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Effect of Paternal Age on Miscarriage Rates in Couples Undergoing In Vitro Fertilization/Intracytoplasmic Sperm Injection

İn Vitro Fertilizasyon/İntrasitoplazmik Sperm Enjeksiyonu Uygulanan Çiftlerde Paternal Yaşın Düşük Oranlarına Etkisi

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ABSTRACT

Aim: The aim of this study was to determine the effect of paternal age on miscarriage rates in couples undergoing in vitro fertilization/intracytoplasmic sperm injection treatment cycles.
Material and Methods: Patients were classified into two groups. The study group consisted of the patients whose pregnancy resulted in a miscarriage (n=73) and a control group in which the patients had a live singleton birth (n=256). Demographic characteristics, treatment indications, duration of infertility, menstruation day 3 follicle stimulating hormone, estradiol, luteinizing hormone, total antral follicle count, anti-Mullerian hormone levels and controlled ovarian stimulation parameters, day of trigger, estradiol and progesterone levels on the day of trigger, the total number of oocytes retrieved, the number of mature oocytes, the number and quality of the embryo, endometrial thickness on the day of trigger, oocyte pick up and embryo transfer, the distance of embryo-fundus and the day of embryo transfer were recorded.
Results: Totally 329 women were included in the present study. The number of patients with the diagnosis of unexplained infertility was statistically significantly higher in the study group than in the control group (p=0.020). Maternal age was found statistically significantly higher in the study group than in the control group (p=0.025). When maternal age increased by 1 unit, the risk of miscarriage increased by 8.7% and those with unexplained infertility had a 75.6% higher risk of miscarriage than those without unexplained infertility.
Conclusion: Paternal age was not associated with miscarriage whereas maternal age and unexplained infertility had a positive correlation with miscarriage rate.
Keywords: Paternal age; miscarriage rate; unexplained infertility; maternal age; IVF/ICSI.

ÖZ

Amaç: Bu çalışmanın amacı in vitro fertilizasyon/intrasitoplazmik sperm enjeksiyonu tedavi siklusları uygulanan çiftlerde paternal yaşın düşük oranları üzerindeki etkisini belirlemektir.
Gereç ve Yöntemler: Hastalar iki gruba ayrıldı. Gebeliği düşükle sonuçlanan hastalar (n=73) çalışma grubunu ve canlı tekil doğum yapan hastalar (n=256) ise kontrol grubunu oluşturdu. Demografik özellikler, tedavi endikasyonları, infertilite süresi, adetin 3. günü folikül uyarıcı hormon, östradiol, lüteinize edici hormon, toplam antral folikül sayısı, anti-Müllerian hormon seviyeleri ve kontrollü ovaryan stimülasyon parametreleri, tetikleme günü, tetikleme gününde östradiol ve progesteron seviyeleri, toplanan toplam oosit sayısı, matur oosit sayısı, embriyo sayısı ve kalitesi, tetikleme, oosit pick-up ve embriyo transfer gününde endometrial kalınlık, embriyo-fundus mesafesi ve embriyo transfer günü kaydedildi.
Bulgular: Bu çalışmaya toplam 329 kadın dahil edildi. Açıklanamayan infertilite tanısı alan hasta sayısı çalışma grubunda kontrol grubuna göre istatistiksel olarak anlamlı derecede daha yüksek idi (p=0,020). Anne yaşının, çalışma grubunda kontrol grubuna göre istatistiksel olarak anlamlı derecede daha yüksek olduğu bulundu (p=0,025). Anne yaşı 1 birim arttığında, düşük yapma riskinin de %8,7 oranında arttığı ve açıklanamayan infertilitesi olanların açıklanamayan infertilitesi olmayanlara göre ise %75,6 oranında daha yüksek oranda düşük yapma riskine sahip olduğu görüldü.
Sonuç: Paternal yaş düşük yapma ile ilişkili olarak bulunmazken, anne yaşı ve açıklanamayan infertilite ile düşük oranı arasında pozitif bir korelasyon olduğu görüldü.
Anahtar kelimeler: Paternal yaş; düşük oranı; açıklanamayan infertilite; anne yaşı; IVF/ICSI.

INTRODUCTION

Pursuing higher education, increasing life expectancy and women having an active role in business management delayed childbearing in developed countries (1). The detrimental effects of advanced maternal age on reproductive outcomes have been thoroughly researched (2-4). In contrast, there was limited information about the effects of paternal age on reproductive outcomes and unfortunately, the results were conflicting. Some studies have shown that the embryo quality, implantation, and live birth rate declined with advanced paternal age (APA), and also reported that abnormal semen parameters, sperm DNA fragmentations, and sperm aneuploidy rates were higher in APA (5-7). On the other hand, other studies did not find any correlation between implantation, miscarriage, and live birth rates with APA (8-10).

