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Original Research Article

## Correlation and discordance of anti-mullerian hormone with follicle stimulating hormone in infertile women with premature ovarian insufficiency and diminished ovarian reserve

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### ABSTRACT

**Background:** The objective of the study was to explore the correlation and discordance of anti-mullerian hormone (AMH) and follicle stimulating hormone (FSH) in the selected population of premature ovarian insufficiency and diminished ovarian reserve.

**Methods:** This was a retrospective analysis of the data obtained from the women who presented to the Gynae Endocrine Clinic of the Infertility unit of the Department of Obstetrics & Gynaecology from 2015 to 2017. Discordance was defined as abnormal basal FSH (>10 IU/l) with assuring AMH (>1 ng/ml). Statistical analysis was done with SPSS version 23.

**Results:** There were 36 women with premature ovarian insufficiency and 35 women with diminished ovarian reserve. The correlations between basal FSH and AMH are not significant. AMH values are relatively higher in younger age groups. There are extreme high outliers in both POI and DOR groups, more in younger age group. The discordance between AMH and basal FSH was more in women categorized to have diminished ovarian reserve, compared to women with premature ovarian insufficiency.

**Conclusions:** Those women who are younger than 35 years and have high FSH combined with reassuring AMH should be counseled with care regarding the prognosis of their treatment.

**Keywords:** Premature ovarian insufficiency, Diminished ovarian reserve, Follicle stimulating hormone, Anti-mullerian hormone

### INTRODUCTION

Premature ovarian insufficiency (POI) is a condition where there is loss of ovarian function before the age of 40. This is characterized by oligomenorrhoea or amenorrhoea, high serum levels of gonadotropins and low estrogen. European Society of Human Reproduction & Embryology recommends for diagnosis of premature ovarian insufficiency an elevated serum follicle stimulating hormone (FSH) level >25 IU/l on two occasions >4 weeks apart.<sup>1</sup> Diminished ovarian reserve (DOR) is a condition where the response to ovarian stimulation or fecundity is reduced due to decline in

quality or quantity of oocytes compared to women of same age.<sup>2</sup> DOR appears to precede POI in most cases.<sup>3</sup>

For couples struggling to become pregnant, treatment of infertility can be financially and emotionally draining. So adequate counselling about the prognosis of treatment is recommended. Ovarian reserve predicts a woman's response to ovulation stimulation. Estimation of ovarian reserve is done by the woman's chronological age, presence of menstrual abnormalities like oligomenorrhoea, amenorrhoea or hypomenorrhoea, serum FSH, estradiol, anti-mullerian hormone (AMH) or transvaginal sonographic finding of antral follicle count. Before

embarking on expensive therapies like gonadotropin stimulation or in vitro fertilization, potential success can be estimated by ovarian reserve markers like serum FSH, AMH or antral follicle count. Age alone is not enough because the relation between age and reproductive capacity is highly variable.<sup>4</sup> AMH is an ovary specific growth factor expressed in the granulosa cells of growing unselected follicles. FSH is secreted from anterior pituitary gland. When ovulation has occurred, the decrease in estrogen signals the increase in serum FSH secretion. FSH potentially recruits a cohort of follicles in the growing pool. FSH levels are low during early follicular development and gradually rise towards ovulation. As the gonadotropin sensitive antral follicles develop, serum levels of estrogen and inhibin B secreted from growing follicles rise and signals the anterior pituitary to discontinue release of FSH. This feedback loop between ovarian hormones and anterior pituitary accounts for the variation in levels of FSH.<sup>5</sup>

Compared to FSH, AMH do not have intra-cycle variation but its levels have been affected by medications like estrogen progesterone pills or GnRH agonists.<sup>5</sup> For obtaining correct antral follicle count high resolution sonographic system is required. Other obstacles include high inter observer and anatomical variations.<sup>6</sup>

Though AMH and FSH correlate, they do not reflect the same ovarian reserve parameters. FSH reflects the last two weeks of follicular development and maturation, when the follicles are gonadotropin sensitive.<sup>5</sup> AMH mostly represents the young, post primordial to pre antral follicle pool going through early stages of folliculogenesis. There may be an occasional discrepancy between the two markers.<sup>7</sup> The objective of the study was to explore the correlation and discordance of AMH and FSH in the selected population of POI and DOR.

## METHODS

This was a retrospective analysis of the data obtained from the women who presented to the Gynae Endocrine Clinic of the Infertility unit of the Department of Obstetrics & Gynaecology from 2015 to 2017. All the women came for treatment of infertility. Those women who had infrequent menstruation or those women who was 30 years or more had estimation of their basal FSH.

