**INTRODUCTION**

Varicocele is the most common treatable cause of male infertility. Molecular changes associated with defective spermatozoa are predominantly due to alterations in the expression levels of their proteins. In varicocele patients, pathophysiological state, compromised testicular function and epididymal dysfunction may change the composition of the seminal plasma. So far, very few studies have focused on the seminal plasma proteome in varicocele men. The main aim of this study was to evaluate the seminal plasma proteome profile in infertile men with varicocele and identify the differentially expressed proteins (DEP) compared to healthy fertile men.

**MATERIALS AND METHODS**

**Semen analysis**

Semen samples were obtained from 10 healthy male donors of proven fertility, and 50 infertile patients (33 unilateral and 17 bilateral). Semen samples were centrifuged for 7 min at 1000 g, and clear seminal plasma was aspirated and stored at -80 °C for proteomic analysis.

**Proteomic analysis of seminal plasma**

Pooled samples from the unilateral varicocele group (n=5), bilateral varicocele group (n=5) and fertile donor group (n=5) were used for proteomic analysis. Proteomic profiling of seminal plasma samples was carried out using a Finnigan LTQ linear ion trap mass spectrometer LC-MS/MS system.

Relative quantification of the proteins was performed by comparing the spectral counts in both varicocele and fertile donor groups and abundance of the proteins i.e. High (H), Medium (M), Low (L), or Very Low (VL) was calculated normalized of spectral counts was done using the normalized spectral abundance factor (NSAF).

**Bioinformatic analysis**

DEPs identified in both study groups were subjected to functional annotation and enrichment analysis using both, publicly available bioinformatic annotation tools and databases such as Ingenuity pathway analysis (IPA) and MetacoreTM to analyze the involvement of DEPs in biological and cellular processes, pathways, cellular distribution, regulatory networks, and protein-protein interactions.

**Western blot validation**

Key DEPs (HSPA2, PRDX2, APOA2 and ACR) were selected for validation in control group (n=6) and varicocele group (n=12).

**RESULTS**

**Figure 1:** The number of proteins identified in unilateral, bilateral varicocele and fertile control and DEPs in the control and varicocele group.

**Figure 2:** Topmost functional enriched network, reproductive system development and function pathway with DEPs in seminal plasma of infertile varicocele patient.

**Figure 3:** Functional analysis of the apolipoprotein A2 (APOA2) that was downregulated in the seminal plasma of infertile varicocele patients with varicocele.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Expression</th>
<th>Relative Fold Change</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR</td>
<td>↓↓</td>
<td>0.10</td>
<td>0.0037*</td>
</tr>
<tr>
<td>HSPA2</td>
<td>↓↓</td>
<td>0.81</td>
<td>0.3861</td>
</tr>
<tr>
<td>PRDX2</td>
<td>↑↑</td>
<td>1.29</td>
<td>0.0474*</td>
</tr>
<tr>
<td>APOA2</td>
<td>↓↓</td>
<td>0.67</td>
<td>0.0373*</td>
</tr>
</tbody>
</table>

*Statistically significant (P<0.05), underexpressed, overexpressed.

**Figure 4:** Upstream transcriptional factors involved in the regulation of DEPs identified in the seminal plasma of varicocele patients (A): transcriptional repressor protein yin yang 1 (YY1) and (B): nucleosome-sensitive element-binding protein 1 (YB-1).

**Figure 5:** Protein expression levels of DEPs selected for validation by Western blot in varicocele group (A): heat shock related 70 kDa protein 2 (HSPA2), (B): peroxidoxin 2 (PRDX2), (C): apolipoprotein A2 (APOA2), (D): acrosin (ACR). Results are expressed as mean ± SEM and in fold variation to control group.

**Table 1:** Validation of DEPs in varicocele patients by western blot

**CONCLUSIONS**

1. **1. Distribution of total and DEPs in unilateral, bilateral and fertile group is shown in Figure 1.

2. **2. Reproductive system development and function was the most enriched network with 15 DEPs (Figure 2).**

3. **3. APOA2 was associated with DNA fragmentation, oxidative stress response, lipid peroxidation, asthenozoospermia, azospermia or oligozoospermia (Figure 3)***

4. **4. HSPA2, eEF1G, HIST-H4, CBRI and ACR were predicted to be under the regulation of transcriptional repressor protein yin yang 1 (YY1) (Figure 4A).**

5. **5. HSPA2, ACR and CD63 were regulated by nuclease-sensitive element-binding protein 1 (YB-1) transcriptional factors (Figure 4B).**

6. **6. HSPA2 and APOA2 was significantly downregulated whereas PRDX2 was significantly upregulated in varicocele patients. ACR was downregulated but was not significant (Table 1 and Figure 5).**

1. Seminal plasma proteome of varicocele patients differs from that of healthy fertile men.

2. Alterations in the expression levels of seminal plasma proteins PRDX2 and HSPA2 may serve as non-invasive biomarkers in varicocele men to determine the patient’s fertility status.

3. **3. APOA2 can serve as a biomarker to predict semen quality of infertile patients with varicocele.**