This study aimed to determine the effect of paternal age on miscarriage rates in couples undergoing in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) treatment cycles.

MATERIAL AND METHODS

This retrospective study was performed at the center of assisted reproduction of Etlik Zübeyde Hanım Women's Health Training and Research Hospital in Ankara, Türkiye, and a total of 329 women were included. The study was approved by the Ethics Committee of Etlik Zübeyde Hanım Women's Health Training and Research Hospital (21.09.2022, 2022/139). Patients were classified into two groups. The study group consisted of the patients whose pregnancy resulted in a miscarriage (n=73) and a control group in which the patients had a live singleton birth (n=256).

Exclusion criteria were as follows: the history of chronic disease, multiple embryo transfer cycles, multiple pregnancies, natural or mild stimulation cycles, severe male factor infertility (azoospermia or total progressive motile sperm count was less than 1 million), and preimplantation genetic diagnosis cycles.

Demographic characteristics (paternal and maternal age, body mass index (BMI), gravidity, abortion, and live birth), treatment indications (male factor, unexplained infertility, and others), duration of infertility, menstruation day 3 (D3) follicle stimulating hormone (FSH), D3 estradiol (E2), D3 luteinizing hormone (LH), D3 total antral follicle count, anti-Mullerian hormone (AMH) levels, and controlled ovarian stimulation parameters (stimulation protocol, total gonadotropin dose (recombinant FSH, Gonal-F® Merck, Germany, human menopausal gonadotropin (hMG), Menopur®, Ferring Pharmaceuticals, Germany), day of trigger, E2 and progesterone levels on the day of trigger, the total number of oocytes retrieved, the number of mature oocytes, the number and quality of embryo (11), endometrial thickness on the day of trigger, oocyte pick up (OPU) and embryo transfer (ET), the distance of embryo-fundus and the day of ET were recorded. The same luteal phase support was used in both groups.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics v.26. Frequency tables and descriptive statistics were referred to interpret the findings. Non-parametric methods were employed for the values that are not normally distributed. In accordance with non-parametric methods, two independent groups were compared through the Mann-Whitney U test method. Pearson chi-square test was resorted to in examining the relationships between two qualitative variables. Binary logistic regression (Backward LR model) was deployed whilst identifying the factors affecting the abortion status. Receiver operating characteristics (ROC) curve analysis was used to detect the performance of maternal age in predicting the risk of miscarriage. A p value of <0.05 was considered to be statistically significant

RESULTS

Totally 329 women were included in the present study. No significant difference was found in the male factor, tubal factor, diminished ovarian reserve, and ovulation induction protocol between the two groups. The number of patients with the diagnosis of unexplained infertility was significantly higher in the study group than in the control group (p=0.020, Table 1).

There was no significant difference in gravidity, abortion, live birth, BMI, paternal age, D3 E2-FSH-LH, total antral follicle count, AMH, the number of cycles, and duration of infertility between the two groups. Maternal age was significantly higher in the study group than in the control group (p=0.025, Table 2).

There was no significant difference in controlled ovarian stimulation parameters between the two groups (Table 3).

As a result of the Backward: LR logistic regression analysis according to the risk of miscarriage, the optimal model is given in Table 4 using all the predictive parameters that could have a significant effect in the univariate analysis. In the current model, it has been determined that maternal age and unexplained infertility are important parameters impacting the occurrence of miscarriage (p=0.032, and p=0.038, respectively). When

Table 1. Comparison of clinical indications and ovulation induction protocols

	Miscarriage (n=73)	**Live Singleton Birth (n=256)**	**p**
Male factor, n (%)	18 (24.7)	75 (29.3)	0.437
Tubal factor, n (%)	5 (6.8)	20 (7.8)	0.784
Unexplained infertility, n (%)	42 (57.5)	108 (42.2)	**0.020**
Diminished ovarian reserve, n (%)	12 (16.4)	52 (20.3)	0.461
Ovulation induction protocol, n (%)	(n=66)	(n=238)	
Long luteal protocol	24 (36.4)	97 (40.8)	
Antagonist protocol	37 (56.1)	130 (54.6)	0.569
Luteal Estradiol + Antagonist protocol	5 (7.5)	11 (4.6)	

