Original Research Article

AMH (Anti- Mullerian Hormone) in Relation to High FSH (Follicle Stimulating Hormone) in Female Subfertility: A Cross Sectional Analysis

Dr. Rupa Shamima Bashar[1], Dr. Shakeela Ishrat[2*], Dr. Rolly Suriya Jahan[3], Dr. Marufa Hossain[4], Dr. Muhammad Jasimuddin[5], Dr. Sheikh Farhana Huda[6]

[1,3]Dr. Rupa Shamima Bashar, Medical Officer, Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[2]Dr. Shakeela Ishrat, Associate Professor, Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[3]Dr. Rolly Suriya Jahan, Medical Officer, Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[4]Dr. Marufa Hossain, Medical Officer, Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[5]Dr. Muhammad Jasimuddin, Consultant, Department of Reproductive Endocrinology & Infertility, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[6]Dr. Sheikh Farhana Huda, Consultant, Department of Gynae Oncology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

Abstract

Background: Aged women who have infrequent menstruation and raised FSH are likely to have diminished ovarian reserve. Serum AMH is a novel marker of ovarian reserve but the test is expensive. The objective of this study is to determine serum AMH level in women who have high FSH and see how AMH and FSH correlate in this group of women. *Methods:* We did an observational cross sectional study of 87 infertile women, age 30-39 years, with oligomenorrhoea and high FSH (>10IU/L). Serum FSH was measured on day 2-4 and AMH was measured on any day of menstrual cycle. The women were divided into three groups based on FSH: 10-20 mIU/mL, 21-40 mIU/mL and >40 mIU/mL. Correlation of AMH with FSH was analyzed in all participants as well as within different groups. *Results:* The mean serum FSH $D_{(2-4)}$ was found 38.8±22.4 mIU/ml, range (min-max) (10.2-93.5) mIU/mL. Not all but 85.1% women had low (≤1.0 ng/ml) serum AMH level. Mean serum AMH was 0.74±0.59 ng/mL, range (min-max) (0.90-2.9 ng/ml). The serum AMH decreased in groups with higher FSH. The one way ANOVA shows that there is significant difference in AMH values between the groups. There is moderate (r = -- 0.537) negative correlation of AMH with FSH. The negative correlation of AMH with FSH is strongest (r = - 0.735) in the group having FSH in the range 10-20mIU/mL. The correlation is comparatively less (r = - 0.519, r = - 0.616) in groups with higher FSH. *Conclusion:* Not all the sub-fertile women with infrequent menstruation and FSH>10 mIU/mL have lower AMH (<1 mg/ml). A significant negative moderate correlation exist between AMH and FSH. The correlation is relatively stronger in the FSH range 10-20 mIU/mL.
Keywords: Anti-Mullerian Hormone, Follicle stimulating hormone, Ovarian reserve markers.

INTRODUCTION

Ovarian reserve refers to the quantity and quality of oocytes available at a given age to produce a dominant follicle later in the follicular phase of the menstrual cycle and ovulate [1]. The number of oocytes in women peak to about 7 millions at 20 weeks of intrauterine life. Only 1-2 millions are present at birth and about 40,000 at puberty. Ovulation occur around 400 times during the reproductive life of women. The remaining oocytes undergo atresia till there is few oocytes around menopause. So there is substantial attrition of oocytes with age. The ovarian reserve decline variably with age in women [2]. Low ovarian

reserve is associated with abnormalities of folliculogenesis, ovulation and oocyte structure and performance [3]. Ovarian reserve can be measured by various markers. There are some indirect measures like early follicular phase follicle stimulating hormone (FSH) and direct measures like anti-mullerian hormone (AMH) [2].

FSH is synthesized and secreted by gonadotrophs of the anterior pituitary gland. FSH is needed to rescue the follicles in the growing follicle pool from atresia, stimulate the follicles to grow, and to select the highest quality follicle from its cohort to mature into ovulatory follicle. Low levels of FSH are found during follicle development, and high levels during ovulation .The variation in levels of FSH results from the feedback loop between the hormones secreted from ovary and pituitary gland. High levels of FSH in aging women results from low levels of estrogen and inhibin available from follicles. Serum FSH levels are measured on D_2 of menstrual cycle to ensure the greatest accuracy possible [4].

