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RESEARCH PAPER

Supplementation of IVM culture media with GDF-9 can potentially enhance oocyte quality, fertilization and embryo development in ICSI procedure

Sareh Ashourzadeh [1], Mohammad Ali Khalili [2,*], Marjan Omidi [1], Stefania A Nottola [3], Azita Faramarzi [4]

[1] Afzalipour Clinical Center for Infertility, Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, Iran

[2] Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

[3] Department of Anatomy, Histology, Forensic Medicine and Orthopedics, University of Rome La Sapienza, Italy

[4] Fertility and Infertility Research Center, Research Institute for Health Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran

Highlights

- Upgrading of in vitro maturation (IVM) in human oocytes as a potential strategy has been considered in assisted reproductive technology (ART).
- IVM medium supplemented with GDF-9 can improve fertilization rate and embryo quality.
- Co-culture of GV stage oocytes with cumulus cells (CCs) can improve IVM medium.
- Oocyte meiotic spindle (MS) assessment with polarized light microscopy provides an early predictor of IVM outcome.

Graphical Abstract

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Abstract

In vitro maturation (IVM) of human oocytes is an important factor in assisted reproductive technology (ART) laboratories. The present study was aimed to investigate the effect of growth differentiation factor 9 (GDF-9) supplemented into IVM medium, and co-culture of cumulus cells (CCs) on IVM outcome. 385 immature oocytes in the germinal vesicle (GV) stage were divided into four groups in terms of IVM culture condition; 1) GV oocytes cultured in standard IVM medium (control), 2) GV oocytes co- cultured with CCs, 3) GV oocytes treated with GDF-9, and 4) GV oocytes co-cultured with CCs and treated with GDF-9, simultaneously. The presence of meiotic spindle (MS) and zona pellucida (ZP) birefringence were assessed in IVM oocytes, followed by intracytoplasmic sperm injection (ICSI). Finally, fertilization rate and embryo development in day 2 embryos were evaluated. The maturation rates of GV oocytes were similar in whole groups following 24-48 h of incubation. A significantly higher rate of oocytes matured in the medium treated with CCs reached into 2 pronuclear (2PN) stage compared to those matured in the standard medium, respectively (76.5% vs. 51.6%, P= 0.01). The percentage of oocytes with visible MS was higher in all treated groups, but this alteration was significant between oocytes matured in medium supplemented with GDF-9 and control group (45% vs. 26.6% respectively, P= 0.05). Co-culturing of GV oocytes with CCs and/or supplementation of the IVM medium with GDF-9 can potentially improve fertilization rate and embryo development. Also, MS assessment provides an early predictor of embryo development.

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* Corresponding author: khalili59@hotmail.com (M.A. Khalili)

Introduction

In vitro maturation (IVM) of human oocytes is a potential alternative to conventional in vitro fertilization (IVF). Retrieval of immature oocytes from unstimulated ovaries, followed by IVM has been proposed to avoid the side effects of exogenous gonadotropin administration (1, 2, 3, 4). The experiences with clinical IVM being controversial in terms of pregnancy and implantation rates as previous studies mentioned that the efficiency of the IVM technique is suboptimal (5, 6). Conversely, more recent studies have shown results from IVM that are comparable to those with IVF cycles (7, 8). Regarding ultrastructural aspects, Coticchio et al., (9) indicated that general ultrastructural organization is unaffected by in vitro conditions. It seems that the outcome of IVM depends on abnormalities of cytoplasmic maturation (10), handling of immature oocytes (11) and culture conditions (5). Dissimilar to the intact oocytes surrounded by cumulus cells (CCs), cumulus-free oocytes are in contact with the surrounding medium (12). Furthermore, until the culture systems for IVM are improved, it is expected that there were differences between the in vivo and in vitro matured oocytes competence (11). In support of this concept for enhancing the IVM efficiency, an optimal culture system is necessary to make this technique clinically acceptable .

Rescue IVM is a procedure in association with immature oocytes that are retrieved from controlled ovarian hyperstimulation (COH) cycles. Veeck et al., (13) showed that these immature oocytes not only were capable of reaching maturation and fertilization in vitro, but also of embryonic development. Interestingly, a recent study by Lee et al., (14) strongly suggested that rescue IVM should become a new treatment option to improve IVF outcomes in low follicular ovarian reserve. However, the rescue immature oocytes are denuded from cumulus-enclosed cells due to evaluation of maturational status and, as such, frequently are cultured in the absence of CCs. Mutual interactions via gap junctions between the oocytes and their surrounding CCs are essential for the development of ovarian follicles in vivo (15). CCs play an important role in the acquisition of oocyte nuclear and cytoplasmic competence and metabolism (16, 17). Besides, it is revealed that the CCs protect the oocyte against oxidative stress and subsequent apoptosis during IVM (18). It was also reported that removal of CCs during maturation has a significant detrimental effect on nuclear maturation, fertilization and developmental competence of oocytes (19). Likely, the CCs are involved in oocyte and embryo development and are regulated by oocyte secreted factors (OSFs) (20). Two well-known growth factors derived from oocytes include growth differentiation factor 9 (GDF-9) and bone morphogenetic protein 15 (BMP-15). OSFs contribute to promoting the proliferation and differentiation of CCs and oocyte maturation through paracrine and autocrine signaling pathways (21). Reports are showing that higher GDF-9 levels in the follicular fluid are significantly related with oocyte maturation and embryo quality (22, 23). In addition, it was confirmed that OSFs are expressed both in oocytes and cumulus CCs (20).

