6. DISCUSSION

In spite of advances in the field of ART, extremes of ovarian response are still a common problem in many of the COS programs. So the accurate assessment of the ovarian reserve and response to gonadotropins is essential for increasing the chances of conception. Ours is the first study of its kind to analyse the cut-off value of AMH to predict the extremes of response in the entire study population as well as both PCOS and Non PCOS groups. There are various studies, which have analyzed the cut-off value of AMH excluding PCOS group.

The outcome of an ART program depends on the age of the female partner. In our study, the mean age was 30.8 years. This changing trend of delaying the first childbirth is attributed to the present clinical situation of delayed marriage, postponing the first childbirth due to various social reasons and availability of advanced ART procedures. Nikolaos et al observed a mean age of 31.9 years whereas Ruma Satwik observed a mean age of 33.14 years.

In our study, 54.1% had duration of infertility beyond 6 years. This might be due to delayed referral to a tertiary care center or seeking delayed medical attention due to present scenario of carrier oriented life. This correlates with Razieh et al and Ashraf et al study. The mean duration of infertility was 7.8 years as in Ruma Satwik et al study.

In our study, primary infertility was common (72.8%). This correlates with Shamila et al study where they observed primary infertility was dominant in south
Discussion

India \(^8\) whereas it was 64\% in Ruma Satwik et al study \(^114\). Among secondary infertility, previous history of abortion was contributing to 67.2\%.

In the etiology of infertility, female factor was the commonest (40.2\%) and of them ovarian factor contributed for 67.8\%. This was the commonest indication for ICSI in our study. In Shamila et al study, the prevalence of female factor ranged from 41 - 45 \% among the various districts of Tamil Nadu \(^8\), whereas male factor (72.4\%) was the commonest indication in Razieh et al study \(^115\).

In our study, 62.6\% were in the overweight and obese groups. This might be due to the present day life style of lack of exercise and the food habits. In a study by Majedah et al on the effect of obesity on the outcome of infertility, they observed obesity to have a negative impact on the outcome of infertility management \(^81\).

In our study we observed the mean AMH was 4.4 ± 3.3 ng/ml and the mean AFC of 12.8 ± 6.0. This correlates with Nikolaos study \(^75\). Correlation of AMH and AFC with age showed a statistically significant negative correlation (r = - 0.303 and - 0.343 respectively) whereas FSH showed a positive correlation (r = 0.28). Van Rooij et al also observed a similar finding of negative correlation of age versus AMH \((r = - 0.30)\) and with AFC \((r = - 0.29)\) \(^118\). Similar observation was made in Ashraf et al study \(^116\). **So as age advances, there is a decrease in AMH and AFC levels but serum FSH levels increases.**
In our study, we found an important observation of early decline in AMH and AFC by 30 years unlike FSH, which started rising only by 35 years. This is very important for counselling a couple who wants to postpone pregnancy for various reasons. So AMH and AFC are early markers of declining ovarian reserve and we cannot rely on serum FSH levels, which are done routinely in many of the ART clinics. Kelton et al also had a similar observation in their study where they observed that, despite a 50% fall in serum AMH levels between 29 - 37 years, there were minimal changes seen in serum FSH levels. So they concluded that serum AMH assessment is superior to serum FSH levels in identifying women with reduced ovarian reserve 18.

In our study 67.5% had agonist protocol for COS. Many of the ART centers prefer to decide the initial dose of gonadotropins based on the age, basal FSH levels and the presence or absence of PCOS. But in our study, the decision for the type of protocol and the initial dose of gonadotropins were based on the age, BMI, ovarian reserve markers and the previous response to stimulation. The average number of days of stimulation was around 12 days.

In our study, we noticed a 3.6 % cancellation of the cycle due to poor follicular response to gonadotropins. The mean number of oocytes retrieved in the total study population was around 13.7 unlike in Razieh et al study, which were only 6.5 115. This may be due to a smaller sample size and inclusion of only poor and normal responders and starting with low dose of gonadotropins in the latter study.
Correlation of ovarian reserve markers with the oocytes retrieved showed a positive correlation with AMH ($r = 0.759$) and AFC ($r = 0.626$) and a negative correlation with FSH ($r = -0.312$). So as the value of AMH and AFC increases, the number of oocytes retrieved also increases. This correlates with Jae Eun Lee study $^{49}$ where $r = 0.70$ with AMH. But in Van Rooij et al study correlation with AMH, AFC and FSH, $r = 0.648, 0.495$ and $-0.395$ respectively $^{117}$ and in Ruma et al study $^{114}$, correlation of AMH with oocytes retrieved was $r = 0.591$.