Women having basal FSH >10IU/l but ≤25IU/l were categorized as having diminished ovarian reserve and women who had basal FSH >25 IU/l as premature ovarian insufficiency. All the women were advised to have serum AMH. Basal FSH was serum FSH done on D2-3 of menstrual cycle. Abnormal FSH level (>10IU/l) was confirmed by repeating the blood test more than 4 weeks apart. Serum AMH was done on any random day.

Clinical history was taken in details with emphasis on associated possible etiology. Autoimmune etiology was suspected when there was associated hypothyroidism, diabetes mellitus or bronchial asthma. Subclinical hypothyroidism was defined as serum TSH level >2.5 mIU/l. Fragile X permutation was suspected when there was history of mental retardation in close male relatives. Previous history of ovarian surgery, contact or personal history of tuberculosis, secondary amenorrhea in female relatives were also noted down.

Discordance was defined as abnormal basal FSH (>10 IU/l) with assuring AMH (>1 ng/ml). Statistical analysis was done with SPSS version 23. AMH and FSH were variables with non-Gaussian distribution, so were analyzed for correlation by Spearman rank correlation test. They were log transformed before plotting for correlation.

## RESULTS

There were 36 women with POI and 35 women with diminished ovarian reserve. Table 1 summarizes the clinical characteristics. There were more women with amenorrhoea and less women with oligo-menorrhoea in POI group compared to DOR.

Table 2 describes the etiological associations, which were more or less similar in two groups. Table 3 shows the endocrine parameters. Serum FSH, AMH and estrogen levels did not have normal distribution and so better described as median with interquartile range.

Table 4 summarizes the correlation coefficient and discordance frequency. The correlations are not significant. The discordance was more in women categorized to have diminished ovarian reserve, compared to women with POI.

**Table 1: Clinical characteristics of the subjects.**

Parameters	POI (n=36)	DOR (n=35)
Age (years)	30.34±5.16 (range 22-39)	31.77±5.02(range 22-39)
Duration of infertility (years)	6.43 ±4.09	8.93±5.97
Primary infertility	66.7%	57.1%
Secondary infertility	25%	42.9%
Age at menarche(years)	12.27±2.69	12.82±1.31
Oligomenorrhoea (cycle<4 months)	50%	85.7%
Amenorrhoea (cycle>4 months)	38.9%	11.4%
Hot flushes	50%	42.9%
Vaginal dryness	38.9%	31.4%

**Table 2: Possible etiology of premature ovarian insufficiency and diminished ovarian reserve.**

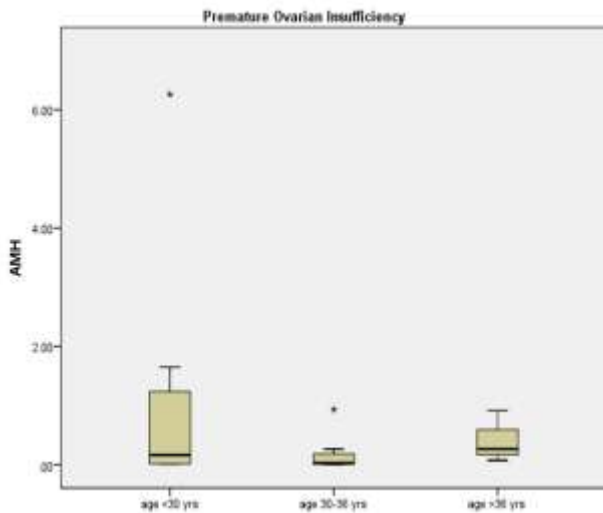
Possible etiology	POI (n=36)		DOR (n=35)	
	number	percentage	number	percentage
Autoimmune diseases	15	41.7	19	54.3
Subclinical hypothyroidism	12	38.3	10	28.6
Family history of secondary amenorrhoea	5	13.9	2	5.7
History suggestive of fragile X syndrome	2	5.6	1	2.9
Ovarian surgery	2	5.6	3	8.6
Contact history of tuberculosis	4	11.1	2	5.7

**Table 3: Endocrine profile of the subjects.**

Parameters	POI (n=36)		DOR (n=35)	
	Median	Interquartile range	Median	Interquartile range
Basal FSH (IU/l)	47.30	35.67-79.27	17.20	14.40-19.91
AMH (ng/dl)	0.16	0.02-0.49	0.50	0.27-0.86
Estrogen(pg/dl)	22.83	15.52-65.00	35.69	16.27-82.56

