Original Article

The Effect of Progesterone on the *In vitro* Maturation and Developmental Competence of Mouse Germinal Vesicle Oocytes

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Abstract

Background: The aim of the present study was to investigate the role of progesterone on the developmental competence of cumulus-oocyte complexes (COCs) and cumulus-denuded oocytes (CDOs) at germinal vesicle (GV) stage.

Materials and Methods: GV oocytes of pregnant mare's serum gonadotropin (PMSG)-primed prepubertal mice were divided into two groups: CDOs and COCs. The oocytes were cultured in TCM199 with different concentrations of progesterone (10, 38, 50 and 100 μM) and without progesterone (controls). The number of oocytes at the GV, germinal vesicle breakdown (GVBD) and metaphase II (MII) stages were counted. *In vitro* fertilization (IVF) of MII oocytes and their development to the blastocyst stage were evaluated.

Results: Significantly different MII rates were observed between the COCs (85%) and CDOs (68%) control groups. The MII rates of 83%, 48%, 14% and 0% for COCs and 65%, 53%, 20% and 0% for CDOs were obtained in TCM199 that contained 10, 38, 50 and 100μM progesterone concentrations, respectively. These MII rates were lower ($p<0.05$) in both COCs and CDOs as compared to their respective control groups, except for 10 μM. The fertilization and blastocyst rates of COCs (83% and 35%, respectively) were higher ($p <0.05$) than those of the CDOs (51% and 5%, respectively) control groups. The fertilization and blastocyst rates in the presence of 10 μM (81% and 36%, respectively) and 38 μM (85% and 30%, respectively) progesterone in COCs and CDOs (52% and 4% for 10 μM; 56% and 4% for 38 μM) were similar to their respective control groups.

Conclusion: Adding progesterone to the medium could not improve maturation of mouse GV oocytes and their development to the blastocyst stage.

Keywords: Germinal Vesicle, Oocytes, Maturation, Progesterone

Introduction

Oocyte maturation is controlled by intraovarian factors such as steroid hormones, cytokines and other growth factors during follicular development (1-5). The role of steroid hormones such as progesterone in follicular development and ovarian function has been demonstrated in some investigations (6-14). It seems that, in some mammals, oocyte maturation and its progression to metaphase II are steroid–dependent. In these experiments, the effects of steroid hormones have been investigated on the different developmental stages of oocytes and follicles such as cumulus oocyte complexes (COCs) or cumulus denuded oocytes (CDOs) which led to controversial findings (6-14).

Progesterone exists in the follicular fluid that contributes to normal mammalian ovarian function. It plays a critical role in ovulation, luteinization, fertilization and maintenance of pregnancy (15, 16). Progesterone is a dominant content of follicular fluid steroids 18 hours after the LH surge (17). The concentration of progesterone in preovulatory follicle fluid may reach levels three fold above those found in circulation. It has been estimated that progesterone in the mouse follicle is present at a concentration of 38 μM (18), but Andersen and Byskove (19) have considered a 10 μM concentration of progesterone as a similar concentration of progesterone in mouse preovulatory follicular fluid. It has been shown that, in vivo levels of progesterone in follicular fluid are closely associated with developmental competence of an oocyte. For example, in humans and rhesus monkeys, high embryonic development and pregnancy frequency have been related (20, 21) to high ratios of proges-

Received: 10 Mar 2009, Accepted: 7 Jun 2009
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Royan Institue
International Journal of Fertility and Sterility
Vol 3, No 1, May-Jun 2009, Pages: 21-28

terone to estrogen in follicular fluid (20, 21). Ryan et al. (22) have reported that the presence of estradiol in maturation medium could adversely affect bovine blastocyst development, but the addition of progesterone in maturation medium improve the frequency of development to blastocyst. While, Silva and Knight (6) have demonstrated that the presence of progesterone or the anti-progestin, mifepristone in *in vitro* maturation (IVM) medium did not influence cleavage rate and progesterone had a significant adverse effect on bovine blastocyst formation rate, which mifepristone could only reverse this effect. In rhesus monkeys, it has been demonstrated that the addition of progesterone and estradiol in the maturation medium of oocytes could improve oocyte developmental capacity (23).