In our study AMH correlated with age, AFC and oocytes retrieved. So AMH levels may reflect the size of antral follicle pool and hence provide an idea about the anticipated number of oocytes retrieved after COS and this correlates with Ashraf et al study $^{116}$.

In agreement with our study, Seifer et al also demonstrated that a high AMH level was associated with more number of oocytes retrieved. So basal AMH and AFC are good tools for counselling the patients $^{118}$. However accurate assessment of AFC depends on the clinician’s expertise, technical properties of the ultrasound machine used, inter cycle and inter observer variability. In contrast, AMH levels are obtained in the laboratory by objective measurements and are free from intra and inter observer variability.

In our study, embryo transfer was done in 77.2%. One of the important reasons for postponing embryo transfer was the risk of OHSS (10.2%). The pregnancy rate in our study was 37.7%. This is in par with the global success rate in many of the IVF centres. In Europe, Devroey et al observed (2004), a pregnancy
Discussion

rate of 27.6% with ICSI \(^{119}\) whereas Gnoth et al observed a clinical pregnancy rate of 47% \(^{69}\).

Most of the studies have classified the response into poor, normal and hyper based on the number of oocytes retrieved. But there is no clear consensus for the number of oocytes retrieved. To label as poor response, various studies have taken the number of oocytes retrieved from 2 to 6. Nelson et al have taken \(\leq 2\) oocytes \(^{63}\), while many studies have taken a cut off of \(\leq 4\) oocytes \(^{117, 120, 18}\). Ficicioglu has taken \(\leq 5\) oocytes \(^{121}\) and \(< 6\) oocytes by Freour \(^{122}\). According to Bologna criteria, to define poor response in IVF, at least 2 of the 3 features must be present i) Advanced maternal age or any of the risk factor for poor ovarian response (POR). ii) Previous POR. iii) Abnormal ORT (AFC < 5 -7 follicles, AMH < 0.5 -1.1ng/ml) \(^{61}\).

In a recent update by La Marca and Sunkara (2014) to predict POR, AMH value should be updated from 0.7 to 1.3 ng/ml with AFC value remaining the same as before \(^{65}\).

In our study, we have taken a cut off value for poor response when oocytes retrieved were \(\leq 3\), normal response when oocytes retrieved were between 4 to 19 and hyper response when oocytes retrieved is \(\geq 20\). This is similar to Jae Eun Lee et al study \(^{49}\).

Accordingly in our study, 13.8% had poor response, 62.2% had normal response and 24% had hyper response. A similar distribution of poor responders of 23%, 63% normal responders and 14 % of hyper responders were observed in
Ruma Satwik et al study\textsuperscript{114} but they had taken the cut off value of more than 15 oocytes retrieved for hyper response.

In our study, majority of the patients (67.6\%) in the poor response group were more than 30 years and there were no patients more than 35 years in the hyper response group. This correlates with Nardo et al study where they also found more women with advanced maternal age in the poor response group\textsuperscript{123}. Similar observations were made in Ashraf et al\textsuperscript{116} Jae Eun Lee et al studies\textsuperscript{49}. This changing trend of postponing pregnancy, which is common in western world, is seen even in India with increase in carrier-oriented couples. Min Hye Choi et al observed a decreasing pattern of age from poor responders to hyper responders\textsuperscript{124}.

In our study, we found 55.2\% of the couples with duration of infertility beyond 5 years, of them around 62\% were in the poor response group. This might be due to the couple planning their first childbirth beyond 30 years for various social reasons. This correlates with Neeta Singh et al study where they found the duration of infertility was more in the poor response group\textsuperscript{125}. This study is similar to our study in being conducted in our Indian population.

In our study, we did not find any significant difference in the type of infertility among the three response groups. This correlates with Ruma Satwik et al study\textsuperscript{114}.

Nardo et al in his study on AMH levels in predicting the ovarian response did not find any significant difference in the distribution of BMI among the three
response groups. Even in our study, we did not observe any significant difference in the distribution of BMI among the three response groups.

Comparison of ovarian reserve markers among the three response groups showed a statistically significant difference. Similar to Ashraf et al study and Muthu Krishna et al study, we found a significantly lower AMH levels in poor response group when compared to normal and high response groups. We also found the baseline FSH levels and not E2 were significantly higher in poor response group compared to normal and hyper response group as in Van Rooij et al study.

The mean AMH in our study for poor response was $0.85 \pm 0.5 \text{ng/ml}$, $3.8 \pm 2.4 \text{ng/ml}$ for normal response and $8.1 \pm 3.0 \text{ng/ml}$ for hyper response. In Jae Eun Lee et al study the corresponding values were $0.94 \pm 0.15 \text{ng/ml}$, $2.79 \pm 0.21 \text{ng/ml}$ and $6.94 \pm 0.9 \text{ng/ml}$ respectively and this is almost similar to our study. Min Hye Choi et al also observed an increasing pattern of serum AMH from $0.9 \pm 0.7$ among poor responders, $2.9 \pm 2.2$ in normal responders to $7.6 \pm 2.9$ among hyper responders and this is comparable with our study. A similar observation was made in La Marca et al study.

In our study, we had nine patients from the poor response group, who did not undergo ICSI in view of the poor follicular growth of them five cycles were cancelled and four cycles were converted into IUI. There were no cycle cancellations in hyper response group.
In our study we found a significantly increased requirement of gonadotropins in the poor response group without much difference in the duration of stimulation. This might be due to the individualisation of the initial dose of gonadotropins. This correlates with Razieh et al study 115.

Nelson et al studied the role of both serum AMH and serum FSH in predicting live birth and extremes of response in IVF cycles. They concluded that age was positively correlated with FSH, and was negatively related to AMH and oocyte yield. In their study, increasing FSH was associated with a reduction in AMH and oocyte yield while AMH demonstrated a remarkably strong correlation to oocyte yield and appeared to be the best predictor of oocyte yield 63. This correlates with our study in AMH and AFC showing a strong positive correlation with oocytes retrieved and FSH showing negative correlation. Comparison of the correlation of AMH and AFC and FSH with oocyte yield showed AMH to be the better predictor than AFC and FSH as in Arce et al study 127.

We also observed a significantly more number of follicles, oocytes retrieved, MII oocytes and oocytes fertilised in hyper response group when compared to normal and poor response groups. But there was no significant difference in the number of patients undergoing embryo transfer and also the pregnancy rate even though the pregnancy rate was better in hyper response group when compared to poor response. Similar observation was made for pregnancy rate in Ashraf et al study 116 and La Marca et al study 126.
The cut off values of AMH to predict the poor response varied as these studies defer, in the uniformity of the AMH assay method and criteria for defining the poor response. Hence there is no clear-cut value till date in predicting the extremes of ovarian response. Various studies quote the cut off value of AMH to predict poor response from 0.1 to 2 ng/ml, this accounts to almost 20-fold variation. So a definitive cut off value to predict the poor response was sought.

To identify the best parameter that would yield optimum combination of false positive and false negative values and for prediction of the number of oocytes retrieved, we used logistic regression and ROC curve analyzes for AMH, AFC and FSH. In our study, for poor response, we found that the AUC for AMH (0.986) was better than that for AFC (0.904) and FSH (0.775) with p = 0.0005. This indicates a good discriminating potential of AMH in predicting the poor response when compared to AFC and FSH. So the optimal cut off value for AMH in our study to predict poor response was 1.25ng/ml or less, which showed a sensitivity of 91.2%, specificity of 96.2% and positive predictive value of 79.5% and a negative predictive value of 98.5%. In our study, we also observed a cut off value of less than 7.7 for AFC and a value more than 7.75mIU/ml for FSH.

Jayaprakasan et al compared three-dimensional ultrasound parameters, antral follicle count (AFC), ovarian volume, and ovarian vascularity indices with AMH for the prediction of poor response to COS during ART. Their results show that AFC and AMH are the most significant predictors of poor response to ovarian stimulation during ART and this correlates with our study.
Budi Wiweko study (2010) correlated with our study, where they found a cut off value of age more than 35 years (ROC \(_{\text{AUC}}\) 0.707), FSH more than 7.25 IU/L (ROC \(_{\text{AUC}}\) 0.765), AFC less than 7.5 (ROC \(_{\text{AUC}}\) 0.843) and AMH of less than 1.4ng/ml (ROC \(_{\text{AUC}}\) 0.846) predict poor ovarian response \(^{128}\).

Like AMH, AFC also showed a considerable variability in agreed cut off values. It ranged from <3 (Chang et al) \(^{66}\) to < 12 (Melo et al) \(^{67}\). A possible reason for this variability is the absence of standardisation of measurement of AFC with difference in the size of follicles from 2 - 6 mm to 2 -10mm and also due to inter observer variation. According to Bologna criteria, the cut off value is taken as < 5 to < 7 \(^{61}\).

The cut off value of FSH was 7.75mIU/ml in our study. Budi Wiweko et al observed a cut off value of more than 7.25 IU/L to predict poor response \(^{128}\). In Bologna criteria, FSH is not included in the criteria due to its various disadvantages like low sensitivity, inter cycle variability and inter lab variability \(^{61}\).

