



Effects of the microfluidic chip technique in sperm selection for intracytoplasmic sperm injection for unexplained infertility: a prospective, randomized controlled trial

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Abstract

Purpose The new-generation spermatozoon selection method, microfluidic technique called Fertile Chip[®] gives the chance to select spermatozoa with lower DNA fragmentation indexes. We aimed to determine the effect of microfluidic techniques for spermatozoon selection in ICSI treatment in patients with unexplained infertility.

Methods This prospective randomized controlled study was conducted at a university hospital. One hundred twenty-two couples with unexplained infertility were included, in which 61 of them were treated with conventional swim-up techniques (control group) and another 61 with the microfluidic technique (study group) for spermatozoon selection in IVF treatment. The fertilization rates and the quality of embryos were the primary outcomes, and clinical pregnancy (CPR) and live birth rates (LBR) were the secondary outcomes of our study.

Results CPR in the study group and control group were 48.3% and 44.8% ($p = 0.35$) and LBR were 38.3% and 36.2% ($p = 0.48$), respectively. The fertilization rates were similar (63.6% and 57.4%, $p = 0.098$). A total number of grade 1 embryos were significantly higher in microfluidic technique group than in control group (1.45 ± 1.62 vs. 0.83 ± 1.03 , $p = 0.01$). There were more surplus top quality embryos leftover to freeze in the study group (0.71 ± 1.48 vs. 0.22 ± 0.69 , $p = 0.02$).

Conclusion Our study showed that the microfluidic technique does not change fertilization, CPR, and LBR during IVF treatment for couples with unexplained infertility. Despite the fact that the total number of grade 1 embryos after ICSI treatment and the surplus number of grade 1 embryos after embryo transfer were higher in the microfluidic technique group, the study was not powered to detect this difference.

Trial registration NCT02488434

Keywords Microfluidic technique · Unexplained infertility · Embryo · ICSI

Introduction

Selecting healthy spermatozoa is requisite for intracytoplasmic sperm injection (ICSI) to achieve higher fertilization rates and to obtain higher quality embryos and live birth rates, which is the goal of in vitro fertilization (IVF). Spermatozoon selection usually depends on conventional density gradient centrifugation or swim-up techniques that require chemical and mechanical

processes which may increase oxygen radical levels in spermatozoa [1]. Elevated oxidative stress in spermatozoa induces DNA base oxidation, increased DNA fragmentation, and eventually cell death [2]. Therefore, new spermatozoon selection methods have been introduced to obtain higher quality spermatozoa to increase fertilized embryo quality. Combined with the selection of spermatozoa with normal morphology, such methods allow selection of spermatozoa with reduced DNA injury and fragmentation rates and higher DNA integrity [3–8]. One new spermatozoon selection method is the microfluidic chip technique called Fertile Chip[®] (Koek Biotechnology, Izmir, Turkey). Although the microfluidic technique was shown to select spermatozoa with a lower DNA fragmentation index, no clinical studies have examined its effect upon embryo quality and pregnancy rates [1, 9]. In contrast to the chemical and centrifuge stages involved in the classical swim-up process for sperm selection, the microfluidic

