Aegean Journal of Obstetrics and Gynecology

Original Article

Experience of our clinic in intrauterine insemination cycles made with microfluidic sperm sorting chips

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A B S T R A C T

Objective: To evaluate the clinical pregnancy rates in intrauterine insemination (IUI) cycles performed with microfluidic sperm sorting chips.
Material and methods: 133 patients who admitted to our tertiary infertility clinic and underwent ovarian stimulation and IUI protocol with microfluidic sperm sorting chip were analyzed respectively in terms of clinical pregnancy rates between January 2016-January 2020. Patients diagnosed with unexplained infertility, mild-moderate male factor, ovulatory dysfunction and mild endometriosis were included. Microfluidic sperm sorting chip was used for sperm preparation. Primary outcome was clinical pregnancy rates and secondary outcomes were the distribution of the rates according to infertile patient's age groups.
Results: Mean female age was 29.86±4.7 years, the mean total motile sperm count was 72.90±63.7 million, mean antral follicle count was 18.1±10.1, the mean total gonadotropin dose used was 897.6±366 IU. The causes of infertility were 54.1% unexplained infertility, 31.6% ovulatory dysfunction, 6% mild endometriosis, 8.3% mild male factor. The clinical pregnancy rate was 19.5% (26/133) as primary outcome. Infertile patients with age of ≤ 25 years have higher pregnancy rates as secondary outcome (25.9%).
Conclusion: Microfluidic sperm sorting chips allow for a practical and rapid sperm preparation with acceptable clinical pregnancy rates. This method is more successful in younger infertile patients.
Keywords: ovulation induction; intrauterine insemination; microfluid sperm sorting; pregnancy rates; microchips

Introduction

Infertility is defined as the absence of pregnancy in a couple in reproductive age despite regular sexual intercourse for at least one year without using any contraceptive method [1]. Intrauterine insemination (IUI) is an easy and cost-effective treatment for infertile couples before assisted reproductive techniques (ART). IUI is based on the hypothesis to increase the chance of pregnancy by increasing the number of motile sperm in the fertilization area and the number of oocyte candidates for fertilization. Generally, it can be performed to couples under 38 years of age, with normal sperm parameters and at least one open fallopian tube, due to unexplained infertility, cervical factor, mild-moderate male factor, stage I and II endometriosis, ejaculatory dysfunction, sexual dysfunction, impotence and vaginismus [2]. There is a need to use advanced sperm selection methods in IUI. Microfluidic chip for sperm preparation is a method that can reduce sperm damage and deoxyribonucleic acid (DNA) fragmentation occuring frequently during traditional sperm preparation methods such as swim-up and density gradient centrifuge (DGC). Sperm samples obtained by this technique have been shown to have

significantly lower reactive oxygen species (ROS) and DNA fragmentation rates compared to conventional methods.
As the channel size of the microchip gets longer, sperms with DNA fragmentation are eliminated. Therefore, when an 8 μm chip is used, DNA fragmentation is significantly reduced compared to swim-up [3].
In this study, we aimed to determine clinical pregnancy rates in IUI cycles performed with microfluidic sperm sorting chips after ovarian stimulation with gonadotropins.

Material and methods

This is a retrospective observational study of infertile women admitted to a tertiary clinic for infertility between January 2016 and January 2020. All procedures performed in the study were in accordance with the ethical standards of national research committee and with the 1964 Helsinki declaration.

135 infertile patients who underwent ovarian stimulation (OS) and IUI cycles were retrospectively analyzed. The tests performed for all women presenting with infertility were as follows: Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol (E2), prolactin, thyroid stimulating hormone (TSH) levels on 3rd day of menstrual cycle, antral

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follicle count with transvaginal ultrasonography (TVUS) and hysterosalpingography (HSG) between 7th and 11th days of cycle. The test performed for male factor was semen analysis after 3-4 days of sexual abstinence.

Female age between 23-42 years (yrs), unexplained infertility, mild-moderate male factor with total progressive motile sperm count >5 million, ovulatory dysfunction detected by irregular menstrual cycle (>35 days) and stage 1-2 mild endometriosis were included to the study. Exclusion criteria were the female age over 43 yrs, bilateral tubal occlusion, male factor with total progressive motile sperm count <5 million, presence of stage 3-4 endometriosis and obtaining three or more follicles above 16 mm during serial TVUS monitoring.