Muthukrishna et al in their study found a cut off value of AMH of 0.2 ng/ml (sensitivity - 87%, specificity - 64%), AFC of 5 (sensitivity - 89%, specificity - 39%), serum FSH value of 10mIU/ml (sensitivity - 87%, specificity - 60%) to predict poor response \(^{120}\). This varied from our study as the AMH was assayed by Immunotech method where the values are up to 40% lower than the present generation assay and difference in AFC could be due to subjective and inter cycle variability.
Jayaprakasan et al (2008) in their prospective study of 135 patients observed a cut off value of AMH of 0.99ng/ml had a sensitivity of 100% and specificity of 73%. In this study AMH was assayed by DSL method. With the conversion to AMH gen II assay it is equivalent to 1.37 ng/ml. This difference in the value from our study might be due to the sample size and the selection criteria as our study included both PCOS and Non PCOS women.

Gnoth et al (2008) in their prospective study evaluated the relevance of AMH in routine IVF program and observed a cut off value of 1.26ng/ml to predict poor response had a sensitivity of 97% and specificity of 62% after adjustment for age. They also observed 98% correct prediction of normal response if levels were above this threshold. They concluded that levels ≤ 1.26 ng/ml are highly predictive of reduced ovarian reserve and should be confirmed by a second line antral follicle count. This cut off value to predict poor response is similar to our study but it varies in the definition of poor response to ≤ 4 oocytes unlike our study where we have considered ≤ 3 oocytes as a cut off for poor response.

Handan et al (2012) in their study evaluated the predictive value of random serum AMH in the assessment of ovarian response in patients with DOR. They observed a serum AMH cut off value of ≤ 1.2 ng/ml for prediction of risk of cycle cancellation, had a sensitivity of 97.3% and specificity of 31.3% with a positive predictive value of 33.9% and negative predictive value of 96.9% in women with high baseline FSH levels. Even though this correlates with our study, it differed from our study in retrospective study design, including patients with DOR only and random serum AMH assay.
Sherif et al (2012) in their prospective study on serum AMH and FSH as predictor of POR in ART observed that serum AMH with an optimal cut-off value of 1.2 ng/ml had a sensitivity of 91.7% and a specificity of 92.5% and is a reliable predictor of poor ovarian response. Serum basal FSH with an optimal cut-off value of 8.9 mIU/ml was of lower value than AMH as a predictor of poor ovarian response with a sensitivity of 83.5% and a specificity of 81.4%. This study correlates with our study in the values, study design, AMH assay method. But it differs in the criteria for poor response taken as < 4 mature oocytes and for hyper response as > 16 mature oocytes in their study 131.

Another study by Mutlu et al (2013) observed a cut off value between poor and normal ovarian response as 0.94ng/ml with sensitivity of 70% and specificity of 86% and for the same cut off with AFC of 5.5 had a sensitivity and specificity of 91% each respectively 132.

Sezai et al (2013) in their prospective study in Japan evaluated the clinical value of day 3 AMH with AFC, FSH and E2 on the day of hCG in the prediction of POR to COS. They observed day 3 AMH and E2 on the day of hCG were more predictive compared to day 3 FSH and AFC. The cut off value of AMH was \(< 2\)ng/ml to predict POR with the sensitivity of 78.9% and specificity of 73.8% 133. This difference in value may be due to ethnic and environmental factors. This study varied from our study in AMH assay by DSL method, criteria for POR as < 3 oocytes retrieved and excluding PCOS group.
Nikolaos et al (2013) had defined the response groups similar to our study and observed a cut off value of AMH of 1.37ng/ml (sensitivity - 74.1%, specificity - 77.5%), AFC of 8 (sensitivity- 2.2%, specificity - 84.6%) for predicting poor response. This is almost similar to our study. Similarly for predicting the hyper response, the cut off value of AMH was 3.52 ng/ml (sensitivity - 89.5%, specificity - 83.8%), AFC was 16 (sensitivity - 80%, specificity - 84.5%). This cut off value of AMH for predicting hyper response is slightly lower than in our study 76.

La Marca et al (2014) reviewed the cut off value of AMH for predicting poor response to gonadotropin stimulation 65. [Table - 49]

