INTRODUCTION

Fertility of females resides in the pool of primordial follicles, they are born with. Amongst these, 30 to 50 follicles are recruited with each menstrual cycle leading to decline in fertility after the age of 30 years. In cases of infertility, evaluation of ovarian reserve (OR) is essential to optimize protocol for assisted reproductive technique (ART) and prediction of response to counsel the couple. Even within comparable ages, wide variability has been reported, concluding age as a weak reflector of ovarian pool. Thus other markers such as Follicle...
Anti Mullerian Hormone (AMH) is recently considered as a unique OR marker, solely secreted by granulosa cells and accurately reflects primordial follicle.\(^8\) It is a glycoprotein dimer belonging to TGF-\(\beta\) family and has potential role in the maintenance of OR.\(^9,10\) Drop in serum AMH reliably indicates decline in ovarian function. Hence, it is now being used to assess ovarian injury induced by chemotherapy, radiation therapy or ovarian surgery.\(^11\) It correlates well with the number of oocytes retrieved after stimulation and reflects excessive follicular growth in women with ovarian hyper stimulation syndrome (OHSS) and PCOS.\(^12,13\) These oocytes retrieved after stimulation, reflects availability of embryos for transfer and hence an optimum response to Long GnRH agonist protocol is said to achieve by retrieval of at least five oocytes.\(^14\)

Therefore, this study aimed to assess reliability of various markers (like FSH, AFC and AMH) in predicting response to intra-cytoplasmic sperm injection (ICSI). Our results propose that regarding number of oocytes retrieved, AMH is a better predictor in comparison to age, BMI, AFC and FSH, particularly in younger infertile population (20 to 35 years).

**METHODS**

In this retrospective cross-sectional analysis, data of 166 infertile females was collected from Australian Concept Infertility Medical Centre (ACMIC). Females booked for the first ICSI treatment from June 2014 to March 2015, aged 20 to 42 year, with regular menstrual cycles, no endocrine disorders or prior ovarian surgery, were included while PCOS were excluded from study. Ethical clearance was obtained from ACMIC. Written consent was waived by Institutional review board as retrospective design could not alter the clinical decision made during ICSI treatment. Furthermore, patients’ records were anonymized prior to analysis to maintain confidentiality. Serum samples were collected on days 2–3 of menstrual cycles one month prior to ICSI treatment. Supernatant fluid was used for the assays maintained at temperature from 2-8 °C.

AMH was measured using AMH Gen 11 Elisa reagent kit (Beckman coulter, ref a79765). FSH and LH were measured using Elecsys reagent kit following the manufacturer protocol. AFC assessment was carried out on day 3, using an Aloka SSD-1000 (Japan) with a 5 MHz transvaginal probe. Follicles measuring <10 mm in diameter were counted in both ovaries to determine the cohort with an inter observer CV <5%.

1 mg of subcutaneous Buserelin Acetate (Suprefact) was initiated on day 21 to achieve adequate ovarian suppression, followed by recombinant FSH (Gonal-f) or hMG (Menogon). hCG (Ovitrelle, 250 μg, Merck Serono) was administered on an adequate E\(_2\) response and two

### Table I: Descriptive statistics of whole cohort and according to responder category.

| Variables | Whole Study Population | Good Responder (5-19 oocyte count) | Bad Responder (<5, >19 oocytes) |
|-----------|------------------------|-----------------------------------|--------------------------------|
|           | n = 166                | n = 90                            | n = 76                          |
| Age (year) | 33.6 ± 6.03            | 31.7 ± 5.4                        | 35.6 ± 6.0                      |
| BMI (kg/m\(^2\)) | 29.3 ± 5.41            | 28.70 ± 5.4                       | 29.8± 5.3                       |
| FSH (IU/L) | 8.5 ± 4.9              | 6.9 ± 3.2                         | 10.2 ± 5.7*                     |
| LH (IU/L)  | 6.9 ± 1.057            | 6.5 ± 1.7                         | 7.4 ± 1.4                       |
| AMH (ng/ml) | 1.6 ± 1.3              | 2.3 ± 0.7                         | 1.4 ± 0.5*                      |
| AFC        | 9.3 ± 4.3              | 10.6 ± 5.1                        | 7.9 ± 2.6 *                     |
| Estradiol (pg/ml) | 46.95 ± 6.3         | 48.0 ± 6.78                       | 45.0 ± 7.3                      |

Data expressed as Mean ± S.D. Mann Whitney U test was used to compare the difference between groups.
or more follicles measuring ≥18 mm. Oocytes were retrieved trans-vaginally and sperms were injected. Embryos were transferred into uterus after 72 hours of insemination. Luteal phase support was given by Pregnyl 1500 IU. Clinical pregnancy was confirmed by sonographic evidence of an intrauterine gestational sac.

