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The modulatory effect *in vitro* of oestradiol-17β, testosterone or cortisol on the output of 17α-hydroxy-20β-dihydroprogesterone by rainbow trout (*Salmo gairdneri*) ovarian follicles stimulated by the maturational gonadotropin s-GtH.

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Summary. The effect of oestradiol-17β, testosterone or cortisol in the incubation medium (1 µg/ml) upon s-GtH-induced 17α-hydroxy-20β-dihydroprogesterone (17α,20β-OH-P) secretion by trout ovarian follicles *in vitro* was investigated in order to elucidate by which mechanism these steroids were able to modulate s-GtH efficiency on the maturation of intrafollicular oocytes with the germinal vesicle initially in subperipheral position.

Oestradiol inhibited significantly 17α, 20β-OH-P output by the follicles of the two females tested at all doses of s-GtH (3.91-500 ng/ml).

Testosterone and cortisol stimulated significantly 17α,20β-OH-P output by follicles only at doses of GtH below 31.25 ng/ml.

The physiological relevance and implications of these findings have been discussed.

Introduction.

In salmonids, the ultimate control of oocyte maturation (resumption of meiosis) by the maturational gonadotropin (GtH) appears to be mediated through follicular synthesis of 17α-hydroxy-20β-dihydroprogesterone (17α,20β-OH-P) (for review see Jalabert, 1976 ; Fostier and Jalabert, 1982 ; Goetz, 1983). 17α, 20β-OH-P, the most potent steroid-inducer of oocyte maturation tested *in vitro* so far (Fostier, Jalabert and Terqui, 1973 ; Jalabert, 1976 ; Duffey and Goetz, 1980 ; Nagahama, Kagawa and Tashiro 1980), is produced *in vitro* by the preovulatory follicle in response to GtH (Fostier *et al.*, 1981 ; Suzuki, Tamaoki and Hirose, 1981 ; Suzuki, Nagahama and Tamaoki, 1981 ; Theofan, 1981). The preovulatory follicle can also produce androgens, oestrogens and corticosteroids (Suzuki, Tamaoki and Hirose, 1981 ; Theofan, 1981 ; Zohar, 1982). From observation of trout plasma profiles of oestradiol-17β and testosterone before ovulation (Fostier *et al.*, 1978 ; Fostier and Jalabert, 1982 ; Scott and Baynes, 1982 ; Scott,

Sumpter and Hardiman, 1983) and considering plasma cortisol levels in maturing female trout (Pickering and Christie, 1981), it is clear that these steroids are present at noticeable levels in blood prior to the surge of 17α,20β-OH-P. From *in vitro* experiments, it was found that oestradiol lowers the efficiency of GtH to induce intrafollicular oocyte maturation, whereas testosterone and cortisol enhance this efficiency (Jalabert, 1975). Similar effects have been shown in the amago salmon with testosterone and deoxycorticosterone, but not with oestradiol-17β (Young, Kagawa and Nagahama, 1982). Since oestradiol-17β and testosterone do not significantly modify the maturational efficiency *in vitro* of 17α,20β-OH-P (Jalabert, 1975), it can be hypothesized that they interfere mostly in the mechanism of GtH action, at the follicular level. Concerning cortisol, which exhibits a positive synergy with either GtH or 17α,20β-OH-P in respect to intrafollicular maturation, it may interact with either the GtH action mechanism in the follicular envelopes or with the 17α,20β-OH-P action mechanism at the oocyte level.

The aim of the present study was to determine if oestradiol, cortisol or testosterone could modulate the secretion of 17α,20β-OH-P induced *in vitro* in rainbow trout follicles by the maturational gonadotropin s-GtH.

Material and methods.

Animals. — Two 3-year old female rainbow trout (*Salmo gairdneri*) weighing about 1.5 kg were used. They were taken from a lot of « hivernal strain » trout (spawning in November-December) reared in the Gournay/Aronde (Oise) fish farm and acclimated for 2 months in the recycled water unit of the laboratory at 12 °C where they were fed with commercial pellets. During the « spawning period », all the fish were anesthetized once weekly by a 0.3 % solution of 2-phenoxy-ethanol in water in order to check the oocyte stage that was determined by examining a few follicles squeezed out by abdominal stripping. The two females were selected when they exhibited oocytes with the germinal vesicle (GV) in subperipheral condition (Jalabert *et al.*, 1976). They were killed by a knock on the head, then completely bled out by puncture in the caudal vein ; the ovaries were removed for subsequent dissection.

