Impact of Sperm Preparation Techniques on the Sperm DNA-Fragmentation, Concentration and Motility

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ABSTRACT
The effect of sperm quality and optimal sperm preparation techniques on assisted reproductive techniques success are still under debate. To assess the effect of sperm preparation technique on sperm DNA fragmentation, motility and concentration. A prospective study conducted at the infertility consultation clinic in Kamal Al-Samarraee Hospital for infertility and IVF, Baghdad / Iraq, included 30 Iraqi infertile males who met the inclusion criteria from them thirty semen samples were collected. Semen samples were fresh, 1.5 ml volume, at least, sperm concentration ≥ 20 x 106 / ml and total sperm motility ≥ 30%. Samples taken after abstaining from ejaculation for 2-5 days. After baseline evaluation and analysis, all the selected semen divided into two sample each of them managed with either pellet or semen swim-up technique. Then we tested for concentration and motility of sperms in addition to DNA fragmentation. Mean DNA fragmentation rate was significantly lower in direct swim-up than pellet swim-up technique, 27.3% ± 7.9% vs. 14% ± 9.6%, (P=0.001). Moreover, it had been significantly. The median sperm concentration was significantly higher in pellet swim-up, 60%, compared to direct-swim-up with 35%, (P. value = 0.001). Other parameters included the progressive, non-progressive motility, morphology and immotility of sperms were not significantly different, (P. value > 0.05). Direct swim-up sperm preparation technique was better than pellet swim-up technique in reduction of sperm DNA fragmentation and sperm concentration where it was higher in pellet than direct swim-up technique.

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1. INTRODUCTION
Infertility is a medical condition that can cause psychological, physical, mental, emotional or medical harm to a patient [1]. There are various etiological factors that contribute to infertility, including male sexual factors, but unexplained infertility is not uncommon [2]. Although semen analysis remains the cornerstone of male infertility assessment, advanced diagnostic tests have been developed to test semen quality and function to improve diagnosis and treatment. The use of assisted reproductive technologies (ART) has improved the chances of infertile couples having biological children [3]. Sperm analysis (SFA) remains important during laboratory testing for male infertility. Sperm analysis is very useful for identifying and
classifying the severity of any male factor [4]. Sperm DNA fragmentation has become a potential causative factor in reproductive failure, and its evaluation has been suggested as a useful adjunct to in vitro methodology for assessing male infertility, especially assisted reproductive technology (ART). There are various methods for obtaining semen, including gradient methods for swimming and sperm DNA fragmentation. Previous literature has shown that the swimming method significantly reduces the rate of sperm DNA fragmentation and may have some predictive value for in vitro fertilization in patients with low sperm DNA integrity [6]. The technique of preparing semen for fertilization of the uterus can also increase the risk of sperm damage, the speed of DNA fragmentation, and the results of the intrauterine device [7]. This question was assessed in a limited number of studies with different teaching techniques in small subgroups. The study noted that no significant effect of semen on DNA fragmentation was found in normal (with centrifugation) and direct (non-centrifugation) swimming techniques [8]. However, others concluded that ball swimming technique had the lowest rates of DNA fragmentation compared to four different methods, including straight swim-up, Pellet swim-up, density gradient, and density gradient followed by swim-up in IVF / ICSI. courses [9]. It is widely believed that the treatment of human sperm adherence to this technique affects the quality of sperm, which in turn affects not only the outcome of fertilization, but also the development of the embryo [10-12]. However, the effects of different methods of sperm production on sperm motility, DNA fragmentation and other factors are still poorly understood. Thus, our goal was to assess the impact of both cooking methods on DNA fragmentation, mobility, and concentration among infertile Iraqi men seeking treatment.

2. Patients and methods
This was a prospective study conducted at the infertility consultation clinic in Kamal Al-Samarraee Hospital for infertility and IVF / Baghdad / Iraq during the period from 1st of Dec. 2020 till 1st of Jun. 2021. We included 30 infertile Iraqi males aged 25-40 years who attained the clinic during the study period and agreed to participate in the study, after full history taking and thorough clinical examination and investigations. Patients who have a chronic disease, pathological varicocele, varicocelectomy, smokers, alcoholic or exposed to environmental toxins were excluded.

Thirty semen samples were collected. Semen samples were fresh, 1.5 ml volume, at least, sperm concentration ≥ 20 x 10^6 / ml and total sperm motility ≥ 30%. Samples taken after abstaining from ejaculation for 2-5 days.