In addition, oocyte quality determines the ability to progress to the MII stage as well as undergoing fertilization and embryo development (17). It was reported that IVM might impose adverse effects on oocyte spindles and chromosome organization, which is associated with the lower developmental capability of the embryos (1). Recently, a non-invasive technique for assessing zona pellucida (ZP) birefringence and meiotic spindles (MS) visualization has been introduced as a prognostic marker for oocyte quality (24, 25). Currently, studies showed that IVM can disturb gene expression and alter matrix protein profiles in CCs (26). Also, exogenous OSFs significantly improved IVM of mouse oocytes (27). With this in view, this study investigated the effect of GDF9 and co-culture of CCs on the development and quality of human oocytes, fertilization and embryo development in rescued ICSI program. Understanding these outcomes can guide the development of an efficient culture condition for advancing the IVM program. To the best of our knowledge, this is the first study that examined the influence of GDF-9 and CCs on IVM outcomes.

Materials and Methods

Study design

This study was approved by the ethics committee of the Research and Clinical Center for Infertility, Yazd, Iran (1622). Signed forms of consent were obtained from participants. In this prospective study, the 385

immature oocytes in the GV stage from 270 women who underwent COH were included. To minimize the confounding factors, the cycles with azoospermic patients and women older than 35 were excluded from the study. After confirming the presence of GV, the oocytes were divided into four groups in terms of IVM culture condition. The presence of MS and ZP birefringence were assessed in IVM oocytes, followed by intracytoplasmic sperm injection (ICSI) performance. Finally, fertilization rate and day 2 embryo development were evaluated (Figure 1).

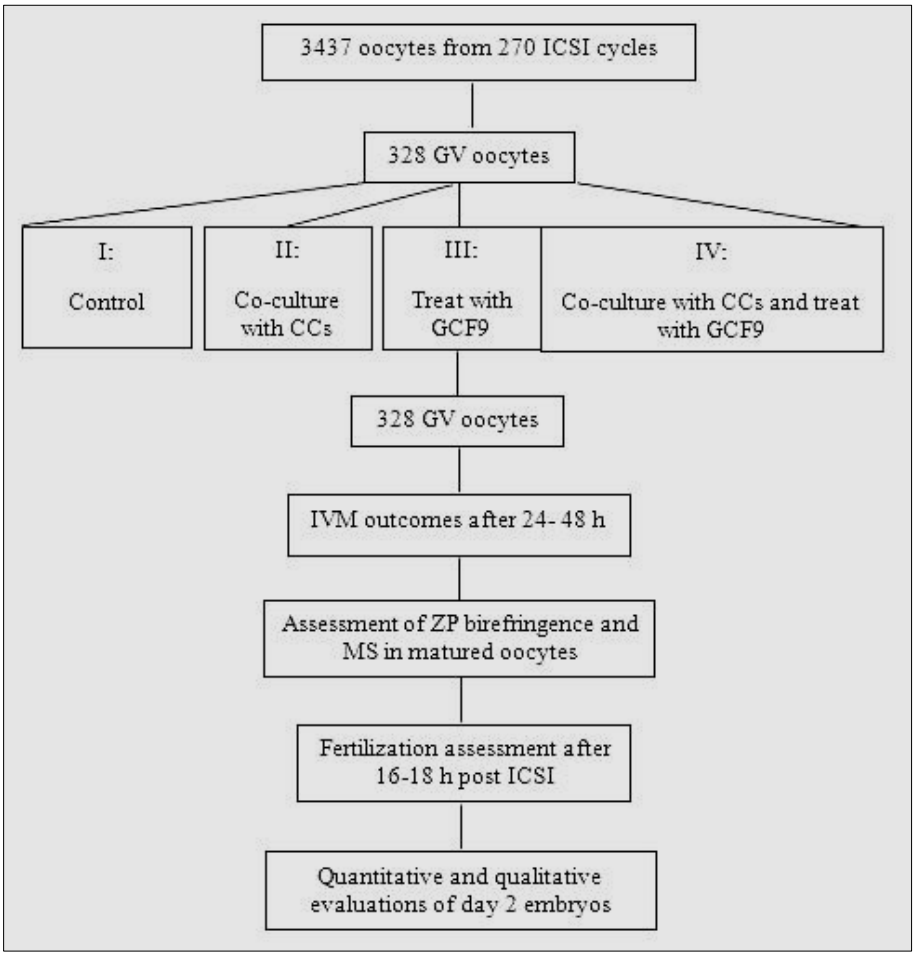

Figure 1. Flowchart of summarized study design.

