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Original Article

The Impact of *In vitro* Oocyte Maturity on Developmental Potential of Embryos Derived from Controlled Ovarian Stimulation Cycles

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Background and Aims: One of the major subjects for improving *in vitro* fertilization (IVF) outcome is the quantity and quality of retrieved oocytes. *In vitro* maturation (IVM) provides an opportunity for using immature oocytes routinely discarded in clinics. This study aimed at evaluating the quality of embryos derived from *in vivo* and rescue *in vitro* matured oocytes.

Materials and Methods: Totally, 462 immature oocytes as cases and 466 mature (MII) oocytes as controls were included for study of their developmental competence. Oocytes underwent intracytoplasmic sperm injection insemination and then denuded oocytes were microscopically assessed regarding cytoplasmic and nuclear maturity and quality.

Results: The morphological assessments showed fertilization rate of 60.9 and 61.4%, the embryo formation rate of 86.7% and 90.9% and arresting rate of 27.3% and 25.6% for the case and control oocytes, respectively. Evaluating embryo quality in the cleavage stage indicated that 63% of the embryos in the case group and 68% of the embryos in the control group were of good quality. There was no significant difference between fertility rate and arresting rate of oocytes matured in both groups, although the embryo formation rate and the quality of embryos differed significantly.

Conclusions: Our findings suggest that IVM is a valuable and practical option for patients who had to cancel IVF treatment cycles because of severe responses or resistance to routine hormonal therapies or those with low functional ovarian reserve.

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Introduction

In the beginning, *in vitro* maturation (IVM) of GV and MI oocytes was applied to overcome the problems associated with the administration of gonadotropin including ovarian hyper-stimulation syndrome (OHSS), high cost of drug and burden of *in vitro* fertilization (IVF) treatment on patients [1, 2]. Alternatively, IVM was used to retrieve immature oocytes for fertility preservation in patients with reproductive cancer and other hormone-sensitive diseases such as polycystic ovarian syndrome (PCOS) [3, 4].

Regarding the fact that about 10-15% of the retrieved oocytes in assisted reproductive technology (ART) cycles are immature, applying them during rescue IVM can help increase the fertility potential [5, 6]. On the other hand, it seems that immature oocytes which do not mature *in vivo*, even with exposure to supraphysiological levels of gonadotrophins in vitro, fail to acquire full developmental competence. There is also concern that aneuploidy rate increases in embryos derived from *in vitro* matured oocytes and low developmental competence of IVM oocytes resulting in the routine lack of application of IVM in IVF center [7-9]. Although some studies have compared morphology, fertility rates, embryo formation rates and pregnancy rates of embryos derived from *in vivo* and in-vitro matured oocytes, there are few investigations regarding developmental competence of immature oocytes. Therefore, we aimed to compare morphology, maturity, fertility and quality of embryos derived from *in vivo* and *in vitro* matured oocytes.

Materials and Methods

Participants

From April 2015 to August 2017, a total number of 98 infertile women referring to Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran, entered into the study. This prospective randomized study was approved by the Ethics Committee of our Institution. Written informed consents were signed by all the women included in our research study. All retrieved oocytes were randomly divided into case (include 462 GV/MI) and control (include 466 MII) groups. The inclusion criteria were: normal ejaculated sperm samples according to World Health Organization criteria [10], oocyte with normal morphology [11], and age < 37 years without history of recurrent pregnancy loss or IVF failure. Women with a background of a chromosomal abnormality or genetic disorder or severe endocrine disorder and also cycles using donor gametes were excluded.

IVF and IVM treatment

All participants in this prospective case-control study were stimulated by the gonadotropin-releasing hormone (GnRH) antagonist [12]. Oocyte aspiration was performed 36 h later transvaginally. Oocytes underwent intracyto-plasmic sperm injection (ICSI) process after enzymatic and mechanical removal of cumulus cells with 80 IU/ml hyaluronidase (Sigma Co, USA). Then, the denuded oocyte was microscopically assessed in terms of

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cytoplasmic and nuclear maturity and quality (Olympus Co, Japan). After washing twice with G-Mops-V1 (Vitrolife), immature oocytes at GV stage were collected for *in vitro* maturation and further comparison with *in vivo* matured oocytes. Included immature oocytes were cultured in blastocyst medium supplemented with 75 IU/L of human menopausal gonadotropin (IVF-M, LG Life Sciences, Jeonbuk-do, Korea) and were incubated 24 h at 37°C in 95% O_2, 6% CO_2 and 90% humidity under paraffin oil (MediCult) [13-15]. After 24 h, the oocyte maturity was assessed again with the observation of the PB1 under an inverted microscope (Nikon Co, Japan) to prepare them for ICSI insemination [3]. About 16-18 h after ICSI, the fertilization rate of oocytes was evaluated and compared between IVM and IVF groups. The fertilized oocytes were then cultured in cleavage medium for 72 h and then, the rate of embryo formation and embryo arresting was compared between the groups [16].

Statistical analysis

The data are presented as mean±standard deviation and percentage according to data normality. Data analysis was performed using SPSS software (version 20). The frequency distributions between the groups were analyzed using appropriate statical tests. All hypotheses were two-sided and $p<0.05$ was considered as significant.

