Turk. J. Vet. Anim. Sci.
30 (2006) 427-434

Research Article

Levels of Some Ovarian Hormones in the Pre- and Post Spawning Periods of *Chalcalburnus tarichi* Pallas, 1811, and the Postovulatory Structure of Follicles

Güler ÜNAL[1,*], Hatice KARAKİŞİ[2], Mahmut ELP[3]

[1]Department of Biology, Faculty of Arts and Science, Yüzüncü Yıl University, 65080 Van - TURKEY

[2]Department of Biology, Faculty of Science, Ege University, 35040 Bornova, İzmir - TURKEY

[3]Department of Fishery, Faculty of Agriculture, Yüzüncü Yıl University, 65080 Van - TURKEY

Received: 22.12.2003

Abstract: The structure of postovulatory follicles and the levels of ovarian 11-dehydrocorticosterone (11-DHC), estradiol-17β (E_2), 17α-hydroxyprogesterone (17α-OH-P), and progesterone (P), before and after spawning, were studied in *Chalcalburnus tarichi*. It was observed that postovulatory follicles are characterized by a highly vascular thecal layer and hypertrophied granulosa cells containing dense smooth endoplasmic reticulum, many large mitochondria with tubular cristae, and lipid droplets. Ovary 11-DHC level was low in the pre- and post-spawning periods (38.3 ± 4.9-22.3 ± 2.6 ng/ml). The level of E_2 was 216.8 ± 31.1 ng/ml before spawning. This hormone declined on the 1st and 5th day after spawning and increased again to the level of 216.8 ± 6.7 ng/ml on the 10th day; however, the E_2 level decreased significantly on the 15th day (61.5 ± 17.8 ng/ml) ($P < 0.01$). The 17α-OH-P level was low (84.5 ± 49.4 ng/ml) before spawning and reached a peak (295.7 ± 52.5 ng/ml) on the 10th postspawning day, but a slight decline was observed on the 15th day (190 ± 21.5 ng/ml) ($P > 0.01$). The P level was low (23.4 ± 18.3 ng/ml) before spawning. Its level began to increase after spawning and reached a peak on the 10th postspawning day (200.7 ± 29.1 ng/ml) ($P < 0.01$), but declined significantly on the 15th day (63.2 ± 19.5 ng/ml) ($P < 0.01$).

The results obtained from this study indicate that: a) In the postovulatory follicles, the granulosa cells produce steroids; b) 11-DHC has no effect before or after spawning; c) E_2 induces spawning; d) 17α-OH-P and P have an effect after spawning. It can be concluded that the apoptotic cells in postovulatory follicles increased on the 15th day, at which time E_2 and P hormones were at their lowest levels after spawning in *C. tarichi*.

Key Words: *Chalcalburnus tarichi*, postovulatory follicle, ovarian steroids

İnci kefali'nin (*Chalcalburnus tarichi* Pallas, 1811) Yumurta Bırakmadan Önce ve Sonraki Periyotta Bazı Ovaryum Hormon Seviyeleri ve Foliküllerin Ovulasyondan Sonraki Yapısı

Özet: Bu çalışmada, inci kefalinde yumurta bıraktıktan sonra foliküllerin yapısı ve yumurta bırakmadan önce ve yumurta bıraktıktan sonra ovaryum 11-dehydrocorticosteroit (11-DHC), östradiol-17β (E_2), 17α-hydroxyprogesteron (17α-OH-P) ve progesteron (P) seviyeleri araştırıldı. Ovulasyondan sonra foliküllerin bol damarlı bir teka tabakası ve bol miktarda düz endoplazmik retikulum, tubüler kristalı çok sayıda mitokondri ve lipit damlaları içeren büyük granüloza hücreleri ile karakterize edildiği gösterilmiştir. Ovaryum 11-DHC seviyesi yumurta bırakmadan önce ve sonra düşüktür (38,3 ± 4,9-22,3 ± 2,6 ng/ml). E_2 seviyesi yumurta bırakmadan önce 216,8 ± 31,1 ng/ml olarak belirlendi. Bu hormon yumurta bıraktıktan sonra 1. ve 5. günde azaldı ve 10. günde tekrar 216,8 ± 6,7 ng/ml'ye yükseldi. Bununla birlikte, E_2 seviyesi 15. günde belirgin olarak düştü (61,5 ± 17,8 ng/ml) ($P < 0,01$). 17α-OH-P seviyesi yumurta bırakmadan önce düşüktü (84,5 ± 49,4 ng/ml) ve yumurta bıraktıktan sonra artarak 10. günde en yüksek seviyeye ulaştı (295,7 ± 52,5 ng/ml) fakat 15. günde hafif bir düşüş gözlendi (190 ± 21,5 ng/ml) ($P > 0,01$). P seviyesi yumurta bırakmadan önce düşüktü (23,4 ± 18,3 ng/ml). Onun seviyesi yumurta bıraktıktan sonra artmaya başladı ve 10. günde maksimum seviyeye ulaştı (200,7 ± 29,1 ng/ml) ($P < 0,01$) fakat 15. günde belirgin olarak düştü (63,2 ± 19,5 ng/ml) ($P < 0,01$).

