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Research Article

Ovarian Follicle Ultrastructure and Changes in Levels of Ovarian Steroids during Oogenesis in *Chalcalburnus tarichi* Pallas, 1811*

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Abstract: The ultrastructure of the ovarian follicle and the levels of the ovarian 11-dehydrocorticosterone (11-DHC), estradiol-17β (E_2), 17α-hydroxyprogesterone (17α-OHP), and progesterone (P) were studied during cortical alveoli, vitellogenesis and oocyte maturation in *Chalcalburnus tarichi*. The microvilli began to form on the oocyte surface during cortical alveoli phase and during vitellogenesis, came into contact in the pore canals of the zona radiata with microvilli formed from granulosa cells. While the special thecal cells posses specific organelles, which are characteristic of steroid-producing cells, namely, mitochondria with tubular cristae, smooth endoplasmic reticulum and lipid droplets, the granulosa cells contain organelles typical for protein-secreting cells. These findings suggest that during vitellogenesis the special thecal cells are the sites of steroid synthesis in the *C. tarichi* ovary.

The levels of 11-DHC and progesterone were measured at lower concentration than other hormones. While there was a decline in 11-DHC level during cortical alveoli phase (from 52.60 ± 6.54 to 20.33 ± 6.74 ng/ml), during vitellogenesis it increased gradually and reached a peak at the end of vitellogenesis (68.90 ± 9.99 ng/ml). At the beginning of maturation phase, it decreased to baseline level (22.90 ± 1.87 ng/ml) and again increased at the end of the maturation (54 ± 0.75 ng/ml). E_2 levels started to increase at cortical alveoli phase and this increase continued during vitellogenesis too. In February, a small decline was noted. However, at the end of vitellogenesis the level of E_2 reached its maximum value (756.10 ± 26.5 ng/ml). At the maturation phase (in April) it decreased to 213.50 ± 25.5 ng/ml and again a slight increase was perceived (in May). 17α-OHP level was 100.10 ± 14.4 ng/ml at the beginning of the cortical alveoli phase and it decreased to 13.40 ± 1.3 ng/ml at the end of the phase. It started to increase during vitellogenesis and reached its maximum value at the end of vitellogenesis (213.90 ± 8.14 ng/ml). At the beginning of the maturation phase (in April) an evident decline was noted (68.00 ± 8.58 ng/ml). It increased again to 142.20 ± 4.45 ng/ml at the end of maturation (in May). P level was measured to 50.90 ± 2.37 ng/ml at the beginning of the cortical alveoli phase (in August), and it decreased to 22.80 ± 0.50 ng/ml at the end of the phase. This low value was kept at the beginning of vitellogenesis, but during vitellogenesis a small increase was noted and at the end of the phase it was measured as 50.80 ± 4.03 ng/ml. This low level is kept during maturation phase too.

These results suggest that in *C. tarichi* none of these hormones (11-DHC, E_2, 17α- OHP and P) is effective during the cortical alveoli phase, E_2, 11-DHC and 17α- OHP are effective during vitellogenesis while P has no effect and 11-DHC and 17α- OHP are effective at the end of maturation while E_2 and P have no role.

Key Words: *Chalcalburnus tarichi*, ovarian follicles, ovarian steroids, ultrastructure

İnci kefalinde (*Chalcalburnus tarichi* Pallas, 1811) Oogenezis Sürecinde Ovaryum Foliküllerinin İnce Yapısı ve Ovaryum Steroid Seviyelerinin Değişimi

Özet: *Chalcalburnus tarichi*'de kortikal alveolar, vitellogenez ve oosit olgunlaşması sırasında, ovaryum folikülünün ince yapısı ve ovaryumdaki 11-dehidrokortikosteron (11-DHC) östradiol-17β (E_2), 17α-hidroksiprogesteron (17α-OHP), ve progesteron (P) seviyeleri incelendi. Kortikal alveolar safha sırasında, genç oosit yüzeyinde mikrovilluslar şekillenmeye başlar ve vitellogenez sırasında, granüloza hücrelerinden şekillenen mikrovilluslar ile zona radiata tabakasındaki kanallar vasıtasıyla bağlantı kurulur. Spesifik teka hücreleri tübüler kristalı mitokondri, düz endoplazmik retikulum ve lipit damlaları gibi steroid salgılayan hücrelerin karakteristik yapılarını içerirken, granüloza hücreleri protein salgılayan hücreler için tipik organelleri içerir (daha fazla granüllü endoplazmik retikulum ve gelişmiş Golgi kompleksi). Bu bulgular, *C. tarichi*'de, vitellogenez sırasında, spesifik teka hücrelerin steroid sentezleyen merkezler olduğunu göstermektedir.