Results

Patients, embryos and Oocytes' information

A total number of 98 infertile women with average age of 29.19±3.05 in the case and of 30.33±4.27 in the control group entered the study. The women were subdivided into four groups based on etiology of infertility in terms of male factor, female factor, both male and female factors and unknown (Table 1). Overall, 480 COC in MII group and 485 COC in GV group were retrieved and after microscopic assessment, 23 GV oocytes and 14 MII oocytes were excluded from the study due to abnormal morphology. Following *in vitro* maturation of GV oocytes, the study was followed by 462 MII oocytes in the case and 466 MII oocytes in the control groups. The MII oocytes were fertilized that led to the formation of 157 and 222 embryos in case and control groups, respectively. Embryonic divisions were followed up, and 43 embryos in the case and 57 embryos in the control group were arrested at 2-8 cells stages (Table 2).

Table 1. Demographic and etiologic characterization of the patients

Variables	Case	Control	P-value
Age (mean±SD)	29.19±3.05	30.33±4.27	0.08
Cause of infertility			
Male factor	36.4*	33.8	
Female factor	43.9	35.3	0.31
Both male and female factor	16.7	20.6	
Unknown etiology	3	10.3	

*Percent

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Developmental competence of mature and immature oocytes

Subsequent to *in vitro* maturation of GV oocytes, the rate of oocyte maturation and fertilization, embryo formation and embryo arresting as well, quality of embryos and stage of arresting in both groups were thoroughly investigated (summarized in tables 2 and 3). Maturation rate of oocytes was 64.2% and 85% in case and control groups, respectively. Although the fertilization rate of the oocytes did not differ significantly between the case (61%) and control (61.4%) groups, the embryo formation rate was significantly higher in the control (90.9%) than in the case (86.8%) group (p=0.001). Moreover, the arresting rate of the embryo was statistically insignificant in the control group in comparison with the cases (p=0.1). Also, there were insignificant differences between groups regarding the stage of arresting, although most of the arrests took place first in 4-cells and then in 6-cells stage. The results revealed no difference between the quality of embryo as 63% of the IVM embryos and 68% of the IVF embryos were of high quality (Table 3).

Table 2. Comparison of oocyte and embryo in the case and control groups

Variables	Control	Case
Total oocyte retrieval	480	485
Included oocytes	466	462
MII oocytes	397	297
2pn	244	181
Total embryo formation	222	157
Total embryo arrest	57	43
High-quality embryos	38	27
Low-quality embryos	19	16

Table 3. Descriptive analysis of maturation, fertilization and embryos development derived from *in vitro* matured oocytes (case) in comparison with *in vivo* matured oocytes (control)

Parameters	Control (MII)	Case	P-value
Maturation rate	85 (397/466)*	64.2 (297/462)	
Fertilization rate	61.4 (244/397)	61 (181/297)	0.614
Embryo formation	90.9 (222/244)	86.8 (157/181)	0.001
Arresting rate	25.6 (57/222)	27.3 (43/157)	0.1
Embryo quality			
Good	68 (150/222)	63 (98/157)	0.03
Poor	32.4 (72/222)	37.5 (59/157)	
2-cell	1	0	
3-cell	2	5	
4-cell	27	18	
5-cell	3	3	0.5
6-cell	15	10	
7-cell	0	1	
8-cell	9	6	

*Percent

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Discussion

Even with some differences in the study design, sample size, infertility etiology and stimulation protocols, similar results were reported by authors. Regarding the fertilization rates, our results are similar to the fertilization rates of 62.1% vs. 64.0% (reported by the Reichman et al. in 2010 or the fertilization rate of 61.7% vs. 61.6% documented by Fesahat et al. in 2018 [17, 18]. Developmental competence of 357 *in vitro* matured oocytes vs. 544 *in vivo* matured oocytes was investigated by Fesahat and colleagues. No significant differences were reported in the fertilization rate, embryo formation, arresting rate and quality of embryo in this study. However, based on our findings, the embryo formation rate and quality of embryo differ slightly. Also, rescue IVM potential in PCOS women was studied and high pregnancy and live birth rates were reported. Therefore, the authors attributed this to over-response or poor-response patients without concern for OHSS risk [19]. A study on PCOs patients demonstrated the opposite results as the clinical pregnancy rate was significantly lower in the IVM group (51.3% vs. 63.5%) [4]. Generally, the implantation potential of human embryos in both group still remains low for the reason that the majority of human IVM embryos are chromosomally abnormal [8]. On the other hand, the usual morphological selection method is not precise and appropriate for evaluating the oocyte and embryo maturity and quality [20, 21]. Another study used IVM in women with low functional ovarian reserve (n=10) in comparison with those with normal functional ovarian reserve (n=25). The findings confirmed that fertilization and embryo formation rates do not differ significantly between groups [22].

Conclusion

Overall, findings of our study and the mentioned research suggests using the rescue IVM treatments in ART especially for women with low functional ovarian reserve, with hormone sensitivity, or with cancer and other diseases including PCOs. Certainly, IVM will improve the available number of oocytes and thus the transferable embryos resulting in higher pregnancy and delivery rates.

Conflict of Interest

There is no conflict of interest.

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