**TABLE 49: AMH cut off values for poor response (La Marca et al) 65**

<table>
<thead>
<tr>
<th>References (et al)</th>
<th>Study design</th>
<th>N</th>
<th>Cut off value</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>AMH assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonilla - Musoles (2012)</td>
<td>Retros</td>
<td>143</td>
<td>1.3</td>
<td>69</td>
<td>64</td>
<td>IBC</td>
</tr>
<tr>
<td>Satwik (2012)</td>
<td>Prosp</td>
<td>198</td>
<td>2</td>
<td>20</td>
<td>98</td>
<td>DSL</td>
</tr>
<tr>
<td>Lee (2012)</td>
<td>Prosp</td>
<td>162</td>
<td>1.08</td>
<td>85.8</td>
<td>78.6</td>
<td>IBC</td>
</tr>
<tr>
<td>Honnma (2012)</td>
<td>Retros</td>
<td>456</td>
<td>1.4</td>
<td>72.2</td>
<td>75.7</td>
<td>IBC</td>
</tr>
<tr>
<td>Arce (2013)</td>
<td>Retros</td>
<td>759</td>
<td>1.68</td>
<td>92</td>
<td>83</td>
<td>AMH GenII</td>
</tr>
<tr>
<td>Polyzos (2013)</td>
<td>Retros</td>
<td>210</td>
<td>1.37</td>
<td>74.1</td>
<td>77.5</td>
<td>AMH GenII</td>
</tr>
</tbody>
</table>

Retros - Retrospective; Prosp - Prospective

All these studies defer in study design, sample size, definition of poor response or the assay method.
Discussion

When we plotted the ROC curve to predict the hyper response, we found for a cut off value of AMH of 5.65ng/ml, with a sensitivity of 86.4%, specificity of 86.6%, positive predictive value of 67.1% and negative predictive value of 95.3%. Again AMH had a better AUC (0.932) when compared to AFC (0.894) and FSH (0.632).

La Marca in his meta analysis showed the cut off values of AMH in various studies (Table 50).

<table>
<thead>
<tr>
<th>References et al</th>
<th>N</th>
<th>Study Design</th>
<th>Cut off value</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>AMH assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoonian (2009)</td>
<td>159</td>
<td>Prosp</td>
<td>4.83</td>
<td>93</td>
<td>78</td>
<td>IBC</td>
</tr>
<tr>
<td>Honnma (2012)</td>
<td>456</td>
<td>Retros</td>
<td>2.46</td>
<td>69</td>
<td>75</td>
<td>IBC</td>
</tr>
<tr>
<td>Anckaert (2012)</td>
<td>731</td>
<td>Retros</td>
<td>4.17</td>
<td>82.5</td>
<td>70.4</td>
<td>IBC</td>
</tr>
<tr>
<td>Lee (2012)</td>
<td>162</td>
<td>Prosp</td>
<td>3.57</td>
<td>94.4</td>
<td>83.3</td>
<td>IBC</td>
</tr>
<tr>
<td>Arce (2013)</td>
<td>759</td>
<td>Retros</td>
<td>3.9</td>
<td>78</td>
<td>67</td>
<td>AMH GenII</td>
</tr>
<tr>
<td>Polyzos (2013)</td>
<td>210</td>
<td>Retros</td>
<td>3.52</td>
<td>89.5</td>
<td>83.3</td>
<td>AMH GenII</td>
</tr>
</tbody>
</table>

Retros - Retrospective; Prosp - Prospective

Again there is difference in the cut off value of AMH to predict the hyper response due to difference in the definition of hyper response, based on oocytes retrieved varying from 15 to 21, and the AMH assay method.

Aflatoonian et al (2009) in their prospective study included 159 women, observed a cut off value of 4.83ng/ml with sensitivity of 93% and specificity of
Discussion

Anti Mullerian hormone as a marker of ovarian reserve and predictor of ovarian response in women undergoing controlled ovarian stimulation for Assisted Reproductive Technology

78% to predict hyper response\textsuperscript{138}. This study varied from our study by a smaller sample size and AMH assayed by IBC method.

Ocal et al (2011) in their retrospective study of 695 women observed a cut off value of AMH of 3.3 ng/ml with a sensitivity of 90% and specificity of 71%. With the conversion to AMH Gen II assay, the corresponding value is 4.6ng/ml\textsuperscript{140}. This study is similar to our study in AMH assay and cut off value almost the same but varies only in the study design.

Usta et al (2012) focused on the clinical usefulness of AMH in predicting the success of IVF. They observed a serum AMH value $\geq 3.5$ng/ml can predict hyper response / OHSS. So AMH assay can reduce the risk of OHSS, optimize treatment burden and maintain pregnancy rates\textsuperscript{141}.