Incubations. — The ovaries were entirely dissected into small clusters of 2 to 6 follicles within the interstitial tissue. Incubations were carried out at 12.5° for 72 h under a renewed atmosphere of 50 % N_2 - 50 % O_2 in vials containing 25 follicles per 4 ml of trout balanced salt solution (TBSS : NaCl 133 mM ; KCl 3.1 mM ; $MgSO_4$ 0.3 mM ; $MgCl_2$ 1 mM ; $CaCl_2$ 3.4 mM ; glucose : 1 g/l ; penicillin ; 100 000 units/l, streptomycin sulfate : 100 mg/l (DIFCO)), buffered by Hepes-NaOH 0.04 M at pH 8. At the beginning of incubation we put into each vial : (1) either 10 µl of ethanol as a control or 10 µl of ethanol containing oestradiol, testosterone or cortisol (400 µg/ml ethanol) to obtain a final steroid concentration of 1 µg/ml and (2) 100 µl of TBSS as a control or 100 µl of TBSS containing the appropriate dose of salmon gonadotropin s-GtH. Nine doses of s-GtH were used to give final concentrations ranging from 3.9 ng/ml to 500 ng/ml.

Each hormonal combination was repeated twice for female 1 and three times for female 2. After 48 h and 72 h, 1 ml of incubation medium was taken from each vial and frozen at − 20 °C until subsequent radioimmunoassay of the amount of 17α,20β-OH-P secreted. After 72 h, the number of mature oocytes was counted in each vial.

Hormones. — The pure salmon maturational gonadotropin s-GtH was prepared in the laboratory by B. Breton, as described by Breton, Prunet and Reinaud (1978). The steroids were purchased from Steraloids (USA).

Bioassay of s-GtH. — The median efficient dose of s-GtH, alone or in combination with steroids, was calculated from the proportion of mature oocytes in each vial, as described by Jalabert, Breton and Billard (1974).

Radioimmunoassay of 17α,20β-OH-P. — RIA was performed as described by Fostier *et al.* (1981). Cross-reactivities, expressed as the ratio of the mass of 17α,20β-OH-P to the mass of steroid required to decrease the bound level of the tracer to half its value without competitor, were : 2 % for 20β-dihydroprogesterone and 1 % for 17α-hydroxy-20α-dihydroprogesterone. Furthermore, 100 ng of progesterone, pregnenolone, 17α-hydroprogesterone, 20α-dihydroprogesterone, 5β-pregnan-3α,17α-diol-20-one, cortisone, 20β-dihydrocortisone or deoxycorticosterone competed less with the tracer than 0.020 ng of 17α,20β-OH-P. There was no competition with as much as 100 ng of 5β-pregnan-3α-ol-20 one, 5β-pregnan-17α-ol-3,20-dione, 11-ketoprogesterone, 11α-hydroprogesterone, testosterone, 11-ketosterone, oestradiol-17β, oestrone, cortisol, corticosterone. A plasma pool was assayed repeatedly to estimate measurement variability. The coefficients of variation were 7.7 % (n = 14) for the assay of the total of the samples from female 1 and 10.0 % (n = 36) for the total of the samples from female 2.

Statistical analysis. — The secretion curves (17α,20β-OH-P secreted in the medium after 48 or 72 h as a function of s-GtH concentration) of steroid treatments and control were compared using covariance analysis applied to the linear portions of curves.

Results.

Figures 1 and 2 show the effect of oestradiol, testosterone or cortisol on the secretion of 17α,20β-OH-P by the follicles of female 1 after 48 h and 72 h, respectively, of incubation *in vitro* under the action of increasing concentrations of s-GtH. In comparison with the control, oestradiol treatment exhibited a clear inhibitory effect (particularly significant for the higher doses of s-GtH) in the range of 31.25 to 500 ng/ml (table 1). On the other hand, the presence of testosterone or cortisol had a stimulatory action only on the lower doses of s-GtH in the range of 3.91 to 31.25 ng/ml. Whereas the increase in the production of 17α,20β-OH-P in the presence of cortisol was already significant after only 48-h incubation, the favourable effect of testosterone needed 72 h to become significant (table 1).

