A Comparison Between the Effects of Global Sperm Washing© and FertiCult Flushing

Media on Certain Sperm Function Parameters of Asthenozoospermic Men

On Journal of Biotechnology Research

February 2017

Vol. 11 No.2

Abstract

The World Health Organization (WHO) and many studies considered the infertility as a disease and so many couples complaining from unsuccessful assisted reproductive technologies procedures to overcome their problem. One of the reasons of this dilemma is the sperm preparation method when no optimum result obtained even by using any of media found globally. However, Global sperm washing©, and FertiCult flushing© media were proved their capability to obtain good results of certain sperm function parameters. Nevertheless, the studies that compare between these media were rare. Therefore, this study aimed to compare between Global sperm washing medium©, FertiCult flushing™ media that used for sperm washing before using the partner sperm for ART procedure. After detecting asthenozoospermia in sixty semen samples, they were divided into two groups according to medium used for sperm activation in vitroGlobal sperm washing medium© (n=31) and FertiCult flushing medium™(n=29) groups. The semen analysis was done after 3-5 days of abstinence as recommended by the manual of WHO (1999). Certain sperm function parameters were recorded. Semen fluid samples were treated with sperm activation media (Global sperm washing medium and FertiCult flushing mediumTM) by using direct swim-up technique for in vitro sperm activation test. A significant (P<0.05) improvement was noticed between the two media regarding active sperm motility grades A and B when using FertiCult flushing medium™ compared to Global sperm washing medium©. Whereas no significant (P>0.05) differences were detected between the two media regarding sperm motility grades C and D. There was no significant (P>0.05) differences in morphologically normal sperm following in vitro activation by using the two media. It is concluded that FertiCult flushing medium™ was better than Global sperm washing medium© in improving active sperm motility of asthenozoospermic men which can be utilized in future for successful of assisted reproduction.

Key words: Global sperm washing medium®, FertiCult flushing medium™, asthenozoospermia

Saad S. Al-Dujaily

Khalid S. Al-Azzawi

Zena Muzher Hussein *

Ban T. Al-An**

Biotechnology Research Center / Al-Nahrain University

Forensic DNA Center Research and Training / Al-Nahrain University

High Institute for Infertility Diagnosis and ART/ Al-Nahrain University

* Zaidi Health Foundation

** Muhsin Health Foundation

E-mail: aldujaily.saad@brc.nahrainuniv.edu.iq.
**Introduction**

Many people assume that infertility is a "man or woman's "problem, but many cases of infertility are a result of a man factor too. Male problems may be a contributing factor in 30 to 50% of couples suffering infertility [1]. There are many factors causing male infertility. The treatment of infertility has undergone phenomenal development and become a highly specialized field involving a multitude of interventions known collectively as assisted reproductive techniques [2]. The successful rate and pregnancy rate of artificial insemination in husband (AIH) varies considerably, and researchers do their best on the stand to base the causes of low success rate of AIH to find the best procedures that improve male fertility, which through the semen must be present in sperm motile, normal morphology, and be able to penetrate an ovum [3].

Sperm preparation and washing are usually performed by different media such as Sage®, In Vitro life®, Global®, FertiCult™, MediCult® and others to wash any semen sample for intrauterine insemination (IUI) and other assisted reproductive technologies (ARTs) procedures. Sperm washing achieves two very important goals. First, it removes most of the seminal plasma from the semen that would otherwise not react well if directly inseminated into the uterus. Second, it concentrates the sperm density into a small volume that is suitable for any assisted reproduction technologies. In most cases, sperm washing may also enhance the forward progression of the sperm or the percent motility [4]. This study was designed to compare between the effects of two cultures media to improve sperm function parameters for sperm preparation and in vitro activation of patient’s semen samples.

