Original Article

Cumulus Cell Role on Mouse Germinal Vesicle Oocyte Maturation, Fertilization, and Subsequent Embryo Development to Blastocyst Stage *In Vitro*

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Abstract

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Objective: The purpose of this study is to investigate the effect of cumulus cells on maturation, fertilization and subsequent development of mouse germinal vesicle oocytes.

Materials and Methods: A total of 470 germinal vesicle (GV) oocytes were obtained from 26 ovaries of 3- 4 week old ICR female mice 48 hours after injection of 5 IU pregnant mare serum gonadotropin (PMSG). Collected oocytes were divided into two groups; group I: GV oocytes without cumulus cells (denuded oocyte), group II: GV oocytes with cumulus cells (cumulus-oocyte complex). The oocytes in both groups were cultured in TCM-199 medium supplemented with 10% fetal bovine serum (FBS) for 22- 24 hours in a humidified atmosphere of 5% CO_2 in air at 37°C. Oocyte maturation was scored under inverted microscope. To do in vitro fertilization, matured oocytes from each group were placed in T6 medium and capacitated spermatozoa were added. Then the fertilized oocytes were cultured and assessed for cleavage to the 2-cell stage 24 hours and production of blastocyst 120 hours after fertilization. Data was analyzed by chi-square test and differences in the values were considerable significant when $p<0.05$.

Results: Maturation, fertilization, cleavage and blastocyst rates in denuded oocytes were: 76.32%, 57.49%, 51.15% and 19.14% respectively. In the cumulus-oocyte complex rates were: 89.41%, 80.76%, 75.58% and 45.62% respectively; all in the cumulus-oocyte complex were significantly higher than those of denuded oocytes ($p<0.05$).

Conclusion: The present study indicates that cumulus cells have important role during maturation, fertilization and subsequent embryo development to the blastocyst stage.

Keywords: Oocytes Maturation, Cumulus Cells, *In Vitro* Fertilization

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Introduction

In vitro maturation (IVM) of germinal vesicle (GV) stage oocytes could potentially provide increased numbers of oocytes for *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), reducing the need for exogenous gonadotrophin treatment and offering an alternative to superovulation. Pregnancies and live births have been achieved following maturation and fertilization *in vitro* of oocytes retrieved from antral follicles (1-3), but clinical pregnancy rates are generally lower than those achieved using standard assisted reproductive techniques (ART).

During the growth and accomplishment of meiotic competence of oocytes (before initiation of meiosis), cumulus cells are responsible for maintenance of nuclear arrest at the germinal vesicle (GV) stage via elevating intercellular cAMP level in the oocytes by transferring an inhibitor signal through gap junctions (4-6). Initiation of meiosis is also related to cumulus function, there are evidences that cumulus cells secrete a meiosis-inducing factor (4, 7). It is generally accepted that the relationship between cumulus cells and oocytes is important not only in the process of oocyte maturation to the metaphase II stage, but also in the cytoplasmic maturation, needed for development of oocytes after fertilization (8-10). The effect of cumulus cells may be due to the local production of glyosaminoglycans, steroid hormones and other

factors that support cytoplasmic maturation; which are responsible for male pronucleus formation, monospermic fertilization and embryonic development (11, 12). Moreover, cumulus cells stabilize the disruption of cortical granules (13). Physical contact between oocyte and cumulus cells has been considered necessary for the transfer of nutrients and essential factors for oocyte development (10, 17).

It has been shown that cumulus denuded oocytes can complete meiotic maturation in rats. In porcine, bovine, pigs, rabbits and humans significantly more cumulus-enclosed oocytes were fertilized with spermatozoa and developed to the blastocyst stage *in vitro* when compared with cumulus-denuded oocytes (14-21).

The present study was conducted to examine whether the presence or absence of cumulus cells during the process of maturation and fertilization affect mouse oocyte nuclear maturation, fertilization, cleavage rates and subsequent development to the blastocyst stage in mouse germinal vesicle oocytes.

Materials and Methods

All chemicals were purchased from Sigma Chemical Co. except for the ones specifically described. All animal experiments were approved by the Institutional Animal Care and Use Committee at Tehran University of Medical Sciences.

