean spotted sea bass *Lateolabrax maculatus* (Jeom-nong-eo). *Aquaculture*, 207:169-183.

Matsuyama M, Fukuda T, Ikeura S, Nagahama Y, Matsuura S. 1994. Spawning characteristics and steroid hormone profiles in the wild female Japanese Sardine *Sardinops melanostictus*. *Fish Sci*, 60:703-706.

Mommsen TP, Walsh PJ. 1988. Vitellogenesis and oocyte assembly. *In*: Hoar WS, Randall DJ (Ed.). *Fish Physiology*. New York, NY: Academic Press. v. XIA, pp. 247-406.

Nagahama Y. 1994. Endocrine regulation of gametogenesis in fish. *Int J Dev Biol*, 38:217-229.

Nagaham Y, Yamashita M. 2008. Regulation of oocyte maturation in fish. *Dev Growth Differ*, 50:195-219.

Nazari RM. 2010. Plasma sex steroid hormones of Persian sturgeon *Acipenser persicus* as influenced by gonad development stages and season. *Int Aquat Res*, 2:49-54.

Pankhurst NW, Stacey NE, Van Der Kraak G. 1986. Reproductive development and plasma levels of reproductive hormones of goldeye, *Hiodon alosoides* (Rafinesque), taken from the North Saskatchewan River during the open-water season Van Der Kraak. *Can J Zool*, 64:2843-2849.

Pankhurst NW, Thomas PM. 1998. Maintenance at elevated temperature retards the steroidogenic and ovulatory responsiveness of rainbow trout *Oncorhynchus mykiss* to luteinizing hormone releasing hormone analogue. *Aquaculture*, 166:163-177.

Pankhurst NW, Hilder PI, Pankhurst PM. 1999. Reproductive condition and behavior in relation to plasma levels of gonadal steroids in the spiny damselfish *Acanthochromis plyacanthus*. *Gen Comp Endocrinol*, 115:53-69.

Paykan Heyrati F, Mostafavi H, Toloee H, Dorafshan S. 2007. Induced spawning of Kutum, *Rutilus frisii kutum* using GnRHa (D-Ala6, Pro9-NEt) combined with domperidone. *Aquaculture*, 265:288-293.

Richter CJJ, Eding EH, Roem AJ. 1985. 17α-hydroxyprogesterone-induced breeding of the African catfish, *Clarias gariepinus*, without priming gonadotropin. *Aquaculture*, 44:285-293.

Sakai N, Iwamatsu T, Yamauchi K, Suzuki N, Nagahama Y. 1988. Influence of follicular development on steroid production in the medaka (*Oryzias latipes*) ovarian follicle in response to exogenous substrates. *Gen Comp Endocrinol*, 71:516-523.

Scott AP, Bye VJ, Baynes SM. 1980. Seasonal variation in sex steroids of female rainbow trout (*Salmo gairdneri* Richardson). *J Fish Biol*, 17:587-592.

Scott AP, Sumpter JP, Hardiman PA. 1983. Hormone changes during ovulation in the rainbow trout (*Salmo gairdneri* Richardson). *Gen Comp Endocrinol*, 49:128-134.

Shimizu A, Aida K, Hanyu I. 1985. Endocrine profiles during the short reproductive cycle of an autumn-spawning bitterling, *Acheilognathus rhombea*. *Gen Comp Endocrinol*, 60:361-371.

Sundararaj BI, Nath P. 1981. Synthesis of vitellogenin and its uptake by the ovary in the catfish, *Heteropneustes fossilis* (Bloch). *Gen Comp Endocrinol*, 43:201-210.

Truscott B, Idler DR, So YP, Walsh JM. 1986. Maturational steroids and gonadotropin in upstream migratory sockeye salmon. *Gen Comp Endocrinol*, 62:99-110.

Tyler CR, Sumpter JP, Witthames PR. 1990. The dynamics of oocyte growth during vitellogenesis in the rainbow trout (*Oncorhynchus mykiss*). *Biol Reprod*, 43:202-209.

Tyler CR, Sumpter JP. 1996. Oocyte growth and development in teleosts. *Rev Fish Biol Fish*, 6:287-318.

Unal G, Karak H, Elp H. 2005. Ovarian follicle ultrastructure and changes in levels of ovarian steroids during oogenesis in *Chalcalburnus tarichi* Pallas,1811. *Turk J Vet Anim Sci*, 29:645-653.

Venkatesh B, Tan CH, Lam TJ. 1990. Steroid hormone profile during gestation and parturition of the guppy (*Poecilia reticulate*). *Gen Comp Endocrinol*, 77:476-483.

Yueh W-S, Chang C-F. 2002. Oocyte maturation-inducing steroids in protandrous black porgy, *Acanthopagrus schlegeli. Comp Biochem Physiol C*, 131:345-353.