The effect of percentage of immature oocytes at the time of oocyte retrieval on ICSI outcomes

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Abstract: Purpose: The goal wastoassesstheeffect of thequantity of immature oocytes at the time of oocyte retrieval on (ICSI) outcomes. **Patients and methods:** A prospective selective clinical study was performed in assisted reproduction unit (Hawa Hospital), Atotalof100ICSIcycleswereincluded, Patientswere divided into two groups; (Group Ⅰ) included patients with the percentage of mature (M2) oocytesmorethan 50%, and (group Ⅱ) includedpatientswiththerateof matureoocytes (M2) lessthan 50% of the total retrievedoocytes. **Results:** There were significant differences for Fertilization rate between both groups; it was 72.21% for (group Ⅰ) and 49.07 % for (group Ⅱ) (P = 0.049). Implantation rate appeared to be highly significant; the implantation rate in (group Ⅰ) was 33.37 %, versus 17.79 % in (group Ⅱ) (P = 0.028). There was a statistical difference between (group Ⅰ) and (group Ⅱ) as regards embryo grading (D3). Also, the rates of clinical pregnancy were significant between groups; 47% of (group Ⅰ) and 33% of (group Ⅱ) patients; (P = 0.04). **Conclusion:** In cases with few retrieved immature oocytes, rates of fertilization may increase.

[Mofeed Fawzy Mohamed, Abd El-Moneim Mohamed Zakria, and Mohamed Ebrahim Hefnawy. **The effect of percentage of immature oocytes at the time of oocyte retrieval on ICSI outcomes.** *Life Sci J* 2018;15(4):12-16]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 2. doi:10.7537/marslsj150418.02.

Keywords: immature oocytes, intracytoplasmic sperm injection.

Introduction

Introduction of intracytoplasmic sperm injection (ICSI) was a great achievement to give the opportunity of having babies. It represented a coalescence of advances in physiology, endocrinology, pharmacology, technology, and clinical care in Assisted reproductive techniques (ART) [1].

In ART, controlled ovarian hyperstimulation (COH) is necessary for inducing the recruitment of multiple follicular developments for harvesting the numerous healthy mature oocytes. In recent years, several COH protocols have been introduced to optimize the ICSI outcomes. Despite optimizing the COH protocols, approximately 20 % of the oocytes remain immature at the GV or MI stages [2].

One of the factors that may affect the ICSI outcomes is related to the number of retrieved oocytes [3,4]. In recent years, some investigators tried to find some predictive values for the number of retrieved immature oocytes in COH program administered for ART outcomes [5].

In addition, the maturity of retrieved oocytes is essential for the success of in vitro fertilization, because mature oocytes are used for ART; while, the remaining immature ones are generally discarded. Although, it is possible to mature these oocytes using in-vitro maturation (IVM) technology, but both pregnancy and implantation rates have been reported very rare [6,7].

Despite the fact that top-quality embryos may be available for transfer during intra-cytoplasmic sperm injection (ICSI) cycles, only a maximum of one-third of the embryos transferred implant finally [8]. There is accumulating evidence that if several embryos are created, and one of them is selected for transfer on the basis ofrespectable morphology, pregnancy rate (PR) and LBR are high [9].

However, there are no strict data about the probable impact of these immature oocytes on maturity and outcome of their mature cohort oocytes in the ART program. It seems that the effect (s) of immature oocyte quantity on ICSI outcomes are scarce. Therefore, this study was designed to evaluate the role of theof immature oocytes at the time of oocyte retrieval on (ICSI) consequences.

Patients and methods

Patient selection

A total of 100 ICSI cycles were involved in this prospective study in an assisted reproduction unit (Hawa Hospital). The females with age less than 35, BMI less than 35 kg/m^2, average menstrualcycleof 21–35 days with both intact ovaries, normal uterus andfallopian tubes on hysterosalpingography and/or hysteroscopywere included. Also, The sperm count of amale partner was more than 0.15 million/ml. The study was conducted after written consent was obtained for each participant in the study.