There are various studies predicting the cut off values of AMH both for poor and hyper response. Nardo et al (2009) in their prospective cohort study, evaluated the clinical value of AMH with AFC and FSH to predict the extremes of response in women undergoing COS, they observed that AMH performed better (ROC\textsubscript{AUC} 0.88) than FSH (ROC\textsubscript{AUC} 0.81) and not AFC (ROC\textsubscript{AUC} 0.81) which had similar ROC\textsubscript{AUC} to predict poor response. Similarly for predicting the hyper response AMH performed better (ROC\textsubscript{AUC} 0.81) than FSH (ROC\textsubscript{AUC} 0.66) and AFC (ROC\textsubscript{AUC} 0.69). The cut off value of AMH of 1.0 ng/ml to predict poor response had a sensitivity of 87% and specificity of 67%. With conversion to AMH gen II assay, the same value is equivalent to 1.39 ng/ml. For hyper response, the cut off value of AMH $> 3.5$ ng/ml had a sensitivity of 88% and specificity of...
70% and with conversion to AMH gen II assay, the same value is equal to 4.8 ng/ml. This study is similar to our study in including both the PCOS and Non PCOS group of women and observed that the prediction of AMH was independent of PCOS.

In Min Hye Choi et al study to investigate whether AMH could predict the poor or hyper response and IVF outcome, found the cut off value of AMH for poor response was 1.05ng/ml (ROC \(_{AUC} = 0.85\), sensitivity of 74% and specificity of 87%). For hyper response, the cut off value of 3.55 ng/ml (ROC \(_{AUC} = 0.91\), sensitivity of 94% and specificity of 81%). So they concluded that serum AMH could be used to predict the number of oocytes retrieved in patients to identify poor and hyper response. This study differed from our study by retrospective study design and the AMH assay done by Immunotech method, which might be the reason for lower AMH values as cut off value.

Joan et al in their multicentric trial predicted the cut off value of AMH in antagonist cycles and observed the AMH cut off values for prediction of poor response were 13 pmol/L [1.82ng/ml](sensitivity 66%, specificity 80%) for hphMG stimulation, and 12 pmol/L [ 1.68 ng/ml] (sensitivity 92%, specificity 83%) for recombinant FSH stimulation. The AMH cut off values for prediction of hyper response were 28 pmol/L 3.92ng/ml] (sensitivity 78%, specificity 67%) for hphMG stimulation and 31 pmol/L [ 4.34ng/ml] (sensitivity 76%, specificity 74%) for recombinant FSH stimulation. This study is similar to our study in AMH assay by Gen II method but the criteria for poor response was \(\leq 3\) oocytes and for hyper response was \(\geq 15\) oocytes.
Jae Eun Lee (2012) in their retrospective study on the clinical application of AMH as a predictor of COS outcome observed a cut off value of AMH of 1.08ng/ml for POR had a sensitivity of 85.8% and specificity of 78.6%. For hyper response the cut off value of 3.57ng/ml had a sensitivity of 94.4% and specificity of 83.3%. This study is similar to our study in defining the criteria of response but differs in AMH assay by IOT method and a retrospective study design. This can explain the differing cut off values.

Nikolaus et al (2013) in their study to predict the cut off value of AMH and AFC observed a cut off value of AMH of 1.37ng/ml for predicting POR had a sensitivity of 74.1% and specificity of 77.5% (AUC - 0.836) and AFC cut off value of 8 with sensitivity of 72.2% and specificity of 84.6% (AUC - 0.830). For predicting the hyper response, the cut off values for AMH were 3.52ng/ml with sensitivity of 89.5% and specificity of 83.8% (AUC - 0.890). For AFC the same cut off values were 16 with the sensitivity of 80% and specificity of 84.5% (AUC - 0.897). They concluded that AMH and AFC are the best predictors for low and excessive response in women treated with corifollitropin alfa in an antagonist protocol.

According to Nikolaou et al young women with poor ovarian response to ovulation induction are at risk of developing early menopause. This confirms that ovarian response to ovulation induction can be used as a surrogate marker for the overall ovarian reserve. As AMH is a better predictor of ovarian response, it is also a better predictor of ovarian reserve as well.
Discussion

From our study, with the AMH value of 1.25ng/ml and below, appeared to be a reasonable cut off to predict poor response, however there is no value of AMH to identify non-response. So with the available evidence, no women can be excluded from the ART program, but this information will be useful for counselling regarding the type of response, avoiding repeated cycles if the women had poor response in the first cycle. Hence this could help in deciding the stimulation protocol and the initial dose of gonadotropins.

Similarly the AMH value of 5.65ng/ml and above can predict hyper response and hence can decide the stimulation protocol, initial dose of gonadotropins.

