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Intracytoplasmic Sperm Injection Cycle Outcomes in Women Aged 40 Years and Over

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ABSTRACT

OBJECTIVE: The main detrimental factor in female fertility is maternal age. Clinical pregnancy and live birth rates decline gradually with age and this decline is dramatical over 40 years. In this study, we examined the in vitro fertilization/intracytoplasmic sperm injection cycle outcomes of women aged 40 years and over.

STUDY DESIGN: This retrospective cohort study included 336 fresh in vitro fertilization/ICSI cycles of women aged ≥40 years. Six groups, stratified by one-year intervals were composed according to age: 40 years; 41 years; 42 years; 43 years; 44 years, and ≥45 years. The primary outcomes were the clinical pregnancy and live birth rates.

RESULTS: The clinical pregnancy rate was 18.6% in 40 years old women and it decreased to 4% in women aged ≥45 years. The live birth rates in women aged 40 and 41 years (10% and 6.1% respectively) were higher than the live birth rates in women aged 42 and 43 years (4.3% and 3.8% respectively). There was no live birth in women aged 44 and over. The miscarriage rate was 46.2% at age 40 and it increased to 100% at age 44 and over.

CONCLUSION: The clinical pregnancy and the live birth rates decreased significantly with every single year after 40 years of age. In patients between the ages of 40-43, the live birth rate is acceptable but starting an in vitro fertilization cycle beyond 43 years of age does not seem to be reasonable.

Keywords: Advanced maternal age, Clinical pregnancy, In vitro fertilization, Live birth

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Introduction

Maternal age is the main determining factor in female fertility and is important in in vitro fertilization (IVF) cycle success. In human, ovarian aging begins at 30 years of age (1) and continues until menopause. The main reason for age-related infertility and decreased IVF success in aged women is diminished ovarian reserve and decreased mitochondrial activity and increased aneuploidy incidence resulting in oocyte and/or embryo incompetence (2). In natural conception cycles; the fertility rate is 95% lower in women aged 40 and 45 years and the miscarriage rate is 34-52% in women aged 40 and over (3). With advanced maternal age not only the fertility capacity is reduced but also the incidence of adverse obstetric and perinatal outcomes are increased (4).

Advanced maternal age is described as women over 35 years of age experiencing pregnancy. In IVF cycles, embryo implantation and live birth rates decline gradually by age but this decline is dramatical over 40 years of age (5). The live birth rate is 5.9%-6.7% per embryo transfer in women undergoing IVF treatment at age 40 years and over (5,6) and it declines to 0.7% in women aged ≥45 years (6) whereas it is 50% in women aged ≤35 years. The cycle cancellation and miscarriage rates are also high in advanced age patients undergoing IVF treatment. The cycle cancellation rate per initiated cycle is 19% at age 40 and increases with every year reaching 55% at age ≥45 (7). The miscarriage rate is 44.8% for women aged ≥40, ranging between 39% at age 40 and 67% at age ≥45 (7).

On the other hand, there is an increasing incidence of advanced age patients attending IVF all around the world as a result of educational, economic, and social issues. In many

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countries, there is legislation on assisted reproductive technologies (ART) and frequently maternal age is the main limiting criteria (8). There are also limits for public funding depending on age. Therefore assessment of IVF outcomes and detailed counseling of an advanced age patient especially over 40 years attending for IVF treatment is important in terms of medical, legal, and financial aspects.

In this study, we aimed to examine the ICSI cycle outcomes of women aged 40 years and over and the change in reproductive outcomes with every single year.

Material and Method

This is a retrospective cohort study and it was conducted at Health Sciences University Etlik Zubeyde Hanim Women's Health Teaching and Research Hospital, ART clinic, Ankara, Turkey. We reviewed the patient files and analyzed 336 fresh cycles of women aged ≥40 years who attended our clinic between January 2007 and October 2019. Six groups, stratified by one-year intervals were composed according to age: 40 years; 41 years; 42 years; 43 years; 44 years, and ≥45 years. We excluded the patients with additional factors affecting fertility such as endometrial, uterine pathologies, endometriosis, or hydrosalpinx and with male factor infertility. The study was conducted in accordance with the Declaration of Helsinki and is approved by the institutional ethics committee (23/06/2021-2021/70). Consent had been obtained from all patients that the data related to the treatment process can be used in scientific research at the beginning of the treatment.

