Assessment of the effectiveness of methods for the selection of sperm with increased DNA fragmentation in ART cycles

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Abstract. Sperm DNA stability is significant in male infertility and favorable reproductive outcomes. Increased DNA fragmentation in spermatozoa can negatively affect the fertilization potential and the kinetics of embryo development. The study aimed to compare traditional and improved high-SDF sperm selection methods and evaluate their impact on the embryological parameters of ART programs. The study included 114 ICSI cycles that used different advanced approaches to sperm selection, including traditional ICSI (control group), magnetic-activated sperm selection, morphological ICSI, and physiological ICSI. The software GraphPad Prism 9.5.1 was used to perform the statistical analysis. The criterion for significance was established at a level of P≤0.05. According to the results, magnetic, physiological, and morphological selection did not increase the fertilization frequency (p=0.1020; p>0.9999; p>0.9999). Magnetic-activated selection of sperm increases the yield of good-quality blastocysts compared to the control group (p = 0.0222); this trend was not observed for physiological and magnetic selection (p > 0.9999; p > 0.9999). The formation of blastocysts of any quality did not exhibit any notable variations (p=0.4139; p>0.999; p>0.999). Among the strategies for selecting sperm with increased DNA fragmentation, magnetic selection is a priority approach for obtaining good-quality blastocysts.

1 Introduction

Infertility is a pressing problem of our time, affecting more than 48.5 million couples worldwide [1, 2]. Despite the constant development of reproductive medicine, the number of couples suffering from infertility is increasing every year.

The identification of the cause of male infertility is currently done through sperm analysis, which is considered the most predictable method. Male infertility can commence from a complex of origins, such as sperm abnormalities, genetic disorders, anatomical irregularities, chronic inflammation, as well as endocrine and immunological abnormalities.
These factors impact the motility, viability, concentration, and other significant characteristics of sperm. Apart from the essential sperm analysis, alternative evaluation techniques exist to assess undefined male infertility, such as estimating anti-sperm antibodies, sperm hyperactivation, acrosomal reaction, hyaluronic acid binding, and sperm DNA fragmentation (SDF) [4].

Sperm DNA assessment is a valuable instrument for estimating the successful progression of spermatogenesis and the genetic material's stability that the offspring will inherit. The stability of sperm DNA plays a crucial role in fertilization, embryo quality, embryo viability, and overall pregnancy progression. The DNA fragmentation index (DFI) has proven to be a beneficial biomarker in diagnosing male infertility. However, it is worth noting that more than 60% of men with unexplained infertility exhibit abnormalities in chromatin structure [5]. Therefore, in cases of unexplained infertility, analyzing sperm DNA can serve as an additional indicator of quality [6].

Several techniques have been devised to address the shortcomings of traditional sperm selection methods and acquire sperm with undamaged DNA. These methods encompass Magnetic Activated Cell Sorting (MACS), Physiological Intracytoplasmic Sperm Injection (PICSI), and Intracytoplasmic Morphological Sperm Injection (IMSI). These improved approaches aim to enhance the quality of selected sperm by ensuring the stability of DNA.

The MACS system is a technique for specifically isolating apoptotic sperm by selectively binding to phosphatidylserine residues present on membranes. Annexin V, a protein with a distinct affinity for this phospholipid, distinguishes the outer surface of apoptotic cells. Recent investigations employing the MACS system have reported decreased apoptotic sperm and DNA fragmentation in samples obtained from ejaculate [7].

IMSI, which stands for Intracytoplasmic morphologically selected sperm injection, is a technique used for selecting sperm with high precision. By utilizing a magnification of ×6000, this method allows for the identification and selection of spermatozoa with the most normal morphology and without vacuoles [8]. Recent research has indicated that IMSI can enhance the selection process and improve pregnancy outcomes, particularly for sperm with low levels of DNA fragmentation [9]. Furthermore, it has been observed that men with teratozoosperma, a condition characterized by abnormal sperm morphology, tend to benefit the most from this approach [10].