Bu çalışmadan elde edilen sonuçlar: a) Ovulasyondan sonraki foliküllerde granuloza hücrelerinin steroit sentezlediğini, b) 11-DHC'nin yumurta bırakmadan önce ve sonra etkili olmadığını, c) E_2'nin yumurta bırakmayı uyardığını, d) 17α-OH-P ve P'un yumurta bıraktıktan sonra etkili olduğunu gösterir. İnci kefalinde, ovulasyondan sonraki foliküllerde apoptotik hücrelerin, E_2 and P'nin yumurta bıraktıktan sonra en düşük seviyede olduğu, 15. günde arttığı sonucu çıkarılabilir.

Anahtar Sözcükler: *Chalcalburnus tarichi*, postovulasyon folikülleri, ovaryum steroidleri

* E-mail: guleru@yyu.edu.tr

Introduction

Chalcalburnus tarichi is an endemic cyprinid species of the Lake Van Basin in Turkey. It spawns once a year and has an ovary of the synchronous type. It is generally accepted that the fish's ovarian follicles, as in other vertebrates, produce steroid hormones. In many fish, it has been shown that the ovarian follicles synthesize estrogens, such as estradiol-17β, estrone and estriol, progestogens, androgens, and corticosteroids (1,2). Ultrastructural and histochemical studies suggest that in some teleosts the special thecal cells (ST cells) and granulosa cells of ovarian follicles are the major cellular sites of ovarian steroidogenesis (3-7). It has been accepted that the induction of final oocyte maturation by gonadotropin in fish ovarian follicules is mediated by maturation-inducing steroids produced by follicle cells (8). In most teleosts, oocyte final maturation and ovulation is mediated by either 17,20β-dihydroxy-4 pregnen-3-one (17,20β-P) (9,10) or 17,20β,21-trihydroxy-4 pregnen-3-one (20β-S) (11,12); however, in *Pagrus major* (13) and *Acipenser transmontanus* (14), it has been reported that both steroids have an effect during final oocyte maturation. Moreover, in some fish, endocrine gland changes during the ovulatory cycle have been observed (15-17). It is reported that the special thecal cells are the sites of steroid synthesis during vitellogenesis and that E_2 induces vitellogenesis and spawning in *C. tarichi* (18).

The aim of the present study was to clarify the histological features of the postovulatory follicles of *C. tarichi* and to determine ovarian estradiol 11-dehydrocorticosterone (11-DHC), estradiol-17b (E_2), 17β-hydroxyprogesterone (17β-OH-P), and progesterone (P) levels in the periods before and after spawning.

Materials and Methods

Mature *C. tarichi* samples were caught in May and June 2000, during their spawning period, from the Karasu River where it enters Lake Van. The fish, which were ready to spawn, were stripped by gently touching their ventral region. These fish were kept in fiberglass tanks (90 x 90 x 250 cm) with a continuous flow rate (1 l/s) at a temperature of 16-18 ºC, with a natural photoperiod. They were fed live zooplankton twice daily. Ovaries from 5 different groups were studied: a) before spawning; b) 1st day after spawning; c) 5th day after spawning; d) 10th day after spawning; e) 15th day after spawning. The fish were anesthetized with 100 ppm 3-aminobenzoic acid ethyl ester (MS-222, Sigma). The ovaries of disected fish were removed immediately and stored in a deepfreeze at –80 ºC for later steroid analysis. The ovary follicles of the fish were fixed on the first day after the spawning for histological study. Fish 4-6 years old, which were 18-20 cm in size, were kept for both hormone analysis and histological 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 were embedded in Epon 812. Thin sections were placed on 1000 mesh copper grids and stained with saturated uranyl acetate followed by lead citrate, and were then examined under a JEOL 100 C electron microscope. For light microscopy, 1 µm thick sections were cut with a Reichert ultramicrotome and stained with methylene blue.

High performance liquid chromatography (HPLC)

Three fish samples from each group and 1-g ovarian tissue samples from each fish were used for hormone analysis. The HPLC analysis was adapted from Shimizu et al. (19), Venkatesh et al. (20), 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) and centrifuged at 8500 xg 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). Next, the samples were passed through Sep-Pak C18 cartridges (Waters) that were preconditioned with 5 ml of distilled water and 5 ml of methanol. Cartridge-adsorbing hormones were eluted with 5 ml of 80% methanol and the eluate was injected into HPLC (Shimadzu, LC-10 AD). Steroids were separated with a chromatographic system under isocratic conditions at a flow rate of 1.5 ml/min in a µBondapak column with acetonitrile:water (53:47, v/v) as the mobile phase. The pressure and wavelength were adjusted 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.

