The Effect of H$_2$O$_2$ and Some Antioxidants on Human Sperm Parameters \textit{in vitro}

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Abstract

This study was performed to determine the effect of H$_2$O$_2$ addition to the semen specimens to stimulate the formation of free radicals, and treated with antioxidants. This study involved ten semen specimens collected from fertile men. Seminal fluid analysis was performed to estimation sperm parameters and Malondialdehyde (MDA) concentration. Also seminal fluid analysis and MDA level was determination after adding of H$_2$O$_2$ and then antioxidants (VE, VC, VE+VC, glutathione and seminal plasma).

The results revealed a significant decrease (p<0.05) in sperm motility percent, grade activity and sperm viability percent, and significant increase (p<0.05) in MDA concentration by addition 0.1% of H$_2$O$_2$ to semen specimens compared to those values with normal saline. The addition of antioxidants (0.6mg/ml VE, 0.4mg/ml Vc + 0.6mg/ml VE, 0.4rng/ml glutathione and seminal plasma) to the semen specimens contain 0.1% H$_2$O$_2$ caused significant increase(p<0.05) in sperm motility percent, grade activity and sperm viability percent , and significant decrease (p<0.05) in MDA level compared to those values in semen samples contain 0.1% H202 alone. While VC caused insignificant differences (p>0.05) in all sperm parameters and MDA concentration.

Introduction

The spermatozoa produced few amount of Reactive Oxygen Species (ROS) in specific physiological condition which are necessary for capacitation, acrosome...
reaction and fertilization (Griveau and Le Lannou, 1997). While the large amount of 
ROS produced by immature sperm and leukocytes caused harmful effects on normal 
spennatozooa as a result of lipid sperm activity (Agarwal et al., 2003). The seminal 
fluid contains molecules with high molecular weight and low molecular weights 
called Antioxidants or Scavengers system protect the seminal fluid from the ROS 
(Pasqualotto et al., 2000).

There are a balance between the ROS production and defense mechanism of 
Antioxidants in male reproductive tract, although may be increased the production of 
ROS (Sikka, 2004), or the reduction of Antioxidants should be caused to Oxidative 
Stress (Momen et al., 1999). The Oxidative Stress considered a very important factor 
in male infertility, because the increasing of Oxidative Stress related negatively with 
normal sperm parameters (Agarwal and Said, 2003).

The goals of this study are showed the relationship between ROS and sperm 
parameters, and trial to repair the defects in sperm parameters result from the ROS by 
Antioxidants addition.

Materials and Methods

Ten semen specimens were collected by masturbation from fertile men after 3-5 
days of sexual abstinence. The specimens were allowed to liquefy at 37°C.

The semen specimens were divided into equal seven splits and adding normal 
saline to the 1st split, 0.1% of H2O2 to the 2nd split, 0.1% H2O2+0.4mg/ml VC to the 
3rd split, 0.1% H2O2+0.6mg/ml VE to the 4th split, 0.1% H2O2+ 0.4mg/ml VC+ 
0.6mg/ml VE to the 5th split, 0.1% H2O2+ 0.4mg/ml glutathione to the 6th split, and 
0.1% H2O2+ seminal plasma to the 7th split.

All splits were incubated at 37°C for 15 minutes. Seminal fluid analysis was 
performed after the incubation, and then each split was centrifuged at 3000 rpm for 15 
minutes to obtain the seminal plasma. MDA concentration was estimation due to the 
technique recorded by Muslih et al., (2002).

The results were statistically analyzed by using F-test and Least Significant 
Difference (LSD) to comparison between the groups (Sorli, 1995).

Results

The addition of 0.1% H2O2 to the semen specimens of fertile men caused 
significant increase (p<0.05) in MDA concentration and significant decrease (p<0.05) 
in sperm motility percent, grade activity, and sperm viability percent compared to 
control (semen specimens with normal saline). While there are insignificant 
differences (p>0.05) in sperm concentration, normal sperm morphology percent and 
leukocytes concentration (Table-1-).

The treated of semen specimens contain 0.1% H2O2 with several antioxidants 
causd significant improvement in sperm quality. The addition separately of 0.6mg/ml of 
VE, 0.4mg/ml of VC + 0.6mg/ml of VE, 0.4mg/ml of glutathione, and seminal 
plasma, caused significant decrease (p<0.05) in MDA concentration and significant 
increase (p<0.05) in sperm motility percent, grade activity, and sperm viability 
percent compared to those values in semen specimens with 0.1% H2O2 only. While 
there are insignificant differences (p>0.05) in sperm concentration, normal sperm 
morphology percent and leukocytes concentration. Also there are insignificant 
differences (p>0.05) in MDA concentration and all sperm parameters mentioned 
above by using 0.4mg/ml of Vc (Table-1-).
Discussion

The results of this study showed that adding of H2O2 to the semen specimens of fertile men caused significant increase of MDA concentration compared to the control, this result may be referred to the increasing of ROS due to adding of H2O2 to the semen specimens caused imbalance between ROS and antioxidants resulting of oxidative stress. The oxidative stress caused increased of lipid peroxidation in seminal fluid and spermatozoa result to increased MDA concentration as end product of lipid peroxidation, this result agreement with Saonka and Kurpisz (2004) study, which are revealed that high concentration of H2O2 activated the lipid peroxidation in semen. The incubation of human spermatozoa with H2O2 caused reduction of Glutathione Peroxidase (GPX) enzyme and Superoxide Dismutase (SOD) enzyme (Griveau et al., 1995).