2 Materials and Methods

The following criteria were used for inclusion in the studies: number of mature oocytes per puncture – at least 5; fertilization method - intracytoplasmic sperm injection (ICSI); sperm selection methods - density gradient centrifugation (control group, without additional selection), magnetically activated cell sorting (MACS), physiological intracytoplasmic sperm injection (PICSI), morphological intracytoplasmic sperm injection (IMSI); DFI from 20% and above.

The exclusion parameters were the following: use of donor material (oocytes or sperm); number of mature oocytes per puncture – less than 5; DFI is less than 20%.

To assess DNA fragmentation, semen smears and staining were performed using a DNA Fragmentation Kit (Sperm Processor Pvt. Ltd., India). The preparation of ejaculate for ICSI was carried out according to WHO recommendations [11]. Spermatozoa were processed by centrifugation in a density gradient of 90% and 45%, followed by washing in a medium (Gynemed, Germany), after which the swim-up procedure was performed. ICSI fertilization was performed using an Olympus IX73 inverted microscope (Olympus, Japan). Magnetic-activated sperm selection was carried out using columns with annexin-V molecules (Miltenyi Biotec, Germany). Falcon manipulation cups (USA) were used for PICSI.
Cultivation until the 5th and 6th days of development took place at a temperature of 37°C and 6% CO2 in ESCO Miri (ESCO Medical, Denmark), ESCO Miri TL (ESCO Medical, Denmark) and Labotect Labo C-Top (Labotect LaborTechnik-Göttingen) incubators GmbH, Germany).

The statistical analysis was conducted using GraphPad Prism 9.5.1 software, and the nonparametric Kruskal-Wallis test was used to confirm significance (p<0.05).

3 Results and Discussion

The study included 114 ICSI cycles performed at IRM from 2020 to 2021. Among the selected cycles, traditional ICSI was performed in 60, and magnetic sorting was performed in 26. The structure of the study groups is presented in Table 1 (Table 1).

<table>
<thead>
<tr>
<th>Cycles (N)</th>
<th>ICSI</th>
<th>MACS</th>
<th>PICS</th>
<th>IMSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDF, %, 95% CI</td>
<td>26.9±3.94 (25.9; 28.0)</td>
<td>27.2±7.66 (24.1; 30.3)</td>
<td>28.3±10.3 (22.4; 34.2)</td>
<td>28.2±7.88 (23.7; 32.8)</td>
</tr>
<tr>
<td>Female Age (Mean, SD, 95% CI)</td>
<td>32.3±5.04 (31.0; 33.6)</td>
<td>31.9±4.48 (30.1; 33.7)</td>
<td>32.3±5.11 (29.3; 35.2)</td>
<td>31.2±4.49 (28.6; 33.8)</td>
</tr>
<tr>
<td>Male Age (Mean, SD, 95% CI)</td>
<td>35.6±5.78 (34.1; 37.1)</td>
<td>35.2±7.74 (32.1; 38.4)</td>
<td>35.0±5.62 (31.8; 38.2)</td>
<td>33.4±3.33 (30.9; 35.9)</td>
</tr>
<tr>
<td>MI (Mean, SD, N, 95% Cl)</td>
<td>10.5±6.19 (8.8; 12.1)</td>
<td>12.0±3.08 629 95% CI (10.7; 13.3)</td>
<td>10.6±4.34 288 95% CI (8.14; 13.2)</td>
<td>11.9±6.65 166 95% CI (8.01; 15.7)</td>
</tr>
<tr>
<td>Fertilization Rate (Mean, SD, N, 95% Cl)</td>
<td>8.5±5.22 (7.15; 9.85)</td>
<td>10.2±3.34 510 81.1% (8.82; 11.6)</td>
<td>8.00±4.28 255 88.5% (5.53; 10.5)</td>
<td>9.79±6.38 112 75.2% (6.10; 13.5)</td>
</tr>
<tr>
<td>Good Quality Blastocyst Rate (Mean, SD, N, 95% Cl)</td>
<td>3.3±2.59 (2.63; 3.97)</td>
<td>4.4±1.35* 198 38.8% (3.84; 4.96)</td>
<td>3.5±2.35 110 43.1% (2.15; 4.85)</td>
<td>3.07±2.2 49 43.7% (1.8; 4.34)</td>
</tr>
<tr>
<td>Blastocyst Rate (Mean, SD, N, 95% CI)</td>
<td>5.7±3.9 (4.73; 6.74)</td>
<td>6.64±2.46 344 67.5% (5.62; 7.66)</td>
<td>5.0±3.31 166 65.1% (3.09; 6.91)</td>
<td>5.21±3.19 70 62.5% (3.37; 7.06)</td>
</tr>
</tbody>
</table>