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11-DHC ve P seviyeleri diğer hormonlardan daha düşük konsantrasyonlarda ölçüldü. 11-DHC seviyesi kortikal alveolar faz sırasında azalırken (52,60 ± 6,54'den 20,33 ± 6,74 ng/ml'ye), vitellogenez sırasında dereceli olarak artarak vitellogenez sonunda en yüksek seviyeye ulaştı (68,90 ± 9,99 ng/ml). Olgunlaşma safhasının başında tekrar bazal seviyeye düştü (22,90 ± 1,87 ng/ml) ve olgunlaşma safhasının sonunda tekrar arttı (54 ± 0,75 ng/ml). E_2 seviyesi kortikal alveolar fazda artmaya başladı ve bu artış vitellogenez boyunca da devam etti. Fakat Şubat'ta küçük bir düşüş görüldü. Bununla birlikte E_2 seviyesi vitellogenez sonunda maksimum seviyeye ulaştı (756,10 ± 26,5 ng/ml), olgunlaşma fazında 213,50 ± 25,5 ng/ml'ye düştü ve Mayıs'ta tekrar bir artış görüldü. 17α-OHP seviyesi kortikal alveolar fazın başında 100,10 ± 14,4 ng/ml, fazın sonunda 13,40 ± 1,3 ng/ml'ye düştü. Onun seviyesi vitellogenez sırasında artmaya başladı ve safhanın sonunda maksimum değere ulaştı (213,90 ± 8,14 ng/ml). Olgunlaşma safhasının başında çok belirgin bir düşüş görüldü (68 ± 8,58 ng/ml) ve olgunlaşma sonunda (Mayıs) tekrar arttı. Progesteron seviyesi, kortikal alveolar fazın başında 50,90 ± 2,37 ng/ml ölçüldü ve fazın sonunda 22,80 ± 0,50 ng/ml'ye azaldı. Bu düşük değer vitellogenezin başında korundu fakat vitellogenez sırasında az bir artışla fazın sonunda 50,80 ± 4,03 ng/ml ölçüldü. Bu düşük seviye olgunlaşma fazında da korundu.

Bu bulgular, *C. tarichi*'de 11-DHC, E_2, 17α-OHP ve P'un kortikal alveolar faz sırasında etkili olmadığını, P'un vitellogenezde etkili olmazken E_2, 11-DHC ve 17α-OHP'nin etkili olduğunu ve E_2 ve P'nin olgunlaşma sonunda (belki ovulasyonda) etkili olmazken 11-DHC ve 17α-OHP'nin etkili olduğunu gösterir.

Anahtar Sözcükler: *Chalcalburnus tarichi*, ovaryum folikülleri, ovaryum steroidleri, ince yapı

Introduction

Chalcalburnus tarichi is an economically important endemic cyprinid species of the lakes Van basin in Turkey. The fish has anadromus behavior and lives in the lake and migrates for spawning to freshwater inlets. Ovarian development stages in *C. tarichi* were determined previously according to age and one-year cycle (1). Teleost oocytes as in other vertebrates are surrounded by two major cell layers as an outer thecal layer and an inner granulosa. Ovarian follicles have the ability to synthesize estrogens such as estradiol-17β, estrone and estriol, progestogens, androgens, and corticosteroids (2,3). In some teleosts, histochemical and ultrastructural studies have shown that the special thecal cells are cellular sources of the sex steroids during the vitellogenesis and oocyte maturation (4-7). Special thecal cells during vitellogenesis and oocyte maturation possess organelles, characteristic of steroid-producing cells, but histochemical reaction for Δ5-3β -hydroxysteroid dehydrogenase activity is negative in *Pagrus major* (7). In the ovarian follicles of *Oncorhynchus rhodurus*, both thecal and granulosa layers are necessary in the production of estradiol-17β in vitro in response to gonadotropins (6,8). The vitellogenic growth and final maturation of teleost oocytes depend largely on the action of ovarian steroid hormones. In many teleosts it has been indicated that plasma estradiol-17β levels increased during the vitellogenic stage but decreased during the maturation stage (9-14). estradiol-17β is known to induce the synthesis and release of vitellogenic protein by the liver (8,9,15,16). However, in *Hiodon alosoides*, estradiol-17β and estrone have not been detectable in any stage of the vitellogenesis (17).

