Original Research

In vitro maturation of human oocytes using culture in alginate matrix

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Abstract: **Introduction:** In vitro maturation (IVM) infertility treatment in women with infertility problems - for example, polycystic ovary syndrome (PCOS) or ovarian hyperstimulation syndrome (OHSS) reduces the amount of gonadotrophin medications, thereby reducing the cost of treatment. This study aimed to investigate the effect of alginate three-dimensional culture on maturity of immature human oocytes and the amount of 8-cell embryos produced by sperm injection to oocyte cytoplasm (ICSI).

Materials and methods: This experimental study was conducted in Mahdieh Infertility Research Center from 2015 to 2017. A total of 70 immature oocytes from the female ovary to candidate for fertilization were selected and divided into two control and experimental groups. In the control group, the basic culture medium (TCM199, FSH 0/75, FBS10 %) and in the experimental group, the basic culture medium with the alginate was used. Then, the mmature oocytes were fertilized by ICSI and 8-cell embryos was evaluated by reverse microscope. Quantitative data was analyzed using the ANOVA statistical method and SPSS software at significant level of a $P<0.05$.

Results: The amount of maturity of immature oocytes to MII metaphase stage and the arrival of the embryos to the 8-cell significantly increased in alginate group compared to the control group ($P<0.001$).

Conclusion: Results demonstrated that alginate can increase the maturity of immature human oocytes and also increases the rate of embryos reaching the 8-cell stage.

Keyword: Oocyte Maturation; In Vitro Maturation; Oocyte; Alginate; Infertility

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1. Introduction

Assisted Reproductive Technologies (ART) is a kind of protocol for ovulation stimulation in order to increase the number of oocytes available for fertilization (1). In Vitro Maturation (IVM) can be applied as an alternative and promising technique for the development of immature oocytes in laboratory conditions for some patients, including those with ovarian hyperstimulation syndrome (OHSS) and polycystic ovary syndrome (PCOS) (2).

IVM is considered as one of the fertility preservation methods to achieve a mature oocyte. Therefore, one of the most important steps in the production of an embryo is maturation of immature oocytes that have been stopped at the stage of I meiosis prophase (3). The simultaneous evolution of the nucleus and oocyte cytoplasm is an important criterion for maturity. Also, the maturation of the oocyte leads to the production of cheap and abundant embryos (4, 5).

The necessity of maturation of oocytes in the laboratory to reduce the problems caused by excess OHSS in recent years has been taken into consideration by embryologists (6). The efficiency of oocyte maturation in the laboratory has been significantly increased in new reproduction techniques (7).

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Laboratory maturity of mammalian oocytes was reported in rabbits by Enzmann and Pincus in 1935 (8), however, in humans, laboratory studies of puberty began with Pincus in 1939 (8). In 1962, Edward showed that mmature human oocytes can be fertilized in vitro culture medium (9) and the first successful birth was reported using this method in 1991 (10). Afterwards, they sought to examine different compounds in the maturity of immature oocytes and increase the amount of IVM culture media. Cultures contained chemicals materials, different amino acids, antibiotics, biological and protein materials (11). Different types of blood serum are added to the environment as supplementary material that play an important role in the culture medium (12). Since 1960, TCM199 was the most important culture medium for mammalian oocyte. Although, currently, few research laboratories tend to use this culture medium by adding serum and other growth factors (13).

The physical and chemical properties of the matrix are considered as the most important factors in the selection of a matrix suitable for the 3D culture system (14). Non-toxicity of the matrix and proper porosity are other critical factors for the 3D culture system (14). Alginate is a natural polymer produced by brown algae that consists of glucuronic acid (a-L-Guluronic acid) and mannuronic acid (b-D-Mannuronic acid) polymers (15). After grafting in the presence of calcium, alginate hydrogel is formed with no need for chemicals, light or temperature. The concentrations used for the alginate hydrogel should allow the exchange of food or hormones to give oocytes (16). Due to its ability to gel and high viscosity of alginates in aqueous solutions, this material is widely used. Alginate is characterized by the ability to produce hydrogel in physiological conditions, mild gel separation for cell retrieval, transparency for microscopic evaluation and gel pore porosity network for the release of nutrients (17).

Because of the importance of oocyte maturation in the culture medium for the treatment of infertility in this research, the effect of alginate was studied as an effective factor on the development of immature oocytes and the development of embryos in the culture medium.

