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The Effects of cumulus cells on in vitro maturation of mouse germinal vesicle stage oocytes

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Abstract

Background: In vitro maturation (IVM) of oocytes is a promising technique to reduce the costs and avert the side-effects of gonadotropin stimulation for in vitro fertilization (IVF). The pregnancy rates from oocytes matured in vitro are much lower than those of in vivo stimulation cycles, indicating that optimization of IVM remains a challenge.

Objective: In this study, we investigated the effect of cumulus cells on maturation and fertilization rate of immature oocytes (Germinal vesicle).

Materials and Methods: Germinal vesicle (GV) oocytes were recovered from 6-8 weeks old Balb C female mice 48hr after injection of 10 IU pregnant mare serum gonadotropin (PMSG). Collected oocytes were divided into two groups. Group A: GV oocytes without cumulus (denuded oocyte). Group B: GV oocytes with cumulus cells (cumulus-oocyte complex). The oocytes in both groups were cultured in TCM-199 medium in a humidified atmosphere of 5% CO_2 in air at 37°C. The maturation, fertilization and developmental rates were recorded after 24hr.

Results: Maturation, fertilization and developmental rates in denuded oocytes (DO) were 65.1%, 68.02%, 78.63% respectively, and in cumulus-oocyte complex (COC) were 78.20%, 85.57% and 85.05%, respectively. The maturation, fertilization and developmental rates of COC were significantly higher than those of DO ($p<0.05$).

Conclusion: The results show that cumulus cells have beneficial effects on maturation, fertilization and cleavage rates of mice oocytes.

***Key Words**: Immature oocyte, In vitro maturation, Mouse, Cumulus cells*

Introduction

In vitro maturation (IVM) of mammalian oocytes has become an efficient method to produce mature oocytes in order to use in assisted reproductive techniques such as In Vitro Fertilization (IVF), Intra cytoplasmic sperm injection (ICSI), and cloning. Induction of ovulation to obtain mature oocytes for IVF has become a routine procedure in many infertility clinics. Some women, however, rather fail to respond to the hormonal stimulation or are at risk of ovarian hyperstimulation (1). In vitro maturation of oocytes offers an alternative to obtain mature oocytes in these cases (2, 3). Immature human oocytes exhibit acceptable meiotic competence to metaphase II (MII), but their subsequent developmental competence remains disappointingly low. Only 40–80% of fertilized IVM oocytes progress through early cleavage, and of those that do cleave and are transferred, 15% implant to form a viable fetus (3-6).

Oocyte maturation is often conceptually divided into nuclear and cytoplasmic processes. Nuclear maturation is a term that refers to the resumption of meiosis from the germinal vesicle (GV) stage and progression to MII. Cytoplasmic maturation is a more general term that refers to other maturational events (not directly related to meiotic progression) that prepares the oocyte for fertilization and preimplantation development (7, 8).

Within the follicle, the regulation of oocyte development and cellular function is dependent on gap junctional communication, paracrine communication and interaction with elements of extracellular matrix (9-11). Cumulus cells play an

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important role on oocyte maturation, since they provide and transfer several known and unknown factors that are essential for normal nuclear and cytoplasmic maturation of oocytes and subsequent embryonic development after fertilization (12-14). Moreover, cumulus cells also are known to play an important role in regulation of meiotic progression of oocytes (15). During the growth and accomplishment of meiotic competence of oocytes (before initiation of meiosis), cumulus cells are responsible for maintenance of nuclear arrest at GV stage via elevating intercellular cAMP level in the oocytes by transferring an inhibitor signal through gap junctions (16, 17). Initiation of meiosis is also related to cumulus-function, as there are evidences that cumulus cells secrete a meiosis-inducing factor (18).

According to the mentioned data, this study was proposed to investigate the effects of cumulus cells on in vitro maturation, fertilization and developmental rates of mouse GV stage oocytes.

Materials and Methods

Collection of GV oocytes

Oocytes were obtained from 6-8 weeks old Blab C female mice. The animal were kept under controlled condition (12hr light, 12hr dark) and fed with water and pellets ad libitum. The mice were stimulated by an i.p. injection of 10 IU pregnant mare serum gonadotropin (PMSG). The animals were killed 44hr later by cervical dislocation and the ovaries were removed into TCM-199 (Sigma) supplemented with 10% fetal bovine serum (FBS). The GV-stage oocytes of ovarian follicle were released by puncturing with a 28G sterile needle under a stereomicroscope. A total of 654 oocytes were obtained from 30 ovaries and they were used for in vitro maturation. The average number of collected oocytes was 21.8 per ovary.

In Vitro Maturation (IVM)

The collected GV-stage oocytes were divided into two groups: Group I: denuded oocytes (DO) (Figure1A) and Group II cumulus oocyte complexes (COC) (Figure1B). Each group were placed in 25µl micro drops TCM-199 supplemented with 10% FBS, 0.23 mM sodium pyrouvate, 50mg/l streptomycin, 60 mg/l penicillin and 1µg/l epidermal growth factor (EGF), over laid with embryo-tested light mineral oil (Sigma) for 24hr in a humidified atmosphere of 5% CO2 at 37ºC. At various intervals from the onset of incubation, oocytes were observed by inverted microscopy and morphological changes in the nucleus or the extrusion of first polar body (MII) were used as the criterion for nuclear maturation of GV-stage oocytes. After IVM, oocytes in group II were exposed to 3 mg/ml hyaluronidase in TCM199 and cumulus cell were removed by repeated pipetting and the matured oocytes were collected for in vitro fertilization.