In some teleost progestogens (especially 17α-hydroxyprogesterone) and corticosteroids induce oocytes maturation (18,19). However, in *Hoplostethus atlanticus,* 17α-hydroxyprogesterone and 11-deoxycortisol have been determined to have no effect on oocyte maturation (20).

In the present study, it was aimed to clarify ultrastructural features of the follicles in the cortical alveoli, vitellogenesis and maturation phases and to determine the levels of the ovarian estradiol-17β (E_2), progesterone (P), 17α-hydroxyprogesterone (17α-OHP) and 11-dehydrocorticosterone (11-DHC) during these phases in *C. tarichi*.

Materials and Methods

Mature *C. tarichi* individuals were caught every month between June 1997 and April 1998 from Lake Van and River Karasu, which flows into Lake Van. Their body weight and length were measured and then they were killed by decapitation. The ovaries were removed immediately and frozen at –80 ºC for steroid analysis. Small pieces of ovary of the three fish in February, May and June according to ovarian development stages defined previously (1) were fixed for ultrastructure study.

Electron microscopy: Ovarian follicles were fixed in Karnovsky's fluid for 2 h at 4 ºC. After washing in 0.2 M Cacodylate buffer (pH 7.4) they were postfixed in 1% OsO_4 for 1 h at 4 ºC and after dehydration embedded in Epon 812. For light microscopy, 1-µm thick sections were cut by Reichert ultramicrotome and stained with

toluidine blue. Thin sections were placed on 1000 mesh copper grids and stained with saturated uranyl acetate followed by lead citrate and examined under a Jeol 100 C electron microscope.

High Performance Liquid Chromatography (HPLC): The three fish samples from each month and 1 g of ovarian tissue from each one were used for hormone analysis. The HPLC analysis was adapted from Shimizu et al. (12), Venkatesh et al. (14), and Battal and Ünal (21). Frozen tissue was powdered in liquid nitrogen and methanol was added. The samples were homogenized in an Ultra Tissue Lysis (Ultrasonic Processor Jenway LTD) and centrifuged at 5000 rpm for 30 min at 4 ºC. The extract was evaporated at 35 ºC and then steroids were redissolved in 0.05 M phosphate buffer (pH 7.6). Then samples were passed through Sep-Pak C18 cartridges (Waters) preconditioned with 5 ml of methanol and 5 ml of distilled water. Cartridge adsorbing hormones were eluted with 5 ml of 80% methanol and the eluation was injected into HPLC (Shimadzu, LC-10 AD). Steroids were separated by chromatographic system under isocratic conditions at a flow rate of 1.5 ml/min: µBondapak column with acetonitrile:water (53:47) as a mobile phase. The pressure and wavelength were 2.8 psi and 254 nm, respectively. The elution pattern of each steroid (11-DHC, E_2, 17α-OHP and P) is shown in Figure 1. All concentrations were expressed as mean ± SE. The data were analyzed by analysis of variance (ANOVA). The significance level for differences was set at 0.01.

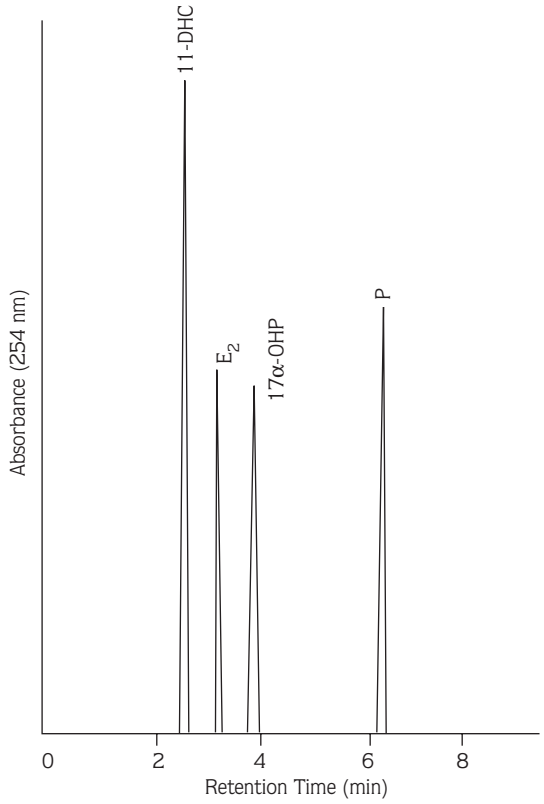

Figure 1. Elution patterns of 11-dehydrocorticosterone (11-DHC), estradiol-17β (E_2), 17α- hydroxyprogesterone (17α-OHP) and progesterone (P) separated using HPLC. Stationary phase: µBondapak column. Mobile phase: acetonitrile:water (53:47). Flow rate: 1.5 ml/min. Detection: uv (254 nm).

