Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2023**; 50(3): 46
https://doi.org/10.31083/j.ceog5003046

Original Research

Comparison of Reproductive Outcomes in ICSI Cycles Using Sperm Chip Technique and Density Gradient Technique in Men with Normal Semen Analysis

Selin Ozaltin[1], Hale Goksever Celik[2,*], Yucel Kocyigit[3], Emre Kar[4], Mete Gungor[1], John Yeh[5], Ercan Bastu[6,7]

[1]Department of Obstetrics and Gynecology, Acibadem Mehmet Ali Aydinlar University Acibadem Maslak Hospital, 34752 Istanbul, Turkey
[2]Department of Obstetrics and Gynecology, Acibadem Mehmet Ali Aydinlar University Acibadem Fulya Hospital, 34752 Istanbul, Turkey
[3]Department of Obstetrics and Gynecology, Acibadem Fulya Hospital, 34349 Istanbul, Turkey
[4]Department of Obstetrics and Gynecology, Istanbul City Hospital, 34480 Istanbul, Turkey
[5]Department of Obstetrics and Gynecology, University of Harvard, Cambridge, MA 02138, USA
[6]Department of Obstetrics and Gynecology, Biruni University, 34010 Istanbul, Turkey
[7]Department of Obstetrics and Gynecology, UMass Chan Medical School, Worcester, MA 01655, USA
*Correspondence: hgoksever@yahoo.com (Hale Goksever Celik)

Academic Editor: Johannes Ott
Submitted: 6 January 2023 Revised: 12 February 2023 Accepted: 13 February 2023 Published: 1 March 2023

Abstract

Background: Microfluidic sperm sorting procedure or sperm chip technique is a chemical-free method of selecting sperm using a disposable chip. It is a new gentle alternative for sperm processing which has been produced to obtain sperm with higher rates of motility and better morphology, as well as to reduce DNA fragmentation in sperm with high DNA fragmentation to nearly undetectable levels. We aimed to evaluate sperm chip techniques on clinical pregnancy rates in patients who underwent intracytoplasmic sperm injection (ICSI). **Methods**: The patients in whom fresh embryo transfer (ET) on Day-3 or 5 after ICSI had been performed were analyzed in this prospective randomized cohort study. **Results**: Of those, 102 patients underwent ICSI with sperm isolated using sperm chip technique (study group) while 111 patients underwent ICSI with sperm isolated using swim-up technique (control group). No significant difference was observed between the groups in terms of fertilization rate in patients who underwent ET on the 3rd or 5th day. In the patients having ET on Day-3, Grade 1 embryos were obtained similarly between the sperm chip group and the control group. Grade 1 embryos transferred on Day-5 were observed significantly more frequently in the study group ($p = 0.050$). However, clinical pregnancy rates did not show significant differences between the groups in patients who were transferred on both the 3rd and 5th days. **Conclusions**: Although sperm selection using by sperm chip technique provides advantage in terms of blastocyst quality, use of this technique does not enhance success in terms of clinical pregnancy. **Clinical Trial Registration:** Approval was obtained from ClinicalTrials.gov with NCT03355937 approval number.

Keywords: infertility; sperm chip; microfluidic sperm sorting; assisted reproductive techniques (ART); *in vitro* fertilization (IVF); intracytoplasmic sperm injection (ICSI); clinical pregnancy rates

1. Introduction

Infertility and subfertility affect a significant proportion of human beings who have a desire to become pregnant. Approximately 12% of the reproductive-aged population (6.7 million couples) in the USA faced impaired fecundity according to statistics published in 2013 by the Centers for Disease Control and Prevention (CDC), of which one-third showed the cause as the man, in another one-third the cause as the woman, leaving the last one-third as unidentified [1]. The leading cause of male infertility is abnormal or lack of sperm, which is usually associated with low sperm motility and flawed sperm function resulting in an inability to fertilize an oocyte [1,2]. In today's practice, one of the best techniques to treat male infertility is a method in assisted reproductive techniques (ART) called intracytoplasmic sperm injection (ICSI). This method in which a single viable sperm is injected directly into the oocyte provides numerous advantages with fewer drawbacks [3]. It decreases the number of sperm needed for fertilization and bypasses *in vivo* barriers including the female genital tract. However, this technique requires more careful selection of the sperm because bypassing the *in vivo* environment also eliminates the natural selection of sperm. ART has recently replaced the natural selection of sperm which has been existent since before the evolution of humans. This is a possible reason why clinical pregnancy and live birth rates are lower than expected, compared to implanted embryo numbers achieved through ART. Thus, a clinically applicable, efficient and inert (not to damage sperm) instrument was needed to find the most fertilizable sperm for ICSI.