2. Method

2.1. Study design and setting

This study was conducted in the Mahdieh Hospital of Tehran for 12 months from 2015 to 2017. The oocytes needed for this study were obtained from IVF female candidates who referred to the hospital and were treated with hormonal medications like FSH and HMG. None of these women's had OHSS. The ovaries of all patients were evaluated by continuous ultrasonography. All of them received 10,000 units of HCG hormone 36 hours before ovulation. During ovulation, some follicles are retrivaled less than 18 mm, which are related to immature oocytes. The oocytes are covered in this stage by granulosa cells and the diagnosis of mature and immature oocytes is difficult that granulosa cells were removed using hyaluronidase enzyme. In fact, immature oocytes are unusable for patients with ethical considerations and patient satisfaction was also obtained.

Germinal vesicle (GV) oocytes obtained during this study were randomly divided into two-dimensional media (n=35) and alginate (n=35). GV of each group were placed in the culture medium and covered with mineral oil (Ovoil, Vitrolife, Sweden). Then, there were placed in a 37 ° C incubator with 5% CO2 for 24 hours. In the next stage, the laboratory maturity stages and restoration of the meiosis were studied by reverse microscope (Olympus, Japan). Oocytes unchanged in the nucleus were considered immature oocyte and oocytes with a polar body were considered mature oocyte. Mature oocytes were used for fertilization using ICSI method and for this process, sperm was used by his spouse. Then, 2 µl of the sperm were placed in a drop of PVP (Vitrolife, Sweden) in plate containing a mature oocyte. After ICSI, fertilized oocytes were examined for 72 hours to 8-cell stage.

Basic culture medium for maturity of GV oocytes was TCM 199 (Gibco), Fetal Bovine Serum (FBS) (Gibco), pen/strep (Sigma), LH and FSH0.075IU/ml (Gonal-F, Sereno). Oocytes in the culture medium inside the 50 microliter droplets, the incubator medium containing 5% with a 95% humidity was placed. After 24 hours, oocytes under microscope were evaluated for determining the stages of growth and maturity.

2.2. Preparation of hydrogel alginate:

Initially sodium alginate (SIGMA) combined with phosphate-buffered saline (PBS) (SIGMA), and after being filtered, a 0.25 µm filter was kept at 4 ° C in the refrigerator. GV oocytes were separately transferred to a 5 microliter drop of sodium alginate. Then, for the formation of calcium and hydrogel grafts, droplets containing GV oocytes were transferred to a calcium bath using a micropipette, combining 140 ML of CaCl2 (MERC) and 50 ML of NaCl (MERC). After 2 minutes, drops of alginate gel were collected from the calcium bath and washed in the medium. Then, each GV was transferred to a drop of alginate by pipettes in 96-well plates.

2.3. Statistical analysis:

Data pertaining to oocyte maturation were analysed by SPSS software performing ANOVA test for control and treatment groups. A significance level of $p<0.05$ was

used throughout this study.

3. Result

Analysis of the resumption and reproductive maturity of immature oocytes in the alginate group compared to the control group showed that 35 immature oocytes (Figure 1) from the control group received 72% metaphase 2 (Figure 2) after 24 hours. Moreover, 35 oocytes in the alginate group received 85/15% metaphase 2 after 24 hours. Statistical analysis revealed that there is a significant increase in the maturity of the samples in the alginate group compared to those in the control group (Figure 3).

The examination of the reproductive maturity of the oocytes 72 hours after the ICSI process showed that fertilized oocytes in the control group were 67% in the 2nd stage of the cell and 33% in the necrosis. Of the fertilized oocytes in the alginate group, 20% of the embryos were in the 2-cell, 28% of the embryos were in the 4-cell and 32% were in the 8-cell stage (Figure 4). Oocyte maturity and fertilization in the in the alginate group than in the control group.

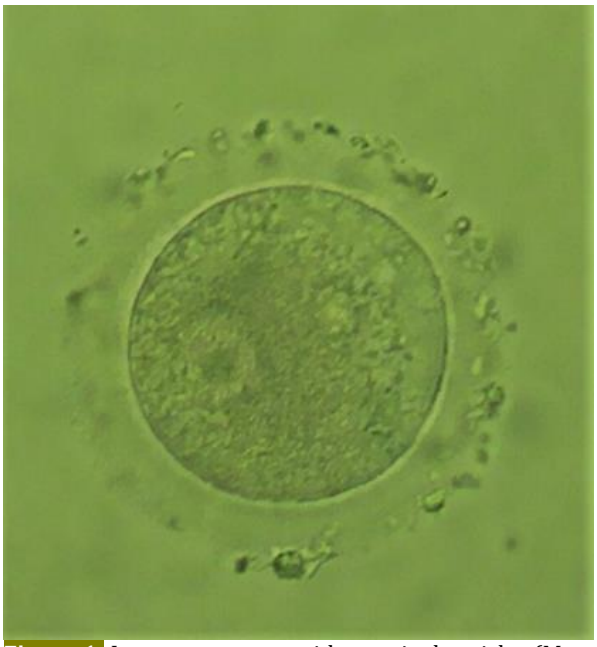

Figure 1: Immature oocyte with germinal vesicles (Magnification x40).