Standard long agonist or antagonist protocols were used for ovarian stimulation. The initial gonadotropin dose in most patients was 225-300 IU/day as human menopausal gonadotropin (hMG) and/or recombinant follicle-stimulating hormone (FSH). The gonadotropin dose was determined and individualized depending on the patient's age and baseline characteristics such as basal serum FSH level, body mass index (BMI), and antral follicle count (AFC). In the antagonist protocol, the GnRH antagonist was initiated according to the flexible protocol; when the leading follicle reached a mean diameter of 12-14 mm. Cycle monitorization until hCG administration was carried out with transvaginal ultrasonography (TVS), serum estradiol (E2), luteinizing hormone (LH), and progesterone level measurements at regular intervals. Oocyte retrieval (OPU) under transvaginal ultrasound guidance was performed after 35.5-36 hours from hCG administration.

For all mature oocytes, intracytoplasmic sperm injection (ICSI) procedure was performed. With the observation of two pronuclei after 18-20 hours from ICSI, fertilization was assessed. Cleavage stage embryos (61-65 hr after ICSI) were graded using the embryo scoring system based on the number, size, and symmetry of the cells and the fragmentation degree (9). The embryo scoring of the blastocyst stage was based on the scoring system proposed by Gardner et al (10).

The absence of follicular development, failure to retrieve oocytes in OPU, fertilization failure, and embryo development arrest were accepted as cycle cancellation.

Following embryo transfer with the guidance of transabdominal ultrasonography, luteal phase support (Crinone 8% gel, Serano, Istanbul) was started for all patients and they were advised to continue it up to 10-12 gestational weeks in case of pregnancy. Increased serum hCG levels measured fourteen days after OPU (hCG > 10 IU/L) were repeated after 2–4 days for the confirmation of hCG doubling levels and TVS was performed 14 days later.

The presence of a positive heartbeat embryo at TVS was defined as clinical pregnancy. Viable delivery of an infant after 22 gestational weeks per embryo transfer was defined as live birth rate and the percentage of pregnancy loss before 20 gestational weeks among all clinical pregnancies was defined as miscarriage rate.

Statistical analysis

The data were analyzed by using the statistical package program SPSS for Windows version 20. Data were expressed as mean ± SD and in percentages. The Kruskal Wallis test for non-parametric numerical data and a One-way ANOVA test for parametric numerical data were conducted. The Chi-square test was used to analyze the relationship between the categorical variables. Investigation of continuous variables was carried out with Kolmogorov-Smirnov/Shapiro-Wilk's tests. Relationships between categorical variables were analyzed by the Chi-square test. A p-value of <0.05 was accepted as statistically significant.

Results

In this study, 336 fresh ICSI cycles of women aged 40 years and over were analyzed. During the study period, it was observed that women aged 40 years and over constituted 6.3% of all patients initiated an IVF cycle at our clinic. There were 106 cycles in 40 years of age group, 58 cycles in 41 years of age group, 44 cycles in 42 years of age group, 41 cycles in 43 years of age group, 29 cycles in 44 years of age group, and 58 cycles in 45 years of age, and above group.

The cycle characteristics are shown in table I. Among all patients, the primer infertility rate was 84.2% and the secondary infertility rate was 15.8%, and there was no statistically significant difference in terms of infertility type between the age groups. The duration of infertility was longer in women aged 45 years and over, but the difference between the age groups was not statistically significant (p=0.06). The number of retrieved oocytes and the number of mature oocytes were compared among the age groups, and both were found to be higher in women aged 40 and 41 years with statistically significant differences (p=0.001). The fertilization rate was similar within the groups.