Another issue with ICSI was the necessity of separation of motile sperm from the rest of the semen. Traditional methods used in common practice for this separation are centrifugation, swim-up, and density gradient centrifu-

gation (DGC). The most commonly used criteria to evaluate the usefulness of these methods are the recovery rates, motility, morphology, and DNA integrity of the sperm.

A newly developed technique named microfluidic sperm sorting or sperm chip technique or microfluidic chip has been added to these techniques recently, and it offers more than just sperm selection. It presents a design that mimics the filtration function of the female genital system, which is responsible for the natural selection of sperm. Unlike the unprocessed samples, sperm preparation methods show higher rates of motility and better morphology, as well as less DNA fragmentation in the collected sperm [4]. This new-generation spermatozoon selection method also gives the chance to select spermatozoa with lower DNA fragmentation indexes. Moreover, when a combination of DGC and swim-up techniques is compared to the microfluidic sperm sorting, the latter was proved to provide lower rates of DNA fragmentation [4,5]. Although conventional sperm preparation techniques can improve sperm function and morphology, they are thought to be limited in preparation of genetically enhanced samples. The microfluidic chip, however, may make sperm selection better in functionality, morphology and genetics [6].

In this study, we aimed to identify the effect of the microfluidic sperm sorting technique on clinical pregnancy rates in ICSI treatment in patients with unexplained infertility.

2. Materials and Methods

2.1 Participants

In this randomized prospective cohort study, data of 213 patients who had undergone ICSI procedure with microfluidic chip or swim-up technique at Acibadem Fulya Hospital for 2 years were analyzed. According to our power analysis results, sample population was selected. Of those, the microfluidic chip technique was used for 102 patients and the swim-up technique was used for 111 patients.

Patients were randomized by giving odd and even numbers to use microfluidic chip technique and swim-up technique. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was taken from all couples in the study. The study was approved by Acibadem Mehmet Ali Aydinlar University Medical Research Ethics Committee (ATADEK 2017-15/16). Approval was obtained from ClinicalTrials.gov with NCT03355937 approval number.

Inclusion criteria were as follows: (1) women aged between 18 and 43 years, (2) regular menstrual cycles (menstrual cycles of 25–34 days), (3) normal body mass index (BMI) of 19.3–28.9 kg/m^2, (4) no metabolic or endocrine disorders, (5) normal hormone panel, (6) normal World Health Organization (WHO) sperm parameters (volume, concentration, total number, total and progressive motility, morphology), (7) couples undergoing the ICSI cycle, (8) normal uterine cavity documented by hysterosalpingography or hysteroscopy, (9) embryo transfers on Day-3 or 5, (10) fresh embryo transfers.

The exclusion criteria were as follows: (1) history of cytotoxic chemotherapy and/or radiotherapy, (2) history of ovarian surgery, (3) patients with sperm count $\leq$1 million/mL, (4) patients undergoing *in vitro* fertilization (IVF) cycle, (5) motile sperm rate $\leq$10%, (6) uterine or endometrial or tubal pathology, (7) frozen/thawed embryo transfers (8) paternal age $>$50 years and (9) women aged $>$43 years.

The demographic and clinical data including age, BMI, infertility duration, prior IVF cycle numbers, and sperm parameters were recorded. The ovarian stimulation parameters such as total gonadotropin dose, stimulation duration, number of oocytes retrieved, fertilization rate, Day-3 and Day-5 quality of embryos obtained after ICSI procedure, grade of embryos at transfer day were analyzed and compared between the study and control groups. Results of ovarian stimulation were also compared between the patients in different groups regarding embryo transfer day. The fertilization rate and quality of embryos were evaluated as the primary outcomes, while clinical pregnancy rate and live birth rates were secondary outcomes.

2.2 Controlled Ovarian Stimulation Protocol (COH)

Antagonist protocol was used for all patients undergoing the fresh ICSI cycle. The COH started with recombinant follicle stimulating hormone (FSH) on the second or third day of the menstrual cycle by evaluation by transvaginal ultrasonography. FSH dose was determined according to the patient's age, BMI, antral follicle count (AFC), hormone levels and previous attempt history [7]. The infertility specialist (author: EB) monitored ovarian follicular development using transvaginal ultrasound at 1–3 day intervals. Patients were treated with a daily 0.25 mg gonadotropin releasing hormone (GnRH) antagonist (Cetrotide, 0.25 mg; Merck KGaA, Darmstadt, Germany) given from stimulation day 6 onwards (fixed regimen). A 250 mg dose of human chorionic gonadotropin (hCG) (Ovitrelle, Merck KGaA, Darmstadt, Germany) was injected to achieve follicular maturation when the leading follicles reached 17–18 mm in diameter. Oocyte retrieval took place 34–36 h after hCG injection and metaphase II (MII) oocytes were fertilized by ICSI and cultured until the day of transfer in a commercially available culture medium. Embryo formation was controlled after 16–18 hours and monitored for replication state until embryo transfer day. Embryos were graded according to the Istanbul Embryo Grading Consensus, including grade 1, 2 and 3 [8].