Table 1 - Sperm parameters and Malondialdehyde (MDA) concentration after H2O2 addition alone and with some antioxidants to the semen Specimens of fertile men.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Saline</th>
<th>H2O2 0.1%</th>
<th>Antioxidants + H2O2 (0.1%)</th>
<th>Glutathione (0.4mg/ml)</th>
<th>Seminar plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>VE (0.6mg/ml)</td>
<td>Vc (0.4 mg/ml)</td>
<td>VE+Vc 0.6+0.4 mg/ml</td>
</tr>
<tr>
<td>Sperm concentration (X106/ml)</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>Sperm motility Percent</td>
<td>75.30±4.42</td>
<td>75.30±4.38</td>
<td>75.20±4.37</td>
<td>74.90±4.39</td>
<td>75.00±5.10</td>
</tr>
<tr>
<td>Sperm grade Activity</td>
<td>58.20±4.19</td>
<td>31.30±3.49</td>
<td>41.9±2.49</td>
<td>32.90±3.18</td>
<td>43.90±2.79</td>
</tr>
<tr>
<td>Sperm viability percent</td>
<td>3.21±0.18</td>
<td>1.74±0.17</td>
<td>2.24±0.13</td>
<td>1.73±0.17</td>
<td>2.63±0.16</td>
</tr>
<tr>
<td>Normal sperm morphology percent</td>
<td>85.10±2.97</td>
<td>56.90±4.11</td>
<td>70.50±3.19</td>
<td>59.20±3.18</td>
<td>75.50±2.92</td>
</tr>
<tr>
<td>Leukocytes concentration</td>
<td>68.30±5.31</td>
<td>67.70±5.08</td>
<td>68.30±5.38</td>
<td>67.80±5.30</td>
<td>69.10±5.30</td>
</tr>
<tr>
<td>MDA concentration</td>
<td>0.70±0.26</td>
<td>0.70±0.26</td>
<td>0.50±0.22</td>
<td>0.60±0.26</td>
<td>0.50±0.16</td>
</tr>
</tbody>
</table>

Number of specimens 10
Different letters indicate to significance (p<0.05)

The present study revealed that semen quality (sperm motility percent, grade activity, and sperm viability percent) were decreased when incubation the semen samples with 0.1% of H2O2. This result may be refer to the role of H2O2 in peroxidation of lipid composed the plasma membrane of spermatozoa result to loss the function of plasma membrane deal with the control the ions transportation result to the defect of sperm motility.

The addition of VE caused significant improvement in sperm parameters and MAD level, these results agreement with other study (Donnelly et al., 1999), revealed that VE addition to the media used for in vitro sperm activation caused decrease the ROS production which are stimulated by addition of H2O2 to the media in Percol gradient technique. Vitamin E can soluble in the lipid, so that it can pass through the plasma membrane of the spermatozoa, and then inhibit the harmful effect of the ROS.
and maintain the nature of spermatozoa, plasma membrane and prevent MDA formation result to increase the sperm motility and spermatozoal efficiency to penetrate the ovum layers.

Agarwal (2004) revealed that the mixing of antioxidants is very benefit for treatment of infertile men. The treatment of fertile patients with VE, VC, and glutathione for two months caused significant improvement in sperm parameters and MDA level. The glutathione posses a protective role for the sperms versus the increasing of lipid peroxidation stimulated by Polynorphonuclear Leukocytes (PML) (Baker et al., 1996).

The seminal plasma contains many enzymatic antioxidants like SOD and GPX, in addition to non-enzymatic antioxidants like VE, VC, glutathione, pyruvate and carnitine (Saleh and Agarwal, 2002). The antioxidants in seminal fluid act to remove the free radicals and protect the spermatozoa from the risk of oxidative stress increasing (Smith et al., 1996). So that the decreasing of MDA level and improvement of sperm parameters with addition of seminal plasma to the semen specimens in our study may be refer to the protective role of antioxidants present in the seminal plasma to reduce the ROS level. In addition to that, the seminal plasma contain several energy sources like fructose and pyruvate which are necessary to maintain the sperm motility and viability.

It was concluded that is very important to adding the antioxidants to the media used for in vitro sperm activation in the cases in which the semen specimens contain high level of ROS.

References: