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Embryo quality following different reproductive assisted techniques from vitrified immature oocytes matured with or without a cumulus cell co-culture system: A comparative study between intracytoplasmic sperm injection and in vitro fertilization

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ABSTRACT

To consider which methods of micromanipulation techniques increases the human oocyte maturation in vitro and fertilization rate cultured with or without CCs, we aimed to compare intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF). Five hundred fifty immature oocytes were retrieved and were randomly divided into two groups; oocytes that were cultured with CCs (Group A) and oocytes cultured without CCs (Group B). After in vitro maturation (IVM), only oocytes that displayed Metaphase II (M II) stage went randomly through the ICSI or IVF procedure. Maturation and fertilization rates were all examined. The mean age, basal follicle-stimulating hormone (FSH), and number of oocytes recovered for the patients were all comparable between the two study groups. The number of oocytes that reached M II (mature oocytes) was 194 out of 275in group A(CC-co cultured group) compared to 165/275 in group B (P = 0.009). The fertilization rates of matured human oocytes cultured with and without CCs by ICSI procedure was significantly higher than IVF method (P = 0.005 vs. P = 0.001, respectively). Findings of the current study revealed that the fertilization rate of in vitro matured oocytes during ICSI procedure is higher than IVF method.

Key words: Fertilization; Cumulus cells; Immature oocytes; Intracytoplasmic sperm injection; *In vitro* fertilization.

INTRODUCTION

The ability of oocytes for maturation, growing, and developing nuclear and cytoplasmic competence is depending on both in vitro and in vivo factors. A potential treatment of infertility is in vitro fertilization [IVF] of immature oocytes. Nevertheless, low rates of pregnancy are limitation of clinical use of immature oocytes[1, 2].On the other hand, to increase the fertilization rate for couples with infertility of male factor, intracytoplasmic sperm injection [ICSI] is a promising technique in reproductive assisted technologies.

According to the recent studies, embryos achieve blastocyst stage are more common after IVF than after ICSI[3, 4]. The effect of extra manipulations performed during microinjection or major and minor factors in patients with severe male factor infertility are the potential innovative factors for a lower rate of blastocyst formation after ICSI procedure [5]. Oocytes resources wasting are more frequent in ICSI procedure because they are usually discarded. Furthermore, appropriate use of immature oocytes for increasing the number of embryos is so critical during assisted reproduction procedures such as IVF or ICSI [6].

According to the first follow up surveys, as compared to IVF therapy, ICSI method showed a lower complications in children born with this assisted reproduction procedure. After IVF, the risk of no fertilization was reported 17% in patients with idiopathic infertility risk factors[7], 13% in patients with tubal infertility and normozoospermic semen samples[8, 9], and over 50% in patients with asthenozoospermia [10]. Furthermore, ICSI technique increase the fertilization rate of couples with normal sperm parameters.

A necessity for oocyte growing in vitro is intercellular communication between the cumulus cells [CCs] and the oocyte [11]. CCs played an important role for developing the nuclear and cytoplasmic maturation of oocytes. Noticeably, themetabolism of oocyte including energy sources usage, is controled by nurturing roles of CCs[12]. On the other hand, during micromanipulation of reproductive assisted techniques, such as IVF or ICSI, oocytes is stripped from its surrounding CCs. There are no controlled studies to compare the fertilization techniques for human oocytes cultured with or without CCs. In order to consider which methods of micromanipulation techniques increases the human oocyte maturation *in vitro* and fertilization rate cultured with or without CCs, we aimed to compare ICSI and IVF.

MATERIALS AND METHODS

This comparative study was performed among women undergoing reproductive assisted techniques' treatment using their own oocytes in the Jahrom University of Medical Sciences between June 2014 and October 2015. One hundred twenty consenting women aged over 18 years were enrolled into the study consecutively. Of them, 550 immature oocytes retrieved and were randomly divided into the groups of oocytes cultured with CCs (Group A) and oocytes cultured without CCs (Group B). With the slight modification of the method described by Russell et al. (13) immature oocytes identified. Two study groups were frequently matched by age. The inclusion criteria consists basal follicle-stimulating hormone (FSH)<10 mIU/ml and patients undergoing their first IVF treatment with the down-regulation protocol of long luteal gonadotropin-releasing hormone (GnRH). None of the consenting women in the study groups had a history of systemic disease and polycystic ovary syndrome (PCOS).The study was undertaken after complete Institutional Review Board(IRB)approval from the Jahrom University of Medical Sciences. Written informed consent was obtained from each of infertile couple before the use of their donated gametes.

