
OUTCOME OF ICSI IN PATIENTS AGED 35 YEARS AND OVER

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ABSTRACT

Objectives: Adequate ovarian response is essential for successful assisted reproduction treatment in ICSI patients, and particularly among older infertile women. This study aimed to evaluate the ovarian response and outcome in ICSI patients aged 35 years and over undergoing ICSI cycles from July 2005 through December 2006 at the assisted reproductive technology (ART) unit, Al-Azhar University. The study included 180 patients classified into two groups; those aged 35 to 40 years (86%) considered to be the first group; and 26 patients aged more than 40 years (14%) are considered the second group. Oocytes retrieved, embryos obtained, and pregnancy outcome were the main outcome measures. Chi-square and t test were used as appropriate. Of the studied women, 158 (88%) reached the oocyte retrieval stage (134 in the first group and 24 in the second group). The mean number of oocytes aspirated per patient was 6.7 ± 4.0 and 3.4 ± 2.0 in the first and second groups respectively with statistically significant difference ($p < .0001$). Excluding negative fertilization patients, the mean number of obtained embryos in the first group ($n=114$) and second group ($n=14$) was 3.7 ± 2.5 and 2.5 ± 12.7 ($p = 0.04$). The pregnancy rate, however was 15% in those aged 35 to 40 years and 21% in those > 40 years, but with statistically significant difference.

Conclusion: This study showed that the number of oocytes retrieved and oocytes obtained to decrease by increasing age in ICSI patients aged 35 years and over. However, the pregnancy rate was slightly higher in older patients suggesting personal variations to play a role. Other studies with large number of patients and from different centers are needed to confirm these results.

INTRODUCTION

Adequate ovarian response is essential for successful assisted reproduction treatment in ICSI patients, and particularly among older infertile women. During recent years, the literature shows to some extent that we are able to predict the ovarian response. The factors which have been investigated as possible predictors include age (Rosenwaks et al., 1995)⁽¹⁾, ovarian volume (Lass et al., 1997; Syrop et al., 1999)^(2,3) number of antral follicles (Ng et al., 2000; Kupesic et al., 2002)^(4,5), assessment of the hormonal markers such as serum follicle stimulating hormone (FSH) (Bancsi et al., 2000)⁽⁶⁾, luteinizing hormone (LH) (Noci et al., 1998)⁽⁷⁾ and estradiol (E2) (Frattarelli et al., 2000)⁽⁸⁾. Cigarette smoking has been also found to predict ovarian response in

some studies (Van Voorhis et al., 1996; El-Nemr et al., 1998)^(9,10). Apart of their sampling variability, most of these published studies included only standard patients who were less than 35 years of age with regular menstrual cycle, and who were treated only with the long protocol. Also, the ovarian response as well as pregnancy outcome in elderly patients over 35 years did not take a considerable attention. From these points of view, this study aimed to study the ovarian response (oocytes retrieved and embryos developed) in ICSI patients aged 35 years and over and in relation to different treatment protocols, and to estimate the pregnancy outcome rate among these patients.

MATERIALS & METHODS

During the period from July 2005 through

December 2006, the infertile women undergoing ICSI cycles at the assisted reproductive technology (ART) unit, International Islamic Center for Population Studies and Researches (IICPSR), Al-Azhar University, and aged 35 years and over were recruited for this study. The medical files and pregnancy outcome of the included patients were reviewed. These women (n=180) were further subdivided into two groups: the first group included those aged from 35 to 40 years (n = 154) (86%), and the second group included those over 40 years (n = 26) (14%). Excluding negative TESE, poor responders, degenerated and ruptured ova, and negative fertilization patients, the main outcome variables were analyzed in only 158 patients (134 patients in the first age group and 24 patients in the second age group) in relation to the number of oocytes retrieved. For the number of embryo obtained and the pregnancy outcome, only 128 patients were analyzed (114 patients in the first age group and 12 patients in second age group) (Table 2). The pregnancy was diagnosed by doing pregnancy test after two weeks from the date of embryo transfer (ET) using the β human chorionic gonadotrophin (β HCG) tests with a diagnostic titer of 20 and more.