Collection of GV oocytes

A total of 470 GV oocytes were obtained from 26 ovaries of 3-4 week old ICR female mice. The animals were kept under controlled conditions (12 hours light:12 hours dark) and fed water and pellets ad libitum. Mice were stimulated by injection of 5 IU pregnant mare serum gonadotropin (PMSG: Teikokuzouki, Tokyo, Japan) intraperitonealy. The animals were killed by cervical dislocation 48 hours later and the ovaries removed and placed in TCM-199 supplemented with 10% fetal bovin serum (FBS). The GV stage oocytes were released by puncturing ovarian follicles with a 28G needle under a stereomicroscope.

IVM

Only those oocytes containing a clearly visible nucleus germinal vesicle were used. The collected GV oocytes were divided into two groups; group I: denuded oocytes (DO), cumulus cells removed by pipeting, (Fig 1A). Group II: cumulus oocyte complexes (COC) (Fig 1B).

Each group was placed in 50 µl microdrops of TCM-199 supplemented with 10% fetal bovine serum (FBS), 0.23 mM sodium pyruvate, 75 mU/ml of follicle-stimulating hormone (FSH), 50 mg/l streptomycin, 60 mg/l penicillin and 1µg/l epidermal growth factor (EGF) over laid with embryo-tested light mineral oil for 22-24 hours in a humidified atmosphere of 5% CO_2 at 37ºC (22). At various intervals from the onset of incubation, oocytes were observed by inverted microscopy and morphological changes in the nucleus (GVBD) or the extrusion of the first polar body (MII) were used as the criterion for nuclear maturation of GV-stage oocytes. The matured oocytes were collected for IVF.

IVF

Sperm was collected from the epididymides of ICR male mice aged 12 weeks. The sperm suspension (1×10^6 motile spermatozoa/ml) was capacitated for 1.5-2 hours in 400 µl of T6 media supplemented with 16 mg/ml BSA.

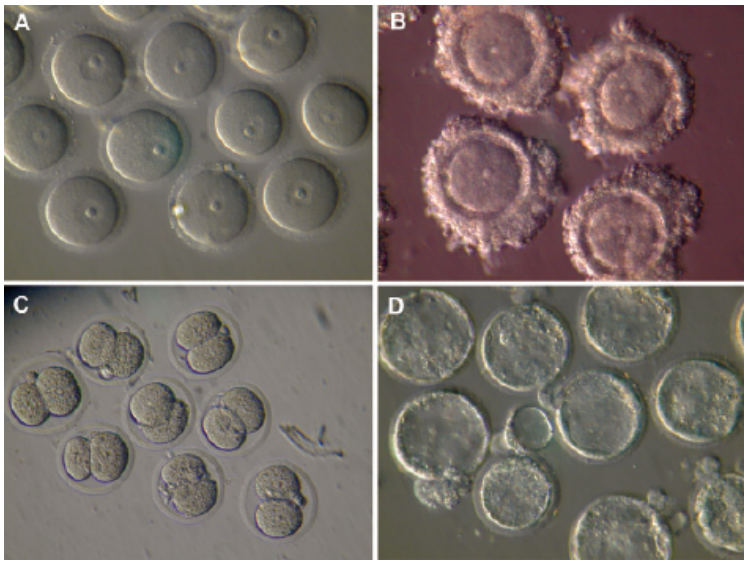

Fig 1: A. DO = Denuded GV oocyte, B. COC = Cumulus oocyte complex, C. 2-cell stage embryos, D. Mouse blastocyst.

In vitro matured (MII stage) oocytes from each treatment group were placed in 0.9 ml T6 and 0.1 ml capacitated spermatozoa was added. After 4-6 hours incubation, the oocytes were washed through three droplets of T6 medium. The oocytes were then cultured in a droplet of T6 (100 µl) under mineral oil. They were assessed for cleavage to the 2-cell stage 24 hours and production of blastocysts 120 hours after fertilization.

Statistical analysis

Collected data were analyzed by chi-square test. *In vitro* maturation, fertilization and embryo development to blastocyst differences were considered significant when ($p<0.05$).

Results

A total of 470 GV oocytes with and without cumulus cells were obtained from 36 ovaries that were used for the DO and COC groups.

In vitro maturation of mouse oocytes

The number of oocytes that arrived to the MII stage, the proportion of oocytes which remained arrested at the GV stage and germinal vesicle breakdown (GVBD) stage in the DO group were significantly greater than the COC group at the 24 hours culture, (Fig 2).
Also as shown in Fig 2, the maturation rate of COC was significantly higher than that of the DO group ($p<0.05$).