Controlled ovarian hyperstimulation and oocyte retrieval

Patients included in the study, as described before (1), were treated by routine COH protocols for IVF using antagonists. COH was achieved by using daily injections of gonadotrophins (rFSH; Gonal-f, Serono Co., Aubnne, Switzerland), followed by a daily dose of GnRH antagonist (0.25 mg of Cetrotide, Serono Laboratories, Geneva, Switzerland), once a leading follicle of 12 mm was observed. Oocytes were retrieved 36-38 h after administration of 10 000 IU of hCG, which was given according to the presence of at least two leading follicles of ≥18 mm. Follicles were aspirated using a 16-gauge single-lumen aspiration needle (Wallace; Smiths Medical International, UK) 150 mmHg pressure.

GV oocytes preparation and IVM

After cumulus-oocyte complex (COC) collection, the COCs were incubated in a culture medium (GIVF, Vitrolife Co., Sweden) for 2-3 h. The CCs were then removed by pipetting the COCs with a pasture pipette. Denuded oocytes were assessed for the nuclear maturation stage. Oocytes lacking first polar body (PB) extrusion were considered immature and candidates for IVM. In IVM procedure, immature oocytes were washed in 3 drops of washing medium (SAGE IVF), then each group of GV oocytes according to their culture

condition incubated in maturation medium (SAGE IVF) supplemented with 75 mIU/ml FSH and 75 mIU/mL LH (Ferring) at 37 °C and 5% CO_2. Study groups including I) GV oocytes cultured in standard IVM medium (SAGE IVF, Trumbull, CT, USA) (control group); II) GV oocytes co-cultured with CCs; III) GV oocytes treated with GDF-9 (200 ng/mL; Sigma- Aldrich Co., Germany), and IV) GV oocytes co-cultured with CCs and treated with GDF-9, simultaneously. In all groups, the base medium was a commercial IVM medium (SAGE IVF, Trumbull, CT, USA). After 24–48 h, the acts were screened for the presence of the first PB by stereomicroscope (Olympus, Tokyo, Japan) to maturation determination.

Cumulus cells treatment

CCs were collected after mechanical removal from the retrieved oocytes at the time of oocyte denudation for ICSI. CCs were washed in Ham's F10 medium (Seromed; Basel, Switzerland) supplemented with 10% human serum albumin (HAS; Red Cross, Brussels, Belgium), then centrifuged for 5 min at 400g. The resulting pellet was resuspended and exposed to a lysis buffer to remove red blood cells (8.29 g/L NH_4Cl+1 g/L KHCO3 + 0.58 g/L EDTA, pH 7.2; 5 min at 37 °C) (28). Subsequently, the suspension washed in Hams' F10 medium supplemented with 10% HSA. This procedure was repeated twice to eliminate all traces of hyaluronidase and debris. Finally, 10µL of suspension was counted in a Neubauer haemocytometer. Cell viability was calculated by Trypan Blue (Merck CO., Germany) exclusion and the samples with >80% viability were included.

Meiotic spindles detection, ZP examination and ICSI

Oocyte imaging and ICSI were performed at the same time. For this purpose, mature oocytes were placed individually in a 4 µL droplet of buffered medium (G-Mops-V1; Vitrolife Co., Sweden) in a glass-bottomed culture dish (WillCo-Dish; Bellco Glass NJ, USA) covered with warm mineral oil (Irvine Scientific) (24). Sperm samples for ICSI were prepared using the density gradient technique (29) and were placed in a central droplet of polyvinylpyrrolidone (PVP) solution (Irvine Scientific, CA). The oocytes were imaged under an inverted microscope (TE300; Nikon, Tokyo, Japan) mobilized with a stage heated to 37 °C and a polarizing optical system (OCTAX PolarAIDE; Octax). This system reveals birefringent structures of ZP and MS, using OCTAX Eyeware software. ZP scoring was automatic and oocytes were classified as having a high (HB) or low (LB) birefringent ZP. For oocytes with detected MS, ICSI was performed after the spindles were placed at a 6 or 12 o'clock position. For oocytes without birefringent spindles, ICSI was performed after placing the first PB at 6 or 12 o'clock position. The injected oocytes were washed twice and cultured in droplets of G1 (Vitrolife co., Sweden) for subsequent analysis.

Fertilization and embryo assessment

Fertilization was checked after 16-18 h post-ICSI. Normally, the oocytes with 2 pronuclei (2PN) and a second PB were judged as a sign of fertilization. Next, fertilized oocytes were washed twice and cultured in fresh cleavage media (G1; Vitrolife co., Sweden). On day 2, cleavage embryos were evaluated according to Hill et al., (30). The embryos were graded as follows: Grade A: equal size blastomeres without fragmentation, Grade B: slightly unequal blastomere, up to 10% cytoplasmic fragments. Grade C: unequal-sized blastomeres up to 50% fragments and large granules. Grade D: unequal blastomeres with significant fragmentation and large black granules. In this study, grades A and B were considered as good quality embryos.

Statistical analysis

Data are presented mean ± SD and odds ratios (OR) with 95% confidence intervals (CI), as appropriate. Data expressed as percentages were compared by the Chi-square and Fisher's exact tests, wherever appropriate. Results were analyzed using SPSS version 18 (SPSS, Chicago, IL, USA). $P< 0.05$ was considered statistically significant.

