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Original Article

Influential effect of age on oocyte morphometry, fertilization rate and embryo development following IVF in mice

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ABSTRACT

Changes of oocyte quality and decreasing of ovarian follicle reserve in advanced age are the major challenges in infertility treatments. One factor associated with aging is the thickness of zona pellucida, which has adverse relation with embryo score. Although, there is no correlation between perivitelline space and granulation with embryo quality, an inversely correlation is observed between these two factors with subsequent embryo quality. The aim was to investigate the influence of age on the oocyte quality, fertilization rate and embryo development following in vitro fertilization setting in mice. NMRI mice (N = 21) were categorized into 3 groups regarding to their ages (groups I–III; 25, 30, 35 weeks old, respectively). Standard in vitro fertilization protocol was conducted for each group. After collection of the oocytes, three points of zona pellucida and perivitelline space diameters were measured in each group and mean values were calculated. Also, the number of oocytes, fertilization rate, the number of cleavage embryos and blastocyst formation were compared among the groups. All the changes were insignificancy age related, as the mean value of zona pellucida and perivitelline space diameters as well as the number of oocytes, rate of fertilization and 2 cells embryos were higher in group I compared to other groups. Also, there was significant difference between some evaluated parameters, such as the number of generated 4 cells cleavage embryos and blastocysts as well as oocytes degeneration rates. The advanced maternal age influenced negatively on the oocyte morphometry and cleavage and blastocyst embryo formation in animal model.

1. Introduction

The age-related reduction in female fertility has been described to several causes containing progressive oocyte diminution, meiotic errors and mitochondrial dysfunction [1]. Furthermore, early embryonic survival, pregnancy outcome, fetal development as well as embryo quality [2] have been affected by oocyte quality acquired during folliculogenesis depending on some aspects, such as maternal age [3]. After fertilization, the embryo is differentiated from an egg with ability of cleavage and morphogenesis [4]. Although, eggs contain equal amounts of DNA paternity and motherhood, but most of the cytoplasm and mitochondria are derived from the oocyte [2].

One of the important factors in in vitro fertilization (IVF) success is the zona pellucida (ZP) glycoprotein that in addition to its role as a sperm receptor, participates in the acrosome reaction [5].The thickness of ZP, affected by maternal advanced age, is related to embryo quality score [6]. It is reported that implantation rate was higher than fertilized oocytes with thinner ZP than those labelled as thick zona [7].

Perivitelline space (PVS) is a gap between the surface of the oocyte and the ZP, an extracellular matrix synthesized by the oocyte. Actually, the PVS appears to play various roles before, during, and after fertilization [8]. Sayaka and associates compared the changes in the size of the mouse oocytes PVS during maturation in vivo and in vitro. Their data confirmed that it was larger in the oocytes matured in vivo in contract with the lower incidence of polyspermy after insemination than in oocytes matured in vitro. They suggested a negative relationship between the size of PVS and the incidence of polyspermy in mouse oocytes due to the presence of larger amount of hyaluronan in larger PVS may also prevent membrane fusion of the sperm and oocytes [9]. To our knowledge, there was no morphorphometric study with the aim of comparing the changes in the size of the mouse oocytes PVS after fertilization in IVF regarding maternal age. Therefore, the aim was to evaluate the effect of age on some morphometric aspects of oocytes, with consequent embryo developmental ability to the blastocyst following IVF protocol in mice. (see Fig. 1)