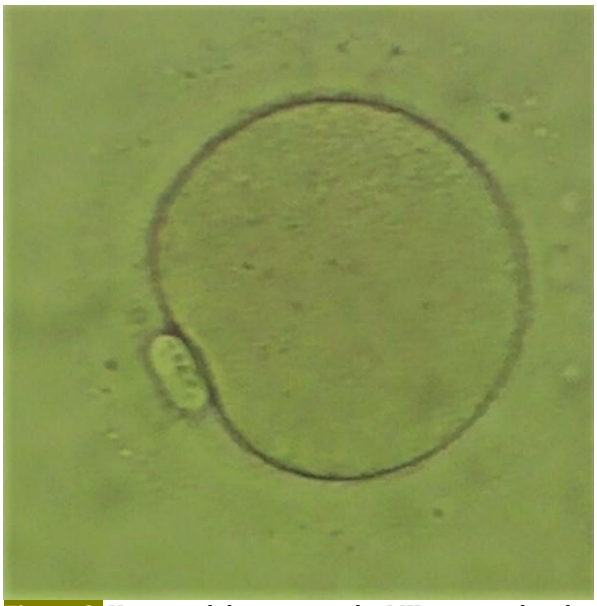

Figure 2: Human adult oocyte in the MII stage with polar body (Magnification x40).

4. Discussion

The results of this study showed that the GV oocyte in a three-dimensional culture of alginate caused a significant increase in the degree of maturity and development of oocytes and increased the fertilization rate. In the alginate group, it was found that the maturity was significantly different from the control group.

Laboratory maturity of human oocytes is a reproductive technology with many benefits. The success of these steps involves the maturity of the nucleus and the cytoplasm (18). When an oocyte grows, it will be able to continue and complete the meiosis. Cytoplasmic changes such as transcription, translation may also occur (19).

The use of gonadotropins as a well-known method to induce growth increases the number of follicles and ovulation in animals and humans (20). In this study, r-FSH hormone was considered as one of the main factors in cytoplasmic and nuclear maturity of immature oocytes. The results of this study showed that hormones can improve the experimental maturity of the oocytes.

Lechniak et al conducted a study on sheep oocysts and reported that in a culture medium containing a gonadotrophin hormone, the oocyte maturation is higher (12). The present study showed that the addition of gonadotropin to the culture medium improve maturity of the oocytes. Bolamba et al. carried out studies on dog oocytes and concluded that the percentage of oocytes stopped at the GV stage is much lower in culture containing gonadotropin (21). They suggested that they have to provide conditions other than the addition of hormones in media culture to improve oocyte maturation (21).

Few studies on the mechanical properties of alginate hydrogel and its effect on development and maturity oocytes have been carried out. Most studies have been focused on the culture of follicles in the alginate environment and have demonstrated that alginates improve ovarian follicular culture conditions (22). Min Xu et 2008 evaluated the follicle growth and oocyte maturation in mice by culture in alginate. They stated that secondary follicle growth and oocyte maturation could be

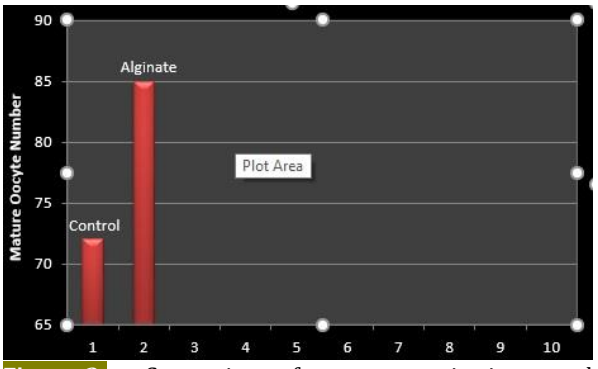

Figure 3: : Comparison of oocyte maturity in control group and gelati (24 h after culture).

