DOI: 10.7860/JCDR/2014/9129.5242

Original Article

Anatomy Section

Influence of Insulin-Like Growth Factor-I on Maturation and Fertilization Rate of Immature Oocyte and Embryo Development in NMRI Mouse with TCM199 and α-MEM Medium

MEHDI AKBARTABAR TOORI[1], ESMAEIL MOSAVI[2], MOHSEN NIKSERESHT[3], MEHRZAD JAFARI BARMAK[4], REZA MAHMOUDI[5]

ABSTRACT

Introduction: In vitro maturation (IVM) of oocytes and subsequent, in vitro fertilization (IVF) for the generation of embryos in the laboratory has important values. Growth factors are a component of a complex system of autocrine and paracrine factors that have a regulatory role in ovarian function and affect oocyte maturation. Therefore, the aim of this study is to evaluate the effect of IGF-I on IVM and IVF of mice oocytes during culture with α-MEM and TCM199 medium.

Materials and Methods: Cumulus oocyte complexes (COCs) and denuded oocyte were obtained from 4-6 week old NMRI mice and underwent in vitro maturation and in vitro fertilization in presence or absence of IGF-I with α-MEM and TCM199.

Result: Maturation rate (79.6%), fertilization rate (87.2%), two cells development rate (79.5%) and blastocyst rate(43.2%) was higher in COCs cultured in α-MEM with IGF-I, while lower maturation rate (50.6%) fertilization rate (61%), two cells development rate (48.8%) and blastocyst rate(14.6%) were seen in cultured denuded oocytes (DOs) in TCM199 without growth factor. As well as, maturation fertilization, two cells development and blastocyst rates in COCs were higher than DOs.

Conclusion: Our findings have shown that IGF-I is involved in the oocyte biology and improve the oocyte maturation, fertilization and embryo development to blastocyst competence in vitro. In addition, it has also shown that cumulus cells are vital for oocyte development when IGF-1 added to the mediums.

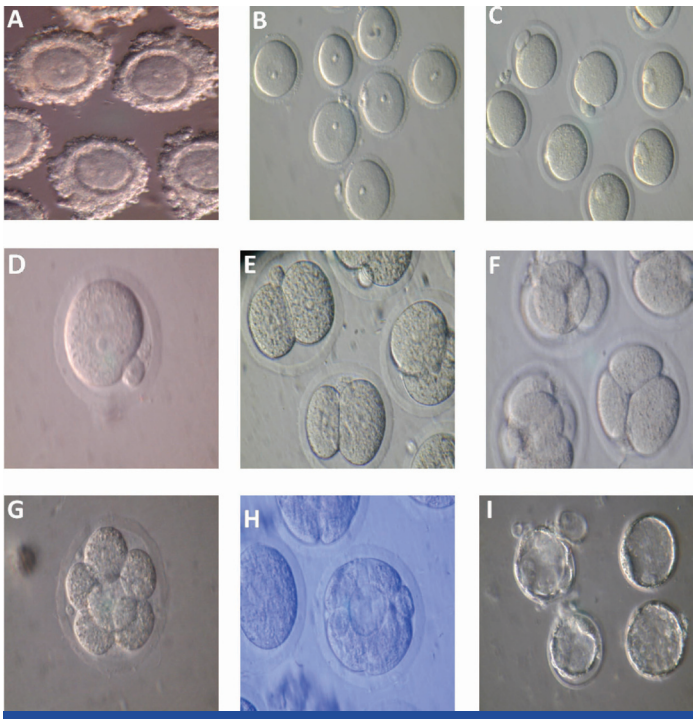
Keywords: Embryo development, Insulin-like growth factor-I, Maturation, Oocyte