The characteristics of the studied women were expressed as mean \pm SD for continuous variables and percent distribution for the categorical variables and compared between the studied groups of women (first group 35-40 years; and second group > 40 years). The outcome variables were also compared between the two groups of the studied women. Chi square, Fischer exact and t tests were used as appropriate.

RESULTS

Table I presents the characteristics of the studied patients by age groups (35-40 years and > 40 years). The mean age of the first group (35-40 years) was

37.0 \pm 1.7 compared with 40.0 \pm 1.3 in the second group (> 40 years). For BMI, basal hormonal profiles, duration and causes of infertility, there were no statistically significant differences in the studied two groups. For ICSI procedure, there were statistically significant differences between the two groups with P value of 0.04 Table II showed also a significant difference regarding the protocol prescribed for each group (p = 0.01); with the long protocol was prescribed more for the studied patients less than 40 years.

Table III displays the mean and SD of retrieved oocytes by age group. There was a statistically high significant difference between the two groups regarding the mean number of oocytes retrieved with the higher mean number was in the first age group (35-40 years). also categorical comparison between the two studied age groups showed a significant difference (p = 0.004).

Table IV displays the mean and SD of embryos obtained by age group. There was a statistically significant difference between the two groups regarding the mean total number of embryos obtained with the higher mean number was in the first age group (35-40 years) (p=0.04). For the embryo grading, however, there was no statistically significant difference between the two studied age groups.

Table VI displays the mean number of embryo transferred by the studied age groups. A border-line significant difference (p = 0.06) regarding the mean total number of embryos transferred with the higher mean was among the age group 35-40 years. Regarding the grading of ET, there was no significant difference in the studied groups.

table VI shows the pregnancy outcome by age groups. Although the positive pregnancy outcome was slightly lower (15%) in the first age group

(35-40 years) compared with 21% in the patients aged over 40 years, no statistically significant difference was observed ($P = 0.43$).

Table VII shows causes of cycle cancellation of the studied patients by age group. No cancellation by negative TESE was found in the older age group (> 40 years); however the negative fertilization in this group was higher compared with first group patients aged from 35 to 40 years with a statistically significant difference ($p=0.03$).

DISCUSSION

Adequate ovarian response is essential for successful assisted reproduction treatment in ICSI patients, and particularly among older infertile women. This study aimed to study the ovarian response (oocytes retrieved and embryos developed) in ICSI patients aged 35 years and over and in relation to different treatment protocols, and to estimate the pregnancy outcome rate among these patients.

This study of 180 patients undergoing intra-cytoplasmic sperm injection (ICSI) cycles was designed to analyze the results of pregnancy outcome after assisted reproductive technology treatment in women aged 35 years old and over. Out of the 180 patients, 158 patients (88%) reached the oocyte retrieval stage, (134 patient in the first group and 24 patients in the second group). The average number of oocytes aspirated per patient was 6.7 ± 4.0 in the first group and 3.4 ± 2.0 in the second group and the total embryos obtained were 3.7 ± 2.5 for the first group and 2.5 ± 1.7 for the second group and the number of these transferred was 2.7 ± 1.1 for the first group and 2.1 ± 1.1 for the second group. These results are in agreement with the results of Ron et al (2002)⁽¹¹⁾; who found that the average number of oocytes

retrieved in patients over 41 years old was 6.7 ± 1.2 , and the number of embryos that were obtained was 2.8 ± 1.6 ; 2.5 ± 0.8 were transferrable, and the pregnancy rate per cycle was 12.4, and he found that the delivery rate was only 4.5% but the delivery rate in our study was not known because of lack of follow up.

