3.1. Introduction:

Antisperm antibody (ASA) can be defined as immunoglobulins of the IgG, IgA and IgM isotype that is directed to various aspects of the spermatozoa (head, tail, midpiece or combination thereof). Irvine, (1998) reported the occurrence of antisperm antibodies (ASA) to human spermatozoa from infertile men was first observed by Rumke and Wilson in the year 1954. Antisperm antibody (ASA) may result in a state of subfertility but would rarely prevent fertility completely in couples (Dondero et al 1979; Marshburn and Kutteh, 1994). A remarkable percentage of infertile couples without any strong etiology for infertility have shown to possess circulating antibodies which are capable of agglutinating spermatozoa (Runke and Hellingc, 1959; Jones, 1979; Soren, 1990). In males, ASAs can be detected in the blood serum, seminal plasma (Mazumdar and Levine, 1998; Hadinedoushan and Ghafourzadeh, 2007). The patient suffering from the antisperm antibodies is quite prevalent in Indian populations (Kapoor et al., 1999; Punekar et al., 2001; Rajeev and Reddy, 2004). The most common causes of ASA include previous genital tract infection, testicular biopsy, testicular trauma, testicular torsion, vasectomy, prolonged use of alcohol, smoking and environmental pollution (Broderick et al., 1989; Koide et al., 2000; Arap et al., 2007).

In most cases, the autoimmunity on testicular molecule resulting from trauma or infectious disease can generate ASA (Naz and Menge, 1994; Mc Donald, 2000; Sakamoto et al., 1995). Mechanisms that can provide the autoimmunity and ASA production are microenvironmental acceleration of Th1 immunity, enhanced secretion of proinflammatory cytokines like IL-1, IFN-γ, TNFα, reduced secretion of anti-inflammatory cytokines such as IL-10 and TGF-β. These mechanisms are associated
with up-regulation of major histocompatibility molecular expression and down-regulation of immune cells through apoptotic mechanism (Naz and Menge, 1994; Sakamoto et al., 1995; Pollanen et al., 1996; Mc. Donald, 2000). Sperm immobilization (Shibahara et al., 1995), inhibition of cervical mucus penetration (Kremer and Jager, 1992), and interference with events that lead to sperm-oocyte binding are some of the mechanisms by which anti-sperm antibodies impede fertilization (Tasdemir et al., 1995; Francavilla et al., 1997).

ASA is thought to impair fertility by inhibiting sperm motility (Caron and Saling, 1991), inability of the sperm to penetrate cervical mucus (D’Cruz et al., 1991), capacitation (Bronson et al., 1982), acrosome reaction (Jaffe and Oates, 1994) or they may also involve the complete cascade resulting in sperm lysis (Jaffe and Oates, 1994; Downie et al., 1997) and can also prevent implantation eventually arresting embryo development (Haas, 1986; Koide et al., 2000). The significance of ASA in infertile men is still in controversial and there is no standardized treatment available till date. Experimental data indicate several possible sites where ASA may interfere with Invitro fertilization (IVF). In this view, the present study was designed to find out the prevalence and relatedness of antisperm antibody which inhibits sperm functional activity among infertile individuals.
3.2 MATERIALS AND METHODS

Sample selection and Antisperm Antibody Assay:

A total of 250 infertile males were recruited from the different IVF centers and hospitals, in and around Mysore for the inability to have a child and for further evaluation and treatment they were recruited in this study. Out of 250 cases 176 cases were subjected for the detection of serum antisperm antibody estimation. Rest other 74 cases were excluded due to technical error during sample processing and storing.

Sperm Agglutination:

Spermatozoa may show head to head, tail to tail or mixed types of agglutination. Agglutination does not refer to a mere aggregation of spermatozoa around cellular debris. The number of both agglutinated and non agglutinated spermatozoa was counted in at least 20 randomly selected optical fields and the incidence of agglutinated spermatozoa was expressed in percentage. Samples showing sperm agglutination were subjected to immunological tests.

