ORIGINAL ARTICLE

# Assessment of ovarian reserve: Anti-Mullerian hormone versus follicle stimulating hormone

**Zehra Jamil, Syeda Sadia Fatima[1], Zahra Cheema[2], Safia Baig[2], Roha Ahmed Choudhary[2]**

Department of Biological and Biomedical Sciences, Faculty of Health Sciences, Medical College, Aga Khan University, [1]Department of Biological and Biomedical Sciences, Aga Khan University, [2]III Year UGME Students, Medical College, Aga Khan University, Karachi, Pakistan

**Background:** This study aimed to evaluate the strength of anti-Mullerian hormone (AMH) and follicle stimulating hormone (FSH) in reflecting the antral follicle count (AFC) in infertile females. **Materials and Methods:** This cross-sectional study was conducted on 160 females, visiting infertility clinic for assisted reproduction. Serum samples collected on the 3rd day of the cycle were assayed for FSH, luteinizing hormone, and AMH while AFC was assessed via transvaginal ultrasound. The study cohort was segregated into three groups based on AFC. **Results:** Chronological age and FSH was significantly high in females with very low AFC ($P < 0.01$ and 0.009, respectively), yet they failed to discriminate patients with normal and higher follicle count ($P = 0.65$ and 0.84). Conversely, AMH reported highly significant difference between very low AFC and with those having either normal AFC ($P = 0.002$) or higher AFC ($P = 0.001$). Moreover, a significant difference in AMH was observed between normal and higher AFC group ($P = 0.04$). **Conclusion:** Compared to female's age and FSH, AMH is superior in clustering study cohort on the bases of antral follicular pool, especially in setups with nonavailability of technological expertise to assess AFC. Incorporation of AMH along with other biomarkers improves estimation of baseline ovarian reserve, required to standardize dose for optimum response; avoiding the risk of failure to retrieve oocyte or inappropriate stimulation leading to ovarian hyperstimulation syndrome. Further prospective studies are required to ascertain its role in predicting the outcomes of ART in such patients.

**Key words:** Anti-Mullerian hormone, antral follicle count, assisted reproduction technique, ovarian reserve

**How to cite this article:** Jamil Z, Fatima SS, Cheema Z, Baig S, Choudhary RA. Assessment of ovarian reserve: Anti-Mullerian hormone versus follicle stimulating hormone. J Res Med Sci 2016;21:96.

## INTRODUCTION

Assessment of the status of an ovarian function is essential to evaluate and plan infertility interventions. In recent times, estimation of ovarian reserve (OR) is the most commonly used criteria to reflect the quality and quantity of oocytes, in turn imitating the fertility potential of a female.[1] With advancing age, a drop in the extent of OR proportionately reflects decline in a female's reproductive capabilities. Hence, its estimation provides an approximation of fertile years left for a woman. Several markers are used to reflect OR in infertility clinics that include patient's age, serum follicle stimulating hormone (FSH), luteinizing hormone (LH), anti-Mullerian hormone (AMH), estradiol levels, antral follicle count (AFC), and ovarian volume.[2,3] These have been summarized in Table 1.

AFC is considered as a gold standard for measurement of OR and is considered necessary before planning assisted reproduction support.[4] It is suggested that an optimum response to infertility assistance is reflected as a retrieval of at least 5 oocytes on ovarian stimulation.[5] Furthermore, an exaggerated AFC (>19) is linked to potential complications such as ovarian hyper stimulation syndrome (OHSS),[3] rendering its evaluation as a better tool for optimization of protocol that may reduce the chances of cycle cancellation. However, it has its own drawbacks such as prerequisite of a skilled operator and latest machinery that reliably assess the count.[6] In addition, its inability to reveal the quality of healthy oocytes

Access this article online

Quick Response Code:

Website: www.jmsjournal.net

DOI: 10.4103/1735-1995.193172



**Address for correspondence:** Dr. Zehra Jamil, Department of Biological and Biomedical Sciences, Faculty of Health Sciences, Medical College, Aga Khan University, Stadium Road, P.O. Box 3500, Karachi-74800, Pakistan. E-mail: zehra.jamil@aku.edu