Main outcome was measured as good responders having at least 5 to 19 oocytes retrieved while bad responders had either less than 5 or more than 19 oocytes retrieved. Positive pregnancies were not considered as the outcome due to limited size of sample and inclusion of cases with male infertility.

Data was analyzed by SPSS version 19 and comparison of variables was done by Mann Whitney U test. Logistic regression determined the predictive value for ovarian response while AUROC analysis was used to see the predictive accuracy of FSH and AMH. Spearman bivariate correlation was applied between AMH and FSH. P value of <0.05 was considered significant.

RESULTS

The mean BMI, FSH and AMH levels of one hundred and sixty-six infertile females recruited in this study is summarized in Table-I. While grouping them according to oocytes retrieved, both AMH and AFC were significantly low in bad responders (p < 0.05) whereas FSH was raised (p < 0.05).

Table-II compares the groups according to AMH cut off, FSH and age. One hundred fifty two females reported low AMH and out of these, only 47.5% responded well while in higher AMH category, 85.7% females had a good response. In these groups, significant difference in AMH was observed (p<0.01) whereas LH, FSH and AFC had insignificant difference. On FSH stratification, significant difference in the levels of LH, AMH and AFC (p <0.05) was observed. Good response was reported by 62.2% of normal while 30.0% of raised FSH group. In age segregated groups, insignificant variance was seen for AMH, LH and FSH.

Next, on binary logistic regression, unadjusted model reported that females with low AMH were 6.66 times more likely to have a poor response than raised FSH (3.66 times). After adjusting for age and BMI, AMH gave even stronger positive significant association with the responder group [OR 15.06 (2.83- 80.01)] versus FSH [OR 4.12 (1.12-9.86)] (Table-III).

Table-II: Biophysical and Biochemical Variables on the basis of AMH, FSH and Age Category.

| Age            | AMH          | FSH          |
|----------------|--------------|--------------|
| 35-42 year     | 20-35 year   | Normal >1.37ng/ml | Low <1.37ng/ml | Normal < 11 IU/L | High >11 IU/L |
| n = 60         | n = 106      | n = 14       | n = 152       | n = 106          | n = 60        |
| Mean ± SD      |              |              |              |                  |              |
| Age (year)     | 40.2 ± 3.5   | 29.9±3.4*    | 33 ± 8.9     | 33.6 ± 5.7       | 32.3 ±5.7     | 36 ± 6.0     |
| BMI (kg/m²)    | 30.1 ± 5.2   | 28.8±5.5     | 33.4 ± 3.3   | 29.8 ± 5.4*      | 29.8 ± 5.1    | 30 ± 5.6     |
| FSH (IU/L)     | 9.1 ± 5.3    | 8.1 ± 4.6    | 5.9 ± 3.3    | 8.8 ± 4.5        | 6.1± 1.9      | 13 ± 5.5*    |
| LH (IU/L)      | 7.3 ± 2.5    | 6.8 ± 1.1    | 4.3 ± 2.5    | 7.2 ± 1.5        | 4.5 ± 2.4     | 11.3 ± 4.4*  |
| AMH (ng/ml)    | 1.5 ± 0.8    | 2.0 ± 0.3*   | 2.8 ± 1.1    | 0.7 ± 0.3*       | 2.2 ± 0.4     | 1.2 ± 0.5*   |
| AFC            | 8.4 ± 2.9    | 9.8 ± 4.8    | 8.0 ± 1.9    | 9.4 ± 4.4        | 8.6 ± 4.6     | 9.7 ± 4.0*   |
| Good Responder%| 36.6         | 58.5         | 85.7         | 47.4             | 62.2          | 30.0         |
| Bad Responder% | 63.3         | 41.5         | 14.2         | 52.6             | 37.7          | 70.0         |

Data expressed as Mean ± S.D. and frequency and percentage wherever applicable. Mann Whitney U test was used to compare the difference between groups. *p<0.05 considered significant.

