PHYSIOLOGY

Are Cumulus Cells Necessary for the Spontaneous Maturation of Germinal Vesicle-Stage Oocytes to Metaphase II

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Purpose: In this study we investigated the need of the support from cumulus cells for germinal-vesicle (GV) oocytes collected from stimulated ovaries to complete their maturation to metaphase II (MII).

Methods: We compared the maturation rate of GV oocytes after coculture with cumulus cells (study group) with their spontaneous maturation in culture medium alone (control group).

Results: Sixty-four and nine-tenths percent of the GV oocytes matured to metaphase II in the coculture group, and of these, 43.5% gave normal 2pn zygotes following intracytoplasmic sperm injection (ICSI), while 73.8% of the GV oocytes spontaneously matured to the MII stage and 30% of these reached the zygote stage after ICSI.

Conclusions: It is probable that a follicular factor is responsible for this arrested maturation in the human and that maturation occurs spontaneously when the oocytes are separated from their follicular fluid environment after collection.

KEY WORDS: in vitro maturation; germinal-vesicle oocytes; intracytoplasmic sperm injection; meiosis inhibitory factor.

INTRODUCTION

After hormonal stimulation, up to 10% of oocytes in programs of intracytoplasmic sperm injection (ICSI) remain arrested at the germinal-vesicle (GV) stage despite exposure to human menopausal gonadotropin or follicle stimulating hormone and human chorionic

gonadotropin (hCG) (1–3). The reason for this arrested maturation in this subpopulation of oocytes is unknown. Although the chromosomal status of in vitro matured oocytes is similar to those matured in vivo (4), little is known about the maturation capacity or viability of these immature human oocytes in vitro. They can be matured in vitro with the aid of hormone-supplemented media (5,6), by means of coculture with Vero cells (7) or granulosa and cumulus cells (1–3). Coculture with granulosa or cumulus cells offers several theoretical advantages. These cells produce pyruvate, steroid hormones, and growth factors such as TGF- α and - β (3,8). Several clinical pregnancies and births have been reported in the literature with these different systems of in vitro maturation after oocyte collection in hormone-stimulated cycles (1,2,5,6). However, there is a lack of controlled studies on the requirements of these immature oocytes for completing their maturation. Various authors have observed a spontaneous maturation capacity of immature mammalian and human oocytes in the past (9,10). In this study, we posed the question whether GV-stage oocytes, collected after hormonal stimulation, need the support of cumulus cells to complete their maturation from GV to metaphase II (MII).

MATERIALS AND METHODS

In our routine ICSI program oocytes were collected by ultrasound-guided vaginal aspiration 34 to 36 hours after hCG. The cumulus cells were removed mechanically in all oocytes 2 to 4 hr after oocyte collection. The corona cells were then removed in modified Earle's medium supplemented by 60 IU/ml of hyaluronidase

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(Type VIII; Sigma; sp act, 320 IU/mg). The nuclear status was subsequently recorded. The GV oocytes were, after the removal of cumulus and corona cells, divided prospectively into two groups for in vitro maturation.

If two or more oocytes from the same patient were at the GV stage, they were cocultured in Earle's medium (components: calcium chloride.H₂O, 0.265 g/L; magnesium sulfate, 0.09767 g/L; potassium chloride, 0.4 g/L; sodium chloride, 6.8 g/L; sodium phosphate, monobasic, 0.122 g/L; D-glucose, 1.0 g/L; Phenol red.Na, 0.011 g/L) together with 10% heat-inactivated patient's serum from blood collected 3 to 4 days before oocyte collection in an incubator at 37°C in an atmosphere of 5% CO₂, 5% O₂, and 90% N₂ in Falcon tubes containing 1 ml of medium with the initially separated cumulus cells in suspension (coculture group).

If only one oocyte in the same patient was at the GV stage, this oocyte was cultured in modified Earle's medium with 10% heat-inactivated patient's serum alone. These oocytes then served as a control group (Earle's medium alone).