**Materials and Methods**

One hundred semen samples were collected from men consulting in Infertility Unit at the Biotechnology Research Center and High Institute of Infertility diagnosis & ART at Al Nahrain University for in vitro Sperm Activation Test (SAT) through the period from September 2015 to March 2016. Following seminal fluid analysis the asthenozoospermic samples were divided into two groups according to culture medium used for in vitro activation, Global sperm washing medium® (n=31) and FertiCult flushing medium™ (n=29) groups. The semen analysis was accomplished after 3-5 days of abstinence as recommended by WHO guidelines [5]. Certain sperm function parameters were recorded namely; sperm concentration million/ml, sperm motility (%) and morphologically normal sperm (%) [5]. All semen samples were activated with sperm preparation media (Global sperm washing® and FertiCult flushing™ media) by using direct swim-up technique for sperm activation test.

The direct swim-up technique was performed either by layering culture medium over the liquefied semen or by layering liquefied semen under the culture medium [6,7]. One ml of liquefied semen samples layering under 1ml of the culture media (Global® or FertiCultTM) and evaluated after 30 minutes of incubation at 37°C.

**Statistical analysis**

Crude data were collected and analyzed using SPSS (Statistical program for social studies, Version 17, Illinois, USA) for descriptive statistics involving means and standard deviation of mean (SD). Paired t- test was used to detect the significant differences of before and after activation between the two groups. P value less than (0.05) was statistically considered as significant [8].

**Results and Discussion**

In Table (1), A significant (P<0.05) decrease was found in sperm concentration (million/ml) of asthenozoospermic men after activation in vitro by Global sperm washing® medium compared to before activation. There was a significant (P<0.05) improvement in active sperm motility grade A after activation (16.87 ± 11.02) compared to before activation (4.70 ± 6.65). The sperm motility grade B was significantly (P<0.05) increased when the semen sample of asthenozoospermic men was activated in vitro by Global® medium (47.48 ± 20.32) compared to before activation (27.41 ± 13.12). There was a significant (P<0.05)
decrement in sperm motility grades C and D following in vitro activation compared to before activation. The percentage of morphologically normal sperm (MNS) was significantly (P<0.05) higher after activation (55.64±11.16) than that of before activation (37.87 ± 8.87).

### Table (1): Certain sperm function parameters of asthenozoospermic men before and after in vitro activation by Global sperm washing<sup>®</sup> medium for 30 minutes incubation using direct swim-up technique

<table>
<thead>
<tr>
<th>Sperm function parameters</th>
<th>Before Activation</th>
<th>After Activation</th>
<th>P – Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Concentration (million/ml)</td>
<td>48.16 ± 23.16</td>
<td>22.48 ± 11.95</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Grade A (%)</td>
<td>4.70 ± 6.65</td>
<td>16.87 ± 11.02</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Grade B (%)</td>
<td>27.41 ± 13.12</td>
<td>47.48 ± 20.32</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Grade C (%)</td>
<td>24.87 ± 7.34</td>
<td>20 ± 7.52</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Grade D (%)</td>
<td>42.67 ± 18.82</td>
<td>13.87 ± 15.90</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Morphologically normal sperm (%)</td>
<td>37.87 ± 8.87</td>
<td>55.64 ± 11.16</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Values were expressed as Mean ± SD.

### Table (2): Certain sperm function parameters of asthenozoospermic men before and after in vitro activation by FertiCult flushing medium™ for 30 minutes incubation using direct swim-up technique

<table>
<thead>
<tr>
<th>Certain Sperm Function Parameters</th>
<th>In vitro activation by FertiCult medium</th>
<th>P – Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Concentration (million/ml)</td>
<td>51.76 ± 25.48</td>
<td>29.53 ± 15.47</td>
</tr>
<tr>
<td>Grade A (%)</td>
<td>8.07 ± 9.26</td>
<td>33.07 ± 13.49</td>
</tr>
<tr>
<td>Grade B (%)</td>
<td>22.73 ± 12.28</td>
<td>33.46 ± 11.80</td>
</tr>
<tr>
<td>Grade C (%)</td>
<td>30.96 ± 10.63</td>
<td>21.15 ± 9.42</td>
</tr>
<tr>
<td>Grade D (%)</td>
<td>38.46 ± 19.90</td>
<td>13.26 ± 9.92</td>
</tr>
<tr>
<td>Morphologically normal sperm (%)</td>
<td>40.11 ± 7.17</td>
<td>58.07 ± 8.0</td>
</tr>
</tbody>
</table>

Values were expressed as Mean ± SD.

