A blood sample is taken from 15 days after embryo transfer and level of bHCG is been checked and pregnancy is confirmed. After 10 days from that the fetal heart beate is checked. The detailed procedure is checked in appendix

3.15 Statistical analysis:

All the results were statistically analysed using a software SPSS system by applying the students “t-test”. The values given to the software are obtained by applying the formula mean ± Standard Deviation. Spearman’s Correlation co-efficient was done between Serum and Follicular fluid AMH and Inhibin B with no. of eggs and mature eggs and also with Sperm DFI with fertilization and cleavage rates in control and infertile groups.

Statistically significance

If ‘t’ value is 1.645 and above, but below 2.327, then p<0.05 – significant at 5% level. If ‘t’ value is 2.327 and above then p<0.01 – significant at 1% level.

RESULTS AND DISCUSSION

The results of the present study “Assessment of Oocyte quality with AMH, Inhibin B in Serum and Follicular fluid and Predicting Pregnancy Outcome with Sperm DNA Fragmentation in Art Cycles” were discussed in the following sequence:

4.1 Study population

4.2 Day 2 hormone profile in serum for control and infertile women

4.2.1 FSH

4.2.2 LH

4.2.3 E2
4.2.4 PRL

4.2.5 P4

4.3 Hormone profile in serum of control and infertile women on the day of oocyte retrieval.

4.3.1 FSH and LH

4.3.2 E2 and P4

4.3.2 AMH and INHIBIN B

4.4 Hormone profile in follicular fluid in control and infertile women

4.4.3.1 FSH and LH

4.4.3.2 E2 and P4

4.4.3.2 AMH and INHIBIN B

4.5 Correlation between serum AMH and INHIBIN B of control and infertile women with no. of eggs and mature eggs

4.6 Correlation between follicular AMH and INHIBIN B of control and infertile women with no. of eggs and mature eggs.

4.7 Semen parameters

4.7.1 Normozoospermia

4.7.2 Teratozoospermia

4.7.3 Asthenoteratozoospermia

4.7.4 Oligoasthenoteratozoospermia

4.7.5 Sperm count

4.7.6 Sperm motility

4.7.7 Sperm morphology

4.8 Sperm DNA fragmentation Index

4.8.1 DFI between raw and processed semen sample in control and infertile men

4.8.2 DFI between control and infertile men
4.9 Correlation between sperm DNA fragmentation Index and fertilization and cleavage rate in control and infertile groups

4.9.1 DNA fragmentation and fertilization rate in control and infertile groups

4.9.2 DNA fragmentation and cleavage rate in control and infertile groups.

4.10 Evaluation of Sperm DFI with fertilization, cleavage rate, embryo quality and development and pregnancy.

4.11 Evaluation of positive and negative patient with DFI, AMH and Inhibin B.

4.1 STUDY POPULATION

The study population consists of 63 patients. Twenty healthy egg donors who had proven fertility were taken as control group for the females. Twenty normozoospermia men were taken as control for men. The remaining 43 patients were the infertile group. The females were infertile due to endometriosis, PCOS, and ovulatory disorders. The men were infertile due to low count, or low motility or low normal morphology.

The age group of females were 34 ± 3.8 and the age group of men were 36.8 ± 4.5 and control group females were of age group 24 ± 2.8.

4.2. Day 2 hormone profile in serum for control and infertile women

The day 2 hormone level in the females plays a vital role in giving an idea about the ovarian reserve which could be used as a guideline to stimulate the females.
This endocrine study is taken on the 2\textsuperscript{nd} or 3\textsuperscript{rd} day of their previous menstrual cycle.

**Table 4**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Control (a) (n=20)</th>
<th>Infertile (b) (n=43)</th>
<th>Groups compared</th>
<th>‘t’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.99 ± 2.37</td>
<td>11.45 ± 19.73</td>
<td>A vs B</td>
<td>1.805*</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>6.30 ± 5.09</td>
<td>7.30 ± 9.77</td>
<td>A vs B</td>
<td>0.502\textsuperscript{ns}</td>
</tr>
<tr>
<td>PRL (ng/ml)</td>
<td>14.49 ± 9.14</td>
<td>18.17 ± 14.81</td>
<td>A vs B</td>
<td>1.126\textsuperscript{ns}</td>
</tr>
<tr>
<td>P4 (ng/ml)</td>
<td>2.41 ± 7.36</td>
<td>12.90 ± 14.22</td>
<td>A vs B</td>
<td>2.425**</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>47.18 ± 45.98</td>
<td>107.9 ± 139.1</td>
<td>A vs B</td>
<td>2.603**</td>
</tr>
</tbody>
</table>

Value are mean ± SD
* - significant at t<0.05  \quad \quad ** - Significant at t<0.01  \quad \quad \text{ns} – Not significant

4.2.1 FSH

The average mean value of serum day 2 FSH in control and infertile women is given in Table 4.

The average mean value of FSH in day 2 serum (11.45± 19.73 mIU/ml) in infertile women was compared with FSH value of control (5.99± 2.37 mIU/ml). It was noted that there was an increase in FSH level in infertile group which was statistically significant at 5% level.

4.2.2 LH
The average mean value of serum day 2 LH in control and infertile women is given in Table 4.

The average mean value of LH in day 2 serum (7.30± 9.77 mIU/ml) in infertile women was compared with LH value of control (6.30 ± 5.09 mIU/ml). It was noted that there was a slight increase in LH level in infertile group which was statistically insignificant.

4.2.3 PRL

The average mean value of day 2 serum PRL in control and infertile women is given in Table 4.

The average mean value of PRL in serum (18.17 ± 14.81ng/ml) in infertile women was compared with PRL value of control (14.49 ± 9.14 ng/ml). It was noted that there was a slight increase in PRL level in infertile group which was statistically insignificant.

4.2.4 P4

The average mean value of serum P4 in control and infertile women is given in Table 4.