INTRODUCTION

In vitro maturation (IVM) of oocytes and subsequent, in vitro fertilization (IVF) for the generation of embryos in the laboratory has important values. One of the indications of these techniques is therapeutic purposes, for example in ovarian hyperstimulation syndrome that cycle cancellation is done [1]. Other use is learning the basic biological actions going on during oocyte maturation, fertilization and early embryonic development. Despite advances in IVM and IVF techniques, optimum condition is required for successful and reliable oocyte maturation which would significantly improve the effectiveness of preimplantation embryonic and fetal development. However, present methods contain supplements like growth factor and/or co-culture with various types of somatic cells due incomplete knowledge of oocyte and embryo metabolism and growth requirements [2]. Growth factors are a component of a complex system of autocrine and paracrine factors that have a regulatory role in ovarian function and affect oocyte maturation [3].

Among them, insulin-like growth factor-I (IGF-I) is involved in the regulation of proliferation, differentiation, and steroidogenesis of follicular somatic cell ([4,5]. In the ovary, IGF-I is the inducer of granulosa cells mitosis [6], even in the lack of follicular stimulating hormone (FSH) [7], and promote the biological activity of FSH [7-8]. Also, studies has demonstrated that IGF-I null mice are infertile and they have several defects such as low expression of FSH receptor and aromatase in granulosa cells. Therefore, it seems that due to lack of amplifier role of IGF-I on granulosa cell FSH responsiveness; they failed to develop normal follicles after the early antral stage [8].

Cumulus cells have been considered to play an important role in oocyte maturation by keeping the oocyte under meiotic arrest, inducing meiotic resumption and by supporting cytoplasmic maturation. These functions have been attributed to their gap junctions and their specific metabolizing capabilities. Physical contact between oocyte and cumulus cells has been considered



[Table/Fig-1]: Different stages of oocyte development and embryo development. A) Germinal vesicle oocyte enclosed cumulus cells. B) denuded germinal vesicle oocyte. C) MII oocyte with first polar body. D) Fertilized oocyte with 2 pronucleus bodies and 2 polar bodies. E) Two cells stage embryo .F) four cells stage embryo. G) Eight cells stage embryo. H) morula stage and I) blastocyst development. Bar = 40 mm.

Treatment	GV (n)	MII	Fertilization	Two cell	Blastocyst
COCS + α- MEM	68	45(66.2%)	34(75.55%)	30(66.7%)	11(24.4%)
DOs+ α- MEM	68	37(54.4%)	24(64.9%)	20(54.05%)	5(13.5%)
COCS +Tcm 199	80	50(62.5%)	35(70%)	31(62%)	12(24%)
DOs+Tcm 199	81	41(50.6%)	25(61%)	20(48.8%)	6(14.6%)
COCS+ IGF-I + α- MEM	98	78(79.6%)[a]	68(78.2%)	62(79.5%)	34(43.6%)[a]
DOs+ IGF-I + α- MEM	100	60(60%)[d]	40(66.7%)[d]	36(60%)[d]	13(21.7%)[d]
COCS + IGF-I + Tcm 199	122	94(77%)[b]	78(83%)	70(74.5%)	35(37.2%)
DOs + IGF-I + Tcm 199	105	60(57.1%)[c]	38(63.3%)[c]	35(58.3%)[c]	11(18.3%)[c]

[Table/Fig-2]: Maturation, fertilization, two cells development and blastocyst rates in COCs and DOs cultured in α- MEM and TCM199 in the presence or absence of IGF-I. *Cumulus oocyte complexes (COCs), denuded oocytes (DOs), insulin-like growth factor-I (IGF-I),; a compared to COCS + α- MEM, P<0.05; b compared to COCS +Tcm 199 P<0.05; c compared to COCS + IGF-I + Tcm 199, p<0.05; d compared to COCS+ IGF-I + α- MEM, p<0.05*

necessary for the transfer of nutrients and factors essential for oocyte development [9-13].