Antimullerianhormone (AMH) is a protein hormone belonging to the transforming growth factor-β family [5]. AMH in female is produced by the granulosa cells of pre antral and early antral ovarian follicles [6, 7]. Production decreases and then stops as follicle grows further. There is almost no AMH in follicles over 8mm. Therefore, the levels are constant and the AMH test can be done on any day of a women's cycle [8]. In women AMH levels are almost undetectable at birth with a subtle increase within the first 2-4 years of age, after that AMH appears to be stable until adulthood but found to decrease as a sign of follicular reserve exhaustion, becoming undetectable at menopause [9]. AMH is solely produced in the growing ovarian follicles, so serum level may be used as a marker for ovarian reserve representing the quality and quantity of ovarian follicle pool [10]. Serum AMH shows negative correlation with age and a positive correlation with antral follicle count at ultrasound and to a lesser extent negative correlation with plasma levels of FSH [11].

At least 15% of couples experience some degree of infertility with all of its feelings and frustrations at some point in their life. Ovarian reserve predicts the response to ovarian stimulation in infertile women. Women having serum FSH levels more than 10mIU/mL have diminished response to ovarian stimulation. Aged women who have infrequent menstruation and raised FSH are likely to have diminished ovarian reserve. Serum AMH is a novel marker of ovarian reserve but the test is expensive, at least 5 times costlier than the measurement of serum FSH. Serum AMH lessthan 1.1 ng/ml is predictive of poor ovarian response (Bologna criteria) [12]. The studies which explored the correlation of AMH and FSH yielded variable results, probably because of the variable characteristics of study participants. No study so far investigated the correlation in the specific population of aged women with infrequent menstruation supposed to have diminished ovarian reserve. The objective of this study is to determine serum AMH level in women who have high FSH and see how AMH and FSH correlate in this group of women. It may help us determine whether the tests are complimentary or supplementary to each other.

METHODS
We did an observational cross sectional study of 87 infertile women, age 30-39 years, with oligomenorrhoea (infrequent menstruation) and high FSH (>10IU/L). Women receiving chemotherapy or radiotherapy at present or before and women with chromosomal anomalies were excluded. FSH was a single serum measurement in the early follicular phase of the menstrual cycle on either day 2, 3, or 4. Serum FSH was measured by immunoassay (Chemiluminescent). AMH was measured at any day of menstrual cycle by the method of ELISA (Beckman Coulter). The women were divided into three groups based on FSH : 10-20 mIU/mL, 21-40 mIU/mL and >40 mIU/mL. Correlation of AMH with FSH was analyze in all participants as well as within different groups.

Statistical analysis was carried out by using the Statistical Package for Social Sciences version 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The quantitative observations were calculated by frequencies and percentages. ANOVA test was used to analyze the continuous variables. Pearson's correlation coefficient was calculated between serum AMH and FSH $D_{(2-4)}$ l. P values <0.05 was considered as statistically significant.

RESULTS
All the participants had oligomenorrhea and high FSH. Mean age was found 33.1±3.2 years (range from 30 to 39 years). It was observed that almost two third (63.2%) patients had primary infertility and mean duration of subfertility was 7.1±5.1 years. Table 1 describes the baseline characteristics of the participants.

The mean serum FSH $D_{(2-4)}$ was found 38.8±22.4 mIU/ml, range (min-max) (10.2-93.5) mIU/mL. Not all but 74(85.1%) patients had low (≤1.0 ng/ml) serum AMH level. Mean serum AMH was 0.74±0.59 ng/mL, range (min-max) (0.90-2.9 ng/ml).

The participants were divided into three groups according to the level of FSH (Table 2). The serum AMH decreased in groups with higher FSH. The one way ANOVA shows that there is significant difference in AMH values between the groups.

Table-1: Baseline characteristics of the participants (n=87)

Parameters	Number of cases	Percentage
Age		
<35	56	64.4
>35	31	35.6
Type of subfertility		
Primary	55	63.2
Secondary	32	36.8
Duration of subfertility (years)		
≤5	38	43.7
6-10	43	49.4
> 10	6	6.9

Table-2: Serum AMH level in different FSH groups (n=87)

Serum FSH (mlU/ml)	Total number	Serum AMH (ng/ml)		P value
		Mean	**±SD**	
10-20	24	1.21	±0.73	
21-40	28	0.66	±0.52	0.001^s
>40	35	0.51	±0.31	

s=significant
P value reached from ANOVA test

Correlation coefficients were used to assess the strength and direction of the linear relationship between AMH and FSH. The higher the correlation in either direction (positive or negative), the more linear is association between variables and the more obvious the trend in scatterplot [13].