**Received:** 26-04-2016; **Revised:** 22-06-2016; **Accepted:** 13-07-2016

**Table 1: Ovarian reserve tests and markers adapted from Jirge, 2011**

| Tests | Ovarian reserve marker |
|---|---|
| Biological | Chronological age |
| Biochemical | FSH |
| | LH |
| | FSH: LH ratio |
| | Inhibin B |
| | Estradiol |
| | AMH |
| | CCCT |
| | GAST |
| | EFORT |
| Biophysical | AFC |
| | Ovarian volume |
| | Ovarian blood flow |
| Histological | Ovarian biopsy |

CCCT=Clomiphene citrate challenge test; GAST=Gonadotropin-releasing hormone agonist stimulation test; EFORT=Exogenous FSH ovarian reserve test; FSH=Follicle stimulating hormone; LH=Luteinizing hormone; AMH=Anti Mullerian hormone; AFC=Antral follicle count

results in counting even those follicles that may not act in response to treatment.[7]

At present, in setups that lack ultrasonography facilities for the assessment of AFC, serum FSH is widely used along with patient's age; based on these markers, patients are either advised to farther wait for natural conception or offered infertility treatment. Various studies imply chronological age as a weak predictor of fertility as even young patients at the times report reduced OR.[8] In regards to FSH, it shows high degree of variability as factors such as exogenous administration of FSH in the form of oral contraceptive pills (OCP) can alter the results obtained.[9] Nowadays, AMH is being preferred over other indicators as its levels are independent of menstrual cycle phases and is fairly easier to estimate through blood sampling.[9]

In this study, we aimed to compare the true accuracy of AMH and FSH in correlating with the number of AFC in infertile population. For this purpose, we divided the cohort into three subgroups based on AFC count; those with <5 follicles (Group A), between 5 and 19 (Group B), and greater than 19 follicles (Group C). We evaluated the strength of AMH and FSH in characterizing the population into sub-groups and witnessed AMH as a superior predictor in distinguishing among them.

## MATERIALS AND METHODS

This cross-sectional study was conducted by means of the data collected from 160 infertile females, aged 20–43 years who visited Australian Concept Infertility Medical Center (ACIMC) during June 2014 to March 2015. Institutional review board of ACIMC granted the exception of Ethical Review consent as this retrospective study could not affect the clinical decision made for infertility treatment. The anonymity of the records was carried out in view of keeping patient confidentiality intact. Besides age, the inclusion criteria also required that participants had intact female reproductive organs and no history of prior ovarian procedures or endocrine dysfunction. Women who had previously received ovarian stimulation treatment or OCP were excluded from the study.

The serum samples were obtained over days 3 of the menstrual cycle from all participants for baseline AMH, FSH, and LH measurements before the commencement of treatment. The hormonal assays were carried out on supernatant fluid maintained between 2 and 8°C. AFCs were assessed via transvaginal ultrasonography by utilization of an Aloka SSD-1000 (Japan) with a 5 MHz probe, on menstrual cycle day 3. Follicular diameter of <10 mm was used as a cutoff while counting in both ovaries to determine the cohort with inter observer coefficient of variation (CV) <5%.

All samples were assayed employing the use of AMH Gen 11 ELISA reagent kit (Beckman coulter, ref a79765) with an analytical sensitivity of 0.57 pmol/L. Intra-assay CV was <5.4% while inter-assay CV was 5.6%. Regarding FSH, samples were assayed utilizing the Elecys reagent kit with intra-assay CV of <3% and inter-assay CV of <6%.

## RESULTS

In our study, the mean body mass index (BMI) of the participants was higher than the South Asia cutoff for obesity, i.e. 25 kg/m$^2$. The mean serum AMH levels were recorded as 1.6 ± 1.37 ng/ml although the mean age was observed as 33.6 ± 6.03 year. The serum FSH levels, as well as AFC of the whole population, was recorded within normal range as listed in Table 2.

Subsequently, the participants were divided into three groups based on their evaluation of the number of antral follicles. Group A comprised individuals with AFC <5 follicles, Group B had a range from 5 to 19 follicles while Group C included participant with AFC >19 follicles. Table 3 presents the biophysical and biochemical variables of the cohort subgrouped according to the AFC criterion.