Kit Principle:

Antisperm antibodies of the patient's sample material will be bind to sperm antigens immobilized on a 96-well-ELISA plate. In a second step, enzyme-linked antibodies directed against human immunoglobulins were added. The antibody concentration in the patient's serum directly correlates with the extinction values of the subsequent photometric measurements.

Specimen Collection: Vein puncture blood was collected from infertile male subjects, blood samples were allowed to clot. The serum was separated by means of centrifugation
at 3000 rpm for 10 minutes. The collected serum was used for detection for antisperm antibody.

Detection of antisperm antibody in blood serum was carried by using ELISA kit (Bioserv Diagnostics, Germany)

**Kit procedure:**

1) 5 µl of blood serum was diluted with 495 µl of dilution fluid provided in the kit. After adding dilution fluid, 50 µl of this mixture was added to the well and incubated for an hour at 37°C.

2) 1X of washing buffer was prepared by using 5 ml of (10X washing buffer) by adding with 45 ml of distilled water.

3) After incubation the wells containing sample were washed thoroughly for 3 times using washing buffer.

4) After washing, 50 µl of enzyme conjugate was added and incubated for 60 minutes at 37°C.

5) After incubation, the wells containing sample were washed thoroughly for 3 times using washing buffer and the wells were tapped until water wipes completely from the sample well.

6) 50 µl of substrate was added and kept for 30 minutes incubated at room temperature.

7) After 30 minutes 50 µl of stop solution was added and the readings were taken at 450 nm under micro plate reader (BIOTEK 800).
3.3. RESULTS

In the present study, the incidence of serum antisperm antibody was depicted in table 1, in which 34% of the infertile males showed positive result for ASA. Table 2 shows the distribution and incidence of ASA among different infertile subgroups, in which, oligozoospermic condition showed highest percentage (46.1%) of serum ASA, followed by aspermic (45.4%), asthenoteratozoospermic (44.9%), asthenozoospermic (41.6%) and azoospermic (40.9%). Rest of the remaining conditions showed less than 30% response. Table 3 furnishes the distribution of different infertile conditions with spermiogram. Sperm motility and viability was not recorded in azoospermic and aspermic conditions. Diminished sperm count was observed in oligospermic and associated oligospermic cases, Sperm motility was found to be decline in all asthenozoospermic and associated asthenozoospermic cases. Viability was decreased in asthenoteratozoospermic and oligoasthenospermic conditions. Whereas, rest of the conditions showed viability above 50%. Abnormal sperm morphology was observed in teratozoospermic and associated teratozoospermic cases. Response of sperm function tests for different infertile conditions with respect to the presence of antisperm antibody was shown in table 4. Except idiopathic condition, all other conditions showed decrease in sperm function tests such HOS, NCD and AR. Incident of antisperm antibody in infertile men with different age group was shown in the table 5 and Figure 1. More of infertile cases were reported in the age group ranging between 31- 40 years followed by 20-30 years. Higher incidence of ASA (45.1%) was found in the age range between 40-50 years followed by other age groups. Response of ASA with respect to smoking habits was shown in table 6. 34% of infertile cases were shown positive for ASA out of which,
38.3% cases were smokers and 61% were nonsmokers. 34.4% of smokers and 65.5% of nonsmokers responded negative for ASA test. Table 7 shows Pearson's correlation study between different sperm parameters, ASA and smoking habit. ASA showed positively significant correlation with agglutination test but negative for different sperm parameters. Smoking as a life style factor was used as a predictor and was analyzed and its effect on the sperm parameters using Pearson's correlation. Pearson's correlation coefficient was not significant ($p>0.05$) in all sperm parameters. Figure 2 illustrates the different grades of sperm agglutination in different infertile males.
Table 1: Incidence of antisperm antibody (ASA) in the infertile males.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total No. of cases</th>
<th>No. of cases with positive for ASA</th>
<th>No. of cases with negative for ASA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertile males</td>
<td>176</td>
<td>60(34%)</td>
<td>116(65.9%)</td>
</tr>
</tbody>
</table>

