THE ROLE OF HYPO-OSMOTIC SWELLING TEST IN RECURRENT ABORTIONS

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Abstract: The role of male factors in abortions has not been studied extensively. We undertook this study to determine if there was any relationship between hypo-osmotic swelling test score and recurrent abortions. This was a cross-sectional case control study conducted at the semen analysis laboratory at a tertiary level referral centre.

The male partners of 25 patients who had three or more first trimester abortions of unknown etiology were chosen as cases. Controls were 26 unmarried volunteers with unknown fertility potential to represent the entire population.

The conventional seminal parameters were studied according to WHO guidelines. The HOS test score were obtained by the method advocated by Jayendran et al. There were no statistically significant differences in mean sperm count, percentage motility and morphology among the cases and controls. We found a low hypo-osmotic swelling test score among the cases [55.7±1.197 (SEM)] than the controls [69.3±1.143 (SEM)] which was statistically significant [P<0.001].

The normal spermatozoal membrane is the prerequisite for the specialized cell-to-cell communications and cell-to-cell binding. In spite of apparently normal semen analysis, subtle membrane defects in the spermatozoa, which could be the cause of defective membrane functions in the embryo effecting miscarriages, can be elucidated by the hypo-osmotic swelling test.

Key words: hypo-osmotic swelling test, sperm function test, recurrent abortions

INTRODUCTION

Recurrent abortions have a multifactorial etiology (1). Spontaneous recurrent abortions is defined as three or more consecutive abortions (2, 3). Abortions have an incidence of 10% (2). Various etiological factors have been demonstrated...
like genetic [50–60%] endocrinological [10–
15%], chorioamniotic separation [5–10%],
anatomical defects [15%], infection [15%],
immunological [3–5%], nutritional and
unknown reasons including sperm factors
[3%] (4). Yet in spite of advanced
investigations the etiology remains
idiopathic (2). The role of male factors in
recurrent abortions remains largely
unknown barring a few cytogenetic
abnormalities (2). Good quality sperms yield
good quality fetus. Any deviation from the
normal or sub-standard sperms will affect
its motility, penetration and decondensation,
ultimately hampering the conception,
implantation and nidation, as one of the
limiting steps in the success of fertility is
the quality of the fertilized ovum (2). Sperm
morphology abnormalities do not seem to
be involved in recurrent abortions (5, 6) and
the functional parameters have not been
studied extensively. Hypo-osmotic swelling
test is the qualitative indicator of the sperm
fertility potential routinely carried out in
most of the Advanced Reproductive
Technology Centers (7). It assesses the
functional integrity of the spermatozoal
membrane (8). The hypo-osmotic swelling
test was claimed to assess an independent
functional characteristic of human
spermatozoa bearing relevance to their
fertilizing capacity (9). Very few researchers
have studied an association between altered
hypo-osmotic swelling test and recurrent
abortions (10, 11, 12). Hence we have tried to find out
any association between altered hypo-
osmotic swelling test score and recurrent
abortions.
0.1 ml of the semen sample with 1 ml of hypo-osmotic solution. The hypo-osmotic solution was prepared by mixing the 1.351 g of fructose with 0.735 g of sodium citrate in 100 ml of distilled water. The sample was incubated at 37° C for half an hour. A slide was prepared by taking one drop of incubated solution and mounting it with a cover slip. This was observed immediately under high power microscope [400 X magnification] (8). The hypo-osmotic swelling test positive sperms i.e. sperms with curled tails were counted in different high power fields, and thus the percentage of hypo-osmotic swelling test positive sperms was calculated. The percentage of initial number of sperms with curled tail was subtracted from the later and thus the final hypo-osmotic swelling test score was obtained.

RESULTS

Of the 30 volunteers, who were considered in the study as the control group, 2 were azoospermic, 5 were oligozoospermic, and 3 had reduced motility. The 2 azoospermics were not included in the study as also 2 with previous history of tuberculosis, and thus remaining 26 subjects formed the control group. The average age of the control group was 24 years. This was significantly lower than the cases, as the group was taken from the unmarried healthy volunteers. Male partners of 25 females with history of recurrent miscarriage were included in the study as cases.

