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Serum 17,20β-dihydroxy-4-pregnen-3-one Levels in Pregnant and Non-pregnant Female Rockfish, *Sebastes schlegeli*, Viviparous Teleost, and its Production by Post-ovulatory Follicles

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ABSTRACT—Changes in serum 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-DP) levels around the gestation time of normal pregnant and experimentally non-pregnant females were investigated in the black rockfish, *Sebastes schlegeli*, a viviparous teleost. The serum 17,20β-DP in both females showed similar changes and levels, increasing from the early to late gestation periods and declining just before parturition in pregnant females and egg-release in non-pregnant females, respectively. These results suggest that the maintenance of high serum levels of 17,20β-DP after oocyte maturation is not correlated with gestation or parturition, but occurs spontaneously in this species. The decline of 17,20β-DP levels prior to egg-release in non-pregnant females tended to occur one week earlier than those prior to parturition in pregnant females, suggesting that both a decline in 17,20β-DP levels in mothers and some response from embryos are needed for a smooth parturition. The post-ovulatory follicles were maintained throughout the gestation period and produced a considerable amount of 17,20β-DP *in vitro* (3.44–6.96 pg/ml/mg tissue), but little estradiol-17β (0.92–1.66 pg/ml/mg tissue). The production of 17,20β-DP tended to be enhanced by the addition of a precursor steroid, pregnenolone, in the pre-, early and mid-gestation periods. These results strongly suggest that the follicle cells in black rockfish have the ability to synthesize 17,20β-DP during the post-ovulatory period, and high serum 17,20β-DP during gestation is supplied by the post-ovulatory follicles, which in *Sebastes* are considered to be functionally homologous to the mammalian corpus luteum.

Key words: viviparous teleost, steroid, gestation, parturition, estradiol-17β

INTRODUCTION

The black rockfish, *Sebastes schlegeli*, is a viviparous teleost belonging to the Scorpaenidae and distributed at the coast of Japan, Korea, and China. Some 330 species of Scorpaenidae have been confirmed, 110 of which are viviparous and mainly concentrated in the genus *Sebastes*. The pattern of pregnancy in *Sebastes* is categorized as an intralumenal gestation type in which eggs are ovulated into the ovarian lumen and embryos are maintained there (Hogarth, 1976). The nutritional relationship between mother and embryos in *Sebastes* is weak. It is described as typical lecithotrophy in which embryos depend mainly on their own egg yolk (Wourms, 1981). Thus, *Sebastes* is considered a primitive group among viviparous teleosts and an interesting one for studying the evolutionary process, including the endocrinological changes from oviparity to viviparity in teleosts.

Concerning female reproductive physiology in black rockfish, the maturational process of gonads (Mori *et al.*, 2003) and the serum profiles of steroid hormones (Nagahama *et al.*, 1991; Mori *et al.*, 2003) have been investigated to date. The results of these studies suggest that vitellogenesis is promoted by estradiol-17β (E2), and oocyte maturation is induced by 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-DP), both of which are steroids synthesized in the ovarian follicles, as is the case in most oviparous teleosts (see Nagahama, 2000). Furthermore, high serum levels of 17,20β-DP during gestation were reported not only in black rockfish but also in other *Sebastes* species (*S. taczanowskii*, Nagahama *et al.*, 1991; *S. rastrelliger*, Moore *et al.*, 2000), suggesting that 17,20β-DP plays an important role in pregnancy. Although the relationship between gonadal development and sex steroids has thus been clarified to a certain extent, there are few detailed studies describing the endocrinological changes during gestation in viviparous rockfish.

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In the present study, detailed changes in serum 17,20β-DP levels around the gestation period of black rockfish were investigated. In particular, in order to examine the roles of 17,20β-DP in gestation and parturition, serum levels of 17,20β-DP in experimentally non-pregnant females were compared with those in normal pregnant females. Although