Patients No. = 29

Table (2), showed that there was a significant (P<0.05) decrease in sperm concentration (million/ml) of asthenozoospermic men following activation in vitro compared to before activation. A significant (P<0.05) increase was noticed in active sperm motility grade A after activation (33.07 ± 13.49) compared to before activation (8.07 ± 9.26). The sperm motility grade B was significantly (P<0.05) improved following in vitro activation of the semen sample of asthenozoospermic men (33.46 ± 11.80) compared to before activation (22.73 ± 12.28). There was a significant (P<0.05) reduction in sperm motility grades C and D after in vitro activation compared to before activation. The percentage of morphologically normal sperm (MNS) was significantly (P<0.05) increased after activation (58.07 ± 8.0) compared to before activation (40.11 ± 7.17).

The comparison between the effects of Global<sup>®</sup> and FertiCult<sup>®</sup> media was shown in Table (3). There was a significant (P<0.05) decrement in sperm concentration after using Global<sup>®</sup> medium (22.48 ± 11.05) compared to FertiCult<sup>®</sup> medium (29.53 ± 15.47). A significant (P<0.05) differences was found between the two media regarding active sperm motility grade A (Global medium=16.87 ± 11.02 and FertiCult=33.07 ± 13.49). The Sperm motility grade B was improved significantly (P<0.05) when using Global<sup>®</sup> medium compared to FertiCult<sup>®</sup> Medium. Whereas no significant (P>0.05) differences were observed between the two media regarding sperm motility grades C and D. There were no significant (P>0.05) differences in MNS following in vitro activation by using the two media (Global<sup>®</sup> medium=55.64 ± 11.16 and FertiCult<sup>®</sup> medium= 58.07 ± 8.0).
The significant reduction in sperm concentration after activation in vitro was observed in both cultures media, may be resulted from inability of the dead and poor grade activity sperm to move up and travel from the lower layer to the upper layer of culture medium. Similar results were recorded by other studies [9,10]. There was an improvement in the percentage of sperm motility (grades A & B) and percentage of morphologically normal sperm after activation. This finding may be due to the fast movement of normal spermatozoa from the lower layer of culture medium as both media constitute of Ca²⁺ and energy supplements to enhance the motility in addition to the impact of some seminal plasma components like leukocytes, and other decapacitation factors which in turn keeping the sperm out of stress factor and reactive oxygen species production that responsible for impaired sperm motility and DNA damage [11,12,13]. This in turn leads to significant reduction of abnormal, non – progressive and immotile sperm (grades C & D) respectively [14,15].

The comparison between the effects of two media showed significant differences in certain sperm function parameters. An increment in sperm concentration and grade A active sperm motility was noticed by using FertiCult medium™ for washing and activating spermatozoa in vitro, while a level of elevation in grade B active sperm motility was noticed when using Global sperm washing® medium for in vitro sperm activation. This in turn may be related to the composition of FertiCult flushing medium™, which contains combination of HEPES, bicarbonate, physiologic salts, glucose, lactate and human serum albumin (4.00g/liter) [16]. HEPES alone in the media as a buffering system increased the buffering capacity and the stability of the pH in the range of (7.2 to 7.6). This allows the media to be better resist fluctuations in the pH resulting from changes of cellular metabolism therefore no CO₂ incubation is required to avoid reduction of pH less than 7.0 [17,18]. Thus, it is concluded from current study that FertiCult sperm washing medium™ has better effect than Global sperm washing® medium on certain sperm function parameters of asthenozoospermic men.

**References**