Cumulus cells could participate in the induction of meiotic resumption and supporting cytoplasmic maturation of oocytes and on the other hand, oocytes could control granulosa cell function through the stimulation of estradiol production and inhibition of progesterone (24). The in vivo specific steroid environment is critical for oocytes to acquire meiotic maturation and developmental competence (25). In this regard little information is known about the participation of steroids, in particular progesterone, on *in vitro* oocyte maturation and fertilization. Therefore, based on the importance of steroid hormones, especially progesterone, as an intrafollicular agent on the maturation of oocytes, the present study was conducted to evaluate the effect of different concentrations of progesterone on *in vitro* maturation and developmental competence of mouse germinal vesicle oocytes and clarify the mode of progesterone effect.

Materials and Methods

Animals

Prepubertal female (24–28 days old) and mature male (8-12 weeks old) National Research Institute (NMRI) mice were used in accordance with the guide for care and use of laboratory animals of Tarbiat Modares University. They were housed under a 12 hour light:12 hour dark regimen (light on at 7:00), at a temperature of 23 ± 3°C and relative humidity of 44 ± 2% for at least one week before use. Animals were provided with food and water, both available *ad libitum*.

Experimental design

To evaluate the effects of progesterone and mifepristone (RU486) as an antagonist of progesterone on the maturation of mouse oocytes at the germinal vesicle stage, our study focused on two groups of cumulus enclosed oocyte complexes (COCs) and cumulus denuded oocytes (CDOs).

Isolation of GV oocytes from mice

All reagents were purchased from Sigma-Aldrich, UK unless otherwise stated. The female mice were primed with an intraperitoneal injection of 7.5 IU pregnant mare's serum gonadotropin (PMSG; Folligon, Intervet, Australia) and then sacrificed 48 hours later by cervical dislocation.

The ovaries were removed and placed in HEPES-buffered TCM199 medium (Gibco-BRL) supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco- BRL), 0.23 mM sodium pyruvate, 50µg/ml penicillin and 75 µg/ml streptomycin. Under the view of a stereomicroscope, the immature GV oocytes were released from large antral follicles by puncturing with a sterile 30 gauge needle. The COCs and CDOs were obtained by repeated pipetting and flushing. Then they were washed in fresh HEPES-buffered TCM199 medium (Gibco-BRL) before being subjected to IVM.

In vitro maturation of GV oocytes

Experiment I:

The maturation medium was TCM199 supplemented with 50 µg/ml penicillin, 75 µg/ml streptomycin, 0.23 mM sodium pyruvate, 10% FBS, 75mIU /ml rhFSH and 10 IU /ml hCG. Different concentrations of progesterone (0, 10, 38, 50 and 100 µM) and 25 µM of mifepristone were added to the maturation medium according to the experimental design.

Progesterone and mifepristone were dissolved in absolute ethanol and diluted appropriately. However, it has recently been demonstrated that the concentrations of progesterone in medium are reduced in response to the high absorbing capacity of paraffin oil or mineral oil (26). Therefore progesterone and mifepristone were dissolved in mineral oil to give the same concentrations as in the medium.

COCs and CDOs were cultured in 5-10 µL drops of maturation medium containing different concentrations of progesterone or 25 µM of mifepristone under mineral oil at 37ºC, 100% humidity in 5% CO_2 for 24 hours. The media without progesterone or mifepristone were considered as controls. At the end of the culture period, the number of degenerated oocytes, oocytes at germinal vesicle (GV), germinal vesicle breakdown (GVBD) and metaphase II (MII) stages were counted using an inverted microscope. The oocytes at MII stages were collected and used for *in vitro* fertilization. Each experiment was repeated at least five times.