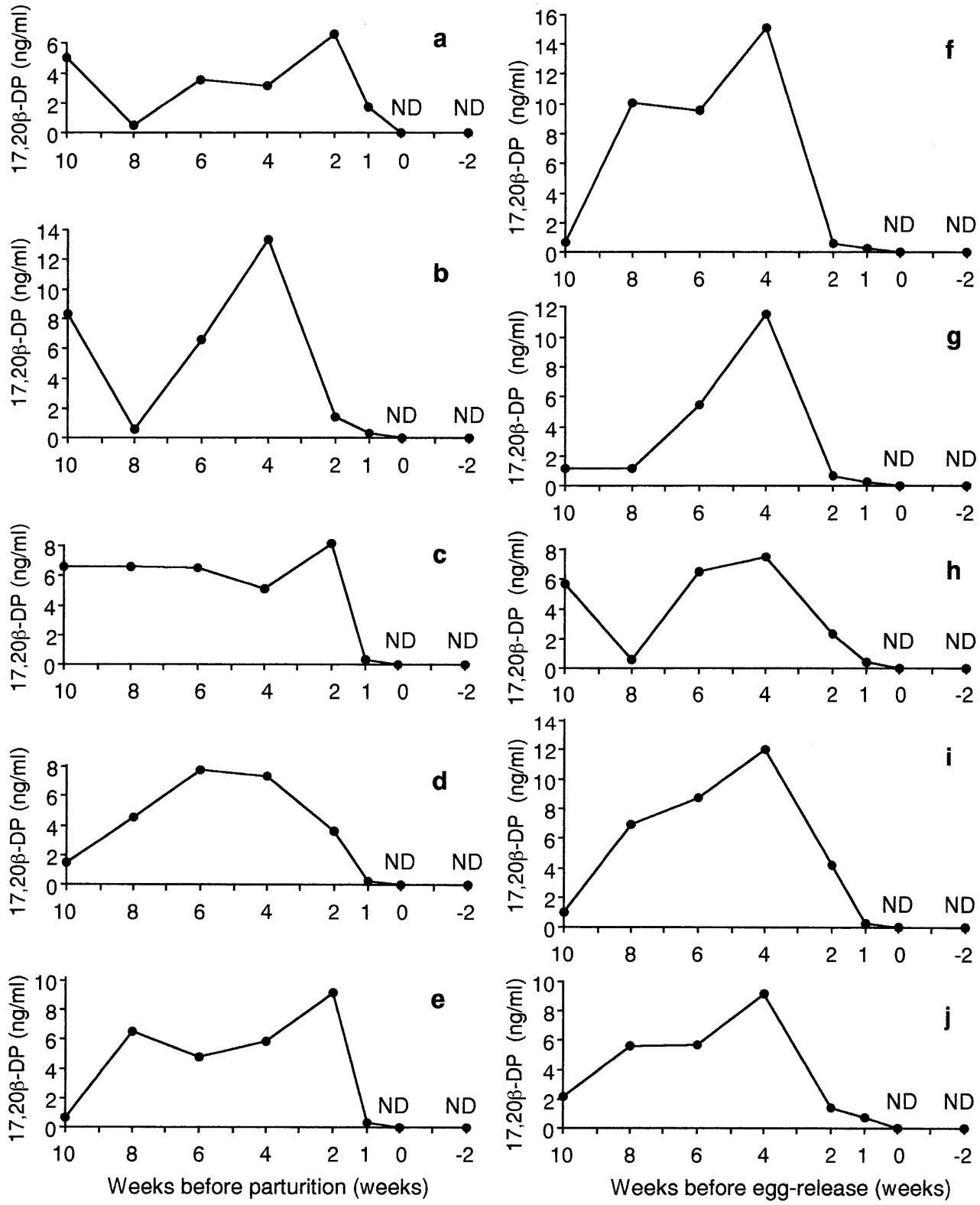

Fig. 1. Changes in the serum levels of 17α,20β-dihydroxy-4-pregnen-3-one during the gestation and postpartum periods in pregnant (a–e) and non-pregnant (f–j) female black rockfish. Separate graphs show the changes in each individual. ND, not detectable.

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the sites for the production of 17,20β-DP during gestation have not been identified, their ultrastructure in *S. taczanowskii* suggests that the post-ovulatory follicles may produce some steroids (Hayashi, 1990). The second aim of the present investigation is to prove the capacity for steroid biosynthesis in post-ovulatory follicles. Morphological changes and the *in vitro* production of 17,20β-DP in post-ovulatory follicles around the gestation period of black rockfish were investigated.

MATERIALS AND METHODS

Fish and serum sample

Seven- to nine-year-old black rockfish (31.8–45.0 cm in standard length, 1.93–2.83 kg in body weight) used in the present study were produced in the aquaria of the Japan Sea-Farming Association, Miyako Station, Iwate Prefecture, Japan.

Five females reared with males and five reared apart from males just before the copulation period (September) were used to monitor the serum 17,20β-DP levels around their gestation time. The females reared with males were confirmed to be carrying embryos by cannulation into the ovary at the start of sampling. All the females were individually identified by intramuscular injection into the back of a passive integrated transponder (PIT) tag (Power Tracker; AVID, USA). Blood sampling was performed at one-week intervals from around the beginning of gestation (March 10) to just after the parturition of all pregnant fish (June 27). Parturition in pregnant fish or egg-release in non-pregnant fish was confirmed by a reduction in the abdomen during the weekly blood sampling. The sampling date when the abdomen of a fish had reduced size was considered the zero week before parturition. About 1 ml of blood was drawn from the caudal vessel with a non-heparinized syringe after anesthetization with ice between 9:00 and 12:00, and each fish was released back into its original pond. Sera were separated by centrifugation at 3000×g for 15 min and stored at −80°C until use.

Females reared with males were used for experiments in *in vitro* steroid production. The experiments were carried out four times (March 30, April 12, April 27, and May 10), and three females were used for each experiment. The fish were killed by decapitation, measured for standard length and body weight, and bled from the caudal vessel with non-heparinized syringes. Sera were separated by centrifugation at 3000×g for 15 min and stored at −80°C for the measurement of steroid levels. The ovaries were dissected and used for culture experiments. Another five females were used for histological examination of ovary after parturition. The fish were reared with males and were killed just after the parturition of all these fish (June 18).

Histological examination

For histological observations of both the ovarian and post-ovulatory follicles, part of the ovary was fixed in Bouin's solution, dehydrated with ethanol, and embedded in paraffin. The specimens were sectioned at a 6-μm thickness and stained with Delafield's hematoxylin and eosin.

The stages of oocytes of fish in their pre-gestation period were identified by histological examination according to the classification of Mori *et al.* (2003). Embryos were classified by microscopic observation according to the classification of Kusakari (1995).

Incubation of follicles

Ovarian follicles including a little connective tissue in maturing females (March 30) or fragments of post-ovulatory follicles including connective tissue and a little immature oocytes in pregnant females (April 12, April 27, and May 10) were separated by tweezers from ovarian tissue in the culture medium. As the culture medium, we used Leivovitz-15 (L-15; Sigma, USA), buffering with 10 mM Hepes (pH 7.5), adding 0.1 mM streptomycin sulfate and 0.075 g/l benzylpenicillin potassium. Twenty separated ovarian follicles or small fragments (each about 50 mg) of post-ovulatory follicles were incubated in individual wells of 24-well plastic tissue culture dishes (Falcon, Lincoln Park, NJ) containing 1 ml of L-15 in the absence (controls) or presence of pregnenolone (0.1, 1, 10 ng/ml) as a precursor. The pregnenolone was dissolved in ethanol and 10 μl was added to 1 ml of incubation medium. There were three replicates for each dose of precursors and for controls. Incubations were carried out at 10°C (equal to the average water temperature during the gestation period) for 12, 24, and 48 hr. After incubation, the medium was frozen at −80°C until assayed for steroids.