The average mean value of FSH in serum (12.90±14.22ng ml) in infertile women was compared with FSH value of control (2.41± 7.36 mIU/ml). It was noted that there was an increase in P4 level in infertile group which was statistically significant at 1% level.

4.2.5 E2

The average mean value of serum E2 in control and infertile women is given in Table 4.
The average mean value of E2 in serum (107.9 ± 139.1 pg/ml) in infertile women was compared with E2 value of control (47.18 ± 9.14 ng/ml). It was noted that there was a increase in E2 level in infertile group which was statistically significant at 5% level.

Abdella et al., 2007 showed that the level of FSH provides a biological marker for ovarian reserve. The higher the FSH level the poorer the ovarian reserve. The number of eggs available in the ovary is certainly reduced and the level of FSH is elevated when womens age was advancing.

Abdalla and Thum (2004) showed that elevated level of FSH are associated with the reduction in pregnancy and live birth. It has been shown that this is primarily due to the reduction in oocyte quantity rather than oocyte quality. Sherman et al., (1975) and Ebbiary et al., (1994) showed that high levels of FSH and LH levels are an indicator of diminished ovarian reserves.

Smotcich et al., (1995) showed high levels of E2 indicate a diminished ovarian function Prestimulation antral follicle count of lesser than 6, lower ovarian volume and diminished stromal blood flow on color Doppler studies, suggest poor ovarian reserve (lass et al., 1999).

Bukulmez et al., (2004) and Kwee et al., (2003) showed the base line FSH, LH and E2 levels are good predictor of ovarian reserve. Muttukrishna et al., (2004) found that levels of baseline FSH were significantly higher in the cancelled group. Jurema et al., (2003) showed that levels of baseline FSH and E2 but not LH were significantly lower in cycles resulting in a normal ovarian response as well as cycles resulting in clinical pregnancy.
Our studies correlated with earlier studies that there was high levels of FSH and LH in the infertile group compared to control group showing the causes of infertility.

4.3 Hormone profile in serum of control and infertile women on the day of oocyte retrieval

The hormones regulating the female reproductive system which in particular takes part in the formation of oocytes, ovulation, fertilization and embryo formation were analysed in serum and follicular fluid collected on the day of oocyte retrieval.

4.3.1 FSH and LH

The average mean value of serum FSH and LH in control and infertile women is given in Table 5.

The average mean value of FSH in serum (6.92 ± 1.91 mIU/ml) in infertile women was compared with FSH value of control (9.81 ± 4.91 mIU/ml). It was noted that there was an decrease in FSH level in infertile group which was statistically significant at 5% level.

Similarly average mean value of LH in serum (0.45±0.31mIU/ml) in infertile women was compared with LH value of control (0.40 ± 0.23mIU/ml). It was noted that there was a slight decrease in LH level in infertile group which was statistically insignificant.

Table 5
Hormone level in serum of control and infertile women on the day Oocyte retrieval

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Control (A) (n=20)</th>
<th>Infertile (B) (n=43)</th>
<th>Groups compared</th>
<th>‘t’ value</th>
</tr>
</thead>
</table>

Assessment of Oocyte Quality with AMH, Inhibin B in Serum and Follicular Fluid and Predicting Pregnancy outcome with Sperm DNA Fragmentation in IVF Cycles
### 4.3.2 E2 and P4

The average mean values of serum E2 and P4 in control and infertile women are given Table 5.

The average mean value of E2 in serum (1782.66±967.95 pg/ml) in infertile women was compared with E2 value of control (1950.97± 846.03 pg/ml). It was noted that there was a slight decrease in E2 level in infertile group which was statistically insignificant.

Similarly average mean value of P4 in serum (18.54±16.09 ng/ml) in infertile women was compared with P4 value of control (10.80± 6.16 ng/ml). It was noted that there was an increase in P4 level in infertile group which was statistically significant at 1% level.

**Figure 62**

**Hormone level in serum AMH of control and infertile women on the day Oocyte retrieval**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Control Mean ± SD</th>
<th>Infertile Mean ± SD</th>
<th>A vs B</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mIU/ml)</td>
<td>9.81 ± 4.91</td>
<td>6.92 ± 1.91</td>
<td>1.94*</td>
<td></td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>0.45 ± 0.31</td>
<td>0.40 ± 0.23</td>
<td>0.63ns</td>
<td></td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>1950.97 ±846.03</td>
<td>1782.66 ±967.95</td>
<td>0.62ns</td>
<td></td>
</tr>
<tr>
<td>P4 (ng/ml)</td>
<td>10.80 ± 6.16</td>
<td>18.54 ± 16.09</td>
<td>1.77*</td>
<td></td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>3.04 ± 1.68</td>
<td>3.66 ± 5.98</td>
<td>0.30ns</td>
<td></td>
</tr>
<tr>
<td>Inhibin B (pg/ml)</td>
<td>63.69 ± 18.87</td>
<td>33.50 ± 9.11</td>
<td>6.22**</td>
<td></td>
</tr>
</tbody>
</table>

Value are mean ± SD

* - significant at t<0.05   ** - Significant at t<0.01   ns – Not significant
4.3.3 AMH and INHIBIN B

The average mean values of serum AMH and INHIBIN B in control and infertile women are given in Table 5 and Figure 62 & 63.

Figure 63

Hormone level in serum Inhibin B of control and infertile women on the day Oocyte retrieval
The average mean value of AMH in serum (3.66 ± 5.98 ng/ml) in infertile women was compared with AMH value of control (3.04 ± 1.68 mIU/ml). It was noted that there was no significant alteration in AMH level.

Similarly average mean value of INHIBIN B in serum (33.50 ± 9.11 pg/ml) in infertile women was compared with INHIBIN B value of control (63.69 ± 18.87 pg/ml). It was noted that there was a decrease in INHIBIN B level in infertile group which was statistically significant at 1% level.