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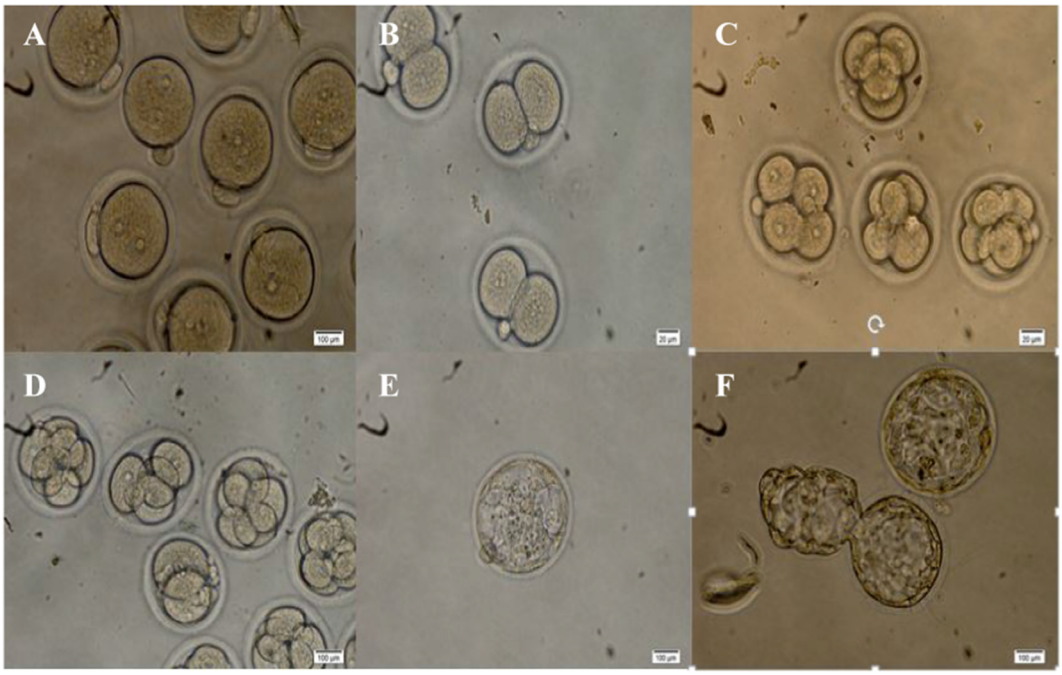

Fig. 1. Embryo development in vitro. (A) 2PN zygote with second polar body (B), 2-cell (C), 4-cell (D), 8-cell (E), morula (F), blastocyst.

2. Materials and methods

2.1. Animals

In this experimental study, 21 adult female NMRI mice were categorized into three groups regarding to their ages (groups I–III: 25, 30, 35 weeks old, respectively), maintained under controlled temperature (25 ± 38 C), proper humidity (50 ± 5%) and a 12 h light/dark cycle. This study was approved by the Ethics Committee of Institute for Reproductive Sciences, Shahid Sadoughi University of Medical Sciences.

2.2. Ovarian stimulation and oocyte preparation

Superovulation was induced by intraperitoneal injections of 10 IU PMSG (Pregnant Mare's Serum Gonadotropin; vwr scientific INC.) and 10 IU hCG (human Chorionic Gonadotropin, CG-10; Sigma) administered 48–50 h apart. The oviducts were cut out 15 h after hCG injection and oocytes were collected by flushing method. Immediately, cumulus-oocyte complexes (COCs) were placed in G-MOPS media. After rinsing 3–5 times, the oocytes from each group were transferred into G-IVF medium at 37 C in an incubator with 5% CO2 and 95% air with high humidity.

2.3. In vitro fertilization and embryo assessment

For IVF, the spermatozoa were collected from 8-12 weeks old mice. The sperms were released into the medium and dispersed for 15 min at 37 C. After dispersion, the sperm concentration was determined to achieve final concentration of $1*10^6$ sperm/mL. The insemination dishes were then incubated for 1–2 h before addition of oocytes. The thawed oocytes from each group were separately transferred to 100 μL droplets of G-IVF medium. After 5 h of incubation with spermatozoa, the oocytes were washed and cultured in G1 medium. Fertilization was determined by the presence of two pronuclei. The progression of embryonic development was monitored every 24 h for 3 days until blastocyst stage.

2.4. Morphometry and morphology evaluations

After fertilization and washing the oocytes, they were placed into G1 medium. Then, we measured thickness of ZP (μm) and PVS (μm) in three different areas of oocytes. Measurements accomplished with inverted microscope equipped with cornus imaging program (Research instruments Ltd. Co., UK). Finally mean values were calculated between groups.

2.5. Statistical analysis

The analyses were performed using SPSS (version 20, USA). The data were analyzed by one way ANOVA fallowed by Post Hoc test. P value of <0.05 was considered significant.

3. Results

A total of 60 oocytes were obtained from 21 stimulated cycles in adult NMRI mice. Retrieved oocytes were categorized into three groups according to the mice age. It was obtained insignificant number of oocytes during stimulated IVF cycles between the groups ($P = 0.66$, Table 1). However, the least degeneration rate of oocytes was seen in group I, compared with other groups (P = 0.04). Also, fertilization rate as well as the number of two cells embryos were statistically not differed between the three groups, although, there was more positive effects at group I than others (Table 1). However, the number of 4 cells embryos in group I were significantly higher compared to the other groups ($P = 0.003$). This revealed detectable arrest rates of embryos from cleavage stage between three groups to blastocyst stage (15, 9 and 9 in groups of I–III, respectively; $P = 0.010$). Also, our data showed that the most significant rates of high quality blastocysts were belonged to group I ($P = 0.00$) despite of insignificancy in oocytes and cleaved embryos ($P \geq 0.05$). Regarding the morphometric assessments of ZP and PVS diameters, zygotes from older mice showed diminished PVS diameter and thicker ZP.