Patients with ages greater than 35 years, BMI greater than 35 kg/m2, anovulation, hydrosalpinx or intrauterine pathology such as submucous fibroid, adenomyosis, intrauterine septum, or adhesions detected by transvaginal ultrasound scan and

hysterosalpingogram, and sperm count of male partner less than 0.15 million/ml were excluded from our study.

Controlled ovarian hyperstimulation protocols (COH)

COH was carried out according to different protocols provided by the staff members of the unit. When at least five follicles (18mm - 23 mm) or more was observed, triggering of ovulation was performed using human chorionic gonadotropin (hCG) (5000–10,000 IU intramuscular), and other medications were interrupted. Timed oocyte retrieval was performed 36 h later. The injected oocytes were washed twice and then individually placed in fresh droplets of G1 covered with mineral oil.

Semen preparation:

The best morphologically well-shaped spermatozoa were selected and evaluated according to the recommendations of the World Health Organization for the microinjection procedure.

Oocyte preparation:

The patients were dividedinto two different groups. (group Ⅰ) included patient with the percentage of mature (M2) oocytes more than 50%, and (group Ⅱ) included patients with the percentage of mature oocytes (M2) less than 50% of the total retrieved oocytes. For every patient, endometrium thickness at the time of hCG administration was of range 8–14 mm; the oocyte quality was investigated immediately before ICSI.

Intracytoplasmic sperm injection procedure

After oocyte aspiration, the oocytes were incubated for about 4 h and then denudation from cumulus cells. Each of the MII oocytes was washed in culture media and before microinjection, and their morphological characteristics were evaluated. For sperm injection, the motile spermatozoa were aspirated.

Assessment of survival, fertilization and embryo development:

The morning after injection (16–22 h later), the oocytes were checked for survival and fertilization. The numbers and aspects of polar bodies and pronuclei were recorded.

3. Results

Regarding maternal age, there was no correlation between both groups; (group Ⅰ) had a mean ± SD of 28± 3.55 while (group Ⅱ) had a mean ± SD of 29 ± 4. (P=0.6). Also, there was no statistical difference between the two groups as regards BMI; (group Ⅰ) had a mean±SD of 26.97±3.5, and (group Ⅱ) had a mean ± SD of 26.5± 3.44. (P=0.26).

There was no statistical difference between the two groupsas regards endometrial thickness at the time of hCG administration; (group Ⅰ) had a mean±SD of 11.3±2.3, and (group Ⅱ) had a mean±SD of 10.53±1.91; (P=0.08). There was no statistical difference between the two groupsas regards follicle stimulating hormone levels; (group Ⅰ) hadamean±SDof6.42±1.78, and (group Ⅱ) had a mean ± SD of6.82± 2.81; (P =0.06).

	Immature oocytes< 50% (group Ⅰ)	**Immature oocytes >50% (group Ⅱ)**	**p.value**
Fertilization rate %	**72.21%**	**49.07%**	**0.049**
Implantation rate %	**33.37%**	**17.79%**	**0.028**

Fertilization rate showed a significant correlation between study groups; it was 72.21% for (group Ⅰ) and 49.07% for (group Ⅱ) (*P* = 0.049). There was a statistical difference between (group Ⅰ) and (group Ⅱ) as regards embryo grading (D3). Both good and fair grades were statistically highly significant. *P* value was 0.002 in good grade and 0.025 in fairgrade. However, the poorgradewas not statistically significant.

There was a highly significant relationship between both groups as regards the implantation rate; (group Ⅰ) was 33.37% versus 17.79% for (group Ⅱ) (*P* = 0.028). A positive β -hCG test followed by fetal heartbeat detection occurred in 47% of (group Ⅰ) and33%of (group Ⅱ) patients. It was statistically significant (*P* =0.04).

4. Discussion

Our clinical study was performed in an assisted reproduction unit (Hawa Hospital). The study included 100 patients, the duration of this study was between March 2016 to December 2017. A detailed medical history was taken, and transvaginal ultrasound scan was done, and controlled ovarian stimulation was done using different protocols according to the unit protocols, for every patient endometrium thickness at the time of HCG administration (8-14 mm), oocyte quality was investigated immediately before ICSI. Evaluation of normal fertilization was identified by the presence of two pronuclei (2PN) at the time of fertilization assessment, 19 hours after ICSI.

