

The factors that effect in vitro fertilization success in women of critical ages: “38-40 years”

İleri yaş kadınlarda tüp bebek başarısını etkileyen faktörler:38-40 yaş

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SUMMARY

Objective: The aim of this study is to assess the factors that affect IVF success in women of critical ages of “38-40 years”.

Method: 468 women aged 38-40 years who were admitted for IVF between January 2013 to December 2017 were enrolled in the study. Ovarian stimulation characteristics, number of oocytes retrieved, number of embryos transferred and pregnancy rates ,cancellation rate ,live birth rate were analyzed.

Results: The overall clinical pregnancy rate was 17.9% . Cancellation rate was 22.4%. The miscarriage rate was 25% (21/84).The live birth rate was 13.4% (63/468).Comparing the patients who did and did not achieve conception, we found statistically meaningful differences in terms of antral follicular count, retrieved oocyte count, number of embryos obtained, embryo count that was transferred,. Logistic regression analysis showed that the following factors were significantly related to higher pregnancy rates: number of embryos transferred, HMG usage in the stimulation protocol, basal FSH and blastocyst transfer.

Conclusions: The most important parameters which predict pregnancy in 38-40 year-old women are the number of transferred embryos, HMG usage in the stimulation protocol, low basal FSH level and blastocyst transfer.

Keywords: Women’s age, IVF outcome, pregnancy rate, number of transferred embryos.

ÖZET

Amaç: Bu çalışmanın amacı 38-40 yaş aralığındaki kadınlarda IVF başarısını etkileyen faktörlerin araştırılmasıdır.

Yöntem: Bu çalışmaya Ocak 2013-Aralık 2017 tarihleri arasında 38-40 yaş aralığında olup IVF için başvuran 468 kadın dahil edilmiştir.Ovarian stimülasyon karakteristikleri,toplanan oosit sayısı, transfer edilen embryo sayısı, siklus iptal oranları, gebelik ve canlı doğum oranları analiz edilmiştir.

Bulgular: Toplam gebelik oranı%17.9 olarak hesaplanmıştır.Siklus iptal oranı%22.4 , gebelik kaybı oranı %25, canlı doğum oranı %13.4 olarak bulunmuştur. Gebe kalan ve gebe kalamayan hasta grupları karşılaştırıldığında antral folikül sayısı,toplanan oosit sayısı, elde edilen ve transfer edilen embryo sayıları anlamlı farklılıklar göstermiştir.Logistic regresyon analizi sonuçlarına göre artan gebelik oranları ile ilişkili faktörler:Transfer edilen embryo sayısı, stimülasyon protokolünde HMG kullanımı, basal FSH değeri ve blastokist transferi yapılmış olmasıdır.

Sonuç: 38-40 yaş arası kadınlarda gebelik başarısını predikte eden en önemli parametreler transfer edilen embryo sayısı, stimülasyon protokolünde HMG kullanımı, bazal FSH seviyesi ve blastokist transferidir.

Anahtar sözcükler: Kadın yaşı, IVF başarısı, gebelik oranı, transfer edilen embryo sayısı

INTRODUCTION

Late marriages and women’s career plans in modern day life result in the postponement of their childbirth plans. However, pregnancy rates

fall towards late 30s, both by spontaneous conception and by assisted reproductive techniques^{1,2}. Age is the single most important factor in achieving pregnancy via assisted reproductive techniques². As patients become

older, conception rates fall. Although there is no universal definition of advanced reproductive age in women, infertility becomes more frequently an issue after the age of 35³.

Starting from age 38, the reduction of ovarian follicles accelerates irreversibly⁴. While some studies claim that advanced reproductive age starts at 37-38, others maintain that it starts after age 40⁵. Therefore, studies generally make a distinction between either under and over 37-38, or under and over 40⁶⁻¹¹.

The aim of the present study is to assess the factors that affect IVF success during the transition period of women life of 38-40 years.

MATERIAL AND METHODS

The women aged 38-40 years who were admitted for IVF from January 2013 to December 2017 were enrolled in the study. Four hundred sixty eight IVF cycles (patients) were evaluated. This cross sectional study was approved by local ethics committee and conducted in accordance with the basic principles of the Helsinki Declaration.

Ovarian stimulation characteristics, number of oocytes retrieved, number of transferred embryos and pregnancy rates were analyzed. IVF indications included tubal factor, hormonal-anovulatory disorder, sub-fertile male factor (≥ 5 million total progressive motile spermatozoa per milliliter), endometriosis or unexplained infertility. Study inclusion criteria were as follows:

- 1- A basal FSH hormone level less than 12 IU/L,
- 2- Patients between 38-40 years of age,
- 3- Patients who have first IVF cycle, and
- 4- Long and microdose flare up protocol with GnRH-a and rFSH.