Table-III: Logistic Regression Analysis for FSH, AMH and AFC.

| Variables | Unadjusted | Adjusted for Age and BMI |
|-----------|------------|--------------------------|
|           | OR         | 95% C.I                  | OR         | 95% C.I                  |
|           | Lower      | Upper                    | Lower      | Upper                    |
| FSH       | 3.66*      | 1.85 7.24                | 4.12*      | 1.72 9.86                |
| AMH       | 6.66*      | 1.44 30.80               | 15.06*     | 2.83 80.01               |
| AFC       | 0.79*      | 0.70 0.89                | 0.81*      | 0.72 0.90                |

Table-I: Main outcome measured as good responders having at least 5 to 19 oocytes retrieved while bad responders had either less than 5 or more than 19 oocytes retrieved. Positive pregnancies were not considered as the outcome due to limited size of sample and inclusion of cases with male infertility.

Data was analyzed by SPSS version 19 and comparison of variables was done by Mann Whitney U test. Logistic regression determined the predictive value for ovarian response while AUROC analysis was used to see the predictive accuracy of FSH and AMH. Spearman bivariate correlation was applied between AMH and FSH. P value of <0.05 was considered significant.
Fig. 1 presents correlation of AMH and FSH with oocytes retrieved in various age groups. AMH depicted stronger positive association in patients <35 year of age ($r=0.245; p=0.012$) versus patients >35 year ($r=0.169; p>0.05$). FSH depicted negative correlation that was likewise higher in patients <35 year of age ($r=-0.415; p<0.001$). In addition, significant negative correlation with bad responders was observed between the oocyte retrieval and AMH ($r = -0.468, p <0.001$), whereas significant positive correlation was seen with FSH ($r = 0.332, p < 0.001$).

Later, ROC analysis observed the discriminatory power of AMH along with FSH and AFC in two responder groups (Table-III). The AUROC for AMH was highest (0.771; $p < 0.05$) in terms of accurately discriminate between good and bad responders. FSH and AFC had a lower discriminatory power (0.692 and 0.690 respectively; $p < 0.05$). The AMH cut-off levels were calculated by the online software MedCalc$^{15}$ for poor ovarian response. A value of 1.37ng/ml was calculated, with a specificity of 75% and a sensitivity of 90% to exhibit higher ability to discriminate between good and bad responders in all the groups. Serum AMH levels of less than 1.37ng/ml was associated with bad response while that of higher than 1.37ng/ml correlated better ovarian reserve.

**DISCUSSION**

Young females are often advised to postpone infertility treatment based on their age and FSH levels. Hence, we explored strength of biomarkers including recently acclaimed AMH in discriminating between good and bad responder. Furthermore, we analyzed ovarian response in sub-groups segregated on the bases of age, FSH levels and AMH cut-off values calculated in our population.

In this study, we report significant correlation of oocytes retrieved with FSH, AMH and AFC while no association with age, BMI or LH. In comparison to FSH and AFC, AMH had a superior role as a solo marker of response. The AUROC for AMH was significantly higher than FSH and AFC ($p <0.05$). Our results revealed that 85.7% patients with normal AMH retrieved more than 5 oocytes. Furthermore, in unadjusted models of binary logistic regression, low AMH showed highest ability to identify bad responder as compared to raised FSH or AFC (6.66
times and 3.66 times respectively). Undoubtedly, 
AFC assessment is substantial to monitor infertility 
treatment however, operator’s variability is its 
limitation.16 As blood tests have marked advantages 
over ultrasound for primary care physicians, AMH 
has a greater efficiency over AFC, especially in 
setups where high class technology is not feasible.

