The effect of percentage of immature oocytes at the time of oocyte retrieval on intracytoplasmic sperm injection outcomes
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Objective
The aim of this study was to evaluate the role of the percentage of retrieved immature oocytes on intracytoplasmic sperm injection (ICSI) outcomes.

Patients and methods
A total of 100 ICSI cycles were included in this prospective evaluation. Patients were divided into two groups. Group A included patients with the percentage of mature (M2) oocytes more than 50%, and group B included patients with the percentage of mature oocytes (M2) less than 50% of the total retrieved oocytes. The impacts of the percentages of GV and MI oocytes were assessed on the rates of fertilization, implantation, embryo grading, and pregnancy rate.

Results
Implantation rate appeared to be significantly high; the implantation rate in group A was 33.2%, versus 17.8% in group B (P= 0.036). Fertilization rate also appeared to be significant; it was 71.63% for group A and 48.58% for group B (P= 0.049). There was statistical difference between group A and group B as regards embryo grading (D3). A positive β-human chorionic gonadotropin test followed by fetal heart beat detection occurred in 46% of group A and in 34% of group B patients. It was statistically significant (P= 0.049).

Conclusion
Our data demonstrated that 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.

Keywords:
immature oocytes, intracytoplasmic sperm injection, infertility

Introduction
Introduction of intracytoplasmic sperm injection (ICSI) was a great achievement in the field of infertility, primarily for couples with male factor infertility [1]. In Assisted reproductive techniques (ART), controlled ovarian hyperstimulation (COH) is necessary for inducing the recruitment of multiple follicular development 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, ~20% of the oocytes remain immature at the GV or MI stages [2].

One of the factors that may affect the ICSI outcomes are related to the number of retrieved oocytes [3,4]. In addition, the maturity of retrieved oocytes is important for the success of in-vitro fertilization, because mature oocytes are used for ART; the remaining immature ones are generally discarded.

Although it is possible to mature these oocytes using in-vitro maturation (IVM) technology, both pregnancy and implantation rates have been reported to be very rare [5,6]. 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 [7]. 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 [8–10]. However, there are no strict data on the probable impact of these immature oocytes on the maturity and outcome of their mature cohort oocytes in ART program. It seems that the effects of immature oocyte quantity on ICSI outcomes are few. Therefore, this study was designed to evaluate the role of the number of retrieved immature oocytes on the mature oocyte count, morphology, rates of fertilization, and embryo development in ICSI cases.

Patients and methods

Patient selection
This prospective clinical study was performed in an assisted reproduction unit (Adam International Hospital). The study included 100 patients who were referred for
ICSI the treatment of infertility, with ages less than 35, BMI less than 35 kg/m², normal menstrual cycle of 21–35 days with both intact ovaries, normal uterus and fallopian tubes on hysterosalpingography and/or hysteroscopy, and sperm count of male partner more than 0.1 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 0.1 million/ml were excluded from our study.

Results

Table 1. Comparison of the two groups as regards the mean age. Group A had a mean ± SD of 27.18 ± 3.66. Group B had a mean ± SD of 29.34 ± 3.78. The study was conducted after written consent was obtained for each participant in the study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
<th>P value</th>
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<tbody>
<tr>
<td>Group A</td>
<td>27.18 ± 3.66</td>
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</tr>
<tr>
<td>Group B</td>
<td>29.34 ± 3.78</td>
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On comparing the two groups, implantation rate appeared to be significantly high; the implantation rate for group A was 33.2 versus 17.8% for group B (P = 0.036). Fertilization rate also appeared to be significant; it was 71.63% for group A and 48.58% for group B (P = 0.049). There was statistical difference between group A and group B 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, poor grade was not statistically significant.

A positive β-hCG test followed by fetal heart beat detection occurred in 46% of group A and in 34% of group B patients. It was statistically significant (P = 0.049).

Discussion

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.

In contrast, 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 show that the retrieval of fewer immature oocytes leads to a higher number of fetal heart beats demonstrating that the percentage of immature oocytes can influence ICSI outcomes. These outcomes showed a strong relationship between low the percentage of immature oocytes and better embryo development.

Discussion

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]. 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 important. One of the influential instances may be 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.
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 [10]. 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 [9].

Embryo grading (D3) was performed before transfer, and calculation of implantation rate was carried out. The primary outcome measures were clinical pregnancy and implantation rates. Clinical pregnancy was defined as a positive β-hCG assay and the presence of at least one gestational sac with fetal heart beat detection by transvaginal ultrasound examination 4 weeks after embryo transfer. Implantation rate was defined as the total number of gestational sacs presenting heart pulsations in relation to 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 A) compared with the patients with the percentage of mature oocytes (M2) less than 50% of the total retrieved oocytes (group B).

The fertilization rate appeared to be significant in group A compared with the patients in group B. There was 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.

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

The influential effects of immature oocytes on the quality and number of mature oocytes in ICSI are still unclear. Wittemer et al. [19] stated that fertilization rate is higher in in vitro fertilization cycles with more than 10% GV oocytes compared with cycles with less GV. They inseminated all cumulus oocyte complexes (including GV, MI, and MII) and compared the outcomes.

In the group with more than 10% GV, they observed more oocytes with higher fertilization rate. Here, we used 2 as the GV cutoff value, and if we had used another strategy for choosing the cutoff value we may have found different results. Moreover, Kok et al. [20] showed that the degree of ovarian response and the fraction of immature oocytes may not affect ICSI fertilization rate. Moreover, Kok et al. [20] showed that the degree of ovarian response and the fraction of immature oocytes may not affect ICSI fertilization rate.

One probable reason for higher fertilization rate in group A may be due to the increase in the number of mature oocytes available for injection. COH has a key 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 are heterogeneity of follicle population at the time of hCG injection [21].

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

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 [24].

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

Recently, de Cásia et al. [26] showed that the number of retrieved oocytes significantly correlated with increasing incidence of cytoplasmic granularity; they also reported that excessive ovarian response had negative effect on oocyte quality.

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.

Acknowledgements
Conflicts of interest
There are no conflicts of interest.

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