The primary outcome measures were clinical pregnancy and implantation rates. Clinical pregnancy was defined as a positive b-hCG assay and the presence of at least one gestational sac with fetal

heartbeat detection by transvaginal ultrasound examination 4 weeks after embryo transfer.

There are some predictors for the number of retrieved immature oocytes, such as the number of 2–6-mm antral follicles, ovarian volume, and peak ovarian stromal blood flow velocity measured with Doppler ultrasound during the follicular phase. Moreover, there have been direct relations between the number of immature oocytes and pregnancy rates following IVM of human oocytes [10].

Many factors may affect the success rate of ICSI, such as maternal age [11], oocyte morphology [12,13], sperm quality [14], ICSI technique [15], injection pipette [16], ICSI operator [17], and quality of transferred embryos [18].

The success of artificial reproductive techniques is highly dependent upon the number and quality of oocytes retrieved [19].

The percentage of high-quality embryos decreased significantly according to the percentage of immature oocytes; the higher the number of retrieved immature oocytes, the lower their chance of becoming an eight-cell embryo with no fragmentation on the third day of development.

Our data showed that 15 % of retrieved oocytes remained immature. Therefore, to maximize the ICSI success rates, the number and quality of MII eggs in stimulated cycles are essential. One of the influential instances maybe the number and the stage of immature oocytes in each cycle. However, the influential effects of immature oocytes on the quality and number of mature oocytes in ICSI are still unclear.

Implantation rate was defined as the total number of gestational sacs presenting heart pulsations about the total number of embryos transferred. The implantation rate appeared to be significantly high in patients with the percentage of mature (M2) oocytes more than 50% (group Ⅰ) compared with the patients with the percentage of mature oocytes (M2) less than 50% of the total retrieved oocytes (group Ⅱ).

The fertilization rate appeared to be significant in (group Ⅰ) compared with the patients in group Ⅱ. There was a significant difference between the two groups in terms of embryo grading in good and fair grades, but there was no significant difference in poor grade in embryo grade.

Wittemer et al. stated that fertilization rate is higher in IVF cycles with more than 10 % GV oocytes compared to cycles with less GV (Wittemer et al., 2000). They inseminated all cumulus-oocyte complexes (including GV, MI, and MII) and compared the outcomes [20].

One probable reason for higher fertilization rate in (group Ⅰ) may be due tothe increase in the number of mature oocytes available for injection. COH has acrucialrole in ART, and high doses of gonadotropins induce simultaneous growth and development of follicles. One of the causes that retrieved oocytes are in different stages of development is heterogeneity of follicles population at the time of hCG injection [21].

A positive β -hCG test followed by fetal heartbeat detection appeared to be significant in patients with the percentage of mature (M2) oocytes more than 50% (group Ⅰ) compared with the patients with the percentage of mature oocytes (M2) less than 50% of the total retrieved oocytes (group Ⅱ).

According to our hypothesis, these immature oocytes may have some negative impacts on the other healthy oocytes through paracrine secretion. It is well known that advanced female age is well correlated with the poor quality of oocytes. One reason may be related to ovarian function, which is decreased with advancing of age as well as the reduction of ovarian response to hyperstimulation.

In addition, one of the most important steps in ART is to harvest healthy mature oocytes, which is related to ART success. Therefore, to maximize the ICSI success rates, the number and quality of MII oocytes in stimulated cycles are important. One of the influential instances may be the number and the stage of immature oocytes in each cycle.

It is reported that follicles may have some effects on each other through paracrine secretion. Transforming growth factor-β operates through paracrine or autocrine mechanisms to regulate follicular development and oocyte maturation [22, 11].

It is mentioned that anti-Mullerian hormone (AMH) inhibits the recruitment of primordial follicles via paracrine activity [23]. Nevertheless, when some oocytes remain immature, in spite of ovarian hyperstimulation, it may be due to the presence of intrinsic defects in the oocytes or even follicles.