4.4 Hormone profile in follicular fluid control and infertile women

The hormones playing a vital role in follicle recruitment, maturation, ovulation and fertilization are analysed in follicular fluid.

4.4.1 FSH and LH

The average mean value of follicular fluid FSH and LH in control and infertile women is given in Table 6.

The average mean value of FSH in follicular fluid (9.02 ± 0.62 mIU/ml) in infertile women was compared with FSH value of control 11.03 ± 1.10 mIU/ml). It was noted that there was a decrease in FSH level in infertile women which was statistically significant at 5% level.

Similarly average mean value of LH in follicular fluid (1.14 ± 2.48 mIU/ml) in infertile women was compared with LH value of control (2.54 ± 3.37 mIU/ml). It was noted that there was a decrease in LH level in infertile women which was statistically significant at 5% level.

Table 6

Hormone level in follicular fluid of control and infertile women
4.4.2 E2 and P4

The average mean value of follicular fluid E2 and P4 in control and infertile women is given in Table 6.

The average mean value of E2 in follicular fluid (3330.67 ± 347.13 pg/ml) in infertile women was compared with E2 value of control (3523.4±316.12 pg/ml). It was noted that there was a slight decrease in E2 level in infertile women which was statistically insignificant.

Similarly average mean value of P4 in follicular fluid (91.6 ± 2.7 ng/ml) in infertile women was compared with P4 value of control (92.34 ± 2.65 ng/ml). It was noted that there was a slight decrease in P4 level in infertile women which was statistically insignificant.
Results and Discussion

Assessment of Oocyte Quality with AMH, Inhibin B, in Serum and Follicular Fluid and Predicting Pregnancy outcome with Sperm DNA Fragmentation in Art Cycles

Figure 64
Hormone level in follicular fluid of AMH in control and infertile women

Figure 65
Hormone level in follicular fluid of Inhibin B in control and infertile women
4.4.3 AMH and INHIBIN B

The average mean value of follicular fluid AMH and INHIBIN B in control and infertile women is given in Table 6 and Figure 64 & 65.

The average mean value of AMH in follicular fluid (8.97± 6.25 ng/ml) in infertile women was compared with AMH value of control (6.71 ±5.01 ng/ml). It was noted that there was a slight increase in AMH values in infertile group which was statistically insignificant.

Similarly average mean value of INHIBIN B in serum (3825.43 ± 198.08pg/ml) in infertile women was compared with INHIBIN B value of control (4120.89 ± 280.94pg/ml). It was noted that there was a decrease in INHIBIN B level in infertile group which was statistically significant at 1 % level.

Intrafollicular concentrations of FSH and LH are affected by their circulating levels: in IVF cycles the serum levels are determined by the amount of exogeneously administered gonodotropins and by the degree of pituitary suppression (relavently reducing the endogeneous gonodotropin secretion)(Revelli 2008)

High concentrations of FSH and LH have been reported to promote oocyte maturation and to be associated with eggs having a high chance of fertilization.(Suchanek et al., 1984 and Cha et al., 1986).

Mendoza et al., (2002) showed that follicular fluid FSH and LH was observed to be consistently higher in follicles containing oocytes that resulted in embryos leading to successful IVF attempts. In the present study low levels of
Results and Discussion

Assessment of Oocyte Quality with AMH, Inhibin B, in Serum and Follicular Fluid and Predicting Pregnancy outcome with Sperm DNA Fragmentation in Art Cycles

FSH and LH in infertile groups compared with the control groups showed that the oocyte quality is low.

Gonadotropins play an important role in the secretion of several substances by granulosa cells (e.g., Hyaluronic acid) in turn affecting oocyte development and maturation. They may also act synergistically with estradiol (E2) in enhancing the oocyte maturation and via cyclic AMP secretion, control oocyte meiosis. Higher levels of gonadotropins would improve these processes and lead to better oocytes, better embryos and improved pregnancy rate (Mendoza et al., 2002).

Tesarik et al., (1997) showed that intrafollicular estrogenic environment is associated with good follicular growth and anti atretia effects. In addition it enhances the cytoplasmic maturation of oocytes via a direct non genomic action at the plasma membrane level, in turn inducing extracellular calcium influx into the cell and a specific pattern of Ca++ oscillations.

Elevated E2 levels and E2/P4 ratio indicate a more advanced stage of oocyte maturation and have been repeatedly found to be associated with a higher chance of achieving pregnancy (Kreiner et al., 1987 and Teissier et al., 2000). This study correlated with the present study where the E2 levels are slightly increased in control groups than infertile groups.

The present study was supported by Enien et al., (1996) that E2/P4 ratios were higher in follicles whose oocytes were fertilized.

Ben-rafael et al., (1987) showed that high progesterone levels in follicular fluid found to have association with post mature oocytes that fertilized abnormally and gave rise to multipronuclear embryos. Kobayashi et al., (1991) and Enien 1995 showed that high levels of P4 was predictive of subsequent...
implantation and pregnancy which correlates with our study where the P4 levels in control group is slightly higher than infertile groups.

Wen et al., (2006) showed that higher levels of inhibin B were associated with the likelihood to retrieve oocytes at the time of oocyte retrieval but not with the IVF outcome, and fertilization competence.

Ocal et al., (2004) found that high levels of inhibin B in follicular fluid were associated with increased fertilization and pregnancy rates. This correlated with our study where there is an increased levels of inhibin B in control groups compared with infertile groups.

Serum levels of AMH between 1.66 and 4.42 ng/ml were found to have high quality oocytes (Ebner et al., 2006) and good quality embryos (Silberstein et al., 2006) which correlated with our study were the serum levels in control group were slightly lower compared to infertile groups.

Takahashi et al., (2007) statement that oocytes were likely to be fertilized when their follicle was able to produce high levels of AMH as follicular fluid AMH levels from follicles with fertilized oocytes are more than 3 times from follicles with non fertilized oocyte which was contrary to Cupisti et al., (2007) who found that AMH level in individual follicles were inversely correlated with the maturation and developmental potential of oocytes which was correlating with our study where there was increased levels of AMH in infertile group compared with control group.