The *post hoc* analysis between AFC and parameters such as age, BMI, FSH, and AMH highlighted the significance of these markers in categorizing the three groups.

Age appeared to be a significant predictor for females having very low number of ovarian follicles ($P < 0.01$) but it failed to differentiate between those with normal or more than 19 follicles ($P = 0.65$) (Groups B and C).

**Table 2: Descriptive statistics of the whole cohort**

| Variables | Whole study population, *n*=160 (mean±SD) |
|---|---|
| Age (year) | 33.6±6.03 |
| BMI (kg/m$^2$) | 29.3±5.41 |
| FSH (IU/L) | 8.5±4.8 |
| LH (IU/L) | 6.9±1.06 |
| AMH (ng/ml) | 1.6±1.37 |
| AFC | 8.8±4.3 |
| Infertility (year) | 7.6±5.6 |

Data expressed as mean±SD. Mann–Whitney U-test was used to compare the difference between groups. **P*<0.05 considered significant. BMI=Body mass index; FSH=Follicular stimulating hormone; LH=Luteinizing hormone; AFC=Antral follicle count; AMH=Anti Mullerian hormone; SD=Standard deviation

**Table 3: Biophysical and biochemical variables on the basis of antral follicle count cut-off**

| Variables | AFC (mean±SD) | | |
|---|---|---|---|
| | <5 (*n*=38) | 5–19 (*n*=78) | >19 (*n*=44) |
| Age (year) | 36.6±5.5* | 33.2±6.5 | 32±4.6 |
| BMI (kg/m$^2$) | 31.9±5.1 | 28.6±5.7 | 29.2±4.5 |
| FSH (IU/L) | 10.1±5.6* | 8.5±5.1 | 7.25±3.4 |
| LH (IU/L) | 7.1±7.6 | 6.3±8.6 | 8.2±5.7 |
| AMH (ng/ml) | 0.5±0.6* | 1.7±1.3* | 2.2±1.7* |
| AFC | 3.5±0.5 | 7.6±1.2 | 14.8±4.7 |

Data expressed as mean±SD. Mann–Whitney U-test was used to compare the difference between groups. **P*<0.05 considered significant. BMI=Body mass index; FSH=Follicular stimulating hormone; LH=Luteinizing hormone; AFC=Antral follicle count; AMH=Anti Mullerian hormone; SD=Standard deviation

The difference in FSH was found to be nonsignificant among patients in Groups A and B ($P = 0.08$) and between Groups B and C ($P = 0.84$). Moreover, it was merely able to significantly differentiate between patients with <5 follicles and those with more than 19 follicles ($P = 0.009$).

AMH was found to be the most comparable predictor to AFC in categorizing the population as there was a significant difference among all three groups. The *P* value was highly significant among patients with low AFC and normal AFC, i.e., Group A and B ($P = 0.002$) as well as between Groups A and C ($P = 0.001$). Furthermore, AMH levels were successful in highlighting the significant difference between Group B and C ($P = 0.04$).

Finally, we performed ordinal regression analysis adjusting for age and BMI to estimate the odds ratio for determining the effect of AMH and FSH on AFC variations. The odds for AMH were 0.14 (95% confidence interval [CI]: 0.03, 0.65 ($P < 0.01$)), while the odds for FSH was 1.42 (95% CI: 0.72, 2.79 [$P = 0.73$]). As compared to FSH, AMH gave a better prediction of variation in the AFC.

## DISCUSSION

In human-assisted reproduction, ovarian response to gonadotropin stimulation is variable, hence, difficult to predict.[10] The evaluation of antral follicles on the transvaginal scan is often used as a gold standard to assess OR, standardize the dose of treatment, and to predict the likelihood of conception with the help of intervention. In this study, we compared the effectiveness of FSH; widely used OR and recently emerged AMH in reflecting the count of antral follicles in infertile females.