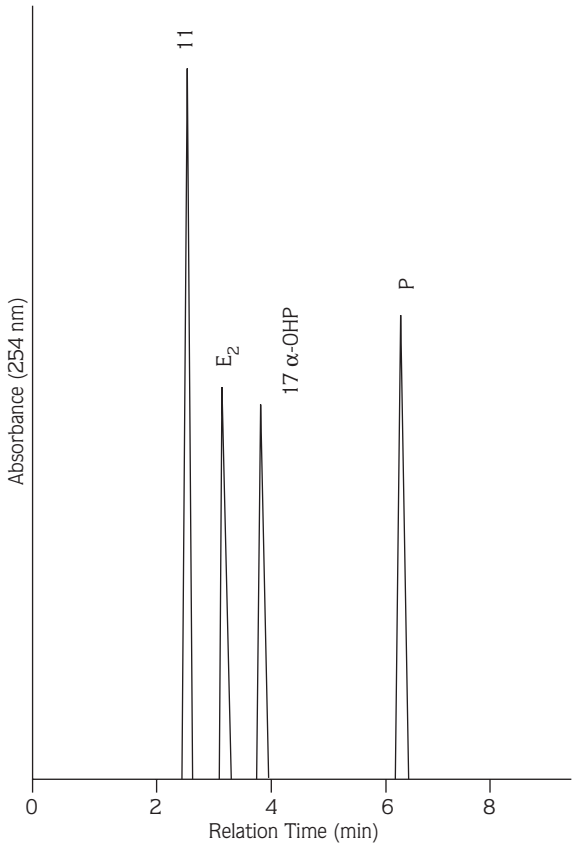

Figure 1. Elution patterns of 11-dehydrocoticosterone (11-DHC), estradiol-17β (E_2), 17β-hydroxyprogesterone (17β-OHP), and progesterone (P) separated using HPLC. Stationary phase: μBondapak column. mobile phase: acetonitrile:water (53:47). flow rate: 1.5 ml/min, detection: uv (254 nm).

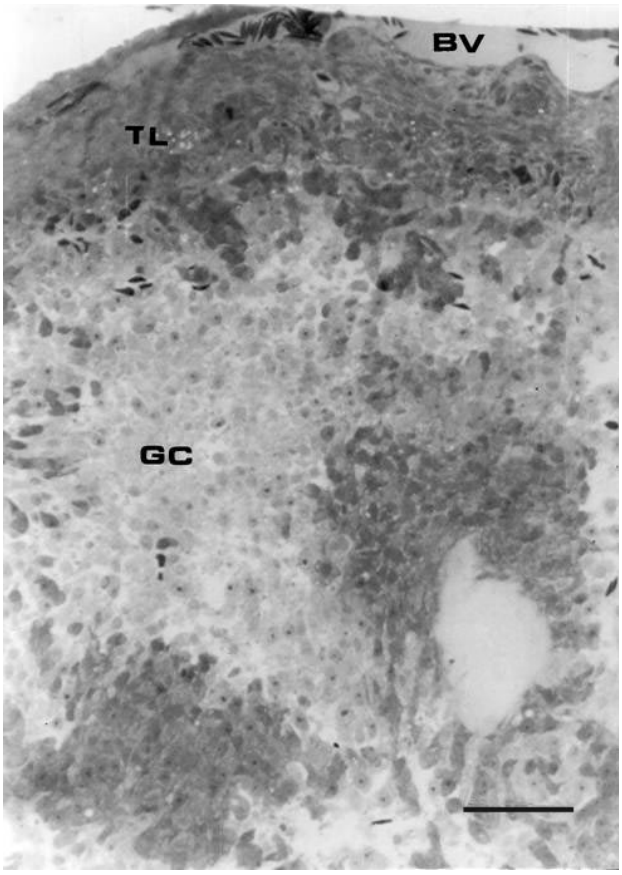

Figure 2. Epon section (1 μm) of a postovulatory follicle 1 day after ovulation. BV: blood vesicle; TL: thecal layer; GC: granulose cells; methylene blue: bar = 60 μm.

All concentrations were expressed as mean ± SE. The data were analyzed by analysis of variance (ANOVA). The significance level for differences was set at P = 0.01.

Results

Histology

The most important feature of postovulatory follicles is the thickness of the thecal layer, which contains many enlarged blood vessels. Postovulatory follicles are also characterized by hypertrophied granulosa cells dispersed in a cavity formerly occupied by oocytes (Figures 2 and 3a). In epoxy sections, the granulosa cells are cuboidal or columnar with cytoplasmic processes projecting into the follicular lumen (Figure 3a). The nucleus, with one prominent nucleolus, is located basally. Some droplets, which stained dark with methylene blue, were observed in the cytoplasm of these cells and in the follicular lumen (Figure 3b). Ultrastructurally, these cells consist of almost smooth endoplasmic reticulum distributed throughout the cytoplasm, with tubules of varying lengths (Figure 4) and mitochondria. The mitochondria were oval or elongated, with tubular cristae. A Golgi apparatus, which was poorly developed, appeared in the basal cytoplasm near the nucleus. Some of the granulosa cells contained lysosome-like bodies.

