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Original Article

The Effect of microfluidic chip technique in sperm selection for recurrent implantation failure

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ABSTRACT

Objective: The microfluidic chip technique gives a chance to select healthy sperm with less deoxyribonucleic acid (DNA) damage. In this study, we aimed to determine the effect of microfluidic techniques on sperm selection in intracytoplasmic sperm injection (ICSI) treatment in patients with recurrent implantation failure.

Materials and Methods: We retrospectively collected data of the patients between 2017 and 2021 from a single center. We analyzed 90 unexplained recurrent implantation failure (RIF) patients, at whom, 45 patients underwent microfluid chip technique, and 45 patients with conventional sperm selection.

Results: Pregnancy rates among the microfluidic chip technique and conventional swim up sperm selection cycles were 36% versus 34%, (p=0,4), clinical pregnancy rates (CPR) were 33% versus 31% (p=0,3) and live birth rates (LBR) were 26% versus 25% (p =0.4).

Conclusion: Our study showed that the microfluidic technique does not change CPR, and LBR during in vitro fertilization (IVF) treatment for couples with recurrent implantation failure.

Keywords: microfluidic chip technique; recurrent implantation failure; sperm selection

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Introduction

According to the estimates of the World Health Organization, 70 million couples worldwide suffer from infertility. According to current information, 40% cases are because of men only. Among all treatment approaches, assisted reproductive treatments (ART) such as intracellular sperm injection (ICSI) have been identified as the best solution to male infertility. Pregnancy rates have also shown it that the key point in ICSI treatment is to select the healthiest sperm. The sperm selection parameter in in vitro fertilization (IVF) and ICSI techniques is sperm motility and morphology. However, it has been observed that current standard techniques do not select sperm with these parameters. In addition, it has been shown that DNA damage occurs in the separation techniques that depend on the density difference. The swimming technique is based on the motile ones floating up to the fresh environment among the sperms in the sediment state. Both techniques are insufficient for semen samples with a high rate of DNA damage. As a new sperm selection method, the microfluidic chip technique has been shown to select sperm with a lower DNA fragmentation index [1]. The sperm travels on the micro channels on their way to the oocyte. In these systems, this environment in fertilization is recreated on a microchip.

Although pregnancy results have increased due to the improvement of technologies that have developed since the first IVF, recurrent implantation failure (RIF), rates are still not underestimated.

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While there is an average of 40-55% success in three IVF cycles, this rate remains between 50-75% in six consecutive IVF treatments.

Today, the definition of RIF can be made in the light of the data as follows; Patients under the age of 40 who do not result in a gestational sac despite receiving fresh or frozen embryos in at least three cycles and at least 4 good quality embryos can be defined as RIF [2-4].

It is thought that sperm quality also affects embryo quality and implantation, and therefore affects pregnancy rates. Especially sperm DNA damage is thought to be effective on poor embryo development [5]. Today, there are various methods and tests to determine sperm DNA damage, and although it has been reported that implantation and pregnancy rates are lower and abortion rates are increased in patients with >30% DNA fragmentation, the clinical value of determining sperm DNA fragmentation index is unclear in the light of current literature.

The aim of this retrospective study was to investigate biochemical pregnancy, clinical pregnancy and live birth rates in the treatment of couples with recurrent implantation failure patients by ICSI using spermatozoa selected by the microfluidic technique.

Material and methods

A retrospective cohort monocenter study was designed for Turkish women in the reproductive age group (18–40 years) with RIF attending infertility clinic at Ege University Faculty

of Medicine. A total of 90 couples were included in the study with 45 couples per group. Informed consent form was taken from the patients included in the study. Ethics committee approval was not obtained because of the retrospective screening.

All couples also underwent ovarian reserve test, pelvic ultrasonography, thrombophilia panel, genetic screening, and sperm analysis.

The inclusion criteria was two unsuccessful IVF/embryo transfer cycle performed under the age of 40 years.