Measurement of steroid hormone levels

Steroids were extracted three times by adding a ten-fold volume of ethyl ether to the serum. The ethyl ether was allowed to evaporate, and the sample was then reconstituted with 100 μl of assay buffer (0.05 M borate buffer, pH 7.8, containing 0.5% BSA and 0.01% thimerosal). Steroid extraction from the incubation medium was not carried out. Levels of estradiol-17β (E2) and 17,20β-DP in the serum and incubation medium were measured by specific enzyme-linked immunosorbent assay (ELISA) according to the method of Asahina *et al.* (1995). Antiserum of 17,20β-DP had

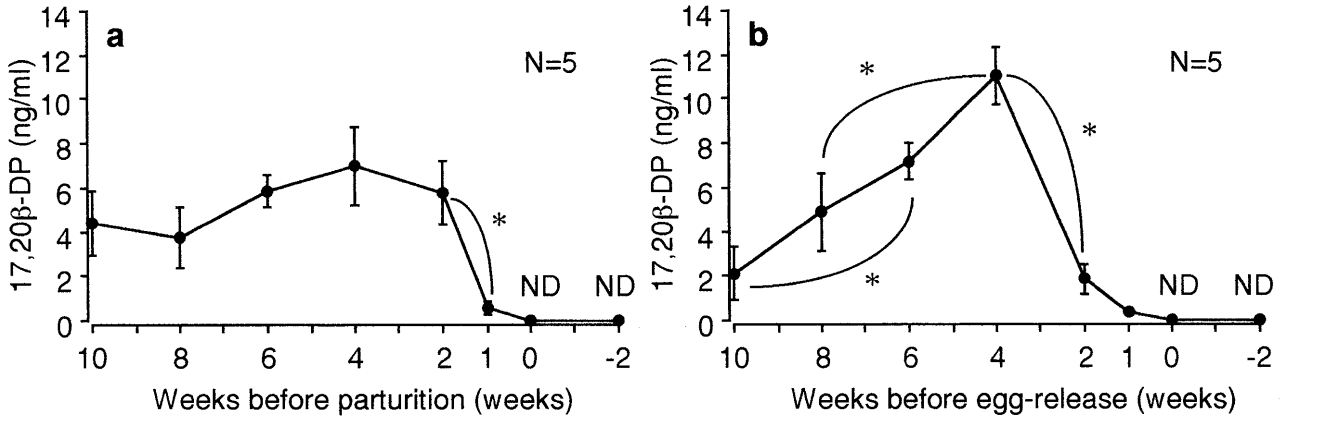

Fig. 2. Changes in the average levels of serum 17α,20β-dihydroxy-4-pregnen-3-one during the gestation and postpartum periods in pregnant (a) and non-pregnant (b) female black rockfish. ND, not detectable. *Significantly different from each other (Turkey-Kramer test, $P<0.05$). $N=5$.

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0.15% of cross reactivity for pregnenolone. Values under the detection limit (50 pg/ml in both steroids) were considered as 50 pg/ml for statistical analysis.

Statistical analysis

All data were presented as mean±SEM and were analyzed by one-way ANOVA to test for the differences among the groups. The means were subsequently compared by Tukey-Kramer or Dunnett test using the Statview 5 program for Macintosh (Abacus Concepts, Inc., Berkeley, CA, USA). Differences were considered statistically significant at $P<0.05$.

RESULTS

Serum 17,20β-DP levels in pregnant and non-pregnant fish

All the females reared with males were delivered between May 12 and June 8. Changes in serum 17,20β-DP levels of individual pregnant females around the gestation period were shown in Fig. 1a–e. Serum levels of 17,20β-DP in each individual varied between 8 and 10 weeks prior to parturition, and reached a stable high (4–8 ng/ml) between 2 and 6 weeks prior to parturition except for one individual shown in Fig. 1b. The 17,20β-DP levels dropped sharply one week prior to parturition, except for the above individual, and were not detected after parturition in any of the fish.

All the females reared apart from males just before the copulation period released unfertilized eggs between May 12 and June 2. Changes in serum 17,20β-DP levels in each of the non-pregnant females during and after the period of holding unfertilized eggs were shown in Fig. 1f–j. Serum levels of 17,20β-DP in each individual varied between 8 and 10 weeks prior to egg-release, and then increased to a maximum 4 weeks prior to egg-release. The levels then declined in 2 weeks and remained low one week prior to egg-release. No 17,20β-DP was detected in the sera of any fish after

Table 1. Ovarian maturity and developmental stages of embryos in the females used in our culture experiment.

Date of experiment	Stage of oocyte	Stage of embryo (No. of Stage)*
30th March	Tertiary yolk globule	–
	Tertiary yolk globule	–
	Migratory nucleus	–
12th April	–	Optic cups, 22–23 somites (20)
	–	Optic cups, 22–23 somites (20)
	–	Optic cups, 22–23 somites (20)
27th April	–	Auditory placodes (21)
	–	Auditory placodes (21)
	–	Pigmentation of retina (25)
10th May	–	Openings of the mouth and anus (28)
	–	Openings of the mouth and anus (28)
	–	Pigmentation of the peritioneal wall (29)

* Development of embryo was divided into 32 stages from mature ovum to hatching according to Kusakari (1995).