Hormone Levels

Ovarian steroid levels according to sampling date are shown in Figure 5. Ovarian 11-DHC level was significantly lower than the other steroids (P > 0.01). Its level was 35.8 ± 4.9 ng/ml before spawning and remained low after spawning (P > 0.01).

Ovarian E_2 level was 216.8 ± 31.1 ng/ml before spawning, and slightly decreased to 184.6 ± 25.6 ng/ml

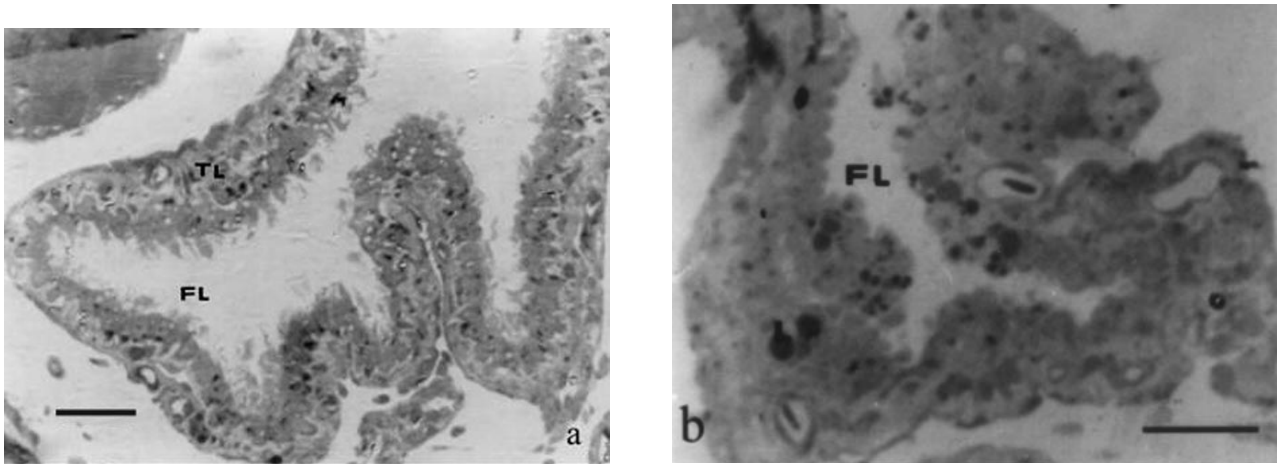

Figure 3. Epon section (1 µm) of a postovulatory follicle 1 day after ovulation. a) Large vacuoles in the thecal layer (TL) and granulosa cells with cytoplasmic processes. FL: follicular lumen. methylene blue: bar = 30 µm. b) Well vascularized thecal layer and stained dark lipid droplets (L). FL: follicular lumen. methylene blue: bar = 30 µm.

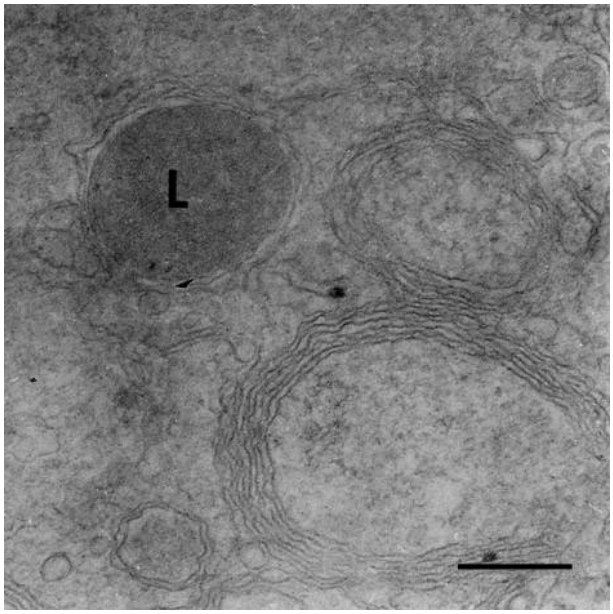

Figure 4. Electron micrograph of granulosa cells 1 day after ovulation. Smooth endoplasmic reticulum and lipid droplet (L). Bar = 0.237 µm.

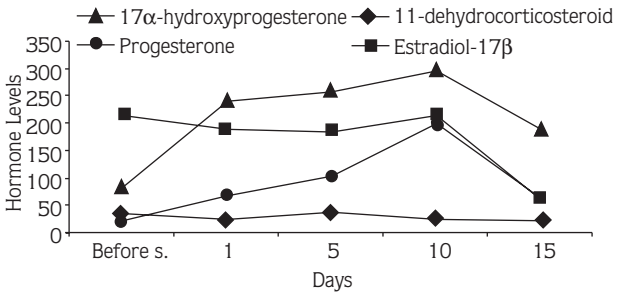

Figure 5. Change in ovary steroid levels before and after spawning (s).

on the 10th day after spawning, but again increased to 216.8 ± 6.7 ng/ml, and decreased dramatically (61.5 ± 17.8 ng/ml) on the 15th day ($P < 0.01$).