Table I: *Basal and in vitro fertilization cycle treatment parameters of women aged ≥40 years*

		40 n=106	**41 n=58**	**42 n=44**	**43 n=41**	**44 n=29**	**≥45 n=58**	***p***
Type of infertility, n(%)	Primer	86 (81.1)	48 (82.8)	41 (93.2)	32(78.0)	25(86.2)	51(87.9)	0.37
	Seconder	20 (18.9)	10 (17.2)	3 (6.8)	9(22.0)	4(13.8)	7(12.1	
Duration of infertility, months		79.2±72.6	87.2±79.6	79.3±76.3	57.8±72.6	49.7±57.6	113.9±114.9	0.066
Body mass index, kg/m^2		26.6±4.2^{c}	27.6±5.8	29.3±8	27.6±4.9	30.2±4.6^{c}	28.9±4.6	**0.010**
Basal FSH level, IU/mL		9.6±5.3^{b,d}	10.7±4.9	9.9±5.4^{i,j}	12.1±6.2^{b,i}	11.1±6.6	11.9±6.4^{d,j}	**0.024**
Basal LH level, IU/mL		5.5±2.7	6.5±4.4	6 ± 6.3	6.2±3.9	4.7±2.8	5.7±3	0.091
Basal E2 level, pg/mL		57±60.9	49.2±33.1	76.9±72.1	64.9±65.6	58.6±24.8	50.6±47.2	0.088
AMH, ng/mL		1.6±1.4	0.7±0.5	0.4±0.3	0.7±0.6	0.8±0.6	0.6±0.6	0.057
Total antral follicle count		6.3±4.3^{a,b,c,d}	7.4±5.5^{e,f,g,h}	4.5±2.8^{a,e}	5±4.9^{b,f}	4.3±3^{c,g}	5.4±4^{d,h}	**0.001**
Stimulation protocol, n(%)	Antagonist	88 (83.0)	45 (77.6)	38 (86.4)	35(85.4)	23 (79.3)	51(87.9)	0.697
	Agonist	18 (17.0)	13 (22.4)	6(13.6)	6(14.6)	6(20.7)	7 12.1)	
Type of gonadotropin, n(%)	rec FSH or hMG	23 (21.7)	13 (22.4)	12 (27.3)	14(34.1)	8(27.6)22	(37.9)	0.249
	rec FSH and hMG	83 (78.3)	45 (77.6)	32 (72.7)	27(65.9)	21 (72.4)	36 (62.1)	
Initial gonadotropin dose, IU		340.1±79.5	345.3±67.3	356.3±65.0	329.3±86.9	337.1±67.0	351.7±83.4	0.621
Duration of ovulation induction, days,		9.5±2.3^{a,b,c,d}	9.7±2.2^{e,f,g,h}	9.3±2.5^{j}	10.5±3^{b,f,i,k,l}	9.1±2.3m^{k}	8.4 ± 2.4^{d,h,l}	**0.006**
Total gonadotropin dose, IU		3193.6±1222.9	3283.9±1006.9	3239.3±1127.5	3501.9±1602.1	2989.3±1039.6	2872.5±934.5	0.294
Number of oocytes retrieved		7.2±4.9^{a,b,c,d}	7.4±6.2^{e,f,g,h}	4±2.9^{a,e}	4.1±2.6^{b,f}	4.6±3.3^{c,g}	4.2±4.4^{d,h}	**0.001**
Number of mature oocytes		5.3± 3.9^{a,b,c,d}	4.9±4.1^{e,f,g,h}	2.8±2.2^{a,e}	2.9±2.4^{b,f}	3.6±2.8^{c,g}	3.3±3.7^{d,h}	**0.001**
Number of fertilized oocytes		2.3±2.2	2.4±3.2	1.5±1.7	1.8±1.8	1.6±1.7	1.8±2	0.326
E2 level on hCG day,pg/mL		1839.8±1180.6^{a,b,c,d}	1718±1598.1	1273±1158.3	1139.7±817.1	971.9±741.1^{c,g}	1049.8±1283.6^{d,h}	**0.001**
Endometrial thickness on hCG day, mm		9.2±1.8	9.2±2.1	9±2.2	8.9±1.8	9.1±2.1	8.5±2.3	0.347
E2 level on OPU day, pg/mL		1087.4±691.2^{a,b,c,d}	986.2±886.9^{e,f,g,h}	700.3±604.9^{a,e}	640.8±449.4^{b,f}	630.1±512.4^{c,g}	666.8±981.6^{d,h}	**0.001**
Endometrial thickness on OPU day, mm		9.3±2.7	9.2±2.2	8.7±2.2	8.6±2.6	9.1±2.4	8.7±2.2	0.474

