

## Comparison of serum anti-mullerian hormone among fertile and infertile normal and diminished ovarian reserve groups

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### Abstract

**Objective:** To compare anti-mullerian hormone among fertile and infertile groups of women in their reproductive age.

**Methods:** This case-control study was conducted at the Dow University of Health Sciences in collaboration with Civil Hospital, Karachi, from October 2011 to October 2012, and comprised fertile non-pregnant and infertile women. Serum anti-mullerian hormone levels were measured in both the infertile and fertile groups. Blood samples to determine anti-mullerian hormone levels were obtained irrespective of their menstrual cycle days. Infertile cases were further divided into two subgroups according to serum anti-mullerian hormone levels, into normal ovarian reserve group and diminished ovarian reserve group. SPSS 18 was used for data analysis.

**Results:** Of the 100 participants, 48(48%) women were fertile controls and 52(52%) were infertile. Of the latter, there were 30(57.69%) in the normal ovarian reserve group and 22(42.31%) in the diminished ovarian reserve group. The mean age of the participants was  $26 \pm 4.026$  years (range: 25-35 years). The mean values for the control, normal ovarian reserve and diminished ovarian reserve groups were  $1.9 \pm 0.16$  ng/ml,  $0.89 \pm 0.47$  ng/ml and  $2.0 \pm 0.6$  ng/ml, respectively ( $p=0.001$ ).

**Conclusion:** Mean concentration of serum anti-mullerian hormone in infertile women with diminished ovarian reserve was significantly lower than that in normal ovarian reserve group and fertile control women.

**Keywords:** Fertile women, Anti-mullerian hormone, Infertility. (JPMA 66: 1060; 2016)

### Introduction

In recent decades, infertility has become a major global issue with medical, economic and psychosocial impact on infertile couples. A large number of infertile population is very anxious and eager to be treated. The prevalence of infertility worldwide is approximately 10-15% whereas in Asia it is around 8-12%. Infertility rate in Pakistan is about 21.9%.<sup>1,2</sup>

The rationale of the study was to assess the ovarian reserve in the infertile women by serum anti-mullerian hormone (AMH), and to opt for better fertility treatments and predict satisfactory outcome during assisted reproductive techniques.

Ovarian reserve is the amount of oocytes present in both ovaries. The conventional tests to determine an individual's ovarian reserve include Day 3 serum follicle-stimulating hormone (FSH) levels and antral follicle count. Recently, another test for measuring AMH levels has also been included as a marker of ovarian reserve.<sup>3</sup>

Mullerian inhibiting substance is the other name for AMH. It is a dimeric glycoprotein and a 140-kilodalton (kDa) hormone. It belongs to the super family of transforming growth factor beta.<sup>4</sup>

Initially, synthesis of AMH occurs as a large precursor molecule. Later on, it is converted into the pre-prohormone and this forms the homodimers. The homodimer, or the mature hormone, then undergoes glycosylation and produces two identical 70-kDa monomer subunits. These monomer subunits are bonded by disulphide-linkage by dimerisation, thus producing a 140-kDa dimer. Each monomer subunit contains two domains; an N-terminal domain and C-terminal domain. AMH acquires its full bioactivity by the N-terminal domain which causes the activation of C-terminal domain.<sup>5</sup> It is mainly produced and secreted by the granulosa cells of ovarian follicles. The levels of serum AMH are hardly detectable at birth, but remains stable throughout the reproductive period. With advancing age, AMH levels start declining. According to recent studies, serum AMH levels represent the quantitative aspect of ovarian reserve. Moreover, its levels strongly correlate with the size of the early growing follicle pool, and due to lack of inter-cyclic variability, it can be regarded as a diagnostic marker to assess ovarian reserve. The ovary secretes AMH directly into the blood; hence it is easy to measure AMH in serum.<sup>6</sup>

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AMH is involved in the process of folliculogenesis. Physiologically, there are two main functions of AMH, i.e. it inhibits the recruitment of primordial follicle cell and it also inhibits the selection of follicles that are under the influence of FSH for dominance.<sup>7</sup>

Female reproductive hormones such as FSH, luteinising hormone (LH) and steroids are involved in late folliculogenesis i.e. during the formation of large antral follicles. Thus, they reflect the peri-ovulatory ovarian activity. On the other hand, AMH is produced by the granulosa cells of early antral follicles and provides information regarding ovarian reserve.<sup>8</sup>