After baseline evaluation and analysis, the semen sample was divided into two samples; Each particle was managed separately. Then, sperm concentration, motility, and DNA fragmentation were monitored. For the semen collection technique, one volume (ml) of sperm medium was poured into a one volume (1 ml) of semen in a 14 ml conical sterile tube. The tube was incubated with 5% CO2 for one hour for 37 seconds. After the incubation period, the dynamics of the sperm concentration was evaluated with the sperm extracted from the cemetery. DNA from semen taken from the cemetery was destroyed. To allow the granules to float, a portion of the fresh sample is carefully mixed with two volumes (ml) of sperm in a sterile disposable 14 mm conical tube and centrifuged for five minutes. The graveyard was then released and the ball came into contact with 300 microns of sperm. The tube was incubated at 37 ° C with 5% CO for one hour. At the end of the procedure, the upper layer was collected for further analysis.

To perform Sperm DNA fragmentation test, a fresh diluted sperm sample is mixed in an agarose micro gel and loaded onto an agarose coated slide. The sperm agarose suspension is exposed to weak acid to denature the DNA and to lyses solution to remove most of the nuclear proteins. Observe of major DNA damage results in the formation of nucleotides with large halos of spreading DNA loops around the central core.
Nucleoids from spermatozoa with fragmented DNA don't show a dispassion halo altogether or the halo is minimal. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 26. The data presented as mean, standard deviation, median and ranges. Independent t-test (two tailed) used to compare the normally distributed continuous variables between both techniques. Paired t-test was used to compare the continuous variables initially and after procedure. A level of $P$ – value less than 0.05 was considered significant.

All ethical issues were approved and verbal consent obtained from all participants Administrative approvals were granted from the institutional review of Kamal Al-Samarraee Hospital for infertility and IVF and from the Council of Arab Board of Medical Specialization.

3. Results
The mean age of the studied group was 33.3 ± 4.1 (range: 25-40). Baseline seminal fluid parameters are shown in (Table 1). Comparison of mean DNA fragmentation percentage according sperm preparation techniques, revealed significant differences ($P=0.001$); pairwise multiple comparisons showed that mean DNA fragmentation in Pellet swim-up technique was significantly increased than its baseline level, 27.3% ± 7.9% vs. 22.3% ± 5.8% (%), respectively, ($P = 0.007$). Conversely, in Direct swim-up technique, it was significantly reduced to 14% ± 9.6% than the baseline level, ($P=0.001$). Moreover, it had been significantly found that the difference in DNA fragmentation between both technique was significant ($P=0.001$), (Table 2).

Further analysis performed to compare seminal fluid parameters across both techniques, results of these comparisons are demonstrated in (Table 3), the median sperm concentration was significantly higher in Pellet swim-up than Direct swim-up technique, 60% vs. 35%, respectively, ($P=0.001$). Other parameters; progressive motility non progressive motility, immotile sperm and abnormal sperm morphology, were not significantly different, ($P>0.05$).

4. Discussion
Although ARTs are widely used and developed in the last years, there still a need to improve their efficiency. According to the reports of reproduction and fertility societies as the American and European, the success rates of ARTs are still suboptimal. Different reasons contributed to failure and low efficiency. Among these factors, those related to selection of spermatozoa and preparation techniques [13]. In last few years, studies that compare sperm preparation techniques mainly focused on recovery rates and sperm parameters like number, motility and morphology. Due to the strong evidence that DNA fragmentation negatively affects the ART outcomes recent studies, concerned with molecular parameters such as DNA fragmentation to get more precise evaluation of different preparation techniques. However, conflicting results were obtained regarding the effect of sperm preparation and selection methods [14]. However, the effect of sperm quality on ARTs success is still not well identified, nonetheless, getting good quality sperms by optimal strategies and preparation technique is inevitable, Hence, we aimed in this study assess and compare the impact two sperm preparation techniques on sperm DNA fragmentation, motility and concentration in 30 fresh samples of semen obtained from 30 Iraqi infertile men who attained the infertility consultation clinic in Kamal Al-Samarraee Hospital for infertility and IVF in Baghdad, Iraq. All participant men were included in the study according to strict selection criteria and standard preparation protocols were applied in both preparation techniques that used in this study. The standardization and strictly followed protocols aimed to control any confounding effect that may deviates our findings and affects the outcome of the study [15].
To best of our information's, the current study considered the first study in our country that compare these preparation techniques from other point of view, previous literatures regarding such comparison are scarce and stated general comparison of different techniques or study the outcome of a single technique without comparing the results with other ones. In the present study, pellet swim-up and direct swim-up techniques were used in sperm preparation and their effects were compared. Age of the studied group ranged between 25 and 40 years. We found that mean DAN fragmentation percent was significantly lower in direct swim-up than both baseline and pellet swim up technique, these findings consistent with that reported by [16]. Who found that Direct swim-up method was preferred and better than other techniques for sperm preparation, where higher rate of motility and reduced rate of DNA fragmentation compared to other methods. Conversely, our findings inconsistent with that of [17]. Who found that pellet swim-up technique was the best for sperm preparation with lower rate of DNA fragmentation than baseline and direct swim-up procedures.