Exclusion criteria were; Uterine malformation, myoma, hydrosalpinx, uterine synechia, female age over 40 years, severe male factor, genetic abnormalities and thrombophilia. The primary outcome was the live birth rate. Secondary outcomes included pregnancy and clinical pregnancy rates. Detection of an intrauterine sac on ultrasonography was defined as a clinical pregnancy and human chorionic gonadotropin (hcg) < 50 ıu/l at 14. day of embryo transfer was defined as a pregnancy. Delivery of an infant after 24 weeks of gestation was defined as live birth. In the study group, the microfluidic technique was used for spermatozoon selection before ICSI. In the control group, the conventional swim-up technique was used for selection of spermatozoa for ICSI. Microtubule chips contain microfluidic channels composed of polymer known as poly (dimethylsiloxane). After the sperm sample is added to the canal entrance, it is placed in an incubator at 37 °C for 30 minutes. Sperm reaching the exit from the microchannels are collected for ICSI. A gonadotropin-releasing-hormone (GnRH) antagonist protocol was used for ovarian stimulation in all patients. 150–450 IU Gonadotropins (follitropin alfa and/or menotropin) were started on day 3 of the menstrual cycle with 0.25 mg GnRH antagonist, Cetrorelix (Cetrotide, Merck, Halle, Germany) started on the cycle day of 7. When the dominant follicle reached 18 mm, ovulation triggering was performed using human chorionic gonadotrophin (hCG). Oocyte pick up was performed 35 h after triggering. One or two embryos were transferred according to our center protocol. The data was collected from the data from medical charts of the patients.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) v.22.0 for Windows (SPSS, Inc., Chicago, IL, USA). Descriptive statistics were used to describe and compare the baseline characteristics of the patient groups. The Shapiro-Wilk Normality test was used to check the normality assumption of the continuous variables. The outcomes of the hysteroscopy+ endometrial scratching and control groups were compared with independent sample t-test or the Wilcoxon runk-sum (Mann-Whitney U) test, according to the normality of data distribution. The descriptive statistics for the continuous variables were calculated with the mean, standard deviation, median, and the interquartile range. A p-value of less than .05 was considered as statistically significant

Results

Out of 110 patients with RIF, 20 were excluded from the study according to the exclusion criteria. In total, 90 unexplained RIF patients who were admitted to our clinic between 2017 and 2019 were included in this retrospective study. Of those, 45 RIF patients the microfluidic technique was used for spermatozoon selection before ICSI, in the control group, the conventional swim-up technique was used for selection of spermatozoa for ICSI.

No statistical difference was observed in the basic characteristic findings of the patients (Table 1).

Cycle characteristics by group were similar between the groups. According to ART cycle outcomes, there were no significant differences in any of the parameters, including a number of retrieved oocytes (8,5±4,1 vs. 8,4 ± 3,8),

Table 1. Baseline characteristics of the groups

Variables	Microfluidic technique (n=45)	Control (n=45)	*p*
	Mean±SD	Mean±SD	
Female Age (years)	29.9 ±4.6	28.6±4.7	0.8
Male Age (years)	32.2±5.2	31.4±3.2	0.7
Body-mass index (kg/m²)	22.4 ±5.3	21.3± 5.3	0.59
Duration of infertility (years)	4.0 ±0.32	4.0 ± 0.44	0.32
Previous IVF cycles	2.6 ±0.9	2.8 ±1.0	0.75
Cause of infertility, %			
Male factor	43.1±4.3	43.9 ±5,7	0.94
Tubal factor	16.9±7.5	15.1 ± 2.5	0.80
Endometriosis	10.8±7.4	8.1±2.6	0.75
Anovulation	6.6±4.2	6.4±7.8	0.85
Combined	6.2±3.3	7±6.7	0.93
Unexplained	15±4.1	18±5.9	0.74
AMH	1.9±1.8	1.9±2.4	0.85

AMH: antimullerian hormon , SD: Standart deviation, IVF: in vitro fertilization

fertilized oocytes (5,1±2,2 vs ±5,8 ±2,1), embryos transferred (1,8±0,6 vs.1,8±0,8) and percentage of top quality embryos (77% vs. 79%) between the groups shown in Table 2.

Table 2. Cycle characteristics by group

Variables	Microfluidic technique	Control	*P*
	Mean±SD	Mean±SD	
Total dose of gonadotropins (IU)	2305±1430	2301±1150	0,16
Number of oocytes retrieved	7,5± 4,1	7,4± 3,8	0,91
Number transferred embryos	1,8±0,6	1,8 ± 1,8	0,66

SD: Standart deviation

Pregnancy rates among the study and control groups were 36% vs. 34%, (p:0,4), respectively. The clinical pregnancy rates were 33% vs. 31%, (p:0,3), and live birth rates were 26% vs 25%, (p:0.4), respectively.

