



## Relation Between Number Of Retrieved Oocytes and Intra Cytoplasmic Sperm Injection (ICSI) Outcome

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### Keywords

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**Abstract:** The implementation of safe and maximally effective ovarian stimulation is a major aim for *in vitro* fertilization (IVF) teams. The goal of controlled ovarian hyperstimulation (COH) is to supply enough oocytes with normal maturation to insure the consequent biological procedures. A variety of different stimulation protocols have been suggested and an individual selection of the correct stimulation protocol is mandatory.

The aim of the present study is to assess whether number of retrieved oocytes in intra Cytoplasmic sperm injection cycles could be an indicator for ICSI outcome. To achieve this target, the present study 650 patients younger than 36 years undergoing controlled ovarian stimulation (COS). We reviewed 650 patients. The study results clearly demonstrate that the aspiration of less than 5 oocytes significantly reduced pregnancy rate. The aspiration of a large number of oocytes (>15) does not lead to an increase of the treatment effect and, at the same time, increases the risk of ovarian hyperstimulation syndrome. The major goal is to obtain 5–15 oocytes as a “gold standard”, connected to optimal pregnancy rate after assisted reproduction (ART).

### Introduction

*In vitro* fertilization (IVF) involves the control of the natural cycle of the female partner to recruit a number of follicles and ultimately retrieve oocytes at a convenient time. This is achieved using varying protocols of pituitary down-regulation and ovarian stimulation. Intracytoplasmic sperm injection (ICSI) is performed only on mature (metaphase II) oocytes, which are usually only a fraction of the retrieved oocytes: 79.9% according to one study (Rattanachaiyanont et al., 1999).

In addition, fertilization rates are between 40% and 70% per injected oocyte (Payne and Matthews, 1995) and complete fertilization failure still occurs. The risk of the latter is dependent on the number of oocytes injected (Flaherty et al., 1998).

In more recent meta-analysis the overall fertilization rate per oocyte that underwent ICSI was 67.5%. (Lauren et al., 2013).

Furthermore one study submitted by Negri .L et al., 2014 reported that Overall 1624 MII oocytes were injected in the ICSI-ejaculated group with a fertilization rate of 60.8%.



The success of IVF treatments depends on the availability of embryos for transfer. Ludwig et al., 1997 suggest that a successful outcome of ICSI is guaranteed if as many oocytes as embryos to be transferred are available.

The number of antral follicles before stimulation and basal FSH levels has been found to be of good predictive value (Ng et al., 2000). So, the present study aimed to assess whether number of retrieved oocytes in intracytoplasmic sperm injection cycles could be an indicator for ICSI outcome. To achieve this target, the present study include 650 patients younger than 36 years undergoing controlled ovarian stimulation (COS). They were subjected to careful history taking, thorough clinical examination, routine laboratory investigations. ICSI procedure was performed according to the standard protocols.

### **Patients and Methods.**

The present study is a retrospective study. It was conducted at Mansoura Integrated Fertility Center. Informed consent was taken from participants before implementation of the study.

The study include 650 women indicated for ICSI. Patients were included on the basis of the following criteria:

#### **Inclusion criteria**

- Patients who fitted the medical definition of infertility "One year of unprotected intercourse but not pregnant".
- No previous intra-cytoplasmic sperm injection (ICSI) cycles.

#### **Exclusion criteria**

- History of ovarian or adnexal surgery.
- Suspicious findings of ovarian malignancy.

#### **Oocyte Retrieval.**

Oocytes retrieval was performed under ultrasound control by the transvaginal route on day 0, 36 h after the injection of Human chronic gonadotrophin (HCG).

Oocytes identification performed under SZ-ST Stereo Microscope, (Olympus, Japan) .

#### **Oocytes Denudation**

The oocytes were denuded of their surrounding cumulus cells 4-6 hours post oocyte retrieval using 80 IU/ml of hyaluronidase in flushing (Fertipro NV,Belgium) for 10 to 15 seconds, and then the oocytes were transferred to Continuous Single Culture™ with Gentamisin ,(Irvine Scientific, Santa Ana, USA) with 10% Serum Substitute Supplement (Irvine Scientific, Santa Ana, USA) or global total medium , (Life GlobalR , USA) for complete removal of the corona cells by repeated aspiration in a finely pulled pipette using Glass Pasteur Pipetten,(Hirschmann Laborgerate, Germany).

