ANIMAL EXPERIMENTATION

In Vitro Stimulation of Cumulus-Cell Expansion by Human Cord Serum in Mouse Oocyte-Cumulus Complexes

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Purpose: The aim of this study was to test whether human cord serum (HCS) containing gonadotropins has an effect on the expansion of oocyte-cumulus complexes (OCCs).

Methods: The concentration of follicle stimulating hormone (FSH) and luteinizing hormone (LH) was measured in HCS by radioimmunoassay (RIA). After short-term culture (4 hr) with or without OCCs, medium containing 0.4% bovine serum albumin (BSA) as control or 10% HCS was collected and analyzed for its concentration of estradiol, progesterone, and testosterone.

Results: The FSH concentration was at the basal level, but the LH level was as high as 142.4 mIU/ml in both natures and denatured serum. Undetectable levels of steroids were observed in control media with or without OCCs. In contrast, a moderate amount of steroid hormones was detected in culture medium containing HCS. OCCs secreted a minute amount of steroid hormones in response to HCS. Similar patterns of cumulus expansion were observable by treatment with HCS, human chorionic gonadotropin (hCG), or HCS plus hCG after 4, 8, or 22 hr of culture. However, no cumulus expansion was observed in controls.

Conclusion: These results suggest that LH in HCS induces cumulus expansion but does not affect the secretion of steroid hormones by OCCs during culture.

KEY WORDS: cumulus expansion; human cord serum; oocyte-cumulus complex; steroid hormones.

INTRODUCTION

The culture of mouse embryos from the zygote to the blastocyst has been used for quality control of culture reagents in human in vitro fertilization (IVF) and embryo transfer (1–7). The mouse embryo culture system has several difficulties, however, including failure of ovulation or fertilization in vivo after ovarian hyperstimulation, failure of mating, and interruption of cleavage by the two-cell-stage block (8–10). Moreover, it is hard to control the quality of the culture medium, serum, and apparatuses in mouse embryo culture system.

It has recently been suggested that the existing embryo culture system can be replaced by in vitro culture of mouse oocyte-cumulus complexes (OCCs), as a means of quality control (11). The quality of the cumulus is often cited as a major criterion in choosing oocytes for fertilization in domestic animals and human IVF protocols, with particular emphasis on the degree of cumulus expansion (12–14). It has also been demonstrated that cumulus cell viability and completion of the first meiotic division are improved by fetal calf serum in bovine and hamster OCCs (15).

Because there are no studies or systematic research about the effect of human cord serum on OCCs, this study was designed to assess whether gonadotropins and steroid hormones in human cord serum may influence cumulus expansion in culture OCCs.

MATERIALS AND METHODS

Collection of Mouse OCCs

ICR female mice 3 to 4 weeks of age were used in this study. At 48 hr after intraperitoneal injection with

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5 IU pregnant mare serum gonadotropin (PMSG; Sigma Chemical Company, St. Louis, MO) to induce follicular growth, mice were sacrificed by cervical dislocation, and ovaries transferred to culture media. After being washed two or three times, the ovaries were placed under a dissecting microscope, and OCCs in the large graafian follicles were collected by puncturing them with a fine needle.

Culture Medium

Ham's F-10 solution (GIBCO, Grand Island, NY) was used as a basic culture medium, which was made of 1 L of HPLC water (Baxter, Muskegon, MI) with Ham's F-10 powder supplemented with penicillin G, 0.075 g (Sigma); streptomycin, 0.075 g (Sigma); calcium lactate, 0.2452 g (Calbiochem, La Jolla, CA); and NaHCO₃, 1.2 g (Sigma). Medium osmolarity was controlled in the range of 280 mOsm/kg. Media were stored at 4°C after filter sterilization (0.22- μ m; Corning, New York). Media were equilibrated by incubation under 5% CO₂, 95% air at 37°C for 15 hr before use.

Human Cord Serum (HCS)

Blood was collected from umbilical veins using disposable syringes after normal delivery. After samples were tested for HIV, hepatitis B, and cytomegalovirus, it was centrifuged at 1000 rpm for 30 min and filter-sterilized with a 0.22- μ m Sartorius (Germany). One milliliter of the serum was kept frozen at -70°C until radioimmunoassay (RIA). Remaining sera were heat-inactivated for 1 hr at 56°C and then kept frozen until used. Each individual sample was kept separately for the assay.

Culture of OCCs

OCCs were collected by capillary micropipette from graafian follicles and washed with the basic medium three or four times. OCCs were cultured in a four-well Multidish (Nunc, Denmark) in the culture medium supplemented either with 0.4% bovine serum albumin (BSA) or with 10% HCS. Twenty-five immature OCCs with compact cumulus cells were cultured in each. In a separate experiment, OCCs were cultured in the presence of human chorionic gonadotropin (hCG; 10 IU) or hCG plus HCS. OCCs were cultured in 50 μ l of medium in a culture dish covered with oil at 37°C in a humid atmosphere of 5% CO₂ and 95% air for the indicated time intervals.