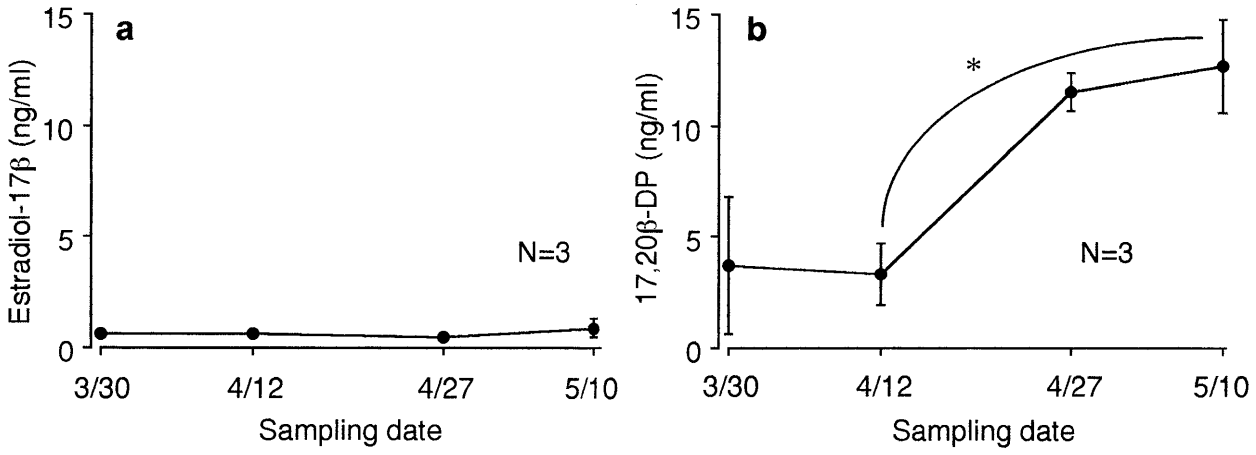

Fig. 3. Changes in serum estradiol-17β (a) and 17α,20β-dihydroxy-4-pregnen-3-one (b) in female black rockfish using *in vitro* culture experiments. *Significantly different from each other (Turkey-Kramer test, $P<0.05$). $N=3$.

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egg-release.

Changes in the average levels of serum 17,20β-DP in pregnant and non-pregnant females were summarized in Fig. 2. The levels in both groups showed similar changes, maintaining high from the early to late gestation periods and declining just before parturition or egg-release. The serum 17,20β-DP levels in non-pregnant females were similar to or a bit higher (about twice the peak level 4 weeks before egg-release) than pregnant females during their egg-holding period. The decline of levels prior to egg-release in non-pregnant females tended to occur one week earlier than those prior to parturition in pregnant females.

Physiological conditions of females used for culture experiments

Stages of developing oocytes or embryos in the ovaries

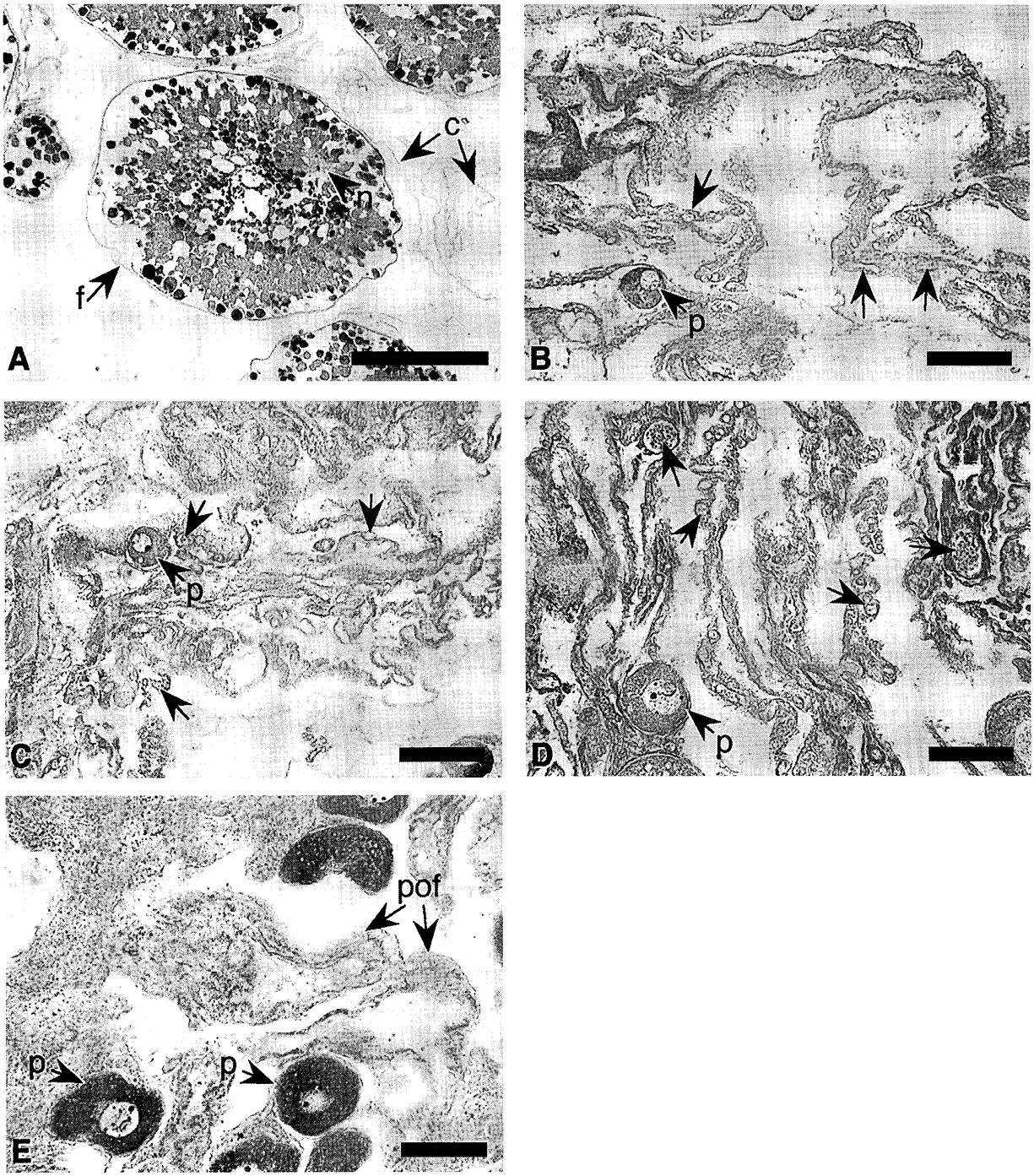

Fig. 4. Histological observation of pre-mature oocyte (A) and post-ovulatory follicles during early (B), mid- (C), and late (D) gestation periods, and after parturition (E) of black rockfish. Arrows indicate blood capillaries in the post-ovulatory follicles. c, connective tissue. f, follicle layer. n, migrating germinal vesicle. p, oocyte at the peri-nucleolus stage. pof, post-ovulatory follicle. Bars represent (A: 500 μm; B, C, D, E: 100 μm).