For Day-3 embryos: a grade-1 embryo was classified as having $<$10% fragmentation with equally sized blastomeres; a grade-2 embryo was classified as having 10–25% fragmentation with equally sized blastomeres; while a grade-3 embryo was classified as having $>$25% fragmentation with blastomeres of various sizes.

Table 1. The comparison of the patients regarding their demographic and clinical characteristics.

Characteristics	All population (n = 213)	Sperm chip group (n = 102)	Control group (n = 111)	p value[a]
	mean ± SD or number (%)			
Maternal age (years)	35.0 ± 5.6	34.9 ± 5.0	35.2 ± 6.2	0.689
Maternal age (years)				
≤35	112 (52.6)	55 (53.9)	57 (51.4)	0.707
>35	101 (47.4)	47 (46.1)	54 (48.6)	
Paternal age (years)	37.2 ± 5.9	37.1 ± 5.4	37.4 ± 6.3	0.715
Paternal age (years)				
≤37	113 (53.1)	58 (56.9)	55 (49.5)	0.285
>37	100 (46.9)	44 (43.1)	56 (50.5)	
Body mass index (kg/m^2)	23.6 ± 3.5	23.4 ± 3.5	23.8 ± 3.5	0.488
Infertility duration (months)	47.6 ± 45.5	51.4 ± 47.6	44.2 ± 43.4	0.295
Previous IVF cycle number	2.5 ± 1.9	3.3 ± 2.2	1.8 ± 1.1	**<0.001**
Previous IVF cycle number				
1	77 (36.2)	20 (19.6)	57 (51.4)	**<0.001**
≥1	136 (63.8)	82 (80.4)	54 (48.6)	
Sperm concentration (million/mL)	32.8 ± 27.7	33.2 ± 26.8	32.6 ± 28.6	0.473
Sperm concentration (million/mL)				
≤10	58 (27.2)	22 (21.6)	36 (32.4)	0.075
>10	155 (72.8)	80 (78.4)	75 (67.6)	

Abbreviations: SD, standard deviation.

[a], $p < 0.05$ accepted as statistically significant; Bold, Statistically significant. Independent sample t-test for continuous variables or chi-square test for categorical variables were used.

For Day-5 embryos: a grade-1 embryo was classified as having many prominent and easily discernible cells that form a cohesive epithelium; a grade-2 embryo was classified as having many easily discernible cells that form a loose epithelium; a grade-3 embryo was classified as having few, difficultly discernible cells.

According to their morphologies, embryos were transferred on the third or fifth days. If there were 4 or more good quality embryos on Day-3, we decided to transfer them on Day-5. Vaginal micronized progesterone (Progestan 200 mg; Koçak, Tekirdağ, Turkey) or vaginal progesterone gel (Crinone 8%, Merck Serono, Italy) was administered daily after the oocyte retrieval.

2.3 Semen Preparation

The couples were informed about the requirement of 2–5 days of abstinence before sample collection. Semen samples were collected by masturbation into a sterile container that was labeled. Samples were incubated at 37 °C for 60 minutes.

2.4 Centrifuge and Swim-Up Technique

The liquefied semen sample was diluted in a one-to-one ratio in a culture medium and centrifuged at 1500 rpm for 10 minutes. Subsequently, the supernatant was discarded and fresh culture medium of 1 mL was laid and incubated for one hour at 37 °C at an angle of 45°. Finally, the supernatant was discarded again, and the remaining sperm were used for ICSI.

2.5 Microfluidic Chip Technique

In this technique, a microfluidic sperm chip (Fertile Chip®, Koek Biotechnology, Izmir, Turkey) was used for sperm sorting. This disposable system contains polymethylmethacrylate and two-sided sticky film that has micro-flow free channels. A sperm sample was added into the 1 mL channel opening and the microfluidic chip was incubated for 30 minutes at 37 °C. Spermatozoa which reached to the exit were collected for ICSI.

2.6 Density Gradient Technique

A two-layer density gradient was applied by layering 90% medium under 50% medium in a conical centrifuge tube. Following fluidification, 1 mL of the semen sample was layered over the top layer and centrifuged at 1400 rpm for 10 minutes.

After centrifugation, most of the leukocytes, dead sperm and other components of the plasma are removed gently. Then the pellet was put into a new sterile tube and resuspended in 3 mL of medium to remove the gradient medium. Centrifugation was repeated. Finally, the pellet was resuspended in a sterile medium.