Data presented as mean±SD. p values with statistical significance (p<0.05) are shown in bold.

a: *Pairwise comparison revealed a statistically significant difference between 40 and 42 years age groups.* **b:** *Pairwise comparison revealed a statistically significant difference between 40 and 43 years age groups.* **c:** *Pairwise comparison revealed a statistically significant difference between 40 and 44 years age groups* **d:** *Pairwise comparison revealed a statistically significant difference between 40 and ≥45 years age groups.* **e:** *Pairwise comparison revealed a statistically significant difference between 41 and 42 years age groups.* **f:** *Pairwise comparison revealed a statistically significant difference between 41 and 43 years age groups.* **g:** *Pairwise comparison revealed a statistically significant difference between 41 and 44 years age groups.* **h:** *Pairwise comparison revealed a statistically significant difference between 41 and ≥45 years age groups* **i:** *Pairwise comparison revealed a statistically significant difference between 42 and 43 years age groups.* **j:** *Pairwise comparison revealed a statistically significant difference between 42 and ≥45 years age groups.* **k:** *Pairwise comparison revealed a statistically significant difference between 43 and 44 years age groups.* **l:** *Pairwise comparison revealed a statistically significant difference between 43 and ≥45 years age groups*

Among 336 cycles, embryo transfer was performed in 189 (56.3%). A total of 147 (43.7%) cycles were canceled, the clinical pregnancy rate was 7.4% per initiated cycle and 13.2% per embryo transfer; the live birth rate was 3.3% per initiated cycle and 5.8% per embryo transfer. When the age groups were analyzed; a significant decrease in clinical pregnancy and live birth rates were observed with each year among increasing age groups (p=0.025). The clinical pregnancy rate was higher in 40, 41 and 42 years of age groups compared with 43, 44, and ≥45 years of age groups, and the difference was statistically significant (p=0.034). The live birth rate was 10% in 40 and 6.1% in 41 years of age groups and there were no live births in 44 and ≥45 years age groups. The miscarriage rate was 46.2% at age 40 years and it increased to 100% at age 44 and above. The difference between miscarriage rates was not statistically significant (Table II).

The analysis of the canceled cycles is demonstrated in Table III. Women aged 44 and over had higher cycle cancellation rates compared with women aged 40, 41, 42, and 43, but only the difference compared with 40 years of age group was statistically significant. Regarding the number of cycles with inadequate response to ovarian stimulation, total fertilization failure, and embryo development arrest, there was no statistically significant difference among the age groups (p=0.98, p=0.053, and p=0.442, respectively).

When the parameters associated with clinical pregnancy in women aged ≥40 years were evaluated with multivariate logistic regression analysis, maternal age (OR=1.86, 95% CI=1.02-3.39) and the number of fertilized oocytes (OR=1.64, 95% CI=1.04-2.60) were identified as independent predictors for clinical pregnancy (Table IV).