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used in the culture experiment were observed and are summarized in Table 1. On March 30, three females had not yet become pregnant, as evidenced by a tertiary yolk globule in two and migratory nucleus stage oocytes in one. By April 12, all females had become pregnant, and the stages of their embryos kept pace with the dates of the experiments (see the number of stages in Table 1). Therefore, in this paper we denote each experimental date as the pre-gestation, early gestation, mid-gestation or late gestation period.

Serum E2 levels in the experimental fish had already been low (0.6±0.1 ng/ml) in the pre-gestation period, and showed no change throughout the gestation period (Fig. 3a). Serum 17,20β-DP levels in the experimental fish had already been high (3.7±3.1 ng/ml) in the pre-gestation period, maintained those high levels in the early gestation period (Fig. 3b), increased further in the mid-gestation period, and reached their highest value (12.7±2.1 ng/ml) in the late gestation period.

Morphology of ovary and follicle

The paired ovaries of black rockfish are located in the dorsocaudal coelomic cavity. Each ovarian lobe fuses caudally following a united oviduct. Ovigerous lamella consisting of connective tissue and oocyte follicles extend from the ovarian hili, which run longitudinally through the dorsal surface of each ovarian lobe (see Moser, 1967).

In the ovary of maturing fish, the ovigerous lamella occupy the whole ovary, and the narrow ovarian cavity is located in the lateral and ventral margins of the ovary. The developing oocytes are covered with a thin follicle layer (Fig. 4A). In the ovary of pregnant fish, the ovigerous lamella shrinks remarkably and is located directly under the ovarian hilus. The developing embryos are maintained in the ovarian cavity, which holds the whole ovary. The post-ovulatory follicles and immature oocytes are located in the shrunken ovigerous lamella. The post-ovulatory follicles thicken remarkably and appear as shrunken pouch-like structures with some blood capillaries on their surface (Fig. 4B, C, D). The structure of these post-ovulatory follicles showed no changes throughout the gestation period. Some immature oocytes at the peri-nucleolus stage were contained in the fragments of post-ovulatory follicles used in the present culture experiments (Fig. 4B, C, D). In the ovary of post-delivery fish, post-ovulatory follicles degenerated and were hard to distinguish from connective tissue (Fig. 4E). Young oocytes at the peri-nucleolus stage increased in number.

Steroid production by ovarian and post-ovulatory follicles *in vitro*

Changes in steroid production by ovarian and post-ovulatory follicles during incubation were shown in Fig. 5. Production of 17,20β-DP without a precursor tended to increase

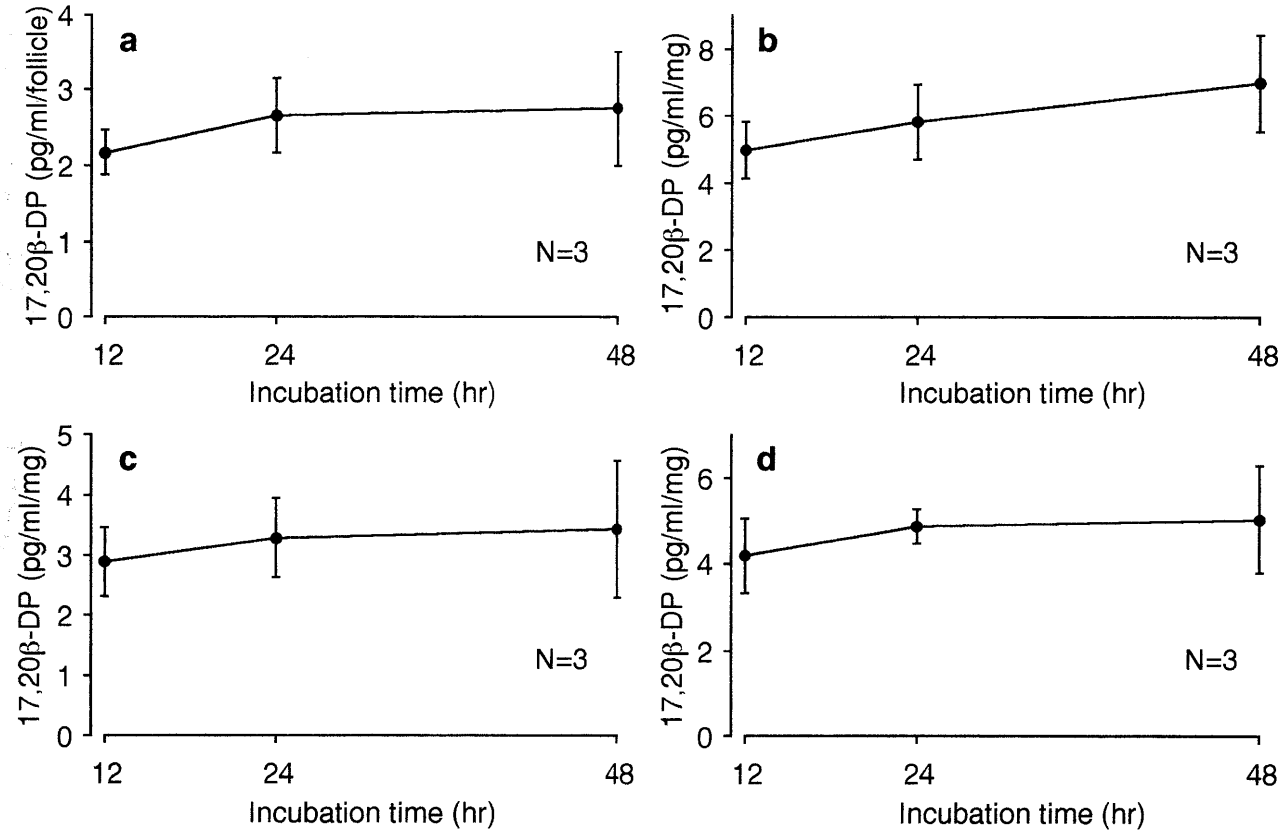

Fig. 5. Effects of incubation time on the *in vitro* production of 17α,20β-dihydroxy-4-pregnen-3-one by the ovarian follicle during the final oocyte maturation (a), and post-ovulatory follicles in early (b), mid- (c) and late (d) gestation in black rockfish. *N*=3.