Ovarian Stimulation (OS)

Daily recombinant follicle stimulating hormone (r-FSH; Gonal-F, Merck Serono, Germany) was used for OS. r-FSH was started on the 3rd day of menstruation with doses varying between 50-150 IU according to the body mass index, age and number of antral follicles in TVUS. A control TVUS was planned on the 5th day of the treatment. Serial TVUS follow-up was done every two or three days to monitor follicle development. One or two ≥18 mm follicle was expected to trigger ovulation. The cycle was canceled in cases with more than 3 follicles ≥ 16 mm. Thus, two patients who developed more than 3 follicles over 16 mm were excluded from the study. IUI was performed 36 hours after the administration of recombinant human chorionic gonadotrophin r-hCG (Ovitrelle, Merck Serono, Switzerland).

Sperm Preparation and IUI

Microfluidic sperm sorting chip was used for sperm preparation. To limit the exposure of semen to changes in ambient temperature, samples were given by masturbation in a nearby special room after 3-4 days of abstinence. A plastic clean, wide-mouthed sterile container was used, which was verified that the ejaculate was non-toxic to spermatozoa. At the end of a 30 minutes liquefaction period at 37ºC in the incubator, 0.85 ml of semen was transferred to a double chamber, free flowing microfluidic sperm sorting chip with a channel size of 8 µm (Fertile Plus, Koek Biotechnology). The first chamber of the microchip is the sample inlet and the liquid channels are separated from the second collection chamber by the microporous membrane. 0.7 ml sperm washing medium was added to the microporous membrane in the outer chamber. The semen transferred to the chip was incubated for 30 minutes at 37°C and then, 0.65 ml of sorted sperm sample was aspirated from the outer chamber of the chip. The sperm sample that had been extracted with a soft-tipped catheter (Bil-Tek, Istanbul, Turkey) into the uterine cavity without touching the fundus by advancing 6 cm from the cervix was transferred to the intrauterine cavity. For luteal support, vaginal progesterone (Crinone gel) daily was started for each patient, starting on the IUI day.

On the 14th day after IUI, all patients were checked with serum beta hCG level. The gestational sac or fetal heart beat on TVUS examination were used to establish the clinical pregnancy. Primary outcomes were clinical pregnancy rates and secondary outcomes were the distribution of the clinical pregnancy rates according to the infertile patient's age groups.

Statistical Method

SPSS 20.0 was used for data evaluation. Data were evaluated as mean value, minimum and maximum.

Results

133 patients with the average age of 29.86 □4.7 yrs and the mean infertility duration of 2.32 yrs included to the study (Table 1). The mean duration of OS with gonadotropins was 9 days. 67.2% of 133 patients were in the first cycle, 28.1% in the second cycle, and 4.7% in the third cycle. 60.9% of these patients were primary infertile and 30.1% were secondary

infertile. 84.2% were nulliparous and 15.8% were multiparous. The mean antral follicle count was 18.1□10.1, the mean total gonadotropin dose used was 897.6□366 IU (Table 1). The mean basal total progressive motil sperm count was 72.90 90 □63.7 million (Table 1).

Table 1. The demographic and clinical characteristics of the infertile couples.

	Average	Min- Max
Age (yrs)	29.86	23-42
Duration of infertility (yrs)	2.32	1-5
Basal TPMSC*	72.900.000	10x106 – 300x106
Gonadotropin dose (IU)	897.6	375-1875
Antral follicle count (n)	18.1	4-45

*TPMSC: Total progressive motile sperm count

The causes of infertility were 54.1% unexplained infertility, 31.6% ovulatory dysfunction, 6% mild endometriosis, 8.3% mild male factor (Table 2).

Table 2. The distribution of infertility causes of the patients.

	Patients applied Microchip
Unexplained (n, %)	72 (54.1)
Ovulatory dysfunction (n, %)	42 (31.6)
Mild Endometriosis (n, %)	8 (6)
Mild male factor (n, %)	11 (8.3)

The clinical pregnancy rate was 19.5% (26/133) as primary outcome (Table 3). The distribution of clinical pregnancy rates according to the infertile patient's age groups are shown as secondary outcomes in Table 3. Infertile patients with age of ≤ 25 yrs have higher pregnancy rates (25.9%) (Table 3).

