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Original Article

Comparing the effects of different in vitro maturation media on IVM outcomes of MI oocytes

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A B S T R A C T

Background: Optimizing in vitro maturation (IVM) media for better assisted in vitro fertilization (IVF) outcome has been the matter of interest in recent years. Commercial media for IVM have been commonly used while, they have several disadvantages, such as limited shelf life and more cost than standard culture media for IVF centers, where IVM services are not routinely performed.
Objectives: This study was performed to compare four different culture media with respect to their supplementary effects to the meiotic progression of metaphase I (MI) oocytes to full maturity in different times. Also, we compared fertilization and embryo developmental rates of oocytes undergoing IVM in stimulated intracytoplasmic sperm injection (ICSI) cycles.
Material and methods: A total of 114 MI oocytes were divided into four groups. They were cultured for 24–48 h at 37 °C at different mediums including; homemade IVM medium (I), cleavage medium (II), blastocyst medium (III) and Sage IVM medium (IV). ICSI was performed for in vitro matured oocytes and the rates of fertilization and embryo development between groups were compared.
Results: There were no significant differences in total maturation, fertilization and embryo development rates between groups I–IV ($p = 0.4$, 0.8, and 0.8, respectively). In addition, this study showed that longer incubation time of MI oocytes did not improve the IVM outcomes.
Conclusion: While the immature oocytes rescued from stimulated cycles based on specific conditions of patients can be useful for an alternative IVM intervention, it seems that different commercial culture media and longer incubation time has no beneficial effects on maturation, fertilization and embryo development on oocytes at MI stage.
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1. Introduction

In vitro maturation (IVM) protocols have been applied for patients undergoing intracytoplasmic sperm injection (ICSI) cycles with controlled ovarian hyperstimulation or non-stimulated cycles [1]. The use of IVM in stimulated cycles particularly has benefits for women with poor response to gonadotropin treatment, diminished ovarian reserve, premature ovarian failure, or for those undergoing oocyte cryopreservation cycles for medical or social indications. The main goal of IVM for these patients would be to maximize the number of mature oocytes available for potential fertilization [2]. Also, some studies demonstrated normal live births from IVM of oocytes during stimulated cycles as well as IVM in nonstimulated cycles [3–6]. Recently, several investigations have focused on culture media optimization in order to improve the IVM techniques, but they have not been clearly analyzed yet [7–9]. In different clinical or research studies, different types of media for IVM have been applied such as human tubal fluid [10], culture medium 199 [11,12], and blastocyst medium [12] besides the commercially available MediCult IVM media (Origio A/S, Jyllinge, Denmark) and Sage IVM media (Cooper Surgical, Trumbull, Connecticut) that are often used with reasonable implantation and pregnancy rates [13]. However, applying these especial formulated commercial media have several disadvantages, such as limited shelf life and more cost than standard culture media for assisted in vitro fertilization (IVF) centers, where IVM services are not routinely performed.

The aim of our study was to compare four different culture media, including specialized IVM/IVF media and our homemade

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culture media, with respect to their supplementary effects to the meiotic progression of metaphase I (MI) oocytes to full maturity in different times. In addition, we compared fertilization and embryo developmental rates of oocytes undergoing IVM in stimulated ICSI cycles.

2. Materials and methods

2.1. Patient selection and oocyte collection

In this prospective experimental study, during 220 ICSI cycles, a total of 130 immature MI oocytes were retrieved from infertile women, aged 23–37 years. Overall 114 out of 130 MI oocytes were included to the study according to morphological assessment as inclusion criteria [14], whereas, 16 MI oocytes were excluded from the study due to either cytoplasmic or extracytoplasmic abnormalities. The estimated sample size ($\alpha = 0.05$, $\beta = 0.2$) in each group was considered 55 based on table of random numbers. Total numbers of immature MI oocytes from each ICSI cycle that have inclusion criteria in the study was used in intended group (Fig. 1). The standard gonadotropin-releasing hormone (GnRH) antagonist stimulation protocols were used for all participants. The written consent form was filled out by the patients who were admitted to Research and Clinical Center for Infertility, Yazd, and Iran.This study was approved by the ethics committee of the authors' institution. Human chorionic gonadotrophin (hCG; 10,000 IU) was injected 36 h before oocyte collection. After enzymatically and mechanically removal of cumulus cells with 80 IU/ml hyaluronidase (Sigma Co, USA), denuded oocytes were assessed for nuclear maturity under the stereo microscope (Olympus Co, Japan). Then, they were washed twice with G-Mops-V1 (Vitrolife) and normal MI oocytes were collected for further use.

2.2. Maturation, fertilization and embryo development

The selected oocytes at MI stage were divided into four groups and cultured in; homemade IVM medium (I), cleavage medium (II), blastocyst medium (III) and Sage IVM medium (IV).They were cultured in groups of 3 oocytes in 25 μL drops of all maturation medium that were supplemented with 75 IU/L of human menopausal gonadotropin (IVF-M, LG Life Sciences, Jeonbuk-do, Korea), cultured for 24 to 48 h at 37 °C in 5% O_2, 6% CO_2 and 90% air with high humidity under paraffin oil (MediCult) and without medium renewal.

The homemade IVM medium was consisting of Ham's F10 (Biochrom Co, Germany) supplemented with 0.75 IU luteinizing hormone (LH) plus 0.75 IU follicle stimulating hormone (FSH) (Ferring Co, Germany) with 40% human follicular fluid (HFF). For HFF preparation, collected HFF in the day of oocyte retrieval was centrifuged at 3500 RPM for 10 min to separate blood and granulosa cells. After inactivation at 56 °C for 30 min, the clear HFF was filtered with 0.22 μm filter and used for media preparation [15]. The oocytes were assessed for maturity after 24 and 48 h by the presence of the first polar body (PBI) under an inverted microscope (Nikon Co, Japan) [16]. ICSI was performed for in vitro matured oocytes and the rates of fertilization and embryo development were compared between four groups. After ICSI, oocytes were cultured in groups of up to 3 in 20 μL drops of cleavage medium (Cook Medical) in groups I, II and III and Sage embryo maintenance medium in group IV in a culture dish covered with paraffin oil. Fertilization was checked 16 h after ICSI and computed in each

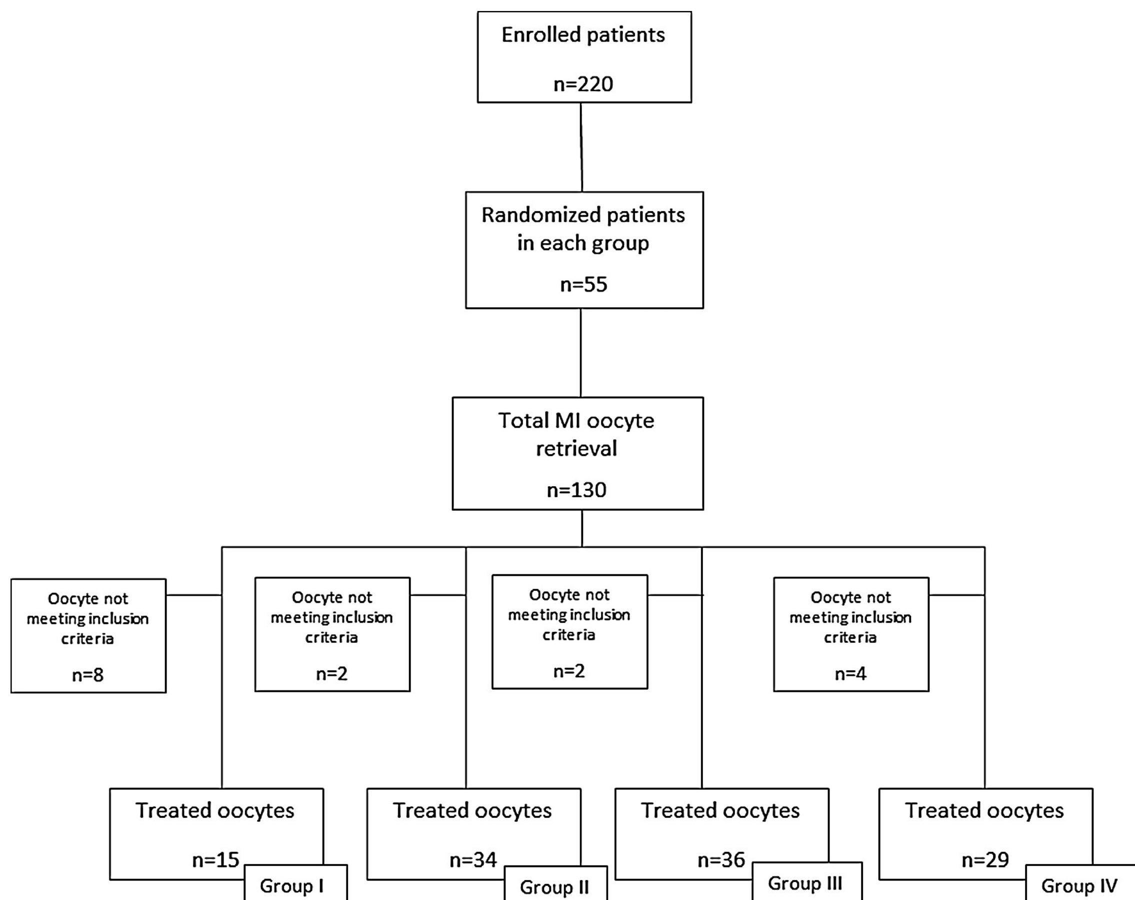

Fig. 1. Flowchart of the patients included in the study.

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group. Then, fertilized oocytes were cultured in maintenance or cleavage medium for 72 h and embryo development were assessed according to embryo grading [17].

2.3. Statistical analysis

The categorical data were presented in frequencies and percentages and Chi-Square test has been used for statistical analysis when more than 25% of table cells have a frequency less than 5%. In tables which data frequency has a smaller amount, Fisher exact test with less power was used. Comparisons of the frequency of data between groups, such as fertilization and developmental rates, were performed using Chi square tests by the SPSS software (Statistical Package for the Social Sciences version 20.0, SPSS Inc., Chicago, IL, USA). P-value < 0.05 was considered to indicate statistical significance.

3. Results

In total, 130 immature oocytes at MI stage were obtained from routine 220 ICSI stimulated cycles. The morphology assessment of oocytes were done using microscope visualization and 16 MI oocytes (12.31%) with abnormal morphology such as shape, dark cytoplasm or diffuse granulation, perivitelline space (PVS), central cytoplasmic granulation and cumulus oocyte complex were excluded from the study. While 114 oocytes (87.69%) were included with normal morphological criteria.

The oocytes were assessed for maturity after 24 and 48 h by the presence of the PBI under an inverted microscope (Fig. 2). MI oocyte has no visible nucleus and has not as yet extruded the PBI while, PBI is clearly visible in the PVS of matured IVM oocyte.

According to IVM media analysis between four groups, there were no significant differences in total maturation, fertilization and embryo development rates between groups I–IV ($p = 0.4$, 0.8 and 0.8 respectively) in 114 MI oocytes with normal morphology (Table 1). In contrast, the maturation rate of MI oocytes was lower at 48 h incubation compare to 24 h in all groups, except group I ($p < 0.01$). However, time duration of IVM did not change fertilization and embryo development rates of matured oocytes in all groups with different media.

4. Discussion

For maturation of oocytes outside the body we must provide optimal environment similar to natural milieu inside the body. Present study aimed to compare the maturation, fertilization and developmental capacity of oocytes involved on IVM program according to different IVM media and maturation times.

As our data showed, different IVM media did not have any significant effects on maturation rates on MI stage oocytes as well as other mentioned variables.

Moschini and colleagues [2] showed that commercially available culture media did not perform significantly better than standard IVF culture media for maturation of both MI and germinal vesicle (GV) stage oocytes rescued from stimulated IVF cases, that is in line with our data. On the other hand, some studies compared the formulation of different media using different sources of oocytes at GV stage from non-stimulated cycles that demonstrated significant differences between different culture media in IVM program. They indicated that some media have supposed to be valuable for IVM because of unique components, such as blastocyst medium [1,18]. It seems that the main reason for this contradiction goes back to oocyte developmental stage (MI stage) that may not need specialized component for reaching to MII. It must be noted that all different maturation media were supplemented with recombinant FSH and hCG, and also containing protein sources [19].

According to the time assessment, the results showed longer incubation time up to 48 h has not superior effects on IVM outcome. Son et al. demonstrated that developmental capacity of

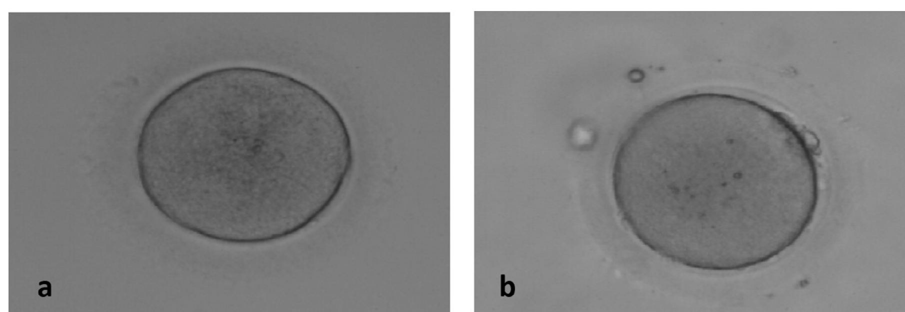

Fig. 2. (a) Denuded MI oocyte before IVM, This oocyte has no visible nucleus and has not as yet extruded the PBI (200× magnification). (b) Denuded MII oocyte after IVM; the PBI is clearly visible in the PVS (200× magnification).