Experiment II:

Reversibility of progesterone effects

The reversibility of progesterone-induced inhibition was evaluated in arrested GV oocytes from the highest concentration of progesterone treatment groups (100 µM). For this purpose arrested GV oocytes were washed twice by media without progesterone and incubated for an additional 24 hours in free progesterone maturation medium. The contents of this medium were described in experiment I. The development and maturation of oocytes were assessed as degenerated, GV, GVBD, and MII.

In vitro fertilization and embryo culture

Mature NMRI male mice were sacrificed and their cauda epididymis were dissected, and placed into a 500-µl drop of T6 medium with 5 mg/ml BSA under mineral oil. The epididymal contents were squeezed out by the use of forceps. The drops containing freshly released spermatozoa were placed in a 37ºC with 5% CO_2 incubator for capacitation. Capacitated sperms were added to fertilization medium which consisted of T6 medium supplemented with 15mg/ml BSA to give a final motile sperm concentration of 1-2 × 10^6/ml. The MII oocytes were then collected from different groups and separately transferred to IVF media. After 4-6 hours, the oocytes were transferred into 10 µl drops T6 medium with 5mg/ml BSA. Embryos were observed daily under an inverted microscope, and the number of embryos that reached 2-cell, 4-cell, morula, blastocyst stages was recorded until 120 hours.

Statistical analysis

All experiments were repeated at least five times. The proportion of oocyte maturation stages, fertilization rates and cleaved oocytes reaching defined developmental stages were subjected to arcsine and then analyzed by two way ANOVA by using SPSS (version 16) software. Tukey's HSD was used as a post hoc test. Differences were considered significant at a level of $p<0.05$.

Results

The maturation rate of germinal vesicle oocytes in the presence of progesterone

After 24 hours culture, the percentage of MII in the COCs group with various concentrations of progesterone (0, 10, 38, 50 and 100 µM) were 85%, 83%, 48%, 14% and 0%, respectively (Table 1) as compared to 68%, 65%, 53%, 20% and 0%, respectively in the CDOs group (Table 2).

The rate of MII in the COCs control group (0 µM of progesterone) was significantly higher than the CDOs control group ($p<0.05$). The MII rates were higher in the control and 10 µM progesterone treatment groups as compared to other concentrations of progesterone in both COCs and CDOs groups ($p <0.05$). Also the 10µM progesterone treatment group did not show a significant difference compared to the control group.

The rates of GVBD in COCs group with different concentrations of progesterone (0, 10, 38, 50 and 100 µM) were 6%, 9%, 24%, 8% and 0%, respectively (Table 1); while these rates in CDOs group in the aforementioned concentrations of progesterone were 12%, 11%, 15%, 13% and 0%, respectively (Table 2).

Table I: The effect of progesterone on in vitro maturation of cumulus oocyte complexes at the germinal vesicle stage

Dosage of P4	No. of GV	GV Arrested (No) % ± SD	GVBD (No) % ± SD	MII (No) % ± SD	Degenerated (No) % ± SD
Control	450	(17) 3.85 ± 1.22	(31) 6.87 ± 2.32	(386) 85.75 ± 3.90	(16) 3.51 ± 1.05
10 µM	482	(24) 5.05 ± 2.08	(43) 9.46 ± 5.64	(403) 83.08 ± 6.00	(12) 2.38 ± 0.69
38 µM	473	(113) 24.41 ± 3.30[a]	(113) 24.08 ± 7.40[a]	(231) 48.06 ± 9.25[a]	(16) 3.44 ± 0.95
50 µM	427	(314) 73.41 ± 2.37[a,b]	(36) 8.74 ± 2.17	(65) 14.96 ± 3.16[a,b]	(12) 2.88 ± 1.20
100 µM	452	(436) 96.31 ± 1.24[a,b,c]	(0) 00 ± 00[a,b]	(0) 0.00 ± 0.00[a,b,c]	(16) 3.50 ± 0.67

P4: progesterone; GV: germinal vesicle stage oocyte; GVBD: germinal vesicle breakdown; MII: metaphase II oocyte. In all cases five experimental replicates were performed.
a: Significant differences compared to control group (0 µM P4) in the same column ($p<0.05$).
b: Significant differences compared to 38 µM concentration of progesterone in the same column ($p<0.05$).
c: Significant differences compared to 50 µM concentration of progesterone within the same column ($p<0.05$).