4.5 Correlation between serum AMH and INHIBIN B of control and infertile women with no. of eggs and mature eggs

4.5.1 AMH
The relationship between AMH levels and no. of eggs and no. of mature eggs in serum are given in Table 7.

### Table 7

**Correlation between Serum AMH and Inhibin B with No. of Eggs and mature Eggs in control and Infertile groups**

<table>
<thead>
<tr>
<th>Egg quantity and quality</th>
<th>Correlation co-efficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
</tr>
<tr>
<td>AMH</td>
<td>Inhibin B</td>
</tr>
<tr>
<td>No. of Eggs</td>
<td>0.3673*</td>
</tr>
<tr>
<td>No. of mature eggs</td>
<td>0.0005ns</td>
</tr>
</tbody>
</table>

* r is significant at 5% level  ns  is not significant

**4.5.2 AMH**

There was a positive correlation between the AMH levels and no. of eggs in the control and infertile group were $r = 0.3673$ ($t=1.667$) and $r = 0.2607$ ($t=1.723$) which was statistically significant ($t<0.05$).

There was no correlation between the AMH levels and no. of mature eggs in control group where $r = 0.0005$ ($t=0.0032$) and $r = 0.0017$ ($t=0.0108$) in infertile group which was statistically not significant ($t<0.05$).

**4.5.2 INHIBIN B**

The relationship between INHIBIN B levels and no. of eggs and no. of mature eggs in serum are given in Table 7

There was a positive correlation between the INHIBIN B levels and no. of eggs in the control and infertile group were $r = 0.4027$ ($T=1.866$) and $r = 0.2897$ ($t = 1.938$) which was statistically significant ($t<0.05$).
There was no correlation between the INHIBIN B levels and no. of mature eggs in control group where \( r = 0.0173 \) (t=0.110) which was statistically insignificant (t<0.05).

There was no correlation between the INHIBIN B levels and no. of mature eggs in infertile group were \( r = 0.0056 \) (t=0.0358) which was statistically insignificant (t<0.05).

4.6 Correlation between follicular fluid AMH and INHIBIN B of control and infertile women with no. of eggs and mature eggs.

Table 8

<table>
<thead>
<tr>
<th>Egg quantity and quality</th>
<th>Correlation co-efficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
</tr>
<tr>
<td></td>
<td>AMH</td>
</tr>
<tr>
<td>No. of Eggs</td>
<td>0.3780*</td>
</tr>
<tr>
<td>No. of mature eggs</td>
<td>0</td>
</tr>
</tbody>
</table>

* - Significant at 5% level  ns - is not significant

4.6.1 AMH

The relationship between AMH levels and no. of eggs and no. of mature eggs in follicular fluid are given in Table 8.

There was positive correlation between the AMH levels and no. of eggs in the control group were \( r = 0.3780 \) (t = 1.713) which was statistically significant (t<0.05).
There was positive correlation between the AMH levels and no. of eggs in the infertile group were \( r = 0.2867 \) (\( t = 1.916 \)) which was statistically significant (\( t<0.05 \)).

There was no correlation between the AMH levels and no. of mature eggs in control and infertile group where \( r = 0 \). and \( r = 0 \) respectively which was statistically not insignificant.

### 4.6.2 INHIBIN B

The relationship between Inhibin B levels and no. of eggs and no. of mature eggs in follicular fluid are given in Table 8.

There was positive correlation between the Inhibin B levels and no. of eggs in the control group were \( r = 0.4296 \) (\( t=2.018 \)) which was statistically significant (\( t<0.05 \)).

There was positive correlation between the INHIBIN B levels and no. of eggs in the infertile group were \( r = 0.4564 \) (\( t = 3.21 \)) which was statistically significant (\( t<0.01 \)).

There was no correlation between the INHIBIN B levels and no. of mature eggs in control group where \( r = 0.0395 \) (\( t = 0.167 \)) which was statistically not significant.

There was no correlation between the INHIBIN B levels and no. of mature eggs in infertile group were \( r = -0.0037 \) (\( t = 0.0156 \)) which was statistically not significant.

AMH is produced by the granulosa cells of early follicles and inhibits the transition of from primordial to primary follicular stage. AMH levels in serum
shown to be proportional to number of small antral follicles. Serum AMH levels decrease with age and are undetectable in post menopausal period. In premature ovarian failure AMH is undetectable or greatly reduced depending of the number of antral follicles in the ovaries. In contrast AMH levels are increased in PCOS patients. (Marca et al., 2008)

Roy mashiach et al., (2007) showed that the degree of maturation of retrieved oocytes as well as fertilization, were not found to correlate with follicular fluid AMH which correlated with the present study.

Freour et al., (2006) showed that anti mullerian hormone is significantly correlated with the no. of eggs collected and is a greatest interest as a negative predictive value for the success of assisted reproductive technology.

Wunder (2008) showed that the AMH and inhibin B levels on the day of oocyte retrieval are correlated to reproductive outcome which are contrary to Guerif et al., (2009) who showed that at the moment serum AMH is a relatively predictive indicator of the ovarian reserve, in terms of quantity but not in terms of quality which is strongly correlated with our study that AMH was significant and positively correlated with the oocyte quantity but not the oocyte quality.

Chang et al., (2002) found that inhibin B in FF may serve as an effective marker of follicular development; he also showed a significant correlation between inhibin B levels in FF and embryo scores on days 2 and 3, so he considered inhibin B a useful predictor of quality of the embryo.

Fried et al., (2003) observed that inhibin B in FF and serum was strongly correlated to the number of oocytes retrieved, but not with the IVF outcome, so
that inhibin could be considered as a marker of ovarian response, but not of oocyte or embryo quality which strongly correlated with our study.

