Original Article

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Assessment of Effective Factors In Recurrent Implantation Failure (RIF) Following Assisted Reproductive Technology (ART)

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ABSTRACT

BACKGROUND AND OBJECTIVE: Despite many advances have been made in the assisted reproduction techniques (ART), implantation rate after embryo transfer has not increased significantly. Therefore, this study aims to determine the factors involved in recurrent implantation failure (RIF) following ART.

METHODS: In this retrospective study, 80 infertile patients with a history of at least 3 recurrent implantation failure (RIF) following assisted reproductive technology (ART) were referred to Fatemezahra Infertility Center in Babol from March 2006 to March 2013 were selected. The control group also included 80 women who became pregnant following the first IVF. Associated factors of infertility, endocrine disorder and endometriosis were recorded and their effect on RIF was assessed in both groups.

FINDINGS: The mean BMI was 26.39±3.63 in the case group and 26.77±4.49 in the control group. Body mass index (BMI) >30 increased the risk of RIF significantly (p=0.001). After adjusting for the effects of other possible confounding factors, the odds ratio of obesity on RIF was 1.09(0.91-1.19) 95% CI which was not significant (p=0.06). There were no significant differences among type and cause of infertility, endocrine abnormalities, uterine malformations, endometriosis and polycystic ovary with RIF.

CONCLUSION: According to our findings, BMI, type and cause of infertility, endocrine disorders, uterine abnormalities, endometriosis, and polycystic ovary were not effective on recurrent implantation failure.

KEY WORDS: *Embryo Implantation*, *Obesity*, *Body Mass Index*, *Infertility*.

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Introduction

According to the WHO definition, infertility refers to infertility after one year of intercourse without the use of contraceptives (1). In developing countries one in four couples suffer from infertility (2). Depending on the causes of infertility in both sexes, various methods, such as drug therapy or the use of assisted reproductive technologies such as in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), are used. IVF is one of the most commonly used techniques in assisted reproduction. In this way, with ovarian stimulating drugs, the ovaries stimulate the growth of the ovum. The ovaries are then fertilized with sperm in vitro. The fertilized ovum are kept in vitro for 2 to 4 days and then the embryos are implanted in the uterus via the cervix (3). Despite advances in IVF technology, implantation success rates vary between 26 and 45% (4). Years after the widespread use of assisted reproductive techniques, especially IVF, an important pathophysiological state in laboratory infertility has been identified, including repeated failure to conceive pregnancy after transplantation, which is termed RIF. Various causes can lead to repeated implantation failure, including maternal factors such as uterine disorders, hormonal and metabolic disorders, infections, immunologic and thrombophilia factors, fetal malformations, maternal age, cigarette smoking, Polycystic ovary syndrome (PCOs), endometriosis and fetal transfer techniques and severe male factor (6, 5). There is no generally accepted definition of repeat embryo failure (RIF).

It is very difficult to provide a precise scientific definition of RIF, as not only infertility authorities have different criteria for RIF, but patients with recurrent fetal implant failure may have a different infertility pathology than other patients (6). Recent definitions have repeatedly defined failure to conceive after 3 times high quality embryo transfer (8). Pathophysiological identification of patients with recurrent implantation failures can guide the treatment of patients and increase the success rate of implantation and increase the chance of fertility in couples (7). Given the importance of this issue and the increasing number of mothers with recurrent in vitro fertilization and few similar studies in this area, this study aimed to investigate some of the potential contributing factors in post-ICSI recurrence failure at Fatemeh Zahra Infertility Center, Babol, Iran.

Methods

This retrospective study after approval by Ethics Committee of Babol University of Medical Sciences was enrolled on 80 women with a history of recurrent fracture failure (RIF) who were treated at the Fatemeh Al-Zahra Infertility Center in Babol from April 2013 to March 2017. This article is approved by Babol University of Medical Sciences under with ethics code of IR.MUBABOL.HRI.REC.1397.118.

Women candidates for ICSI with history of 3 times failed embryo transfer (high quality) mean that no pregnancy occurred after three times of embryo transfer (6). In the case group 80 women with successful pregnancy (observation of live fetus in vaginal ultrasound) in the first ICSI treatment period were selected as control group. For random selection of control group, a random number of one to 10 was selected and K = 2 samples were taken and 80 files were included in the study for comparison and excluded if they contained incomplete and lacking information (9). All patients were treated with Long GnRH agonist protocol.

Patients' medications were from a single company and the dosage varied according to age and BMI. All patients received 1-2 embryos on day 3 after puncture of grade A and B. Fetal transplantation was performed by a qualified embryologist and gynecologist based on the same procedure with the same catheter type. Patients in terms of anthropometric indices, (weight, BMI), cause of infertility, type of infertility, menstrual status, uterine and tubal myoma and abnormality, history of previous pregnancy, endocrine hormone levels (FSH, LH, PRL, TSH), cigarette smoking, endometriosis, polycystic ovary syndrome, hypertension, diabetes mellitus, thyroid disorders and other underlying diseases were studied and data were entered into the questionnaire.