Table 2: The effect of progesterone on the in vitro maturation of cumulus denuded oocytes at germinal vesicle stage

Dosage of P4	No. of GV	GV Arrested (No) % ± SD	GVBD (No) % ± SD	MII (No) % ± SD	Degenerated (No) % ± SD
Control	506	(53) 10.45 ± 1.90	(60) 12.00 ± 5.38	(347) 68.55 ± 2.24	(46) 8.98 ± 3.09
10 µM	471	(55) 11.76 ± 1.41	(55) 11.62 ± 2.81	(310) 65.94 ± 1.50	(51) 10.67 ± 1.89
38 µM	474	(101) 20.76 ± 2.85[a]	(71) 15.42 ± 3.48	(256) 53.73 ± 1.98[a]	(46) 10.07 ± 1.94
50 µM	496	(275) 55.07 ± 3.12[a,b]	(68) 13.81 ± 2.95a	(105) 20.68 ± 1.75[a,b]	(48) 10.10 ± 2.47
100 µM	540	(479) 88.55 ± 2.8[a,b,c]	(0) 0.00 ± 0.00	(0) 0.00 ± 0.00[a,b,c]	(61) 11.44 ± 2.80

P4: progesterone; GV: germinal vesicle stage oocyte; GVBD: germinal vesicle breakdown; MII: metaphase II oocyte.
a: Significant differences compare to control group (0 µM P4) in the same column (p<0.05).
b: Significant differences compare to 38 µM concentration of progesterone in the same column (p<0.05).
c: Significant differences compare to 50 µM concentration of progesterone in the same column (p<0.05).

There was a significant difference (p<0.05) between oocytes that were cultured in the presence of 38µM and 100µM of progesterone compared to the other concentrations of progesterone and the higher rate of GVBD oocytes were observed in the presence of 38µM of progesterone than that of 100µM (p <0.05) in both COCs and CDOs groups.
The rates of oocytes that arrested at the GV stage after 24 hours culture in the COCs group with different concentrations of progesterone (0, 10, 38, 50 and 100µM) were 3%, 5%, 24%, 73% and 96%, respectively (Table 1) Those of the CDOs group were 10%, 11%, 20%, 55% and 88%, respectively (Table 2). In the presence of 0µM and 10µM concentrations of progesterone in maturation medium, the proportion of oocytes that arrested at the GV stage in the CDOs group were more than COCs group (p <0.05). In the presence of 38µM concentration of progesterone in maturation medium, there was no significant difference between the arrested GV rate of CDOs and COCs, while in the presence of 50µM and 100µM progesterone concentrations the proportion of oocytes that arrested at the GV stage in COCs group were more than that of the CDOs group (p <0.05). There was a significant interaction between the effect of progesterone and the presence or absence of cumulus cells on MII and arrested GV rate (p <0.05). The rate of degenerated oocytes was not significantly different in the control and progesterone treated oocytes in both of the COCs and CDOs groups (Tables 1 and 2).