Hormone Assay

Before and after heat inactivation, the levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in human cord serum were measured by RIA kit (Amerlex-M, USA). The concentrations of estradiol, progesterone, and testosterone in HCS and media were measured by conventional RIA technique as described previously (16, 17). Antibodies for estradiol, progesterone, and testosterone were gifts from Dr. Y.-D. Yoon (Han Yang University, Seoul).

Morphological Assessment of Cumulus Expansion and Oocyte Maturation

After culture, the degree of cumulus expansion was examined by microscopic visualization (18) and classified into three categories (i) closely packed compact cells (-), (ii) less compact cells (;pl), and (iii) expanded cells located radially away from the oocyte (pl;pl). The nuclear state of the oocyte was also studied. After the culture period in each medium, the oocytes were denuded of surrounding cumulus cells by repeated pipetting. The denuded oocyte was fixed and stained as described previously (19).

Statistical Analysis

One-way analysis of variance (ANOVA) followed by the multiple-comparison method was used to analyze the data. A *P* value of <0.05 is considered statistically significant.

RESULTS

Gonadotropin and Steroid Hormone Concentrations in HCS

As shown in Table I, levels of FSH were very low in HCS. However, levels of LH were comparatively high. Heat inactivation did not affect gonadotropin levels.

As shown in Table II, concentrations of progesterone were much higher than those of estradiol and testosterone irrespective of heat inactivation.

Effect of HCS on Steroid Production

To test whether LH in HCS affects steroid production by OCCs, OCCs were cultured in the presence of HCS. Estradiol, progesterone, and testosterone concentrations were essentially undetectable in OCCs cul-

Table I. Concentrations of Gonadotropins in Human Cord Serum

Gonadotropin	No. of individuals	Natured (mIU/ml) ^a	Denatured (mIU/ml) ^b	P value ^c
LH	18	140.4 ± 9.1	142.4 ± 8.7	0.521
FSH	18	2.1 ± 0.1	1.5 ± 0.1	0.491

^aEach individual sample was centrifuged at 100 rpm for 30 min. One milliliter of the serum was kept frozen at -70°C without heat inactivation.

^bSerum was heat-inactivated for 1 hr at 56°C.

^cOne-way ANOVA followed by the multiple-comparison method.

Table II. Concentrations of Sex Steroids in Human Cord Serum

Steroid	No. of individuals	Natured (ng/ml) ^a	Denatured (ng/ml) ^b	P value ^c
Estradiol	18	9.76 ± 0.56	8.42 ± 0.560	0.0997
Progesterone	18	146.14 ± 4.7	138.78 ± 4.58	0.257
Testosterone	18	0.69 ± 0.02	0.65 ± 0.02	0.171

^aEach individual sample was centrifuged at 100 rpm for 30 min. One milliliter of the serum was kept frozen at -70°C without heat inactivation.

^bSerum was heat-inactivated for 1 hr at 56°C.

^cOne-way ANOVA followed by the multiple-comparison method

tured in medium containing BSA as shown in Table III. However, levels of estradiol, progesterone, and testosterone in HCS cultured with or without OCCs were quite high. A minute increase in steroid hormone levels was observed in HCS cultured with OCCs compared to the levels in HCS cultured without OCCs.

Cumulus Expansion and Oocyte Maturation by HCS

In the control OCCs cultured in BSA, there was no expansion of OCCs in any culture period (Table IV and Fig. 2), similar to OCCs before culture (Fig. 1). However, cumulus cell expansions of OCCs appeared to occur partially in the group treated with 10 IU hCG 8 hr after culture (Fig. 3), and 90.6% were expanded 22 hr after culture (Table IV and Fig. 4).

OCCs that were cultured for 8 hr in the presence of HCS showed a partial expansion of the cumulus similar to that observed in hCG (Fig. 5). A marked cumulus expansion could be seen after 22 hr of culture in the presence of HCS (77.5%; Table IV and Fig. 6). Co-treatment with HCS and hCG showed no additive

Table III. Concentrations of Sex Steroids After 4 hr of Culture in Two Media Containing BSA or HCS

Culture medium ± OCC ^a	No. of cultures	Estradiol (pg/ml)	Progesterone (pg/ml)	Testosterone (pg/ml)
Ham's F-10 + 0.4% BSA ^b	6	—	—	—
Ham's F-10 + 0.4% BSA +OCC	6	—	—	—
Ham's F-10 + 10% HCS ^c	6	117.1 ± 11.2	3507 ± 560.3	29.0 ± 9.7
Ham's F-10 + 10% HCS + OCC	6	124.1 ± 12.4	3948 ± 820.1	31.2 ± 11.9

^aWith or without 25 OOCs cultured in the medium.

^bOCCs cultured in Ham's F-10 supplemented with 0.4% BSA.

^cOCCs cultured in Ham's F-10 supplemented with 10% HCS.