2.7 Embryo Transfer and Outcome Measurements

During the study period, one embryo was transferred to patients <35 years of age in their first two IVF attempts; two embryos were transferred only after first 2 or more failed IVF attempts. In patients who are aged above 35 years, either one or two embryos could be transferred re-

Table 2. Comparison of the patients regarding their ovarian stimulation parameters and results.

Characteristics	All population (n = 213)	Sperm chip group (n = 102)	Control group (n = 111)	p value[a]
	mean ± SD or number (%)			
Total gonadotropin dose (IU/L)	1884.9 ± 786.2	1908.4 ± 796.1	1863.3 ± 779.8	0.677
Stimulation duration (days)	9.1 ± 2.0	8.7 ± 1.8	9.4 ± 2.2	**0.011**
Stimulation duration (days)				
≤9	134 (62.9)	74 (72.5)	60 (54.1)	**0.005**
>9	79 (37.1)	28 (27.5)	51 (45.9)	
Number of oocytes retrieved	10.1 ± 7.0	9.7 ± 6.2	10.4 ± 7.6	0.429
Number of oocytes retrieved				
≤10	133 (62.4)	66 (64.7)	67 (60.4)	0.513
>10	80 (37.6)	36 (35.3)	44 (39.6)	
Metaphase II oocyte number	6.8 ± 5.2	7.0 ± 5.1	6.6 ± 5.3	0.633
Metaphase I oocyte number	1.0 ± 1.4	0.9 ± 1.2	1.1 ± 1.6	0.358
Day-1 pronucleus	5.3 ± 4.1	5.5 ± 3.8	5.1 ± 4.4	0.566
Day-2 pronucleus	4.9 ± 3.9	4.7 ± 3.2	5.0 ± 4.4	0.460
Number of obtained embryos after ICSI procedure	1.5 ± 0.5	1.5 ± 0.5	1.6 ± 0.5	0.070
Embryo transfer day				
Day-3	88 (41.3)	36 (35.3)	52 (46.8)	0.087
Day-5	125 (58.7)	66 (64.7)	59 (53.2)	
Grade of embryos transferred on Day-3				
Grade 1	46 (52.3)	16 (44.4)	30 (57.7)	0.145
Grade 2	24 (27.3)	9 (25)	15 (28.8)	
Grade 3	18 (20.5)	11 (30.6)	7 (13.5)	
Grade of embryos transferred on Day-5				
Grade 1	83 (66.4)	50 (75.8)	33 (55.9)	**0.050**
Grade 2	18 (14.4)	8 (12.1)	10 (16.9)	
Grade 3	24 (19.2)	8 (12.1)	16 (27.1)	
Clinical pregnancy rate (%)	42.3 (90/213)	41.2 (42/102)	43.2 (48/111)	0.760

Abbreviations: SD, standard deviation; ICSI, intracytoplasmic sperm injection.

[a], $p < 0.05$ accepted as statistically significant; Bold, Statistically significant. Independent sample t-test for continuous variables or chi-square test for categorical variables were used.

gardless of previous IVF attempts, in accordance with the Turkish legislation of embryo transfer policy.

On the 10th day following embryo transfer, serum beta-hCG level was measured. If the beta-hCG level was >10 mIU/mL, it was considered as positive and patients with such levels were regarded as chemically pregnant. Clinical pregnancy was confirmed by the presence of a gestational sac on the 10th day after the positive result. Ongoing pregnancy was defined as the presence of fetal cardiac activity beyond 12 weeks of amenorrhea.

2.8 Statistical and Power Analysis

IBM SPSS Statistics (IBM Corp. Released in 2017. IBM SPSS Statistics for Windows, Version 25.0, Armonk, NY, USA) was used for statistical analysis. The sample size was calculated to prevent type-II errors. Inclusion of a minimum of 204 couples was calculated in the study with at least 102 couples per group, for a power analysis with a 0.5% error margin and 90% reliability. Categorical variables were expressed as numbers and percentages, whereas continuous variables were expressed as mean and standard deviation. Comparison of continuous variables between groups was performed, and the Student's t test was used when the assumption for the precondition of a parametric distribution was met. The chi-square test was used for comparison of categorical variables. To determine risk factors affecting clinical pregnancy, logistic regression analysis was used to evaluate variables found to be statistically significant by univariate analysis. For the multivariate analysis, the possible factors identified by univariate analyses were further entered into the logistic regression analysis to determine independent predictors of patient outcome. A p-value of 0.05 was considered as statistically significant.