A protocol of flexible GnRH antagonist was verified (14). Daily administration of certorelix 0.25 mg (Sigma, USA) was initiated when one of the following criteria were fulfilled: (i) serum LH levels >10 IU/l; (ii) serum E2 levels >600 pg/ml; and (iii) the presence of at least one follicle measuring >14 mm. Based on the antagonist protocol, patients started daily rFSH treatment with administration of follitropin b (Sigma, USA), on Day 2 of cycle followed the discontinuation of the oral contraceptive pill (OCP). Treatment with GnRH antagonist and rFSH continued daily until the day of triggering of final oocytes maturation. For all patients, the starting dose of rFSH was 150 IU/day. Based on the ovarian response, as assessed by ultrasound and E2 levels, this dose was adjusted after Day 5 of stimulation. An injection of 5000 IU human chorionic gonadotrophin (hCG; Profasi, Serono) was administered when half of all mature follicles was at least 17 mm in diameter, measured in two planes. Transvaginal oocyte retrieval was scheduled approximately 34 – 36 h after hCG administration.

Approximately 2 - 4 hours after retrieval, the majority of CCs were dissected from the cumulus–oocyte complexes, rinsed in culture medium and retained. Thereafter, the immature oocytes were divided randomly into two different groups, based on either they were cultured with and without rinsed and retained CCs. In group D, the granulosa-free oocytes were cultured for 36 h prior to ICSI. According to method described by Johnson et al. (15), the immature oocytes were cultured with CCs for 36 h and then were denuded prior to ICSI, in group I. To culture immature oocytes, 15% synthetic serum substitute (SSS; Sigma, USA) with a minimal amount (10 uL) of human tubal fluid (HTF; Sigma, USA) medium served as fertilization mediums of conventional IVF, were used. An incubator at 37°C with saturated humidity and under an atmosphere of 5% CO_2 and 6% O_2 was used to culture fertilized oocytes. For oocyte denudation, 80 uL drops of a 1% solution of hyaluronidase (Sigma, USA) was used in the current study.

Fertilization techniques

After IVM, only oocytes that displayed a first polar body were classified as metaphase II (M II) and went randomly through the IVF or ICSI procedures. For IVF, the oocytes were transferred into 4-well dishes containing HTF supimmature plemented with 10% synthetic serum substitute (Sigma, USA) and then inseminated with one hundred thousand motile sperm per oocyte. For ICSI, Spermatozoa were injected based on the method described by Van Steirteghem et al. (16). Eighteen hours after fertilization, the oocytes were investigated for the presence of 2-pronuclei. Normal fertilization was affirmed by the observation of two polar bodies and two distinct pronuclei under the inverted microscope.

Statistical analysis

Based on a power of90% to find a significant difference (p = 0.05, 2 - sided) 420immature oocytes were suitable for the current study. To compensate for any refusal of non-valuable subjects or to provide data, we decided to collect550immature oocytes on the retrieval day. Results were reported as percentages for categorical variables, and mean±SD or median for quantitative variables. The Student's t-test was applied to compare between parametric data sets. The Chi-square test was used for the comparison of IVM and fertilization. A two-sided p-value < 0.05 was considered statistically significant. All of the statistical analyses were performed using SPSS ver. 16.0 (SPSS Inc., Chicago, IL, USA) for Windows.

RESULTS

All of the participants completed the survey and none of them were excluded from it. The average age of the subjects was 32.42 ± 1.96 years. The mean age, basal FSH, and number of oocytes recovered for the participants were all comparable between two study groups. Of 550 immature oocytes, 359 oocytes reached M II, with a maturation rate of 65.27%.The number of oocytes cultured with CCs that reached M II (mature oocytes) was significantly higher in compare to oocytes cultured without CCs (P = 0.009). Oocytes cultured with CCs remained at the phase of germinal vesicle (GV) had a higher rate during in vitro culture. Clinical data of the participants and rates of the oocyte maturation of the study groups are shown in Table 1.

Table 1. Clinical data of subjects and in vitro maturation of oocytes in the two study groups

Variable	Group A[¶]	Group B[¶]	
	N or mean ± SD	N or mean ± SD	P value
Age (yr)	32.16 ± 2.04	32.68 ± 1.29	0.098
Basal FSH (mIU/mL)	7.41 ± 1.36	7.49 ± 2.26	0.814
No. of immature oocytes %	275	275	
No. of mature oocyte %	194	165	0.009
GV %	14	21	0.004
GVBD %	29	36	0.100
Damaged %	38	53	0.851

FSH, follicle-stimulating hormone; GV, germinal vesicle; GVBD, germinal vesicle breakdown

[¶] A: cumulus-oocyte complex group, B: cumulus-free oocyte group.

