THE JOURNAL OF OBSTETRICS AND GYNECOLOGY OF INDIA

The Journal of Obstetrics and Gynecology of India (March–April 2014) 64(2):130–133
DOI 10.1007/s13224-013-0482-3

ORIGINAL ARTICLE

Anti-Mullerian Hormone: A New Marker of Ovarian Function

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Received: 5 June 2013 / Accepted: 14 October 2013 / Published online: 3 December 2013

Abstract
Objectives The aim of this study was to determine day 3 Serum AMH, FSH, LH, Estradiol (E_2), Inhibin B levels, ovarian volume, and antral follicular count to assess ovarian function.
Methods This study was conducted on 130 infertile women between age 18 and 43 years. Day 3 Serum AMH level was estimated by sandwich enzyme immunoassay; Serum FSH, S. LH, S. E_2, by solid-phase two-site chemiluminescent immunometric assay; Inhibin B by ELISA; and Ovarian volume and AFC, by transvaginal ultrasonography.
Results With advancing age, Serum AMH level ($p < 0.0001$), AFC ($p < 0.05$), ovarian volume (>0.05), and Inhibin B (>0.05) were decreased, and Serum FSH ($p < 0.05$), LH ($p > 0.05$), and E_2 ($p < 0.05$) were increased. Serum AMH level was 4–6.8 ng/ml with optimal fertility in 26.15 % cases and 2.2–4.0 ng/ml with satisfactory fertility in 53.85 % cases. Serum AMH levels were more strongly correlated with AFC ($p < 0.0001$) and ovarian volume ($p < 0.0001$).
Conclusion Serum AMH levels were more robustly correlated with AFC than FSH, LH, E_2, and Inhibin B on day 3 of the cycle. This suggested that serum AMH might be taken as single test to reflect ovarian reserve.

Keywords Anti-Mullerian hormone · Antral follicle count · Follicle stimulating hormone · Lutenizing hormone · Estradiol

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Introduction

The ovarian reserve constitutes one of the most important factors affecting ovulation. By "ovarian reserve," we basically mean the size of the ovarian follicle pool and the quantity of the oocytes therein. Many efforts have been made since the beginning to assess the ovarian reserve. Previously, a composite test consisting of early follicular serum levels of FSH, Inhibin B, and Estradiol (E_2) was used. Inhibin B and E_2 are produced by early antral follicles in response to FSH, having the classical feedback loop of pituitary gonadal axis. With the decline of the follicle pool, serum levels of Inhibin B and E_2 decrease leading to a rise in serum FSH level. Because these factors are part of feedback system, their serum levels are not independent of each other, and hence, they have to be measured collectively. Separately, their levels are poor predictors of ovarian reserve because their levels vary widely by assay, laboratory, population, and reproductive aging.

So far, AFC (follicles of 2–10 mm size), which quantifies the number of antral follicles in the ovary by ultrasonography on day 3 of menstrual cycle, best predicts the

quantitative aspect of ovarian reserve. However, it might be sometimes difficult for the patient to get ultrasound done on a specific day; additionally, it requires for the patient measurement of the AFC by additional transvaginal ultrasound examination during early follicular phase.

Therefore, in search of a better, time-independent parameter, serum anti-Mullerian hormone (AMH) emerged as a promising test to assess the ovarian reserve. AMH or Mullerian inhibiting substance (MIS) is a glycoprotein hormone, with a molecular weight of 140 kDa, and produced by granulosa cells in ovarian follicles from 36 weeks of gestation until menopause. It is first made in the primordial follicle stage but the highest production is in the preantral and small antral stages (<4-mm diameter) of folliculogenesis. During these stages, follicles are microscopic and cannot be seen by ultrasonography, thus limiting their ability to be counted by ultrasound. Production of AMH gradually decreases as the follicle grows further and then finally stops once the follicle reaches 8-mm diameter. AMH levels do not change significantly throughout the menstrual cycle. Normal serum AMH level range is 2–6.8 ng/ml (14.28–48.55 pmol/l) in any phase of the cycle. In recent years, accumulated data indicate that serum AMH may fulfill the requirements to be the best test to predict ovarian reserve.