Nuclear maturation was recorded after 24, 48, and 72 hr. If no maturation occurred after 72 hr, the oocytes were discarded.

When the oocytes in the coculture group reached the MII stage, they were injected with preserved or fresh husbands' sperm by a standard ICSI procedure as described by Palermo *et al.* (11).

Twenty-four hours after the ICSI procedure, the oocytes were observed and pn recorded and all oocytes were cryopreserved by a rapid PROH procedure if a fresh transfer occurred in this cycle. If no transfer occurred, the embryos from in vitro matured oocytes were transferred at the two- to four-cell stage, 2 days after collection. We recorded the number of oocytes at the germinal vesicle stage, the number of matured metaphase II oocytes, their activation rate, and the number of cryopreserved zygotes.

RESULTS

From October 1996 to December 1997, 131 oocytes were submitted to in vitro maturation in the coculture group and 42 in the group with Earle's medium alone. The results are shown in Table I. Of 131 GV oocytes, 85 (64.9%) matured to MII in the coculture group, and 31 of 42 (73.8%) in the control group. There was no statistical evidence of a difference in the maturation success rates for the two groups of patients ($P = 0.347$

Table I. Results of In Vitro Maturation of GV Oocytes by Coculture with Cumulus Cells and Culture Medium Alone

Group	No. GV oocytes	No. MII oocytes	No. 2pn
Coculture	131	85 (64.9%)*	30 (43.5%)
Control group (medium alone)	42	31 (73.8%) (n.s.)	10 (32.3%)

* $P = 0.347$, Fisher's exact test.

by Fisher's exact test). Thus the ratio of maturation rates was estimated as 1.137 in favor of the control group, with 95% confidence limits on this ratio estimated as 0.867 and 1.393, limits which, of course, embrace the null hypothesis value of 1.0. Thirty oocytes (43.5%) were successfully activated by ICSI in the coculture group. A similar activation rate by ICSI was observed in the spontaneous group, 10 of 29 (30%). All but two zygotes from the coculture group were cryopreserved. Both zygotes developed to morphologically grade I four-cell embryos and were transferred at day 4 after collection because the patient had no prior transfer from initially fertilized MII oocytes. A clinical pregnancy occurred but unfortunately ended in an abortion after 9 weeks of gestation.

DISCUSSION

The reason that a population of human oocytes remains at the GV stage after hormonal stimulation is unknown. They can, however, be matured in vitro by hormone-supplemented media or by coculture. Pregnancies have been described after transfer of in vitro matured oocytes that had been fertilized with ICSI. Spontaneous in vitro maturation in mammals was first observed in the rabbit by Pincus over 50 years ago (9). The primary oocyte resumed meiosis spontaneously when liberated from its follicle and placed in a suitable culture medium alone. In this study we found no statistical difference between the spontaneous maturation rate in the absence of cumulus cells and the maturation rate of human oocytes cocultured with cumulus cells. The spontaneous maturation rate without cumulus cells is the same as that reported in the literature for the various other maturation systems. It would be interesting to study the time delay from hCG to the expulsion of the second polar body. This parameter should reflect the velocity of the meiotic cell cycle. As in the case for the faster-dividing embryos, faster meiotic cycles may indicate developmental competence. The success

rate of activation of these in vitro matured oocytes after intracytoplasmic injection seems to be lower than that of fresh MII oocytes. One explanation for this may be that, in about 50% of the cases, we used 2-day-old preserved sperm with nearly complete loss of any motility for the injection, which reduces the success rate.

It is probable that a follicular fluid factor is responsible for the maturation arrest in the human oocyte and that maturation occurs spontaneously after separation of the oocytes from their follicular fluid after oocyte collection. We do not know what this meiosis inhibitory factor is in the human, but a likely candidate is hypoxanthine as found in the mouse (12).

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