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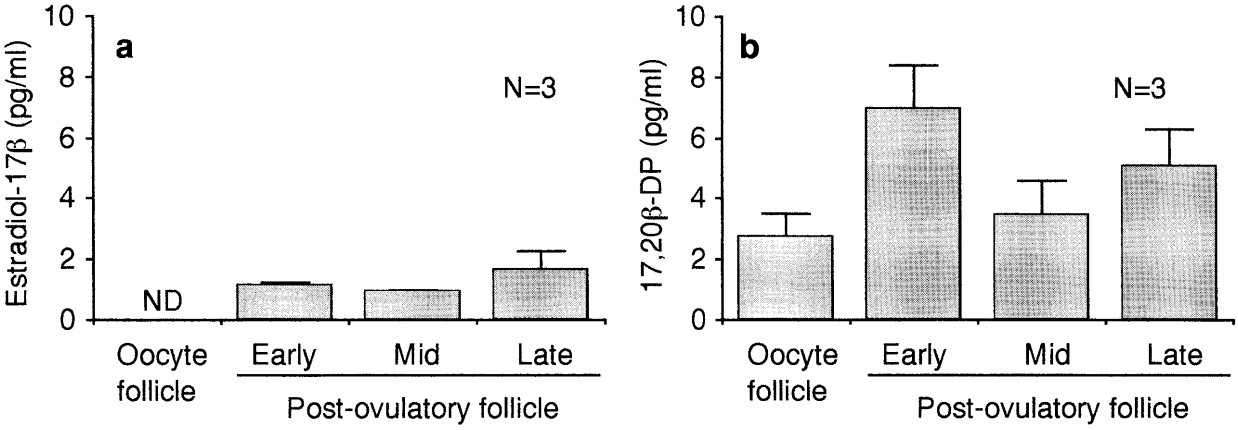
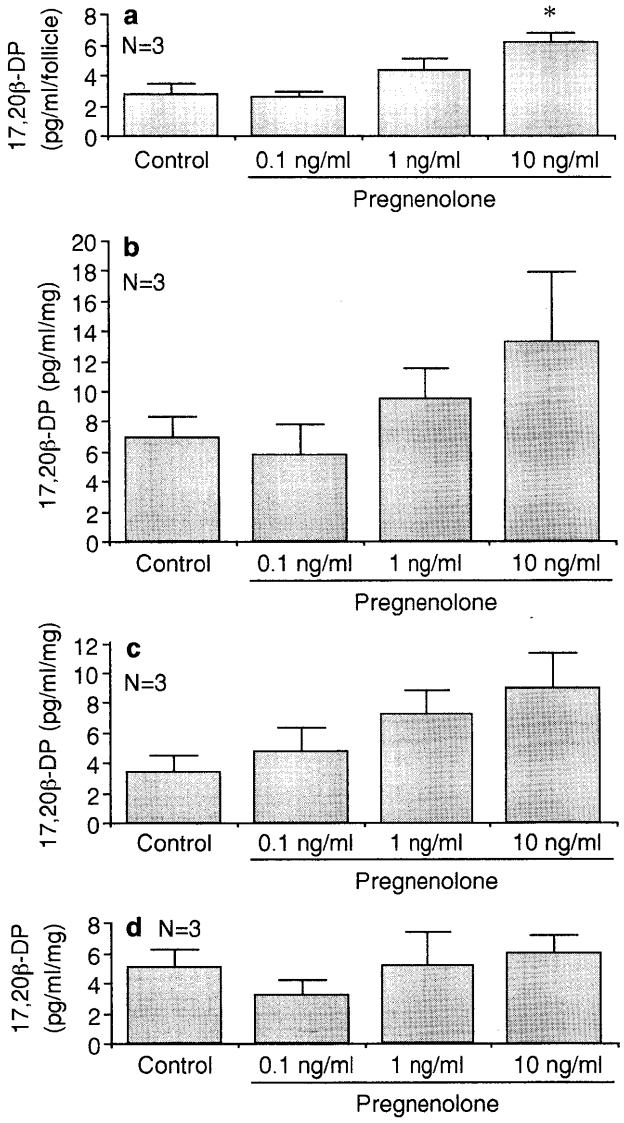


Fig. 6. *In vitro* production of eastradiol-17β (a) and 17α,20β-dihydroxy-4-pregnen-3-one (b) by the ovarian follicle during the final oocyte maturation, and post-ovulatory follicles during early, mid- and late gestation periods in female black rockfish. ND, not detectable. *N*=3.



over time, and showed a maximum at 48 hr of incubation in all experimental periods. Production of 17,20β-DP with a precursor in the incubation medium also tended to increase with time in all experimental periods (data not shown). Production of E2 with or without a precursor was very low but tended to increase over time in all experimental periods, as did that of 17,20β-DP (data not shown). Therefore, the subsequent results of steroid production shown were those at 48 hr of incubation.

Changes in the production of E2 and 17,20β-DP by ovarian and post-ovulatory follicles without a precursor (control) throughout the experimental period were shown in Fig. 6. Production of E2 by ovarian follicles was under the detection limit in the pre-gestation period. Production of E2 by post-ovulatory follicles was low (0.92–1.66 pg/ml/mg tissue) and showed no changes throughout the gestation period (Fig. 6a). On the other hand, production of 17,20β-DP by ovarian follicles was comparatively high (2.75 pg/ml/follicle) in the pre-gestation period. Production of 17,20β-DP by post-ovulatory follicles was also high (3.44-6.96 pg/ml/mg tissue), exhibiting no significant changes throughout the gestation period (Fig. 6b). Production of steroids by post-ovulatory follicles was 1.5–4 times higher in 17,20β-DP than in E2 throughout the gestation period.

