The Impact of Woman's Age on the Success of ART Applications

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Summary

Objective: Diminished fecundity with increasing woman's age is well documented. In this study we addressed the question of whether there is an effect of woman's age on Assisted Reproductive Technology (ART) outcomes by using data which were obtained from patients who underwent ART applications at the Family Planning and Infertility Research and Treatment Center, Ege University, Bornova, Izmir, Turkey.
Design: The number of retrieved oocytes, mature oocytes, fertilized oocytes, transferred embryos, embryo quality, fertilization rates and pregnancy rates were determined in the different age groups of patients who underwent ART applications.
Materials and Methods: 309 cycles (= 309 patients) consisting of IVF cycles without andrologic factor and ICSI cycles were evaluated. The patients were divided into five groups according to age as following: (1) 22-29, (2) 30-34, (3) 35-37, (4) 38-39, (5) 40-44. These different age groups were compared according to the required gonadotropin hormone dosage for ovulation induction, cancellation rate of cycles, number of retrieved oocytes, mature oocytes, fertilized oocytes, transferred embryos, fertilization rates, embryo quality and pregnancy rates.
Results: The cancellation rate of cycles were found to be statistically significant higher in the older age groups (1,8 % in the group 1 and 26,7% in the group 5; $p \leq 0,05$). The number of retrieved oocytes, mature oocytes, fertilized oocytes and transferred embryos were also found significantly lower. The quality of embryos did not show any significant difference between these groups. The clinical pregnancy rates were evidently found to be high in women ≤ 38 years (20,8 % vs. 10,1 %).
Conclusions: As a conclusion we have found that while the number of retrieved oocytes, mature oocytes, fertilized oocytes, transferred embryos and the pregnancy rates were decreasing significantly with aging of patients; the cancellation rate of cycles were increasing. Taking over 38 years as a cut-off, we observed poor ART outcomes in women ≥ 38 years.

Keywords: Woman's age, IVF, ICSI, ART outcomes

Introduction

An increase in anovulatory cycles has been found in women over the age of 40 years and in perimenopausal women who experienced a sudden break in menstrual cyclicity after years of regular cycles (Metcalf et al., 1981). Pregnancy rates are known to decline with increasing age after standard IVF-ET treatment and after IVF-ET using intracytoplasmic sperm injection (ICSI) (Piette et al., 1990; Abdelmassih et al., 1996).

Nevertheless, it is still somewhat controversial whether the woman's age during Assisted Reproductive Technologies (ART) treatment has an effect on oocyte production, oocyte quality, or uterine receptivity. Sauer et al. (1992), Abdalla et al. (1993) , Navot et al. (1994), and Check et al. (1994) found an effect of the woman's age on oocyte quality, but no or only a negligible effect on uterine receptivity.

Chetkowski et al. (1991) using a mathematical model were able to demonstrate a significant age-related drop in both embryo quality and uterine receptivity. Although some authors have observed decreased pregnancy rates in women older than 40 years as compared with younger patients, others have found no such differences (Navot et al., 1994; Check et al., 1994; Flagmini et al., 1993). Thus although some models seem to be valid, some controversy still exists as to whether or not the uterus ages with the ovary.

Many IVF programs restrict the age of the woman to < 40 years as a requirement for acceptance. Explanations for these restrictions include the expected decline in fertility potential with the increased maternal age, the increased incidence of miscarriages, and the higher risk of chromosomal abnormalities. Investigators have reported maternal age as a cause of lower success rate, higher abortion rate, and a higher cancellation rate per cycle (Padilla et al., 1989).

The purpose of this study is to evaluate the effect of woman's age on ART outcomes in patients who underwent ART applications at the Family Planning and Infertility Research and Treatment Center, Ege University, Izmir, Turkey. The intention was to provide prognostic information that would be valuable to both clinicians and patients.

Material and Methods

Our data consist of women attending the ART program at the Family Planning and Infertility Research and Treatment Center, Ege University, Turkey, in the period between 1997 June and February 1998. 309 women (= 309 cycles) undergoing the IVF program without an andrologic factor or ICSI program, were induced by short or long protocol GnRHa and gonadotropins.

112 cycles out of 309 cycles were induced by short protocol GnRHa combined with FSH and HMG where as 197 cycles were induced by long protocol GnRHa combined with FSH and HMG. 177 cycles of the long protocol were started from follicular phase and 20 cycles were started from luteal phase.