3. Results

A total of 213 patients who met the eligibility criteria were included in the study. Table 1 shows the comparison between two groups in terms of patients' demographic and clinical characteristics. The mean maternal age was comparable between the sperm chip and control groups (34.9 ± 5.0 and 35.2 ± 6.2, respectively ($p = 0.689$)). The mean age of men was also similar between the groups (37.1 ± 5.4 and

Table 3. Comparison of the patients according to different embryo transfer days and whether spermchip is used or not.

Characterists	Embryo transfer on Day-3			Embryo transfer on Day-5		
	Sperm chip group (n = 36)	Control group (n = 52)	*p* value[a]	Sperm chip group (n = 66)	Control group (n = 59)	*p* value[a]
	mean ± SD or number (%)			mean ± SD or number (%)		
Day-1 pronucleus	3.7 ± 2.5	4.9 ± 4.2	0.114	6.4 ± 4.1	5.3 ± 4.5	0.183
Day-2 pronucleus	3.1 ± 2.2	4.6 ± 4.3	**0.044**	5.5 ± 3.4	5.5 ± 4.5	0.969
Number of obtained embryos after ICSI procedure	1.5 ± 0.5	1.6 ± 0.5	0.599	1.5 ± 0.5	1.6 ± 0.5	0.054
Grade						
Grade 1	16 (44.4)	30 (57.7)	0.145	50 (75.8)	33 (55.9)	**<0.050**
Grade 2	9 (25)	15 (28.8)		8 (12.1)	10 (16.9)	
Grade 3	11 (30.6)	7 (13.5)		8 (12.1)	16 (27.1)	
Clinical pregnancy rate (%)	38.8 (14/36)	42.3 (22/52)	0.255	48.5 (32/66)	44.1 (26/59)	0.621
Miscarriage rate (%)	11.1 (4/36)	9.6 (5/52)	0.343	10.6 (7/66)	8.4 (5/59)	0.434
Live birth rate (%)	27.8 (10/36)	32.6 (17/52)	0.164	37.8 (25/66)	35.6 (21/59)	0.712

Abbreviations: SD, standard deviation; ICSI, intracytoplasmic sperm injection.

[a], $p < 0.05$ accepted as statistically significant; Bold, Statistically significant. Independent sample *t*-test for continuous variables or chi-square test for categorical variables were used.

Table 4. Predictive factors to determine clinical pregnancy rate in ICSI procedure.

Factor	Univariate analysis			Multivariate analysis		
	Odds ratio	95% Confidence interval	*p* value[a]	Odds ratio	95% Confidence interval	*p* value[a]
Maternal age (years)	0.60	0.34–1.03	0.064	0.72	0.33–1.57	0.409
Paternal age (years)	0.72	0.42–1.24	0.238	1.05	0.49–2.24	0.900
Sperm concentration (million/mL)	1.16	0.63–2.14	0.639	1.41	0.74–2.69	0.302
Sperm chip use	0.92	0.53–1.58	0.760	0.91	0.52–1.60	0.736
Number of oocytes retrieved	2.31	1.31–4.08	**0.004**	2.19	1.19–4.04	**0.012**

Abbreviations: ICSI, intracytoplasmic sperm injection.

[a], $p < 0.05$ accepted as statistically significant; Bold, Statistically significant. Univariate and multivariate linear logistic regression analysis were used.

37.4 ± 6.3, respectively, p = 0.715). Body mass index (kg/m^2) was evaluated between study and control groups (23.4 ± 3.5 *vs* 23.8 ± 3.5, respectively, p = 0.488).

When sperm concentrations were compared, both the sperm chip and control groups had similar results (33.2 ± 26.8 and 32.6 ± 28.6 million/mL, respectively, p = 0.473).

Differences were detected between two groups regarding COH parameters and results, as shown in Table 2. Total FSH doses administered in the sperm chip and control groups were 1908.4 ± 796.1 IU/L and 1863.3 ± 779.8 IU/L, respectively (p = 0.677). The average number of stimulation days was statistically shorter for the sperm chip group than the control group (8.7 ± 1.8 days and 9.4 ± 2.2 days, respectively, p = 0.011). The mean number of retrieved oocytes and MII oocyte number were also similar between the groups (p = 0.429 and p = 0.633, respectively). The mean number of Day-1 fertilization of oocytes did not differ significantly between the groups (5.5 ± 3.8 and 5.1 ± 4.4, p = 0.566). The mean total number of embryo obtained after ICSI between study *vs* control group (1.5 ± 0.5 *vs* 1.6 ± 0.5, p = 0.070) and embryo transfer day did not show significant difference between Day-3 and Day-5. When subgroup analysis was carried out between the groups in regards to embryo development, although the grade of embryos transferred on Day-3 was comparable between the groups, grade 1 embryos were transferred significantly more in the sperm chip group on Day-5 transfers (75.8% *vs* 55.9, p = 0.050) (Table 2).