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Table 1
Mean ± SD of oocyte morphometry, fertilization and different developmental status of embryos during IVF of oocytes in three experimental groups.

Groups				
Variable	I	II	III	p-value
Oocytes	16.43 ± 6.02	14.14 ± 6.34	12.14 ± 3.22	0.66
Degenerated oocytes	2.82 ± 0.74	3.2 ± 1.4	6.06 ± 2.18	0.04[a]
ZPthickness (μm)	6.7 ± 1.3	6.7 ± 0.74	7.04 ± 1.19	0.81
PVS (μm)	6.6 ± 1.98	5. 3 ± 1.12	4.7 ± 1.81	0.13
Oocyte diameter (μm)	61.7 ± 5.3	60.5 ± 2.4	63.5 ± 1.07	0.93
Fertilization	5.28 ± 4.23	4.43 ± 0.95	3.00 ± 0.76	0.36
2 cells embryos	5.28 ± 4.23	4.43 ± 2.51	3.00 ± 0.75	0.36
4 cells embryos	16.50 ± 0.56	10.00 ± 0.78	6.50 ± 0.9	0.003[a]
Blastocysts	15.00 ± 1.03	9.00 ± 2.3	9.00 ± 0.12	0.01[a]
Degenerated embryos	2.86 ± 2.48	6.28 ± 2.49	7.86 ± 8.74	0.22

[a] There is a significant difference between groups ($p < 0.01$). Values are presented as mean ± SD.

4. Discussion

Decrease of ovarian follicle reserve in advanced age is a major challenge in infertility treatment. It was shown that in advanced age, the level of estradiol and inhibin B was diminished; while, the FSH level is increased [10]. This phenomenon causes to decrease follicular response as well as the quality and the number of the oocytes. On the other hand, some studies have reported that the supplementation of estradiol can ameliorate the response of ovary to hormone replacement therapy [11]. Besides, in mice, it was shown that with age, the amount of AMH reduces and lead to the decrease of ovarian growing follicle which is related to the number of the primordial follicle [12].

Our findings revealed that there is reasonable downfall in retrieved oocytes number as well as higher significant oocytes degeneration rates in group III compared with others. One study on assessed the influence of the age related changes on the ultrastructure of the follicles in human ovaries. They concluded that detectable morphological changes related with age in several cell organs, such as mitochondria, Golgi complex. This could be the cause of atresia on initiation of follicular growth because of the substantial increase in metabolic requirements [13]. Also, it is established that in the female either human or other mammalians, the number of oocytes decreases [14] with age that is in agreement with our findings.

Furthermore, we observed that positive increasing on ZP diameters in regards to maternal age despite of insignificancy. The objective of morphometrical assessment helps to choose the oocytes as well as zygotes with higher developmental potential before embryo transfer. Following IVF, fertilized oocytes can assess in more details on the aspect of the extracytoplasmic structures, such as the ZP, first polar body and PVS [15]. The relationship between ZP structure and aging is a controversial issue. In contrast with our findings, Valeri et al. showed that there is a significant positive correlation between thickness of the ZP and women's age [16]. While, some studies have suggested reversed correlation between ZP thickness and patients age [17].

Our data showed that the rate of fertilizations were similar between the three groups ($p = 0.364$). Influence of age on the fertilization rate is controversial in the literature. There are indications of both a reduced fertilization rate as well as an uninfluenced impact by age [16]. One of the reasons for the age related poor quality of oocytes and embryos, is that the expression of oocyte genes, in a variety of major functional categories including cell cycle regulation, cytoskeletal structure, energy pathways, transcription control, and stress responses, are influenced by maternal age [1]. One of the major limitations of the present work was lack of pregnancy following by embryo transfer in mice with different age categories in regard with morphometric and their morphological quality scoring that should be considered in further study.

5. Conclusion

The advanced age influenced the oocyte morphometry and cleavage & blastocyst embryo formation. Although, the role of oocyte aging as one of the important factors in the failure of the ART is verified, but new predictive noninvasive methodologies, such as time laps monitoring and advanced genetic testing can have beneficial advantages. The couples with advanced age may benefit from these high technologies in ART program.

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Compliance with ethical standards

The authors declare that they have no competing interests. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving animal subjects were approved by all authors and the ethics committee of Research and Clinical Center for Infertility, Yazd, Iran.

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