Lack of an inhibitory influence of oocytes upon luteinization of porcine granulosa cells in culture

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Nalbandov (1972) suggested that the ovum produces a "luteostatic" substance which prevents luteinization of granulosa cells. This suggestion was derived from three lines of observation: (1) surgical ovariectomy *in vivo* induced luteinization and increased the output of progesterone into the ovarian venous blood of rabbits (El-Fouly, Cook, Nekola & Nalbandov, 1970) and pigs (Nalbandov, 1972); (2) rat granulosa cell monolayers cultured with only a few oocytes luteinized, whereas those cultured in the presence of many ova retained their granulosa cell morphology (Nekola & Nalbandov, 1971); and (3) oocytic degeneration on the 3rd day of culture of rat follicles coincided with morphological luteinization of the mural granulosa (Stoklosowa & Nalbandov, 1972).

To test this hypothesis porcine granulosa cells were cultured with and without the addition of cumulus-enclosed porcine oocytes. Granulosa cells and oocytes were collected from medium and large follicles (3–12 mm) of pig ovaries removed at a local slaughterhouse. Within 20–30 min after death the ovaries were excised and placed in 0.9% (w/v) NaCl containing 100 i.u. penicillin/ml, and 100 µg streptomycin/ml and kept at room temperature until culture 3–5 h later. Details of granulosa cell and oocyte collection have been described elsewhere (Channing & Ledwitz-Rigby, 1975; Tsafiriri & Channing, 1975a, b). About 1×10^6 granulosa cells were placed in compartments of Multiwell Tissue Culture plates (Falcon, Oxnard, California) containing glass coverslips and grown in 0.5 ml medium 199 with Earle's salts and 25 mM-Hepes buffer (Grand Island Biological Co., New York) supplemented with 15% pig serum (GIBCO), 1 mM-L-glutamine (GIBCO), 0.03 mM-sodium pyruvate (Eastman Organic Chemicals, Rochester, New York), 2.5 mM-sodium lactate (Sigma Chemical Co., St. Louis, Missouri), 5 µg crystalline bovine zinc insulin (Lilly Laboratories, Indianapolis, Indiana)/ml, and 50 µg Gentamicin (Schering Diagnostics, Port Reading, New Jersey)/ml. The pig serum used had an LH activity (in terms of NIH-oLH-S1 units) of < 2 ng/ml as assayed by a radioligand displacement assay (Channing, 1974). The serum contained 66 pg total oestrogens/ml as measured by radioimmunoassay (Channing & Coudert, 1976). The progesterone content of the serum was 3.8–4.1 ng/ml, using a modification (Channing & Coudert, 1976) of the radioimmunoassay developed by Thorneycroft & Stone (1970). Morphological luteinization and the number of cells in the cultures were assessed as described elsewhere (Channing, 1970a, b). To some of the cultures cumulus-enclosed oocytes (50/culture) and/or ovine LH (NIH-LH-S19; 5 µg/ml) were added. Each treatment combination was tested in eight replicate cultures. At 12-h intervals the medium was changed and progesterone was determined by radioimmunoassay using an antiserum against 11 α -hydroxyprogesterone-BSA conjugate.

The progesterone secretion rate by granulosa cell cultures with and without oocytes and LH during the 12 h intervals is presented in Table 1. To ascertain the effect of oocyte presence and of LH addition on the progesterone secretion rate by the cultures, two-way analysis of variance (Sokal & Rohlf, 1969) was performed. LH increased the rate of progesterone accumulation ($P < 0.001$) measured at 12, 36, 48, 60 and 72 h, whether or not oocytes were present. The presence of oocytes (within their cumuli) caused an unexpected increase rather than a decrease in the rate of progesterone secretion, and morphological luteinization of the granulosa cells was not prevented. Progesterone secretion was increased significantly at 48, 60, 72 and 96 h. The increase in progesterone accumulation in oocyte-rich cultures may have been due to synthesis by the cumulus cells, since rabbit cumulus cells have been shown to luteinize and secrete progesterone in culture (Nicosia & Mikhail, 1975). The present results

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Table 1. Effect (mean $\pm$ S.E.M.) of cumulus-enclosed oocytes (50/culture) and LH (5 μ g/ml) on progesterone secretion (pg/cell/day) by porcine granulosa cell cultures

Time (h)	Controls			LH		
	—Oocytes	+Oocytes	P	—Oocytes	+Oocytes	P
12	73.0 $\pm$ 8.5	89.0 $\pm$ 11.0	N.S.	50.5 $\pm$ 3.0	110.0 $\pm$ 8.0	<0.001
24	83.0 $\pm$ 8.0	102.0 $\pm$ 7.0	<0.05	110.0 $\pm$ 4.0	75.0 $\pm$ 8.0	<0.001
36	168.0 $\pm$ 15.0	143.0 $\pm$ 22.0	N.S.	110.5 $\pm$ 14.0	199.5 $\pm$ 16.0	<0.001
48	703.0 $\pm$ 111.0	924.0 $\pm$ 86.0	<0.01	486.5 $\pm$ 45.0	1140.0 $\pm$ 70.0	<0.001
60	1168.0 $\pm$ 100.7	2593.0 $\pm$ 967.0	<0.001	1044.0 $\pm$ 56.0	1479.5 $\pm$ 117.0	<0.001
72	639.0 $\pm$ 104.0	1050.0 $\pm$ 89.0	<0.001	578.0 $\pm$ 69.0	1105.0 $\pm$ 102.0	<0.001
84	593.0 $\pm$ 101.0	671.0 $\pm$ 116.0	N.S.	543.5 $\pm$ 117.0	120.5 $\pm$ 95.0	N.S.
96	374.0 $\pm$ 61.0	672.0 $\pm$ 96.0	<0.01	439.0 $\pm$ 90.0	581.0 $\pm$ 87.0	N.S.
108	197.0 $\pm$ 35.0	184.5 $\pm$ 41.0	N.S.	155.5 $\pm$ 39.0	226.0 $\pm$ 34.0	N.S.
120	143.0 $\pm$ 31.0	148.0 $\pm$ 30.0	N.S.	85.5 $\pm$ 28.0	205.0 $\pm$ 23.0	<0.01

do not support the view that oocytes from medium- and large-sized follicles exert a luteostatic influence on the surrounding granulosa cells. Under these culture conditions the oocytes stay viable and mature to the first polar body within 48 h (Tsafiriri & Channing, 1975a, b).

Further support for lack of oocyte secretion of a luteinization inhibitor was obtained by Lindner *et al.* (1974) who observed that ovariectomy of explanted rat follicles did not result in an increase of progesterone accumulation in the presence or absence of LH. Nicosia (1972) failed to observe any inhibitory influence of oocytes upon morphological luteinization or progesterone secretion by rabbit granulosa cells. The spontaneous luteinization observed in cultures of preovulatory granulosa cells obtained from equine (Channing, 1969), monkey (Channing, 1970a), porcine (Channing, 1970b), rabbit (Nicosia, 1972) and rat (Fischer & Kahn, 1972) follicles must therefore be explained by a mechanism other than removal of the egg from the granulosa cells. Ledwitz-Rigby *et al.* (1977) have observed that addition of follicular fluid obtained from small- and medium-sized follicles can inhibit spontaneous luteinization of porcine granulosa cells. It is therefore likely that there is a luteinization inhibitor present in follicular fluid which originates from somewhere in the ovary other than the oocyte.

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