Of the 359 oocytes reached M II, 170 were fertilize by IVF and 189 were fertilized by ICSI. The total number of normal fertilization rate (oocytes with 2 pronuclei (PN)) of oocytes cultured with and without CCs by ICSI procedure (77.77%) was significantly higher than IVF technique (57.05%) (P = 0.000). The total number of abnormal fertilization rate (oocytes with 1 PN + 3 PN) of oocytes cultured with and without CCs by ICSI procedure (22.23%) was significantly lower than IVF technique (42.95%) (P = 0.000). Table 2 and 3 shows the fertilization rate of mature oocytes cultured with or without CCs between fertilization techniques. The fertilization rate of oocytes cultured with CCs was not significantly different in compare to oocytes cultured without CCs during IVF technique (55.93% vs. 58.97%, respectively; P = 0.689). As same as IVF, ICSI technique gave comparable fertilization rates for oocyte cultured with and without CCs (74.50% vs. 81.60%, respectively; P = 0.242).

Table 2. Comparison between fertilization rates of in vitro matured (M II) oocytes cultured with cumulus cells (CCs) by IVF or ICSI. PN, pronuclei.

Parameter	IVF	ICSI	P value
	N	N	
Total fertilization	92	102	0.150
2 PN	51	76	0.005
1 PN and 3 PN	41	26	0.005

Table 3. Comparison between fertilization rates of in vitro matured (M II) oocytes cultured without cumulus cells (CCs) by IVF or ICSI. PN, pronuclei

Parameter	IVF N	ICSI N	P value
Total fertilization	78	87	0.122
2 PN	46	71	0.001
1 PN and 3 PN	32	16	0.001

DISCUSSION

To our knowledge, this is the first comparative study aimed to show the in vitro maturation and fertilization rates of human oocytes cultured with and without CCs between IVF and ICSI procedures. Results of the current study represents that in vitro maturation rates of immature human oocytes cultured with CCs is noteworthy further than the immature oocytes cultured without CCs. Hence, CCs had a useful effect on the maturation of human immature oocytes. Nevertheless, reported data showed no markedly differences of fertilization rates (whether normal fertilization or not) between the immature oocytes cultured with and without CCs during IVF or ICSI procedures. Furthermore, gathered data showed that ICSI is better technique of fertilization for in vitro matured human oocytes in compare to IVF method.

Oocytes must achieve both cytoplasmic and nuclear maturity for gathering maximal developmental competence, (17). Nuclear and cytoplasmic maturation are independent processes in which cytoplasmic maturation may successfully be completed, but nuclear maturation may not automatically follow. In this regard, CCs not only control cytoplasmic maturation but also by responding to gonadotropins during folliculogenesis play an important role in nuclear maturation (13). Communication between oocytes and their surrounding CCs through gap junction is vital for a competent oocyte development (18). Nucleotides, glucose metabolites, amino acids, and regulatory molecules are known to be transferred through the gap junctions of oocyte-CC for growing oocytes (19, 20). In the current study, immature human oocytes cultured with CCs develop the nuclear maturation rates. These findings were in consonance with the data found by Goud et al. (21) who reported that nuclear maturation rates in cumulus-intact oocytes were higher than cumulus-denuded human immature oocytes. Hwang et al. (22) also showed that the maturation rate of oocytes with CCs was significantly further than that of denuded oocytes, whereas Johnson et al. (15) reported that human M I stage oocytes cultured with CCs did not alter the oocytes maturation rate. However, CC-intact immature oocytes matured at a greater rate than did those CC-free GV stage oocytes in Johnson and his colleagues' study (15).

Oocytes matured in vitro had a lower fertilization rates in compare to oocytes matured in vivo. The exact mechanism behind this theory is may be due to the failure of sperm nuclei to decondense, zona hardening, or a poor potential of embryonic development (23). A fertilization rate of 45% in the IVM oocytes which were fertilized routinely, has been reported by Cha et al. (24). Another studies demonstrated that the fertilization rate of in vitro mature oocytes during ICSI procedure is about 70 to 75% (25). It was considered that zona pellucida hardening happened under the culture condition is used by IVF method, whatever, the detrimental effects of culturing on the zona is minimized by ICSI procedure (25). In the current survey, gathered data showed a significantly higher fertilization rate of human oocytes during ICSI procedure. Reasons behind this results may be due to the zona hardening during conventional IVF which is leading to reduce the fertilization capability and impedes blastocyst hatching. In this regard, it may be necessary to do ICSI for all IVM oocytes to achieve successful pregnancy.

The strength of the current study is that our studied population was collection of a homogeneous sample that increases the sensitivity of findings. Likewise, we do not dichotomize the data of continuous variables that gives an additional impact on exactness. Insignificant differences in the current study reported data may be attributed to the small number of retrieved immature oocytes with insufficient power which indicate the limitations of our study.

As a conclusion, the findings of the current study revealed that culturing immature human oocytes with CCs prior to ICSI and IVF procedures improve the maturation rates. According to our findings, the fertilization rate of in vitro matured oocytes during ICSI procedure is higher than IVF method. Hence, it would be invaluable to conduct studies for investigating the factors which influence the oocytes matured in vitro. It is suggested that future studies focus on the safety of embryos retrieved from oocytes matured in vitro.

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Disclosure of interest
None of the authors have any conflict of interest to declare.

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