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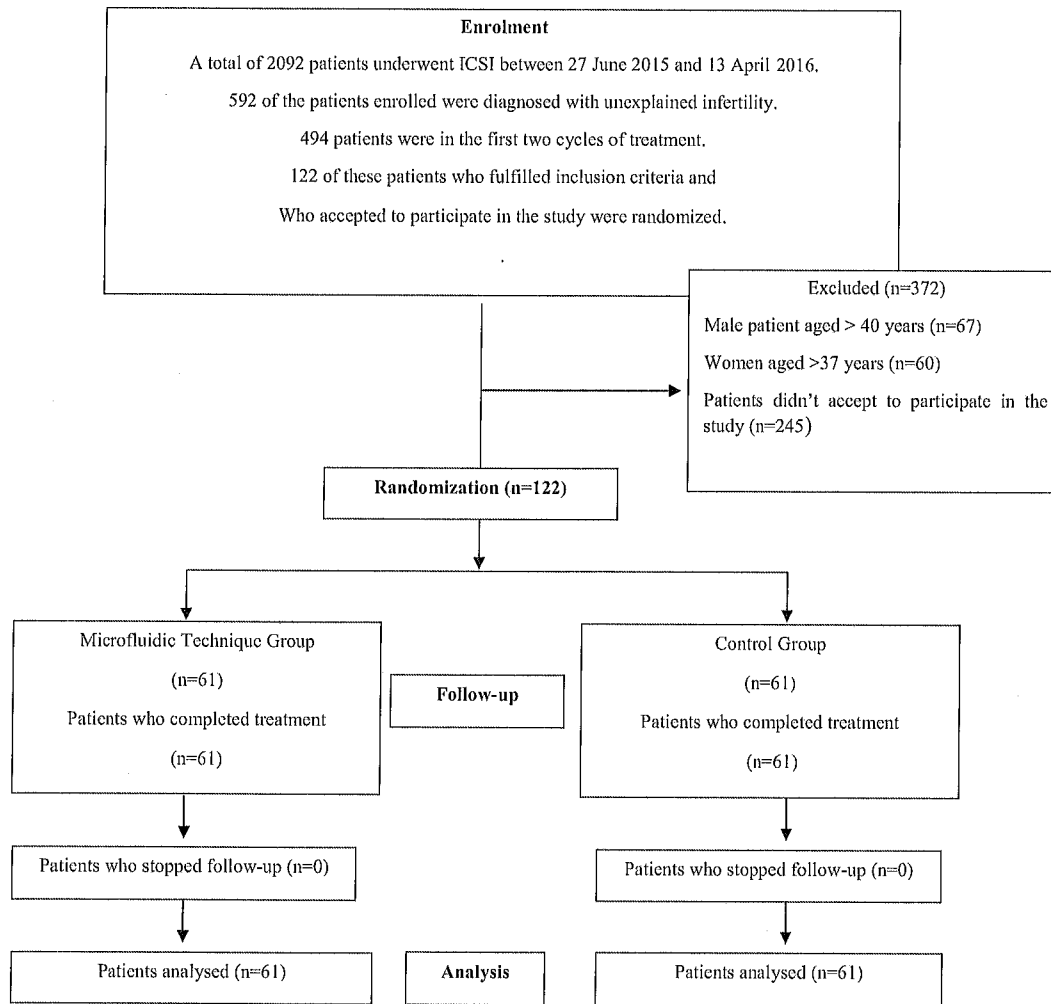


Fig. 1 The patient selection flowchart

deliveries that resulted in a live born neonate, expressed per 100 embryo transfers.

Conventional swim-up technique

In the conventional swim-up technique, all semen samples were liquefied in a 37 °C incubator for 1 h. The liquefied semen sample was then diluted 1:1 with culture medium and centrifuged for 10 min at 1500 rpm. Thereafter, the supernatant was discarded and 1 ml of fresh culture medium was layered above the pellet. The tube was inclined at 45° and incubated for 1 h (37 °C, 6% CO₂). After incubation, the supernatant was ready for ICSI and transferred into an empty tube.

Microfluidic technique

Fertile Chip[®] contains microfluidic channels created with polymethylmethacrylate and double-sided adhesive film.

Inlet and outlet ports were created by cutting holes through the polymethylmethacrylate (Fig. 2). For sperm sorting, the microfluidic channel was first filled with medium and the outlet port was filled with medium followed by a thin layer of mineral oil to avoid medium evaporation. After liquefaction of the sperm sample, 1 ml was added to the channel inlet, and the microfluidic chip was then placed into an incubator at 37 °C for 30 min. Healthy spermatozoa in the sample swam through the microchannels from the inlet up to the outlet and were collected for ICSI as described [16].

Statistical analysis

Statistical analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) software. Categorical variables were expressed as numbers and percentages, whereas continuous variables were expressed as mean and standard deviation (min–max, where applicable). Comparison of

Table 1 Main patient characteristic properties

	Microfluidic technique (<i>n</i> = 61)	Control group (<i>n</i> = 61)	<i>p</i>
Female age	28.61 ± 2.96	28.21 ± 3.31	0.49
Male age	32.74 ± 3.72	32.82 ± 3.73	0.90
Duration of infertility (years)	5.23 ± 3.49	4.36 ± 2.94	0.14
BMI (kg/m ²)	24.19 ± 3.55	24.33 ± 4.37	0.86
Sperm count (× 10 ⁶ /ml)	66 ± 37	58 ± 41	0.27
A + B motility (%)	57.08 ± 14.84	54.70 ± 14.37	0.37
Antral follicle count (<i>n</i>)	7.97 ± 2.02	7.72 ± 2.18	0.52