The cause of infertility was divided into four categories based on the factors involved in infertility: female, male, female-male and unknown. Patients' BMIs were also classified according to the WHO classification into three groups of normal (under 25), overweight (25- 29.9), obesity (30 and above) (10). All patients with intramural myoma greater than 5 cm in size who had a compressive effect on the endometrium initially had a myomectomy and then entered the treatment cycle.

Tubal and uterine abnormalities were defined based on the findings of hysterosalpingography and tubular or arcuate obstruction. In case of uterine septum, the patients underwent hysteroscopy and the abnormalities were resolved and the patient was entered the treatment cycle. In this study, the type of infertility was defined

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based on its definition as primary and secondary infertility. Primary infertility is defined as infertility that occurs after one year of unprotected intercourse, and secondary infertility refers to cases in which a woman has had at least one pregnancy but is now unable to conceive (12). Data were analyzed by SPSS 18 software using t-test, chi-square and logistic regression. P<0.05 was considered significant.

Results

Eighty patients with a history of recurrent implantation failure and 80 Patients who had successful pregnancy with the first ICSI as the control group participated in the present study.

The mean age of the study group was 29.42±5.27 years and the mean age of the control group patients was 30.52±5.54 years, the youngest being 20 years and the highest being 43 years. The mean BMI was 26.39 ±3.63 in the control group and 26.77± 4.49 in the control group with the lowest weight of 45 kg and the highest weight of 98 kg.

After BMI classification, the probability of repeated failure of obese individuals in reproductive aids was higher and this relationship was statistically significant (p = 0.001). For other variables, there was no significant difference between the study and control groups (Table 1). There was no significant difference between FSH, LH, PRL and TSH levels in study and control groups (Table 2).

Table 1. Examination of baseline characteristics of patients in the two groups study and control

Group Variable	Study N(%)	Control N(%)	P-value
Classification of patients based on BMI			
normal	32(40)	25(31.2)	
Overweight	26(32.5)	44(55)	0.001
obese	22(27.5)	11(13.8)	
Cause of infertility			
man	38(47.5)	40(50)	
woman	13(16.2)	15(18.8)	
unknown	7(8.8)	6(7.5)	0.92
both	22(27.5)	19(23.7)	
Infertility type			
primary	58(72.5)	59(73.8)	0.85
secondary	22(27.5)	21(26.3)	
Menstrual pattern			
regualr	65(81.3)	64(80)	0.84
irregular	15(18.7)	16(20)	

Table 2. Assessment of hormonal levels in two groups of study and control

Group Variable	Study Mean±SD	Control Mean±SD	P-value
FSH(mIU/ml)	6.71±3	6.76±2.99	0.92
LH(mIU/ml)	6.69±4.68	6.43±5.2	0.74
PRL(mlU/L)	84.88±14.5	81.01±15.5	0.73
TSH(mIU/ml)	3.13±8.95	3.49±8.72	0.8

Table 3 shows that there was no significant relationship between myoma, tubal abnormalities, endometriosis, polycystic ovary syndrome, diabetes, hypertension, and thyroid dysfunction with repeated implantation failure (Table 3). In data analysis to compare mean body mass index (BMI) in different groups using binary regression test, possible confounders (cause and type of infertility, menstrual pattern, myoma, tubal and uterine disorders, endometriosis, Polycystic ovary syndrome, diabetes, hypertension and thyroid dysfunction) were eliminated and mean weight was adjusted accordingly. The results showed that by eliminating the confounders, the odds ratio of body mass on repeated implantation failure with a 95% confidence interval was 1.09 (0.91-1.19) which was not significant (p=0.06).

Table 3. Comparison of characteristics of patients in study and control groups

Variable	Group	Study N(%)	Control N(%)	P-value
Myoma	Yes	6(7.5)	79(8.8)	0.77
	No	74(92.5)	73(91.2)	
Uterine and tubal disorders	Yes	16(20)	19(23.8)	0.7
	No	64(80)	61(76.2)	
Endometriosis	Yes	5(6.3)	3(3.8)	0.47
	No	75(93.7)	77(96.2)	
Poly cystic ovarian syndrome	Yes	18(22.5)	26(32.5)	0.16
	No	62(77.5)	54(67.5)	
Diabetes	Yes	2(2.5)	3(3.8)	0.65
	No	78(97.5)	77(96.2)	
Hypertension (≥ 140.9 mmHg)	Yes	2(2.5)	2(2.5)	1
	No	78(97.5)	78(97.5)	
Thyroid function disorders	Yes	10(12.5)	9(11.3)	0.8
	No	70(87.5)	71(88.7)	

Discussion

In this study, preliminary statistical analysis clearly showed that obesity significantly caused repeated failure of ART cycles, but after adjusting for the effects of other possible confounding variables, there was no

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relationship between BMI and failure of patients with a history of repeated implantation failure. The results of this study are in line with the results of several studies that showed no significant relationship between BMI and repeated failure of IVF cycles (13). Even Kupka and colleagues found the highest success rate of embryo transfer in ICSI courses in couples in which both husband and wife were obese, and the lowest success rate was when only the woman was obese. Analysis of their data shows that a combination of a relatively obese, normal-weight male is positively associated with success in IVF and ICSI.