Table 2: Incidence of antisperm antibody in different infertile subgroups.
(n=Number of subjects, ASA=Antisperm antibody, OAT=Oligoasthenoteratozoospermia)

<table>
<thead>
<tr>
<th>Infertile subgroups</th>
<th>n=176</th>
<th>%</th>
<th>No. of cases positive for ASA (n=60)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthenozoospermia</td>
<td>12</td>
<td>6.25</td>
<td>5</td>
<td>41.6</td>
</tr>
<tr>
<td>Asthenoteratozoospermia</td>
<td>9</td>
<td>5.11</td>
<td>4</td>
<td>44.9</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>44</td>
<td>25</td>
<td>18</td>
<td>40.9</td>
</tr>
<tr>
<td>Ejaculation failure</td>
<td>11</td>
<td>6.25</td>
<td>5</td>
<td>45.4</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>15</td>
<td>8.50</td>
<td>4</td>
<td>24.6</td>
</tr>
<tr>
<td>OAT</td>
<td>19</td>
<td>10.79</td>
<td>5</td>
<td>26.3</td>
</tr>
<tr>
<td>Oligoasthenozoospermia</td>
<td>15</td>
<td>8.52</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Oligozoospermia</td>
<td>13</td>
<td>7.38</td>
<td>6</td>
<td>46.1</td>
</tr>
<tr>
<td>Oligoteratozoospermia</td>
<td>9</td>
<td>5.11</td>
<td>2</td>
<td>22.2</td>
</tr>
<tr>
<td>Teratozoospermia</td>
<td>29</td>
<td>16.47</td>
<td>8</td>
<td>27.5</td>
</tr>
</tbody>
</table>
Table 3: Distribution of semen profile in different infertile subgroups with respect to the cases with serum antisperm antibody. (n=Number of subjects, OAT=Oligoasthenoteratozoospermia)

<table>
<thead>
<tr>
<th>Infertile conditions</th>
<th>n=60</th>
<th>Count (Mean±SE)</th>
<th>Motility (Mean±SE)</th>
<th>Morphology (Mean±SE)</th>
<th>Viability (Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthenozoospermia</td>
<td>5</td>
<td>31.0±7.3</td>
<td>31.0±7.3</td>
<td>29.4±6.9</td>
<td>54.0±7.4</td>
</tr>
<tr>
<td>Asthenoteratozoospermia</td>
<td>4</td>
<td>32.1±5.0</td>
<td>32.1±5.0</td>
<td>19.0±6.8</td>
<td>49.0±8.8</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>18</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Ejaculation failure</td>
<td>5</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>4</td>
<td>58.8±15.6</td>
<td>58.8±15.6</td>
<td>47.5±3.2</td>
<td>77.5±3.2</td>
</tr>
<tr>
<td>OAT</td>
<td>5</td>
<td>5.6±0.6</td>
<td>5.6±0.6</td>
<td>35.0±4.1</td>
<td>54.2±4.0</td>
</tr>
<tr>
<td>Oligoasthenozoospermia</td>
<td>3</td>
<td>4.6±0.8</td>
<td>4.6±0.8</td>
<td>18.3±1.6</td>
<td>35.0±11.5</td>
</tr>
<tr>
<td>Oligozoospermia</td>
<td>6</td>
<td>12.7±2.5</td>
<td>52.7±2.5</td>
<td>37.6±4.2</td>
<td>50.0±7.7</td>
</tr>
<tr>
<td>Oligoteratozoospermia</td>
<td>2</td>
<td>16.5±1.5</td>
<td>56.5±1.5</td>
<td>15.0±5.0</td>
<td>59.5±10.5</td>
</tr>
<tr>
<td>Teratozoospermia</td>
<td>8</td>
<td>38.5±5.05</td>
<td>58.5±5.0</td>
<td>13.3±3.8</td>
<td>55.00±6.2</td>
</tr>
</tbody>
</table>