In patients with a serum E2 level < 70 pg/ml and no follicular cyst > 15 mm in the transvaginal ultrasonography (TVUS);

Triptorelin 0.5 mg (Decapeptyl, Ferring) and HMG (Pergonal - Serono ; Humegon – Organon) and FSH (Metrodin – Serono) were started on the 2nd day of menstrual cycle, as short protocol application. These patients received Triptorelin 0.5 mg/day for 5 days continuing with Triptorelin 0.1 mg/day until day of HCG administration.

In the long protocol application, 20 patients received Triptorelin 0.5 mg/day s.c starting from the midluteal phase in the previous cycle (day 21), and 177 patients from the early follicular phase (day 2) in the same cycle. Triptorelin 0.5 mg/day was given for 7 days and continued by 0.1 mg/day for 7 days s.c.. Down regulation by using only GnRHa for 14 days was continued by vaginal ultrasonography and serum E2 level detection. If the serum E2 levels were < 70 pg/ml and TVUS revealed no follicular cyst > 15 mm in diameter, ovulation induction with HMG and FSH was started. Triptorelin 0.1 mg/day was continued until the day of HCG administration.

The dose of gonadotropin hormone was individualized according to patient's age, baseline hormone levels and previous stimulation history or response to stimulation. Cycles were monitored by TVUS (Kretz, Combison 310, 5 mHz transvaginal probe, 240°, Austria) and serum E2 levels. Follicular maturation was completed by the administration of 10000 IU HCG (Pregynl 5000 IU– Organon; Profasi 2000 IU– Serono), when at least two follicles reached a diameter ≥ 17-18 mm. Oocyte retrieval was performed by the guidance of TVUS 36 hours after HCG administration. Oocyte were cultured in Ham's F-10 culture medium. Mature oocytes were inseminated by sperms prepared by the swim up technique 4-6 hours later. The ICSI procedure was performed according to the method described by Van Steirteghem et al. (1993). Fertilization was observed 16-22 hours after insemination and cleaved embryos were transferred 48-56 hours after oocyte retrieval.

Luteal phase support was given by administrating progesterone vaginal suppositories (Utrogestan 100 mg , 2x2) or progesterone ampoules (25 mg , 1x2 i.m.) daily and HCG (1500-2000 IU) on days +1, +4, +7, +9 after embryo transfer. Serum HCG levels were detected on day +12 after embryo transfer. A clinical pregnancy was defined as serum HCG levels > 1000 IU/ml and intrauterine gestational sac documented by TVUS.

The patients were divided into five groups according to their ages as following: **group 1**=22-29 years, **group 2**= 30-34 years, **group 3**= 35-37 years, **group 4**= 38-39 years, **group 5**= 40-44 years. The required gonadotropin hormone dosage for ovulation induction, cancellation rate of cycles, number of retrieved oocytes, mature oocytes, fertilized oocytes, transferred embryos, fertilization rates, embryo quality and pregnancy rates were compared between these different age groups.

Endocrinologic parameters were evaluated by using Automated Chemiluminescence System (ACS 180 plus, Ciba Corning).

Statistical analysis was evaluated by using t-test, X^2 , Fisher's exact test, Kruskal-Wallis test and Mantel-Haenszel test for linear association, with significance of $p< 0.05$.

Results

We evaluated 309 ART cycles in 309 patients. Controlled ovarian hyperstimulation (COH) was performed by using short protocol GnRHa and FSH+HMG combinations in 112 cycles (36.2%) and long protocol in 197 cycles (63.75%). Follicle puncture was performed in 276 (89.3%) of the cases, and the number of cancellation rate of cycles was 31 (10%) (in two patients no oocyte could be obtained). ART was performed in 267 (86.4%) of the cases. IVF was performed in 148 (47.9%) cases where as ICSI was performed in 119 (38.5%) cases. Nothing was performed in 9 (13.6%) of the cases due to either and/or lack of spermatozoa, mature oocyte available. The total number of patients with embryo transfer were 230 (74.4%). In 37 of the cases no cleavage was detected.

The average age in our study group was (mean ± semean) 33.75 ± 0.25 (min: 22, max: 44). The total number of clinical pregnancy was 57 and clinical pregnancy rate per cycle was 18.4%. Clinical pregnancy rate per follicle puncture and per embryo transfer were respectively 20.6% and 24.7%.

Table 1 : Characteristics of patients in the different age groups.