This combination is more likely to be found in couples with a higher social status. Increased pregnancy rates in this group may also be related to other factors, such as quality of life associated with higher social status (14). One limitation of this study was the lack of access to literacy and economic income of the participants in their profile, which as a confounding has not been evaluated. The following studies do not confirm the results of our studies. Luke et al showed that higher BMI was associated with lower incidence of clinical pregnancy. However, in the study of Luke et al., other confounders such as race and ethnicity were considered as confounding effects of BMI on repeated implantation failure (15).

A study by Bellver et al showed that although there was no significant difference in the number of oocytes, fertilization success and even the number of transmitted embryos between the groups, the incidence of implantation, pregnancy and live birth in the group is significantly reduced with high body mass index (16). Another important factor that is discussed in the incidence of repeated implantation failure in various studies and as a factor associated with high body mass in repeated implantation failure is smoking. Unfortunately, in our study, female patients probably did not respond correctly to tobacco use because of the culture of social shame. But several studies have shown that smoking cessation can have very beneficial effects on the success of implantation in cases of recurrent IVF failure. In a meta-analysis of 17 studies, Waylen et al. found that the incidence of birth per cycle was significantly lower in smokers than in non-smokers women and the incidence of IVF failure was significantly higher in smokers (17).

The results of this study showed that neither of the two factors of infertility and sex (male and female) could play an important role in the recurrent failure of IVF. Regular or irregular menstruation has also had no effect on recurrent failure. Uterine myomas have always been the source of gynecological disorders in women. In the present study, 7.9% of the study group and 8.8% of the control group had uterine myomas, which was not significant. Farhi et al. in their study showed that uterine myomas can only affect IVF results when the uterine space is completely deformed by the myoma (18).

In the present study, large myomas with a compressive effect on the endometrium or caused deformity of the uterus were removed before entering the treatment cycle, which probably did not result in a significant difference between the two groups. Also in this study 25% of control group and 7.5% of study group had tubal and uterine abnormalities as Arcuate uterine, but their difference was not statistically significant. In the present study, patients whose uterine septum was diagnosed by pre-ART evaluations, uterine septum was removed by hysteroscopy. Most studies evaluated the small uterine septum and it has not been introduced as a large clinical trial (13). In one study, Moini et al. showed that structural abnormalities including Arcuate uterus, dorsum, and septal uterus were found in 10.9% of cases of repeated implantation failure (19). Mollo et al. demonstrated that uterine septum removal significantly improved fertility outcomes, indicating an important role of uterine anomaly in repeated implantation failure (20).

In our study, 3.8% of the control group and 6.3% of the study group had endometriosis that failed IVF cycles. Although the rate of failure was higher in the endometriosis group, this difference was not statistically significant. Bukulmez et al. in the study showed that there was no significant difference in the failure rate of IVF cycles between the endometriosis group and the control group. The number of oocytes obtained, the success rate of fertilization, the number and quality of transferred embryos were similar between the two study and control groups and there was no significant difference. They concluded that the presence and extent of endometriosis had no effect on implantation rate and clinical pregnancy in IVF patients (21). Lutuc et al. in their study showed that IVF is used in resistant cases of endometriosis, but implantation failure rates are high (22).

In this study diabetes, hypertension and thyroid disease and hyperprolactinemia were not significantly different between control and study groups. However, mostly because patients in both groups were treated for the above disorders during ICSI. Baker et al.'s study also showed that abnormal TSH levels were associated with a higher probability of fertility failure, although the difference was not statistically significant. Newer

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studies have focused on the role of AntiTPO Ab (23). In a study of people with recurrent miscarriage, hyperprolactinemia has been one of the causes of recurrent miscarriage (24). In the study of polycystic ovary syndrome and its role on repeated failure of fetal implantation, our results showed that the presence of PCOs does not significantly increase recurrent IVF failure. In a study by Urman et al., it was reported that this disease had no significant negative effect on the outcome of IVF cycles (25).

There was no significant difference between the control and study groups in the levels of LH and FSH hormones in our study. A study by Zebitay et al showed that the number of oocytes obtained was inversely and significantly correlated with the level of FSH and age of the patients, while it was not significantly correlated with the level of LH (26). Other causes including maternal factors such as metabolic diseases, infections, immunological factors and thrombophilia can lead to repeated implantation failure (6). Various studies have emphasized the important role of thrombophilia on recurrent implantation failure (27). One of the limitations of this study was the lack of access to immunological and thrombophilia tests. Since factors such as the quality of transmitted embryos and the quality of sperm and oocytes used can have a direct and significant impact on the success of IVF cycles, all patients were transferred to Grade A and B embryos to consider the effect of embryo quality in both groups. In a study of factors associated with pregnancy by Basirat et al., infertility duration was also effective in incidence of pregnancy (28). The results of this study indicate that BMI, type and cause of infertility, endocrine abnormalities, uterine malformations, endometriosis, polycystic ovary syndrome are not effective in repeated failure of fetal implantation. Removing the effect of more confounders in future studies can be helpful to confirm the results.

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