Results

A total of 3437 oocytes were collected in ICSI cycles, of which 385 oocytes (11.2%) were found to be at the GV stage. Of these, 328 GV oocytes were available for randomization in different study groups. The other GV-stage oocytes were discarded due to their abnormal characteristics (e.g. abnormal cytoplasm appearance, vacuoles, etc.). There were no significant differences in the general conditions between patients in the groups (Table 1).

Table 1. Comparison of general information of patients between groups with different maturation medium (MM) condition.

General indicator	Control	MM treated with CCs	MM supplemented with GDF-9	MM treated with CCs and GDF-9	P-value
Age (y)	62.3 ± 4.12	28.41 ± 3.46	29.32 ± 3.52	30.61 ± 4.11	NS
Etiology of infertility					
Male factor (%)	92	89.4	83.3	85.4	NS
Other factors (%)	8	10.6	16.7	14.6	NS
No. of GV oocytes	100	66	66	96	

NS: Non significant.

Outcomes of oocytes post IVM

The maturation rates of GV-stage oocytes following 24- 48 h of incubation either in the standard medium or in the same medium supplemented with GDF-9 or co-cultured with CCs, are presented in Table 2. An insignificant rate of maturation was seen between the groups. However, the proportion of IVM oocytes in the control group was higher than in other groups. The oocytes reaching the MII stage following IVM underwent ICSI, and the laboratory outcomes are presented in Table 2. A significantly higher rate of the oocytes matured in the medium treated with CCs reached the 2PN stage compared with those matured in the standard medium, respectively (76.5% vs. 51.6%, P= 0.01). Regarding the embryo formation, a superior rate of cleavage was noted in treated groups as compared to the control group. Moreover, there was a significant difference in the rate of good quality embryos between the control and co-culture with CCs groups, respectively (37.5% vs. 66.7%, P= 0.05).

Table 2. Comparison of laboratory outcomes between groups with different MM condition.

Laboratory outcomes	Control	MM treated with CCs	MM supplemented with GDF-9	MM treated with CCs and GDF-9
IVM oocytes (%)	64.100 (64)	34.66 (51.5)	40.66 (60.6)	54.96 (56.3)
Fertilized oocytes (%)	33.64 (51.6) [a]	26.34 (76.5) [b]	25.40 (62.5)	33.54 (61.1)
Embryos (%)	24.33 (72.7) [c]	21.26 (80.8)	22.25 (88)[d]	28.33 (84.8) [e]
Good quality embryos (%)	9.24 (37.5) [f]	9.21 (42.9)	14.21 (66.7) [g]	12.28 (42.9)

a vs. b: Odds ratio (95% CI)=0.32 (0.13-0.83) P= 0.01

c vs. d: Odds ratio (95% CI)=0.429 (0.20-0.92) P= 0.02

c vs. e: Odds ratio (95% CI)=0.442 (0.19-1.02) P= 0.05

f vs. g: Odds ratio (95% CI)=0.3 (0.08-1.02) P= 0.05

Polyscope analysis

The percentage of oocytes with visible MS was higher in all treated groups, but this difference was significant between the oocytes matured in medium supplemented with GDF-9 and control group respectively (45% vs. 26.6%, P= 0.05). Furthermore, data revealed that the number of IVM oocytes with high ZP birefringence was higher in the control group as compared to each of the other groups (Table 3). In a subanalysis, the fertilization rate and embryo development were assessed and compared between groups in matured oocytes

with visible spindle and high ZP birefringence (Tables 4, 5). In the population of oocytes with detected MS, fertilization rate was lower in the control compared with treated groups, and these differences were significant between control with group II and IV respectively (52.9% vs. 70%, P= 0.045 and 52.9% vs. 71.4%, P= 0.045). Regarding the rate of good quality embryos, there were insignificant differences between groups in neither detected spindle, nor high ZP birefringence in oocytes.

Table 3. Comparison of spindle visualization and ZP birefringence in matured oocytes, between groups with different MM condition.

Polscope variables	Control	MM treated with CCs	MM supplemented with GDF-9	MM treated with CCs and GDF-9
Spindle visualization				
Visible (%)	17.64 (26.6)[a]	10.34 (29.4)	18.40 (45)[b]	14.52 (26.9)
No visible (%)	47.64 (73.4)	24.34 (70.6)	22.40 (55)	38.52 (37.1)
ZP birefringence				
High (%)	40.64 (62.5)[c]	18.34 (53)	18.40 (45)	19.52 (36.5)[d]
Low (%)	24.64 (37.5)	16.34 (47)	22.40 (55)	33.52 (63.5)

a vs. b: Odds ratio (95% CI) = 0.442 (0.19-1.08) P=0.05
c vs. d: Odds ratio (95% CI) = 0.345 (0.16-0.74) P=0.005

Table 4. Comparison of laboratory outcomes of matured oocytes with visible spindle, between groups with different MM condition.