The maturation of oocytes in the presence of mifepristone

When mifepristone was added solely to the maturation medium of COCs, the percentage of degenerated oocytes, arrested GV, GVBD and MII were 2%, 3%, 8% and 84%, respectively, while these rates in the CDOs group were 10%, 12%, 13% and 64%, respectively. Statistically significant differences were not observed between mifepristone treatment and control (data were shown previously) within both of the COCs and CDOs groups (Table 3). When COCs and CDOs oocytes were cultured in maturation medium containing progesterone (100µM) and mifepristone (25µM); 96% and 89% of oocytes arrested at the GV stage, respectively. None of the GV oocytes reached to GVBD and MII in both of the COCs and CDOs groups (Table 3).

Reversibility of oocyte in the absence of progesterone

To test whether the inhibitory effects of progesterone were due to toxic effects and to ensure that the arrested GV oocytes could resume their meiosis; reversibility experiments were performed on arrested GV oocytes that had been cultured in the presence of 100µM of progesterone. In the COCs group, after being cultured for a further 24 hours in progesterone-free medium, 3% of arrested GV oocytes were degenerated, 4% of those remained at the GV stage, 9% of those reached GVBD and the rest (82%) could resume meiosis and reach the MII stage. While, in CDOs group, 5% of arrested GV oocytes were degenerated, 6% of those remained at GV stage, 8% of those reached GVBD and the rest (78%) were capable to resume meiosis and reach the MII stage (Table 4). Significant differences were not observed between COCs and CDOs groups in reversibility after a further 24 hours culture in the absence of progesterone (p>0.05).

Table 3: Effect of mifepristone (RU486) on in vitro maturation of oocytes at germinal vesicle stage

Groups	Treatment	No. of GV	GV Arrested (No) % ± SD	GVBD (No) % ± SD	MII (No) % ± SD	Degenerated (No) % ± SD
COC	Control	450	(17) 3.85 ± 1.22	(31) 6.87 ± 2.32	(386) 85.75 ± 3.90	(16) 3.51 ± 1.05
COC	RU486	413	(15) 3.50 ± 1.05	(35) 8.35 ± 3.63	(351) 84.96 ± 4.43	(12) 2.84 ± 1.47
COC	RU486+P4	440	(423) 96.13 ± 1.30[b]	(0) 00 ± 00[b]	(0) 0.00 ± 0.00b	(17) 3.86 ± 1.30
CDO	Control	506	(53) 10.45 ± 1.90[a]	(60) 12.00 ± 5.38[a]	(347) 68.55 ± 2.24[a]	(46) 8.98 ± 3.09[a]
CDO	RU486	451	(55) 12.27 ± 1.42[a]	(60) 13.25 ± 3.72[a]	(289) 64.13 ± 3.53[a]	(47) 10.34 ± 1.33a
CDO	RU486+P4	504	(453) 89.83 ± 0.71[a,b]	(0) 0.00 ± 0.00[a,b]	(0) 0.00 ± 0.00[a,b]	(51) 10.16 ± 0.71[a]

P4: progesterone; RU486: mifepristone; GV: germinal vesicle stage oocyte; GVBD: germinal vesicle breakdown; MII: metaphase II oocyte; COC: cumulus oocyte complex; CDO: cumulus denuded oocyte
a: Significant differences compare to COC groups in the same column (p<0.05).
b: Significant differences compare to control group in the same column (p<0.05).

Table 4: Reversible ability of arrested oocytes at germinal vesicle in the presence of 100µM of progesterone after 24h

Groups	No. of GV arrested	Degenerated (No) % ± SD	GV Arrested (No) % ± SD	GVBD (No) % ± SD	MII (No) % ± SD
COC	436	(17) 3.89 ± 1.31	(19) 4.37 ± 1.00	(39) 9.04 ± 4.49	(361) 82.69 ± 4.68
CDO	472	(27) 5.75 ± 1.35	(33) 6.85 ± 1.76	(39) 8.60 ± 2.56	(373) 78.77 ± 3.53

P4: progesterone; GV: germinal vesicle stage oocyte; GVBD: germinal vesicle breakdown; MII: metaphase II oocyte; COC: cumulus oocyte complex; CDO: cumulus denuded oocyte.
There were no significant differences between COC and CDO groups (p<0.05).