One study detected enlarged PVS as a sign of degeneration/postmaturity in unfertilized human oocytes from an ultrastructural point of view [24]. Also, it is suggested that the extracytoplasmic dysmorphisms (e.g., wide PVS) should be considered only a phenotypic heterogeneity of the retrieved oocytes [25].

Wide PVS may be seen because of overmaturity of cytoplasm at the time of hCG injection. So, PVS enlargement may be related to increased maternal age, and oocyte aging [26,27].

Our data demonstrated that the percentage of immature oocytes might be useful for prognosis of ICSI outcome. Accordingly, if the percentage of immature oocytes was higher than 50%, the fertilization was significantly lower. This fact may be related to the possibility that, when immature oocytes are numerous at the pick-up, ovarian follicles may, in general, be less responsive to the ovarian stimulation.

This study shows that low percentage of

immature oocytes is a statistically significant predictor of the individual implantation rate even after adjustment for common variables known to be associated with implantation. The strength of the effect is similar to the difference in implantation rate between the two groups.

Recently, **de Cassia et al.** showed that the number of retrieved oocytes significantly correlated with increased incidence of cytoplasmic granularity [28]. They also reported that excessive ovarian response had a negative effect on oocyte quality. In the present study, we tried to omit the effect of various ovarian stimulation protocols on oocyte quality, as the patients received long protocol for ovarian stimulation, but the various ovarian response and intrinsic variations between the patients may be one reason for seeing these abnormalities.

The cause of oocyte morphological abnormalities is probably multifactorial, so larger studies are in need to investigate the relationship between the probable effects of retrieved immature oocytes on afeature of mature cohort oocytes.

In conclusion, the percentage of immature oocytes may be useful in the prognosis of ICSI outcome. If the percentage of immature oocytes is higherthan 50%, the fertilization was significantly lower.

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References

1 Palermo GD, Cohen J, Rosenwaks Z. Intracytoplasmic sperm injection: a powerful tool to overcome fertilization failure. Fertil Steril 1996; 65:899–908.
2 Rienzi L, Ubaldi F, Anniballo R, Cerulo G, Greco E. Preincubation of human oocytes may improve fertilization and embryo quality after intracytoplasmic sperm injection. Hum Reprod. 1998;13(4):1014–9.
3 McAvey B, Zapantis A, Jindal SK, Lieman HJ, Polotsky AJ. How many eggs are needed to produce an assisted reproductive technology baby: is more always better? Fertil Steril. 2011;96 (2):332–5.
4 Yoldemir T, Fraser I. The effect of retrieved oocyte counts on pregnancy outcomes in an assisted reproduction program. Arch Gynecol Obstet. 2010;281(3):551–6.
5 Jee BC, Ku SY, Suh CS, Kim KC, Lee WD, Kim SH. Serum anti- Müllerian hormone and inhibin B levels at ovulation triggering day can predict the number of immature oocytes retrieved in in vitro fertilization cycles. J Korean Med Sci. 2008;23(4):657–61.
6 Lin YH, Hwang JL. In vitro maturation of human oocytes. Taiwan J Obstet Gynecol. 2006;45(2):95–9.
7 Nazari S, Khalili MA, Esmaielzadeh F, Mohsenzadeh M. Maturation capacity, morphology and morphometric assessment of human immature oocytes after vitrification and in-vitro maturation. Iranian J Reprod Med. 2011;9(3):209–16.
8 Jarvela I, Sladkevicius P, Kelly S, Ojha K, Campbell S and Nargund G (2005). Evaluation of endometrial receptivity during in-vitro fertilization using three-dimensional power Doppler ultrasound. Ultrasound Obstet Gynecol., 26: 765–9.
9 Tiitinen A, Halttunen M, Harkki P, Vuoristo P and Hyden-Granskog C (2001). Elective single embryo transfer: the value of cryopreservation. Hum Reprod. 16(6):1140–1144.
10 Jayaprakasan K, Deb S, Batcha M, Hopkisson J, Johnson I, Campbell B, Raine-Fenning N (2010). The cohort of antral follicles measuring 2– 6 mm reflects the quantitative status of ovarian reserve as assessed by serum levels of anti-Müllerian hormone and response to controlled ovarian stimulation. Fertil Steril.; 94(5):1775–81.
11 Halvaei I, Khalili MA, Soleimani M, Razi MH. Does maternal age have any effect on the rates of fertilization and embryo development in ICSI cycles? Iran J Reprod Med 2011; 9 (Suppl 1):49.
12 Khalili MA, Mojibian M, Sultan AM. Role of oocyte morphology on fertilization and embryo formation in assisted reproductive techniques. Middle East Fertil Soc J 2005; 10:72–77.
13 Xing X, Zhao H, Li M, Sun M, Li Y, Chen ZJ. Morphologically abnormal oocytes not capable of fertilization despite repeated strategies. Fertil Steril 2011; 95:2435. e5–2435.e7.
14 Strassburger D, Friedler S, Raziel A, Schachter M, Kasterstein E, Ron-el R. Very low sperm count affects the result of intracytoplasmic sperm injection. J Assist Reprod Genet 2000; 17:431–436.
15 Blake M, Garrisi J, Tomkin G, Cohen J. Sperm deposition site during ICSI affects fertilization and development. Fertil Steril 2000; 73:31–37.
16 Svalander P, Forsberg AS, Jakobsson AH, Wikland M. Factors of importance for the establishment of a successful program of intracytoplasmic sperm injection treatment for male infertility. Fertil Steril 1995; 63:828–837.
17 Shen S, Khabani A, Klein N, Battaglia D. Statistical analysis of factors affecting

fertilization rates and clinical outcome associated with intracytoplasmic sperm injection. Fertil Steril 2003; 79:355–360.
18 Hsu MI, Mayer J, Aronshon M, Lanzendorf S, Muasher S, Kolm P, Oehninger S. Embryo implantation in vitro fertilization and intracytoplasmic sperm injection: impact of cleavage status, morphology grade, and number of embryos transferred. Fertil Steril 1999; 72:679–685.
19 Van der Vorst, M., Joris, H., Van Steirteghem, A. et al. (1997) Correlation between ongoing pregnancy rates and the number of cumulus–oocyte complexes retrieved in agonist-HMG-stimulated ICSI cycles. Hum. Reprod.,12, Suppl., P-101.
20 Wittemer C, Ohl J, Bettahar-Lebugle K, Viville S, Nisand I. A quantitative and morphological analysis of oocytes collected during 438 IVF cycles. J Assist Reprod Genet. 2000;17(1):44–50.
21 Kim BK, Lee SC, Kim KJ, Han CH, Kim JH. In vitro maturation, fertilization, and development of human germinal vesicle oocytes collected from stimulated cycles. Fertil Steril 2000; 74:1153–1158.
22 Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle: a review. Hum Reprod Update. 2012;18(1):73–91.
23 Durlinger A, Visser JA, Themmen A. Regulation of ovarian function: the role of anti-Mullerian hormone. Reproduction. 2002;124(5):601–9.
24 Motta PM, Nottola SA, Micara G, Familiari G. Ultrastructure of human unfertilized oocytes and polyspermic embryos in an IVF–ET program a. Ann N Y Acad Sci. 1988;541(1):367–83.
25 Balaban B, Urman B. Effect of oocyte morphology on embryo development and implantation. Reprod Biomed Online. 2006;12(5):608–15.
26 Miao Y, Ma S, Liu X, Miao D, Chang Z, Luo M, Tan J. Fate of the first polar bodies in mouse oocytes. Mol Reprod Dev. 2004;69(1):66–76.
27 Xia P. lntracytoplasmic sperm injection: correlation of oocyte grade based on polar body, perivitelline space and cytoplasmic inclusion with fertilization rate and embryo quality. Hum Reprod. 1997; 2:1750–5.
28 de Cassia SFR, de Almeida Ferreira Braga DP, Semiao-Francisco L, Madaschi C, Iaconelli Jr A, Borges Jr E. Metaphase II human oocyte morphology: contributing factors and effects on fertilization potential and embryo developmental ability in ICSI cycles. Fertil Steril. 2010;94(3):1115–7.

4/3/2018