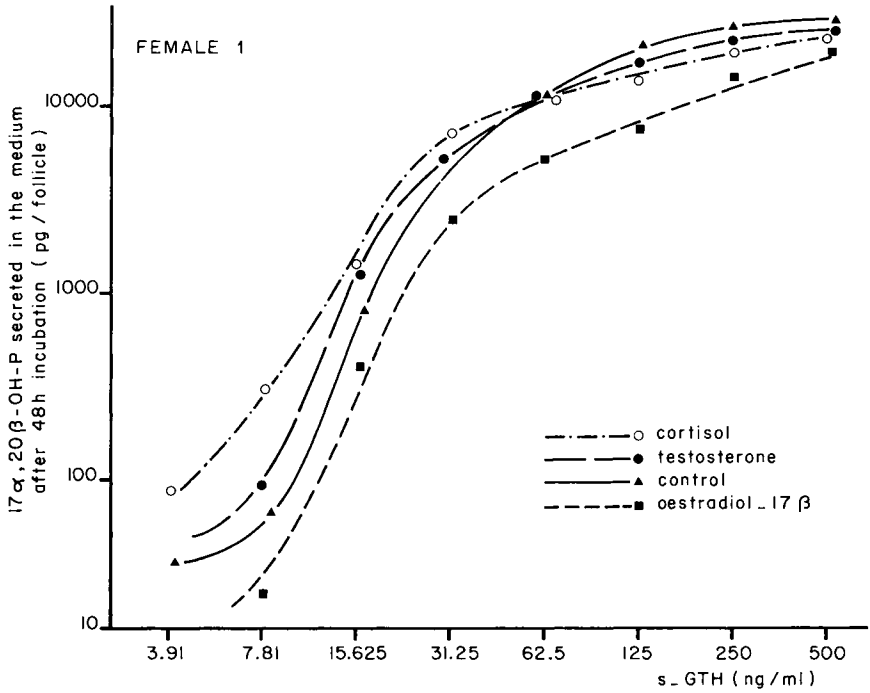

FIG. 1. — *Amount of 17α, 20β-OH-P secreted into the incubation medium after 48-h incubation as a function of the concentration of s-GtH in the medium* (female 1).

TABLE 1

(Female 1) : A comparison of the secretion curves (17α, 20β-OH-P as a function of GtH concentration) *of steroid treatments and control.*

		Concentration of s-GtH (ng/ml)				
		3.91	7.81	15.62	31.25	500
Oestradiol-17β	48 h	inhibition (NS)			inhibition (***)	
	72 h	inhibition (NS)			inhibition (**)	
Testosterone	48 h	stimulation (NS)			NS	
	72 h	stimulation (*)			NS	
Cortisol	48 h	stimulation (*)			NS	
	72 h	stimulation (***)			NS	

Significance of the difference : NS = not significant ; *, **, *** = significant at the probability levels of 0.05, 0.01, 0.001, respectively.

