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Rainbow trout granulosa cells were isolated just before spontaneous oocyte maturation (according to Jalaber, unpublished data) and cultured in a synthetic medium (L15 type without phenol red).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).

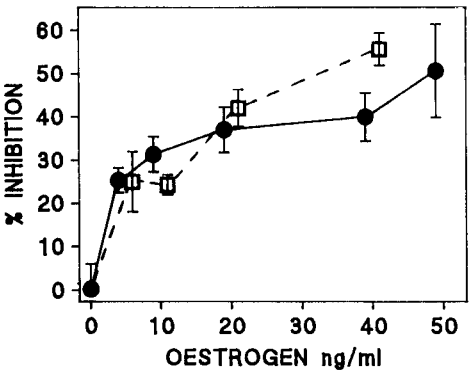

Fig 1. Inhibition of granulosa cells 17,20-OHP production by oestrogens. Each point is mean of triplicates ± SD. (●—●—● E2; □----□---□ DES).

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

atresia, IGF-I and IGF-II concentrations did not change but free IGF-binding activity markedly increased. Western ligand blotting analysis of follicular fluid showed that small molecular weight (< 35 kD) BP levels increased 2-fold in atretic follicles, whereas BP3 (MW = 44–42 kDa) levels were slightly reduced in small ones (2–4 mm diameter). These results suggest that IGF-binding protein levels are locally controlled in follicles during growth and atresia.

A role for gonadotropins in rescuing or protecting follicles from atresia has been previously proposed by an intrafollicular regulation of atresia could also be involved. To test this hypothesis, the action of follicular fluid from normal, early atretic and late atretic follicles was studied comparatively on degeneration and proliferation of granulosa cells *in vitro*. In 24-h cultures, the pycnotic index (% of pycnotic cells) of granulosa cells cultured with follicular fluid (1% final concentration clearly increased when fluid from early and late atretic follicles was used (x 1.6 and x 1.3 respectively). This effect was partially reversed when cells were cultured with IGF-I. Moreover, IGF-I-enhanced proliferation of granulosa cells (assessed by measurement of the labelling index –% of labelled cells- of cells incubated for 2 h with ^{3}H-thymidine at the end of the culture) was lowered or inhibited by follicular fluid from atretic follicles. These results suggest that, *in vivo*, factors present in follicular fluid could modulate the degeneration and the proliferation of granulosa cells; the high levels of the small molecular weight IGF-binding proteins in atretic follicles may be particularly involved in this process.

An experimental model for studying the kinetics of the degenerative changes underlying follicular atresia has been proposed in the ewe. At the end of the follicular phase of a synchronized cycle, animals were hypophysectomized and ovaries and recovered at various times after hypophysectomy. Histological examination of the ovaries showed that the rate of atresia of follicles larger than 0.3 mm diameter was not clearly increased until 36 h after hypophysectomy and the number of mitoses in follicles between 0.3 and 2 mm diameter remained unchanged. Functional studies accompanying this experimental model are required to improve understanding of the mechanisms leading to atresia in ovine follicles.

IGF I autoregulation in porcine granulosa cells *in vitro*. F Hatey, V Dupouy, I Langlois, A Bonnet, P Mulsant, F Grasser *(INRA, Laboratoire de Génétique Cellulaire, Auzeville 31326 Castanet-Tolosan Cedex, France)*

In the pig ovary, the growth factor IGF I is produced by and acts on granulosa cells. In these cells, IGF I production is increased by gonadotropins both *in vivo* and *in vitro*. Thus, an autocrine pathway has been proposed. Moreover, IGF I and FSH have been shown to synergize in the production of cyclic AMP, progesterone and oestradiol.

Using inhibitors of transcription and translation in cell culture *in vitro*, whe have recently shown that the gonadotropin stimulation of IGF I production results, at least in part, from an increased transcription and is not dependent on newly synthesized proteins. In view of the autrocrine mechanism, the question then arises as to an autoregulation of IGF I production, that is, does IGF I regulate its own synthesis? To answer this question, we treated primary cultures of granulosa cells in a serum-free, insulin-free medium with either IGF I, FSH or both. We then examined, by Northern analysis, the effect of such treatment on IGF I mRNA levels.

IGF I or FSH alone had only a slight effect, if any, on IGF I mRNA levels (< 2-fold). By contrast, simultaneous treatment by both IGF I and FSH resulted in a dramatic increase (up to 30-fold) in IGF I mRNA level. The same synergy was observed in progesterone production.

When cells were treated with variable amounts of IGF I (3–100 ng/ml) in the presence of 100 ng/ml FSH (fig 1A), a dose-dependent increase in the IGF I mRNA level seemed to occur. If IGF I concentration was fixed to 100 ng/ml and FSH concentration varied (10 to 300 ng/ml), accumulation of mRNA occurred when FSH concentration was 30 ng/ml or higher (fig 1B).

Our results thus suggest that IGF I may be autoregulated as it increases its own mRNA level in the presence of FSH. This further indicates that the IGF I – FSH synergy is also active on the autocrine loop of IGF I.