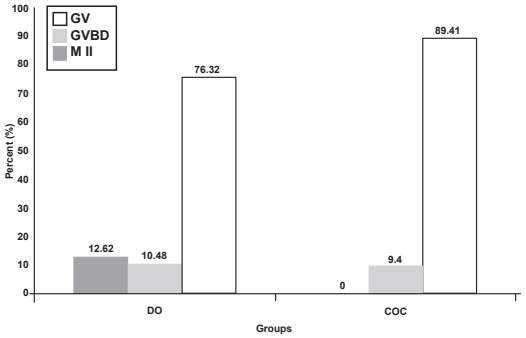

Fig 2: Maturation, GVBD and degeneration rate of mouse GV oocytes after 24 hours culture.
DO= Denuded oocyte, COC = Cumulus oocyte complex, GV= Germinal vesicle oocyte. GVBD= Germinal vesicle breakdown, MII=metaphase II.
Significant differences between DO and COC are indicated by an asterisk ($p<0.05$).

In vitro fertilization and development to blastocyst stage

The number of fertilized oocytes, 2-cell stage after 24 hour culture (Fig1 C) and blastocysts after 120 hours culture (Fig1 D) are shown in Fig.3. As shown in this figure, fertilization and cleavage rates, and the subsequent development to blastocyst stage was significantly higher in the COC group than that of the DO group ($p<0.05$).

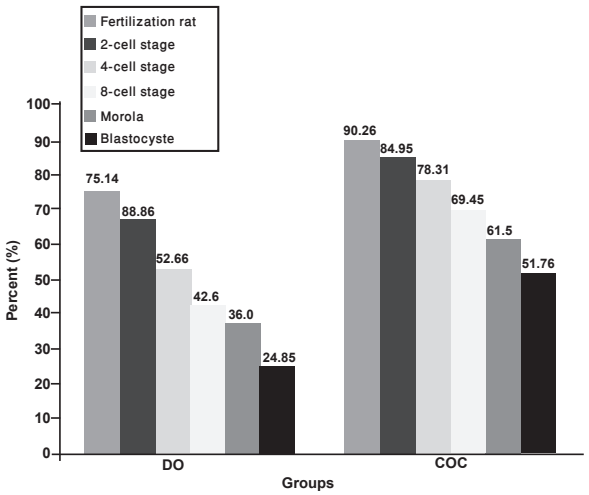

Fig 3: Fertilization, cleavage and balastocyst rate of mouse GV stage oocytes after in vitro maturation. Significant differences between DO and COC are indicated by an asterisk ($p<0.05$).

Discussion

The results of this study showed that mouse GV oocytes with cumulus cells had a significantly higher maturation, fertilization, cleavage and blastocyst rate than oocytes without cumulus cells. The beneficial effects of cumulus cells on oocyte maturation and development were reported in different species (18-28). In the company of our results, Warriach et al. (18) reported that buffalo oocytes with cumulus cells had a significantly higher maturation rate than oocytes without cumulus cells. Also, Chauhan et al. (24) have found a significantly different IVM rate (85%, 54% and 26%) for grade1 (>5 layers of cumulus cell), grade 2 (<4 layers of cumulus cells) and grade 3 (without cumulus cells) buffalo oocytes. The subsequent fertilization and cleavage rates in oocytes with cumulus cells were better than buffalo oocytes without cumulus cells.
In contrast to these studies, Asada et al. (25) reported that the presence of cumulus cells in mink whales did not affect the maturation rate of oocytes. In porcine, it is unclear whether the presence or absence of cumulus cell during IVF are suitable for fertilization and subsequent development of oocytes. Also Marchal et al. have not found any differences in the rates of total and normal fertilization between porcine oocytes denuded before and after IVF (29). Gil et al. reported that the efficiency of fertilization and embryonic development was lower in oocytes with cumulus cells than in oocytes without cumulus cells (30). It has been suggested that the attachment of cumulus cells to oocytes during IVF enhances oocyte penetrability by secreting substances that promote penetration (10, 26) and acrosome reaction of sperm by favoring sperm capacitation (27). However, de-

nuded mouse oocytes are still widely used for IVF in many research laboratories. The results of our study confirm the positive effects of cumulus cells on IVM, fertilization and subsequent developmental capacity of mouse germinal vesicle oocytes.

Conclusion

This study indicates that the presence of cumulus cells during culture are responsible for oocyte nuclear maturation, fertilization, cleavage and subsequent development to blastocytes.

Acknowledgments

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