There is moderate (r = -- 0.537) negative correlation of AMH with FSH (Figure-1). The negative correlation of AMH with FSH is strongest (r = -- 0.735) in the group having FSH in the range 10-20mIU/mL. The correlation is comparatively less (r = -- 0.519, r = -- 0.616)) in groups with higher FSH (Figure 2-4).
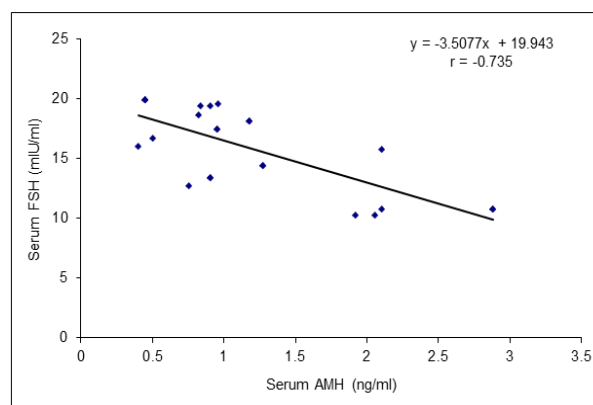

Fig-2: Scatter diagram showing significant (strong) negative correlation between serum AMH with serum FSH D$_{(2-4)}$ level 10-20 mlU/ml (Pearson's correlation coefficientr=--0.735).
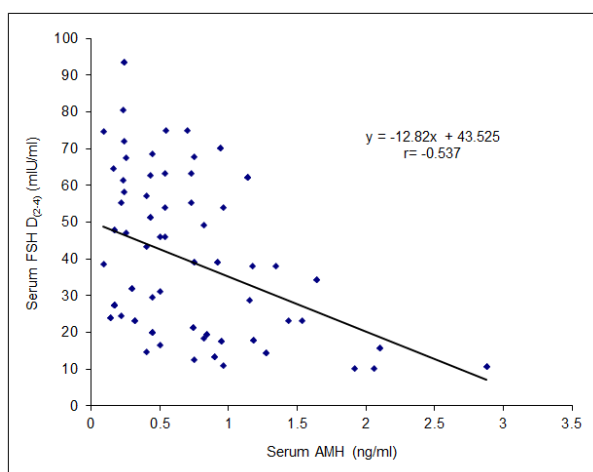

Fig-1: Scatter diagram showing significant (moderate) negative correlation between serum AMH with serum FSH D$_{(2-4)}$ level (Pearson's correlation coefficient r=--0.537).
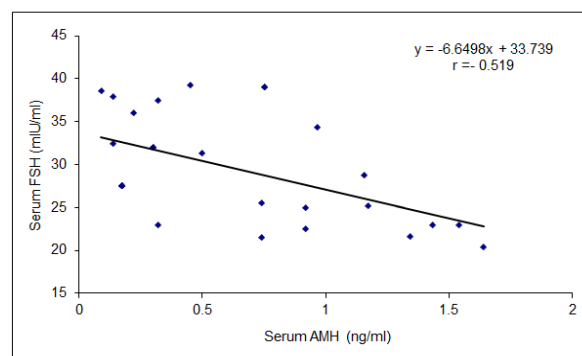

Fig-3: Scatter diagram showing significant (moderate) negative correlation between serum AMH with serum FSH D$_{(2-4)}$ level 20-40 mlU/ml (Pearson's correlation coefficient r= --0.519)
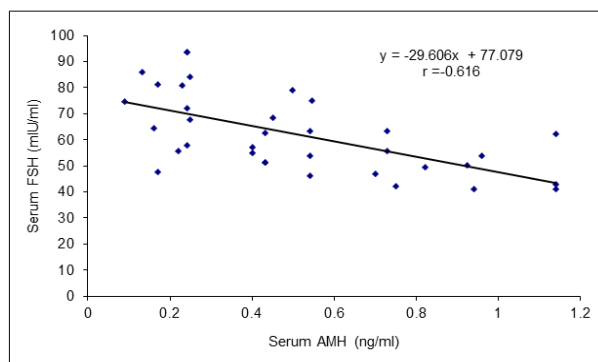
y = -29.606x + 77.079
r =-0.616