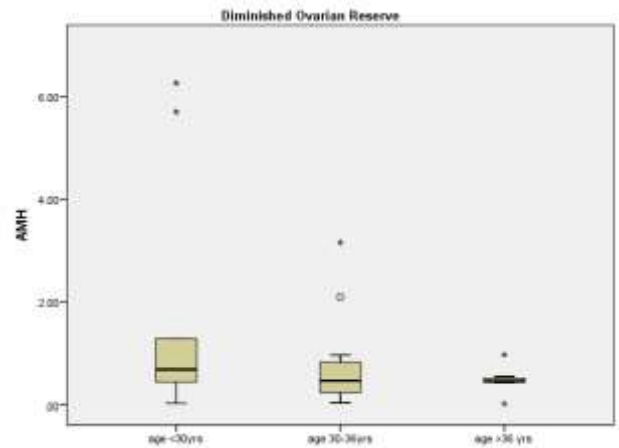
**Table 4: Correlation and discordance of basal FSH and AMH.**

	POI	DOR
Spearman's rho	-0.343	-0.220
Discordance	11.1% (4/36)	17.1% (6/35)

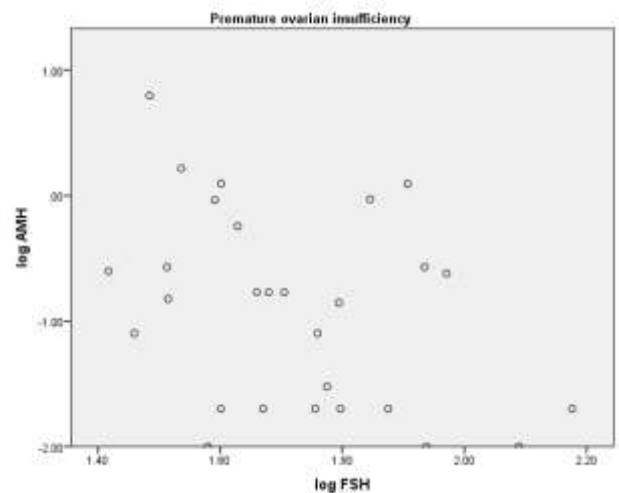


**Figure 1: Distribution of AMH levels in different age groups of POI.**

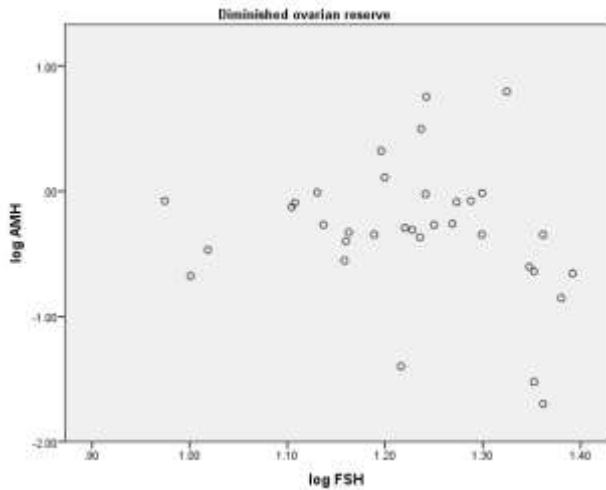
Figure 1 and 2 display box and whisker plots of distribution of AMH in different age groups namely the women less than 30 years, 30-36 years and >36 years. The boxes indicate interquartile range while the intersecting line through the box indicate the median value. AMH values are relatively higher in younger age groups. There are extreme high outliers in both POI and DOR groups, more in younger age group. Figure 3, 4 show scatterplots of correlation of AMH with FSH in women with POI and DOR.



**Figure 2: Distribution of AMH levels in different age group of DOR.**



**Figure 3: Correlation of AMH and FSH in POI.**



**Figure 4: Correlation of AMH and FSH in DOR.**

## DISCUSSION

The objective of the study was to explore the correlation and discordance of serum FSH and AMH levels in women with diminished ovarian reserve and premature ovarian insufficiency. We found no significant correlation between the FSH and AMH. There is discordance between AMH and FSH, more frequent in women of younger age and in women with diminished ovarian reserve than in women with premature ovarian failure.