Previous studies have shown that serum inhibin B is believed to be of predictive value in monitoring ovarian stimulation treatment for IVF (Hayes et al., 1998 and Elder- Geva et al., 2000).

Takahashi et al., (2008) showed that there was no correlation between the ratio of high quality grade embryos and either serum AMH or inhibin B levels. There was a strong correlation between serum E2 and inhibin B level and no. of oocytes which correlates with our present study.

4.7 Semen parameters

Semen analysis was performed in all the men and they were categorized under the following category

4.7.1 Normozoospermia

In semen analysis when the count was > 20 M/ml and motility with rapid progression (a) was 25% or both rapid (a) and slow (b) was 50% and normal morphology is 30% were considered as normozoospermia and served as control groups.

4.7.2 Teratozoospermia.

In semen analysis if they have normal count (>20M/ml) and normal motility (a-25% or a+b-50%) but normal morphology is <30% they were considered as teratozoospermia

4.7.3 Asthenoteratozoospermia
In semen analysis if they have normal count (>20M/ml) and less motility (<a+b-50%) and normal morphology <30% they were considered as asthenoteratospermia.

4.7.4 Oligoasthenoteratozoospermia

In semen analysis if they have less count (<20M/ml) and less motility (<a+b-50%) and normal morphology <30% they were considered as oligoasthenoteratospermia.

4.7.5 Sperm count

The sperm count of control and infertile men are discussed in Table 9 and Figure 66.

Table 9

<table>
<thead>
<tr>
<th>Count category</th>
<th>Average in percentage</th>
<th>Groups compared</th>
<th>‘t’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normozoospermia (A) (n = 20)</td>
<td>86.05 ± 15.53</td>
<td>A vs B</td>
<td>3.74**</td>
</tr>
<tr>
<td>Teratozoospermia (B) (n = 16)</td>
<td>71.67 ± 12.92</td>
<td>A vs C</td>
<td>2.82**</td>
</tr>
<tr>
<td>Astheno Teratozoospermia (C) (n = 18)</td>
<td>69.17 ± 10.82</td>
<td>A vs D</td>
<td>13.35**</td>
</tr>
<tr>
<td>Oligoastheno Teratozoospermia (D) (n = 9)</td>
<td>11.19 ± 7.08</td>
<td>B vs C</td>
<td>14.05**</td>
</tr>
</tbody>
</table>

Value are mean ± SD

* - significant at t<0.05  ** - Significant at t<0.01  ns – Not significant
When the average mean sperm count of normozoospermia (86±15.53) was compared with teratozoospermia(71.67±12.92), it was observed that the sperm count decreased in teratozoospermia and was statistically significant at 1% level.

When the average mean sperm count of normozoospermia (86±15.53) was compared with asthenoteratozoospermia(69.17±10.82), it was observed that the sperm count decreased in asthenoteratozoospermia and was statistically significant at 1% level.

![Figure 66](image)

**Sperm Count in M/ml**

When the average mean sperm count of normozoospermia (86±15.53) was compared with oligoasthenoteratozoospermia (11.19±7.08), it was observed that the sperm count decreased in oligoasthenoteratozoospermia and was statistically significant at 1% level.
When the average mean sperm count of teratozoospermia (71.67±12.92) was compared with asthenoteratozoospermia (69.17±10.82), it was observed that the sperm count decreased in asthenoteratozoospermia and was statistically significant at 1% level.

When the average mean sperm count of teratozoospermia (71.67±12.92) was compared with oligoasthenoteratozoospermia (11.19±7.08), it was observed that the sperm count decreased in oligoasthenoteratozoospermia and was statistically significant at 1% level.

When the average mean sperm count of asthenoteratozoospermia (69.17±10.82) was compared with oligoasthenoteratozoospermia (11.19±7.08), it was observed that the sperm count decreased in oligoasthenoteratozoospermia and was statistically significant at 1% level.

Martin et al., (2008) demonstrated that men with severe oligozoospermia (< 1 M/ml) have a highest frequency of sperm chromosome abnormalities and may have the greatest risk of producing a chromosomally abnormal child after undergoing ICSI.

4.7.6 Sperm motility

The sperm motility of control and infertile men is discussed in Table 10 and Figure 67.

<table>
<thead>
<tr>
<th>Count category</th>
<th>Average in percentage</th>
<th>Groups compared</th>
<th>‘t’ value</th>
</tr>
</thead>
</table>

Table 10

Sperm motility in percentage
**Results and Discussion**

Assessment of Oocyte Quality with AMH, Inhibin B, in Serum and Follicular Fluid and Predicting Pregnancy outcome with Sperm DNA Fragmentation in Art Cycles

<table>
<thead>
<tr>
<th>Normozoospermia (A) (n = 20)</th>
<th>Teratozoospermia (B) (n = 16)</th>
<th>Astheno Teratozoospermia (C) (n = 18)</th>
<th>Oligoastheno Teratozoospermia (D) (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>73.25 ± 5.57</td>
<td>64.07 ± 7.20</td>
<td>40.50 ± 4.23</td>
<td>35.00 ± 9.84</td>
</tr>
</tbody>
</table>

* - significant at t<0.05  ** - Significant at t<0.01  ns – Not significant

<table>
<thead>
<tr>
<th>A vs B</th>
<th>A vs C</th>
<th>A vs D</th>
<th>B vs C</th>
<th>B vs D</th>
<th>C vs D</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.80**</td>
<td>17.55**</td>
<td>8.37**</td>
<td>11.31**</td>
<td>7.95**</td>
<td>1.94*</td>
</tr>
</tbody>
</table>

Value are mean ± SD

When the average mean sperm motility of normozoospermia (73.25± 5.57) was compared with teratozoospermia(64.07±7.20), it was
observed that the sperm motility was decreased in teratozoospermia and was statistically significant at 1% level.