Results

The Cortical Alveoli Phase: The ovary of mature *C. tarichi* contains oocytes in various stages of development. The spawning occurs during May-June. After ovulation, oocytes that will be ovulated next year develop from cortical alveoli. This phase is marked by the appearance of the cortical alveoli in the cytoplasma (Figure 2a). The lipid droplets accumulate in the cytoplasm until October. In this phase the oocytes are still covered with one layer of follicle cells. The zona radiata externa (ZE) appears. The microvilli from the oocyte surface appear and penetrate the ZE (Figure 2b).

Table. Ovary steroid levels of corticol alveoli (c.a.), vitellogenic (vit.) and maturation (mat.) phase in *Chalcalburnus tarichi* (ng/ml). Values are mean ± SE.

	c.a.		vit.			mat.		c.a.
Steroids/Dates	Sep. 26 1997	Oct.13. 1997	Dec. 5. 1997	Feb.26. 1998	Mar. 26. 1998	Apr. 1998	May 1998	Aug.24 1998
11-dehydrocorticosterone	20.33 ± 6.74	20.00 ± 4.62	45.75 ± 5.90	58.42 ± 8.0	68.90 ± 9.99	22.90 ± 1.87	54.00 ± 0.75	52.60 ± 6.54
Estradiol-17β	208.70 ± 17.3	286.00 ± 5.20	415.75 ± 61.3	294.95 ± 26.2	756.10 ± 26.5	213.50 ± 25.5	349.60 ± 30.6	166.40 ± 20.8
17α-hydroxyprogesterone	13.40 ± 1.3	78.80 ± 16.1	111.40 ± 34.4	127.00 ± 12.6	213.90 ± 8.14	68.00 ± 8.58	142.20 ± 4.45	100.10 ± 14.4
Progesterone	22.80 ± 0.50	21.50 ± 3.06	44.10 ± 13.2	62.35 ± 4.89	50.80 ± 4.03	45.90 ± 1.74	53.10 ± 12.2	50.90 ± 2.37

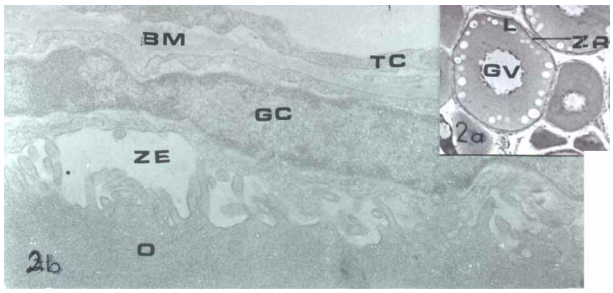

Figure 2a. Light micrograph of an oocyte in the cortical alveoli phase in *C. tarichi*. Lipid droplets (L) are present under the (ZR) zona radiata. GV, germinal vesicle. x70.

Figure 2b. Electron micrograph of follicular cells and zona radiata of an oocyte in the cortical alveoli phase in *C tarichi*. ZE, zona radiata externa. GC, granulosa cell; TC, thecal cell; BM, basement membrane; O, ooplasm. x24,900

Hormone Levels: Changes in 11-DHC, E_2, 17α-OHP and P levels are shown in the Table. In August E_2, 11-DHC, P and 17α-OHP levels were 52.6 ± 6.54, 166.4 ± 20.8, 100.1 ± 14.4 and 50.9 ± 2.37 ng/ml, respectively. In September, 11-DHC and E_2 levels declined. The levels of these hormones in September were 20.33 ± 6.74, 208.70 ± 17.3, 13.40 ± 1.3 and 22.80 ± 0.50 ng/ml, respectively. The changes in 11-DHC and E_2 were not significant ($P > 0.01$) while changes in P and 17α-OHP were significant statistically ($P < 0.01$).