Table 3. Pregnancy and livebirth outcomes

Variables	Microfluidic technique	Control group	*p*
Pregnancy	36%	34%	0,4
Clinical pregnancy	33%	31%	0,3
Live birth	26%	25%	0,4

Discussion

In this retrospective study, we investigated the effectiveness of the microfluidic chip technique in patients with recurrent implantation failure. Embryo quality is one of the important factors in patients with recurrent implantation failure. Obtaining healthy sperm, possible effects during sperm selection procedures, affect embryo quality. In this study, in which the microfluidic chip was used as the sperm selection method, we did not detect a significant difference in pregnancy outcomes.

The use of microfluidic chip technology, which was designed at Stanford University at first, has found use in ART cycles as well as in many biomedical fields [6-7].

Matsuura et al. [8], in their ICSI study on porcine cells, found that the time to get healthy sperm in the group with poor sperm quality was shorter in the microchip group. In our study, we also found that the laboratory team took less time to apply microchip for the observation of sperm selection before ICSI, although it was not reflected in the conclusion part.

Sperm DNA damage is an issue that has been emphasized in recurrent IVF failure cases in recent years. When the rate of sperm DNA damage exceeds 30%, the chance of pregnancy decreases. Schulte et al. stated that sperm DNA damage is less in microchip technique compared to conventional methods [9]. however, pregnancy outcomes were not evaluated in the same study. Although we did not measure sperm DNA damage in this study, we did not detect any difference in live birth outcomes.

In the microfluidic technique method, sperm selection can be done at low cost, and more importantly, it can be done more objectively because it also eradicates the margin of human error.

The micro channels of the microfluidic chip technique are like the paths followed by sperm in the natural reproductive system, and the most progressive sperm reach the end of the micro-channel and sperm with lower DNA damage can be selected.

Yetkinel et al., in their randomized controlled study, compared the conventional method and the microchip method in unexplained infertility cases [10]. In the study, fertilization rates and the rate of freezing the remaining embryos after fresh transfer were found to be higher in the microchip group. However, no difference was found in pregnancy outcomes in this study, parallel to our study.

In the classical ICSI method, the embryologist has a 7% probability of degenerating the oocyte. In order to increase fertilization rates in recent years, the microchip method, which is thought to increase fertilization rates, can be preferred instead to the ICSI method, which is widely used in our country, thus eliminating the risk of oocyte degeneration. Whether such studies will be performed, or will lead to a conversion of ICSI to microfluidic IVF/CI cycles, might be highly questionable because current use of ICSI in non-male factor cases is on the rise [11].

The selection of a motile and a morphologically normal sperm is an integral step in ARTs. A healthy and high-quality sperm with high DNA integrity and proper structure can increase the pregnancy rate and live birth. An incompetent and unhealthy sperm can result in failure in reproduction or affect the health of the offspring. In order to overcome the incompetence of the conventional techniques in mimicking the natural sperm selection process, microfluidic sperm selection chips allow creating more naturally relevant environments leading to the selection of high-quality sperms. The female reproductive organ is very complex. As opposed to the traditional approaches, microfluidic chips and microfluidics devices can be designed to create a bioinspired environment for the sperm to increase the selectivity. The microfluidic chips are often fabricated to mimic the natural environment for the sperms or include micro channels that separate motile and healthy ones from the defected ones.

When the literature is examined, it is seen that this method increases fertilization rates, but its effect on pregnancy outcomes is not clear. In our study, no difference was found in terms of live birth outcomes when both methods were compared.

Current microfluidics chips for sperm selection are either passive or active. In passive devices, the selection process utilizes the sperm motility. The micro channels are designed such that the sperms with higher motility swim to a side channel. In some designs, two streams flow alongside each other; one being the sample and the other a clean buffer. The sperms with high motility swim to the clean buffer. These high-quality sperms are then collected from these channels.

The strengths of our study were that it was single-centered and the study populations were homogeneous, while its retrospective nature was its limitation.

In conclusion, our study indicates that the microfluidic technique does not change live birth, and clinical pregnancy rates during IVF treatment for couples with recurrent implantation failure.

Disclosure

Authors have no potential conflicts of interest to disclose.

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