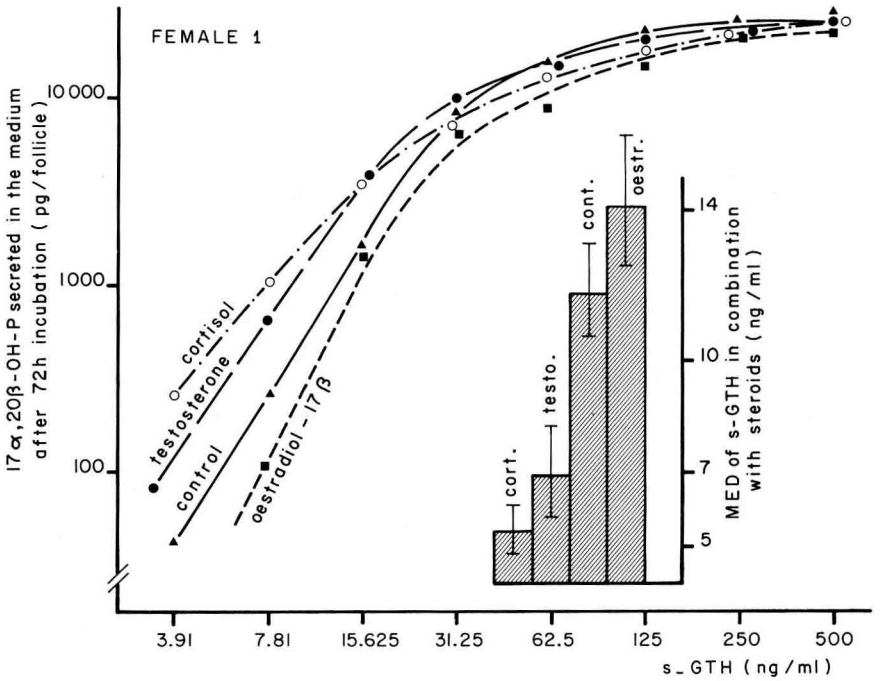

FIG. 2. — *Same as figure 1 after 72-h incubation.* The histogram on the right represents the median efficient dose (MED) of s-GtH for oocyte maturation (morphological criterion) in the presence of cortisol, testosterone, oestradiol or in the control (female 1).

These data fit well with the maturational response of the oocytes incubated within the follicles, as shown by the MED of s-GtH for oocyte maturation in the presance of steroids (fig. 2) ; this response was significantly lowered by cortisol and testosterone and increased by oestradiol (but not significantly in female 1).

In the experiment run with female 2 (fig. 3, table 2), the effect of oestradiol and testosterone was confirmed in the same experimental conditions. The inhibitory effect of oestradiol on 17α,20β-OH-P secretion was already significant

TABLE 2

(Female 2) : Same legend as Table 1.

		Concentration of s-GtH (ng/ml)			
		3.91	31.25	125	500
Oestradiol-17β	48 h	inhibition (***)		inhibition (*)	
	72 h	inhibition (***)			
Testosterone	48 h	stimulation(**)		NS	
	72 h	stimulation (*)		NS	

after 48 h and became very highly significant after 72 h, whatever the dose of s-GtH ; this effect corresponded to a significant increase in the MED of s-GtH for oocyte maturation. The effect of testosterone upon the MED of s-GtH was not significant in female 2, but a stimulatory effect could be observed on 17α,20β-OH-P secretion in response to the lower doses of GtH (up to 31.25 ng/ml).

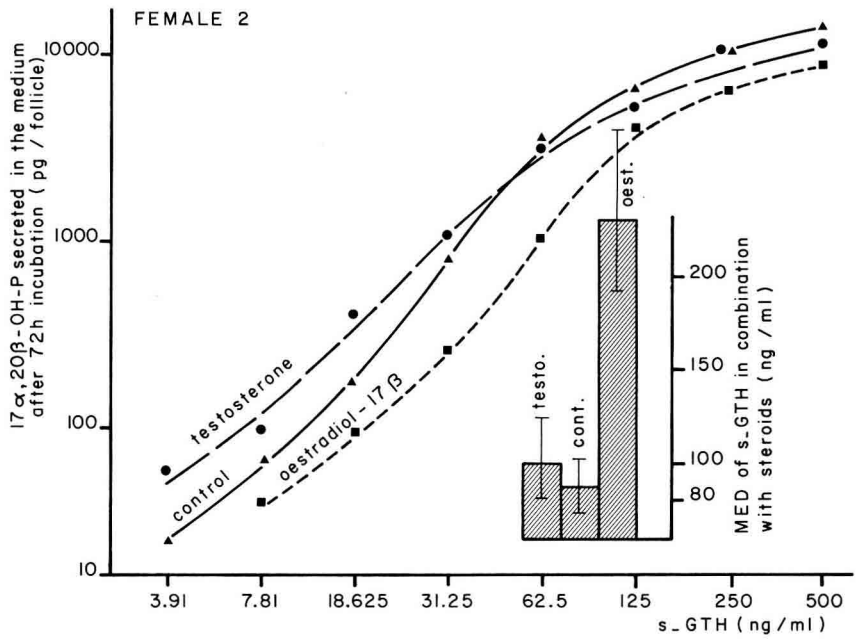

FIG. 3. — *Same as figure 2 but concerns female 2.*

Discussion.