Before the spawning, ovarian 17α-OH-P level was significantly lower (84.5 ± 49.4 ng/ml) than after spawning. After spawning, this level increased rapidly to 242 ± 1.6 ng/ml ($P < 0.01$) and peaked on the 10th day (295.7 ± 52.5 ng/ml). 17α-OH-P level slightly decreased 15 days after spawning (190 ± 21.5 ng/ml) ($P > 0.01$).

The ovarian P level was also low (23.4 ± 18.3 ng/ml) before spawning ($P < 0.01$). After spawning, it gradually increased to a prominent peak (200.7 ± 29.1 ng/ml) on the 10th day ($P < 0.01$). The high value was followed by a sharp decline on the 15th day (63.2 ± 19.5 ng/ml) ($P < 0.01$).

Discussion

Postovulatory follicles in *C. tarichi* the first day after the spawning were characterized by a highly vascularized thecal layer and hypertrophied granulosa cells. The follicular layer, which was shown to synthesize the steroid hormone in postovulatory follicles, varies among

teleost fish (4,22). In the white-spotted salmon (*Salvelinus leucomaenis*), pink salmon (*Oncorhynchus gorbuscha*), and coho salmon (*Oncorhynchus kisutch*), the special thecal cells in the thecal layer possess organelles characteristic of steroid production (4,5). In tilapia, *Oreochromis mossambicus*, although the granulosa cells in postovulatory follicles increase in size and organelle content, the primary site of steroidogenesis seems to be the special thecal cells, which show an intense 3β-HSD reaction (6). Matsuyama et al. (7) found that the cells that possess organelles characteristic of steroid-producing cells show negative histochemical reactions for 3β-HSD activity in *Pagrus major*. However, in goldfish, *Carassius auratus*, 3β-HSD activity is positive, not only in the granulose cells, which possess organelles characteristic of steroid-producing cells, but also in special thecal cells (3). In *C. tarichi*, the granulosa cells in postovulatory follicles 1 day after spawning showed an ultrastructure essentially similar to those in *Carassius auratus* (3), such as smooth endoplasmic reticulum, large mitochondria with tubular cristae, and lipid droplets. According to ultrastructural studies, granulosa cells (steroid-producing cells) are where steroids are produced in *C. tarichi*. Nevertheless, histochemical experiments are necessary to determine the exact role of granulose cells.

Goswami and Sundararaj (23) reported that corticosteroids are terminal hormones, which act to induce oocyte maturation and ovulation in catfish, *Heteropneustes fossilis*. In orange roughy, 11-DOC has also been measured in the plasma of female and male fish, but no change in relation to reproductive development has been reported (24). Corticosteroids in brook trout, *Salvelinus fontinalis*, and yellow perch, *Perca flavescens*, are ineffective in germinal vesicle breakdown and ovulation. Similarly, in the present study, 11-DHC was detected in the ovaries of *C. tarichi*, but its level did not change after spawning.

In *C. tarichi,* ovarian E_2 level showed high values before and after spawning, but it decreased dramatically on the 15th day after spawning. E_2 is produced primarily during vitellogenesis. In many teleosts, it has been shown that E_2 induces the synthesis and secretion of vitellogenic protein by the liver (5,17,19). E_2 levels and gonadosomatic index values are correlated during vitellogenesis (25). In our previous study (18), the production of ovarian steroid hormones during vitellogenesis and oocyte maturation was observed. E_2 level peaked during vitellogenesis and then decreased slightly, and this high level was maintained during oocyte maturation. In salmonids, plasma E_2, which was observed at high levels during vitellogenesis, decreased to very low levels during oocyte maturation and ovulation (15,25), whereas, in bitterling, *Acheilognathus rhombea*, high levels of E_2 observed during vitellogenesis were maintained during oocyte maturation and ovulation (19). The high level of E_2 is explained by the requirement of continuous vitellogenesis during the short reproductive cycle of this species. High levels of E_2 are known to prevent apoptosis in fish (26). According to our results, in *C. tarichi,* apoptosis in postovulatory follicles is dense on the 15th day after ovulation.