A



B



Figure 1. In vitro maturation, fertilization, and cleavage of mouse germinal vesicle (GV) oocytes. (**A**) Cumulus oocyte complex. The attached cumulus–corona cells can be seen. (**B**). magnification: ×320: B×200. Bar=32 µm.

In Vitro Fertilization (IVF)

Sperms were collected from epididymes of blab C male mice aged 12 weeks. The sperm suspension (1 $\times 10^6$ motile spermatozoa/ml) was capacitated for 2hr in one ml T6 media supplemented with 16 mg/ml BSA. The in vitro matured MII stage oocytes from each treatment group were placed into 0.9 ml T6 and 0.1 ml capacitated spermatozoa was added to it. After 5hr incubation, the oocytes were washed through three droplets of T_6 medium. Then, the oocytes were

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cultured in a droplet of T_6 (25µl) under mineral oil. They were assessed for cleavage to the 2-cells stage after 24hr.

Statistical analysis

Collected data were analyzed by chi-square test. The differences in the values of maturation, fertilization and developmental rates, were considered significant when $p<0.05$.

Results

In vitro maturation of mouse oocytes

Table I shows the number of oocytes arrived to MII stage (Figure 2C) after 24hr culture. The maturation rate of COC was significantly higher than that of DO ($p<0.05$).



Figure 2C. Denuded GV stage oocyte. magnification: ×320: B×200. Bar=32 µm.

In vitro fertilization and development of mouse oocytes

As shown in Table II, the number of fertilized oocytes (Figure 3D) were significantly higher in COC than those of DO ($p<0.05$).But the difference between the number of fertilized oocytes which developed to 2-cells stage (Figure 3E) was not significant ($p>0.05$) in two groups.

D



E



Figure 3D&E. Completion of the second meiotic division (MII) after 24h culture. **(D).** A fertilized 2-pronuclear zygote (**E**). A cleaved embryo at 24h post fertilization. All pictures taken under Hoffman contrast optics. Original magnification: ×320: B×200. Bar=32 µm.

Table I: Maturation rate of mouse oocytes after 24hr culture. DO = Denuded oocyte, COC = Cumulus oocyte complex, GV= Germinal vesicle oocyte. GVBD= Germinal vesicle breakdown, MII=metaphase II. Significant differences between DO and COC are indicated by an asterisk ($p<0.05$).

Group	No. of GV stage oocytes	Final stage oocyte maturation		
		GVBD (%)	MII (%)	Degeneration (%)
A (DO)	264	25 (9.5)	172 (65.1)	67 (27.4)
B (COC)	390	24 (6.1)	305 (78.2)	61 (15.7)

Table II: Fertilization and developmental rates of mouse oocytes. Significant differences between DO and COC are indicated by an asterisk ($P<0.05$).

Group	Matured (MII) oocytes	Fertilized Oocytes (%)	Degenerated Oocytes (%)	2-cell stage Embryos (%)	Degenerated Embryos (%)
A (DO)	172	117 (68.02)	55(31.97)	92 (78.63)	25(31.97)
B (COC)	305	261(85.57)	44(14.42)	222(85.05)	39(14.95)

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Discussion

Cumulus cells have been considered to play an important role in oocyte maturation by keeping the oocyte under meiotic arrest, inducing meiotic resumption and by supporting cytoplasmic maturation. These functions have been attributed to their gap junctions and their specific metabolizing capabilities (12-15). Physical contact between oocyte and cumulus cells has been considered necessary for the transfer of nutrients and factors essential for oocyte development (16-19). In addition, dissociated cumulus cells have been reported to produce paracrine factors, which resume meiosis in denuded oocytes (20). The beneficial effects of cumulus cells on oocyte maturation and early development were reported in different species (12, 14, 19-23). Our study confirmed these mentioned results and demonstrated that maturation and fertilization rates were significant by higher in cumulus-oocyte complex compared with denuded oocytes ($p<0.05$). But there was no significant difference in cleavage rates. However, the obtained data in this study are different whit some other published results. For example Nishi *et al* (2003) investigated on the mice GV oocytes and reported that maturation, fertilization and developmental rates of DO were 64.4, 51.7 and 43.2%, and cumulus-oocyte complex (COC) were 76.4, 70.5 and 50.5%, respectively. The maturation rate (MII) and fertilization rate (2PN) of COC was significantly higher than that of DO ($p<0.05$) (25). On the other hand, Aono *et al* (2003) investigated on murine GV oocyte and reported that the maturation (MII) and cleavage rate (2-cell) of COC were 90.2%, 94.1% respectively (25). In our study, these amounts are higher than Nishi *et al* investigation in both COC and DO, and lower than that of Aono *et al*. This difference maybe is due to the animal, oocyte collection method and medium that used.

Conclusion

In conclusion, our study indicates that in vitro maturation and fertilization and cleavage rates of mouse cumulus-oocyte complex (COC) are more successful than denuded oocytes. Further studies should explore the factors in cumulus cells that are responsible for these results. We think by exploring and extracting these factors and using them in culture media, oocytes maturation and embryonic preimplantation development in vitro can be improved.

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