Laboratory outcomes	Control	MM treated with CCs	MM supplemented with GDF-9	MM treated with CCs and GDF-9
Fertilized oocytes (%)	9.17 (52.9) [a]	7.10 (70) [b]	11.18 (61.1)	10.14 (71.4) [c]
Embryos (%)	6.9 (66.7) [d]	3.7 (42) [e]	9.11 (81.8)	10.10 (100) [f]
Good quality embryos (%)	4.6 (66.7)	1.3 (33.3)	6.9 (66.7)	4.10 (40)

a vs. b: Odds ratio (95% CI) = 0.21(0.04-1.01) P= 0.045
a vs. c: Odds ratio (95% CI) = 0.22 (0.05-1.00) P= 0.045
d vs. f: Odds ratio (95% CI) = 0.68 (0.42-1.05) P= 0.047
e vs. f: Odds ratio (95% CI) = 0.42 (0.18-1.01) P= 0.006

Table 5. Comparison of laboratory outcomes of matured oocytes with high birefringence ZP, between groups with different MM condition.

Laboratory outcomes	Control	MM treated with CCs	MM supplemented with GDF-9	MM treated with CCs and GDF-9
Fertilized oocytes (%)	23.40 (57.5) [a]	15.18 (83.3) [b]	13.22 (59.1) [c]	11.19 (57.9) [d]
Embryos (%)	16.23 (69.6) [e]	12.15 (80)	13.13 (100) [f]	9.11 (81.8)
Good quality embryos (%)	6.16 (37.5)	5.12 (41.7)	8.13 (61.5)	5.9 (55.6)

a vs. b: Odds ratio (95% CI) =0.18 (0.04-0.90) P=0.024
b vs. d: Odds ratio (95% CI) =5. 4 (0.96-30.8) P=0.042
b vs. c: Odds ratio (95% CI) = 5.1 (0.93-28.5) P=0.045
e vs. f: Odds ratio (95% CI) = 0.7 (0.53-0.91) P=0.027

Discussion

In the present study, we aimed to noticeably improve the competence of IVM of immature oocytes in the ICSI program using the advanced culture system. Approximately, 5-15% of aspirated oocytes during an IVF/ICSI cycle remain at the GV stage at the time of oocyte retrieval (5). These immature oocytes from COH cycles are usually discarded, although they failed to complete nuclear maturation because of resistance or less

response to hormonal stimulation (31) or they may be at an earlier developmental stage (32). Lee and colleagues (14) observed high in vitro oocyte maturation, fertilization and embryo development rates following COH. They also experienced pregnancy and live birth from IVM- generated oocytes (14). However, many are still capable of undergoing maturation and fertilization, if appropriate conditions are present in vitro (1).

Our study presented that supplementation of the maturation medium with GDF-9 or co-culturing with CCs did not improve the maturation rate of human GV oocytes in the IVM program. However, Ben-Ami et al., (4) reported that epidermal growth factors (EGF; Areg and Ereg) significantly improved the maturation rate of human GV oocytes. In conflict with our findings, others reported the increased nuclear maturation rate of GV oocytes cultured with GCs (5, 33, 34). Noticeably, in the aforementioned studies, it was applied the granulusa-intact human GV oocytes, whereas we co-cultured the denuded GV oocytes with pooled and processed CCs derived from other COCs. Besides, our data are not in line with the work of Johnson et al., (34), who reported that GV oocytes cultured with CCs matured at a significantly higher rate than did GV oocytes cultured without CCs. Recently, data of mouse oocytes undergoing IVM revealed an enhanced maturation rate using intact COCs. However, IVM outcomes did not improve in GV oocytes co-cultured with CCs (35).

The basal IVM rate of GV oocytes collected from COH cycles is in the wide range between different studies due to several factors (36). Our data showed that the IVM rate seen over 24- 48 h in the control group (64%) is comparable with previous studies (1, 24). Interestingly, the IVM rate is lower (approximately 36%) after a 24 h period of culture (4, 32). According to our study, co-culturing GV oocytes with CCs results in an increased fertilization rate. Our findings are similar to those of the others, in terms of the optimistic effect of CCs on fertilization rate (5, 34). Recently, Sudiman et al., (21) noticed no improvement in mouse embryo development after the addition of GDF-9 to IVM media. Moreover, we observed that the embryo formation was enhanced when the immature oocytes were cultured in 3 different treated groups compared to controls. Overall, we have shown that there is an obvious advantage for treatment of GV oocytes with CCs and GDF-9 during IVM in fertilization and developmental competence yield. Additionally, it was not observed better results for fertilization rate and embryo formation when co-cultured with CCs and GDF-9 were applied simultaneously.
Two types of intracellular communication of gap junctions and paracrine factors between oocyte and surrounded CCs have been described (37). In the gap junctional mode, the CCs are attached to the oocyte through long microvilli that cross through the ZP to contact the oolemma (38). Moreover, a part of the bidirectional communication between CCs and oocytes is served via signaling molecules in a paracrine manner (21). The fact that OSFs can act even without direct contact between CCs and oocytes (39), suggesting the feasibility of CCs in a co-culture system. On the other hand, several disadvantages for the application of somatic cell co-culture have been discussed, including the risk of contamination, technical problems and involvement of unidentified factors (40).