In our study, the mean age of infertile patients was found to be approximately 33 years. As chronological age is used as a marker of OR since many years, we scrutinized its strength in reflecting the follicular pool. Expectedly, higher age correlated with patients whom AFC had drastically declined, suggesting deterioration in the ovarian pool as female ages. However, it failed to segregate between women with healthy counts of follicles or an overblown AFC that is critical to reduce undesired effects of treatment. Literature too recommends age as a week reflector of the reserve as it is widely reported to differ even among age-matched population.[11] Even though age does influence the fecundity of a female but its utility as a marker of OR can only be substantiated while synergistically using it along other biochemical and biophysical markers.[12] Undoubtedly, baseline OR status has an extensive role in infertility management. Primarily, it is decisive in suggesting either to wait for natural conception or to proceed for assisted reproduction. Furthermore, it assists in the standardization of doses that may lead to satisfactory response, avoiding the risk of inadequate reaction resulting in failure to retrieve a decent number of oocytes. In extreme cases, OR might predict the chances of inappropriate ovarian stimulation leading to potentially fatal complication termed as OHSS.[13] Thus, finest prediction of the ovarian pool is essential for grander results of ART. To this end, in secondary care hospital where technical expertise is not available to assess AFC, serum FSH is commonly used as an OR markers other than age, LH, inhibin, and ovarian volume.[6]

Regarding mean AFC of our study group, we reported approximately nine follicles per patient. However, while segregating the participants into three groups based of their AFC, 24% reported < 5 antral follicles which indicated determent in their ovarian pool. Likewise, 27.5% population was at the risk of hyperresponsiveness as they testified more than nineteen antral follicles on initial assessment. AFC is considered as the first test of choice in evaluating infertile patients as it reflects the baseline capability of ovarian pool to respond to treatment.[11] This promises for timely identification of women with shortened reproductive life span, requiring immediate intervention. Moreover, it leads to appropriate counseling of expected poor responders and in setting apart those females who may experience enhanced responsiveness.[13] Contrary to this, AFC evaluation has its own limitations. These include the requirement of latest equipment and skilled personnel, biasness due to

operator's variability, incapability to visualize follicles in female with ovarian cysts or prior surgery, and counting both healthy and atretic follicles as capable to respond to treatment.[14] Therefore, we compared the strength of FSH; mostly commonly used OR in secondary care clinics and AMH; recently acclaimed as the best solo marker to reflect ovarian pool, in segregating infertile population. As blood test has a clear advantage of sample collection and avoidance of human error, these tests may be easily used in setups lacking the sophisticated technology to assess AFC.[14]

In this investigation, we found that serum FSH was incapable of significantly indicating sub-groups with varied AFC. Although mean FSH levels in patients with very low AFC were higher (10.1 IU/L) than the other two sub-groups as shown in Table 3, they were still within the normal range (<11 IU/L). This suggests that FSH may identify individuals only once considerable the loss of ovarian function has already occurred. Furthermore, the only significant difference reported in our study was among FSH levels of patients with <5 follicles and those with more than 19 follicles; however, it failed to segregate those with a high chance of life-threatening OHSS. There have been discrepancies in the literature regarding the role of FSH as an accurate OR. Undoubtedly, it is the most widely used OR marker, but there is ample evidence to state that FSH levels begins to derange lately; thus, lone assessment of FSH or along with chronological age is losing their strength as timely and true indicators of OR.[15]

Interestingly, we found that serum AMH most accurately clustered the study cohort on the bases of antral follicular pool. The patients with low AFC had significantly lower mean AMH levels in comparison to normal as well as higher follicle count ($P = 0.002$ and 0.001, respectively). Perhaps the most striking finding in our investigation is that AMH was the only parameter that differentiated between the normal AFC and higher AFC group ($P = 0.044$). This raises the possibility of AMH's clinical value in identifying patients with likelihood to develop hyperresponse to ovarian stimulation on the standard doses of gonadotropins.[16] Timely, identification of such patients would prevent the drastic complications of the unfortunate phenomenon OHSS. It may also have the potential to screen for polycystic ovarian syndrome as women with this condition are likely to have higher follicle counts, which is well correlated with AMH levels as our study has shown.[17] Our study strongly supports the addition of AMH assessment to evaluate woman before making decisions pertaining to infertility intervention.

As ethnic variation has been reported in the levels of AMH across the various population, this is the first study that compares the strength of FSH and AMH in reflecting the follicle count in Pakistani population. A collection of retrograde data is one of our study's limitation; however, it provides ample evidence to support further studies highlighting the role of AMH as a robust OR marker. As ethnicity affects the ovarian pool as well as its responsiveness to ovarian stimulation, local studies are required to further strengthen the diagnostic role of AMH in reflecting a response to treatment in various ovarian dysfunctions.

