Study into the iron content of seminal plasma in normal and infertile subjects

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The iron content in seminal plasma of normal (n19), oligozoospermic (n11), azoospermic (n12), oligoasthenozoospermic (n19), and asthenozoospermic (n17) subjects was estimated by using atomic absorption spectrophotometer. The concentration of iron in normal seminal plasma varied from 265 to 365 μg%. The source of iron in seminal plasma seems to be the adnexal glands and not spermatozoa, as azoospermic semen also contained it. A statistically highly significant difference was seen when normal was compared with azoospermia and with asthenozoospermia. The necessary average wastage of iron through semen is calculated as 2.52 μg/day. This value is highly variable according to the seminal volume and frequency of ejaculation.

KEY WORDS: Normal, Pathological, Seminal plasma Iron, Fertility, Infertility

Accepted: September 7, 2011

INTRODUCTION

An optimum composition of seminal plasma is essential for the viability and function of spermatozoa. The roles played by various electrolytes and metals in semen have been studied for four decades (1-2). The presence of certain elements believed to be non-existent has been found with the help of highly sophisticated methods (3-8). Various workers have estimated certain electrolytes and metals in semen or in seminal plasma and explained the probable mechanisms involved in the activity of the sperm (2,8). Acceptability of those mechanisms could not be confirmed because of the want of detailed study on the semen composition. Iron is one of such elements. In this study therefore, an attempt has been made to find its concentration and postulate its role on the viability of spermatozoa, if any.

MATERIALS AND METHODS

Healthy subjects belonging to the 21-35-year age group were included in the study. Normal samples were from proven fertile subjects and also from subjects who reported to infertility clinics, but their semen was normal under routine examination. The subjects who were diagnosed as infertile were otherwise healthy by clinical examination. The samples were collected by masturbation into sterile, wide mouthed glass bottles. They were examined routinely, under the microscope, within 20 minutes after collection.
RESULTS

Results of the present study are given in Table I.

DISCUSSION

For almost half a century several groups of workers have been trying to establish the level and function of various electrolytes and metals in normal human semen and its possible involvement in seminal pathology (10-22). The importances of few of these elements were discussed in lines of conception and contraception (23-29). The continuing studies revealed the details of otherwise unknown elements. Iron was one of such elements. Iron was present in erythrocytes as metalloporphyrins and functions in oxygen carriage, and also found in all body cells in the form of enzymes concerned with biological oxidation. The presence of iron in spermatozoa as well as in seminal plasma is known (8,30), although its role in the germ cells may be considered of a similar nature as in cells elsewhere, its function in seminal plasma is a matter of conjuncture. The total iron in the blood plasma is $100 \text{ mg}\%$ (31) while that in seminal plasma was found as $315 \text{ mg}\%$ in our study (Tab. I).

In whole semen, the concentration of iron was reported as $510 \text{ mg}\%$ (32). This high concentration of iron in seminal fluid must be accounted for by an active secretion from the genital tract. Its presence in azoospermia corroborates this, and the amount is $296.25 \text{ mg}\%$, which, therefore, confirms the contribution of it from the genital tract. The transferrin, iron-binding protein, has also decreased in azoospermic seminal plasma (33). The genital tract, particularly the prostate, having been attributed as a source of another metal, namely, zinc (15,16), a line of thought in the same direction for iron, is not out of place. Therefore, to account for the higher concentration ($315 \text{ mg}\%$) in normal seminal fluid compared to that in azoospermia, we should search for an additional source. The presence of iron in the spermatozoa is established (30); it will be tempting to find the additional source in them. The release of zinc from the sperm was reported earlier (12). This may be true for iron also. Moreover, it was reported by Hafez and Prasad (34) that about 50% of the germ cells were disintegrated in the anterior region of the epididymis. This obviously releases iron, which accounts for the high iron content in the normal seminal plasma.

It is not known if the iron in seminal plasma is just an

They were classified and placed under one of the groups given below (9).

<table>
<thead>
<tr>
<th>Group</th>
<th>Sperm count in Percentage of millions/mL motile sperms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&gt;40 &gt;60</td>
</tr>
<tr>
<td>Oligozoospermia</td>
<td>&lt;40 &gt;60</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>Sperm was not present in deposit even after centrifugation (10000 rpm x 10).</td>
</tr>
<tr>
<td>Oligoasthenozoospermia</td>
<td>&lt;40 &lt;60</td>
</tr>
<tr>
<td>Asthenozoospermia</td>
<td>&gt;40 &lt;60</td>
</tr>
</tbody>
</table>

Seminal plasma was separated from samples by centrifugation (2000 rpm x 10) and kept at -20°C till further processing was done for iron analysis. All glassware used in this study was immersed in 6 M nitric acid overnight, and passed through water, distilled water, deionized water, distilled and redistilled deionized water before use, to exclude metallic contamination. Also for the same purpose, nowhere during the whole procedure, samples, chemicals and glassware were brought into contact with any metal.