Table 5: The developments of embryo derived from MII oocytes in COC and DO groups

Groups	Dosage of P4	No. of MII Oocyte	PN (No) % ± SD	2-Cell (No) % ± SD	4-Cell (No) % ± SD	Morula (No) % ± SD	Blastocyst (No) % ± SD
COC	Control	386	(322) 83.45 ± 3.82	(289) 74.82 ± 3.78	(212) 55.13 ± 3.23	(164) 42.39 ± 3.95	(137) 35.33 ± 3.89
COC	10 µM	403	(321) 81.64 ± 4.75	(287) 70.77 ± 2.31	(219) 54.37 ± 1.68	(169) 41.41 ± 4.04	(149) 36.31 ± 4.02
COC	38 µM	231	(195) 85.39 ± 3.97	(168) 73.30 ± 5.29	(124) 53.34 ± 3.47	(88) 37.19 ± 3.33	(74) 30.77 ± 5.08
CDO	Control	347	(176) 51.08 ± 5.23	(134) 38.61 ± 4.41	(80) 23.10 ± 3.43	(38) 10.89 ± 2.67	(20) 5.69 ± 2.92
CDO	10 µM	310	(161) 52.40 ± 5.35	(108) 34.96 ± 2.88	(63) 19.95 ± 3.64	(26) 8.10 ± 3.02	(15) 4.53 ± 2.82
CDO	38 µM	256	(144) 56.90 ± 4.58	(95) 37.27 ± 2.78	(57) 21.63 ± 3.31	(24) 8.55 ± 4.02	(14) 4.95 ± 3.66

COC: cumulus oocyte complex; CDO: cumulus denuded oocyte; MII: metaphase II oocyte; PN: pronucleus stage.
The fertilization rates and developmental rates to blastocyst stage were based on the number of MII oocyte.
There were significant differences between CDO compare to COC groups in the same column in each concentrations of progesterone (p<0.05).

The fertilization rate of oocytes

The fertilization rate of MII oocytes in the COCs group in the presence of 0, 10 and 38 μM of progesterone were 83%, 81% and 85%, respectively and those in the CDOs group were 51%, 52% and 56%, respectively. There were significant differences between COCs and CDOs groups ($p < 0.05$; Table V). Moreover, there were no significant differences among treatment groups within COCs and CDOs groups ($p > 0.05$).

The rate of embryos that reached 2-cell, 4-cell, morula and blastocyst stages in COCs control group were 75%, 55%, 42% and 35%, respectively and in the presence of 10 μM of progesterone were 70%, 54%, 41% and 36%, respectively; while in the presence of 38 μM of progesterone were 73%, 53%, 37% and 30%, respectively. The rate of embryos that reached the 2-cell, 4-cell, morula and blastocyst stages in CDOs control group were 38%, 23%, 10% and 5%, respectively and in the presence of 10 μM of progesterone were 34%, 19%, 8% and 4%, respectively; while in the presence of 38 μM of progesterone were 37%, 21%, 8% and 5%, respectively.

There were no significant differences in the developmental rate of embryos in the presence of different concentrations of progesterone within both of the COCs and CDOs groups ($p > 0.05$; Table 5). The developmental rates to blastocyst were higher in COCs than CDOs groups ($p < 0.05$).

Discussion

In this study, the effects of different concentrations of progesterone on the maturation and fertilization rates of germinal vesicle oocytes and their development to blastocyst stage in COCs and CDOs groups were evaluated. Data reported here demonstrate that the maturation rates and development to blastocyst stage were significantly higher in COCs than CDOs control groups. This finding implicates a crucial role of cumulus cells in the acquisition of developmental competence by oocytes. This is in agreement with the reports of Ge et al. (27, 28), who showed that removal of cumulus cells before IVM compromises the developmental competence of mouse oocytes. It seems that cumulus cells produce factors that act on oocyte development. Byskov et al. (4), have reported that cumulus cells secrete diffusible heat stable substances in response to FSH which activate the resumption of meiosis via the paracrine way. Importance of cumulus cells in resumption of oocyte meiosis were supported by evidences that showed segregation of connection between the oocyte and surrounded cumulus cells by gap junction inhibitors, which prevented oocyte maturation (29-31). It was demonstrated that paracrine interactions exist between the oocyte and granulosa cells within developing follicles (32, 33).