The Vitellogenenic Phase: The yolk globules appear in the peripheral cytoplasm between the lipit droplets and soon after fill in oocyte cytoplasm. With the growth of the oocyte, zona radiata (ZR) increases in thickness (Figure 3) and in November, February and March measured 11.05, 20.54 and 34.06 µm, respectively. The zona radiata consists of an outer (ZE) and an inner layer (ZI). However, the ZE begins to develop with the appearance of cortical alveoli (Figures 2a,b). With the accumulation of yolk globules, lamellar ZI begins to form. The ZE appears electron-dense and compact. With the growth of the oocyte the thickness of TI increases. Microvilli from both the granulosa cells and the oocyte travers the ZR and form the pore channels (Figure 4). The ovarian follicule is composed of two main cell layers: an inner granulosa layer and an outer thecal layer. These layers are separated by thick basement membrane (Figure 5). The thecal layer is composed of theca externa and theca interna cells. A small number of special thecal (ST) cells in thecal interna can be separated easily. These cells are larger than the ordinary thecal cells and contain a centrally located nucleus. ST cells consisted of the smooth endoplasmic reticulum distributed throughout the cytoplasm and the lipid droplets (Figure 6). These cells include generally round mitochondria with tubular cristae. The polysome, relatively less well-developed rough endoplasmic reticulum and the Golgi complex appear in the cytoplasm. A single layer of flattened granulosa cells surrounds the oocytes. They have a centrally located and flattened nucleus with occasionally invaginated outline. One prominent nucleolus was found. The specialized connections between the granulosa cells appeared. A well-developed rough endoplasmic reticulum consists of parallel arrays of cisternae and is oriented near the nucleus (Figure 7). A small amount of smooth endoplasmic reticulum and free ribosome and a lot of mitocondria were also present throughout the cytoplasm.

Hormone Levels: The ovary 11-DHC level was low (20.0 ng/ml) in October, gradually increased during the vitellogenic stage ($P < 0.01$) (45.75 ± 5.90 ng/ml in December and 58.42 ± 8 ng/ml in February) and reached a peak (68.9 ± 9.99 ng/ml) on 20 March (Table). E_2 level in October and December was 286.0 ± 5.2 and 415.75 ± 61.3 ng/ml, respectively. Its level decreased to 294.95 ± 26.2 ng/ml in February but this decline was insignificant ($P > 0.01$). An evident increase was observed in March ($P < 0.01$) and it reached a peak (756.10 ± 26.5 ng/ml). 17α-OHP level was 78.80 ± 16.1 ng/ml on 13 October and its level gradually increased during vitellogenesis and reached a peak on 26 March (213.90 ± 8.14 ng/ml) ($P < 0.01$). P level was the lowest in the beginning of the vitellogenic phase (13 October) (21.50 ± 3.06 ng/ml), increased to 44.10 ± 13.2 ng/ml in December and 62.35 ± 4.89 ng/ml in February. In March, it decreased to 50.80 ± 4.03 ng/ml. The changes in P level during vitellogenesis were not significant.

Maturation Phase: This phase continued for about one month. During the egg maturation, granular structure of material changed, the granules were united, and germinal vesicle migration started. The thickness of the granulosa and theca layers increased and the ZR became more opaque (Figure 8).

Hormone Levels: A decline in all hormone levels was noted during progression from vitellogenesis to maturation phase. At the beginning of maturation (on 24 April), the 11-DHC, E_2, 17α-OHP and P levels were 22.90 ± 1.87, 213.50 ± 25.5, 68 ± 8.58 and 45.90 ±

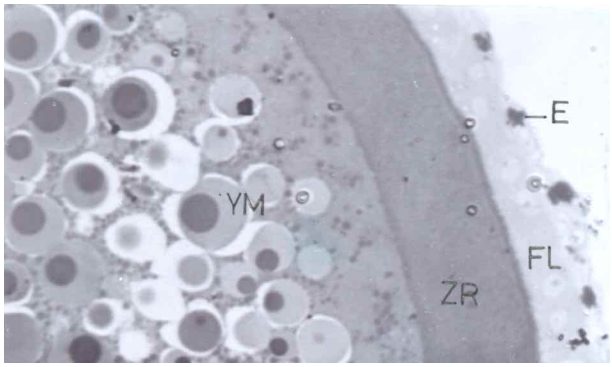

Figure 3. Light micrograph of a oocyte in the vitellogenic phase from 1 µm epon section in *C. tarichi*. YM, yolk material; ZR, zona radiata; FL, follicular layer, E, eritrocyte. x480.

Figure 4. Electron micrograph of the zona radiata in the vitellogenic oocyte *C. tarichi*. ZE, zona radiata externa; ZI, zona interna; GC, granulosa cell; O, ooplasm. x4600.