Culture conditions and culture media components are one of the key factors regulating the quality of oocytes in IVM cases. The culture system can affect and even modulate the meiotic regulation of mammalian oocytes (5, 41). On the other hand, it has been reported that high-quality embryos are obtained following IVM if normal oocytes are used (42). Moreover, Omidi and associates (24) have recently demonstrated a lower rate of spindles visualization in IVM oocytes than in vivo matured oocytes. The data generated from the present study revealed that the spindle visualization rate is higher when the IVM medium was treated with GDF-9, and regarding ZP birefringence intensity, the high birefringent ZP oocytes did not differ between groups. Also, we observed a higher tendency in fertilization rate in detected spindle within oocytes in all treated groups compared to the control group. Kilani and colleagues (43, 44) found that the fertilization rate would reduce significantly under in vivo matured oocytes without visible MS. In contrast to their findings, we previously did not notice any significant differences in the fertilization rate between two groups of oocytes in regards to spindle detection (1). Furthermore, our data confirmed that there is a tendency for more fertilization, embryo formation and the number of good quality embryos in high birefringent oocytes that were matured in the treated medium than the

control group. In general, more studies suggested that assessment of birefringent structures normality is a predictive value for the chances of fertilization, embryo development and pregnancy. However, this study has assessed, for the first time, the fertilization and embryo development in matured oocytes in different conditions of IVM medium in relation to birefringent structure features.

Conclusion

It is concluded that co-culturing the immature oocytes with CCs and/or supplementation of the IVM medium with GDF-9 is potent in the improvement of fertilization and embryo development. We also propose using polarized light microscopy as a predictor of good quality oocytes and selection of the best embryo. Further studies are needed by sibling oocytes in different groups, also the oocytes from IVM cycles to verify the findings generated from this study.

References

1. Ashourzadeh S, Khalili MA, Omidi M, Mahani SN, Kalantar SM, Aflatoonian A, Habibzadeh V. Noninvasive assays of in vitro matured human oocytes showed insignificant correlation with fertilization and embryo development. Arch Gynecol Obstet 2015; 292(2): 459-463. https://doi.org/10.1007/s00404-015-3644-y
2. Khalili MA, Nottola SA, Shahedi A, Macchiarelli G. Contribution of human oocyte architecture to success of in vitro maturation technology. Iran J Reprod Med 2013; 11(1): 1-10. ISSN: 16806433
3. Shahedi A, Khalili MA, Soleimani M, Morshedizad S. Ultrastructure of in vitro matured human oocytes. Iran Red Crescent Med J 2013; 15(12). https://doi.org/10.5812/ircmj.7379
4. Ben-Ami I, Komsky A, Bern O, Kasterstein E, Komarovsky D, Ron-El R. In vitro maturation of human germinal vesicle-stage oocytes: role of epidermal growth factor-like growth factors in the culture medium. Hum Reprod 2011; 26(1): 76-81. https://doi.org/10.1093/humrep/deq290
5. Goud PT, Goud AP, Qian C, Laverge H, Van der Elst J, De Sutter P, Dhont M. In-vitro maturation of human germinal vesicle stage oocytes: role of cumulus cells and epidermal growth factor in the culture medium. Hum Reprod 1998; 13(6): 1638-1644. https://doi.org/10.1093/humrep/13.6.1638
6. Son WY, Lee SY, Yoon SH, Lim JH. Pregnancies and deliveries after transfer of human blastocysts derived from in vitro matured oocytes in in vitro maturation cycles. Fertil Steril 2007; 87(6): 1491-1493. https://doi.org/10.1016/j.fertnstert.2006.11.027
7. Chian RC, Buckett WM, Tan SL. In-vitro maturation of human oocytes. Reproductive biomedicine online. 2004; 8(2): 148-166. https://doi.org/10.1016/S1472-6483(10)60511-1
8. Le Du A, Kadoch IJ, Bourcigaux N, Doumerc S, Bourrier MC, Chevalier N, Fanchin R, Chian RC, Tachdjian G, Frydman R, Frydman N. In vitro oocyte maturation for the treatment of infertility associated with polycystic ovarian syndrome: the French experience. Hum Reprod 2005; 20(2): 420-424. https://doi.org/10.1093/humrep/deh603
9. Coticchio G, Dal Canto M, Fadini R, Mignini Renzini M, Guglielmo MC, Miglietta S, Palmerini MG, Macchiarelli G, Nottola SA. Ultrastructure of human oocytes after in vitro maturation. Mol Hum Reprod 2016; 22(2): 110. https://doi.org/10.1093/molehr/gav071
10. Lin YH, Hwang JL, Huang LW, Mu SC, Seow KM, Chung J, Hsieh BC, Huang SC, Chen CY, Chen PH. Combination of FSH priming and hCG priming for in-vitro maturation of human oocytes. Hum Reprod 2003; 18(8): 1632-1636. https://doi.org/10.1093/humrep/deg335
11. Piquette GN. The in vitro maturation (IVM) of human oocytes for in vitro fertilization (IVF): is it time yet to switch to IVM-IVF? Fertil Steril 2006; 85(4): 833-835. https://doi.org/10.1016/j.fertnstert.2005.08.069
12. Miki H, Ogonuki N, Inoue K, Baba T, Ogura A. Improvement of cumulus-free oocyte maturation in vitro and its application to microinsemination with primary spermatocytes in mice. J Reprod Develop 2006; 52(2): 239-248. https://doi.org/10.1262/jrd.17078