According to the data, magnetic, physiological, and morphological selection did not increase the fertilization frequency (p=0.1020; p>0.9999; p>0.9999, respectively). Magnetic-activated selection of sperm increases the yield of good-quality blastocysts compared to the control group (p = 0.0222); this trend was not observed for physiological and magnetic selection (p > 0.9999; p > 0.9999, respectively). There were also no significant differences in the formation of blastocysts of any quality (p=0.4139; p=0.999; p>0.999 for magnetic, physiological, and morphological selection, respectively, Fig. 1).
Several studies have demonstrated that SDF can have detrimental effects on patients in ART programs. Specifically, three articles have reported a decrease in the fertilization rate of oocytes when sperm with fragmented DNA is used [12-14]. Tello-Mora et al. found that an increase in SDF during ART cycles is inversely related to the blastocyst yield and can result in developmental delays [15]. Additionally, two other studies have shown that increased DNA fragmentation negatively impacts the kinetics of embryo development and leads to a decrease in the quality of the resulting blastocyst [16, 17]. Vončina et al. further confirmed that higher levels of DNA fragmentation in sperm are associated with decreased rates of clinical pregnancy and live birth [12].

4 Conclusion

Magnetic selection is a priority approach for obtaining good-quality blastocysts among the strategies for selecting sperm with increased DNA fragmentation in ART. As previously shown, this approach also has advantages for a cohort of patients with unselected DNA fragmentation [18].

A healthy child's birth relies heavily on preserving sperm DNA stability [6]. Numerous studies have emphasized the substantial influence of SDF on male infertility and reproductive outcomes [19]. Enormous SDF can negatively influence the fertilization capacity of sperm cells. However, it is essential to note that sperm with defective DNA can still retain their ability to fertilize.

Multiple variables can provide an enormous DNA fragmentation index (DFI) [20]. These factors include varicoceles, infections in the accessory glands, progressing age, lifestyle preferences, obesity, occupational and environmental elements, specific medications, radiation exposure, and heat exposure [21]. These circumstances may influence DFI by inducing defective spermatogenesis, incomplete apoptosis, or an elevation in the generation of reactive oxygen species (ROS) [6].

In such cases, substituting protaminized sperm DNA with the primary histones required for accurate DNA replication falls short. As a result, the embryo has the potential to halt its growth or experience difficulties in implantation to the uterus, ultimately resulting in a spontaneous termination of the pregnancy during a subsequent phase. On the other hand, if the oocyte's inherent DNA repair mechanisms can restore a genetically stable genome, it enables the occurrence of normal syngamy and subsequent embryonic development [22].
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Authors’ contribution


References


17. F. Anbari, M. A. Khalili, A. Agha-Rahimi, B. Maleki, A. Nabi, and N. Esfandiar, Does sperm DNA fragmentation have negative impact on embryo morphology and morphokinetics in IVF programme? Andrologia, 52(11), e13798 (2020). https://doi.org/10.1111/and.13798