When the average mean sperm motility of normozoospermia (73.25±5.57) was compared with asthenoteratozoospermia (40.50±4.23), it was observed that the sperm motility was decreased in asthenoteratozoospermia and was statistically significant at 1% level

When the average mean sperm motility of normozoospermia (73.25±5.57) was compared with oligoasthenoteratozoospermia (35.00±9.84), it was observed that the sperm motility was decreased in oligoasthenoteratozoospermia and was statistically significant at 1% level

When the average mean sperm motility of teratozoospermia (64.07±7.20) was compared with asthenoteratozoospermia (40.50±4.23), it was observed that the sperm motility was decreased in asthenoteratozoospermia and was statistically significant at 1% level.

When the average mean sperm motility of teratozoospermia (64.07±7.20) was compared with oligoasthenoteratozoospermia (35.00±9.84), it was observed that the sperm motility was decreased in oligoasthenoteratozoospermia and was statistically significant at 1% level.

When the average mean sperm motility of asthenoteratozoospermia (40.50±4.23) was compared with oligoasthenoteratozoospermia (35.00±9.84), it was observed that the sperm motility was decreased in oligoasthenoteratozoospermia and was statistically significant at 5% level.
Bostofte et al., (1983 and 1984) showed that the motility of ejaculated spermatozoa has long been recognized as an important functional characteristic that must be evaluated as an integral part of semen analysis. The likelihood of achieving a pregnancy increases with decreased proportion of immotile spermatozoa and with increasing quality of sperm progression which correlated to our study.

4.7.7 Sperm morphology

Sperm morphology of control and infertile men is discussed in Table 11 and Figure 68.

When the average mean sperm normal morphology of normozoospermia (29.95± 3.30) was compared with teratozoospermia (27.00±17.59), it was observed that the sperm normal morphology was decreased in teratozoospermia and was statistically insignificant.

Table 11

<table>
<thead>
<tr>
<th>Count category</th>
<th>Average in percentage</th>
<th>Groups compared</th>
<th>‘t’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normozoospermia (A) (n = 20)</td>
<td>29.95 ± 3.30</td>
<td>A vs B</td>
<td>0.71 ns</td>
</tr>
<tr>
<td>Teratozoospermia (B) (n = 16)</td>
<td>27.00 ± 17.59</td>
<td>A vs C</td>
<td>11.21 **</td>
</tr>
<tr>
<td>Asthenoteratozoospermia (C) (n = 18)</td>
<td>17.72 ± 3.23</td>
<td>B vs C</td>
<td>2.12 *</td>
</tr>
<tr>
<td>Oligoasthenoteratozoospermia (D) (n = 9)</td>
<td>10.78 ± 2.77</td>
<td>C vs D</td>
<td>5.30 **</td>
</tr>
</tbody>
</table>

Table 11: Sperm morphology in percentage

Value are mean ± SD
Assessment of Oocyte Quality with AMH, Inhibin B, in Serum and Follicular Fluid and Predicting Pregnancy outcome with Sperm DNA Fragmentation in Art Cycles

When the average mean sperm normal morphology of normozoospermia (29.95±3.30) was compared with asthenoteratozoospermia (17.72±3.23), it was observed that the sperm normal morphology was decreased in asthenoteratozoospermia and was statistically significant at 1% level.

When the average mean sperm morphology of normozoospermia (29.95±3.30) was compared with oligoasthenoteratozoospermia (10.78±2.77), it was observed that the sperm normal morphology was decreased in oligoasthenoteratozoospermia and was statistically significant at 1% level.

When the average mean sperm morphology of teratozoospermia (27.00±17.59) was compared with asthenoteratozoospermia(17.72±3.23), it was...
Results and Discussion

Assessment of Oocyte Quality with AMH, Inhibin B, in Serum and Follicular Fluid and Predicting Pregnancy outcome with Sperm DNA Fragmentation in ART Cycles

observed that the sperm normal morphology was decreased in asthenoteratozoospermia and was statistically significant at 5% level.

When the average mean sperm morphology of teratozoospermia (27.00±17.59) was compared with oligoastheno teratozoospermia (10.78±2.77), it was observed that the sperm normal morphology was decreased in oligoasthenoteratozoospermia and was statistically significant at 1% level.

When the average mean sperm morphology of asthenoteratozoospermia (17.72±3.23) was compared with oligoasthenoteratozoospermia (10.78±2.77), it was observed that the sperm normal morphology was decreased in oligoasthenoteratozoospermia and was statistically significant at 1% level.

Sperm morphology in terms of the proportion of normal forms has been shown to be significantly related to in vivo conception (Bostofte 1984), in vitro fertilization (Kruger et al., 1986, 1988) in vitro tests of sperm function (Aitken et al., 1982) and the assessment of acrosomal normality is predictive of IVF success (Jeulin et al., 1986)

Our study correlated with calogero et al., (2001) found teratozoospermia groups have sperm aneuploidy rate similar to that oligiasthenoteratospermia patients have high no. of abnormal spermatozoa and suggested that teratozoospermia was a critical parameter associated with aneuploidy.

Cretzee et al., (1998) concluded in their closing remarks that normal sperm morphology had a role to play in diagnosis of male fertility potential. Low morphology resulted in low fertilization rate and embryo development as of it our study where oligozoospermia cases have very low normal forms.

4.8 Sperm DNA fragmentation Index

4.8.1 In raw and prepared sample in control and infertile men

...
Sperm DNA assessment was performed in the smears made from raw semen and then also with the processed semen after performing ICSI procedure fragmentation is discussed in Table 12 and Figure 69.