13. Veeck LL, Wortham Jr JE, Witmyer J, Sandow BA, Acosta AA, Garcia JE, Jones GS, Jones Jr HW. Maturation and fertilization of morphologically immature human oocytes in a program of in vitro fertilization. Fertil Steril 1983; 39(5): 594-602. https://doi.org/10.1016/S0015-0282(16)47052-4
14. Lee HJ, Barad DH, Kushnir VA, Shohat-Tal A, Lazzaroni-Tealdi E, Wu YG, Gleicher N. Rescue in vitro maturation (IVM) of immature oocytes in stimulated cycles in women with low functional ovarian reserve (LFOR). Endocrine 2016; 52(1): 165-171. https://doi.org/10.1007/s12020-015-0744-1
15. Guigon C, Cohen-Tannoudji M. Reconsidering the roles of female germ cells in ovarian development and folliculogenesis. Biol Aujourdhui 2011; 205(4): 223-33. https://doi.org/10.1051/jbio/2011022
16. Su YQ, Sugiura K, Wigglesworth K, O'Brien MJ, Affourtit JP, Pangas SA, Matzuk MM, Eppig JJ. Oocyte regulation of metabolic cooperativity between mouse cumulus cells and oocytes: BMP15 and GDF9 control cholesterol biosynthesis in cumulus cells. Development 2008; 135(1): 111-121. https://doi.org/10.1242/dev.009068
17. Sánchez F, Romero S, De Vos M, Verheyen G, Smitz J. Human cumulus-enclosed germinal vesicle oocytes from early antral follicles reveal heterogeneous cellular and molecular features associated with in vitro maturation capacity. Hum Reprod 2015; 30(6): 1396-1409. https://doi.org/10.1093/humrep/dev083
18. Tatemoto H, Sakurai N, Muto N. Protection of porcine oocytes against apoptotic cell death caused by oxidative stress during in vitro maturation: role of cumulus cells. Biol Reprod 2000; 63(3): 805-810. https://doi.org/10.1095/biolreprod63.3.805
19. Wongsrikeao P, Kaneshige Y, Ooki R, Taniguchi M, Agung B, Nii M, Otoi T. Effect of the removal of cumulus cells on the nuclear maturation, fertilization and development of porcine oocytes. Reprod Domest Anim 2005; 40(2): 166-170. https://doi.org/10.1111/j.1439-0531.2005.00576.x
20. Zhao SY, Qiao J, Chen YJ, Liu P, Li J, Yan J. Expression of growth differentiation factor-9 and bone morphogenetic protein-15 in oocytes and cumulus granulosa cells of patients with polycystic ovary syndrome. Fertil Steril 2010; 94(1): 261-267. https://doi.org/10.1016/j.fertnstert.2009.03.014
21. Sudiman J, Ritter LJ, Feil DK, Wang X, Chan K, Mottershead DG, Robertson DM, Thompson JG, Gilchrist RB. Effects of differing oocyte-secreted factors during mouse in vitro maturation on subsequent embryo and fetal development. J Assist Reprod Genet 2014; 31(3): 295-306. https://doi.org/10.1007/s10815-013-0152-5
22. Li Y, Li RQ, Ou SB, Zhang NF, Ren L, Wei LN, Zhang QX, Yang DZ. Increased GDF9 and BMP15 mRNA levels in cumulus granulosa cells correlate with oocyte maturation, fertilization, and embryo quality in humans. Reprod Biol Endocrinol 2014; 12(1): 1-9. https://doi.org/10.1186/1477-7827-12-81
23. Gode F, Gulekli B, Dogan E, Korhan P, Dogan S, Bige O, Cimrin D, Atabey N. Influence of follicular fluid GDF9 and BMP15 on embryo quality. Fertil Steril 2011; 95(7): 2274-2278. https://doi.org/10.1016/j.fertnstert.2011.03.045
24. Omidi M, Khalili MA, Ashourzadeh S, Rahimipour M. Zona pellucida birefringence and meiotic spindle visualisation of human oocytes are not influenced by IVM technology. Reprod Fertil Dev 2014; 26(3): 407-413. https://doi.org/10.1071/RD13001
25. Omidi M, Khalili MA, Nahangi H, Ashourzadeh S, Rahimipour M. Does women's age influence zona pellucida birefringence of metaphase II oocytes in in-vitro maturation program? Iran J Reprod Med 2013; 11(10): 823.
26. Dunning KR, Lane M, Brown HM, Yeo C, Robker RL, Russell DL. Altered composition of the cumulus-oocyte complex matrix during in vitro maturation of oocytes. Hum Reprod 2007; 22(11): 2842-2850. https://doi.org/10.1093/humrep/dem277
27. Gilchrist RB, Ritter LJ, Cranfield M, Jeffery LA, Amato F, Scott SJ, Myllymaa S, Kaivo-Oja N, Lankinen H, Mottershead DG, Groome NP. Immunoneutralization of growth differentiation factor 9 reveals it partially accounts for mouse oocyte mitogenic activity. Biol Reprod 2004; 71(3): 732-739. https://doi.org/10.1095/biolreprod.104.028852