Materials and Methods

The study was conducted on 130 infertile patients, aged between 18 and 43 years, over a period of 12 months. The infertile women, who had regular, menstrual cycles of 21–35 days with no current or past diseases affecting ovaries or gonadotrophin or sex steroid secretion, clearance or excretion; a body mass index (BMI) of 18–27 kg/m^2, on no current hormone therapy; and adequate visualization of ovaries during transvaginal ultrasound scanning. Complete hemogram, blood sugar estimation, Thyroid profile, Liver function test, Kidney function test, VDRL, HIV1 and 2, HBsAg, urine routine and microscopic examination, and Montoux test were done. Hysterosalpingography was done on day 8 or 9 of menstruation to see patency of tubes. Ultrasonography was done on day 3 of menstrual cycle to measure ovarian volume and for antral follicles count. The cases with endocrinological disorders, abnormal liver, and kidney function tests were excluded from the study.

Study Protocol

Serum AMH; FSH, LH, E_2, and Inhibin B were estimated for venous samples on day 3 of menstrual cycle at ~9:00 h. Serum AMH estimation was done by sandwich enzyme immunoassay for in vitro quantitative measurement in human serum. Normal level, corresponding to normal ovarian reserve, was 2.0–6.8 ng/ml. Serum FSH, LH, E_2 levels were measured by solid-phase, two-site, chemiluminescent immunometric assay. Patient test results were determined automatically by the system software using the smoothing "spline" math model. Inhibin B was tested by enzyme-linked immunosorbant assay (ELISA) method. Normal values in serum considered were for FSH 1.4–9.9 mIU/ml LH, 1.0–90.0 mIU/ml, and E_2 are 30–400 pg/ml. Normal value for Inhibin B was >45 pg/ml. Transvaginal sonography was done on day 3 of menstrual cycle, using the 7.5 MHz transvaginal probe, to assess antral follicular count and total ovarian volume. Antral follicle count was done by scanning the ovary from the outer to inner margin. All follicles measuring 2–10 mm size were counted in both the ovaries. The sum of both counts was "Antral follicular count." Normal AFC was taken if it was more than 12. The volume of the ovaries was assessed by measuring the diameter of the contour in three perpendicular directions and applying the equation of volume of an ellipsoid (D1 × D2 × D3 × 0.523). Total ovarian volume was then obtained by sum of the volumes of left and right ovary. Normal reference level was 9–11 cm^3. Finally, Correlations of day 3 "serum AMH" and day 3 "serum FSH, LH, E_2, and inhibin B" levels were found out with antral follicular count and ovarian volume. The correlation was found to be statistically significant, if on analysis p value was <0.05.

Observation

The cases were divided into four groups according to age. The maximum infertile women were of age between 21 and 30 years (Table 1). Age-dependent loss of fertility has been described due to decreasing follicle pool. However, this fact may be variable individually. Hence, for the assessment of the follicle pool, among the biochemical markers, Serum FSH and Serum E_2 showed statistically significant variations (p value <0.05). Serum level at day 3 FSH increased with the advancing age. Serum E_2 level was also found to increase with the increasing age. Though the results may look to be contradicting the fact of negative

Table 1 Age distribution

Group	Age in years	No. of cases	% of cases	Mean ± SD
1	<20	19	14.62	18.33 ± 0.594
2	21–30	76	58.46	26.07 ± 2.25
3	31–40	22	16.92	35.09 ± 2.06
4	>40	13	10.00	42.66 ± 1.11
Total		130	100	27.67 ± 2.04

Table 2 Correlations of AFC, ovarian volume, S. FSH, S. LH, S. Inhibin B, S. E_2, and S. AMH with different age groups