Exclusion criteria were:

- 1- Patients younger than 38 years and older than 40 years,
- 2- Patients with severe male factor (<5 million motile spermatozoa per milliliter and which required TESE),
- 3-Patients with endocrine disorders and systemic disease,
- 4-Women with uterine anomaly or uterine fibroids and hydrosalpinges, and
- 5-Frozen-thawed cycles .

Ovulation induction:

Ovarian stimulation was performed by one of the following protocols depending on previous or estimated ovarian response:

1- Long desensitizing protocol using the gonadotropin-releasing hormone (GnRH) agonist Lucrin (Lucrin Daily ®, Abbott, Johannesburg) 15 u /day was given subcutaneously from the mid-luteal phase of the preceding cycle (days 21-23). Gonadotropins were started on day 2 of the cycle.

2- In microdose flare protocol, the treatment started with oral contraceptives. Two days after the end of oral contraceptive, GnRH analogs were administered at microdoses (leuprolide acetate 50µg) subcutaneously twice a day. Gonadotropins were started 2 days later.

Ovulation induction protocols involved human menopausal gonadotropin (HMG) (Menogon, Ferring Pharmaceuticals) or Recombinant Fsh (Gonal F, Merck Serono), or a combination of both. Dose alterations were performed on the 4th day of stimulation and continuing days according to the sonographic findings and circulating estradiol (E₂) levels. Ovulation was induced by a subcutaneous injection of 250 µg recombinant human chorionic gonadotropin (hCG) (Ovitrelle®, Merck Serono) when at least 3 follicles reached a diameter of 18 mm. Oocyte pick up (OPU) was performed 34-36 hours after hCG injection. ICSI was performed for all metaphase II oocytes. Embryo transfer was performed under ultrasound guidance on day of 2,3 or 5 for patients. Embryo transfer was determined based on the American Society for Reproductive Medicine (ASRM) guidelines¹² and performed using Wallace catheter (Edwards-Wallace Catheter; Marlow Technologies, Willoughby, OH). All patients received intravaginal progesterone (Crinone gel) support. Clinical pregnancy was defined as visualization of gestational sac by vaginal ultrasound.

Miscarriage was defined as a pregnancy lost before 20 weeks of gestation. Live birth was defined as a pregnancy resulting in a delivery of a viable infant. Cancellation was defined as patients' cycle did not reach oocyte retrieval or in whom oocyte retrieval was performed but no oocytes were retrieved or fertilization failure .

Statistical analysis :

Data analysis was performed by using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, United States). While, continuous data were shown as mean \pm standard deviation, otherwise, number of cases and percentages were used for nominal variables. Continuous variables were compared by Student's t or Mann Whitney U test, where applicable. Nominal data were analyzed by Chi-square or Fisher's exact test, where appropriate. Multiple Backward LR Logistic Regression Analyses were used for determining the predictors which mostly affected on pregnancy. Any variable whose univariable test had a p value <0.05 was accepted as a candidate for the multivariable model along with all variables of known clinical importance. Odds ratio and 95% CIs for each variables were also calculated. A p value less than 0.05 was considered statistically significant.

RESULTS

Four hundred sixty eight IVF cycles (patients) were evaluated. Their mean age was 38.7 ± 0.77 . The distribution of patients with respect to reason for infertility was as follows: Unexplained 69.3%, male factor 16.7%, tubal factor 7.1%, endometriosis 3.8% and mixed factor 3.2%. The overall clinical pregnancy rate (PR) was 17.9% (84/468). The miscarriage rate (MR) was 25% (21/84). The live birth rate (LBR) was 13.4% (63/468). Cancellation rate (CR) was 22.4%. Of the 468 patients, 27 (5.7%) did not reach oocyte retrieval; of the 25 (5.3%) in whom oocyte retrieval was performed, no oocytes were retrieved; and fertilization failure occurred in 53 (11.3%) patients. Other clinical characteristics of the patients are presented in Table 1.

Table 1: Characteristics of IVF cycles

Number of patients	468
Age(years)	38.7 ± 0.77
Cancellation rate	22.4% (105/468)
Number of gonadotropin ampules	44.0 ± 13.5
Length of stimulation(days)	10.1 ± 2.0
Peak estradiol level on day of HCG administration(pmol/l)	1965.5 ± 1097.8
Number of follicles >14 mm on day of HCG administration	6.7 ± 4.1
Number of oocytes retrieved	6.3 ± 4.2
Fertilization rate	64.3 ± 31.7
Number of embryos transferred	3.0 ± 2.6
Clinical pregnancy rate per patient	17.9% (84/468)
Miscarriage rate	25% (21/84)
Live birth rate per patient	13.4% (63/468)

Data are expressed as mean \pm SD or n(%).