28. Vanhoutte L, Nogueira D, De Sutter P. Prematuration of human denuded oocytes in a three-dimensional co-culture system: effects on meiosis progression and developmental competence. Hum Reprod 2009; 24(3): 658-669. https://doi.org/10.1093/humrep/den420
29. World Health Organization. WHO laboratory manual for the Examination and processing of human semen. Switzerland. WHO press; 2010.
30. Hill GA, Freeman M, Bastias MC, Rogers BJ, Herbert III CM, Osteen KG, Wentz AC. The influence of oocyte maturity and embryo quality on pregnancy rate in a program for in vitro fertilization-embryo transfer. Fertil Steril 1989; 52(5): 801-806. https://doi.org/10.1016/S0015-0282(16)61034-8
31. Farhi J, Nahum H, Zakut H, Levran D. Incubation with sperm enhances in vitro maturation of the oocyte from the germinal vesicle to the M2 stage. Fertil Steril 1997; 68(2): 318-322. https://doi.org/10.1016/S0015-0282(97)81522-1
32. Kim BK, Lee SC, Kim KJ, Han CH, Kim JH. In vitro maturation, fertilization, and development of human germinal vesicle oocytes collected from stimulated cycles. Fertil Steril 2000; 74(6): 1153-1158. https://doi.org/10.1016/S0015-0282(00)01617-4
33. Jahromi BN, Mosallanezhad Z, Matloob N, Davari M, Ghobadifar MA. The potential role of granulosa cells in the maturation rate of immature human oocytes and embryo development: A co-culture study. Clin Exp Reprod Med 2015; 42(3): 111-117. https://doi.org/10.5653/cerm.2015.42.3.111
34. Johnson JE, Higdon HL, Boone WR. Effect of human granulosa cell co-culture using standard culture media on the maturation and fertilization potential of immature human oocytes. Fertil Steril 2008; 90(5): 1674-1679. https://doi.org/10.1016/j.fertnstert.2007.06.017
35. Rahiminia T, Faramarzi A, Khoradmehr A, Khalili MA. Cumulus co-culture system does not improve the in-vitro maturation (IVM) of oocytes in mice. Middle East Fertil Soc J 2016; 21(2): 109-113. https://doi.org/10.1016/j.mefs.2015.10.003
36. Combelles C, Cekleniak N, Racowsky C, Albertini D. Assessment of nuclear and cytoplasmic maturation in in-vitro matured human oocytes. Hum Reprod 2002; 17(4): 1006-1016. https://doi.org/10.1093/humrep/17.4.1006
37. Eppig JJ. Intercommunication between mammalian oocytes and companion somatic cells. Bioessays 1991; 13(11): 569-574. https://doi.org/10.1002/bies.950131105
38. Motta PM, Makabe S, Naguro T, Correr S. Oocyte follicle cells association during development of human ovarian follicle. A study by high resolution scanning and transmission electron microscopy. Arch Histol Cytol 1994; 57(4): 369-394. https://doi.org/10.1679/aohc.57.369
39. Hussein TS, Froiland DA, Amato F, Thompson JG, Gilchrist RB. Oocytes prevent cumulus cell apoptosis by maintaining a morphogenic paracrine gradient of bone morphogenetic proteins. J Cell Sci 2005; 118(22): 5257-5268. https://doi.org/10.1242/jcs.02644
40. Vajta G, Peura TT, Holm P, Paldi A, Greve T, Trounson AO, Callesen H. New method for culture of zona-included or zona-free embryos: The Well of the Well (WOW) system. Mol Reprod Dev 2000; 55(3): 256-264. https://doi.org/10.1002/(SICI)1098-2795(200003)55:3<256::AID-MRD3>3.0.CO;2-7
41. Downs SM, Mastropolo AM. Culture conditions affect meiotic regulation in cumulus cell-enclosed mouse oocytes. Mol Reprod Dev 1997; 46(4): 551-566. https://doi.org/10.1002/(SICI)1098-2795(199704)46:4<551::AID-MRD13>3.0.CO;2-Z
42. Mikkelsen AL, Lindenberg S. Morphology of in-vitro matured oocytes: impact on fertility potential and embryo quality. Hum Reprod 2001; 16(8): 1714-1718. https://doi.org/10.1093/humrep/16.8.1714
43. Kilani S, Cooke S, Tilia L, Chapman M. Does meiotic spindle normality predict improved blastocyst development, implantation and live birth rates? Fertil Steril 2011; 96(2): 389-393. https://doi.org/10.1016/j.fertnstert.2011.05.023

44. Montag M, Schimming T, Köster M, Zhou C, Dorn C, Rösing B, Van Der Ven H, Van der Ven K. Oocyte zona birefringence intensity is associated with embryonic implantation potential in ICSI cycles. Reprod Biomed Online 2008; 16(2): 239-244. https://doi.org/10.1016/S1472-6483(10)60580-9

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