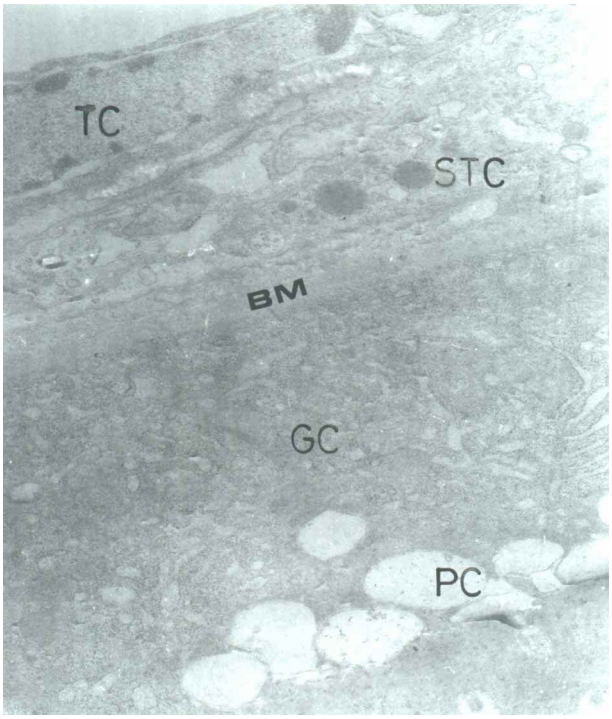

Figure 5. Electron micrograph of follicular cells of a vitellogenic oocyte *C. tarichi*. TC, thecal cell; STC, special thecal cell; BM, basement membrane; PC, pore channel; GC, granulosa cell. x12,500

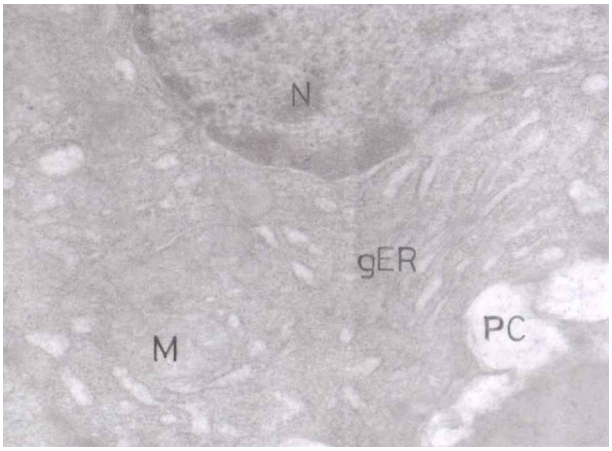

Figure 7. Electron micrograph of the granulosa cell in a vitellogenic oocyte *C. tarichi*. N, nucleus; M, mitochondria; gER, granuler endoplasmic reticulum; PC, pore channel. x19,800

Figure 6. Electron micrograph of the special thecal cell of a vitellogenic oocyte *C. tarichi*. Lipid droplets (L), the smooth endoplasmic reticulum (S) and mitochondria (M). x19,800.

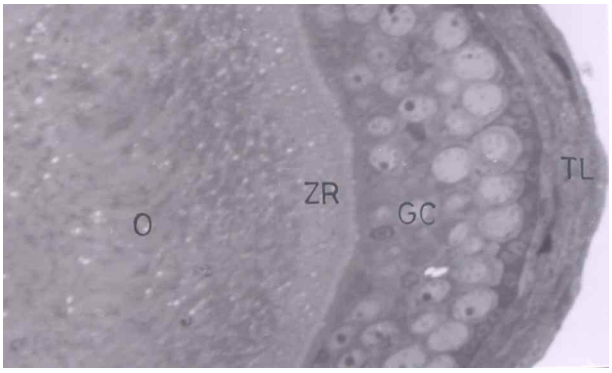

Figure 8. Light micrograph of follicular layers and oocyte in the maturation phase from 1 µm epon section *C. tarichi* . ZR, zona radiata; GC, granulosa cell; TL, thecal layer; O, ooplasm. x525

1.74 ng/ml, respectively. The decreases in 11-DHC, E_2 and 17α-OHP levels were significant ($P < 0.01$) while the decrease in P level was not significant ($P > 0.01$). Generally, the ovary steroid levels showed an increase at the end of the maturation phase. 11-DHC level increased from 22.90 ± 1.87 to 54 ± 0.75 ng/ml ($P < 0.01$), E_2 level from 213.50 ± 25.5 to 349.60 ± 30.6 ng/ml ($P > 0.01$) and 17α-OHP level from 68 ± 8.58 to 142.20 ± 4.45 ng/ml ($P < 0.01$) and P level from 45.90 ± 1.74 to 53.10 ± 12.2 ng/ml ($P > 0.01$). During the cortical alveoli, vitellogenesis and oocyte maturation the measured ovary steroid levels are shown in Figure 9 and the Table.