Table 2. Comparison of demographic and obstetric characteristics

	Miscarriage (n=73)		Live Singleton Birth (n=256)		p
	Mean±SD	**Median [min-max]**	**Mean±SD**	**Median [min-max]**	
Gravidity	0.37±0.76	0 [0-4]	0.50±0.89	0 [0-5]	0.331
Abortion	0.23±0.51	0 [0-2]	0.27±0.65	0 [0-5]	0.912
Live birth	0.04±0.20	0 [0-1]	0.11±0.41	0 [0-3]	0.195
Maternal age (year)	29.78±3.40	30 [21-35]	28.77±3.52	28 [20-38]	**0.025**
Paternal age (year)	32.25±4.31	32 [25-45]	31.81±3.24	32 [25-41]	0.774
BMI (kg/m^2)	26.68±5.93	25.6 [16.6-50.0]	25.83±4.91	25.2 [15.8-42.7]	0.320
D3 FSH (mIU/mL)	7.42±3.68	6.8 [1.0-25.4]	7.45±3.42	7.0 [0.0-33.6]	0.634
D3 LH (mIU/mL)	5.48±2.67	4.9 [0.5-11.7]	5.28±3.54	4.6 [0.1-25.0]	0.171
D3 E2 (pg/mL)	47.23±23.03	44.5 [5.0-126.0]	44.77±21.60	41.0 [3.0-134.0]	0.360
Total antral follicle count	15.62±9.38	13 [2-36]	14.51±8.17	13 [0-33]	0.622
AMH (ng/mI)	4.53±4.56	3.1 [0.1-15.0]	3.17±3.70	2.1 [0.1-18.1]	0.096
Cycle number	1.77±1.09	1 [1-6]	1.61±0.91	1 [1-6]	0.374
Duration of infertility (month)	69.82±44.10	60 [7-180]	59.98±36.39	48 [2-204]	0.374

BMI: body mass index, D3: day 3, FSH: follicle stimulating hormone, LH: luteinizing hormone, , E2: estradiol, AMH: anti-Mullerian hormone, SD: standard deviation

Table 3. Comparison of controlled ovarian stimulation parameters

	Miscarriage (n=73)		Live Singleton Birth (n=256)		p
	Mean±SD	**Median [min-max]**	**Mean±SD**	**Median [min-max]**	
Total doses of gonadotropins (IU)	2322.95±989.74	2100 [925-5300]	2145.67±824.09	2025 [600-4725]	0.400
Day of trigger progesterone (ng/mL)	1.05±0.66	0.9 [0.2-2.6]	0.92±1.33	0.7 [0.1-14.6]	0.127
Day of trigger E2 (pg/mL)	3061.66±2721.96	2525.0 [653.0-19372.0]	2529.29±1611.89	2184.0 [248.9-11041.7]	0.146
Day of trigger	10.52±1.92	10 [7-16]	10.44±1.73	10 [7-18]	0.794
Day of OPU E2 (pg/mL)	1984.46±1739.88	1524.3 [16.8-11681.0]	1647.54±1075.96	1299.6 [149.0-6112.9]	0.247
Day of OPU Progesterone (ng/mL)	8.23±5.95	7.1 [0.6-29.2]	7.33±4.89	6.1 [0.5-36.5]	0.320
Day of ET E2 (pg/mL)	1796.11±1476.91	1469.0 [178.8-9317.0]	1607.47±1173.42	1325.7 [183.8-6663.5]	0.432
Day of ET Progesterone (ng/mL)	73.70±47.26	60.0 [3.1-205.0]	77.48±54.71	60.0 [3.2-387.3]	0.620
Follicle number (15-17 mm)	4.74±4.18	4 [0-25]	4.00±2.83	4 [0-16]	0.462
Follicle number (≥17 mm)	4.03±3.50	3 [0-23]	3.53±2.56	3 [0-18]	0.507
Total oocyte count	13.16±7.58	11 [1-33]	12.26±6.33	11 [2-34]	0.689
Mature oocyte count	9.89±5.85	9 [1-26]	9.46±5.04	9 [1-30]	0.867
Grade 1 embryo	0.93±0.89	1 [0-3]	0.71±0.60	1 [0-3]	0.133
Grade 2 embryo	0.49±0.69	0 [0-3]	0.34±0.52	0 [0-2]	0.137
Grade 3 embryo	0.18±0.45	0 [0-2]	0.09±0.31	0 [0-2]	0.058
Endometrial thickness day of trigger (mm)	9.97±2.27	9.5 [6.5-20.0]	10.24±1.82	10.2 [6.5-16.0]	0.143
Endometrial thickness day of OPU (mm)	9.69±2.48	9.6 [3.2-17.0]	10.04±2.29	9.9 [3.6-18.0]	0.362
Endometrial thickness day of ET (mm)	10.13±2.14	9.8 [5.0-17.0]	10.54±2.31	10.1 [4.6-19.3]	0.150
Distance of embryo-fundus	9.39±4.83	9.7 [0.5-24.9]	8.90±3.81	8.4 [0.1-20.6]	0.456
Day of embryo transfer	3.99±1.02	3 [3-6]	3.88±1.00	3 [2-5]	0.439

E2: estradiol, OPU: oocyte pick up, ET: embryo transfer, SD: standard deviation

maternal age (year) increased by 1 unit, the risk of miscarriage increased by 8.7% and those with unexplained infertility had a 75.6% higher risk of miscarriage than those without unexplained infertility.