Characteristics	Group 1	Group 2	Group 3	Group 4	*p* value
Antral follicular count	16.22 ± 4.00	13.31 ± 3.00	9.26 ± 1.17	6.21 ± 0.92	<0.05
Ovarian volume (ml)	8.5 ± 1.08	7.31 ± 0.93	7.21 ± 0.96	7.41 ± 0.46	>0.05
FSH (mIU/ml)	4.77 ± 0.53	6.09 ± 0.59	6.48 ± 0.28	6.80 ± 0.23	<0.05
LH (mIU/ml)	3.86 ± 0.59	5.54 ± 0.59	5.59 ± 0.33	6.58 ± 0.36	>0.05
Inhibin B (pg/ml)	81.98 ± 1.20	77.9 ± 5.58	77.34 ± 0.95	75.43 ± 0.71	>0.05
E_2 (pg/ml)	18.48 ± 3.14	39.72 ± 11.84	54.20 ± 3.57	59.96 ± 4.61	<0.05
AMH (ng/ml)	5.56 ± 0.99	3.25 ± 0.70	2.61 ± 1.20	0.20 ± 0.11	<0.0001

feedback loop, the fact, however, is that Serum E_2 level basically depicts follicular growth rather than the number of antral follicle. Elevation in FSH and decrease in Inhibin B result in advanced follicular growth at the end of preceding luteal phase, in response to which day 3 serum E_2 levels are typically higher in older women with advanced reproductive aging, but Serum AMH level had highly significant reduction with the increasing age (<0.0001), even more than correlation of AFC with advancing age (p value <0.05) as depicted in Table 2. 34 patients were having serum AMH level 4.0–6.8 ng/ml, and 70 patients had 2.2–4.0 ng/ml. All of them ovulated on giving ovulation inducing drugs. Hence, it can be reemphasized that serum AMH ranging between 2.2 and 6.8 ng/ml predicts optimal to satisfactory ovarian reserve patients. Four cases had high level of AMH, and their ultrasonography revealed polycystic ovaries. In spite of having higher levels of AMH, these patients had low fertility. This mandates ultrasonographic picture of the ovary in the work up of ovarian reserve. PCOS patients had large number of antral follicles secreting high amount of AMH. Subfertility in this group of patients is due to other coexisting hormonal imbalances. In eight cases where AMH levels were <0.3 ng/ml, the fertility was said to be very low (Table 3). A noticeable reduction in the number of early antral follicles characterizes the decline of ovarian function that results from relative follicular attrition. Antral follicle count is so far the best predictor of the ovarian reserve. AFC <4 definitely predicts poor ovarian reserve, and >12 indicates good ovarian reserve. The strength of correlations between the number of early antral follicles and other biochemical markers of the ovarian reserve were compared. As the number of AFC increased, ovarian volume also increased ($p < 0.05$), serum FSH decreased ($p < 0.001$), serum LH decreased ($p < 0.001$), serum inhibin B increased ($p < 0.05$), serum E_2 decreased ($p < 0.05$), and serum AMH increased ($p < 0.0001$). All the parameters assessed in this study showed statistically significant correlation, but Serum AMH level was the most strongly correlated with antral follicle count (p value <0.0001) (Table 4).

Table 3 Serum AMH levels and fertility

AMH (ng/ml)	Number of cases	% of cases
High level (>6.8)	4	3.08
Optimal fertility (4–6.8)	34	26.15
Satisfactory fertility (2.2–4.0)	70	53.85
Low fertility (0.3–2.2)	14	10.77
Very low/undetectable (0.0–0.3)	8	6.15
Total	130	100

Discussion

The present study was designed to evaluate the direct relationship between peripheral AMH levels and the ovarian follicular status on day 3 of menstruation and to compare the strength of correlations between the number of antral follicle count and hormonal parameters implicated directly or indirectly in the eventual stages of folliculogenesis. It was observed that serum AMH levels are closely related to early antral follicle count, with a relationship that was remarkably more intense than those obtained with serum levels of inhibin B, E_2, FSH, and LH. These results not only corroborate to but also expand clinical data reported previously by other investigators. Outcome of the present study revealed that with the assessment of ovarian function and reserve, we can identify those patients who are destined to fail. Age, as an ovarian function marker, was found to have prognostic value in general infertile population because age showed strong correlation with the all markers of ovarian function like AMH, AFC, E_2, and FSH. AMH can screen the present status of ovarian function in general subfertile population because it has a role in both the processes of initial and cyclical recruitments as well as in women entering controlled ovarian stimulation and ART program. AMH better reflected the continuous decline of follicle pool with age than the other markers, it appears to be the best marker of gradual dwindling of follicle numbers and ovarian volume. AMH gives the most