Discussion

In this species, the microvilli began to form on the oocyte surface in cortical alveoli phase as Matsuyama et al. (7), and Takashima and Hibiya (22) indicated a close contact between microvilli processes of the granulosa cells and oocyte surface was maintained only during vitellogenesis. Therefore, in *C. tarichi*, the transport of the yolk material to oocyte is probably via these pore channels by diffusion and active transport at the molecular level and by endocytosis as suggested by Selman and Wallace (23) and Matsuyama et al. (7). It is generally known that chorion is formed either by the oocyte itself or both the oocyte and the follicle cells. In *Oryzias latipes* it was shown that the major glycoprotein constituent of the inner layer of chorion called ZI was constructed and this substance was produced in the liver in response to estrogen (24,25). In *C. tarichi*, as the red sea bream (7) in which the ZE was formed before the vitellogenic phase initiated, the ZI began to form in the vitellogenic phase and its thickness increased remarkably during vitellogenesis.

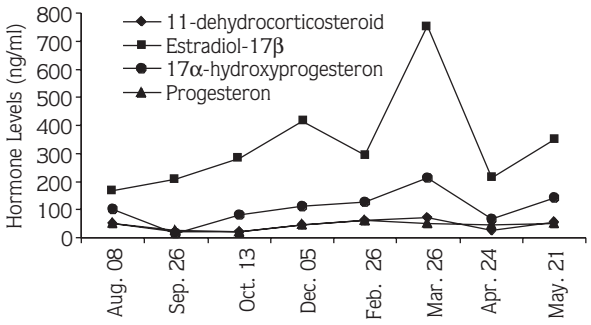

Figure 9. Changes in the ovary steroid levels at cortical alveoli (Aug. and Sep.), vitellogenenesis (Oct., Dec., Feb. and Mar.) and oocyte maturation (Apr. and May) phases in *C. tarichi.*

The ovarian E_2 levels were measured to increase during vitellogenesis. This result is the same in *Oryzias latipes* (26). It may be suggested that as shown in *O. latipes* and *Pagrus major,* the E_2 affects ZI formation in *C. tarichi*.

The granulosa cells and ST cells are the major sites of steroid synthesis in the teleost ovary (6). They are steroid-producing cells that are characterized by ultrastructural features such as mitocondria with typical tubular cristae, smooth endoplasmic reticulum and lipid droplets. As described in *Carassius auratus* (4), *Oncorhynchus kisutch* and *O. gorbuscha* (5), *Salvelinus leucomaenis* (8) and *P. major* (7), in the ST cells of *C. tarichi* these features were also found. In some fish, the electron microscope observations on ST cells are also supported with histochemical results (4,6,8). However, in *P. major*, these observations are not supported histochemically; histochemical reactions for Δ5-3β-hydroxysteroid dehydrogenase (3β-HSD) activity are negative in ST cells during vitellogenesis and maturation (7).

Ovarian development in the *C. tarichi* is synchronous. Spawning occurred once a year in May-June. The highest level of ovary E_2 was observed at the end of vitellogenesis when the oocytes were the largest in size. In many teleost species, plasma E_2 concentrations have been reported to be high during the main vitellogenic growth period of the oocyte development (10,12,14). However, the granulosa cells of vitellogenic oocytes of the *P. major* (7) and two salmonids (5) contain organelles typical of protein-secreting cells. In the *P. major* (7), the histochemical reactions for 3β-HSD activity are negative. Similarly, the granulosa cells of vitellogenic oocytes of the *C. tarichi* contained features suggestive of protein synthesis, i.e. rough endoplasmic reticulum, Golgi complex and mitochondria (Figure 8). In *O. rhodurus* both thecal and granulosa layers are necessary for E_2 production in vitro (27). Therefore in *C. tarichi* the possible involvement of granulosa cells in steroid production cannot be totally excluded. In vitro, biochemical and physiological experiments are necessary to determine the exact role of the granulosa cells in *C. tarichi.*