The present study results showed that fertilization rates of COCs control group were significantly higher than CDOs control group. It may be due to some changes in the zona pellucida which influence the penetration of sperm. Ducibella et al. (34, 35) have demonstrated that zona pellucida hardening occurs during in vivo and *in vitro* oocyte maturation. It is due to a premature cortical granules reaction (36). Cumulus cells could prevent zona hardening and result in higher fertilization in COCs than CDOs (34, 35).

Other results of the present study show that progesterone could not improve maturation rates of germinal vesicles in COCs and CDOs groups at any concentrations which were tested than compared to control groups. On the other hand, the presence of 10 μM progesterone in maturation medium could not exert any effect on maturation rate in COCs and CDOs groups. It seems that a low concentration of progesterone was inactive in this maturation medium with high protein content (10% FBS). This may be due in part to the high protein binding capacity of progesterone (11). When levels of progesterone concentration in the culture medium were increased from 10 to 100 μM, the maturation rate decreased in a dose dependent pattern. This is in agreement with other studies that showed some steroids could inhibit mouse oocyte maturation in a dose dependent manner (11). In contrast to the present study's results, some investigators showed that the maturation rate of oocytes increased in the presence of progesterone. For example, Jamnongjit et al. (37, 38) indicated that testosterone or progesterone and epidermal growth factor (EGF), through their own receptors, could induc meiotic resumption in mouse oocytes on the IVM and the effect of these steroids could have been inhibited by respective specific receptor antagonists. Moreover, the inconsistent results reported here may be due to different experimental strategies that have been used. Our results indicated that the progesterone antagonist (mifepristone) which occupies progesterone receptors could not reverse the inhibitory effect of progesterone on oocyte maturation. It seems that the mechanism which interferes in the inhibition of mouse oocyte maturation by progesterone is not receptor mediated. However, it has been shown that steroid hormones could directly bind to some enzymes (39) and progesterone, estradiol and testosterone could inhibit cAMP phosphodiesterase (PDE) activity via binding to

the purine-binding site of this enzyme (13).
In this study, the effect of progesterone in inhibition of meiotic resumption is more effective in COCs than CDOs. It seems that there is intensive interaction between oocytes and surrounding cumulus cells (24, 40, 41). The present experiment results show that progesterone in concentrations similar to that of preovulatory follicular fluid (10 and 38 μM) (18, 19) did not improve the fertilization rate and development to blastocyst stage in both of the COCs and CDOs groups. The results of this study were inconsistent with that found in the porcine (42), bovine (43), ovine (44) and primate (23) oocytes. It seems that, the conflicting results were due to differences in time intervals between gonadotropin stimulation and resumption of meiosis; which in some species is prolonged. It seems that steroids are beneficial for developmental competence of oocytes in these mammals.

Conclusion

The results of the present study suggest that cumulus cells improved the developmental competence of mouse oocytes; but the presence of progesterone in maturation medium could not improve maturation and fertilization rates of mouse GV oocytes and their development to the blastocyst stage in both COCs and CDOs groups. Therefore, there is no need to add progesterone to the medium during IVM of mouse oocytes.

Acknowledgements

This work was conducted and funded partially by the Faculty of Medical Sciences, Tarbiat Modares University, Tehran and College of Veterinary Medicine, Urmia University, Urmia, Iran. The authors thank Dr. Mohammad Asghari for his statistic advice. There is no conflict of intereset in this article.

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