To gain insight into this process, we evaluated the effect of IGF-I on IVM and IVF of mice oocytes during culture with α-MEM and TCM199 medium which are more common mediums in assisted reproductive technology. We also examined the role of IGF-I on fertilization rate and preimplantation embryo development. Our hypothesis was that these events are enhanced by the addition of IGF-I to the culture media with germinal vesicle oocytes with and without cumulus cells.

MATERIAL AND METHODS

Collection and maturation of oocytes [Table/Fig-1A-I]

Experiments were performed using 4-6 week old NMRI mice. Animals were handled according to the Guide for Care and Use of Laboratory Animals (Yasuj University of medical sciences) and maintained in a 12h light: 12h dark photoperiod under constant temperature and relative humidity. Adequate Food and water were provided. For ovarian stimulation 7.5 IU pregnant mare serum gonadotropin was injected intraperitoneally. After 48h the animals were sacrificed by cervical dislocation, and then ovaries were removed from animals and transferred to HEPES buffered human tubal fluid (HTF) medium supplemented with 5mg/ml bovine serum albumin (BSA). Cumulus oocyte complexes (COCs) [Table/Fig.-A] and Denuded oocytes (DOs) [Table/Fig.-1B] were harvested. They were in germinal vesicle stage; and then randomly divided in 8 groups including: 1) DOs cultured in TCM199 without supplement; 2) DOs cultured in TCM199 supplemented with 5 ng/ml IGF-I; 3) DOs cultured in α-MEM without supplement; 4) DOs cultured in α-MEM supplemented with 100 ng/ml IGF-I; 5) COCs cultured in TCM199 without supplement; 6) COCs cultured in TCM199 supplemented with 100 ng/ml IGF-I; 7) COCs cultured in α-MEM without supplement; 8) COCs cultured in α-MEM supplemented with 5 ng/ml IGF-I. All media contain 100IU penicillin, 100ng/ml streptomycin, 75 mIU/ml FSH and 3mg/ml fetal bovine serum (FBS), 1% insulin transferring selenium (ITS). COCs and manually denuded oocytes were cultured in incubator with 5% CO_2 at 37ºC. After 24 h mature (MII) oocytes were fertilized with sperm.

In vitro fertilization and development

Male NMRI mice of 12 weeks age were killed by cervical dislocation and then spermatozoa was harvested from cauda epididymis into IVF medium T6 supplemented with 4 mg/ml BSA. The sperms were hanged in 200 µl droplet of the IVF medium covered with mineral oil and incubated at 37ºC for 1- 2 hrs in humidified atmosphere of 5% CO_2 and 37ºC for capacitation. Mature oocytes from each group (n=15-20) were separately placed in 200 µl droplet of IVF medium under mineral oil. Sperm mixture (10-20 µl) was added to each droplet to achieve a concentration of 1-2 × 10^6 motile sperm/ml. After coincubation for 5h at 37ºC, the oocytes were then taken away and rinsed in fresh T6 medium and put in a 5% CO_2 incubator at 37ºC. After 6- 8 h, embryos were observed by using phase-contrast inverted microscope, and embryos were classified as pronucleus (PN) stage because two distinct pronuclei and a second polar body were seen. After 24h and 120h, embryos were observed for 2-cell stage and blastocyst development respectively. A total of 722 oocytes were obtained from 40 ovaries and they were used for in vitro maturation. The average number of collected oocytes was 18 per ovary.

STATISTICAL ANALYSIS

Data were analyzed by SPSS version 17. We calculated the proportion of maturation rate of GV oocyte to MII and embryo development from MII to the blastocyst stage and then compared the proportion rate of each stage in different groups in a 2x2 tables and then summarized the results in [Table/Fig-2]. Chi-square test was used to compare the groups in terms of maturation, fertilization and developmental rate. The differences in the proportions were considered significant when p<0.05.

