BRIEF COMMUNICATIONS

Inhibitory effect of follicular fluid on RNA synthesis of rat granulosa cells *in vitro*

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Follicular fluid, the composition of which has been shown to be nearly the same as that of plasma, contains many substances believed to be secreted by the thecal and/or granulosa cells, e.g. steroids, particularly oestradiol-17 β and progesterone (Short, 1962), pituitary hormones (McNatty, Hunter, McNeilly & Sawers, 1975) and mucopolysaccharides (Zachariae & Jensen, 1958). The proteins in follicular fluid are the same as those of plasma, although of different concentrations (Desjardins, Kirton & Hafs, 1966; Shalgi, Kraicer, Rimón, Pinto & Soferman, 1973; Menezo & Testart, 1975). Follicular fluid is probably, therefore, only partly a transudate from the plasma; its role in follicular growth and ovulation has been discussed extensively by Edwards (1974). Although no specific antigenic substance in follicular fluid has yet been demonstrated, it has been suggested that granulosa cell luteinization is controlled by an inhibitory factor in the fluid (El-Fouly, Cook, Nekola & Nalbandov, 1970; Channing, 1973) and Moore *et al.* (1975) found an inhibition of the activity of RNA-polymerase of rat Yoshida ascites cells by bovine follicular fluid. Bernard (1975) showed that pig follicular fluid prevents morphological differentiation of rat granulosa cells *in vitro*, but does not alter their progesterone secretion. To investigate further the presence and nature of a 'luteinization inhibiting factor' in follicular fluid we compared the incorporation of [3 H]uridine into the total RNA by rat granulosa cells after incubation in either serum or bovine follicular fluid.

Follicular fluid was collected at the slaughterhouse from cow follicles 3–15 mm in diameter, centrifuged and kept at -30°C until used. Half of this fluid and dialysed fetal calf serum (GIBCO) were incubated at 37°C in dextran-charcoal buffer for 2 h to eliminate all the oestrogens (unpublished data) and most of the other steroids present (Bernard, 1975). As described by Bernard (1975), granulosa cells were removed from immature Wistar rats 51 h after injection of 30 i.u. PMSG, but just before the critical period of endogenous LH release (Strauss & Meyer, 1966). The cells were placed in equal quantities in plastic scintillation vials, for maintenance in culture without mitotic divisions (Channing & Ledwitz-Rigby, 1975), and incubated for 22 h in 1 ml medium, at 37°C in an atmosphere of 5% CO_2 in air. The medium consisted of 50% of the 'control medium' (90% TC 199; 10% dialysed fetal calf serum; antibiotics: see Bernard, 1975) and of 50% of either oestrogen-free dialysed fetal calf serum or untreated ('total') or oestrogen-free follicular fluid. In three experiments ovine LH (activity: $2.26 \times \text{NIH-LH-S1}$) was added to all three media to give a final concentration of 1 $\mu\text{g/ml}$.

After incubation, 20 μCi [3 H]uridine (sp. act. 21 Ci/mmol: Commissariat à l'Energie Atomique)/ml were added for 30 min to each vial. The cells were then centrifuged, rinsed twice in unlabelled uridine and treated with 1 ml cold (4°C) 5% trichloroacetic acid (TCA) for 20 min, followed by a rinse in cold 2.5% TCA. After centrifugation the cell pellet was solubilized in 0.5 ml Soluene 100 (Packard Instruments Inc.) for 30 min at 60°C . The 5% dried TCA solution and the Soluene were counted with 10 ml toluene PPO-POPOP scintillation fluid in a Beckman LS 100 counter. Because the radioactivity of the Soluene gave an estimate of the uridine incorporation into TCA-insoluble material, i.e. that containing the RNA, and the radioactivity of the TCA-soluble fraction gave an estimation of free uridine, the results are expressed by the ratio of the TCA-insoluble fraction/total uridine uptake (free + incorporated uridine). This gives a percentage estimation of the RNA synthesis by the granulosa cells.

The results are shown in Table 1. The levels of RNA synthesis by cells incubated in the control medium varied in different experiments, perhaps because of metabolic differences in the granulosa cell samples. There was a significant reduction in the radioactivity of the TCA-insoluble fraction

when granulosa cells were cultured in the presence of follicular fluid. RNA synthesis was also decreased by up to 30%. There was no significant difference between the effects of total and oestrogen-free follicular fluid. The inhibition does not seem to be due to a cytotoxic effect of the follicular fluid because the cells exhibited the same degree of viability, as judged by the trypan-blue staining test, whatever the type of incubation. The radioactivity of the TCA-insoluble fraction, and the levels of RNA synthesis were not affected by the concentration of LH in the medium.