	Woman's age (year)				
	22-29	30-34	35-37	38-39	40-44
No. of patients	57 (18.4%)	113 (36.6%)	70 (22.7%)	39 (12.6%)	30 (9.7%)
Etiology of infertility (%)					
Male	54.4	35.4	31.4	20.5	23.3
Tubal	26.3	42.5	51.4	46.2	56.7
Endometriosis	3.5	4.4	1.4	7.7	6.7
Tubal + Endomeriosis	3.5	1.8	2.9	5.1	0
Male + Endometriosis	1.8	2.7	2.9	2.6	6.7
Idiopatic	7	8	7.1	15.4	6.7
Tubal + Male	3.5	5.3	2.9	2.6	0

Among the 309 cases in our study group, the main cause of infertility was male factor in 108 (35%), tubal in 134 43.4%), endometriosis in 13 (4.2%), tubal + endometriosis in 8 (2.6%), male + endometriosis in 9 (2.9%), unexplained in 26 (8.4%) and tubal + male in 11 (3.6%).

Fertilization rates were calculated for IVF and ICSI separately. These rates were found as 54.12% for IVF applications and 53.39% for ICSI applications. There was no statistically significant difference between these rates (t test, $p > 0.05$) and therefore these two groups were evaluated together.

Distribution rates of etiology of infertility according to age groups of the patients are shown in **Table 1**.

There was significant difference between these age groups in cancellation rates ($p < 0.05$). The cancellation rates were increasing with the age of patients. While the cancellation rate was 1.8% in 22-29 years group, this rate increased to 26.7% in 40-44 years group. The amount of HMG and FSH ampoules used for ovulation induction were increasing with age of patients as shown in **Table 2** ($p < 0.05$).

The number of retrieved oocytes, mature oocytes and fertilized oocytes were significantly decreasing in older age groups ($p < 0.05$). Difference of the number of transferred embryos between these age groups were also found statistically significant as (mean ± semean) 2.70 ± 0.23 in group 1 and 1.13 ± 0.23 in group 5. There was no difference in the quality of embryos ($p > 0.05$).

Follicle puncture rates per cycle and embryo transfer rates per cycle were found higher in the younger age groups. Clinical pregnancy rates per embryo transfer was found 26.5% in group 1, as this rate was 5.5% in group 5 (*Table 3*). We

Table 2: Laboratory characteristics of the age groups (mean ± semean)

	Woman's age (year)					P
	22-29	30-34	35-37	38-39	40-44	
Amount of HMG Ampoules	17.6±0.5	18.7±0.9	19.9±0.9	23.6±2.3	23±2.9	< 0.05
Amount of FSH Ampoules	13.3±0.7	12.7±0.7	15.3±1.2	18.9±1.9	22.8±2.9	< 0.05
No. of retrieved oocytes	9.6±0.6	7.7±0.4	5.5±0.5	5.3±0.7	2.8±0.6	< 0.05
No. of fertilized oocytes	3.6±0.3	3.1±0.2	2.2±0.3	2.8±0.5	1.3±0.3	< 0.05
Cancellation rates of cycle %	1.8 (1)	6.2 (7)	15.7 (11)	10.3 (4)	26.7 (8)	< 0.05
No. of mature oocytes	6.5±0.4	5.5±0.3	4±0.4	4.1±0.5	2.2±0.6	< 0.05
No. of transferred embryos	2.7±0.2	2.3±0.2	1.8±0.2	2±0.2	1.1±0.2	< 0.05
Grade I embryo rates %	65	73.8	70.8	61.3	55.6	> 0.05

Values in parentheses are the number of cases.

Table 3: The outcome of treatment cycles according to the age groups who underwent ART applications.

	Woman's age (year)				
	22-29	30-34	35-37	38-39	40-44
Embryo transfer rates per cycle %	85.9	74.3	68.5	79.4	60
Follicle puncture rates per cycle %	98.2	92.9	82.8	89.7	73.3
Embryo transfer rates per follicle puncture %	87.5	80	82.7	88.5	81.8
Clinical pregnancy rates per cycle %	22.8 (13)	22.1 (25)	17.1 (12)	15.4 (6)	3.3 (1)
Clinical pregnancy rates per follicle puncture %	23.2	23.8	20.6	17.1	4.5
Clinical pregnancy rates per embryo transfer %	26.5	29.7	25	19.3	5.5

Values in parentheses are the number of cases.