Table 2: Comparison between achieved clinical pregnancy and non-pregnant groups

	Clinical pregnancy group (n=84)	No pregnancy group (n=384)	Odds Ratio	Wald	p Value	%95 Confidence Interval	
						Min	Max
Patient age(year)	38.3±0.5	38.7±0.7	0.852	4,977	0.016	0,207	18,269
Antral follicle count	8.1±4.1	5.9±2.9	1,696	0,554	0.012	0,422	6,811
Basal FSH level(mIU/mL)	7.9±1.8	9.2±2.0	0,857	11,584	0.001	0,784	0,937
Basal E2 level(pg/mL)	41.2±18.7	55.0±33.3	0,259	9,977	0.013	0,112	0,599
Number of gonadotropin ampules used	38.1±11.1	45.3±13.6	0,446	3,819	0.011	0,199	1,002
Length of stimulation(day)	9.6±2.0	10.2±2.0	1,307	3,673	0.011	0,967	4,171
Peak E2 level on the day of HCG administration(pg/mL)	2292.1±1062.6	1892.9±1093.6	1,590	8,403	<0,001	1,286	1,965
Number of follicles >14 mm ,on day of HCG administration	8.8±4.0	6.2±3.9	0,805	5,491	0.013	0,672	0,965
Number of oocytes retrieved	9.1±4.2	5.7±3.9	0,981	6,878	0.015	0,967	0,995
Fertilization rate(%)	70.4±22.9	62.9±33.3	0,999	5,548	0.082	0,999	1,000
Number of embryos obtained	4.8±2.6	2.6±2.5	1,348	5,117	0.014	1,041	1,746
Number of embryos transferred	1.9±0.1	1.4±0.6	0,909	0,086	0.019	0,479	1,723
Peak E2 levels/number of follicles>14 mm, on day of HCG	277.7±104.1	340.9±154.5	3,046	6,179	0.011	2,203	4,627
Peak E2 levels/number of oocytes retrieved	291.4±149.7	404.5±255.2	9,247	10,689	0.013	2,437	35,084

*Significant at $p < 0.05$. Data are expressed as mean \pm SD .

Comparing the patients who did and did not achieve conception, we found statistically meaningful differences in terms of antral follicular count, basal FSH level, basal E2 level, gonadotropin dose and kind, protocol type, stimulation duration, Peak E2 level on the day of HCG administration, number of follicles >14 mm on day of HCG administration, retrieved oocyte count, number of embryos obtained, embryo count that was transferred, transfer day, Peak E2 levels/number of follicles>14 mm on day of HCG administration and Peak E2 levels/number of oocytes retrieved (Table 2).

No differences were found between the groups as regards BMI, duration of infertility, reasons for infertility, basal LH levels, HCG day endometrial thickness, HCG day progesterone levels and fertilization rates. With respect to the type of

gonadotropin used, pregnancy rates in patients who only received HMG (15.3%) and HMG plus rFSH (21.2%) were statistically higher than in those who received only rFSH (4.5%) ($p=0.04$). While 21% (60/282) of the patients who were administered long protocol achieved conception, only 12.9% (24/186) of the patients who were administered microdose flare-up protocol achieved it. When compared, long protocol patients had meaningfully higher pregnancy rates ($p=0.024$). When the patients were evaluated with respect to transfer day, pregnancy rate in patients who had transfer on day 5 was 62.5% (15/24), and the rates in patients who had transfer on days 2 and 3 were 18.5% (30/162) and 22% (39/177), respectively. The pregnancy rate in patients who had transfer on day 5 was statistically meaningful ($p=0.014$).

Logistic regression analysis showed the following factors were significantly related to higher pregnancy rates: number of embryos transferred, HMG usage in the stimulation protocol, basal FSH and blastocyst transfer.

DISCUSSION

In this study, we assessed the factors that influence IVF success among patients aged between 38-40. As a result, the most important parameters associated with high pregnancy rates seemed to be the number of embryos transferred, HMG usage in the stimulation protocol, basal FSH and blastocyst transfer.

In IVF cycles, advanced age has negative effects such as decreased response to COH, lower fertilization and implantation rates, and high cancellation rates¹³.

In most studies, pregnancy rates were found to be significantly lower among patients over 35 years and even lower among those aged over 40^{14,15}. Further, despite clinical identification of pregnancy, 14% of patients fail to achieve live birth under the age of 35; 19% will fail to achieve it between ages 35-37; 25% between ages 37-40; and 40% after age 40¹⁶.