RESULT

Statistical analysis has shown [Table/Fig-2] that higher maturation rate[Table/Fig-1C] (79.6%), fertilization rate [Table/Fig-1D] (87.2%), two cells development rate [Table/Fig-1E] (79.5%) and blastocyst rate [Table/Fig.-1I] (43.2%) was seen in COC cultured in α–MEM with growth factor, while lower maturation rate (50.6%) fertilization rate (61%), two cells development rate (48.8%) and blastocyst rate (14.6%) were seen in cultured DOs in TCM199 without growth factor. As well as, maturation, fertilization, two cells development and blastocyst rates in COCs were higher than DOs. Meanwhile maturation, fertilization, two cells development and blastocyst rates was higher in α-MEM medium than TCM199 medium in all groups but they weren't significant.

Comparison of maturation, fertilization rates, development of two cells and blastocyst stage with or without added IGF-1

Maturation, fertilization, two cells development and blastocyst rates were higher in medium with growth factor than medium without growth factor. However, significant differences were shown in maturation rate of COCs cultured in TCM199 (p<0.05) and blastocyst rate of COCs cultured in α-MEM (p<0.05) [Table/Fig.-2].

Comparison of maturation, fertilization rates, development of two cell stage and blastocyst stage in COCs and DOs.

Maturation rate was significantly higher in COCs cultured in α-MEM with growth factor (p<0.05) and COCs cultured in TCM199 with growth factor groups (p<0.05) than other groups. As well as, fertilization rate was higher in COCs than DOs in same condition. Meanwhile these differences were significant in COCs cultured in α-MEM with growth factor group (p<0.05) and COCs cultured in TCM199 with growth factor group (p<0.05). Development to two cells and blastocyst stage was higher in COCs than DOs in same condition. Two cells development rate was significantly increased in COCs cultured in α-MEM with growth factor (p<0.05) and

COCs cultured in TCM199 with growth factor groups ($p<0.05$) in comparison to other groups. Blastocyst rate was significantly higher in COCs cultured in α-MEM with growth factor ($p<0.05$) and COCs cultured in TCM199 with growth factor groups ($p<0.05$) than other groups [Table/Fig.-2].

Comparison of maturation, fertilization rates, and development of two cells and blastocyst stage in MEM and TCM199.

Maturation, fertilization, two cells development and blastocyst rates were higher in α-MEM than TCM199, but they weren't significant.

Comparison of effect of growth factor on maturation, fertilization rates, development of two cells and blastocyst stage in COCs and DOs.

Growth factor has significantly increased the maturation rate in COCs cultured in α-MEM medium ($p<0.05$) and TCM199 ($p<0.05$) compared to DOs. As well as fertilization rate was significantly higher in COCs cultured in α-MEM medium ($p<0.05$) and TCM199 ($p<0.05$) supplemented with growth factor.

Growth factor has significantly increased the two cells development rate in COCs cultured in both α-MEM medium ($p<0.05$) and TCM199 ($p<0.05$) compared to DOs. Meanwhile growth factor has significantly increased the blastocyst rate in COCs cultured in both α-MEM medium ($p<0.05$) and TCM199 ($p<0.05$) compared to Dos [Table/Fig.-2].

Comparison of maturation, fertilization rates, and development of two cells and blastocyst stage in DOs in presence or absence of growth factor.

Maturation, fertilization, two cells development and blastocyst rates were higher in denuded oocyte cultured in presence of growth factor (60%, 66.7%, 60%, 21.7% respectively) than in denuded oocyte cultured in the absence of growth factor (54.4%, 64.9%, 54.02%, 13.5% respectively), but they were not significant [Table/Fig.-2].

Comparison of maturation, fertilization rates, and development of two cells and blastocyst stage in COCs in presence or absence of growth factor.

