Human granulosa-lutein cell in vitro production of progesterone, inhibin A, inhibin B, and activin A are dependent on follicular size and not the presence of the oocyte

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Objective: To investigate inhibin A, inhibin B, activin A, and P production by cultured granulosa cells (GCs) and what relationship this hormone production has to fertility.
Design: Luteinized GCs from individual follicles were cultured, and inhibin A, inhibin B, activin A, and P production were measured by ELISA at 24 and 72 hours.
Setting: Research laboratory and university hospital.
Patient(s): Fifteen women who undertook an IVF-ICSI program, yielding 58 follicles.
Intervention(s): Individual follicular aspiration and preparation of GCs for culture.
Main Outcome Measure(s): Inhibin A, inhibin B, activin A, and P production; oocyte retrieval; and fertility outcome.
Result(s): Inhibin A, inhibin B, and P continued to be secreted by GCs in vitro, and activin A levels were detected only marginally in 56% of cultures. The rate of production also was dependent on the size of follicle from which the GCs originated but not on oocyte presence or ability to fertilize. Granulosa cell stimulation with hCG had no effect on inhibin A but increased P and decreased inhibin B production.
Conclusion(s): A marked effect of luteal differentiation appears to be the inhibition of inhibin B production in response to hCG stimulation. Luteinized GC function, with respect to inhibins, activin A, and P production, was not influenced by the presence or absence of an oocyte and did not correlate with fertility outcome. However, follicle size did influence rates of local hormone production. (Fertil Steril® 2008;89:1406–13. ©2008 by American Society for Reproductive Medicine.)

Key Words: Progesterone, inhibins, activin A, granulosa-lutein cells, follicular maturation

There is a direct interdependence between the oocyte and its surrounding granulosa cells (GCs) throughout oocyte development. Granulosa cell support is fundamental to providing the oocyte with nutrients and growth regulators (1). Conversely, it has been suggested that oocytes promote the proliferation and differentiation of GCs via the secretion of one or more paracrine factors (2–7).

This symbiosis was noted in the 1970s, when viability of the oocyte in vivo was shown to be dependent on the presence of an adequate number of GCs at each stage of follicular maturation (8). In vitro, it has been demonstrated that oocytes cannot develop properly in isolation from their GCs. Thus, GCs have been assessed morphologically, immunohistochemically, and biochemically by examining different variables in an attempt to provide further insight into the dynamics of follicular development (8–12).

Although numerous studies have been performed to investigate GC function by detecting different hormones and cytokines during each stage of follicular development, most experiments were based on cells that were isolated from pooled follicular fluid samples (13–18), which cannot be correlated to specific cell populations harvested from individual follicles for which fertilization rates can be established.

In this study, preovulatory luteinized GCs taken from individual follicles were cultured, and in vitro production of inhibin A, inhibin B, activin A, and P was measured. In addition, their inhibins, activin A, and P secretion responses to hCG stimulation also were examined.

MATERIALS AND METHODS

Subjects

Fifty-eight follicular fluid samples were collected from 15 women who were undergoing controlled ovarian stimulation, for IVF–intracytoplasmic sperm injection (ICSI) at the

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TABLE 1

Descriptive and comparative analysis of inhibin A, inhibin B, P, and activin A productions at basal 24-hour and 72-hour cultures and 72-hour cultures with hCG stimulation.

Parameter	24 h	72 h	72 h + hCG 0.1 IU/L	72 h + hCG 0.5 IU/L
No. of samples	58	58	58	58
Inhibin A				
Median (pg/mL)	19[a]	47[a]	60	67
IQR	14–37	26–111	28–104	38–122
Inhibin B				
Median (pg/mL)	41[b]	57[b,c]	44[c]	33[c]
IQR	23–78	20–122	16–78	16–64
Progesterone				
Median (nmol/L)	880[d]	5,448[d,e]	10,438[e]	10,235[e]
IQR	551–1,682	1,518–10,016	4,210–18,585	4,059–18,119
Activin A				
Median (ng/L)	ND	ND	0.08	0.12
IQR	ND–0.12	ND–0.20	ND–0.14	ND–0.20

Note: All differences assessed by Friedman's two-way analysis, with statistical significance set at $P<.001$. IQR = interquartile range; n = number of samples.

[a] Significant difference between 24-h and 72-h cultures in basal inhibin A production.

[b] Significant difference between 24-h and 72-h cultures in inhibin B production.

[c] Significant difference between hCG-treated and -untreated 72-h cultures in inhibin B production.

[d] Significant difference between 24-h and 72-h cultures in basal P production.

[e] Significant difference between hCG-treated and -untreated 72-h cultures in P production.

Wen. Inhibin, activin, and progesterone production by granulosa-lutein cells. Fertil Steril 2008.

Fertility Centre, Saint Bartholomews' Hospital (London, UK). All couples who were undergoing treatment for tubal or male-factor infertility, endometriosis, polycystic ovary syndrome, and other ovulatory disorders were excluded. The ages of patients varied from 29 to 38 years; their follicular diameters varied from 11.2 mm to 35 mm. Serum levels of LH and FSH were determined on day 3 of the treatment cycle. Written consent was obtained from each patient, and the study was approved by the East London and The City Health Authority Research Ethics Committee.

Ovarian Stimulation and Follicular Fluid Aspiration

Multiple follicular development was stimulated with SC injections of recombinant FSH (Puregon; Organon, Cambridge, Cambridgeshire, UK) and highly purified urinary FSH (Metrodin; Serono, Welwyn Garden City, Hertfordshire, UK) after a long GnRH agonist stimulation protocol ranging from 10–14 days and monitored by transvaginal ultrasound measurements. Both dose and the duration of ovarian stimulation were dependent on the serum E_2 level and the ultrasound scan result; no significant difference was observed among all patients. Follicular fluid aspiration followed the protocol, as described elsewhere (19).

Isolating and Harvesting Granulosa-Lutein Cells From Individual Follicles

Cultures were established from follicular fluid samples that were collected as described elsewhere (19). After oocyte retrieval, they were transported immediately to the research laboratory, where each sample was processed by centrifuge at 200 × *g* for 10 minutes. The cell pellet was resuspended in 4 mL of medium (RPMI-1640 with glutamine [2 mM] and $NaHCO_3$ [2.5 g/L]; Sigma, Poole, Dorset, UK) supplemented with 10% fetal calf serum (GibcoBRL, Paisley, UK) and 1% (vol/vol) antibiotic containing 10,000 μg/mL of penicillin G sodium and 25 μg/mL of streptomycin (GibcoBRL) and again was processed by centrifuge at 200 × *g* for 10 minutes.

The pellet was then resuspended in 5 mL of medium and then incubated at 37°C for 30 minutes with 0.2% hyaluronidase (80 IU/mL; Medicult, Surrey, UK) for cell dispersion. After this period, the sample again was processed by centrifuge at 200 × *g* for 10 minutes, and the pellet was resuspended with 2 mL of medium and layered over 4 mL of 50% Percoll (Pharmacia, Amersham, UK). This was processed by centrifuge at 250 × *g* for 20 minutes to separate the luteinized granulosa-lutein cell buffy coat from the red blood cells. The cells were removed with a pipette and washed with 8 mL of medium (RPMI-1640). After a final centrifugation at 250 × *g* for 10 minutes, the pellet was resuspended, and the cell number was determined with a hemocytometer. Cell viability was assessed by trypan blue exclusion (mean ± SD, 91.5% ± 7.0%).

Cell Culture

Cells then were plated in triplicate wells, at a concentration of 10,000 cells per well, in 24-well plates (Corning, Corning,