Studies about ovarian reserve have yielded various cut off values for age. Saldeen found a mean age of 37 in the poor responder group⁶, while Fontaine concluded it was 36¹⁷. Meden-Vrtovec evaluated patient groups aged under and over 38¹⁸. As can be seen, studies mostly choose to investigate patients under and over 37-38 or under and over 40⁷⁻¹¹.

Our clinical pregnancy rate is 17.9%. This rate is similar to the rate (18%) of the patients over 38 in the study of Abdalla¹⁹. This rate is 40% for those patients under 38. LBR also decreases with the age increase. This rate is 32.4% for the normoresponder women under 38 and 12.1% for the women over 38¹⁹. In our study, this rate was found as 13.4%.

In the study of Abdalla, while CR before OPU was 4% for the normoresponder patients who are under 38, this rate was 10% for those who are over 38¹⁹. In the study of Saldeen, while CR after OPU was 13.2% for the normoresponder patients who are over 37, this rate was 10.5 % for those who are under 37⁶. CR increases by the age. Decrease of quality of oocyte and embryo due to the aging leads to reduced pregnancy rate²⁰. With the age increased, lower ovarian response is one

of the most effective factors accountable for the non-development of oocytes²¹. In fertilization failure, failed oocyte activation and maternal chromosome defects come to the foreground²². Our cancellation rate was higher than these studies.

Miscarriage rate also increases by age because of the DNA fragmentation and apoptotic deaths increase²³. Furthermore, aneuploidy in oocytes also increases²⁴. In the study of Abdalla, MR was found 20% for the patients under 38 however this rate increased to 35% for those over the age 38. In our study, our MR was found as 25%. This rate is nearer to that of the patient group under 38 in the study of Abdalla¹⁹.

Klipstein reported in a study conducted on a group of patients aged 40 years and older that the most important factor that increases pregnancy rates was a high number of embryos to be transferred and embryos suitable for cryopreservation²⁵. Opsahi showed that the number of transferrable embryos predicted successful pregnancy outcomes among patients aged 39 and older with normal ovarian reserve(26). In our study too, logistic regression showed that the most important factor that increases pregnancy rates was the number of embryos transferred. In contrast to our study, Valeva claims that single and double embryo transfer did not result in different pregnancy outcomes in the 36-39 age group²⁷.

The effects of HMG usage or adding LH to FSH on IVF outcomes are debatable. Fabregues reports that his patients with a mean age of 38 did not have increased ovarian response to LH addition in assisted reproductive technologies²⁸. On the other hand, Marrs and Humaidan claim that LH addition increased positive IVF outcomes in patients aged 35 and over^{29,30}. In our study of 38-40 year-old patients, pregnancy rates with the usage of HMG alone or joint usage of HMG and rFSH were statistically higher than the rates in patients in whom rFSH was used alone. Logistic regression analysis also showed that HMG usage was the second most important parameter after the number of embryos transferred in stimulation protocol.

It is known that as basal FSH levels increase, ART success decreases, however, there is not an exact cut-off value to discriminate normal or high FSH³¹. FSH and age were assessed together in ART cycles and age was reported to be the single most important factor in IVF outcome. Under the age of 38, FSH elevation did not affect pregnancy and live birth rates, while after the age of 38, FSH

elevation statistically decreased these rates. This study also claimed that fertilization rate was independent of FSH elevation and age(18). In Akende's study, cancellation rate was minimal among younger age groups, while it increased rapidly after age 35 and had a more significant relationship with FSH in advanced ages³². In this study, the age of patients in cancelled cycles was more advanced and basal FSH levels were statistically higher. Age and FSH values were correlated in some studies^{32,33}. In our study too, basal FSH value was found to be one of the most important parameters predicting pregnancy in the 38-40 age group.

In our study, blastocyst transfer was found to more meaningfully predict pregnancy when compared to 2nd and 3rd day transfers. Similar to our study, Milki found blastocyst transfer to be more effective than 3rd day transfer in his 2002 study⁹.

Strength of this study is high patients number and this is the first study in literature that investigated the factors that effect in vitro fertilization success in women of advanced ages in Turkish population. Most important limitation of the study is that, IVF procedures is realized in various IVF clinics, it would be more valuable if all procedure were take places in same center.

In conclusion; the age of the patient is an important factor for assessment of infertility treatments and it is our major reference point that determines stimulation protocol, drug type and dose to be selected . While the patient group aged between 38- 40 carry certain similar characteristics to young patients regarding response to IVF, they exhibit characteristics of advanced age patients regarding other aspects. We are of the opinion that the most important parameters which predict pregnancy in 38-40 year-old women are the transfer of a high number of embryos, HMG usage in the stimulation protocol, low basal FSH level and blastocyst transfer. In order to find an advanced age cut-off value, it may be appropriate to conduct a study to investigate the ages of 38,39,40 separately.

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