The kit manufacturers reference range interprets AMH levels as - “Healthy women, below 38 years with normal follicular status at day 3 of the menstrual cycle, have AMH levels of 2.0 - 6.8 ng/ml”. The reference range for AMH quoted is

<table>
<thead>
<tr>
<th>Ovarian Fertility potential</th>
<th>pmol/L</th>
<th>ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal Fertility</td>
<td>28.6 - 48.5</td>
<td>4.0 - 6.8</td>
</tr>
<tr>
<td>Satisfactory Fertility</td>
<td>15.7 - 28.6</td>
<td>2.2 - 4.0</td>
</tr>
<tr>
<td>Low fertility</td>
<td>2.2 - 15.7</td>
<td>0.3 - 2.2</td>
</tr>
<tr>
<td>Very Low / undetectable</td>
<td>0.0 - 2.2</td>
<td>0.0 - 0.3</td>
</tr>
<tr>
<td>High Level</td>
<td>&gt; 48.5</td>
<td>&gt; 6.8</td>
</tr>
</tbody>
</table>
They also mention that the interpretation guide provided are only suggestions based on multiple published studies, which may have refinement of the ranges in the near future.

From all these data in our study, it is clear that AMH and AFC can be used in everyday practice for the prediction of poor and hyper response in patients undergoing COS. Among the two AMH is a better predictor of ovarian response than AFC and FSH based on AUC values.

PCOS is a common endocrine disorder affecting up to 10% of women in the reproductive age group. Since AMH is secreted by granulosa cells of early developing follicles and its serum levels have been shown to correlate with the small antral follicle count. The vast majority of the studies assessing the performance of AMH in the prediction of the ovarian response exclude women with PCOS. So there are not many studies comparing the AMH levels in PCOS and Non PCOS groups. In our study, we aimed to find the cut off value of AMH to predict the extremes of response in both PCOS and Non PCOS groups.

In our study, the study population were subdivided into PCOS and Non PCOS groups based on the Rotterdam’s criteria. Accordingly 31.3 % were in the PCOS group and 68.7% were in the Non PCOS group. In Non PCOS group, women were significantly older than the PCOS group. This could be due to early evaluation of infertile couple presenting with ovulatory disorders, as it is one of the indication for early evaluation of infertile couple. This correlates with Tina et al, Dewailly et al and Priya Bhide et al studies where women in the
Non PCOS group were of advanced age than in the PCOS group. However Pigny et al study \(^9\) and Terhi et al \(^1\) did not show any significant difference in the age distribution among the PCOS and Non PCOS groups.

We did not observe any significant difference in the duration of infertility and BMI, which is similar to Dewailly study \(^\text{145}\) and Sezai et al study \(^\text{133}\). But Terhi et al showed significantly more obese women in the PCOS group \(^\text{102}\). Similarly Priya Bhide observed women with more BMI in PCOS group in a study \(^\text{146}\).

Pigny et al observed a significantly high LH only in PCOS group when compared to Non PCOS group \(^\text{98}\) where as in our study, we observed a significantly more FSH in Non PCOS group and significantly high LH levels in PCOS group but no significant difference in estradiol levels in both the groups. This high FSH in Non PCOS group might be due to more advanced age of the female partner and high LH in PCOS group can be explained by the pathophysiology in PCOS. Priya Bhide et al \(^\text{146}\) also observed similar observation as in our study.

Sub fertile PCOS women secrete significantly more AMH per antral follicle than in PCOM and Non PCOS women \(^\text{146}\). Similarly in our study, we observed nearly a 2 to 3-fold increase in the AMH secretion in PCOS group and almost 2 - fold increase in antral follicle count. Terhi et al also observed that serum AMH was always 2 to 3 fold higher and remained elevated until 40 years of age in PCOS subjects \(^\text{102}\). Pigny et al also observed a 2 to 3 fold increase in AMH in growing follicles of PCOS patients \(^\text{98}\). Sezai et al also observed a significant increase in mean AMH levels in PCOS group \(^\text{133}\).
In our study, we observed a significantly less requirement of gonadotropins in PCOS group when compared to Non PCOS group. This might be due to the individualisation of the initial dose of gonadotropins with no significant difference in the number of days of stimulation. We also observed a significantly more number of follicles, oocytes retrieved, mature oocytes, oocytes injected and fertilised in the PCOS group. This can be explained by more number of antral follicles getting recruited during the stimulation in PCOS group.

Amer et al, observed significantly greater amount and longer duration of stimulation in patients with high AMH than those with low AMH \(^{147}\). But we did not observe these findings in our study.

In the PCOS group, we did not have any patient belonging to the poor response group whereas all patients with poor response were in the Non PCOS group. So we compared between the normal and hyper response groups of PCOS and Non PCOS groups.

There are few studies comparing the PCOS and Non PCOS group, but there are no studies similar to our study comparing the 3 response groups in both PCOS and Non PCOS group.

In our study, there was no significant difference in the age distribution, duration of infertility and BMI among the normal and hyper response subgroups of PCOS and Non PCOS groups. Terhi et al in their study on AMH levels in the late reproductive age group compared PCOS and Non PCOS groups. They did not find any significant difference in the age distribution but observed a significant
difference in the BMI with more obese patients in the PCOS group\textsuperscript{102}. This difference in observation might be due to subgroup analysis rather than between the PCOS and Non PCOS groups overall. Amer et al compared the poor and good responders in PCOS group and they did not observe any significant difference in the age, BMI and duration of infertility\textsuperscript{147}.