In *C. tarichi* firstly, the changes in the ovary steroid hormone levels during the cortical alveoli, vitellogenesis and maturation phases of the oocyte development were described. In *C. tarichi* 11-DHC and P levels were measured lower than other hormones. A small decline in the level of 11-DHC was noted during the cortical alveolar phase ($P > 0.01$). 11-DHC level increased gradually during vitellogenesis and reached a maximum value at the end of the phase (68.90 ± 9.99 ng/ml). At the beginning of the maturation phase, a decline in the 11-DHC level was observed again but it increased to 54 ± 0.75 ng/ml at the end of the phase ($P < 0.01$). While in *H. fossilis* (19) the corticosteroids are basic hormones that induce maturation and ovulation, 11-deoxycortisol and its 20β-reduced derivatives (17, 20β, 21-P) are significant in spawning female goldfish (28). Goetz and Bergman (18) stated that in brook trout and yellow perch, the 11-deoxycorticosteroids induce GVBD. However, in Orange Roughy (*Hoplostethus atlanticus*) these hormones are present in the plasma of both sexes but their concentration does not change with reproductive development (20). In *C. tarichi* 11-DHC may affect vitellogenesis and similar to in *H. fossilis* (19) and goldfish (28), it may have a role during or at the end of maturation.

In many teleosts, plasma E_2 levels are high during the vitellogenesis (10-14). Similar results were also found in *C. tarichi*. The increase in ovary E_2 levels correlated with oogenesis. In *C. tarichi* the diameter of the oocytes during cortical alveoli, vitellogenesis and maturation phases has been measured as 369.8 ± 9.2, 773.2 ± 8.9 and 957.5 ± 8.8 µm, respectively (1). E_2 level increased from 166.40 ± 20.8 ng/ml to 208.7 ± 17.3 ng/ml during cortical alveoli but this was not significant ($P > 0.01$). Its level increased during vitellogenesis and reached a peak concentration at the end of the phase (756.10 ± 26.5 ng/m). A slight decline was noted in February. This decline was not significant ($P > 0.01$) and it may be related to individual features or nutrition. The vitellogenin is synthesized in the liver in response to estrogens especially E_2, transported via blood and converted into yolk (8,15,29). It has been reported that E_2 regulated the lipid and carbohydrate metabolism in goldfish (30) and increased serum calcium and phosphoprotein phosphorus levels in *Salmo gairdneri* (9). In *C. tarichi*, the highest value of E_2 indicates the stimulation of vitellogenin synthesis in the liver prior to active accumulation of yolk globules in the oocytes.

Progestins, especially 17α,20β-diOH-P, have been reported to be very effective in inducing oocyte maturation in some teleosts (14,31-33). Asahina et al. (34) stated that some C21-steroids especially 17α-OHP and 17α-20β DHOP have prominent effects on growth of ovipositor. In *C. tarichi* changes in 17α-OHP level observed in each phase of oocyte development had common features. During the cortical alveoli phase, 17α-OHP level decreased from 100.10 ± 14.4 to 13.40 ± 1.3 ng/ml ($P < 0.01$). Its level increased gradually during vitellogenesis and reached its maximum value at the end of vitellogenesis (213.90 ± 8.14 ng/ml). At the beginning of the maturation phase a clear decline was noted but at the end of the phase it increased to 142.20 ± 4.45 ng/ml ($P < 0.01$). These results suggest that 17α-OHP has no effect on the cortical alveoli phase and at the beginning of maturation but is effective during vitellogenesis and at the end of maturation. Both 17α-OHP and 17α,20β-diOH-P have been reported to increase prominently at the time of oocyte maturation and ovulation in *C. auratus* (35) and Kanehira bitterling (12). However, generally the 17α,20β-diOH-P has been considered to be the main maturation-inducing steroid. The major role of 17α-OHP is regarded as a precursor of 17α,20β-diOH-P rather than a direct inducer of oocyte maturation in *Salmo salar* (36), *Plecoglossus altivelis* (37) and *Oryzias latipes* (13). In general, 17α-OHP and/or 17α,20β-diOH-P seem to be the main maturation-inducing steroids in some teleosts, except *Heteropneustes fossilis* (19) and *H. atlanticus* (20). In *H. atlanticus* (20), 17α-OHP has not been detectable in the plasma of any fish at any time. In *C. tarichi*, 17α-OHP may play a role as an indirect inducer on oocyte maturation (as a precursor of 17α,20β-diOH-P).

It is known that progesterone reaches its maximum concentration at the ovulation or after ovulation (38). In *C. tarichi* ovarian P level decreased from 50.90 ± 2.37 ng/ml to 22.80 ± 0.50 ng/ml at the end of cortical alveoli phase ($P < 0.01$) but the changes observed during vitellogenesis and maturation phase were not significant ($P > 0.01$). In our unpublished paper, P level was measured on the 15th day after spawning as 200.7 ± 29.1 ng/ml. It suggests that P was not affected directly before ovulation.

In conclusion, this study investigated the effects on oocyte development of some ovary steroids in *C. tarichi*.

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