Fertilization rate of embryos transferred on Day-3 and Day-5 were compared between the groups and no significant difference was observed (Day-3: 3.7 ± 2.5 *vs* 4.9 ± 4.2 (p = 0.114) and Day-5: 6.4 ± 4.1 *vs* 5.3 ± 4.5 (p = 0.183)).

Clinical pregnancy rate did not show significant difference between the groups (Day-3: 38.8% *vs* 42.3% (p = 0.255) and Day-5: 48.5% *vs* 44.1% (p = 0.621). Miscarriage rate did not show significant difference between the groups (Day-3: 11.1% *vs* 9.6 % (p = 0.343) and Day-5: 10.6 % *vs* 8.4 % (p = 0.434).

Live birth rate did not show significant difference between the groups (Day-3: 27.8% *vs* 32.6% (p = 0.164) and Day-5: 37.8% *vs* 35.6% (p = 0.712)) (Table 3).

The number of oocytes retrieved was a significant predictor for clinical pregnancy (p = 0.004). However, maternal age, paternal age and sperm concentration did not predict clinical pregnancy (Table 4). Additionally, this association with the number of oocytes retrieved for clinical pregnancy was found to be still significant after adjustment of confounding factors in multivariate linear regression analysis (p = 0.012).

4. Discussion

In our study, a relatively recent technique, the sperm chip technique, was compared to one of the traditional approaches. The ICSI procedures were performed with males having normal semen analysis. Although use of the sperm chip technique contributed to the development of good quality embryos reaching to the blastocyst stage, clinical pregnancy rates were not found to be significantly higher in the sperm chip group and use of this technique did not increase clinical pregnancy rates in the ICSI procedure.

There are several reports in the literature evaluating the factors that affect ART success. Clinical variables such as maternal age, total sperm count, total motile sperm count, sperm motility, embryo quality and sperm selection method are among these predictors of IVF/ICSI treatment success [9,10].

The sperm chip is a technique developed as an alternative to other sperm preparation techniques and best imitates natural sperm selection in the female genital tract. Sperm can be prepared with this method to use during intrauterine insemination (IUI), IVF and ICSI procedures. In other words, the sperm chip technique was developed to imitate the female genital system and provide great convenience for clinicians, especially in male infertility cases and recurrent unsuccessful previous ART attempts [11–13]. A recent study by Ozcan *et al.* [14] showed that use of microfluidic sperm-sorting chip for sperm selection in infertile cases with male factor might improve IVF success rates.

For patients with oligozoospermia, ICSI is a vital technique since only a few sperm are adequate for the procedure. In ICSI, the process of oocyte selection is disregarded and fully left to the ICSI performer [15]. As clinical success following ICSI depends on obtaining the healthiest and the most viable sperm cells, sperm chip technique enables us to select sperm having the best DNA quality and being the least exposed to free oxygen radicals.

Another factor for predicting success is the sperm DNA Fragmentation Index (DFI). It was shown that high DFI is associated with failed pregnancies following IVF/ICSI cycles [16–18]. Although another study published later showed that high DFI had no effect on fertilization rate, quality of embryo or clinical pregnancy rate, it was indicated that the spontaneous abortion rate was statistically higher in those with abortion of over 27% [19]. It was also shown that DFI had little to no effect on fertilization and early embryo development. However, it had an obvious negative effect on blastocyst development phase and embryo implantation [20,21]. When ejaculate is added into the sperm chip channels, sperm with the least DNA fragmentation will sort at the highest percentage and therefore will have the highest chance for fertilization [22]. Thus, the most clinically usable, highly motile sperm with nearly undetectable levels of DNA fragmentation could be selected by sperm chip technique [4,23]. It is possible to speculate that sperm chip techniques may increase clinical pregnancy rate by eliminating the negative effect of DFI by selecting healthy sperm. In other words, the number of good quality embryos that can reach the blastocyst stage was increased significantly by the use of techniques. Success in obtaining

good quality embryos with the use of sperm chip techniques may be explained by the lower exposure of the sperm to oxidant factors. In traditional methods, during processing of the sperm sample, the protocol may extend to 2 hours and this causes a prolonged exposure to the free oxygen radicals already produced as a result of the procedure. On the other hand, a sperm chip technique that lacks centrifugation and is completed in one step protocol only takes 30–45 minutes [24]. Reduced procedure time results in an important drop in the sample's exposure time to free oxygen radicals, enabling prevention of possible DNA damage [25].