In our study the mean FSH, AMH and AFC showed significant difference between the normal responses subgroup of PCOS and Non PCOS but in hyper response group of PCOS and Non PCOS, only for AMH and AFC showed significant difference. This indicates that AMH and AFC are better predictors of hyper response in the PCOS group. Terhi et al\textsuperscript{102} and Amer et al\textsuperscript{147} studies also showed a significant difference in AMH levels among the PCOS and Non PCOS groups.

In our study we observed less number of days of stimulation in the PCOS group of both normal and hyper response subgroup, which was more significant in the hyper response subgroup of PCOS and Non PCOS groups. This could be again due to individualisation of the initial dose of gonadotropins and less dose of gonadotropin requirement in the PCOS group of both normal and hyper response subgroups.

In our study, we observed a significant difference in the number of follicles, oocytes retrieved, MII oocytes, oocytes injected and fertilised among the normal responders of PCOS and Non PCOS group. But the same were not significant in the hyper response subgroup of PCOS and Non PCOS. This could be
due to more number of antral follicles in the normal responders of PCOS group, which may not be of much difference in the hyper responders of PCOS and Non PCOS groups. Even though the number of patients undergoing embryo transfer was significant in the hyper response group, there was no significant difference in the pregnancy rates of normal and hyper response subgroups of PCOS and Non PCOS.

In our study as we did not have poor response subgroup in the PCOS group, the cut off values of ovarian reserve markers to predict poor response in the Non PCOS group remained the same as poor response group of the entire population. Among the hyper response group, the cut off value of AMH in the PCOS group was 6.85ng/ml (ROC AUC 0.722) with a sensitivity and specificity of 66.7% and 68.7% respectively. In the non PCOS group was 4.85ng/ml (ROC AUC 0.959) with sensitivity and specificity of 85.7% and 89.7% respectively. When compared to AFC and FSH, AMH is a better predictor of ovarian response in both PCOS and NON PCOS group.

In Dewailly et al study, the threshold values of AMH and AFC for the diagnosis of polycystic ovaries were 35pmol/L (5ng/ml) and 19 respectively. They also observed that serum AMH level is more reliable than the follicle number for the diagnosis of PCOM with a sensitivity and specificity of 97% and 92% respectively. Sezai et al observed a cut off value of AMH of 3.94ng/ml with 89.8% specificity and 80% sensitivity for PCOS diagnosis.
In Pigny et al study on whether serum AMH is a surrogate to AFC in the definition of PCOS, found a cut off value of 60 pmol/L had a better sensitivity and specificity of 67% and 92% to define PCOS. They also observed a 3 - fold higher AMH levels in PCOS patients. So they concluded that serum AMH is an accurate marker and offers a good diagnostic potency and could be used instead of AFC as diagnostic criteria of PCOS 148.

Amer et al observed a cut off value of AMH of 4.7 ng/ml to have a sensitivity of 100% and specificity of 58% in predicting poor response to hMG ovarian stimulation in PCOS patients 147. They also observed a good response rate in women with AMH < 4.7 ng/ml and 100% poor response rate in women with AMH > 10.2 ng/ml. This does not correlate with our study as we did not observe any patients with poor response in the PCOS group and our study did not have many patients with AMH > 10.2ng/ml. This cut off values are applicable to AMH assay by Uscan method which is 50% of the values obtained by IOT and Gen II assay which are more widely used in clinical practice.

In contrast Seifer et al 119 and Van Rooij et al 117 observed that high AMH levels are known to predict hyper response to gonadotropins in women without PCOS. So what is considered high AMH level in normal women would be an average level in PCOS women.

Dewailly et al observed a serum AMH > 35 pmol/L (> 5ng/ml) appears to be more sensitive and specific than AFC > 19 and they concluded that serum AMH should be included in the current diagnostic classification for PCOS 145.
6.1 STRENGTHS

- Study design - Prospective Cohort study
- All patients enrolled continued till the end of the study
- First study of its kind analysing a) The infertile population (both PCOS and Non PCOS) b) PCOS and Non PCOS groups separately.
- Estimating the optimal cut off value of AMH to predict the response - Poor / Hyper
- Predicting the cut off values of AMH for PCOS /NON PCOS group for extremes of response.

6.2 LIMITATIONS

- Smaller sample size to extrapolate to the entire Indian population.
- Couples attending the outpatient unit of the department of Reproductive Medicine at Sri Ramachandra University only were included.