Table 12

DNA fragmentation index between raw and processed semen samples in control and infertile men

<table>
<thead>
<tr>
<th>Groups</th>
<th>Raw(A)</th>
<th>Processed (B)</th>
<th>Groups compared</th>
<th><em>t</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normozoospermia)</td>
<td>10.74 ± 2.52</td>
<td>7.59 ± 4.15</td>
<td>A vs B</td>
<td>3.304**</td>
</tr>
<tr>
<td>Terato</td>
<td>17.00 ± 5.09</td>
<td>11.44 ± 4.16</td>
<td>A vs B</td>
<td>2.389**</td>
</tr>
<tr>
<td>Asthenoterato</td>
<td>15.44 ± 7.48</td>
<td>11.22 ± 6.62</td>
<td>A vs B</td>
<td>1.74*</td>
</tr>
<tr>
<td>OAT</td>
<td>22.78 ± 2.77</td>
<td>18.44 ± 3.35</td>
<td>A vs B</td>
<td>3.02**</td>
</tr>
</tbody>
</table>

Value are mean ± SD

* - significant at t<0.05  ** - Significant at t<0.01

Figure 69

DNA fragmentation index between raw and processed semen samples in control and infertile men
The average mean value of processed semen sample (7.59 ± 4.15%) was compared with raw semen sample (10.74±2.52) in control groups it was found that there was an decrease in DFI in processed semen sample which was statistically significant at 1% level.

The average mean value of processed semen sample (11.44±4.16%) was compared with raw semen sample (17.00±5.09) in teratozoospermia it was found that there was an decrease in DFI in processed semen sample which was statistically significant at 1% level.

The average mean value of processed semen sample (11.22±6.62%) was compared with raw semen sample (15.44±7.48) in asthenoteratozoospermia and it was found that there was an decrease in DFI in processed semen sample which was statistically significant at 5% level.

The average mean value of processed semen sample (18.44 ± 3.35%) was compared with raw semen sample (22.78 ± 2.77%) in oligoasthenoteratozoospermia and it was found that there was a decrease in DFI in processed semen sample which was statistically significant at 1% level.

Tomlinson et al., 2001 showed that simple density gradient centrifugation can enrich the sample both with morphologically normal forms and spermatozoa with improved nuclear integrity. Our present study correlates with this study indicating that there was a significant improvement in sperm DNA damage in processed semen compared with raw semen in control and infertile groups.
4.8.2 Sperm DFI in control and infertile men

The sperm DFI of control and infertile men is discussed in Table 13 and Figure 70.

Table 13

<table>
<thead>
<tr>
<th>Count category</th>
<th>Average %</th>
<th>Groups compared</th>
<th>‘t’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normozoospermia (A)</td>
<td>7.59 ± 4.15</td>
<td>A vs B</td>
<td>3.60**</td>
</tr>
<tr>
<td>Teratozoospermia (B)</td>
<td>11.44 ± 4.16</td>
<td>A vs C</td>
<td>2.79**</td>
</tr>
<tr>
<td>Astheno Teratozoospermia (C)</td>
<td>11.22 ± 6.62</td>
<td>A vs D</td>
<td>8.58**</td>
</tr>
<tr>
<td>Oligoastheno Teratozoospermia (D)</td>
<td>18.44 ± 3.35</td>
<td>B vs C</td>
<td>0.08ns</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B vs D</td>
<td>4.37**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C vs D</td>
<td>2.96**</td>
</tr>
</tbody>
</table>

Value are mean ± SD

* - significant at t<0.05   ** - Significant at t<0.01

Figure 70

Sperm DFI in percentage
When the average mean sperm DFI of normozoospermia (7.59 ± 4.15%) was compared with teratozoospermia (11.44 ± 4.16%), it was observed that the sperm DFI was increased in teratozoospermia and was statistically significant at 1% level.

When the average mean sperm DFI of normozoospermia (67.59 ± 4.15%) was compared with asthenoteratozoospermia (11.22± 6.22%), it was observed that the sperm DFI was increased in asthenoteratozoospermia and was statistically significant at 1% level.

When the average mean sperm DFI of normozoospermia (7.59 ± 4.15%) was compared with oligoasthenoteratozoospermia (18.44 ± 3.35%), it was observed that the sperm DFI was increased in oligoasthenoteratozoospermia and was statistically significant at 1% level.

When the average mean sperm DFI of teratozoospermia (11.44 ± 4.16%) was compared with asthenoteratozoospermia (11.22 ± 6.22%), it was observed that the sperm DFI was statistically insignificant between the two groups.

When the average mean sperm DFI of teratozoospermia (11.44 ± 4.16%) was compared with oligoasthenoteratozoospermia (18.44 ± 3.35%), it was observed that the sperm DFI was increased in oligoasthenoteratozoospermia and was statistically significant at 1% level.

When the average mean sperm DFI of asthenoteratozoospermia (11.22 ± 6.22%) was compared with oligoasthenoteratozoospermia...
(18.44 ± 3.35), it was observed that the sperm DFI was increased in oligoasthenoteratozoospermia and was statistically significant at 1% level.

4.9 Correlation between sperm DNA fragmentation index and fertilization and cleavage rate in control and infertile groups

Sperm DFI was correlated with the fertilization and cleavage rate of control and infertile groups is discussed in Table 14.

Table 14
Correlation between DNA fragmentation index and fertilization and cleavage rate in control and infertile groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Correlation co-efficient (r)</th>
<th>Correlation co-efficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DFI vs fertilization rate</td>
<td>DFI vs cleavage rate</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terato</td>
<td>-0.0776</td>
<td>0.0225&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Asthenoteratozoospermia</td>
<td>0.052&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.050&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>OAT</td>
<td>-0.536&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-0.584&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* - significant at 5% level  
ns – r is not significant

4.9.1 DNA fragmentation and fertilization rate in control and infertile groups

The sperm DNA fragmentation with fertilization rate given in Table 14.

The fertilization did not seem to be influenced by sperm fragmentation were the r = - 0.0776 (t = 0.330) for control groups r = 0.052 (t=0.220) teratozoospermia r = 0.004 (t=0.0256) asthenoteratozoospermia.
The fertilization rate was negatively correlated with sperm DFI in oligoasthenoteratozoospermia were $r = -0.536$ ($t = 1.672$) which is statistically significant.