The reality of a physiological role for oestradiol, testosterone or cortisol *in vivo,* as suggested by our results, could be questioned, considering the dose applied *in vitro* (1 µg/ml) which is far higher than the blood levels. As no data were available concerning steroid levels and availability *in situ* within the ovarian follicle in fish, this dose was chosen because it was expected to be high enough to ensure good penetration through the follicular envelopes in conditions of static incubation *in vitro* without perfusion. Besides, each of the considered steroids had already been shown to be ineffectual alone in inducing intrafollicular oocyte maturation at that level (Jalabert *et al.,* 1973). In mammals, androgens and oestrogens are found in the follicular fluid at much higher levels than in the plasma (Chang *et al.,* 1976 ; Eiler and Nalbandov, 1977 ; Fortune and Hansel, 1979). In amphibians, the high level of testosterone found in *Xenopus* ovaries treated by GtH *in vitro* led Fortune and Tsang (1981) to the hypothesis that endogenous testosterone could trigger meiotic maturation *in vitro.* Moreover, we

know that cortisol and oestradiol can significantly modify the MED of s-GtH for intrafollicular oocyte maturation in trout even at a concentration of 40 ng/ml (Jalabert and Fostier, 1983) which is close to plasma levels during vitellogenesis (Billard *et al.*, 1978).

It can be hypothesized that the amount of 17α,20β-OH-P measured in the incubation medium after 48 and 72 h was related to the total amount actually synthesized by the follicle. On the one hand the possibility that metabolic transformations of 17α,20β-OH-P could occur during incubation cannot be ruled out (Suzuki, Tamaoki and Hirose, 1981), but this is probably a minor phenomenon, if any, considering the kinetics of 17α,20β-OH-P accumulation already observed in the incubation medium after s-GtH stimulation (Fostier *et al.*, 1981) ; on the other hand, most of the amount of 17α,20β-OH-P synthesized *in vitro* after s-GtH stimulation has been shown to be released into the surrounding medium (Fostier and Jalabert, 1983).

The above arguments lead us to conclude from the present data that oestradiol, testosterone and cortisol are actually able to modulate the secretion of 17α,20β-OH-P by trout ovarian follicles in response to GtH. This would explain why these steroids modulate, in the same way, the morphological maturational response to GtH of oocytes incubated within their follicle (Jalabert, 1975). Moreover, in the present experiment where overall follicular sensitivities (evaluated by the MED of s-GtH for intrafollicular oocyte maturation) are very different in the two females, it is crucial to note that the significance of the modulatory ability of any steroid on the MED of maturational s-GtH is strictly related to the significance of the modulatory effect of the same steroid on 17α, 20β-OH-P secretion in response to a dose of s-GtH in the range of the control MED. This observation supports our hypothesis that oestradiol and testosterone, which do not interfere with 17α,20β-OH-P maturational action *in vitro,* act essentially at the follicular level in trout. In contrast, some observations in other fishes and amphibians favour the hypothesis that the oocyte itself is a target for oestradiol or testosterone which would interfere directly with the maturation-inducing steroid (MIS) at the oocyte level. This appears to be the case in catfish, where oestradiol or testosterone inhibits oocyte maturation induced by cortisol, the hypothetical MIS (Sundararaj, Goswami and Lamba, 1979), and in such amphibians as *Rana pipiens* where progesterone-induced germinal vesicle breakdown (GVBD) can be inhibited by oestradiol even in denuded oocytes (Lin and Schuetz, 1983). One cortisol action site also appears to be located at the follicular level, but the possibility remains of a synergic action with 17α,20β-OH-P at the oocyte level. Such a synergic action between 17α,20β-OH-P and deoxycorticosterone at that level in the amago salmon has also been suggested (Young, Kagawa and Nagahama, 1982).