The finding of this study suggested a better ovarian response, as indicated by oocytes retrieved and oocytes obtained in ICSI patients less than 40 years where the mean number of oocytes retrieved and obtained were higher in patients 35-40 years compared with the older age group of more than 40 years with a statistically significant difference. These results are consistent with other studies reporting increasing age to decrease significantly the mean number of oocytes retrieved and obtained (Seifer et al., 1999; Barnhart and Osheroff, 1998; Scott and Hofmann, 1995; Piette et al., 1990)⁽¹²⁻¹⁵⁾. According to Scott and Hofmann (1995)⁽¹⁴⁾, the declining of the ovarian response with age is a biological and not just a chronological function.

Also, this study found the ovarian response to be affected by the prescribed protocols with the long protocol being the best. This finding is also reported in other studies (Seifer et al., 1999)⁽¹²⁾. In that study, women with long protocol showed good ovarian response with a considerable number of oocyte retrieved and oocyte obtained. In this study, 25% of women aged 35-40 years received long protocol compared with only 28% of women aged > 40 years. This may explain the significant difference observed regarding the ovarian response in the two studied age groups.

A remarkable feature of the present study is that, unexpectedly, the pregnancy rate was found to increase slightly among patients over 40 years compared with those aged 35-40 years. This result

appeared inconsistent with the finding of Creus et al (2000)⁽¹⁶⁾ who found the increasing age to decrease the pregnancy rate and concluded that age is an important predictor of pregnancy outcome. Apart from the expected personal variation, it has been reported that older women with good ovarian response, producing more than three embryos suitable for transfer, have a pregnancy rate similar to younger patients. Cycles yielding three embryos or fewer have a poor prognosis. In the present study, although the mean number of ET was higher in the first group, the grading of embryos transferred was nearly the same in the studied two groups. Consistent with the present study finding regarding the pregnancy rate, Nikolettos et al (2000)⁽¹⁷⁾ reported that the great majority of the total pregnancies, in their retrospective study, resulting in women aged between 40 and 42 years. Furthermore, the women aged 35-40 years in this study were slightly obese with their mean BMI 30.1 ± 3.4 compared with those women > 40 years (mean BMI was 29.4 ± 4.1). Overweight and obesity have been reported in some studies to decrease the positive pregnancy outcome (Clark et al, 1998; Swieten et al, 2005)^(18,19). Also, the ICSI TESE procedure was higher in the first group compared with the second group with statistically significant differences. ICSI TESE is known to be associated with lower pregnancy rates.

Unlike other studies, this study has included only women in their first treated cycles and this may decrease the bias that may result when the study includes women in their first cycle treatment together with those with previous cycle treatments.

In summary, this study showed that the number of oocytes retrieved and oocytes obtained to decrease by increasing age in ICSI patients aged 35 years and over. However, the pregnancy rate was slightly higher in older patients suggesting personal variations

to play a role. Other studies with large number of patients and from different centers are needed to confirm these results. Researchers concluded that ICSI performed produced no clinical pregnancy among women greater than 45 years and no deliveries in women greater than that or equal to 44 years, and every time the statistics are figured for women over 40 years, it just keeps coming up the same, we just do not seem to get any better in treating this group of women, but we keep trying.

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Table I: Characteristics of the studied patients by age.

Variable	Age group 35 - 40 years (n = 154) (86.0 %)	Age group > 40 years (n = 26) (14.0 %)	P. Value
Age in years (mean ± SD)	37.0 ± 1.7	2.0 ± 1.3	< .0001
BMI (kg / m ²) (mean ± SD)	32.1 ± 3.4	29.4 ± 4.1	0.50
BMI (Kg / m ²)			
< 25	10 (6.0)	1 (4.0)	
25 - < 30	63 (41.0)	9 (35.0)	
> = 30	70 (45.0)	11 (42.0)	
Missing data	11 (8.0)	5 (19.0)	0.24
Hormonal profiles (mean ± SD)			
FSH	6.9 ± 3.9	8.4 ± 6.1	0.22
LH	5.3 ± 2.9	5.2 ± 3.0	0.90
PRL	14.5 ± 9.2	14.0 ± 10.7	0.80
E ₂	65.0 ± 54.3	71.2 ± 50.6	0.60
Main cause of infertility			
Male	76 (49.0)	14 (54.0)	
Female			
1 st Y	62 (40.0)	7 (27.0)	
2 nd Y	16 (11.0)	5 (19.0)	0.27
Duration of infertility in years (mean±SD)	11.5 ± 6.1	11.3 ± 7.9	0.90
Procedure			
ICSI / ejaculate	112 (72.0)	23 (88.0)	
ICSI / TESA	37 (24.0)	1 (4.0)	0.04
ICSI / PESA	5 (4.0)	2 (8.0)	