While comparing AMH with the most commonly 
used marker FSH, segregation on the basis of 
FSH showed significant inverse correlation with 
AMH (Table-II). Therefore, we suggest AMH as 
its substitute in cases of deranged FSH production 
as discussed earlier. Being an “acyclic” marker, 
AMH assessment is considered reliable anytime 
throughout menstrual cycle17 although few studies 
do report converse findings.18

To further reinforce our results, AMH cut-off 
value for our population was calculated as 1.37ng/ 
ml with the strength of 75% specificity and 90% 
sensitivity to correctly predict the response. We 
found that 87% patients having more than 1.37ng/ 
ml AMH reported higher oocyte retrieval. Similarly, 
mean AMH of patients who conceived on ICSI was 
observed as 1.78 ± 0.95ng/ml. However, due to the 
limitation of sample size and inclusion of couples 
with male infertility, we cannot debate on wider 
role of AMH in predicting pregnancy outcomes. 
Various cut-off are reported across the globe. An 
Indian study has reported serum AMH levels of 
less than 1.4ng/ml as suggestive of poor ovarian 
response to stimulation.19 Here, it is important 
to emphasize that these cut-offs need to be used 
with caution as in our study we witnessed one 
patient reporting good response even with serum 
AMH of 0.6ng/ml. European study quoted AMH 
levels of as low as 0.8 µg/l as sufficient to reflect 
healthy ovarian response.20 This might be 
explained as although low AMH reflects decline in OR, but 
even few oocyte of good quality may still respond 
to gonadotropin stimulation. Thus, we advocate its 
capability in counselling infertile couples, selecting 
treatment protocol and tentatively predicting 
chances of pregnancy.21

Next, we assessed AMH as a predictor of oocyte 
retrieval in two different groups based on age. 
In our study there was a stronger correlation in 
patients younger than 35 years (p<0.001, Fig.1 A 
and B). This further supports role of AMH to predict 
ART outcome in younger population that might 
be misjudged due to early age and a normal FSH. 
Studies do report that in population of similar age 
group, wide variations of OR have been testified in 
individuals.3 This explains the variation observed 
in AMH levels but its assessment along with other 
baseline investigations seems to be of additional 
value in screening young infertile patients with a 
decreased OR. Contrary to this, David H et al. 
reported significant correlation of AMH and oocyte 
retrieval in older infertile women.22 This difference 
could possibly be due to population stratification or 
selection criteria. Our study further reports similar 
results between FSH levels and oocyte retrieval as 
it was likewise found to correlate better in younger 
population (p<0.001, Fig.1 A and B). On the other 
hand, AFC showed higher correlation in the older 
group. Our results reported insignificant difference 
in AMH of patients with varied BMI, thus 
suggesting that it reflects true ovarian response 
irrespective of the patient’s BMI as also supported 
by earlier work.23

In conclusion, we support the role of AMH in 
reflecting the number of oocytes collected for 
successful ART. For the first time, we are reporting 
AMH levels in Pakistani infertile population 
and predict a cut-off value of 1.37ng/ml that 
discriminate good and bad responders. We strongly 
suggest incorporation of AMH evaluation in 
baseline assessment of ovarian reserve, especially 
in younger infertile patients as timely IVF treatment 
can improve the pregnancy outcomes in these 
populations. As our sample size was limited and 
localized, further longitudinal studies are required 
to reassess the cut-off values. Furthermore, studies 
focusing on association of serum AMH with viable 
pregnancy outcomes will increase the support for 
AMH as a predictive marker of live births in ART 
clinics.

ACKNOWLEDGEMENT

The authors sincerely thank Dr. Syed Sajjad 
Hussain, Director of ACIMC for his support in 
conducting this study.

Declaration of interest: The authors declare that 
they have no conflict of interest.

Funding: None.

REFERENCES

1. Broekmans FJ, Knauff EA, te Velde ER, Macklon NS, Fauser 
BC. Female reproductive ageing: current knowledge and 
future trends. Trends Endocrinol Metab. 2007;18(2):58-65.
2. Hansen KR. Predicting reproductive age with biomarkers of 
ovidian reserve--how (and what) are we measuring? Semin 
Reprod Med. 2013;31(6):416-426.
3. 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(6):1519-1524.

4. Nelson SM. Biomarkers of ovarian response: current and future applications. Fertility and Sterility. 2013;99(4):963-969.

5. Abdalla H, Thum M. An elevated basal FSH reflects a quantitative rather than qualitative decline of the ovarian reserve. Human Reproduction. 2004;19(4):893-898.