Changes in the production of 17,20β-DP by ovarian and post-ovulatory follicles with pregnenolone throughout the experimental period were shown in Fig. 7. The production of 17,20β-DP tended to increase by the addition of pregnenolone in a dose-dependent manner from the pre- to mid-gestation periods, increasing significantly 2.3-fold upon the addition of 10 ng/ml pregnenolone in the pre-gestation

Fig. 7. Effects of the association of pregnenolone (0.1, 1 and 10 ng/ml) with the *in vitro* production of 17α,20β-dihydroxy-4-pregnen-3-one by the ovarian follicles during final oocyte maturation (a), and post-ovulatory follicles during early (b), mid- (c) and late (d) gestation periods of female black rockfish. *Significantly different from control (Dunnett test, $P<0.05$). *N*=3.

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period (Fig. 7a). However, 17,20β-DP production, despite the addition of pregnenolone, did not tend to increase dose-dependently in the late gestation period (Fig. 7d).

DISCUSSION

17,20β-DP is one of the maturation-inducing hormones (MIH) in most oviparous teleosts (Nagahama, 1997), and is believed also to be the MIH in the viviparous rockfish, *Sebastes*. This is because this steroid has the strongly inducing effect of final oocyte maturation and is synthesized by follicles with the addition of human chorionic gonadotropin in the white-edged rockfish, *S. taczanowskii* (Takemura *et al.*, 1989), and also induces an increase in serum levels during oocyte maturation in the white-edged rockfish and black rockfish (Nagahama *et al.*, 1991; Mori *et al.*, 2003). On the other hand, 17,20β-DP or 17,20β-dihydroxyprogesterone is suggested to play an important role in gestation in several rockfishes, because the serum levels of this steroid remain high during the gestation period (Nagahama *et al.*, 1991; Moore *et al.*, 2000; Mori *et al.*, 2003). In the present study, we demonstrated detailed changes in the serum 17,20β-DP profiles of each black rockfish during gestation. The serum levels of 17,20β-DP were already high 8 to 10 weeks prior to parturition compared to their basal levels during the immature and vitellogenic periods (Mori *et al.*, 2003), further increasing and reaching their highest levels 2 to 4 weeks prior to parturition, rapidly declining in pre-parturition, and dropping beneath the detection limit after parturition. A similar pattern was observed in the fish used for culture experiments in the present study. These results confirm that the serum 17,20β-DP levels in black rockfish increased in the early to mid-gestation period and remained high in the late gestation period.

To clarify whether or not high serum levels of 17,20β-DP during gestation are due to the presence of embryos in the ovary, we investigated the serum profiles of 17,20β-DP in non-pregnant females reared separately from males since the pre-copulatory period (September). Such non-pregnant females released unfertilized eggs at the same time that normal pregnant females delivered. The levels of 17,20β-DP in non-pregnant females changed in a way almost identical to those in pregnant females during the corresponding gestation period. These results indicate that the increase in serum 17,20β-DP levels after oocyte maturation is related to the lack of copulation stimulation, the fertilization of mature eggs, and the progress of embryonic development in the ovary. It is thought that the maintenance and increase of 17,20β-DP production after oocyte maturation may occur spontaneously in this species.

In the present study, pregnant females were delivered of embryos within one week after an abrupt decrease in serum 17,20β-DP levels. Similar changes in 17,20β-DP around parturition were observed in the same species by Nagahama *et al.* (1991) who speculated that one of the possible roles of 17,20β-DP is to inhibit contractions of the ovarian muscle to release the embryos (Nagahama *et al.*, 1991). On the other hand, non-pregnant female released unfertilized eggs a little later than the release of embryos in pregnant females, i.e., within two weeks after the decline of 17,20β-DP levels in the present study. These results suggest that both a decline in 17,20β-DP levels in the mother and some response from the embryo are needed for smooth parturition.

In general, post-ovulatory follicles of oviparous teleosts are not homologous to the mammalian corpus luteum in terms of their steroid hormone production, and degenerate soon after ovulation in most cases (Nicholls and Maple, 1972; Nagahama *et al*, 1976, 1978; Lang, 1981; Van den Hurk and Peute, 1985). The post-ovulatory follicles of the medaka, *Oryzias latipes*, the Japanese whiting, *Sillago japonica*, and the sea bream, *Pagrus major*, which are all daily spawners, degenerate within one day after ovulation (Kagawa and Takano, 1979; Matsuyama *et al.*, 1988, 1990). On the other hand, the post-ovulatory follicles of the black rockfish in our study remained at the dorsal ovarian hilus throughout the gestation period, and degenerated soon after parturition. In white-edged rockfish, it has been observed ultrastructurally that the granulosa cells of post-ovulatory follicles retained their normal form throughout the gestation period, showing mitochondria with tubular cristae and a smooth-surfaced endoplasmic reticulum suggestive of steroid-producing activity (Hayashi, 1990). Thus, it is thought that the post-ovulatory follicles of viviparous *Sebastes* have the ability to synthesize steroids throughout the gestation period. In the present study, we tried to experimentally verify the possibility of steroid biosynthesis by the post-ovulatory follicles during the gestation period.

Productions of 17,20β-DP by ovarian follicles and post-ovulatory follicles were higher than those of E2 throughout the experimental period, reflecting the serum levels of both steroids. The production of 17,20β-DP tended to be enhanced in a dose-dependent manner with a precursor steroid, pregnenolone. These results strongly suggest that follicle cells have the ability to synthesize 17,20β-DP not only during oocyte maturation but also during post-ovulation, and that the high levels of serum 17,20β-DP during gestation were due to synthesis and secretion by the post-ovulatory follicles in *Sebastes*. In some oviparous teleosts, their marked capacity for steroid production in young post-ovulatory follicles just after ovulation has been suggested by enzyme-histochemical and ultrastructural studies as well as by serum steroid levels (Lambert and van Oordt, 1974; Nagahama *et al.*, 1976; Kagawa *et al.*, 1981). In the amago salmon, *Oncorhynchus rhodurus*, the *in vitro* culture of follicle cells indicates that post-ovulatory follicles produce a large amount of progesterone soon after ovulation (Nagahama and Kagawa, 1982). However, old post-ovulatory follicles a long time after ovulation showed no enzymatic activity for steroid biosynthesis in the zebrafish, *Brachydanio rerio*, or the white-spotted char, *Salvelinus leucomaenis* (Lambert and van Oordt, 1974; Kagawa *et al.*, 1981), and

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produced neither E2 nor progesterone by *in vitro* culture in amago salmon (Nagahama and Kagawa, 1982). Thus, the pronounced capacity for steroid production in the post-ovulatory follicles of *Sebastes* is a special mechanism unique to viviparous species, and is considered to be a functionally homologous phenomenon by which the post-ovulatory follicles become a corpus luteum and continue to secrete hormones in mammals.