Diminished ovarian reserve (DOR) is regarded as one of the major causes of infertility.<sup>9</sup> AMH is regarded as an excellent marker of the ovarian reserve, a measure of the biological age of the ovaries and a diagnostic marker of ovarian dysfunction.<sup>10</sup> Moreover, in DOR, serum AMH levels are found to be reduced earlier before any increase in baseline FSH levels.<sup>11</sup> AMH testing is particularly beneficial for younger women because DOR is often overlooked in this age group, leading to an incomplete diagnosis of infertility.<sup>12,13</sup>

Assessment of ovarian reserve by serum AMH levels can be determined with greater specificity and sensitivity in women of reproductive age rather than by determination of FSH together with other ovarian reserve markers. This is due to the fact that AMH acts in paracrine fashion and is not under the influence of negative feedback mechanisms of hypothalamic-pituitary-gonadal (HPG) axis. It can be measured on any day of the menstrual cycle.<sup>14,15</sup>

This study was planned to include AMH as part of the ovarian reserve tests in our population besides the conventional markers like FSH and antral follicle count.

## Patients and Methods

This case-control study was conducted at the Institute of Basic Medical Sciences, Dow University of Health Sciences (DUHS) in collaboration with Gynaecology and Obstetrics Unit-II, Civil Hospital, Karachi, from October 2011 to October 2012, and comprised fertile non-pregnant and infertile women. The patients were selected from outpatient department (OPD) and bench work was carried out at the Dow Diagnostic Research and Reference Lab (DDRRL), Ojha Campus.

The sample size was estimated by using the OpenEpi software with expected sensitivity of 83.5%, specificity of 79% and prevalence of female infertility as 0.065 at 95% confidence interval (CI) and 5% estimated error

(d=5%, CI=95%). Primary infertile women with no history of previous pregnancy and with normal semen analysis of their husbands and patent fallopian tubes on the basis of hysterosalpingography were included.

Secondary sub-fertile females with history of previous pregnancy, with normal report of husband's semen analysis, patent fallopian tubes and no history of pelvic inflammatory disease or endometriosis were also included.

Fertile and infertile women aged above 35 years and having the history of blocked fallopian tubes, ovarian malignancy, previous pelvic surgeries, drugs interfering with fertility like oestrogen antagonists and male infertility factor, were excluded.

For estimation of serum AMH levels, all selected subjects' blood samples were drawn by venipuncture in serum separator tubes. Blood samples were taken from both the fertile and infertile groups for AMH levels on any day of the menstrual cycle. Approximately 3ml of blood was collected by venipuncture in separate gel tubes, centrifuged and serum collected and frozen in aliquots at -20° Celsius. The samples were stored temporarily at the Dow Collection Point and were then transferred to the Microbiology Lab at the DDRRL for further storage. Serum AMH levels were determined by enzyme-linked immunosorbent assay (ELISA), using Human AMH Elisa kit (CDN-E 1350) at DDRRL.