Table 4: ART outcomes in women < 38 years according to women ≥ 38 years

	Woman's age (year)		P
	< 38 years	= 38 years	
No. of patients	240	69	
Amount of HMG Ampoules	18.80 ± 0.52	23.31 ± 1.81	< 0.05
Amount of FSH Ampoules	13.62 ± 0.51	20.57 ± 1.66	< 0.05
No. of retrieved oocytes	7.52 ± 0.30	4.21 ± 0.48	< 0.05
Cancellation rates of cycle %	7.9 (19)*	17.4 (12)*	< 0.05
No. of mature oocytes	5.30 ± 0.24	3.28 ± 0.43	< 0.05
No. of transferred embryos	2.25 ± 0.12	1.66 ± 0.18	< 0.05
Grade I embryo rates %	70.7	59.2	> 0.05
Clinical pregnancy rates per cycle %	20.8 (50)*	10.1 (7)*	< 0.05

* Values in parentheses are the number of cases.

have determined statistically significant difference in pregnancy rates per cycle between the age groups, as higher in group 1 (22.8%) and lower in group 5 (3.3%) ($p < 0.05$).

We found a significant drop in pregnancy rate starting at the age of 38. Taking over 38 years as a cut-off, we compared these parameters between women over 38 years and under 38 years. We observed poor outcomes in women ≥ 38 years as shown in **Table 4**. While the cancellation rate of cycles were increasing significantly in women over 38 years, the embryo quality was decreasing. Pregnancy rates per cycle were found two times lower in women ≥ 38 years as to women < 38 years (10.1% vs 20.8%).

Discussion

ART is nowadays widely used for the treatment of infertility and in several countries, national success rates and outcomes are published regularly. Nevertheless, the availability, consequences, and costs of IVF continue to stimulate discussion and disagreement as does the effectiveness of treatment.

In our study we investigated the effect of woman's age on the success of ART. Age is well known to be most important factor affecting the outcome of fertility treatment and particularly ART (Tan et al., 1992; Roseboom et al., 1995). Several studies have addressed the effect of woman's age on the success of ART treatment (Sauer et al., 1992; Abdalla et al., 1993; Navot et al., 1994; Check et al., 1994; Flagmini et al., 1993).

Padilla et al. (1989) reported that patients who were 30 to 36 years of age had ongoing pregnancy rates per embryo transfer between 16% and 24%, whereas patients aged 37 and 38 years had ongoing pregnancy rates of 9% and 11%, respectively. Dor et al. (1996) also reported that women ≥ 40 years of age have a significantly lower cumulative pregnancy rate and various female infertility factors did not influence this rate.

Preutthipan et al. (1996) indicated that maternal age especially when greater than 35 years old adversely affects the clinical pregnancy and the spontaneous abortion rate. And also two studies on standard IVF report a gradual decrease in clinical pregnancy and live birth rates after the age of 34 years and a steeper decline in the pregnancy rate after the age of 37 years than before the age of 37 years (Piette et al., 1990; Tan et el., 1992). Stolwijk et al. (1997) showed there is no aging effect of the uterus but that there is an aging effect of the oocyte in women of > 40 years.

As mentioned in most of the other studies, our results also demonstrate a negative effect of the age on the ART applications. While the cancellation rate of cycles were found to be statistically significantly higher in the older age groups, the number of retrieved oocytes, mature oocytes, fertilized oocytes and transferred embryos were found significantly lower. But the quality of embryo did not show any significant difference between the age groups.

In our study, we also found that embryo transfer rate per cycle (85.9% in group 1 and 60% in groups) and clinical pregnancy rate per embryo transfer (26.5% in group 1 and 5.5% in group 5) were decreasing with aging of patients. The clinical pregnancy rates per cycle were determined two times lower in the age of over 38 years old than in women < 38 years (10.1% vs 20.8%).

As we found, some authors reported that fertilization and embryo cleavage rates were not effected adversely by the age (Hull et al., 1996). And they also found that implantation rates were significantly lower in the age group of 40-44 years.

Large general reports of IVF-ET treatment show accelerated decline in pregnancy rates after 37 or 38 years (FIVNAT, 1993). This is also the age when the rate of follicular atresia accelerates and may be related to ovarian capacity being reduced to a critical number of follicles and increasing preponderance of originally defective oocytes. Comparative studies of pregnancy and miscarriage rates from the same own or donated oocytes from the same cohort to women of different ages indicate the primary importance of oocyte quality rather than uterine receptivity to explain the age-related decline in female fecundability (Abdalla et al., 1993; Navot et al., 1994).

In conclusion, our study shows that the woman's age has a negative effect on ART success. A sharp reduction in pregnancy rates occur particularly in women > 38 years. Although there is no reduction in embryo quality; ovarian capacity and oocyte quality (the number of mature oocytes) declines in older age patients.

The age of women who underwent ART applications should therefore be taken into account in an assessment of ART results as does for the effectiveness of ART treatment. And this can be used to predict the outcome of treatment with the other individual patient's characteristics.

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