In many teleosts, it has been reported that progestins, especially 17α-OH-P, 17α,20β-diOH-P, and 17,20β-21-P, have a major effect on oocyte maturation and ovulation (27-29). Each one of these may have an effect in different species. In goldfish, *Carassius auratus*, both 17α-OH-P and 17α,20β-diOH-P increase significantly at the time of oocyte maturation and ovulation (30). In the same fish, however, plasma 17α,20β-diOH-P levels show a peak before ovulation, but plasma levels of 17α-OH-P were not determined (16). Hence, in the same fish, Kime et al. (31) reported that 17α,20β-diOH-P, 11-deoxycortisol, and 17,20β-21-P may have an effect, depending on the maturation state of the ovaries or the method used to stimulate oocyte maturation. Nonetheless, 11-deoxycortisol and the 20β-reduced derivative (17, 20β-21-P) may be significant in spawning goldfish since 17,20β-diOH-P has been found in only low levels (as sulfate). It is generally accepted that, in salmonids, 17,20β-diOH-P has an effect on oocyte maturation and it is called a maturation-inducing steroid (MIS) (15,11). Yet, in trout, *Salmo gairdneri*, both 17α,20β-diOH-P and 17α-OH-P were measured at the time of ovulation (32). Goetz and Bergman (33) showed that in brook trout, *Salvelinus fontinalis*, and yellow perch, *Perca flavescens*, 17α,20β-diOH-P induced ovulation at a lower concentration than both 17α-OH-P and 11-deoxycortisol, and had a strong influence during oocyte maturation. In the white sucker, *Catostomus commersoni*, 17α-P and 17,20-P levels are low in prespawning fish and their levels are the highest in fish that have ovulated (34). The peak values of 17α,20β-diOH-P are higher than those of 17α-OH-P during ovulation in bitterling, *Acheilognathus rhombea* (19). The

major role of 17α-OH-P is considered to be as a precursor of 17α,20β-diOH-P rather than a direct inducer of oocyte maturation and ovulation (35-37). The effects of steroids in oocyte maturation and ovulation have been determined by in vitro experiments (36). 17α-OH-P is the immediate precursor to 17α,20β-diOH-P in ovaries, testes, and head kidneys of the Atlantic salmon, *Salmo salar* (37). Sakai et al. (35) have also shown that 17,20β-P levels before spawning increased, depending on the exogenous addition of 17α-OH-P in vitro. In *C. tarichi*, the level of 17α-OH-P was low before spawning. According to the results mentioned above, it may be concluded that 17α-OH-P has an indirect effect on this species and it may be a precursor of 17α,20β-diOH-P.

In our previous study (18), ovarian progesterone was low level during the cortical alveoli, vitellogenesis, and oocyte maturation phases. In the present study, these low levels were also maintained relatively before spawning. Moreover, in *Fundulus heteroclitus*, steroid levels in media were measured after incubation with progesterone, pregnenolone, and 25-hydroxycholesterol of the isolated follicles and the substrates added to follicle cultures caused an elevation even above the basal levels of E_2, 17α-OH,20β-DHP, and testosterone secretion. In all cases, the incidence of oocyte maturation and the accumulation of steroids in the medium are dependent upon the concentration of added precursor (38). Matsuyama et al. (7) have shown that progesterone has no effect on germinal vesicle breakdown (oocyte maturation). When ayu, *Plecoglossus altivelis*, both treated and untreated with a salmon gonadotropin, were incubated with ^{14}C-labeled progesterone and 17α-OH-P in the presence of NADH, metabolites of progesterone were observed in the ovaries (36). We can conclude that progesterone has no effect on ovulation in *C. tarichi*, but according to the above-mentioned studies its metabolites may have an influence. The progesterone level reached a peak on the 10th day after ovulation and then decreased dramatically to basal levels just on the 15th day. To the best of our knowledge, no study about the effect of progesterone on apoptosis in fish has been published. In one study carried out in cow luteal cells, aminoglutethimide added to culture media was shown to block progesterone synthesis, and an increase in apoptosis was observed during this block. In another study, adding progesterone to the culture media prevented apoptosis (39). Similar to this, it can be noted that in the present study, in *C. tarichi*, apoptosis increased on the 15th day after ovulation when E_2 and P levels were low; however, the effect of these hormones should be studied further in vitro and in vivo.

In conclusion, this study investigated steroid secretory cells after ovulation and some ovary hormones levels before and after spawning in *C. tarichi*. Granulosa cells are where steroid synthesis in postovulatory follicles occurs. 11-DHC has no effect during spawning or postspawning. E_2 induces ovulation. 17β-OH-P and P have an effect after spawning. It may be suggested that the decline in the levels of E_2 and P increases apoptosis in postovulatory follicles.

Acknowledgments

This study was supported by Yüzüncü Yıl University Research Fund (Project 97/FED-044). The authors wish to thank Dr. Peyami Battal, who analyzed the samples by HPLC.

