Production of prostaglandin F by rabbit granulosa cells and thecal tissue

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Prostaglandin production within the Graafian follicle may be of importance in relation to steroidogenesis and ovulation (see Lindner *et al.*, 1974). In culture, human and rabbit follicles synthesize prostaglandin (PG) F, and this capacity can be stimulated by exogenous gonadotrophins (Plunkett, Moon, Zamecnik & Armstrong, 1975; Moon, Zamecnik & Armstrong, 1974). However, the cell type responsible for this prostaglandin production has not been established. Previous studies showed that granulosa cells from preovulatory follicles of the rabbit secreted PGF when cultured as a monolayer for 10 days (Challis, Erickson & Ryan, 1974), but the activity of thecal tissue was not examined. In the present study we have compared the capacity of granulosa and thecal cells to produce PGF in tissue culture, and have observed the effects on this capacity of exogenous gonadotrophin, arachidonic acid and oestradiol.

Tissue was obtained from non-pregnant sexually mature New Zealand White rabbits (2.0–3.5 kg). The animals were killed by cervical dislocation, and the ovaries removed and placed at 4°C in McCoy's 5a culture medium (Erickson, Challis & Ryan, 1974). Granulosa cells were isolated and cultured in 5 ml McCoy's 5a medium (modified) for 2 days in a humidified 95% air/5% CO₂ incubator at 37°C. The details of this procedure and the composition of the medium have been published previously (Erickson *et al.*, 1974). Each culture contained the granulosa cells from 4 preovulatory Graafian follicles (1000–1200 µm diameter). Thecal tissue from 4 preovulatory follicles was cultured as monolayer sheets of cells. However, as judged by serial sections of isolated pieces of theca it proved impossible to obtain this tissue completely free of granulosa cells. Each set of cultures was incubated as controls, or with the addition of one or more of the following; 2.5 i.u. LH/FSH (Pergonal: Cutter Laboratories, California)/ml; 1 mM-N⁶O^{2'}-dibutyryl adenosine-3':5'-cyclic monophosphoric acid (dbcAMP); 1 µg oestradiol-17β (Steraloids)/ml; 1 µg arachidonic acid (Sigma)/ml. The media were collected after 2 days, and stored at –20°C until analysed for PGF using a previously evaluated procedure (Challis, Davies & Ryan, 1973). Solvent blanks were generally < 10 pg/tube and have not been deducted. The interassay recovery of PGF added to culture medium at a concentration of 1 ng/ml was 1.09 ± 0.18 (S.D.) ng/ml (*n* = 9).

The results are shown in Table 1. The mean concentration of PGF in granulosa cell cultures was increased significantly by the addition of arachidonic acid or oestradiol but not with LH/FSH (*t* test). This amount of added LH/FSH induced luteinization and increased progesterone secretion (Erickson *et al.*, 1974). On a per follicle basis, the granulosa generally produced more PGF than the thecal tissue, and in the cultures treated with arachidonic acid (Group IV) the difference between the tissues was highly significant (*P* < 0.01).

These studies show that granulosa cells and thecal tissue from preovulatory follicles of the rabbit produce PGF in tissue culture, and the increment in PGF produced after addition of arachidonic acid suggests the presence of an active PG synthetase system. In general, the granulosa cells produced more PGF than the theca, even allowing for the possibility that the thecal cultures were contaminated with some granulosa cells. However, PGs of the E series were not measured in this study, and the

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Table 1. Mean $\pm$ S.E.M. concentrations of prostaglandin F in cultures (no. in parenthesis) of rabbit granulosa cells and thecal tissue after 2 days

Group	Addition to medium	Conc. PGF (ng/culture)	
		Granulosa cells	Thecal tissue
I	None (see text)	19 $\pm$ 4 (5)	8 $\pm$ 0.4 (7)
II	LH/FSH	41 $\pm$ 19 (4)	17 $\pm$ 4 (5)
III	dbcAMP	13 $\pm$ 3 (4)	14 $\pm$ 2 (4)
IV	Arachidonic acid	**122 $\pm$ 28 (4)	***32 $\pm$ 7.5 (6)
V	Oestradiol	*38 $\pm$ 5 (6)	12 $\pm$ 3 (4)
VI	Arachidonic acid + oestradiol	67 $\pm$ 31 (4)	58 $\pm$ 23 (3)
VII	Arachidonic acid + oestradiol + LH/FSH	53 $\pm$ 17 (3)	45 $\pm$ 6 (2)

Compared with respective control groups, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

results may not reflect total synthetase activity because the ratio of PGE to PGF is known to vary in the different compartments of the human ovary (Challis *et al.*, 1976).

Ovulation in the rabbit is associated with an increase in the concentration of primary PGs in the preovulatory follicles (Yang, Marsh & LeMaire, 1973; LeMaire, Yang, Behrman & Marsh, 1973) and a peak of oestradiol secretion occurs in the rabbit 90–120 min after coitus (Hilliard & Eaton, 1971), some 3 h before the PG increase (Yang *et al.*, 1973). The present results suggest that the granulosa cells may be a major source of the PG, the production of which could be steroid (oestrogen)-mediated.

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