Growth factor has increased the maturation rate of cultured COCs in both α-MEM (77.6% vs. 66.2%) and TCM199 (77% vs.62.5%) media supplemented with growth factor versus media without growth factor. This difference was significant in TCM199 medium. Fertilization rate of cultured COCs increased in both α-MEM (87.2% vs. 75.55%) and TCM199 (83% vs.70%) media supplemented with growth factor versus media without growth factor, however these changes weren't significant. Meanwhile two cells development and blastocyst rate were higher in presence of growth factor than in absence of growth factor and this difference was significant in blastocyst rate in α-MEM medium ($p<0.05$) [Table/Fig.-2].

Comparison of medium effect on maturation, fertilization rates, and development of two cells and blastocyst stage in COCs and denuded oocyte

Although the rates of maturation, fertilization, two cells and blastocyst development were higher in α-MEM groups than TCM199 groups, the differences were not significant [Table/Fig.-2].

DISCUSSION

Our results have shown that presence of cumulus cells enhance the oocyte maturation from GV to MII, fertilization rate and embryo development; however DOs have low level in these events. This finding confirmed the fact that cumulus cells induce meiosis [14] and enhance cytoplasmic maturation [15]. Moreover, DOs are less able to survive because there is no harmony between nuclear and cytoplasmic maturation. In mammals, there is an important physical contact between oocytes and follicular somatic cells via granulosa cell extensions known as transzonal projections (TZPs). TZPs have important roles in oocyte development, including exchange of the metabolite by gap junction, heterocellular adhesion, regulation of cell cycle and bidirectional signaling [16,17].

On the other hand in oocyte maturation process meiosis-regulating molecules like cAMP is secreted by adjacent cumulus cells and through gap junction access to ooplasm and exert paracrine actions [18].

In addition, cumulus cells secrete progesterone that enhance sperm capacitation and penetration. As well as cumulus cells change the oocyte cytoplasm and zona pellucida to increase normal fertilization rate [19].

Moreover cumulus cells removal leads to precocious TZPs retraction and subsequently loss of cortical stability and loss of eccentric position of GV oocytes. The significance of eccentrically positioned GV is assisting in preservation of maternal transcript and vital organelles by separation of these components from developing spindle [20].

Our findings have shown that the maturation, fertilization rate and embryo development were increased in presence of IGF-1 and cumulus cells. These results are consistent with those of previous reports and studies asserting that IGF-I stimulates maturation in Xenopus oocytes [21], bovine oocyte and fertilization in vitro [22], and supports rabbit blastocyst development [23] and human oocyte and blastocyst development [24]. It is concluded that IGF-I have an important role in the nuclear maturation and cytoplasmic maturation of oocytes and exert its effect through cumulus cells.

In addition, IGF-I have many functions in reproductive system. It plays a potent mitogen role for granulosa cells and acts as a biological enhancer for FSH activity in the ovary [25] including; amplification of the luteinizing hormone (LH) receptor induction by FSH; improved FSH mediated granulosa cells differentiation, potentiating the action of FSH to raise adenylate cyclase which leads to cAMP production [26, 27]. Finally IGF-I affect the P-450 synthesis and then stimulates aromatase activity of human granulosa cells [28].

CONCLUSION

Our findings have shown that IGF-I is involved in the oocyte biology and improving the oocyte maturation, fertilization and embryo development to blastocyst competence in vitro. In addition, it has also shown that cumulus cells are vital for oocyte development when IGF-1 added to the medium.

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PARTICULARS OF CONTRIBUTORS:
1. Social Determinants of Health Research Center, Yasuj University of Medical Sciences, Yasuj, Iran.
2. Student Research Committee, Yasuj University of Medical Sciences, Yasuj, Iran.
3. Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran.
4. Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran.
5. Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Reza Mahmoudi,
Cellular and Molecular Research center, Yasuj University of Medical Sciences, Yasuj, Iran
Phone : 00989122022407, E-mail : rezamahmoudi5@gmail.com, rmahmoudi40@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Mar 02, 2014**
Date of Peer Review: **Jun 25, 2014**
Date of Acceptance: **Aug 11, 2014**
Date of Publishing: **Dec 05, 2014**