Table 1. Effect of bovine follicular fluid on RNA synthesis (ratio of TCA-insoluble fraction/total uptake $\times 100$) by rat granulosa cells incubated in various media for 22 h

Exp.	Control medium		Total follicular fluid		Oestrogen-free follicular fluid	
	Radioactivity of TCA-insoluble fraction*	RNA synthesis	Radioactivity of TCA-insoluble fraction*	RNA synthesis	Radioactivity of TCA-insoluble fraction*	RNA synthesis
1	65 $\pm$ 5	30 $\pm$ 2.1	8.4 $\pm$ 1	11 $\pm$ 3.4	14 $\pm$ 2	16 $\pm$ 2.3
2	443 $\pm$ 11	46.6 $\pm$ 2.7			163 $\pm$ 13	35.6 $\pm$ 1.9
3	746 $\pm$ 13	36.7 $\pm$ 2.5	363 $\pm$ 6	23.8 $\pm$ 2.0	453 $\pm$ 9	29.4 $\pm$ 1.0
4	516 $\pm$ 18	61.2 $\pm$ 0.6	187 $\pm$ 9	56.8 $\pm$ 1.1		
5	485 $\pm$ 32	28.1 $\pm$ 1.8	169 $\pm$ 31	21.5 $\pm$ 0.9		
6	440 $\pm$ 45	38.7 $\pm$ 1.2	101 $\pm$ 11	28.3 $\pm$ 6.0		
7	393 $\pm$ 31	36.4 $\pm$ 0.3	108 $\pm$ 12	24.3 $\pm$ 0.9		
8	272 $\pm$ 26	30.8 $\pm$ 4.2	94 $\pm$ 8	21.3 $\pm$ 3.7		
<i>With LH</i>						
1	546 $\pm$ 21	45.2 $\pm$ 2.1	106 $\pm$ 12	33.3 $\pm$ 1.5	176 $\pm$ 18	37.8 $\pm$ 1.2
2	388 $\pm$ 42	35.1 $\pm$ 1.2	80 $\pm$ 6	24.5 $\pm$ 3.5		
3	666 $\pm$ 12	35.8 $\pm$ 1.4	280 $\pm$ 32	25.6 $\pm$ 1.7		

Results are means ($\pm$ S.E.M.) of 3 experiments.

* Counts/min/vial $\times 10^{-2}$.

Ledwitz-Rigby, Stetson & Channing (1973) have shown that the number of LH/HCG receptors of pig granulosa cells is not altered by preincubation with follicular fluid, although the LH-induced stimulation of cyclic AMP, a step preceding the luteinization, is affected by follicular fluid but not by follicular fluid + aminophylline. This suggests that follicular fluid may act on a step between the accumulation of cyclic AMP and the formation of specific messenger RNAs for luteinization. Furthermore, LH stimulates the adenyl cyclase activity in the granulosa cells (Kolena & Channing, 1972), but it has been shown to have no stimulatory effect on in-vitro RNA synthesis by prepubertal rat ovaries (Jarlstedt, Nilsson, Hamberger & Ahrén, 1973) and to regulate protein synthesis of Graafian follicles at a translational level in the rabbit (YoungLai, 1975; Mills, 1975) and the rat (Lieberman *et al.*, 1975). Our results agree with these findings, since LH affected neither the level of RNA synthesis of the cells incubated in the control medium, nor the inhibitory effect of follicular fluid on this synthesis.

Our data also show that the effect of follicular fluid on RNA synthesis does not seem to be influenced by the presence of oestrogens in it. The small but not significant reduction of inhibition observed with the oestrogen-free follicular fluid may have been due to a slight inactivation of the treated follicular fluid. The oestrogens of follicular fluid strongly stimulate the secretion of progesterone by rat granulosa cells *in vitro*, although they do not influence its inhibitory effect on morphological luteinization of the cells (Bernard, 1975). This finding, and the data of the present study, lend support to the hypothesis that luteinization may involve regulation of at least two processes, namely progesterone synthesis and morphological changes. It is therefore suggested that there is a 'luteinization inhibitor' (El-Fouly *et al.*, 1970) present in follicular fluid which regulates the DNA-dependent RNA synthesis required to initiate luteinization.

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