References

1. Reinboth, R.: Hormonal control of the teleost ovary. Am. Zoologist, 1972; 12: 307-324.
2. Wingfield, J.C., Grimm, A.S.: Seasonal changes in plasma cortisol, testosterone and oestradiol-17β in the plaice, *Pleuronectes platessa* L. Gen. Comp. Endocrinol., 1977; 31: 1-11.
3. Nagahama, Y., Chan, K., Hoar, W.S.: Histochemistry and ultrastructure of pre-and post-ovulatory follicles in the ovary of the goldfish, *Carassius auratus*. Can. J. Zool., 1976; 54: 1128-1139.
4. Nagahama, Y., Clarke, W.C., Hoar, W.S.: Ultrastructure of putative steroid-producing cells in the gonads of coho (*Oncorhynchus kisutch*) and pink salmon (*Oncorhynchus gorbuscha*). Can. J. Zool., 1978; 56: 2508-2519.
5. Kagawa, H., Takano, K., Nagahama, Y.: 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., 1981; 218: 315-329.
6. Smith, C. J., Haley, S.R.: Evidence of steroidogenesis in postovulatory follicle of the tilapia, *Oreochromis mossambicus*. Cell Tissue Res., 1987; 247: 675-687.

7. Matsuyama, M., Nagahama, Y., Matsuura, S.: Observations on ovarian follicle ultrastructure in the marine teleost, *Pagrus major*, during vitellogenesis and oocyte maturation. Aquaculture, 1991; 92: 67-82.

8. Nagahama, Y.: Endocrine control of oocyte maturation. In: Norris, D.O., Jones, R.E. (Eds.) Hormones and Reproduction in Fishes, Amphibians, and Reptiles. Plenum Press, New York, 1987; 171-202.

9. Scott, A.P., Inbaraj, R.M., Vermeirssen, E.L.M.: Use of a radioimmunoassay which detects C_{21} steroids with a 17,20β-dihydroxyl configuration to identify and measure steroids involved in final oocyte maturation in female plaice (*Pleuronectes platessa*). Gen. Comp. Endocrinol., 1997; 105: 62-70.

10. Wright, R.S., Hunt, S.M.V.: A radioimmunoassay for 17α,20β-dihydroxy-4-pregnen-3-one: Its use in measuring changes in serum levels at ovulation in Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), and rainbow trout (*Salmo gairdneri*). Gen. Comp. Endocrinol., 1982; 47: 475-482.

11. Scott, A.P., Sumpter, J.P., Kime, D.E., Rolfe M.S.: Changes in 17α, 20β, 21-trihydroxy-4-prenen-3-one membrane receptor concentrations in ovaries of spotted seatrout during final oocyte maturation. Proceeding of the Fourth International Symposium on the Reproductive Physiology of Fish. FishSymp 91, University of East Anglia, Norwich, U.K., 7-12 July, 1991; 1-6.

12. Pinter, J., Thomas, P.: Induction of ovulation of mature oocytes by the maturation-inducing steroid 17, 20β,21-trihydroxy-4-pregnen-3-one in the spotted seatrout. Gen. Comp. Endocrinol., 1999; 115: 200-209.

13. Ohta, K., Yamaguchi, S., Yamaguchi, A., Gen, K., Okuzawa, K., Kagawa, H., Matsuyama, M.: Biosynthesis of steroids in ovarian follicles of red seabream, *Pagrus major* (Sparidae, Teleostei) during final oocyte maturation and the relative effectiveness of steroid metabolites for germinal vesicle breakdown in vitro. Comp. Biochem. Physiol. B Bicohem. Mol. Biol., 2002; 133; 45-54.

14. Webb, M.A.H., Feist G.W., Trant, J.M., Van Eenennaam, J.P., Fitzpatrick, M.S., Schreck, C.B., Doroshov, S.I.: Ovarian steroidogenesis in white sturgeon (*Acipenser transmontanus*) during oocyte maturation and induced ovulation. Gen. Comp. Endocrinol., 2002; 129: 27-38.

15. Scott, A.P., Sumpter, C.P., Hadiman, P.A.: Hormone changes during ovulation in the rainbow trout (*Salmo gairdneri* Richardson). Gen. Comp. Endocrinol., 1983; 49: 128-134.

16. Kobayashi, M., Aida, K., Hanyu, I.: Hormone changes during ovulation and effects of steroid hormones on plasma gonadotropin levels and ovulation in goldfish. Gen. Comp. Endocrinol., 1987; 67: 24-32.

17. Kobayashi, M., Aida, K., Hanyu, I.: Hormone changes during the ovulatory cycle in goldfish. Gen. Comp. Endocrinol., 1988; 69: 301-307.

18. Ünal, G., Karakişi, H., Elp, M.: Ovarian follicle ultrastructure and changes in levels of ovarian steroids during oogenesis in *Chalcalburnus tarichi,* Pallas 1811. Turk J. Vet. Animal. Sci., 2005; 29: 645-653.

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

20. Venkatesh, B., Tan, C.H., Lam, T.J.: Blood steroid levels in the goldfish: Measurement of six ovarian steroids in small volumes of serum by reverse-phase high-performance liquid chromatography and radioimmunoassay. Gen. Comp. Endocrinol., 1989; 76: 398-407.

21. Battal, P., Ünal, Ü.: Hayvansal hormonların izokratik yüksek basınçlı sıvı kromatografisi (HPLC) yöntemiyle Belirlenmesi. II. Ulusal Kromatografi Kongresi Kitapcığı, 2001; 138-145.