Maternal age in relation to the risk of miscarriage was determined as 28.5 year with a sensitivity of 67.1% and a specificity of 50.4% (area under the curve (AUC): 0.586; SE=0.037; 95% CI: 0.513-0.658; p=0.026, Figure 1).

DISCUSSION

In the current study, we observed that paternal age had no major effect on the miscarriage in couples undergoing IVF/ICSI while maternal age and the diagnosis of unexplained infertility were associated with increased miscarriage rate.

It was demonstrated that one of the most common causes of miscarriage was aneuploidies (12). Vagnini et al. (13) reported that sperm DNA damage increased with paternal age, and Garcia-Ferreyra et al. (14) also revealed supporting the previous study that sperm DNA damage increased with APA and genetic screening was necessary for patients whose paternal age is >50 years of age. The study by Tiegs et al. (15) investigated that APA did not affect IVF pregnancy outcomes when the euploid embryo was transferred. In addition, APA is associated with increased sperm DNA fragmentation that leads to impaired implantation and increases the miscarriage rate (16). APA predisposes to the formation of de novo mutations by increasing the replication errors in the germ line with an increase of reactive oxygen species and decreasing in antioxidant capacity. This situation is aggravated by the deterioration of DNA repair mechanisms with age (17). As opposed to the mentioned studies, a fair number of other studies showed that there was no correlation between miscarriage rates and APA as in agreement with the present study (18-20).

In one study by Stone et al. (21) the sperm concentration, the rate of normal morphology, and motility declined as paternal age progressed. In accordance with this, ICSI is gaining popularity particularly in preventing the detrimental effect of APA on pregnancy outcomes by improving the fertilization rate (9). In the present study, ICSI was used in all cycles hence, the detrimental effect of sperm abnormalities on the pregnancy outcomes was eliminated.

When the literature was reviewed, it appeared that IVF failure can be caused by many factors, but clearly, the fetal loss was greatly affected by advanced maternal age and the rate of age-related aneuploidy boosted with increasing maternal age (22,23). In the present study, the cut-off value of maternal age for risk of miscarriage was found as 28.5 years.

Unexplained infertility is indeed a “gordian knot” in IVF that forms nearly a third of infertility patients (24), and the lack of understanding of its pathogenesis has made it a mysterious phenomenon.

In accordance with the present study, Fuchinoue et al. (25) reported that miscarriage and unexplained infertility had a relation with each other and determined that natural killer 22 (NK22) cells were elevated in the peripheral blood and endometrium of women with unexplained infertility and women suffering from recurrent miscarriage. In addition, Ran et al. (26) revealed that the core gene of the unexplained infertility and recurrent miscarriage had similar functions such as regulation of immune cells and cellular secretion.

Table 4. Logistic regression analysis of factors for the prediction of a miscarriage

	OR	95% CI	p
Maternal age (year)	1.087	1.007 - 1.172	**0.032**
Unexplained infertility	1.756	1.032 - 2.988	**0.038**

OR: odds ratio, CI: confidence interval, CCR=77.8%, $\chi^2_{(8)}$=10.330; p=0.243

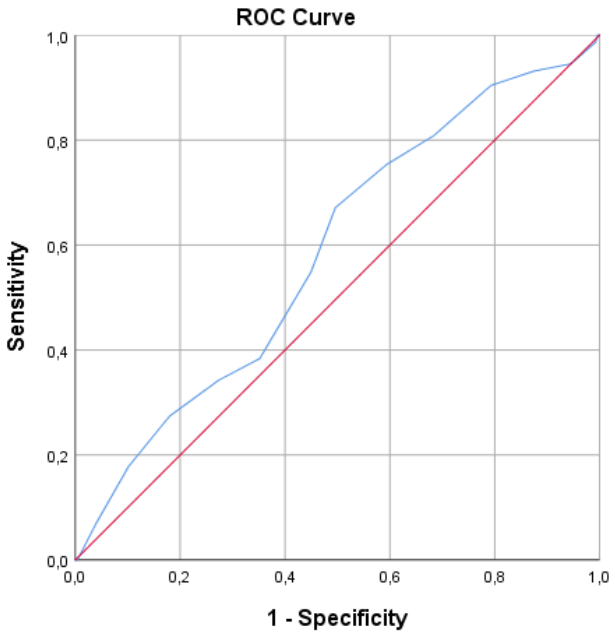

Figure 1. Receiver operating characteristics curve of maternal age for the risk of miscarriage

CONCLUSION

In conclusion, paternal age was not associated with miscarriage whereas maternal age and unexplained infertility had a positive correlation with miscarriage rate. Larger studies are needed to confirm the results of this study.

Ethics Committee Approval: The study was approved by the Ethics Committee of Etlik Zübeyde Hanım Women’s Health Training and Research Hospital (21.09.2022, 139).

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