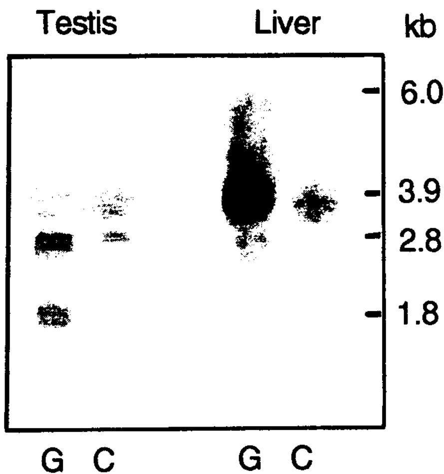

Fig 1. Hybridization of salmon IGF-I cDNA (pb388 PCR product from Duguay *et al; Mol Endocrinol*, in press) to a Northern blot of total RNA (20 μg) from rainbow trout testis and liver. Tissues were obtained 11 h after a single injection of trout growth hormone (G) or vehicle (C). On the right of the figure the size (in kb) of the different IGF-I transcripts found in salmon liver by Cao *et al* (1989; *Mol Endocrinol* 3, 2005-2010) and Sakamoto and Hirano (in press).

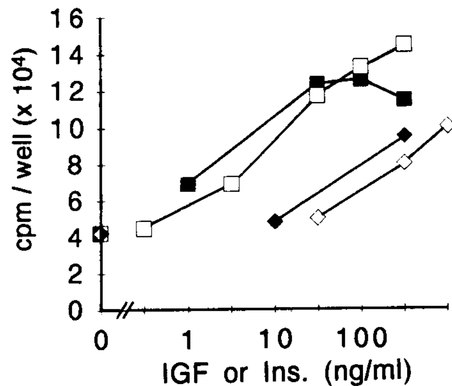

Fig 2. Effect of increasing concentrations of rhIGF I and II, s- and b-insulin on *in vitro* ^{3}H-T incorporation (incubation for 3 d; 8 μCi/ml ^{3}H-T and 3rd day) by trout germ cells (0.7 x 10^6 Go + Ci/well). Mean ± SD (n = 4). —■— IGF-I; —□— IGF-II; —◆— s-insulin; —◇— b-insulin.

Effects of recombinant human IGF-I and IGF-II (gift from Ciba-Geigy) and of salmon (gift from Dr E Plisetskaya) and bovine insulin on ^{3}H-thymidine (^{3}H-T) incorporation by spermatogonia (Go) and primary spermatocytes (Cl), cultured either in the absence or in the presence of Sertoli cells (SC), were examined *in vitro*. IGF-I (ED_{50} 3.5-12 ng/ml) and IGF-II (ED_{50} 27-38 ng/ml) stimulated ^{3}H-T incorporation by Go+Ci, in a dose-dependent manner (fig 2). A hundred- to 300-fold higher concentration of s- or b-insulin was required to produce an effect similar to that of IGF-I. In the presence of SC, the responsiveness of Go/Cl was decreased (ED_{50} 120 ng/ml). Combinations of various concentrations of IGF-I, IGF-II and insulin suggest that the effects of these molecules should be mediated through a unique IGF receptor.

Inhibition of production of maturation inducing steroid in rainbow trout granulosa cells: effect of oestradiol on gonadotropin stimulated 20 β-hydroxysteroid dehydrogenase activity. A Fostier, H Baek *(INRA, Laboratoire de Physiologie des Poissons, Campus de Beaulieu, 35042 Rennes Cedex, France)*

In most teleost species investigated, 17-hydroxy-20 β-dihydroprogesterone (17, 20-OHP) was the most effective maturation inducing steroid (MIS). Gonadotropin type 2 (GtH 2) is known to stimulate the 20 β-hydroxysteroid dehydrogenase activity (20βHSDH) in granulosa cells, the key enzyme converting 17-hydroxyprogesterone (17-OHP) into 17,20-OHP (Jalabert *et al*, 1991, *In: Vertebrate Endocrinology: Fundamental and Biomedical Implications*. Academic Press, NY, 4A, 23-90). High doses of oestradiol (E2), which is synthesized by granulosa cells during vitellogenesis, have been shown to depress 17,20-OHP production by ovarian follicles (Jalabert and Fostier, 1984;

Reprod Nutr Dev 24, 127-136). The present study was designed to investigate the effect of physiological doses of oestradiol on the 20β-HSDH activity of granulosa cells.