One of the most important indications for the sperm chip technique use is recurrent IVF failures. Yildiz *et al*. [13] found a significant improvement in the fertilization rates in infertility patients who had previously failed more than twice in fertility treatment. In contrast, we did not obtain increased success regarding fertilization rate comparing to controls although our study population included the patients with previous failure histories rather than patients coming to our clinic for the first time.

Despite many existent studies showing the potential of sperm chip technique microscopically when it comes to sperm morphology, motility and DNA integrity, there are not many studies in which clinical pregnancy rates were reported [4,6,26,27]. In a recent randomized controlled study with 122 patients, grade 1 embryo count was found to be significantly higher in sperm chip group, similar to our results. This study failed to show a significant difference in clinical pregnancy or live birth rates [28]. Similarly, we found that use of sperm chip contributes positively to the quality of embryo to be transferred on the 5th day but did not increase the clinical pregnancy rate.

Relatively small sample size and the undetermined cumulative pregnancy rate per patient due to failed reaching of all the frozen embryos data (even if the total and transferred embryo counts were similar) are the main limitations of our study.

In addition, we did not investigate the effect of sperm motility on fertilization and clinical pregnancy rates because sperm motility might cause a potential bias favoring the sperm chip group. Thus, we included patients with similar sperm motility and we only found that the number of oocytes retrieved was a significant predictor of clinical pregnancy rates, even after adjusting multivariable regression models for potential confounders.

5. Conclusions

The sperm chip techniques were found as clinically useful for increasing the development of good quality embryos that reach the blastocyst stage. However, we found no increase in clinical pregnancy rates. Further research in a larger study population is necessary to determine whether sperm chip technique would be an option to increase success in ICSI treatment.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

SO—protocol/project development; data collection or management, manuscript writing/editing; HGC—protocol/project development, data collection or management, data analysis, manuscript writing/editing; YK—protocol/project development, data collection or management; EK—protocol/project development, data collection or management; MG—manuscript writing/editing; JY—manuscript writing/editing; EB—protocol/project development, data collection or management, data analysis, manuscript writing/editing. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was taken from all couples in the study. The study was approved by Acibadem Mehmet Ali Aydinlar University Medical Research Ethics Committee (ATADEK 2017-15/16). Approval was obtained from ClinicalTrials.gov with NCT03355937 approval number.

Acknowledgment

The authors would like to thank the participants of this study and to staff of Acibadem Fulya Hospital Embryology Laboratory for their help to access the data.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Chandra A, Copen CE, and Stephen EH. Infertility and impaired fecundity in the United States, 1982–2010: data from the National Survey of Family Growth. US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics: California. 2013.

[2] Gunasheela S. AZ Encycylopedia on Male and female Infertility an Infertility Manual. 1st edn. Jaypee Brothers Medical Publishers: New Delhi. 2005.

[3] Lee SH, Lee JH, Park YS, Yang KM, Lim CK. Comparison of clinical outcomes between *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) in IVF-ICSI split insemination cycles. Clinical and Experimental Reproductive Medicine. 2017; 44: 96–104.

[4] Quinn MM, Jalalian L, Ribeiro S, Ona K, Demirci U, Cedars MI, *et al*. Microfluidic sorting selects sperm for clinical use with reduced DNA damage compared to density gradient centrifuga-