Fig-4: Scatter diagram showingsignificant (moderate) negative correlation between serum AMH with serum FSH D$_{(2-4)}$ level >40 mlU/ml. (Pearson's correlation coefficient r= --0.616)

Table-4: Correlation of AMH with FSH in different studies

Studies	AMH ng/ml Mean, SD	FSH mIU/mL Mean, SD	Correlation coefficient
Our study, Bangladesh, 2021	0.74±0.59	38.8±22.4	-0.537
Bala *et al*, India, 2014	1.18±0.58	9.10±2.51	-0.448
Goksedef *et al*, Turkey, 2010	2.23±1.90	6.81±3.35	-0.299
Singer *et al*, USA,2009	0.8±0.5	10.7±6.9	-0.42

DISCUSSION

Follicle stimulating hormone (FSH) and anti-Mullerian hormone (AMH) represent the two most frequently utilized laboratory tests in determining ovarian reserve (OR). This observational study was carried out with an aim to estimate serum AMH in the specific population of infertile women who were aged 30-39 years, had infrequent menstrual cycle and high FSH, and to determine the correlation with FSH. There was moderate negative correlation of AMH with FSH (correlation coefficient -5.37).Other studies[5,12-13] which explored the correlation of AMH and FSH are summarized in table4.

Singer *et al*., [5] did a retrospective cohort study on eighty one women in preparation for an IVF cycle of levels of FSH and AMH before the start of ovulation induction. They found that serum AMH and log converted FSH levels were negatively correlated (R^2=0.42). The linear regression model for FSH based on random AMH suggested that an AMH level of 0.5 ng/ml is predictive of a baseline level of 12.1 mIU/ml. This is in agreement of our finding that 85.1% of our women with FSH higher than 10 mIU/mL had an AMH <1ng/ml. Bala *et al*., [14] studied 75 infertile women aged 30-40 years and found significant inverse relationship serum AMH and FSH concentration. Goksedef *et al*., [15] evaluated 141 infertile women prior to their first treatment cycle and found poor correlation. The participants had regular menstrual cycles, in contrast to our participants who had infrequent menstrual cycles above the age 30.AMH levels are significantly higher in normal weight women with PCOS and in normal weight ovulatory women. Serum AMH levels are not correlated with serum FSH levels in women with PCOS or in ovulatory women [16].

Barbakadze *et al*., [17] identified the correlation between AMH and FSH in different age groups of infertile women. The correlation was significant in age group <35 years (r = - 0.41) and age group 35-40 years (r = -0.55) but not in age group 41-46 years. Iverson *et al*., [4] demonstrated a relationship of chronological age with AMH and FSH. There was a 33% increase in FSH levels from the <30 age group to the 31-36 age group and another 26% increase at >36 age group. The levels of AMH decreased 40% from <30 to 31-36 age group but 4% in >36 age, much less

compared to the increase in FSH. Moreover the receiver operating curve (ROC) had mean level of FSH 9.53 in range of AMH <1. This finding is in conformity with the finding of our study that negative correlation of AMH with FSH is relatively weaker in higher FSH group and AMH >1ng/ml does not necessarily follow FSH>10 mIU/mL.

We had study participants who were 30-40 years, had infrequent cycle and baseline FSH more than 10 mIU/mL. They were supposed to have diminished functional ovarian reserve. Gleicher *et al*., [18] studied 544 consecutive infertile women in their first IVF cycles. Women less than 38 years, with high (>95% CI for age) AMH combined with high (>95% CI for age) FSH had relatively favorable in vitro fertilization (IVF) outcomes. So the negative correlation of AMH and FSH cannot be unequivocally decided in this specific population.

Barad *et al*., 19 compared AMH versus FSH in predicting IVF outcomes like oocytes retrieved and clinical pregnancies achieved. For prediction of retrieval of more than four oocytes AMH values of 0.5 ng/ml has the maximal sensitivity and specificity (87% sensitivity sand 84% specificity) compared to 65% sensitivity and 82% specificity of baseline FSH values of 12 mIU/mL. For prediction of ongoing clinical pregnancy, the AMH values of 1.0 ng /ml had maximal sensitivity and specificity (62% sensitivity and 75% specificity) compared to 62% sensitivity and 40% specificity of baseline FSH 11 mIU/mL. The threshold of FSH (10 mIU/mL) we decided was less, and the stronger correlation in the range of FSH (10-20 mIU/mL) can be explained by the above findings.