Table 4: Distribution of sperm function test in different infertile subgroups with respect to the cases with antisperm antibody.(n=Number of subjects, OAT=Oligoasthenoteratozoospermia, HOS=Hypo-osmotic swelling test, NCD=Nuclear chromatin decondensation test, AIT=Acrosome intactness test)

<table>
<thead>
<tr>
<th>Infertile conditions</th>
<th>n=60</th>
<th>HOS (Mean±SE)</th>
<th>NCD (Mean±SE)</th>
<th>AIT (Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthenozoospermia</td>
<td>5</td>
<td>50.0±8.0</td>
<td>61.2±10.7</td>
<td>52.0±1.0</td>
</tr>
<tr>
<td>Asthenoteratozoospermia</td>
<td>4</td>
<td>46.0±10.7</td>
<td>61.2±11.9</td>
<td>30.4±4.7</td>
</tr>
<tr>
<td>Azoospermia</td>
<td>18</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Ejaculation failure</td>
<td>5</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>4</td>
<td>76.75±2.3</td>
<td>68.2±11.2</td>
<td>62.50±11.8</td>
</tr>
<tr>
<td>OAT</td>
<td>5</td>
<td>53.0±7.6</td>
<td>59.8±8.8</td>
<td>44.0±8.1</td>
</tr>
<tr>
<td>Oligoasthenozoospermia</td>
<td>3</td>
<td>43.3±7.2</td>
<td>40.0±15.2</td>
<td>34.3±10.2</td>
</tr>
<tr>
<td>Oligozoospermia</td>
<td>6</td>
<td>54.3±4.0</td>
<td>62.0±12.61</td>
<td>43.3±13.1</td>
</tr>
<tr>
<td>Oligoteratozoospermia</td>
<td>2</td>
<td>47.5±2.5</td>
<td>51.0±11.0</td>
<td>44.0±4.0</td>
</tr>
<tr>
<td>Teratozoospermia</td>
<td>8</td>
<td>47.1±8.8</td>
<td>60.1±7.8</td>
<td>57.5±6.0</td>
</tr>
</tbody>
</table>
Table 5: Age wise distribution of infertile condition with respect to positive and negative response for antisperm antibody.

<table>
<thead>
<tr>
<th>Age wise distribution (Years)</th>
<th>20-30 years</th>
<th>31-40 years</th>
<th>41-50 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cases for ASA</td>
<td>14(31.9)</td>
<td>33(32.7)</td>
<td>14(45.1)</td>
</tr>
<tr>
<td>Negative cases for ASA</td>
<td>30(68.1)</td>
<td>68(32.7)</td>
<td>17(54.9)</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>101</td>
<td>31</td>
</tr>
</tbody>
</table>

Figure 1: Age wise distribution of infertile condition with respect to positive and negative response for antisperm antibody.
Table 6: Distribution of positive and negative response of antisperm antibody with respect to smoking habits.

<table>
<thead>
<tr>
<th>Age wise distribution (Years)</th>
<th>No. of cases (n=176)</th>
<th>Infertile smokers</th>
<th>Infertile nonsmokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cases for ASA</td>
<td>60(34%)</td>
<td>23(38.3%)</td>
<td>37(61%)</td>
</tr>
<tr>
<td>Negative cases for ASA</td>
<td>116(65.9%)</td>
<td>40(34.4%)</td>
<td>76(65.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>176</td>
<td>63(35.7%)</td>
<td>113(64.2%)</td>
</tr>
</tbody>
</table>
Table 7: Pearson correlation between different sperm parameters and serum antisperm antibodies.

