

# ステロイドホルモンによるマダイ卵母細胞の生体外卵成熟誘起

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### Induction of Oocyte Maturation *in vitro* by Steroid Hormones in the Red Sea Bream *Pagrus major*

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A number of steroids have been shown to induce oocyte maturation in teleosts *in vitro*.<sup>1)</sup> Among them,  $17\alpha$ ,  $20\beta$ -dihydroxy-4-pregnen-3-one ( $17\alpha$ ,  $20\beta$ -diOHprog) is one of the most potent steroids.<sup>1,2)</sup> More recently, this steroid has been identified as the natural maturation-inducing hormone in the amago salmon *Oncorhynchus rhodurus*.<sup>3)</sup> These investigations, however, have been limited to several salmonid fishes and some freshwater fishes; there have been few studies on marine fishes.<sup>4,5)</sup> The present study used *in vitro* incubation techniques to determine the relative effectiveness of six steroids in inducing germinal vesicle breakdown (GVBD) in follicle-enclosed oocytes of red sea bream *Pagrus major*, an economically important marine teleosts in Japan.

Three-year-old *P. major* were held under natural ambient conditions in an indoor fiber reinforced plastic holding tank (30 kl) with free flowing sea water and an additional recirculating pump. The water temperature was approximately 16°C, similar to the natural sea water temperatures during breeding season. Under these conditions, most fish spawn in the early evening (17:00–19:00) every day during the breeding season (early April to mid-May). Fish were killed in the early afternoon and then oocytes in the tertiary yolk stage with 10–20 oil droplets were isolated from ovarian pieces. Oocytes in more advanced stages had already undergone ovulation. Experiments were conducted in late April. Isolated oocytes were incubated in plastic tissue culture dishes (24 wells) (Costar) containing a L-15 (GIBCO) culture medium (320 mOs/kg H<sub>2</sub>O) (10 oocytes/ml/well). Incubates contained various doses of progesterone,  $17\alpha$ -hydroxyprogesterone ( $17\alpha$ -OHprog),  $17\alpha$ ,  $20\beta$ -diOHprog, testosterone, estradiol-17 $\beta$  and deoxycorticosterone (DOC) (Sigma). Controls consisted of groups of oocytes incubated in hormone-free media. Three replicates were made of each treatment. Incubation was carried out at 18°C for 24 h. Following this incubation period, oocytes were transferred to a clearing solution<sup>6)</sup> and inspected for GVBD. Oocytes which had undergone GVBD were always transparent. In this study, the occurrence of GVBD was used as the criterion of oocyte maturation.

In this study all steroids, with the exception estradiol-17 $\beta$ , induced GVBD *in vitro* in follicle-enclosed oocytes

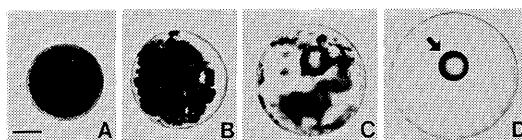


Fig. 1. Time course of *P. major* oocyte changes during  $17\alpha$ ,  $20\beta$ -diOHprog-induced oocyte maturation *in vitro*. The bar represents 200  $\mu$ m. Oocytes were incubated with 0.1  $\mu$ g/ml  $17\alpha$ ,  $20\beta$ -diOHprog for 0 (A), 8 (B), 10 (C) and 12 (D) h. Arrow indicates an oil drop.

of *P. major*. A time course study on oocyte size changes during  $17\alpha$ ,  $20\beta$ -diOHprog (0.1  $\mu$ g/ml)-induced oocyte maturation indicated that steroid treatment markedly increased oocyte size, probably due to hydration (Fig. 1).

Table 1 shows the percentage GVBD induced by various steroids.  $17\alpha$ ,  $20\beta$ -DiOHprog was the most effective steroid, inducing 96.3% at a concentration of 0.1 ng/ml.  $17\alpha$ -OHprog and DOC are the other steroids effective at this concentration, inducing about 10% GVBD. Progesterone induced 20.7% GVBD at a concentration of 1 ng/ml. Testosterone was less effective, while estradiol-17 $\beta$  was totally ineffective. No GVBD occurred in the hormone-free medium. These results on the relative effectiveness of various steroids for the induction of GVBD in follicle-enclosed oocytes of *P. major* is in good agreement with previous reports that  $17\alpha$ ,  $20\beta$ -diOHprog is the most effective steroidal inducer of oocyte maturation *in vitro* in a number of teleost species.<sup>1,2)</sup> The present results, however, only suggest that  $17\alpha$ ,  $20\beta$ -diOHprog is involved in oocyte maturation of red sea bream, since supportive physiological and biochemical data are lacking.

#### References

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Table 1. Effects of various steroids on germinal vesicle breakdown in *P. major* oocytes *in vitro*

Treatment	Concentration (ng/ml)						
	1000	100	10	1	0.1	0.01	0
Progesterone	100*	100	100	20.7	0	0	0
$17\alpha$ -Hydroxyprogesterone	96.2	92.9	100	52.0	10.7	0	0
$17\alpha$ , $20\beta$ -Dihydroxy-4-pregnen-3-one	100	100	100	96.2	96.3	0	0
Deoxycorticosterone	100	100	96.2	20.7	10.0	0	0
Testosterone	100	68.0	10.7	0	0	—	—
Estradiol-17 $\beta$	0	0	0	—	—	—	—
Control							0

\* % Germinal vesicle breakdown.

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