Hussain *et al.*, [20] analyzed the relationship between AMH and FSH of 107 women undergoing first IVF/ICSI. Concordance of both normal or both abnormal AMH/FSH was found in 57% whereas 43% had discordant values, one hormone normal and the other abnormal. Similar discordance between AMH and LH was found in other studies [21-23] as well. The finding supports the absence of strong correlation (correlation coefficient >8) between the two hormones.

Izharetal [24] applied the poor response criteria of NICE guidelines to evaluate infertile women with infrequent menstruation for 'occult' premature ovarian in suffinency (FSH ≥ 8.9 or AMH ≤0.7 ng/mlor total AFC≤ 4). The kappa statistic of agreement between FSH and AMH was 0.76 (substantial) while that between AFC and AMH was 0.93 (almost perfect). So AMH was more correlated with AFC than FSH. The observation supports the significant but moderate correlation between AMH and FSH.

Clinicians have long used high baseline FSH levels for their diagnoses of poor ovarian reserve prior to any interventions. Anti-Mullerian hormone levels are more expensive to measure but have been credited as more reliable biomarkers for ovarian reserve. Considering the moderate correlation between AMH and FSH, we should not rely on FSH alone in evaluation of poor ovarian reserve. Moreover, stronger correlation of AMH with FSH in 10-20 mIU/mL range and weaker correlation in higher FSH range should be taken into consideration during counselling women suspected of poor ovarian reserve.

LIMITATIONS
Women with infrequent menses include those with polycystic ovary syndrome. Our study did not decidedly exclude the rare PCOS women with age 30-40 years, infrequent menstruation and high FSH. The moderate correlation may have the confounding effect of inadvertent inclusion of PCOS women. The study was conducted on small size in a selected center, so cannot be generalized for wider population. Larger multicenter studies are required.

CONCLUSION
Not all the subfertile women with infrequent menstruation and FSH>10 mIU/mL have lower AMH (<1 mg/ml). A significant negative moderate correlation exist between AMH and FSH. The correlation is relatively stronger in the FSH range 10-20 mIU/mL.

ACKNOWLEDGEMENT
Our gratitude goes to the FCPS trainees in Reproductive Endocrinology and Infertility who provided service in the Gynae Endocrine Clinic, the women who have been part of our study and to those who supported the investigation facilities in the allied Departments of Bangabandhu Sheikh Mujib Medical University.

DECLARATION
Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES
1. Baird, D. T., Collins, J., Egozcue, J., Evers, L. H., Gianaroli, L., Leridon, H., Sunde, A. Templeton, A., & Van Steirteghem, A. (2005). Fertility and aging. Hum Reproduction. 11: 261-267.
2. Roudebush, W. E., Kivens, W. J., & Mattke, J. M. (2008). Biomarkers of ovarian reserve. Biomarker insights, 3, BMI-S537.
3. Battaglia, D. E., Goodwin, P., Klein, N. A., & Soules, M. R. (1996). Fertilization and early embryology: Influence of maternal age on meiotic spindle assembly oocytes from naturally cycling women. Human reproduction, 11(10), 2217-2222.
4. Iverson, A., Younis, A., Butler, W., & Roudebush, W. (2011). Inverse Correspondence of AMH and FSH levels in Women Presenting for Infertility Treatments. Journal of the South Carolina Academy of Science, 9(2), 1-4.
5. Singer, T., Barad, D. H., Weghofer, A., & Gleicher, N. (2009). Correlation of antimüllerian hormone and baseline follicle-stimulating hormone levels. Fertility and sterility, 91(6), 2616-2619.
6. La Marca, A., Broekmans, F. J., Volpe, A., Fauser, B. C., & Macklon, N. S. (2009). Anti-Müllerian hormone (AMH): what do we still need to know?. Human Reproduction, 24(9), 2264-2275.
7. Durlinger, A. L., Kramer, P., Karels, B., de Jong, F. H., Uilenbroek, J. T. J., Grootegoed, J. A., & Themmen, A. P. (1999). Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. Endocrinology, 140(12), 5789-5796.
8. Advanced Fertility Center of Chicago. (2011). Anti-Mullerian Hormone Testing of Ovarian Reserve available on www. advanced fertility. com/amh-fertility test. (visited on 2011 Oct 26).
9. La Marca, A., Stabile, G., Artensisio, A. C., & Volpe, A. (2006). Serum anti-Mullerian hormone throughout the human menstrual cycle. Human reproduction, 21(12), 3103-3107.
10. Te Velde, E. R., & Pearson, P. L. (2002). The variability of female reproductive ageing. Human reproduction update, 8(2), 141-154.
11. de Vet, A., Laven, J. S., de Jong, F. H., Themmen, A. P., & Fauser, B. C. (2002). Antimüllerian hormone serum levels: a putative marker for ovarian aging. Fertility and sterility, 77(2), 357-362.