EXPERIMENT I

Rainbow trout granulosa cells were isolated just before spontaneous oocyte maturation (according to Jalaber, unpublished data) and cultured in a synthetic medium (L15 type without phenol red). GtH 2 and 17-OHP were added to the medium at a concentration of 50 ng/ml. Oestrogen was added to yield different media with E2 (0, 5, 10, 20, 40, 50 and 500 ng/ml) or diethylstilbestrol (DES: 5, 10, 20 and 40 ng/ml). Each treatment was performed in triplicate. Cell viability was controlled by erythrosin staining. Cells and culture media were collected after 48 h of culture (12 °C). Samples were analyzed for 17,20-OHP by RIA. The conversion of 17-OHP to 17,20-OHP was inhibited by both types of oestrogen (fig 1). There was a total inhibition with the highest dose of E2 (50 ng/ml, not shown).

EXPERIMENT 2

Granulosa cells were cultured for 48 h in the presence of GtH, with or without E2 (20 ng/ml).

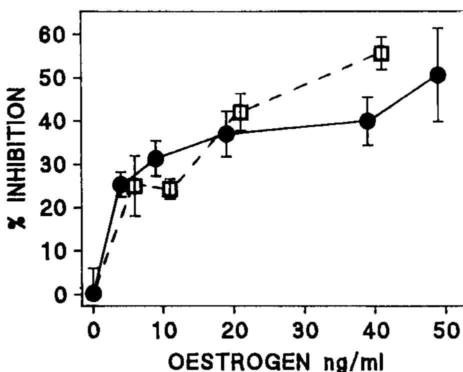

Fig 1. Inhibition of granulosa cells 17,20-OHP production by oestrogens. Each point is mean of triplicates ± SD. (●—●—● E2; □---□---□ DES).

At the end of the culture period, ^{3}H-17-0H-P was added. After a 4-h incubation period, the reaction was stopped by adding ethanol, unlabelled 17-OHP and 17,20-OHP and ^{14}C-17,20-OHP. The samples were extracted and subjected to thin layer chromatography in order to determine ^{3}H-17,20-OHP/^{14}C-17,20-OHP ratios. These ratios decreased significantly in the presence of E2 thus showing inhibition of 20βHSDH activity (control = 8.02 ± 1.5 SD; E2 = 4.52 ± 0.04 SD; $n = 3$).

In conclusion, physiological doses of E2 decrease secretion of 17,20-OHP by rainbow trout granulosa cells. This effect is exerted by inhibition of 20βHSDH activity. Such a paracrine regulation may prevent MIS secretion if vitellogenesis is not completed.

Functional changes throughout follicular atresia in the ewe. D Monniaux, P Monget, JC Mariana, A Nicolle, C Pisselet, Y Cognié, N Poulin, J Fontaine, P Durand *(INRA, Station de Physiologie de la Reproduction des Mammifères Domestiques, URA CNRS 1291, 37380 Nouzilly, France)*

Atresia is the ultimate fate of 99.9% of ovarian follicles, although mechanisms leading to follicular degeneration remain obscure.

In sheep, aromatase activity in granulosa cells is lost in atretic follicles but changes in thecal cell steroidogenesis are still unknown. In Romanov ewes, androstenedione secretion by thecal tissue of 213 individual follicles of various sizes (2–7 mm diameter) and degrees of atresia (assessed by microscopical examination of smears of granulosa cells) were measured in short-term incubations (4 h). Androstenedione secretion was generally low. Levels were higher than the limit of detection of the assay (2.5 ng/tube) for 25% of normal follicles, 15% of early atretic follicles and only 7% of late atretic follicles. Nevertheless, these results suggest that androstenedione synthesis by thecal cells decreases during atresia.

To assess their possible involvement in atresia, IGF (insulin-like growth factor) and IGF-binding protein (BP) levels were measured in follicular fluid of the same individual follicles in which steroidogenesis was studied. Throughout