The oocytes were then rinsed and incubated in the culture media under mineral oil ,(Irvine Scientific, Santa Ana, USA) at 37°C, 6% CO<sub>2</sub> and 5% O<sub>2</sub> in incubator,(Heraeus incubator ,Kendro Laboratory Product, Hanau, Germany),until the time of injection (after few minutes), which was done for oocytes at the metaphase II stage.

#### **Intra Cytoplasmic Sperm Injection (ICSI)**

Only mature oocytes with a visible first polar body by the time of ICSI procedure were microinjected. During all the time of the current study, the ICSI conditions were identical (equipments,media, and culture conditions).

#### **Embryo transfer**

Embryos were transferred on day 3 or day 5 after oocytes retrieved with cook Catheter,(Cook Medical ,USA).

ICSI were performed at a private center for assisted reproduction, Mansoura Integrated Fertility Center, ( MIFC ), (Mansoura, Egypt) between January 2010 and April 2013.

### Statistical analysis

Data obtained from the present study were computed using SPSS versions 17 under the platform of Microsoft Windows 7. Continuous data were expressed in the form of mean  $\pm$  SD while categorical data were expressed in the form of count and percent. Comparison of continuous data was performed utilizing student t test or one way ANOVA, while categorical data were done using Chi-square test. Relation between variables were investigation by Pearson's correlation coefficient. P value less than 0.05 was considered statistically significant.

### Results

Results of the present study are shown in the following tables:

**Table-1:** Frequency distribution of retrieved oocytes number.

	No	%
$\leq 5$	42	6.5
6-15	493	75.8
> 15	115	17.7

This table shows that 42 women (6.5 %) had  $\leq 5$  oocytes while 493 women (75.8 %) had 6-15 oocytes and 115 women (17.7 %) had > 15 oocytes.

**Table-2:** Relation between oocytes number and embryo quality.

	$\leq 5$ (n=42)	6-15 (n=493)	> 15 (n=115)	Chi-square test	
				X <sup>2</sup>	P
Good	7 (16.7%)	88 (17.9%)	25 (21.7%)	1.06	0.82
Fair	20 (47.6%)	255 (51.7%)	55 (47.8%)		
Poor	15 (35.7%)	150 (30.4%)	35 (30.4%)		

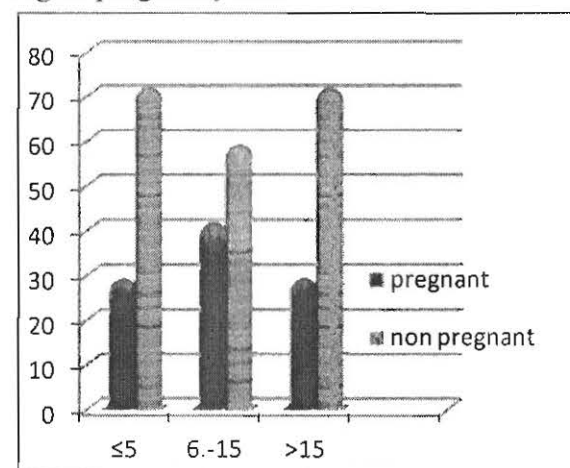
This table shows no statistically significant relation between number of retrieved oocytes and embryo quality.

**Table-3:** Comparison between pregnant and non pregnant women regarding frequency distribution of retrieved oocytes count.

	Pregnancy rate(38.2 %)	Non-pregnancy rate(61.8 %)	Chi-square test	
			X <sup>2</sup>	p
$\leq 5$	12/42 (28.6%)	30/42 (71.4%)		
6-15	203/493 (41.2%)	290/493 (58.8%)	11.9	0.008
> 15	33/115 (28.7%)	82/115 (71.3%)		

This table shows a significant higher pregnancy rate of retrieved oocytes 6-15 when compared with the pregnancy rate of  $\leq 5$  and > 15 retrieved oocytes.