tion with swim-up in split semen samples. Human Reproduction. 2018; 33: 1388–1393.
[5] Akerlöf E, Fredricson B, Gustafsson O, Lundin A, Lunell NO, Nylund L, *et al*. Comparison between a swim-up and a Percoll gradient technique for the separation of human spermatozoa. International Journal of Andrology. 1987; 10: 663–669.
[6] Shirota K, Yotsumoto F, Itoh H, Obama H, Hidaka N, Nakajima K, *et al*. Separation efficiency of a microfluidic sperm sorter to minimize sperm DNA damage. Fertility and Sterility. 2016; 105: 315–321.e1.
[7] Bastu E, Buyru F, Ozsurmeli M, Demiral I, Dogan M, Yeh J. A randomized, single-blind, prospective trial comparing three different gonadotropin doses with or without addition of letrozole during ovulation stimulation in patients with poor ovarian response. European Journal of Obstetrics, Gynecology, and Reproductive Biology. 2016; 203: 30–34.
[8] Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Human Reproduction. 2011; 26: 1270–1283.
[9] Zanetti BF, Braga DPDAF, Setti AS, Iaconelli A, Jr, Borges E, Jr. Predictive factors for biochemical pregnancy in intracytoplasmic sperm injection cycles. Reproductive Biology. 2019; 19: 55–60.
[10] Vaegter KK, Lakic TG, Olovsson M, Berglund L, Brodin T, Holte J. Which factors are most predictive for live birth after in vitro fertilization and intracytoplasmic sperm injection (IVF/ICSI) treatments? Analysis of 100 prospectively recorded variables in 8,400 IVF/ICSI single-embryo transfers. Fertility and Sterility. 2017; 107: 641–648.e2.
[11] Bukatin A, Denissenko P, Kantsler V. Self-organization and multi-line transport of human spermatozoa in rectangular microchannels due to cell-cell interactions. Scientific Reports. 2020; 10: 9830.
[12] Oseguera-López I, Ruiz-Díaz S, Ramos-Ibeas P, Pérez-Cerezales S. Novel Techniques of Sperm Selection for Improving IVF and ICSI Outcomes. Frontiers in Cell and Developmental Biology. 2019; 7: 298.
[13] Yildiz K, Yuksel S. Use of microfluidic sperm extraction chips as an alternative method in patients with recurrent *in vitro* fertilisation failure. Journal of Assisted Reproduction and Genetics. 2019; 36: 1423–1429.
[14] Ozcan P, Takmaz T, Yazici MGK, Alagoz OA, Yesiladali M, Sevket O, *et al*. Does the use of microfluidic sperm sorting for the sperm selection improve in vitro fertilization success rates in male factor infertility? The Journal of Obstetrics and Gynaecology Research. 2021; 47: 382–388.
[15] Speyer BE, Pizzey AR, Ranieri M, Joshi R, Delhanty JDA, Serhal P. Fall in implantation rates following ICSI with sperm with high DNA fragmentation. Human Reproduction. 2010; 25: 1609–1618.
[16] Larson-Cook KL, Brannian JD, Hansen KA, Kasperson KM, Aamold ET, Evenson DP. Relationship between the outcomes of assisted reproductive techniques and sperm DNA fragmentation as measured by the sperm chromatin structure assay. Fertility and Sterility. 2003; 80: 895–902.
[17] Virro MR, Larson-Cook KL, Evenson DP. Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in *in vitro* fertilization and intracytoplasmic sperm injection cycles. Fertility and Sterility. 2004; 81: 1289–1295.
[18] Xue LT, Wang RX, He B, Mo WY, Huang L, Wang SK, *et al*. Effect of sperm DNA fragmentation on clinical outcomes for Chinese couples undergoing in vitro fertilization or intracytoplasmic sperm injection. The Journal of International Medical Research. 2016; 44: 1283–1291.
[19] Lin MH, Kuo-Kuang Lee R, Li SH, Lu CH, Sun FJ, Hwu YM. Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. Fertility and Sterility. 2008; 90: 352–359.
[20] Tesarik J. Paternal effects on cell division in the human preimplantation embryo. Reproductive Biomedicine Online. 2005; 10: 370–375.
[21] Frydman N, Prisant N, Hesters L, Frydman R, Tachdjian G, Cohen-Bacrie P, *et al*. Adequate ovarian follicular status does not prevent the decrease in pregnancy rates associated with high sperm DNA fragmentation. Fertility and Sterility. 2008; 89: 92–97.
[22] Asghar W, Velasco V, Kingsley JL, Shoukat MS, Shafiee H, Anchan RM, *et al*. Selection of functional human sperm with higher DNA integrity and fewer reactive oxygen species. Advanced Healthcare Materials. 2014; 3: 1671–1679.
[23] Shui L, Bomer JG, Jin M, Carlen ET, van den Berg A. Microfluidic DNA fragmentation for on-chip genomic analysis. Nanotechnology. 2011; 22: 494013.
[24] Hughes CM, Lewis SE, McKelvey-Martin VJ, Thompson W. The effects of antioxidant supplementation during Percoll preparation on human sperm DNA integrity. Human Reproduction. 1998; 13: 1240–1247.
[25] Mortimer D. Sperm preparation techniques and iatrogenic failures of *in-vitro* fertilization. Human Reproduction. 1991; 6: 173–176.
[26] Samuel R, Feng H, Jafek A, Despain D, Jenkins T, Gale B. Microfluidic-based sperm sorting & analysis for treatment of male infertility. Translational Andrology and Urology. 2018; 7: S336–S347.
[27] Schulte R, Chung YK, Ohl DA, Takayama S, Smith GD. Microfluidic sperm sorting device provides a novel method for selecting motile sperm with higher DNA integrity. Fertility and Sterility. 2007; 88: S76.
[28] Yetkinel S, Kilicdag EB, Aytac PC, Haydardedeoglu B, Simsek E, Cok T. Effects of the microfluidic chip technique in sperm selection for intracytoplasmic sperm injection for unexplained infertility: a prospective, randomized controlled trial. Journal of Assisted Reproduction and Genetics. 2019; 36: 403–409.