12. Ferraretti, A., La Marca, A., Fauser, B. C. J. M., Tarlatzis, B., Nargund, G., Gianaroli, L., & ESHRE working group on Poor Ovarian Response Definition. (2011). ESHRE consensus on the definition of 'poor response'to ovarian stimulation for in vitro fertilization: the Bologna criteria. Human reproduction, 26(7), 1616-1624.

13. Mukaka, M. (2012). Statistics corner: A guide to appropriate use of correlation in medical research. Malawi Med. J., 24(3), 69-71.

14. Bala, J., Agrawal, Y., Seth, S., Goyal, V., & Kumar, P. (2014). Correlation between anti-Mullerian and follicle-stimulating hormone in female infertility. International Journal of Health & Allied Sciences, 3(4), 232-232.

15. Göksedef, B. P., Idiş, N., Görgen, H., Asma, Y. R., Api, M., & Çetin, A. (2010). The correlation of the antral follicle count and Serum anti-mullerian hormone. Journal of the Turkish German Gynecological Association, 11(4), 212-215.

16. Georgopoulos, N. A., Saltamavros, A. D., Decavalas, G., Piouka, A., Katsikis, I., & Panidis, D. (2010). Serum AMH, FSH, and LH levels in PCOS. Fertility and sterility, 93(3), e13.

17. Barbakadze L, Kristesashville J, Khonelidze N, TsagareshvilliG . The correlation of anti-mullerianhormone , follicle stimulation hormone and antral follicle count in different age groups. Int j FertilSteril 2015; 8(4):393-398

18. Gleicher, N., Kim, A., Kushnir, V., Weghofer, A., Shohat-Tal, A., Lazzaroni, E., ... & Barad, D. H. (2013). Clinical relevance of combined FSH and AMH observations in infertile women. The Journal of Clinical Endocrinology & Metabolism, 98(5), 2136-2145.

19. Barad, D. H., Weghofer, A., & Gleicher, N. (2009). Comparing anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH) as predictors of ovarian function. Fertility and sterility, 91(4), 1553-1555.

20. Hussain, M., Cahill, D., Akande, V., & Gordon, U. (2013). Discrepancies between antimullerian hormone and follicle stimulating hormone in assisted reproduction. Obstetrics and gynecology international, 2013.

21. Zhang, Y., Xu, Y., Xue, Q., Shang, J., Yang, X., Shan, X., ... & Zeng, C. (2019). Discordance between antral follicle counts and anti-Müllerian hormone levels in women undergoing in vitro fertilization. Reproductive Biology and Endocrinology, 17(1), 1-6.

22. Wang, S., Zhang, Y., Mensah, V., Huber, W. J., Huang, Y. T., & Alvero, R. (2018). Discordant anti-müllerian hormone (AMH) and follicle stimulating hormone (FSH) among women undergoing in vitro fertilization (IVF): which one is the better predictor for live birth?. Journal of ovarian research, 11(1), 1-8.

23. Gleicher, N., Weghofer, A., & Barad, D. H. (2010). Discordances between follicle stimulating hormone (FSH) and anti-Müllerian hormone (AMH) in female infertility. Reproductive Biology and Endocrinology, 8(1), 1-7.

24. Izhar, R., Husain, S., Tahir, S., & Husain, S. (2017). Occult form of premature ovarian insufficiency in women with infertility and oligomenorrhea as assessed by poor ovarian response criteria. Journal of reproduction & infertility, 18(4), 361.