Table 3. The distribution of clinical pregnancy rates according to the infertile patient's age groups.

Age Groups	Clinical pregnancy is not achieved (n, %)	Clinical pregnancy is achieved (n, %)
18-25	20 (74.1)	7 (25.9)
26-30	39 (81.2)	9 (18.8)
31-35	34 (82.9)	7 (17.1)
36-42	14 (82.4)	3 (17.6)
Total	107 (80.5)	26 (19.5)

Discussion

Success in IUI is possible by selecting quality gametes. Classical methods, DGC and swim-up, and a newer method, microfluidic channel system can be used in the selection of good quality sperm. We reached a clinical pregnancy rate of 19.5% by using the microfluidic sperm sorting chip in our infertility clinic.

In classical methods, sperms are separated on the basis of sedimentation or migration, but motility and morphology characteristics of the sperm cannot be selected [4, 5]. Also, features of sperm such as DNA integrity, membrane maturation, and apoptotic properties cannot be determined. Therefore, there has been a need to develop new sperm selection methods [6]. Motility is an important parameter for evaluating sperm quality. In the DGS method; it is known that ROS increase due to the centrifugation of the processes and therefore DNA structure is disrupted [7, 8]. There may be variations in the number of motile sperm obtained in the flotation method [9, 10, 11]. One of the methods developed for sperm selection that can prevent sperm losses and DNA damage occuring in traditional sperm preparation methods is the sperm sorting chip [12]. When the sperm samples obtained by the sperm chip method were compared with the flotation method, it was shown that there were significantly low ROS and DNA fragmentation rates. As the channel size of the microchip gets longer, the sperm with DNA fragmentation is left behind and its passage through the channel is prevented. Therefore, when 8 µm chip is used;

DNA fragmentation is significantly reduced compared to flotation method [3]. This system contains a chip with microchannel that resembles sperm's intrauterine, cervical and vaginal canal microenvironment. In the microchip, a combination of 1.5 mm thick Polymethylmethacrylate (PMMA) and 50 micron thick double-sided adhesive (DSA) film form microfluidic channels. A lensless charge-coupled device (CCD) is integrated to observe sperm movement within the microfluidic channel. Microfluidic channel medium was supplemented with serum and pre-filled with fresh human tubal fluid (HTF) medium. The sperm sample is loaded with a pipette at the top channel entrance of the column. Sperms are expected to swim through certain lengths of canal systems. The floating sperms are collected and used for ICSI or IUI. Due to CCD, it is possible to record the shadow movement of the sperm by monitoring it [12]. It allows the microfluidic device to be used without the need for an easy-to-use laboratory environment [13].

Clinical pregnancy rates in patients who underwent IUI after ovulation induction were examined in the meta-analysis of ESHRE's Capri Workshop Group and the pregnancy rate ranges between 11.4% and 12.6% [14]. In our study, the clinical pregnancy rate is 19.5% with microfluid sperm sorting chips which clearly shows the success of the method.

Yetkinel et al. compared the microfluid sperm sorting chips with swim-up technique in patients with unexplained infertility in a single-center prospective randomized controlled trial [15]. There was no significant difference between two groups in terms of fertilization, clinical pregnancy and live birth rate (p=0.098, p=0.35, p=0.42 respectively) [15]. However, the total number of Grade I embryos undergoing ICSI using microfluidic sperm sorting chips was significantly higher than the swim-up group (p=0.01) [15]. In our study, we used the microfluid sperm sorting chips method in IUI cycles and found an acceptable clinical pregnancy rates.

Merviel et al. reported clinical pregnancy rate as 13.5% and continuing pregnancy rate as 11.7% in OS+IUI cycles with gonadotropins in a single center, retrospective study including 1038 cycles of 353 couples [16]. They concluded that woman age under 30 with cervical infitility, ovulatory dysfunction or mild male factor with a total motile sperm over 5 million spermatozoa has the best chance during IUI cycles. In our study, the characteristics of infertility causes were similar and average total motile sperm count was over 5 million. We also demonstrated that the clinical pregnancy rates are higher in younger infertile patients, especially between 20-25 ages. Merviel et al. stated that two follicles >16 mm during TVUS follow-up with an E2 level >500 pg/mL on the day of hCG administration present ideal situation [16]. In our study, we also performed serial TVUS follow-up to determine one or two follicles >16 mm and used the hCG to ovulation trigger.