## CONCLUSION

AMH improves the estimation of baseline OR, required to predict optimum ovarian response during assisted reproduction. Compared to female age and FSH alone, AMH has a superior role in projecting accurate antral follicle pool, especially in setups where technological expertise to assess AFC is not available. Incorporation of AMH along with other biomarkers constitutes a better model for the prediction of ovarian response. Further prospective studies are required to ascertain its role in predicting the outcomes of ART in such patients.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## AUTHORS' CONTRIBUTIONS

- Conceived and designed the experiments: ZJ
- Performed the experiments: ZC, SB, RAC
- Analyzed the data: SSF, ZJ
- Contributed reagents/materials/analysis tools: ZJ
- Wrote the manuscript: ZJ, SSF, ZC, SB, RAC
- All authors approved the final manuscript for publication.

## REFERENCES

1. te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update 2002;8:141-54.
2. Kalaiselvi V, Saikumar P, Prabhu K, Krishna G. The anti mullerian hormone – A novel marker for assessing the ovarian reserve in women with regular menstrual cycles. J Clin Diagn Res 2012;6:1636-9.
3. Jirge PR. Ovarian reserve tests. J Hum Reprod Sci 2011;4:108-13.
4. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: Practical recommendations for better standardization. Fertil Steril 2010;94:1044-51.
5. Ubaldi F, Vaiarelli A, D'Anna R, Rienzi L. Management of poor responders in IVF: Is there anything new? Biomed Res Int 2014;2014:352098.
6. Nelson SM. Biomarkers of ovarian response: Current and future applications. Fertil Steril 2013;99:963-9.
7. Coccia ME, Rizzello F. Ovarian reserve. Ann N Y Acad Sci 2008;1127:27-30.

8. El-Toukhy T, Khalaf Y, Hart R, Taylor A, Braude P. Young age does not protect against the adverse effects of reduced ovarian reserve – an eight year study. Hum Reprod 2002;17:1519-24.
9. Kunt C, Ozaksit G, Keskin Kurt R, Cakir Gungor AN, Kanat-Pektas M, Kilic S, *et al.* Anti-mullerian hormone is a better marker than inhibin B, follicle stimulating hormone, estradiol or antral follicle count in predicting the outcome of *in vitro* fertilization. Arch Gynecol Obstet 2011;283:1415-21.
10. Peluso C, Fonseca FL, Rodart IF, Cavalcanti V, Gastaldo G, Christofolini DM, *et al.* AMH: An ovarian reserve biomarker in assisted reproduction. Clin Chim Acta 2014;437:175-82.
11. La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: From theory to practice. Hum Reprod Update 2014;20:124-40.
12. Lee TH, Liu CH, Huang CC, Hsieh KC, Lin PM, Lee MS. Impact of female age and male infertility on ovarian reserve markers to predict outcome of assisted reproduction technology cycles. Reprod Biol Endocrinol 2009;7:100.
13. Iliodromiti S, Anderson RA, Nelson SM. Technical and performance characteristics of anti-müllerian hormone and antral follicle count as biomarkers of ovarian response. Hum Reprod Update 2015;21:698-710.
14. Jamil Z, Fatima SS, Ahmed K, Malik R. Anti-mullerian hormone: Above and beyond conventional ovarian reserve markers. Dis Markers 2016;2016:5246217.
15. Toner JP, Seifer DB. Why we may abandon basal follicle-stimulating hormone testing: A sea change in determining ovarian reserve using antimüllerian hormone. Fertil Steril 2013;99:1825-30.
16. Iliodromiti S, Kelsey TW, Wu O, Anderson RA, Nelson SM. The predictive accuracy of anti-müllerian hormone for live birth after assisted conception: A systematic review and meta-analysis of the literature. Hum Reprod Update 2014;20:560-70.
17. Köninger A, Koch L, Edimiris P, Enekwe A, Nagarajah J, Kasimir-Bauer S, *et al.* Anti-mullerian hormone: An indicator for the severity of polycystic ovarian syndrome. Arch Gynecol Obstet 2014;290:1023-30.