**4.9.2 DNA fragmentation and cleavage rate in control and infertile groups**

The sperm DNA fragmentation with cleavage rate given in Table 14.

The cleavage rate did not seen to be influenced by sperm DNA fragmentation $r = 0.0225$ ($t = 0.144$) not significant in control groups $r = 0.050$ ($t = 0.050$) not significant in teratozoospermia and $r = 0$ not significant in asthenoteratozoospermia.

The cleavage rate was negatively correlated with sperm DFI in oligoasthenoteratozoospermia were $r = -0.584$ ($t = 2.018$) which is statistically significant ($t<0.05$)

**4.10 Evaluation of Sperm DFI with fertilization, cleavage rate embryo quality, embryo development and pregnancy**

Infertile men with poor semen motility and morphology have increased DNA fragmentation compared with individuals with normal semen parameters (Lopes et al., 1998; Irvine et al., 2000 and Zini et al., 2001). These studies correlated with our results where the oligoasthenoteratozoospermia and asthenoteratozoospermia have low normal forms and high sperm DNA fragmentation index.

Impairments of sperm characteristics were associated with an increase in the proportion of sperm with DNA fragmentation confirming the results of recent studies (sun et al., 1997; Lopus et al., 1998; Gandini et al., 2000; Irvine et al., 2000 and Yonglai et al., 2001).
In our present study for a threshold value above 12% we found a significant negative relationship between sperm DNA fragmentation index and the fertilization in disagreement with some authors (Tomlinson et al., 2001 and Morrisey et al., 2002) but in agreement with others who found sperm DNA fragmentation rate negatively correlated with fertilization rate in ICSI programme (Lopes et al., 1998, Host et al., 2002). Similar to our present study, DNA fragmentation index did not vary for fertilization >40% but increased for fertilization rate <40%.

It is possible that if DNA fragmentation is low, the oocytes are able to repair the damaged sperm DNA (Sakkas et al., 1996 and Ahmadi et al., 1999b) but their capabilities are overloaded in cases of high level of sperm DNA fragmentation index.

Sakkas et al., (1996) postulated that damaged sperm DNA might contribute to failure of sperm DNA decondensation after ICSI, thus resulting in fertilization failure.

Host et al., (2000) assumed that in ICSI the operator tries to select a motile and as far as possible, a morphologically normal spermatozoon, so this till has an improved chance of also having an intact DNA however it can be agreed that a spermatozoon can be considered as normal, and the same time have damaged DNA (Lopes et al., 1998 and Tomlinson et al., 2001).

Several authors showed that in a poor quality sperm population, DNA damage is found at a high level (Sun et al., 1997, Lopes et al., 1998, Gandini et al., 2000, Irvine et al., 1998 and Younglai et al., 2001) however, such sperm with DNA damage would have very few chances of fertilizing the oocyte if...
the IVF procedure was used. But with ICSI, where the choice of the spermatozoon to be injected is made according to very criteria and in case of very poor sperm characteristics, it is even possible to select one normal motile sperm so the risk of injecting one spermatozoon with impaired DNA is high. This correlates with our study where the fertilization rate is negatively correlated with DNA fragmentation index in oligoasthenoteratozoospermia groups where it has low count, low motility, low normal forms and high DFI.

In conclusion our study indicates that the proportion of sperm with DFI influences fertilization rate for a threshold value above 10% and good quality embryos was derived when the DFI was < 10 % which correlates with Benchaib et al., (2003). No pregnancy was obtained if > 20 % of selected sperm were Acridine orange (AO) positive, this factor may have a good predictive value in cases of successive failures of implantation for apparently good quality embryos. DNA fragmentation was higher than 10% the embryo development was lower than 70% whereas this could reach >80 % when DFI<10 %.

Our study was correlated with other studies (Benchaib et al., 2003; Bonde et al., 2003; Seli et al., 2004; Virro et al., 2004 and Henkel et al., 2003, 2004) that fertilization by sperm with fragmented DNA results in poor embryonic development, decreased implantation, lower pregnancy rates and recurrent pregnancy losses.

4.11 Evaluation of positive and negative patients with DFI, AMH and INHIBIN B

A total of 63 patients were taken for the study. For the women the mean age was 34.2±3.8 years. Control patients for females were healthy egg donors of age 24±2.8. Causes of female infertility were tubal deterioration, dysovulation endometriosis and PCOS and uterine anomaly. For the men the mean age was
36.8± 4.5. According to WHO there were 20 normoozoospermia who served as control and 18 were asthenoteratozoospermia, 9 were oligozoospermia and 16 were teratozoospermia.

The mean (±) SD quantity of FSH administered to the patients was 2367± 1561, the average time of stimulation was 11.1±1.8 days and the estradiol rate at the day of puncture of follicles was 1950±846.03pg/ml.

Pickup were performed in which each patient had an average of 6 – 12 oocytes. Sperm DFI assessment was performed on both raw and processed semen sample collected on the day of oocyte retrieval. ICSI procedure was done with all the oocytes. All the females had good and bad fertilization and cleavage. An average of 1 to 3 embryos was transferred to each patient and they were given luteal support.

After 15 days of embryo transfer a β hCG test was performed for all the patients. Out of 63 patients 31 patients got pregnant which was 49.2% pregnancy rate which is a good success rate. Out of 31 patients 6 patients had missed abortions out of which 2 patients were severe oligoasthenoteratozoospermia cases. The 26 patients are till date ongoing pregnancies.

The pregnancy was achieved when the DFI was < 15 % for all the patients. The two missed abortion patients had DFI more than > 15 %. So DFI could serve as a predictor of IVF outcome. So the acridine orange method used to identify sperm DNA damage is cheap and easy method. The Sperm DNA damage test has to be included as an additional parameter with the routine semen analysis