Table II: *Comparison of in vitro fertilization intracytoplasmic sperm injection cycle outcomes among age groups*

	40 (n=106)	41(n=58)	42 (n=44)	43 (n=41)	44 (n=29)	≥45 (n=58)	*p*
Embryo transfer rate	70(66.0)[c,d]	33(56.9)	23(52.3)	26(63.4)	12(41.4)[c]	25(43.1)[d]	**0.035**
Clinical pregnancy rate	13(18.6)[b,c,d]	5(15.2)[f,g,h]	3(13.0)[i,j,k]	2(7.7)[b,f,i]	1(8.3)[c,g,j]	1(4.0)[d,h,k]	**0.034**
Live birth rate	7(10.0)[a,b,c,d]	2(6.1)[e,f,g,h]	1(4.3)[a,e,j,k]	1(3.8)[b,f,l,m]	0(0.0)[c,g,j,l]	0(0.0)[d,h,k,m]	**0.046**
Miscarriage rate	6(46.2)	3(60.0)	2(66.7)	1(50.0)	1(100)	1(100)	0.809

Data presented as n(%). p values with statistical significance (p<0.05) are shown in bold.
a: *Pairwise comparison revealed a statistically significant difference between 40 and 42 years age groups.* ***b:*** *Pairwise comparison revealed a statistically significant difference between 40 and 43 years age groups.* ***c:*** *Pairwise comparison revealed a statistically significant difference between 40 and 44 years age groups.* ***d:*** *Pairwise comparison revealed a statistically significant difference between 40 and ≥45 years age groups.* ***e:*** *Pairwise comparison revealed a statistically significant difference between 41 and 42 years age groups.* ***f:*** *Pairwise comparison revealed a statistically significant difference between 41 and 43 years age groups.* ***g:*** *Pairwise comparison revealed a statistically significant difference between 41 and 44 years age groups.* ***h:*** *Pairwise comparison revealed a statistically significant difference between 41 and ≥45 years age groups.* ***i:*** *Pairwise comparison revealed a statistically significant difference between 42 and 43 years age groups.* ***j:*** *Pairwise comparison revealed a statistically significant difference between 42 and 44 years age groups.* ***k:*** *Pairwise comparison revealed a statistically significant difference between 42 and ≥45 years age groups* ***l:*** *Pairwise comparison revealed a statistically significant difference between 43 and 44 years age groups.* ***m:*** *Pairwise comparison revealed a statistically significant difference between 43 and ≥45 years age groups*

Table III: *The analysis of the canceled cycles of the age groups*

	40 (n=106)	41(n=58)	42 (n=44)	43 (n=41)	44 (n=29)	≥45 (n=58)	*p*
Cancellation rate	36(33.9)[a,b]	25(43.1)	21(47.7)	15(36.6)	17(58.6)[a]	33(56.9)[b]	**0.025**
No ovarian response	16(15.1)	9(15.5)	12(27.3)	5(12.2)	4(13.8)	17(29.3)	0.098
Total fertilization failure	10(9.4)	8(13.8)	5(11.4)	2(4.9)	8(27.6)	11(19.0)	0,053
Embryo development arrest	10(9.4)	8(13.8)	4(9.1)	8(19.5)	5(17.2)	5(8.6)	0.442

Data presented as n(%). . p values with statistical significance (p<0.05) are shown in bold
a: Pairwise comparison revealed a statistically significant difference between 40 and 44 years age groups
b: Pairwise comparison revealed a statistically significant difference between 40 and ≥45 years age groups

Table 4: *The multivariate logistic regression analysis of the parameters associated with clinical pregnancy of women aged ≥40 years*

	B	*p*	OR	95% CI	
				Lower	Upper
Maternal age	-0.624	0.041	1.866	1.026	3.390
Body mass index	-0.073	0.383	0.930	0.789	1.095
Basal FSH level	0.030	0.647	1.030	0.908	1.169
Basal E2 level	0.000	0.993	1.000	0.978	1.023
Total antral follicle count	0.100	0.258	1.105	0.929	1.314
Ovulation_Induction_Duration	0.029	0.881	1.029	0.707	1.497
E2 level on hCG day	0.000	0.581	1.000	0.999	1.001
Number of mature_oocytes	-0.230	0.243	0.795	0.540	1.169
Number of fertilized oocytes	0.498	0.043	1.645	1.041	2.601

Discussion

Fertility decreases as woman ages, and although the success of an IVF treatment cycle depends on many factors, maternal age is the main predictor. With advanced age, the clinical pregnancy and live birth rates decrease dramatically especially over the age of 40. The main reason for fertility decline related to age is diminished ovarian reserve and oocyte and/or embryo incompetence due to decreased mitochondrial activity and increased aneuploidy incidence (2). The incidence of adverse obstetric and perinatal outcomes is also increased in advanced age patients (4).