Discussion

In this randomized controlled trial, comparison of spermatozoon selection by the microfluidic and conventional swim-up techniques demonstrated that the fertilization, pregnancy, and live birth rates of the couples were similar in both groups. The embryo quality is overall affected by multiple factors in addition to the possible effects by the processing and selection methodologies of sperm. Here, we changed the sperm processing method and demonstrated a statistically significant difference in the study group compared to the control. The total number of grade 1 embryos after ICSI treatment and the surplus number of grade 1 embryos after embryo transfer were higher in the microfluidic technique group. Furthermore, the rate of embryo freezing (for extra embryos) after embryo transfer was higher in the microfluidic group. Despite all, the study was not powered to detect this difference.

This is the first clinical study that shows potential effects of the microfluidic technique in the selection of spermatozoa for ICSI. The primary outcomes of our study are fertilization rate and embryo quality. Few studies have investigated new-generation sperm selection techniques using embryo quality. Gianaroli et al. suggested that sperm selected with an inverted microscope produced higher embryo quality and ongoing pregnancy rates compared with classical ICSI, but fertilization rates were similar [4]. In contrast, Balaban et al. found no difference in the quality of embryos or live birth rates for IVF treatment using intracytoplasmic morphologically

selected sperm injection (IMSI) compared with classical ICSI [17]. In our study, we found higher number of grade I embryos in the study group but we did not find any significant differences in terms of fertilization, pregnancy, and live birth rates between the microfluidic techniques compared with classical ICSI. In the meta-analysis of magnetic-activated cell sorting (MACS) (another new-generation sperm selection method) compared with classical ICSI, authors suggested that the pregnancy rate was higher in the MACS group (RR = 1.50, 95% CI 1.14–1.98). However, this study did not include data about the quality of embryos or the fertilization rate [18].

Although the levels of estradiol and progesterone on the day of hCG were statistically different in our study, we did not find any significant difference in regression analysis of these parameters. The days of embryo transfer were one of the third, fourth, or fifth days. Although different days of embryo transfer might affect the pregnancy and live birth rates, the transfer days were homogeneously distributed in the two groups.

Spermatozoon selection by the microfluidic technique was made objectively and successfully with lower cost and without the human margin of error of the conventional swim-up technique. It is an easy, less time-consuming procedure with high clinical applicability and repeatability. Instead of the chemical and centrifugation stages involved in the conventional swim-up technique which impairs sperm DNA integrity by increasing oxygen radical levels in the spermatozoa, the microfluidic selection technique mimics the natural routes that selects healthy spermatozoa traveling through the cervix,

Table 2 Characteristic properties of the ICSI cycle

	Microfluidic technique (<i>n</i> = 61)	Control group (<i>n</i> = 61)	<i>p</i>
FSH baseline dose (IU)	217.01 ± 58.92	209.79 ± 64.65	0.519
Induction duration (days)	9.05 ± 1.44	8.82 ± 1.83	0.439
Total GnRH α dose (IU)	2605.90 ± 1391.47	2086.87 ± 778.79	0.849
hCG day serum estradiol level (pg/ml)*	2605.90 ± 1391.47	2125.20 ± 1137.82	0.040*
hCG day serum progesterone (ng/ml)*	1.14 ± 0.65	0.92 ± 0.43	0.032*
hCG day endometrium (mm)	10.82 ± 1.88	10.54 ± 1.92	0.403
Number of follicles after induction (<i>n</i>)	13.30 ± 4.97	13.43 ± 5.47	0.885
Oocytes picked up (<i>n</i>)	16.62 ± 7.30	15.45 ± 8.43	0.415
Metaphase II oocyte number (<i>n</i>)	12.79 ± 5.73	11.70 ± 7.29	0.363

**p* < 0.05

unexplained infertility. Embryo quality after ICSI was one primary outcome of our study, and the higher number of grade 1 embryos supports the need for further studies of the microfluidic technique. Already we have planned larger scale studies dealing with couples with abnormal sperm morphological parameters, such as the male factor, or groups with high DNA fragmentation rates to further investigate the clinical effects of the microfluidic selection method.

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