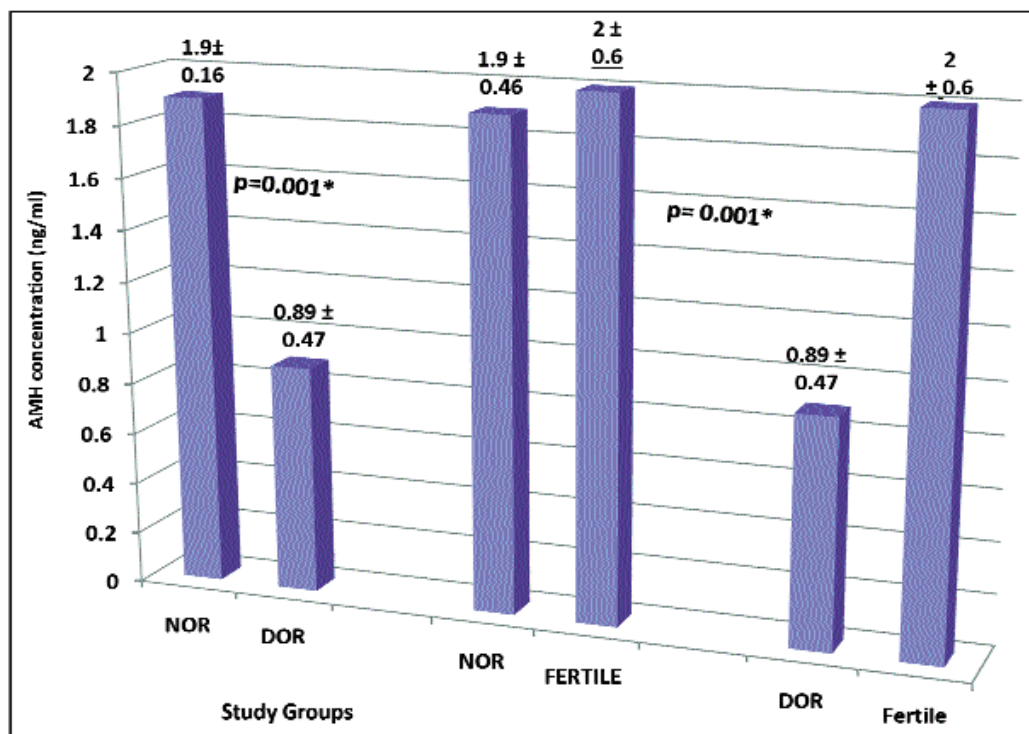
Infertile female subjects were grouped according to their serum AMH levels into Normal Ovarian Reserve (NOR) group (>1.2ng/ml) and DOR group (< 1.2 ng/ml).<sup>9</sup>

SPSS 18 was used for data analysis. Analysis of variances (ANOVA) was done to compare the mean values for NOR, DOR and fertile groups.  $P < 0.05$  was considered significant. Games-Howell post-hoc test was applied to measure the differences of mean AMH levels between various groups.

## Results

Of the 100 participants, 48(48%) women were fertile controls and 52(52%) were infertile. Of the latter, there were 30(57.69%) in the NOR group and 22(42.31%) in the DOR group. The mean age of the participants was  $26 \pm 4.026$  years (range: 25-35 years). The mean values for the control, NOR and DOR groups were  $1.9 \pm 0.16$  ng/ml,  $0.89 \pm 0.47$  ng/ml and  $2.0 \pm 0.6$  ng/ml, respectively ( $p = 0.001$ ) (Table).

Since homogeneity of variances were unequal, Games-Howell post-hoc test was applied which showed significant difference between NOR and DOR groups, and



NOR: normal ovarian reserve  
 DOR: diminished ovarian reserve  
 NOR: DOR as P-value < 0.05\*  
 DOR: Fertile controls as P-value < 0.05\*  
 AMH: Anti-mullerian hormone

Figure: Games Howell Post Hoc Test for the comparison of AMH level among the study groups.

Table: ANOVA between NOR, DOR & fertile controls.

Groups	N	Mean±SD	P Value
NOR†	30	1.90±0.16	
DOR††	22	0.89±0.47	0.001**
Fertile Controls	48	2.0±0.6	

\*\*P value is significant at < 0.05

NOR: Normal ovarian reserve

DOR: Diminished ovarian reserve.

between DOR and fertile groups ( $p=0.001$ ) (Figure).

## Discussion

Infertility is a global issue and needs to be assessed at an earlier stage for better options of fertility treatments. There are various conventional tests available for ovarian reserve assessment, including the basal Day 3 FSH levels and early antral follicle count by transvaginal ultrasound. Apart from these tests, AMH has also been proved as one of the most reliable markers for ovarian reserve

assessment.<sup>16</sup>

This study was designed to compare mean AMH levels in both fertile and infertile females. There is no uniform reference range for interpreting AMH levels with regard to ovarian reserve, largely because different laboratories use different assays. Gnath et al. determined AMH levels of less than 1.26 ng/ml as DOR regardless of age.<sup>9</sup> Ebner et al. and Tremellen et al. determined an AMH of < 1.7 ng/ml and < 0.8 ng/ml as reduced ovarian reserve.<sup>17,18</sup>

We divided infertile subjects into two subgroups on the basis of AMH levels which were consistent with the findings of another study in which 97% of infertile women were

identified with a reduced ovarian reserve, with a cut-off level <1.26 ng/ml of AMH.<sup>9</sup>

## Conclusion

Assessment of DOR in infertile women with AMH would predict better options for fertility treatments.

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**Conflict of Interest:** No

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