<table>
<thead>
<tr>
<th></th>
<th>Count</th>
<th>Motility</th>
<th>Viability</th>
<th>Agglutination</th>
<th>Antibodies</th>
<th>Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>Pearson Correlation</td>
<td>1</td>
<td>0.628**</td>
<td>0.614**</td>
<td>0.164*</td>
<td>-0.095</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.031</td>
<td>0.210</td>
</tr>
<tr>
<td>Motility</td>
<td>Pearson Correlation</td>
<td>1</td>
<td>0.773**</td>
<td>0.013</td>
<td>-0.078</td>
<td>-0.015</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
<td>0.000</td>
<td>0.861</td>
<td>0.304</td>
</tr>
<tr>
<td>Viability</td>
<td>Pearson Correlation</td>
<td>1</td>
<td></td>
<td>0.098</td>
<td>-0.099</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
<td>0.198</td>
<td>0.194</td>
<td>0.968</td>
</tr>
<tr>
<td>Agglutination</td>
<td>Pearson Correlation</td>
<td></td>
<td></td>
<td>1</td>
<td>0.386**</td>
<td>-0.044</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
<td>0.567</td>
</tr>
<tr>
<td>Antibodies</td>
<td>Pearson Correlation</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>-0.119</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.118</td>
</tr>
<tr>
<td>Smoking</td>
<td>Pearson Correlation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).
Figure 2: Grades of sperm agglutination in different infertile subjects

Plate A: Grade 1 agglutination,
Plate B: Grade 2 agglutination,
Plate C: Grade 3 agglutination,
Plate D: Grade 4 agglutination,
3.4. Discussion:

During initiation of spermatogenesis, sperm-specific antigen first appears at the time of puberty. Since such sperm specific antigens are not present during development of immunological tolerance, these proteins are potential targets for an immune response and therefore generate antisperm antibodies (Dana and Alan, 1996; Koide et al., 2000). 8-21% of infertile males were found to be associated with the production of antisperm antibody (Dana and Alan, 1996; Reza et al., 2009). In our study, a total of 176 infertile males were investigated, out of which 60 (34 %) subjects showed positive response for ASA in their blood serum. Our results are compared and were similar with the results reported by Husted and Hjort, (1975) and Kapoor et al., (1999). In our study, 46.1% of oligozoospermic and 40.9% of azoospermic were associated with ASA Jones, (1979) has reported a strong association between sperm count and antibody occurrence stated that auto-immunity to sperm antigens can be related to infertility in men by an association with abnormal spermatogenesis resulting in oligozoospermia or azoospermia. In our study, higher incidence of ASA was found in asthenospermic and associated asthenozoospermic conditions. This may be due to sperm immobilizing antigens which may decrease the motility (Turek, 1997). It is also evident in earlier result the association of ASA with decreased motility.

In the present study, significant correlation was observed with sperm agglutination and ASA. But all other sperm function test does not show significant relation between ASA. Earlier studies also showed the correlation of ASA with sperm agglutination hence in our study also it is evident the earlier hypothesis holds good (Francavilla et al., 2007). Ludwikowski et al., (2004) have showed the presence of
increased number of antisperm antibodies in smoking patients, in our study we found 38.3% of the infertile smokers have responded positively for ASA. Our results holds good with the previous study done by Omu *et al.*, (1997) where, 39.3% of the infertile smokers were with positive for the presence of ASA. One of the possible mechanisms of antisperm antibody production by cigarette smoking may be caused by the impaired action of the suppressor/helper T lymphocytes and/or other immunosuppression factors (McClain *et al.*, 1995).

Many studies tried to correlate age as a factor influencing on male fertility and incidence of ASA. Recent report showed increased incidence of ASA in the younger age than the older age (Svetoslav *et al.*, 2002). But in our study, the incidence of ASA was high in age group between 41-50. But, still it’s in controversial to hypothesis the influence of age and production of ASA. The age group between 31-40 in our study was found to be maximum number, from this we can understand that one of the possible reasons could be due to late marriages. Further more investigation of circulating ASA has a prognostic value in diagnosing infertility.