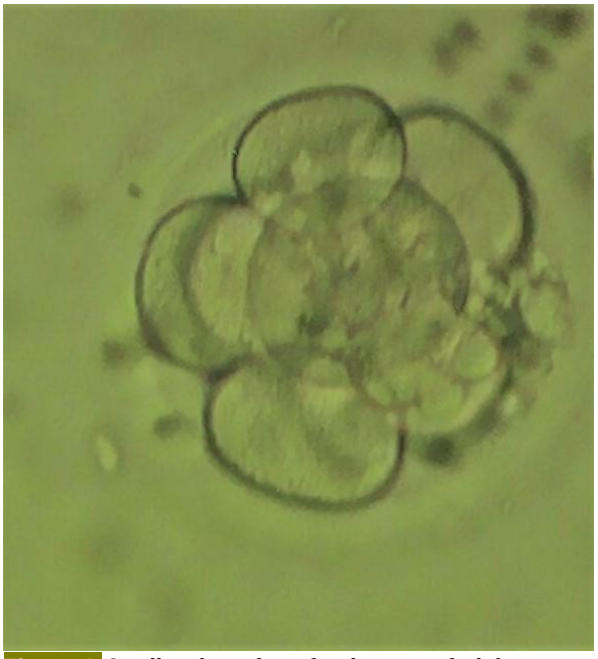

Figure 4: 8-cell embryo from fertilization of adult oocytes in culture medium by ICSI (Magnification x40).

successful in alginate culture (22). Stouffer et all 2011 studied on secondary follicle growth and oocyte maturation in in alginate. They demonstrated that culture conditions can support secondary follicle growth to the antral stage and oocyte maturation to the MII stage (23). Laronda et all evaluated human oocytes in three-dimensional coculture and reported that using 3D media provides better conditions for oocyte maturation (24). In the present study, the maturity of premature oocytes in alginate culture media is higher than that of two-dimensional medium and results are consistent with previous studies. No 8-cell embryos were observed in the control group, however, in the alginate group 32% reached the 8-cell stage. These results indicate that alginate improves the growth and development of fetal divisions.
These results can be discussed with regard to the physical properties of the hydrogel and the exchange of environmental compositions via hydrogel. The mechanical properties of the hydrogel depend on a variety of factors, such as the volume of the polymer and its bonds. In addition, the hydrogel constructs a lattice structure of alginate around oocytes and thus allows the exchange of essential hormones and proteins for the development of oocyte (15).
Further studies are also suggested in order to achieve optimal culture medium for oocyte laboratory maturity, development of the primary embryo and recognition of the exact mechanism.

5. Conclusion

The results of this study showed that using alginate can increase oocyte maturity and improve the embryo. Further studies are required on immature oocytes, and if possible, the embryos from these oocytes are transferred to the uterus and the pregnancy rate is also examined.

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7. Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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9. Author's contributions

Farhang Abed project development; Fereshte Aliakbari; Data collection and data analysis, Manuscript writing; Mohamad Ali Hossini Manuscript editing.

10. Reference

1. Farquhar C, Marjoribanks J. Assisted reproductive technology: an overview of Cochrane Reviews. Cochrane Database of Systematic Reviews. 2018(8).
2. Hatırnaz Ş, Ata B, Hatırnaz ES, Dahan MH, Tannus S, Tan J, et al. Oocyte in vitro maturation: A sytematic review. 2018.
3. Kedem A, Yerushalmi GM, Brengauz M, Raanani