22. Nagahama, Y., Kagawa, H., Young, G. Cellular sources of sex steroids in teleost gonads. Can. J. Fish. Aquat Sci., 1982; 39: 56-64.

23. Goswami, S.V., Sundararaj, B.I.: Temporal effects of ovine luteinizing hormones and desoxycorticosterone acetate on maturation and ovulation of oocytes of the catfish, *Heteropneustes fossilis* (Blonch): An in *vivo* and in *vitro* study. J. Exp. Zool., 1971; 178: 457-465.

24. Pankhurst, N.W., Conroy, A.M.: Endocrine changes during gonadal maturation and spawning in the orange roughy (*Hoplostethus atlanticus* Collett) a teleost from the Midslope waters of New Zealand. Gen. Comp. Endocrinol., 1988; 70: 262-273.

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

26. Janz, D.M., Van Der Kraak, G.: Suppression of apoptosis by gonadotropin, 17β-estradiol, and epidermal growth factor in rainbow trout preovulatory ovarian follicles. Gen. Com. Endocrinol., 1997; 105: 186-193.

27. Goetz, F.W., Theofan, G.: In vitro stimulation of germinal vesicle breakdown and ovulation of yellow perch (*Perca flavescens*) oocytes: Effects of 17α-hydroxy-20β-dihydroprogesterone and prostaglandins. Gen. Comp. Endocrinol., 1979; 37: 273-285.

28. Nagahama, Y., Hirose, K., Young, G., Adachi, S., Suzuki, K., Tamaoki, B.: Relative in vitro effectiveness of 17β-20β-dihydroxy-4-pregnen-3-one and other pregnene derivatives on germinal vesicle breakdown in oocytes of ayu (*Plecoglossus altivelis*), amgo salmon (*Onchorhynchus rhodurus*), rainbow trout (*Salmo gairdneri*) and goldfish (*Carassius auratus*). Gen. Comp. Endocrinol., 1983; 51: 15-23.

29. Lee, W.K., Yang, S.W.: Relationships 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 maculates* (Jeom-nong-eo). Aquaculture, 2002; 207: 169-183.

30. Kobayashi, M., Aida, K., Hanyu, I.: Radioimmunoassay for silver carp gonadotropin. Bull. Japan. Soc. Sci. Fish., 1985; 51: 1085-1091.

31. Kime, D.E., Scott, A.P., Canario, A.V.M.: In vitro biosynthesis of steroids, including 11-deoxycortisol and 5α-pregnane-3β, 7α,17,20β-tetrol, by ovaries of the goldfish *Carassius auratus* during the stage of oocyte final maturation. Gen. Comp. Endocrinol., 1992; 87: 375-384.

32. Scott, A.P., Sheldrick, E.L., Flint, A.P.F.: Measurement of 17α,20β-dihydroxy-4-pregnen-3-one in plasma of trout (*Salmo gairdneri,* Richardson): Seasonal changes and response to salmon pituitary extract. Gen. Comp. Endocrinol., 1982; 46: 444-451.

33. Goetz, W.F., Bergman, H.L.: The effects of steroids on final maturation and ovulation of oocytes from brook trout (*Salvelinus fontinalis*) and yellow perch (*Perca flavescens*). Biol. Reprod., 1978; 18: 293-298.

34. Scott, A.P., MacKenzie, D.S., Stacey, N.E.: Endocrine changes during natural spawning in the white sucker, *Castostomus commersoni*. II. Steroid hormones. Gen. Comp. Endocrinol., 1984; 56: 349-359.

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

36. Suzuki, K., Tamaoki, B., Hirose, K.: In vitro metabolism of 4-pregnenes in ovaries of a freshwater teleost, the ayu (*Plecoglossus altivelis*): Production of 17α,20β-dihydroxy-4-pregnen-3-one and its 5β-reduced metabolites, and activation of 3β-and 20β-hydroxysteroid dehydrogenases by treatment with a fish gonadotropin. Gen. Com. Endocrinol., 1981; 45: 473-481.

37. Sangalang, G.B., Freeman, H.C.: In vitro biosynthesis of 17α, 20α,20β-dihydroxy-4-pregnen-3-one by the ovaries, testes, and head kidneys of the Atlantic Salmon *Salmo salar*. Gen. And Comp. Endocrinol., 1988; 69: 406-415.

38. Petrino, T.R., Peter Lin, Y.W., Wallace, R.A.: Steroidogenesis in *Fundulus heterolitus*. I. Production of 17α-hydroxy,20β-dihydroprogesterone, testosterone and 17β-estradiol by prematurational follicles *in vitro*. Gen. Comp. Endocrinol., 1989; 73: 147-156.

39. Rueda, B.R., Hendry, I.R., Hendry III, W.J., Stormshak, F., Slayden, O.D., Davis, J.S.: Decreased progesterone levels and progesterone receptor antagonists promote apoptotic cell death in bovine luteal cells. Biol. Reprod., 2000; 62: 269-276.