In the present study, the serum levels of 17,20β-DP and the *in vitro* production of 17,20β-DP without a precursor did not always correlate during the gestation period. On the other hand, the production of 17,20β-DP with or without a precursor showed a correlation with serum 17,20β-DP levels. In our preliminary studies, the 17,20β-DP production of post-ovulatory follicles was not affected by the addition of human chorionic gonadotropin or forskolin, the activators for adenylate cyclase (data not shown). These facts may suggest that some unknown factors regulate the production of precursor and/or enzymatic activities for 17,20β-DP production.

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REFERENCES

Asahina K, Kambegawa A, Higashi T (1995) Development of microtiter plate enzyme-linked immunosorbent assay for 17α,20β-21-trihydroxy-4-pregnen-3-one, a teleost gonadal steroid. Fish Sci 61: 491–494

Hayashi S (1990) Studies of the ultrastructural changes of ovarian tissue with oogenesis and pregnancy in white-edged rockfish, *Sebastes taczanowskii*. Master's Thesis, Hokkaido University, Hakodate, Japan (in Japanese)

Hogarth PJ (1976) Viviparity. Arnold, London

Kagawa H, Takano K (1979) Ultrastructure and histochemistry of granulosa cells of pre- and post-ovulatory follicles in the ovary of the medaka, *Oryzias latipes*. Bull Fac Fish Hokkaido Univ 30: 191–204 (in Japanese)

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

Kusakari M (1995) Studies on the reproductive biology and artificial juvenile production of kurosoi *Sebastes schlegeli*. Sci Rep Hokkaido Fish Exp Stn 47: 41–124 (in Japanese)

Lambert JGD, van Oordt PGWJ (1974) Ovarium hormones in teleosts. Fortscher Zool 22: 340–349

Lang I (1981) Electron microscopic and histochemical study of post-ovulatory follicles of *Perca fluciatillis* L. (Teleostei). Cell Tissue Res 22: 201–212

Matsuyama M, Adachi S, Nagahama Y, Matsuura S (1988) Diurnal rhythm of oocyte development and plasma steroid hormone levels in female red sea bream, *Pagrus major*, during the spawning season. Aquaculture 73: 357–372

Matsuyama M, Adachi S, Nagahama Y, Maruyama K, Matsuura S (1990) Diurnal rhythm of serum steroid hormone levels in the Japanese whiting, *Sillago japonica*, a daily-spawning teleost. Fish Physiol Biochem 8: 329–338

Moore RK, Scott AP, Collines PM (2000) Circulation C-21 steroids in relation to reproductive condition of a viviparous marine teleost, *Sebastes rastrelliger* (grass rockfish). Gen Comp Endocrinol 117: 268–280

Mori H, Nakagawa M, Soyano K, Koya Y (2003) Annual reproductive cycle of black rockfish, *Sebastes schlegeli*, in captivity. Fish Sci 69: 910–932

Moser HG (1967) Reproduction and development of *Sebastodes paucispinis* and comparison with other rockfishes off Southern California. Copeia 1967: 773–797

Nagahama Y (1997) 17α,20β-Dihydroxy-4-pregnen-3-one, a maturation-inducing hormone in fish oocytes: mechanism of synthesis and action. Steroids 62: 190–196

Nagahama Y (2000) Gonadal steroid hormones: major regulators of gonadal sex differentiation and gametogenesis in fish. In "Proceedings of the 6th International Symposium on the Reproductive Physiology of Fish" Ed by B Norberg, OS Kjesbu, GL Taranger, E Andersson, SO Stefansson, Bergen, Norway, pp 211–222

Nagahama Y, Kagawa H (1982) *In vitro* steroid production in the postovulatory follicles of the amago salmon, *Oncorhynchus rhodurus*, in response to salmon gonadotropin. J Exp Zool 219: 105–109

Nagahama Y, Chang K, Hoar WS (1976) Histochemistry and ultrastructure of pre- and post-ovulatory follicles in the ovary of the goldfish, *Carassius auratus*. Can J Zool 54: 1128–1139

Nagahama Y, Clarke WC, Hoar WS (1978) Ultrastructure of putative steroid-inducing cells in the gonads of coho (*Oncorhynchus kisutch*) and pink salmon (*Oncorhynchus gorbuscha*). Can J Zool 56: 2508–2519

Nagahama Y, Takemura A, Takano K, Adachi S, Kusakari M (1991) Serum steroid hormone levels in relation to the reproductive cycle of *Sebastes taczanowskii* and *S. schlegeli*. Env Biol Fish 30: 31–38

Nicholls TJ, Maple G (1972) Ultrastructural observations on possible sites of steroid biosynthesis in the ovarian follicular epithelium of two species of cichlid fish, *Cichlasoma nigrofasciatum* and *Haplochromis multicolor*. Z Zellforsch Mikrisk Anat 128: 317–335

Takemura A, Takano K, Yamauchi K (1989) The *in vitro* effects of various steroid hormones and gonadotropin on oocyte maturation of the viviparous rockfish, *Sebastes taczanowskii*. Bull Fac Fish Hokkaido Univ 40: 1–7

Van den Hurk R, Peute J (1985) Functional aspects of the postovulatory follicle in the ovary of the African catfish, *Clarias gariepinus*, after induced ovulation: an ultrastructural and enzyme-histochemical study. Cell Tissue Res 240: 199–208

Wourms JP (1981) Viviparity: the maternal-fetal relationship in fishes. Am Zool 21: 473–515

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