Table 4 Correlations of AFC with ovarian volume(ml), S. FSH (mIU/ml), Serum LH (mIU/ml), S. Inhibin B(pg/ml), S. E2 (pg/ml), and Serum AMH(ng/ml)

AFC (no.)	Mean ± SD	No. of cases	Ovarian volume (ml)	S. FSH (mIU/ml)	S.LH (mIU/ml)	S. Inhibin B (pg/ml)	S. E_2 (pg/ml)	S. AMH (ng/ml)
<4	2.82 ± 0.73	8	5.95 ± 3.85	6.67 ± 0.90	6.1 ± 0.64	43.81 ± 35.3	59.77 ± 13.65	1.26 ± 1.79
4–7	6.34 ± 0.76	15	6.10 ± 2.98	7.02 ± 2.09	5.73 ± 1.30	75.61 ± 39.87	57.12 ± 14.03	1.49 ± 1.64
8–12	9.19 ± 1.58	82	7.56 ± 1.66	6.20 ± 0.84	5.47 ± 0.75	56.10 ± 34.49	55.35 ± 24.97	4.78 ± 2.52
>12	16.32 ± 1.79	25	7.88 ± 0.68	5.16 ± 1.32	4.28 ± 1.15	78.23 ± 25.32	26.84 ± 16.27	3.15 ± 0.98
p value			<0.05	<0.001	<0.001	<0.05	<0.05	<0.0001

reliable reflection of individual reproductive aging. In accordance with the present study, Van Rooij et al. [1] studied the relationship between AMH levels and ovarian response during ovarian stimulation for IVF on 130 patients. They found that serum AMH levels were highly correlated with the number of antral follicles ($r = 0.77$, $p < 0.01$) and the number of oocytes retrieved ($r = 0.57$, $p < 0.01$). A negative association was found between AMH levels and poor ovarian response (fewer than 4 oocytes or cycle cancelation or 0.82, 95 % CI 0.75–0.90, $p < 0.01$). The post GnRH rise in FSH and LH levels did not influence AMH values and concluded that poor response in IVF, indicative of a diminished ovarian reserve, associated with reduced baseline serum AMH concentrations. Fanchin et al. [2] studied day 3 Serum levels of AMH, Inhibin B, E_2, FSH, LH, and the number of early antral follicles estimated to compare the strengths of hormonal–follicular correlations; they also found that Serum AMH levels were more strongly correlated with follicular count than were with other markers. Ernest et al. [3] found that AFC was the ovarian reserve marker that was significantly different among different age groups. Muttukrishna et al. [4] found that the patients, who had canceled IVF treatment cycle, had AMH levels lower than the detection limits, while FSH levels were significantly high and Inhibin B were 50-folds lower compared with the patients who completed treatment. Muttukrishna et al. [5] postulated that there was no significant change in AMH levels on ovarian stimulation with gonadotropins, referring to AMH level as independent marker, and not altered by FSH or LH even in a normal menstrual cycle. Van Rooij et al. [6] found that Serum AMH concentrations showed the best consistency, with AFC. The FSH and inhibin B showed only modest consistency, whereas E_2 showed no consistency at all, and it was concluded that serum AMH represented the best endocrine marker to assess the age-related decline of reproductive capacity. La Marca et al. [7] investigated that AMH exhibits a relatively stable expression during the menstrual cycle, making it an attractive determinant of ovarian activity. Knauff et al. [8] concluded that in comparison with inhibin B and AFC, AMH was more consistently correlated with the clinical degree of follicle pool depletion in young women presenting with elevated FSH level.

Conclusion

Serum AMH levels were more robustly correlated with Antral Follicle Count than Serum FSH, LH, Inhibin B, and E2 at day 3 of cycle. S.AMH is superior marker of ovarian reserve because it is highly associated with the number of antral follicle, and has little cycle variability and decline throughout the reproductive life span. This suggests that AMH, a new marker, may reflect ovarian function better than the usual hormone markers.

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