Iron content was estimated by using atomic absorption spectrophotometer of Perkin and Elmer (Model 305), at Physical Research Laboratory, Ahmedabad. Seminal plasma 0.5 mL was diluted to 5 mL with 0.1% lanthanum solution. Lanthanum solution was prepared from analytical grade of lanthanum oxide (La 2O3) of Loba Chemicals limited, Bombay. Standard solutions were prepared from iron powder (Analytical grade, British Drug House, Bombay) in the range of 0.1, 0.2, 0.5 and 1 microgram per mL, after dissolving with minimum quantity of nitric acid (Analar, British Drug House, Bombay). Lanthanum solution (0.1%) was used for dilution. Blank was prepared from 1.0 mL of double distilled deionized water diluted to 10 mL with 0.1% lanthanum solution.

A mixture of acetylene gas and air was preferred for the production of flame and a hollow cathode lamp for iron was used in the instrument for estimation. Wavelength of light was kept at 248.3 µm and the slit opening was set at 0.2 nm. Each sample was injected for 10 seconds duration and the concentration of iron in it was recorded in the form of graph, automatically, and from this the concentration of iron in 100 mL was calculated.
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Excretory product or it has a role to play in the activity of the sperms and therefore in fertility. The active secretion of the iron from the genital tract could not be with any purpose. Our observations presented in Table I showed that in oligoasthenozoospermia and asthenozoospermia the mean concentration of iron was less than normal. The difference between normal concentration and asthenozoospermia was statistically highly significant, proving the importance of iron for normal motility; Gaffuri et al. (32) also showed the decreased level of iron in oligoasthenozoospermia. However, a linear relationship between iron in seminal plasma and sperm motility was not found in the present study.

On the contrary, three isolated cases in the astheno groups (Tab. I) show the level of iron in seminal plasma as high as 440, 665 and 690 mg%, and in all these three cases motility was less than 10%. The higher concentration of iron was likely to be responsible for the reduced motility. It was well established from the experimental studies of Loewit (35-36) that the increased iron in the surrounding medium made the spermatozoa immotile. Findings by Gaffuri et al. (32) also supported this observation of ours.

Therefore, it could be concluded that an optimum physiological range for iron is necessary for a normal sperm motility, like sodium, potassium (28), and calcium (37). Any alteration in iron concentration in seminal plasma on either side of this range is likely to affect motility adversely; a greater number of cases should be studied before a definite conclusion is passed.

Lastly, one should not fail to take into account the loss of iron from the body through ejaculations. The literature mentioned the source of excretion of iron as through urine, sweat and desquamation of intestinal epithelium in general and through menses in women (38). The necessary wastage of iron through semen can be calculated as 2.52 mg/day, if the average of 3 ejaculations per week is 2 mL. This quantity, however, is highly variable according to the volume of ejaculate and its frequency.

Disclaimers

The authors have no proprietary interest with regard to this article.
Written informed consent was obtained from the patients before clinical examination.

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**TABLE I - CONCENTRATION OF IRON IN NORMAL AND PATHOLOGIC GROUPS AND THE RELEVANT RESULTS WHEN COMPARED WITH EACH OTHER**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Average count in Mill/mL</th>
<th>Average Motility (%)</th>
<th>Range of iron (µg%)</th>
<th>Mean iron (µg %)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normozoospermia</td>
<td>19</td>
<td>129.63</td>
<td>67.63</td>
<td>265-365</td>
<td>315.00</td>
<td>9.36</td>
</tr>
<tr>
<td>2. Oligozoospermia</td>
<td>11</td>
<td>14.50</td>
<td>60.00</td>
<td>310-365</td>
<td>323.18</td>
<td>5.62</td>
</tr>
<tr>
<td>3. Azoospermia</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>240-340</td>
<td>296.25</td>
<td>7.61</td>
</tr>
<tr>
<td>4. Oligoasthenozoosperm</td>
<td>18</td>
<td>10.03</td>
<td>33.33</td>
<td>240-390(a)</td>
<td>308.06</td>
<td>9.60</td>
</tr>
<tr>
<td>5. Asthenozoospermia</td>
<td>15</td>
<td>58.68</td>
<td>41.33</td>
<td>265-340(b)</td>
<td>293.30</td>
<td>5.54</td>
</tr>
</tbody>
</table>

Comparison

<table>
<thead>
<tr>
<th>Comparison</th>
<th>&quot;P&quot; Value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 v/s 2</td>
<td>&gt;0.1*</td>
<td>-</td>
</tr>
<tr>
<td>1 v/s 3</td>
<td>&lt;0.001</td>
<td>**</td>
</tr>
<tr>
<td>1 v/s 4</td>
<td>&gt;0.1</td>
<td>-</td>
</tr>
<tr>
<td>1 v/s 5</td>
<td>&lt;0.001</td>
<td>**</td>
</tr>
</tbody>
</table>

n=number of cases
(a) One case of 440 and
(b) Two cases of 665 and 690 µg % were observed

*student 't' test-Non significant
**highly significant
REFERENCES