H, Orvieto R, Hourvitz A, et al. Outcome of immature oocytes collection of 119 cancer patients during ovarian tissue harvesting for fertility preservation. Journal of assisted reproduction and genetics. 2018;35(5):851-6.
4. Bertoldo MJ, Walters KA, Ledger WL, Gilchrist RB, Mermillod P, Locatelli Y. In-vitro regulation of primordial follicle activation: challenges for fertility preservation strategies. Reproductive biomedicine online. 2018.
5. Mohsenzadeh M, Khalili M, Tabibnejad N, Salehi-Abargouei A, Karimi-Zarchi M, editors. Impact of vitrification on human oocytes before and after in vitro maturation (IVM): a systematic review and meta-analysis. HUMAN REPRODUCTION; 2018: OXFORD UNIV PRESS GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
6. Zhu J-Q, Liu Y, Zhang J-H, Liu Y-F, Cao J-Q, Huang Z-T, et al. Cadmium exposure of female mice impairs the meiotic maturation of oocytes and subsequent embryonic development. Toxicological Sciences. 2018.
7. Tiwari M, Prasad S, Tripathi A, Pandey AN, Ali I, Singh AK, et al. Apoptosis in mammalian oocytes: a review. Apoptosis. 2015;20(8):1019-25.
8. Pincus G, Enzmann EV. The comparative behavior of mammalian eggs in vivo and in vitro: I. The activation of ovarian eggs. Journal of Experimental Medicine. 1935;62(5):665-75.
9. Horn EC. Extranuclear histone in the amphibian oocyte. Proceedings of the National Academy of Sciences of the United States of America. 1962;48(2):257.
10. Cha KY, Koo JJ, Ko JJ, Choi DH, Han SY, Yoon TK. Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture in vitro and their transfer in a donor oocyte program. Fertility and sterility. 1991;55(1):109-13.
11. Khashavi Z, Karimzadeh M. Comparison of Unstimulated In Vitro Maturation and Stimulated: In Vitro Fertilization in Women with Poly Cystic Ovarian Syndrome. Journal of Research in Medical Sciences. 2004;9(6):260-3.
12. Accardo C, Dattena M, Pilichi S, Mara L, Chessa B, Cappai P. Effect of recombinant human FSH and LH on in vitro maturation of sheep oocytes; embryo development and viability. Animal reproduction science. 2004;81(1-2):77-86.
13. de Matos DG, Gasparrini B, Pasqualini SR, Thompson JG. Effect of glutathione synthesis stimulation during in vitro maturation of ovine oocytes on embryo development and intracellular peroxide content. Theriogenology. 2002;57(5):1443-51.
14. Cortvrindt R, Smitz J, Van Steirteghem A. Ovary and ovulation: In-vitro maturation, fertilization and embryo development of immature oocytes from early preantral follicles from prepuberal mice in a simplified culture system. Human Reproduction. 1996;11(12):2656-66.
15. West ER, Xu M, Woodruff TK, Shea LD. Physical properties of alginate hydrogels and their effects on in vitro follicle development. Biomaterials. 2007;28(30):4439-48.
16. Kreeger PK, Deck JW, Woodruff TK, Shea LD. The in vitro regulation of ovarian follicle development using alginate-extracellular matrix gels. Biomaterials. 2006;27(5):714-23.
17. Brito I, Silva G, Sales A, Lobo C, Rodrigues G, Sousa R, et al. Fibrin–alginate hydrogel supports steroidogenesis, in vitro maturation of oocytes and parthenotes production from caprine preantral follicles cultured in group. Reproduction in Domestic Animals. 2016;51(6):997-1009.
18. Segers I, Mateizel I, Van Moer E, Smitz J, Tournaye H, Verheyen G, et al. In vitro maturation (IVM) of oocytes recovered from ovariectomy specimens in the laboratory: a promising "ex vivo" method of oocyte cryopreservation resulting in the first report of an ongoing pregnancy in Europe. Journal of assisted reproduction and genetics. 2015;32(8):1221-31.
19. Dahan MH, Tan SL, Chung J, Son W-Y. Clinical definition paper on in vitro maturation of human oocytes. Human Reproduction. 2016;31(7):1383-6.
20. Wei S, Gong Z, Zhao H, Liang H, Lai L, Deng Y. Equine chorionic gonadotropin influence on sheep oocyte in vitro maturation, apoptosis, and follicle-stimulating hormone receptor and luteinizing hormone receptor expression. Genet Mol Res. 2016;15(4):1-12.
21. Bolamba D, Russ KD, Harper SA, Sandler JL, Durrant BS. Effects of epidermal growth factor and hormones on granulosa expansion and nuclear maturation of dog oocytes in vitro. Theriogenology. 2006;65(6):1037-47.
22. Xu M, West E, Shea LD, Woodruff TK. Identification of a stage-specific permissive in vitro culture environment for follicle growth and oocyte development. Biology of reproduction. 2006;75(6):916-23.
23. Xu J, Lawson M, Yeoman R, Pau K, Barrett S, Zelinski M, et al. Secondary follicle growth and oocyte maturation during encapsulated three-dimensional culture in rhesus monkeys: effects of gonadotrophins, oxygen and fetuin. Human Reproduction. 2011;26(5):1061-72.
24. Laronda MM, Duncan FE, Hornick JE, Xu M, Pahnke JE, Whelan KA, et al. Alginate encapsulation supports the growth and differentiation of human primordial follicles within ovarian cortical tissue. Journal of assisted reproduction and genetics. 2014;31(8):1013-28.