Ovarian reserve is an indirect measure of the women's reproductive age, or the remaining reproductive lifespan as opposed to chronological age.<sup>5</sup> Ovarian reserve is assessed in infertile women for several reasons. The ovarian reserve markers help predict the response to controlled ovarian stimulation, facilitate appropriate pre-treatment counseling, help decide individualized modified treatment protocol in an attempt to maximize success. Besides FSH and AMH, antral follicle count is another ovarian reserve marker.<sup>6</sup> The age-related decrease in female fertility is attributable to the waning of the quantity and quality of oocytes in the ovaries. But the relation of age and the ovarian reserve markers is highly variable.<sup>8</sup>

Ovarian reserve reflects the finite amount of oocytes within primordial follicles. Folliculogenesis begins when follicles leave this primordial pool to become primary follicle and then antral follicle. Anti-Mullerian hormone is a dimeric glycoprotein hormone of transforming growth factor beta family, secreted by the granulosa cells of the recruited follicle cohort of primary, pre-antral and early antral (<2mm, not visible by transvaginal ultrasonogram) or upto 4 mm, follicles.<sup>9</sup> Most of the follicles in ovary are primordial follicles. AMH begins to be significantly expressed after a potential FSH recruitment and transition of primordial follicles to primary follicles have occurred. Antral follicles (2-9 mm

follicles visible by transvaginal ultrasonogram) are responsive to FSH stimulation. The ovaries and the brain communicate through a feedback loop that exists between the hormones of ovaries released from developing follicles (estrogen and inhibin B) on one hand and the gonadotropin releasing hormones of hypothalamus and gonadotropins (FSH) on the other hand. High basal FSH is associated with poor ovarian reserve.

AMH is produced by the Sertoli cells of early differentiating testes in male fetus. AMH has a primary role in regression of mullerian duct and continues to be expressed after development of male genital tract is complete. AMH is not detected in women until puberty and reaches its highest level at age 24.5 years.<sup>10</sup> AMH is an ovary specific growth factor and the blood level of AMH reflects the size of the primordial pool. AMH expression in granulocytes of growing primary, pre antral and early antral follicles is independent of HPO axis and because of continuous non cyclic growth of small follicles in the ovary, it does not fluctuate throughout the cycle. Serum AMH levels are more tightly correlated with antral follicle count than age or serum FSH.<sup>6</sup> AMH can be used in early assessment of diminished ovarian reserve which precedes premature ovarian insufficiency in most women.<sup>3</sup>

Estimation of serum AMH is costly (at least 5 times that of FSH) and lacks international assay standard.<sup>6</sup> Serum FSH estimation is technically easier and costs less, but has significant intra-cycle and inter-cycle variation. This can be resolved by taking the blood sample in early follicular phase or day 2-4 of the cycle and repeating the test in next cycle or >4 weeks apart as appropriate. AMH estimation can be undertaken any time of the cycle. For cost effective benefits, we screened the women who had oligo-menorrhoea or amenorrhoea or who were  $\geq 30$  years for poor ovarian reserve with basal serum FSH, two times >4 weeks apart. When both the FSH levels were >10 IU/l, we did serum AMH. We considered serum AMH levels <1 ng/dl suggestive of DOR.

The study compares the clinical, etiological and endocrine features of women with POI and DOR. Though the two conditions are distinct from each other, when they were the presenting in infertile women, we explored them to see if one was the continuum of the other. There were similarities in clinical presentations and etiological associations. Amenorrhoea was more common in premature ovarian insufficiency. Besides autoimmunity one important finding was the association of fragile X pre-mutation in both groups. Permutation in FMR1 gene is the X-linked cause of premature ovarian insufficiency. The FMR1 gene contains a unstable trinucleotide CGG repeats in the 5' unsaturated region and the full mutation (>200 CGG repeats) is the most common inherited form of mental retardation in males, the fragile X syndrome. The prevalence of premature ovarian insufficiency in the women carrying the so-called permutation (between 55-200 CGG repeats) is 12-28%.<sup>3</sup> The women with

diminished ovarian reserve, formerly called premature ovarian aging should be screened for etio-pathogenesis as they may have genetic or autoimmune factors similar to that of premature ovarian insufficiency.<sup>11</sup>

Studies have demonstrated that AMH predicts IVF outcome parameters such as oocyte yield, number of embryos and chemical or clinical pregnancy better than FSH. This is explained by the fact that AMH reflects the recruited primordial and pre-antral follicle population and FSH mostly reflects late stage follicles in the gonadotropin sensitive stage. AMH therefore better reflects the follicle pool. Follicle recruitment declines and follicles reaching gonadotropin sensitivity also decline in numbers with advancing reproductive age. In general, infertile population undergoing IVF, there is a moderate inverse correlation between AMH and FSH.<sup>12,13</sup> But the correlation was not significant in our study. The reasons may be the selected population of premature ovarian insufficiency and diminished ovarian reserve or small sample size.

