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; microfluidic sperm sorting; pregnancy rates; microchips

I N T R O D U C T I O N

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 occurring 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.

M A T E R I A L  a n d  m e t h o d s

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...
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 896.7 ± 366 IU (Table 1). The mean basal total progressive motil sperm count was 72.90 ± 63.7 million (Table 1).

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

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Mean- Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.86</td>
<td>23-42</td>
</tr>
<tr>
<td>Duration of infertility (yrs)</td>
<td>2.32</td>
</tr>
<tr>
<td>Basal TPMSC*</td>
<td>72.900.000</td>
</tr>
<tr>
<td>Gonadotropin dose (IU)</td>
<td>897.6</td>
</tr>
<tr>
<td>Antral follicle count (n)</td>
<td>18.1</td>
</tr>
</tbody>
</table>

*TPMSC: Total progressive motive 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.

<table>
<thead>
<tr>
<th>Causes</th>
<th>Patients applied Microchip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexplained (n, %)</td>
<td>72 (54.1)</td>
</tr>
<tr>
<td>Ovulatory dysfunction (n, %)</td>
<td>42 (31.6)</td>
</tr>
<tr>
<td>Mild Endometriosis (n, %)</td>
<td>8 (6)</td>
</tr>
<tr>
<td>Mild male factor (n, %)</td>
<td>11 (8.3)</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Clinical pregnancy is not achieved (n, %)</th>
<th>Clinical pregnancy is achieved (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-25</td>
<td>20 (74.1)</td>
<td>7 (25.9)</td>
</tr>
<tr>
<td>26-30</td>
<td>39 (81.2)</td>
<td>9 (18.8)</td>
</tr>
<tr>
<td>31-35</td>
<td>34 (82.9)</td>
<td>7 (17.1)</td>
</tr>
<tr>
<td>36-42</td>
<td>14 (82.4)</td>
<td>3 (17.6)</td>
</tr>
<tr>
<td>Total</td>
<td>107 (80.5)</td>
<td>26 (19.5)</td>
</tr>
</tbody>
</table>

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 floatation method [9, 10, 11]. One of the methods developed for sperm selection that can prevent sperm losses and DNA damage occurring 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 floatation 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 Poly(methylmethacrylate) (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=0.989, 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 infertility, ovulatory dysfunction or mild male factor with a total motile sperm over 5 million spermatoozoa 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 strengths 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