Moreover, in earlier study, stated that swim-up methods had higher rate of DNA fragmentation compared to other procedures [18]. In the current study, sperm concentration was significantly lower in direct swim-up than pellet swim-up technique. Found that mean concentration in swim-up procedure was lower than that with density gradient processing [18]. In the present study, no significant differences were reported between both techniques regarding other seminal fluid analysis parameters. Found that direct swim-up procedure was significantly better than density-gradient centrifugation followed by swim-up in term of improving the progressive motility, but, both techniques were not significantly different in other parameters, however, recommended the use of direct swim-up technique because it required less time and labor for sperm preparation.

Furthermore, found that direct swim-up was significantly reduced DNA fragmentation, but not significantly different than gradient sperm preparation method [19].

However, the conflicting results obtained in previous studies could be attributed to different factors, hence we suggested further studies with larger sample size and multiple centers taking into account comparison of other techniques to get good evidence and evaluation.

5. Conclusion
Direct swim-up sperm preparation technique was better than pellet swim-up technique in reduction of sperm DNA fragmentation. Both sperm preparation technique were not significantly different in all seminal fluid analysis parameters except concentration where it was higher in pellet than direct swim-up technique. However, further studies with larger samples and multiple centers which involve other preparation techniques are highly suggested.

6. References


**Table 1.** Baseline seminal fluid analysis parameters of the studied group (N=30)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD*</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>3.6</td>
<td>1.1</td>
<td>2 – 5.2</td>
</tr>
<tr>
<td>Liquefaction time (minutes)</td>
<td>22.7</td>
<td>8.1</td>
<td>10 – 30</td>
</tr>
<tr>
<td>Sperm Concentration (x 10^6/ml)</td>
<td>56.2</td>
<td>16.2</td>
<td>35 – 90</td>
</tr>
<tr>
<td>Progressive Motility (%)</td>
<td>42.1</td>
<td>17.0</td>
<td>5 – 65</td>
</tr>
<tr>
<td>Non progressive Motility (%)</td>
<td>15.5</td>
<td>9.0</td>
<td>5 – 40</td>
</tr>
<tr>
<td>Total Motility (%)</td>
<td>57.7</td>
<td>16.1</td>
<td>30 - 80</td>
</tr>
<tr>
<td>Immotile Sperm (%)</td>
<td>42.3</td>
<td>16.1</td>
<td>20 – 85</td>
</tr>
<tr>
<td>Abnormal sperm morphology (%)</td>
<td>89.7</td>
<td>13.7</td>
<td>40 - 97</td>
</tr>
</tbody>
</table>

All patients had gray and normal viscous seminal fluid

**Table 2.** Comparison of DNA fragmentation percent across different preparation technique and baseline levels

<table>
<thead>
<tr>
<th>DNA fragmentation percent</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Overall P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>22.3</td>
<td>5.8</td>
<td>6.0</td>
<td>54.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Pellet swim-up</td>
<td>27.3</td>
<td>7.9</td>
<td>4.0</td>
<td>45.0</td>
<td></td>
</tr>
<tr>
<td>Direct swim-up</td>
<td>14.0</td>
<td>9.6</td>
<td>2.0</td>
<td>42.0</td>
<td></td>
</tr>
</tbody>
</table>

**Post-hoc (LSD) multiple comparisons**

<table>
<thead>
<tr>
<th>Pair 1</th>
<th>Pair2</th>
<th>Mean Difference (Pair1-Pair2)</th>
<th>SE of difference</th>
<th>95% Confidence Interval of the Difference</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pellet swim-up</td>
<td>Baseline</td>
<td>5.00</td>
<td>1.87</td>
<td>1.390 - 8.540</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Table 3. Comparison of seminal fluid analysis parameters between both preparation techniques

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pellet swim-up Median (IQR)</th>
<th>Direct swim-up Median (IQR)</th>
<th>P. value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Concentration (%)</td>
<td>60.0 (50-87)</td>
<td>35.0 (25-50)</td>
<td>0.001</td>
</tr>
<tr>
<td>Progressive Motility (%)</td>
<td>65.0 (55-70)</td>
<td>60.0 (40-70)</td>
<td>0.789</td>
</tr>
<tr>
<td>Non progressive Motility (%)</td>
<td>5.0 (5 – 5)</td>
<td>4.0 (0-10)</td>
<td>0.935</td>
</tr>
<tr>
<td>Immotile Sperm (%)</td>
<td>30.0 (25-40)</td>
<td>35.0 (30-50)</td>
<td>0.796</td>
</tr>
<tr>
<td>Abnormal sperm morphology (%)</td>
<td>85.0 (85-92)</td>
<td>90.0 (87-91)</td>
<td>0.796</td>
</tr>
</tbody>
</table>

IQR: Inter-quartile range for median
*non parametric median test, Yates' Continuity Correction, Chi-Square, used in comparison

Figure (1): 1. fragmented DNA sperm (abnormal), 2. nonfragmented DNA sperm (normal).