Values are means ± SE.

Table IV. Cumulus Expansion And Oocyte Maturation of OCCs in Four Media

Culture medium	No. of cultures	Culture time											
		4 hr				8 hr				22 hr			
		Expansion (%)				Expansion (%)				Expansion (%)			
		—	+	++	% MI ^b	—	+	++	% MI	—	+	++	% MI ^c
Ham's F-10 + 0.4% BSA	6	100	0	0	100	100	0	0	100	100	0	0	50.3 ± 28
Ham's F-10 + 10% HCS	6	100	0	0	100	79.4 ± 40 ^a	20.6 ± 40	0	100	0	22.5 ± 18.3	77.5 ± 18.3	54.6 ± 23.4
Ham's F-10 + 10IU hCG	6	100	0	0	100	0	100	0	100	0	9.4 ± 13.9	90.6 ± 14.4	45.0 ± 23.3
Ham's F-10 + 10% HCS + 10IU hCG	6	100	0	0	100	5.5 ± 13.4	94.5 ± 13.4	0	100	0	2.5 ± 3.8	97.5 ± 3.5	50.5 ± 17.9

^aValues are means ± SEM.

^bPercentage metaphase I oocytes.

^cPercentage metaphase II oocytes.

Fig. 1. OCCs (prophase I) with a germinal vesicle and compact cumulus cells before culture. Original magnification, $\times 200$.

Fig. 2. OCCs cultured in Ham's F-10 supplemented with 0.4% BSA for 22 hr showing no cumulus expansion. Original magnification, $\times 200$.

Fig. 3. OCCs cultured in Ham's F-10 supplemented with 10 IU hCG for 8 hr showing partial cumulus expansion. Original magnification, $\times 200$.

effect on cumulus expansion (Figs. 7 and 8). However, as shown in Table IV, there was no difference in oocyte maturation among different groups, and the first polar body appeared in the range of 45–54.6% after 22 hr of culture.

DISCUSSION

The oocyte of mammalian graafian follicle is surrounded by several layers of compact cumulus cells, known as the oocyte–cumulus complex. The cumulus

Fig. 4. OCCs cultured in Ham's F-10 supplemented with 10 IU hCG for 22 hr showing marked expansion of the cumulus. Original magnification, $\times 200$.

Fig. 5. OCCs cultured in Ham's F-10 supplemented with 10% HCS for 8 hr showing partial cumulus expansion. Original magnification, $\times 200$.

cells are interconnected by an extensional network of gap junctions (20–22), which function in the mediation of nutritional support, electric coupling (23), and transport of messenger molecules from follicle cells to

the oocyte. The LH surge induces the loss of gap junctions, resulting in the resumption of oocyte meiotic maturation by reducing the transport of meiosis inhibitory substances from the somatic to the germinal

Fig. 6. OCCs cultured in Ham's F-10 supplemented with 10% HCS for 22 hr showing marked expansion of the cumulus. Original magnification, $\times 200$.

Fig. 7. OCCs cultured in Ham's F-10 supplemented with 10% HCS plus 10 IU hCG for 8 hr showing partial cumulus expansion. Original magnification, $\times 200$.

Fig. 8. OCCs cultured in Ham's F-10 supplemented with 10% HCS plus 10 IU hCG for 22 hr showing complete cumulus expansion. Original magnification, $\times 200$.

compartment of the follicle (21, 24–29). In addition, the cumulus cell secretes hyaluronic acid, which causes expansion (30–36), emphasizing the role of cumulus expansion in the subsequent development of the oocyte.

This study demonstrates that HCS can induce cumulus expansion of mouse OCCs cultured in vitro. Because HCS contains high levels of LH and steroid hormones, the effect of HCS on expansion might be due to the presence of these hormones. However, HCS did not stimulate steroid production by OCCs, probably due to the saturation levels of steroids present in HCS. Schuetz *et al.* (36), however, have demonstrated that treatment with HCS stimulates OCC production of steroids, suggesting that steroid hormones are secreted from OCCs by the stimulation of LH in HCS.

The pattern of cumulus-cell expansion induced by hCG treatment was basically similar to that with HCS, and somewhat more remarkable expansion was observed in the medium supplemented with HCS and hCG together ($P < 0.01$). These results might indicate that LH in HCS is a major factor that causes cumulus-cell expansion.

In the present study, oocyte maturation occurred in OCCs cultured either with or without HCS or hCG. It is well-known that gonadotropins induce oocyte maturation (37, 38), but the reason for the induction of maturation without gonadotropins in the present study is presently unknown.

In summary, the present study demonstrates that LH in HCS induces cumulus expansion but does not affect the secretion of steroid hormones by OCCs during culture. Based on the present findings, it is possible that culturing conditions before and at the time of fertilization as well as for the normal embryo development after fertilization could be anticipated by measuring the level of oocyte maturation and cumulus expansion. Therefore culturing OCCs with HCS might provide a model quality-control system for IVF.

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