**Fig-1:** pregnancy rate in the studied women.



### Discussion

The present study aimed to assess whether number of retrieved oocytes in intra Cytoplasmic sperm injection cycles could be



an indicator for ICSI outcome. To achieve this target, the present study include 650 patients younger than 36 years undergoing controlled ovarian stimulation (COS). They were subjected to careful history taking, thorough clinical examination, routine laboratory investigations. ICSI procedure was performed according to the standard protocols.

The numbers of oocytes obtained per cycle were classified into 3 groups  $\leq 5$ , 6-15, and  $> 15$  retrieved oocytes.

In our study, we found that pregnancy rate was significantly high with number of retrieved oocytes 6-15 when compared with the pregnancy rate of  $\leq 5$  and  $> 15$  retrieved oocytes.

This was further shown by the higher frequency of oocytes count equal to or less than 5 in non-pregnant women. These data find support in the study of *Melie et al.*, (2003) who aimed to determine the number of oocytes sufficient for satisfactory fertilization and pregnancy rates in intra cytoplasmic sperm injection cycles.

The numbers of oocytes obtained per cycle were classified into groups A, B, C, and D, consisting of 110, 124, 96, and 122 cycles, with 1-5, 6-10, 11-15,  $>15$  oocytes retrieved from each patient in each group, respectively. The chance of not obtaining a viable oocyte for injection was highest in group A (5.5%). Most fertilization failures occurred in group A (11.8%). Total cleavage failure occurred in the greatest percentage of cycles in group A (3.6%) with a significantly lower mean number of embryos (1.9 +/- 1.7) being transferred.

The clinical pregnancy rate was also lowest in group A (7.1%) compared with groups B (25.8%), C (20.8%), and D (23.8%).

Previous studies looking at the relationship between the number of eggs and pregnancy rates have reported inconsistent results in showing that pregnancy rates increased with an increasing number of eggs (Meniru and Craft, 1997), best pregnancy rates being obtained with number of eggs of 10-15 (Kably Ambe et al., 2008), or 7-16

(Molina Hita Ma. del M et al., 2008). Furthermore, these studies involved small numbers and were reported from single centres, which limited their generalizability. (Sunkara et al., 2011) Provide vital information on predicting the LBR on the basis of eggs retrieved in women of different age groups, this study suggest that around 15 eggs may be the optimal number to aim for in a fresh IVF cycle in order to maximize treatment success while minimizing the risk of OHSS which is associated with high number of eggs of  $>18$  (Lyons et al., 1994; Verwoerd et al., 2008; Lee et al., 2010).

### Conclusion

Our study showed that the number of retrieved oocytes could be used as an indicator for ICSI outcome.

The aspiration of 5 or less oocytes was related to significant reduction of success rate. At the same time production of a large number of oocytes ( $>15$ ) did not lead to an increase of the treatment effect.

We propose that a "golden standard" for optimal results of treatment is retrieved of 6 to 15 oocytes.

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## العلاقة بين عدد البويضات الناتجة وتأثيرها على نتائج عملية الحقن المجهري

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الحقن المجهري هو أسلوب مخبري جديد ومتطور يهدف الى علاج حالات العقم التي يكون فيها الحيوان المنوي غير قادر على إختراق جدار البويضة لتلقيحها، وفي هذه الحالة يحقن الحيوان منوي مباشرة داخل البويضة. تتضمن تقنية الحقن المجهري على تنشيط المبيضين بحقن الهرمونات لإنتاج أكبر عدد ممكن من البويضات. تهدف هذه الدراسة لتحديد العدد الامثل من البويضات الذي يحقق افضل النتائج ولا يؤدي الى حدوث اثاره مفرطه للمبايض " OHSS ". اظهرت الدراسة التي تمت على 650 سيدة خضعت لعملية الحقن المجهري وتقل اعمارهن عن 36 سنة ان نتائج الحمل تقل بشكل ملحوظ في السيدات اللاتي كانت استجابة التبويض 5 بويضات او اقل بينما كانت نتائج الحمل مرتفعه لدى السيدات اللاتي كانت استجابة التبويض بين 5-15 بويضه ولم يؤدي زيادة عدد البويضات عن 15 بويضه الى زياده ملحوظه في نتائج الحمل. توصي الدراسة باعتبار العدد من 5-15 المعيار الذهبي لعدد البويضات الذي يحقق افضل النتائج وتجنب حدوث إثارة المبايض المفرطة «OHSS»