In the last decades, advanced age patients attending IVF clinics have been increased markedly. In many countries, there is legislation on assisted reproductive technologies (ART) and frequently maternal age is the main limiting criteria (8). There are also limits for public funding depending on age. Therefore, IVF treatment of an advanced age patient is not only a medical problem but also has legal and financial aspects.

The results of studies comparing the efficacy of different ovarian stimulation protocols on IVF outcomes in advanced age patients are controversial. ICSI outcomes were found to be better with the long protocol compared to the antagonist protocol in women aged 40 years and over (11,12). Lambalk et al concluded in their systematic review and meta-analysis that there was no difference in ongoing pregnancy rates between the antagonist and agonist protocols in poor responder patients (13). In our study, 83.3% of the patients were stimulated with the antagonist protocol and 16.7% with the long agonist protocol.

As a result of poor ovarian response to controlled ovarian hyperstimulation, the number of cycles resulting with embryo transfer is decreased by age (5-7). The fertilization rate is decreased and embryo development is impaired in advanced age patients. In our study, embryo transferred cycles also decreased by age, from 66% among women at 40 years of age to 41.4% among women aged 44. Cycle cancellation because of inadequate follicular development or no mature oocytes retrieved in OPU and total fertilization failure was higher among women aged 45 years and above compared with 40 years of age group.

After ≥40 years of age, an additional single year is important in live birth and miscarriage rates (14). There are studies concluding that 43 is the cut-off age which is statistically significant for lower clinical pregnancy and live birth rates (6,7,14). The live birth rate per embryo transfer was found to be ≤5% in women aged ≥43 years and 0.7% in women aged ≥45 years, whereas clinical pregnancy rate was 22.3% and live birth rate was 12.9% in women aged 40 years (6). Similar to these studies, clinical pregnancy rates were higher in 40, 41, and 42 years age groups compared with other age groups in our study. Live birth rates were 10.0% in 40, 6.1% in 41 years of age groups whereas it was 4.3% in 42 and 3.8% in 43 years of age groups. In many studies live birth was not achieved in women aged ≥45 years (5,14,15). In Ron-El et al.'s study, there were no live births beyond age 43 (16). In our study, there were no live births at 44 years of age and beyond.

Miscarriage rates are shown to be increased with age due to increased incidence of aneuploidy. Cimadomo et al. concluded in their study that the aneuploidy rate in blastocysts is >90% in women over 44 years of age (2). Ubaldi et al advised 45 years to be a threshold for IVF treatment with autologous oocytes as there were no euploid embryos available in patients older than 45 years (17). In our study, 56% of clinical pregnancies resulted in miscarriage in women aged ≥40 years. The miscarriage rate increased with age from 46.2% at age 40 years to 100% at age 44 years and above. Preimplantation genetic screening (PGS) is advised to decrease miscarriage rates due to chromosomal abnormalities and to improve live birth rates for advanced age IVF patients. Lee et al reported that the live birth for PGS-fresh embryo transfer cycles (45%) was significantly higher than fresh embryo transfer cycles without PGS (15.8%) in women aged 40-43 (18). On the other hand, there are limitations for PGS especially in patients ≥43 years of age, as the number of developing embryos is low.

In conclusion, the live birth rate with ICSI is low in women aged 40 years and older, and every single year after 40 years of age decreases the chance. The live birth rate is acceptable between ages 40 to 43 years, but starting an IVF cycle beyond 43 years of age does not seem to be reasonable and feasible.

Declarations: Consent had been obtained from all patients that the data related to the treatment process can be used in scientific research at the beginning of the treatment.
Availability of data and materials: The data supporting this study is available through the corresponding author upon reasonable request.
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