Table 1
Nuclear maturation, fertilization and embryo formation rate of MI oocytes after 24 to 48 h cultured in different IVM media.[*]

Group	24 h			48 h			Total		
	Maturation	Fertilization	Embryo development	Maturation	Fertilization	Embryo development	Maturation	Fertilization	Embryo development
I	53.3 (8/15)	50 (4/8)	100 (4/4)	20 (3/15)	66.6 (2/3)	100 (2/2)	73.3 (11/15)	54.5 (6/11)	100 (6/6)
II	52.9[a] (18/34)	50 (9/18)	88.8 (8/9)	2.9[ab] (1/34)	100 (1/1)	100 (1/1)	55.8[b] (19/34)	52.6 (10/19)	90 (9/10)
III	61.1[a] (22/36)	68.1 (15/22)	86.6 (13/15)	11.1[ab] (4/36)	50 (2/4)	100 (2/2)	72.2[b] (26/36)	65.3 (17/26)	88.2 (15/17)
IV	51.7[a] (15/29)	66.6 (10/15)	90 (9/10)	13.7[ab] (4/29)	50 (2/4)	100 (2/2)	65.5[b] (19/29)	63.1 (12/19)	91.6 (11/12)
p-Value	0.8	0.5	0.8	0.2	0.7	1	0.4	0.8	0.8

[*] Note: data are presented as percentage (ratio number). Homemade IVM medium (I), cleavage medium (II), blastocyst medium (III) and Sage IVM medium (IV) cultured for 24 to 48 h at 37 °C.
[a] MI maturation rate has significant difference at 24 h incubation compare to 48 h incubation in each media group ($p < 0.01$).
[b] MI maturation rate has significant difference at 48 h compare to total time duration in each media group ($p < 0.01$).

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oocytes collected following HCG-priming in an IVM program is correlated with their maturation time [9]. They suggested that oocytes that reach faster to metaphase II in an IVM program have better embryonic developmental competence. The study by Mikkelsen [20] also showed a beneficial effect of FSH priming on pregnancy and implantation rates of immature oocytes by limiting the maturation time. Whereas, these rates were much lower when the maturation time was extended to 48 h that is in agreement with the present results. Furthermore, in stimulated ICSI cycles, immature oocytes were better to culture for 24–30 h and oocyte can be injected for further culturing after extrusion of the PBI [1]. Also, one study reported the most percentage of maturity in human GV oocytes without cumulus cells after IVM in stimulated cycles during 24–26 h [21] that were in line with the present study. From genetic point of view, Zhang et al. demonstrated that, when embryos were categorized based on maturation time of oocytes in IVM cycles, embryos derived from oocytes that matured 48 h after collection had a higher chromosomal abnormality rates compared with embryos derived from in vivo matured oocytes and to embryos derived from oocytes that matured in the first 24 h after collection [22]. They concluded that embryos derived from 24 h matured oocytes should be preferentially transferred in IVM cycles.

One of our study limitations was unequal number of immature oocytes in each media owing to diversity in the number of immature oocytes obtained from each ICSI cycle. But, it was tried to minimize errors by considering various aspects such as; sample size calculation, inclusion criteria for oocyte selection and performing ICSI and technical assay by one expert. Because, immature oocytes retrieved from stimulated ART cycles are usually discarded and don't use for patients, therefore, it is not possible to follow up the transfer outcomes of these oocytes and further study should be done to ascertain implantation and pregnancy potential of these embryos.

In addition, most of research studies used immature GV oocytes from natural IVF cycles or Cesarean sections [7,8] and therefore they were not compatible with our findings.

Another point to be mentioned regarding the performance of IVM program for special cases is the assessments of chromosomal abnormalities in comparison to in vivo matured oocytes that showed incoherence among studies [23–25].

In one study, the majority of the oocytes reaching the MII stage by IVM showed a normal unmethylated pattern comparing with GV and MI oocytes [26]. It seems that IVM efficiency should be measured by epigenetic reprogramming during meiosis, fertilization, implantation development, and live birth rates based on culture media and timing that now remains uncertain [2] and was another limitation of this study.

5. Conclusion

It seems that different commercial culture media as well as longer incubation time has no beneficial effects on maturation, fertilization and embryo development on oocytes at MI stage undergoing IVM, while the immature oocytes rescued from stimulated cycles based on specific conditions of patients can be useful for an alternative IVM intervention.

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