OEstradiol-17β generally appears to play an inhibitory role in the regulation of oocyte meiotic maturation in different classes of vertebrates : mammals (Racowsky and McGauchey, 1982) amphibians (Schuetz, 1972 ; Baulieu *et al.*, 1978 ; Lin and Schuetz, 1983) with a contradictory result in *Xenopus laevis* (Hanocq-Quertier and Baltus, 1981) and fish (Jalabert, 1975 ; Sundararaj, Goswami and Lamba, 1979 ; Theofan, 1981). In *Rana pipiens* (Spiegel, Jones and

Snyder, 1978) and in the brook trout, *Salvelinus fontinalis* (Theofan, 1981), œstradiol inhibits 3β-hydroxysteroid dehydrogenase. The physiological role of such an inhibitory œstradiol-17β effect on GtH-induced progestin secretion would be to avoid premature meiotic maturation before completion of vitellogenesis when œstradiol-17β levels are high.

Testosterone induces maturation *in vitro* in intrafollicular oocytes of the medaka when used alone (Hirose, 1972) or shows positive synergy with GtH in trout (Jalabert, 1975). However, it was found to inhibit oocyte intrafollicular maturation induced by either cortisol, LH or pregnenolone in the catfish, *Heteropneustes fossilis,* exactly like œstradiol (Sundararaj, Goswami and Lamba, 1979). Such a contradiction might result from differences in aromatase activity which could depend on the ovarian stage and/or the species.

The positive action of cortisol upon GtH-induced 17α,20β-OH-P secretion in trout in the present study can be compared to some extent to the positive synergic effect of glucocorticoids on the FSH-stimulated production of progesterone by rat granulosa cells *in vitro* (Adashi, Jones and Hsueh, 1981).

In conclusion, our data lead to the assumption that the modulatory effect of oestradiol, testosterone or cortisol an GtH-induced 17α,20β-OH-P secretion *in vitro* in the present experiment is probably representative of the physiological regulation occuring *in vivo.* Finally, such a regulatory role for these steroids is coherent with our present knowledge of the profiles of their secretion *in vitro* by s-GtH-stimulated follicles (Fostier *et al.,* 1981 ; Zohar, Breton and Fostier, 1982), and of their plasma profiles around spawning time. Thus, preovulatory decrease of the œstradiol level (Fostier *et al.,* 1978), transitory rise in testosterone level (Fostier and Jalabert, 1982) and overall increase of corticosteroids (Pickering and Christie, 1981) certainly contribute to the progressively increased ability of the follicle to secrete 17α,20β-OH-P in response to GtH.

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Résumé. *Effet modulateur de l'œstradiol-17β, de la testostérone ou du cortisol* in vitro *sur la libération de la 17α-hydroxy-20β-dihydroprogestérone par des follicules ovariens de Truite arc-en-ciel* (Salmo gairdneri) *induite par la gonadotropine maturante s-GtH.*

L'effet de l'adjonction au milieu d'incubation d'œstradiol-17β, de testostérone ou de cortisol (1 μg/ml) sur la libération de 17α, 20β-OH-P *in vitro* par des follicules ovariens de Truite (contenant initialement des ovocytes au stade de la vésicule germinative en position subpériphérique) a été étudié afin de préciser par quel mécanisme ces stéroïdes sont capables de moduler l'efficacité de la GtH sur la maturation ovocytaire intrafolliculaire *in vitro.*

L'œstradiol-17β s'est révélé capable de déprimer la sécrétion de 17α, 20β-OH-P par les follicules des deux femelles restées, même lorsque l'augmentation de la dose de GtH efficace à 50 % (DE_{50} ou MED) pour induire la maturation ovocytaire (critères morphologiques) n'était pas significative chez l'une des femelles. Cet effet dépresseur est observé pour toutes les doses de GtH essayées (de 3,91 à 500 ng/ml).

La testostérone et le cortisol ont stimulé de façon significative la sécrétion de 17α, 20β-OH-P, mais seulement pour les doses de GtH inférieures à 31,2 ng/ml, alors que la diminution de DE_{50} de la GtH pour la maturation ovocytaire n'était pas toujours significative.

Ces résultats apparaissent comme représentatifs de la situation physiologique, compte tenu des connaissances récentes sur l'évolution des profils plasmatiques des stéroïdes considérés pendant la période périoculatoire chez la Truite.

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