Table II: Distribution of the studied patients by age group and protocol.

Variable	Age group 35 - 40 years (n = 154) (86.0 %)	Age group > 40 years (n = 26) (14.0 %)	P. Value
Protocol			
Long	81 (52.0)	8 (28.0)	
Short	54 (35.0)	15 (60.0)	
Low cost	18 (12.0)	1 (4.0)	
Natural	1 (1.0)	2 (8.0)	0.01

Table III: Mean and SD of retrieved oocytes by age group*.

Variable	Age group 35 - 40 years (n = 134)	Age group > 40 years (n = 24)	P. Value
Oocytes retrieved (mean \pm SD)	6.7 \pm 4.0	3.4 \pm 2.0	< .0001
Categories :			
< 5	57 (43.0)	19 (79.0)	0.004
5 - 10	55 (41.0)	4 (17.0)	
> 10	22 (16.0)	1 (4.0)	

* Excluding negative TESA and poor responder patients.

Table IV: Distribution of embryos obtained by age group*.

Variable	Age group 35 - 40 years (n = 114)	Age group > 40 years (n = 14)	P. Value
Total embryos obtained (mean \pm SD)	3.7 \pm 2.5	2.5 \pm 1.7	0.04
Embryo (grade A) (mean \pm SD)	3.1 \pm 2.4	2.4 \pm 1.2	0.12
Embryo (grade B) (mean \pm SD)	1.5 \pm 0.8	1.5 \pm 1.0	0.96

* Excluding negative TESA, poor responders, degenerated and ruptured ova, and negative fertilization patients

Table V: Distribution of embryos obtained by age group*.

Variable	Age group 35 - 40 years (n = 114)	Age group > 40 years (n = 14)	P. Value
Total embryos transferred (mean \pm SD)	2.7 \pm 1.1	2.1 \pm 1.1	0.06
Total embryos transferred (categories)			
One embryo	19 (16.0)	5 (36.0)	0.15
Two embryos	27 (24.0)	3 (21.0)	
Three and more embryos	68 (60.0)	6 (43.0)	
Embryo transfer (grade A) (mean \pm SD)	2.5 \pm 1.1	2.1 \pm 1.0	0.30
Embryo transfer (grade B) (mean \pm SD)	1.2 \pm 0.4	1.0 \pm 0.01	0.50

* Excluding negative TESA, poor responders, degenerated and ruptured ova, and negative fertilization patients

Table VI: Distribution of pregnancy outcome by age group*.

Pregnancy outcome	Age group 35 - 40 years (n = 114)	Age group > 40 years (n = 14)	P. Value
Negative pregnancy outcome	98 (85.0)	11 (79.0)	
Positive pregnancy outcome	16 (15.0)	3 (21.0)	0.43

* Excluding negative TESA, poor responders, degenerated and ruptured ova, and negative fertilization patients

Table VII: Causes of cycle cancellation by age group.

Variable	Age group 35 - 40 years (n = 40)	Age group > 40 years (n = 12)	P. Value
Negative TESA	11 (27.0)	0 (0.0)	
Degenerated and rupture ova	16 (40.0)	5 (42.0)	
Poor response	9 (23.0)	2 (16.0)	
Negative fertilization	4 (10.0)	5 (42.0)	0.03