The effects of microfluidic sperm sorting and DGC methods on the ongoing pregnancy rates in OS+IUI cycles were compared in a single center retrospective cohort study of 265 patients [17]. They stated that clinical pregnancy rates were 18.04% with microfluidic sperm sorting method whereas it was 15.15% with DGC method and it was not statistically significant different (p=0.21) [17]. However, they found significantly higher ongoing pregnancy rates in the microfluidic sperm sorting group than the DGC group (15.03%, 9.09% respectively; p=0.03) [17]. In addition, it was also determined that the microfluidic sperm sorting method significantly increased the sperm concentration and the motility compared to the DGC method (p=0.00) [17]. Our clinical pregnancy rate with microfluidic sperm sorting method were 19.5% and this result is similar to Gode et al.'s data.

The strengthens of the study is to demonstrate an easy, practical method which is acceptable technique proven by outcomes. The limitations are its retrospective design, the small population and the lack of a comparison group. Further studies with larger population should be performed to compare this technique with other sperm preparation techniques.

Conclusion

This study demonstrates that microfluidic sperm sorting chips during IUI have acceptable clinical pregnancy rates, its practicality and ease of use provides clinical advantages. This method is more successful in younger infertile patients. It is obvious that prospective studies with larger population are needed.

Disclosure

Authors have no potential conflicts of interest to disclose.

References

[1] Practice Committee of American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. Fertil Steril 2008; 90:S60.

[2] National Institute for Health and Care Excellence. Fertility: assessment and treatment for people with fertility problems (CG156). London: National Institute for Health and Care Excellence (UK); 2015.

[3] 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. Adv Healthc Mater. 2014 Oct; 3(10): 1671–9.

[4] Akerlof 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. Int J Androl 1987;10:663–669.

[5] Le Lannou D, Blanchard Y. Nuclear maturity and morphology of human spermatozoa selected by Percoll density gradient centrifugation or swim-up procedure. J Reprod Fertil 1988, Nov;84(2):551-6.

[6] Boomsma CM, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination. Cochrane Database of Systematic Reviews 2019, Issue 10. Art. No.: CD004507.

[7] Aitken RJ, Clarkson JS. Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm preparation techniques. J Androl 1988; 9, 367-376.

[8] Zini A, Finelli A, Phang D, Jarvi K. Influence of semen processing technique on human sperm dna ıntegrity. Urology 2000; 56, 1081-1084.

[9] Englert Y, Vandenbergh M, Rodesch C, Bertrand E, Biramane J, Legreve A. Comparative auto-controlled study between swim-up and percoll preparation of fresh semen samples for ınvitro fertilization. Hum. Reprod 1992; 7, 399-402.

[10] Smith S, Hosid S, Scott L. Use of postseparation sperm parameters to determine the method of choice for sperm preparation for assisted reproductive technology. Fertil Steril 1995; 63, 591-597.

[11] Ricci G, Perticarari S, Boscolo R, Montico M, Guaschino S, Presani G. Semen preparation methods and sperm apoptosis: swim-up versus gradient-density centrifugation technique. Fertil Steril. 2009, 91, 632-638.

[12] Henkel RR and Schill WB. Sperm preparation for ART. Reprod Biol Endocrinol. 2003 Nov 14;1:108.

[13] Zhang X, Khimji I, Gurkan UA, Safaee H, Catalano PN, Keles HO, et al. Lensless imaging for simultaneous microfluidic sperm monitoring and sorting. Lab Chip, 2011; 11, 2535-2540.

[14] ESHRE Capri Workshop Group. Intrauterine insemination. Hum Reprod Update May-June 2009; 15(3):265-77.

[15] 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. J Assist Reprod Genets. 2019 Mar;36(3):403-409.

[16] Merviel P, Heraud MH, Grenier N, Lourdel E, Sanguinet P, Copin H. Predictive factors for pregnancy after intrauterine insemination (IUI): an analysis of 1038 cycles

and a review of the literature. Fertil Steril. 2010 Jan; 93(1):79-88.

[17] Gode F, Bodur T, Gunturkun F, Tamer B, Pala I, Isik AZ. Comparison of microfluid sperm sorting chip and density gradient methods for use in intrauterine insemination cycles. Fertil Steril. 2019 Nov; 112(5): 842-848.