6. Kuohung W, Mark D Hornstein. Evaluation of female infertility. http://www.uptodate.com/home. Accessed October 22, 2015.

7. Colao A, Di Somma C, Pivonello R, Faggiano A, Lombardi G, Savastano S. Medical therapy for clinically non-functioning pituitary adenomas. Endocrine-related Cancer. 2008;15(4):905-915.

8. Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegoed JA, et al. Control of Primordial Follicle Recruitment by Anti-Müllerian Hormone in the Mouse Ovary 1. Endocrinology. 1999;140(12):5789-5796.

9. Kalich-Philosoph L, Roness H, Carmely A, Fishel-Bartal M, Ligumsky H, Paglin S, et al. Cyclophosphamide triggers follicle activation and “burnout”; AS101 prevents follicle loss and preserves fertility. Science Translational Medicine. 2013;5(185):185ra62-185ra62.

10. Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegoed JA, et al. Control of Primordial Follicle Recruitment by Anti-Müllerian Hormone in the Mouse Ovary 1. Endocrinology. 1999;140(12):5789-5796.

11. Anderson RA, Rosendahl M, Kelsey TW, Cameron DA. Pretreatment anti-Müllerian hormone predicts for loss of ovarian function after chemotherapy for early breast cancer. Euro J Cancer. 2013;49(16):3404-3411.

12. Broer S, Dölleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. Hum Reprod Update. 2011;17(1):46-54.

13. Ubaldi F, Vaiarelli A, D’Anna R, Laura Rienzi L. Management of poor responders in IVF: is there anything new? Bio Med Res Int. 2014(2014), Article ID 352098, 10 pages. doi: 10.1155/2014/352098

14. Verberg M, Eijkemans MJ, Macklon NS, Heijnen EM, Baart EB, Hohmann FP, et al. The clinical significance of the retrieval of a low number of oocytes following mild ovarian stimulation for IVF: a meta-analysis. Hum Reprod Update. 2009;15(1):5-12.

15. Available from: https://www.medcalc.org/. Accessed September 14, 2015.

16. Carlsson IB, Scott JE, Visser JA, Ritvos O, Themmen AP, Hovatta O. Anti-Müllerian hormone inhibits initiation of growth of human primordial ovarian follicles in vitro. Hum Reprod. 2006;21(9):2223-2227.

17. Hehenkamp WJ, Looman CW, Themmen AP, de Jong FH, Te Velde ER, Broekmans FJ. Anti-Müllerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. J Clin Endocrinol Metab. 2006;91(10):4057-4063.

18. Lindhardt Johansen M, Hagen CP, Johanssen TH, Main KM, Picard JY, Jørgensen A, et al. Anti-Müllerian hormone and its clinical use in pediatrics with special emphasis on disorders of sex development. Int J Endocrinol. 2013(2013), Article ID 198698, 10 pages. doi: 10.1155/2013/198698 2013.

19. Singh N, Malik E, Banerjee A, Chosdol K, Sreenivas V, Mittal S. Anti-Müllerian Hormone: Marker for Ovarian Response in Controlled Ovarian Stimulation for IVF Patients. A first pilot study in the Indian population. J Obstet Gynecol India. 2013;63(4):268-272.

20. Hamdine O, Eijkemans M, Lentjes E, Torrance H, Macklon N, Fauser B, et al. Ovarian response prediction in GnRH antagonist treatment for IVF using anti-Müllerian hormone. Human Reproduction. 2015;30(1):170-178.

21. Jamil Z, Fatima SS, Ahmed K, Malik R. Anti-Müllerian Hormone: Above and Beyond Conventional Ovarian Reserve Markers. Disease Markers. 2016;2016:5246217. doi: 10.1155/2016/5246217.

22. Barad DH, Weghofer A, Gleicher N. Comparing anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH) as predictors of ovarian function. Fertility and Sterility. 2009;91(4):1553-1555.

23. Parveen N, Sheikh S, Javed S. Relationship of body mass index with ovarian reserve status of infertile women measured by anti-müllerian hormone level. Pak J Physiol. 2014;10(1-2):25-27.

Author’s Contribution:

ZJ conceptualized the study, designed and drafted the manuscript. SSF, SA and RR drafted the manuscript. FA critically reviewed the manuscript. All the authors approved the final version of the paper.