We observed a higher mean sperm density in the control group, but statistically the difference was not significant [P<0.05]. All other conventional semen parameters like percentage motility, and percentage normal sperm morphology were statistically similar in cases and controls.

The mean hypo-osmotic swelling test score for control group was 69.3 ± 1.1432 [standard error of mean] while that for cases it was 55.7 ± 1.1977 [standard error of mean]. This difference of mean between these two groups was statistically highly significant [student ‘t’ test; P<0.001] (see Table I).

### TABLE I: Comparison of age and different seminal parameters in controls and cases.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>Cases</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N = 26</td>
<td>N = 25</td>
</tr>
<tr>
<td>Age</td>
<td>24±0.642</td>
<td>29.2±0.725</td>
</tr>
<tr>
<td>Sperm count (millions/ml)</td>
<td>66.9±8.092</td>
<td>62.3±7.153</td>
</tr>
<tr>
<td>Percentage motility</td>
<td>70±2.053</td>
<td>67.1±2.212</td>
</tr>
<tr>
<td>Percentage of sperms with normal morphology</td>
<td>63.8±2.603</td>
<td>61±1.791</td>
</tr>
<tr>
<td>Hypo-osmotic swelling test score in percentage</td>
<td>69.3±1.143</td>
<td>55.7±1.197</td>
</tr>
</tbody>
</table>
DISCUSSION

Even up to the recent times the semen analysis was done in the same age old fashion, and it is only from the last few years that the steps have been taken adopting advanced techniques. The role of male factors in recurrent abortion remains largely unexplored as only a few studies have been conducted on the subject (10, 11, 12). The spermatozoal factors account for about 3% of all recurrent abortions (2). It has been shown in various studies that inspite of apparently normal semen parameters, the male factor can be responsible for early pregnancy loss (10, 12). This may be a consequence of functional spermatozoal abnormalities resulting in sub standard embryo (12).

Although, minor chromosomal abnormalities do not result in gross morphological defects of the sperm (14), the functional defects in the spermatozoa may be a result of such chromosomal abnormalities causing subtle alteration in the membrane constitution. These defects can be detected by hypo-osmotic swelling test, as it is the test of functional integrity of the cell membrane.

The hypo-osmotic swelling test can detect minute alterations in the constitution of the sperm membrane. When the cell wall is functionally intact it acts as the semipermeable membrane. In the hypo-osmotic swelling test, as the fluid in the vicinity of the sperm is hypo-osmotic in relation to the intracellular fluid, there occurs entry of the hypo-osmotic solution inside the sperm membrane causing the tail of the sperm to swell. This swollen tail curls around itself showing a typical “curling.” The curling is thus the sequelae to the swollen tail. Curling indicates that the sperm membrane is functionally intact. This test was first advocated by Jayendran et. al (8). And now a days is considered as one of the integral parts of the infertility protocol in most of the Advanced Reproductive Technology Centers. Hypo-osmotic swelling test is proving to be a pioneer in the armamentarium against male infertility and in future will be indelible in selecting the sperms for ICSI [Intra-cytoplasmic sperm injection] and IUI [Intrauterine insemination] (15, 16). In our study we observed a significantly low hypo-osmotic swelling test scores in the male partners of females with history of recurrent miscarriage.

Both the groups were comparable except for the age. The mean age for the control group was significantly lower, as the controls were the bachelor volunteers, but there is no association between hypo-osmotic swelling test score and age of the subject (9, 17, 18). The normal spermatozoal membrane is the prerequisite for the specialized cell-to-cell communications and cell-to-cell binding. Studies have shown that cell-to-cell binding precedes the penetration of the ovum by the sperm (19). Thus any subtle defects in the membrane will not only hamper the process of fertilization, but also will be responsible for the subsequent abnormal membrane function in the embryo. The abnormal membrane function of the embryo may result in abnormal implantation, thus predisposing to early pregnancy complications (12).

Few studies have shown that in some animals (20) and human (21) paternally derived proteins are expressed in the embryo at the preimplantation stage indicating a role of male factor in.
implantation. The same minor cytogenetic abnormalities, which result in sperm membrane defects, may be responsible for abnormal expression of these paternally derived proteins.

REFERENCES