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

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Abstract: Purpose: The goal was to assess the effect of the quantity 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), A total of 100 ICSI cycles were included, Patients were divided into two groups; (Group I) included patients with the percentage of mature (M2) oocytes more than 50%, and (group II) included patients with the rate of mature oocytes (M2) less than 50% of the total retrieved oocytes. **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.

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 of respectable 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 the of 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 menstrual cycle of 21–35 days with both intact ovaries, normal uterus and fallopian tubes on hysterosalpingography and/or hysteroscopy were included. Also, The sperm count of a male 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/m^2 , 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 divided into 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 \pm SD of 28 ± 3.55 while (group II) had a mean \pm 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 \pm SD of 26.97 ± 3.5 , and (group II) had a mean \pm SD of 26.5 ± 3.44 . ($P=0.26$).

There was no statistical difference between the two groups as regards endometrial thickness at the time of hCG administration; (group I) had a mean \pm SD of 11.3 ± 2.3 , and (group II) had a mean \pm SD of 10.53 ± 1.91 ; ($P=0.08$). There was no statistical difference between the two groups as regards follicle stimulating hormone levels; (group I) had a mean \pm SD of 6.42 ± 1.78 , and (group II) had a mean \pm SD of 6.82 ± 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 fair grade. However, the poor grade was 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) and 33% 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 IVF 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 to the increase in the number of mature oocytes available for injection. COH has a crucial role 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 a feature 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 higher than 50%, the fertilization was significantly lower.

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