AMH and FSH are generally useful in predicting response to ovarian stimulation but existing evidences are not clear about their utility in predicting live birth. There may be occasional discrepancy between the two markers, which is evident in our study as well. Some women in the cohort going for IVF are found to have elevated levels of both FSH and AMH.<sup>7</sup> This is confusing because abnormally elevated serum FSH levels usually indicate abnormally low functional ovarian reserve whereas high AMH suggests the opposite. In young women less than 35 years, abnormally high serum FSH combined with abnormally high AMH do not reflect poor functional poor ovarian reserve because during IVF they do not have low oocyte yield and have favorable outcomes.<sup>7,14-16</sup>

There may be an elevated FSH (FSH>10 IU/l) but reassuring AMH (>0.6 ng/ml), or normal age specific AMH with abnormal FSH. However women in this group had higher oocyte yield during in vitro fertilization compared to those with random AMH levels<0.6ng/dl but lower oocyte yield than those with concordant and normal age specific AMH and FSH.<sup>7</sup> Analysis of a large IVF data comprising 13,964 cycles revealed that in discordant groups a reassuring AMH (AMH≥1 ng/dl, FSH>10 IU/l) suggest a better likelihood of live birth compared to reassuring FSH (FSH<10 IU/l, AMH<1ng/dl) in the older (37-40 years) women.<sup>14</sup>

The ovarian reserve markers are associated with live birth in an age dependent manner. The estimated probability of live birth decreases as age increases, given the same AMH and FSH. Geiser et al investigated 366 infertility patients excluding polycystic ovary syndrome for the clinical significance of concordance and discordance of age specific values of AMH and FSH. They had 33.1% women with discordance in that age specific AMH normal with age specific abnormally high FSH. Young women with diminished ovarian reserve had excellent

oocyte yields whether AMH and FSH or whether only AMH was in normal range. They had clearly reduced oocyte yields if only FSH was in normal range or both hormones were abnormal. Elevated FSH in younger (<34 years) females are much less ominous than in older women and this observation only applies if AMH is still in normal range. This implies superiority of AMH over FSH in reflecting ovarian reserve in younger patients. Also, in the age group 34-42 years, normal AMH is better than normal FSH in predicting better oocyte yield. Once AMH levels are abnormal, oocyte yields are low in comparison even if FSH is normal. At advanced age (>42 years) normal FSH better predicts higher oocyte yields than AMH. Ovarian reserve assessments are most difficult at youngest and oldest ages. There is widening ranges of normal FSH and AMH values at both age extremes. That may be because of greater heterogeneity of ovarian function at very young and old ages. At younger ages, abnormally elevated FSH levels are not that significant in presence of good AMH levels. In older women (>40 years) AMH is not that much specific in predicting poor response to stimulation when FSH is in assuring levels.<sup>16</sup>

The higher prevalence of discordance in women with diminished ovarian reserve in comparison to premature ovarian insufficiency in our study may be a reflection of higher prevalence of discordance in women of younger reproductive age. The heterogeneity of ovarian function at extremes of age may explain the lack of significant correlation between FSH and AMH in our study population.

Few women with premature ovarian insufficiency have serum AMH levels in normal range despite minimal to nil follicles on biopsy.<sup>11</sup> So low plasma AMH value cannot be the absolute, one and only predictor of non-responder status to controlled ovarian stimulation. In contrast extremely high FSH is associated with high risk of low oocyte yield and cycle cancellation. Psychological morbidity can be minimized by allowing adjustment with patients' expectations and by individualization of therapeutic strategies. Older women with low AMH will yield fewer oocytes than younger women with same AMH.

There is enhanced intra-ovarian production of AMH in polycystic ovary syndrome.<sup>17</sup> Serum AMH concentration decrease over time in both normo-ovulatory and PCOS women, though the decrease is less pronounced in the later.<sup>18</sup> The change in ovarian markers with increasing age is more obvious in women in their thirties. There is increasing FSH, decreasing AMH and decreasing antral follicle count and ovarian volume.<sup>19</sup>

### **Limitations**

Limitations of the study include analysis of retrospective data and relatively small sample size. Further studies should be undertaken with larger sample size taking into

account the ovarian response to gonadotropin stimulation during intrauterine stimulation and in vitro fertilization.

## CONCLUSION

The baseline FSH have been used for many years to predict response to ovarian stimulation and IVF success. Studies on women undergoing IVF have suggest that compared to FSH, AMH is a better marker of the women's response to controlled ovarian stimulation. Following cost benefit analysis, FSH can be used for screening women for diminished ovarian reserve or premature ovarian insufficiency for counseling, while AMH can be reserved for those having high FSH or those who agree to go for gonadotropin stimulation. Those women who are younger than 35 years and have high FSH combined with reassuring AMH should be counselled with care regarding the prognosis of their treatment.

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