Serum Anti-Mullerian Hormone is available test to predict ovarian follicular Status In Infertile Women

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Abstract— In the ovary, Anti-Mulllerian hormone (AMH) is produced by the granulosa cells of early developing follicles. The aim of our study was to assess the value of the AMH as a test to predict ovarian damage in groups of infertile women. Ninety seven infertile women were divided into four groups: (G1=29) with regular menstrual cycle, (G2=30) with prolonged or irregular cycles, (G3=21) with secondary amenorrhea and (G4=17) with polycystic ovarian syndrome or PCOS. A control group of fertile women was included in the study. AMH levels were higher in fertile women than in G1, G2 and G3, but in PCOS women (G4) AMH was higher than in fertile group, reflecting the increased number of small antral follicles in these categories of patients. FSH levels in infertile group were higher in G3 than in G2 and G1 (p=0.002); in fertile women, FSH was (9.2±3.0 UI/ml). There was negative correlation between AMH and age (r=-0.40; p=0.00) and between AMH and FSH, in infertile group. A positive correlation was also found between AMH levels and antral follicle count (AFC) (r=0.6; p=0.00).Our findings suggest that the measurement of AMH seems to be an ideal test for ovarian status which can be assessed in different group of infertile women.

Keywords— Anti-Mullerian hormone / Antral follicles count / Infertility / Ovarian reserve / PCOS

1 Introduction

Anti-müllerian hormone (AMH) is a glycoprotein traditionally known for its role in male sexual differentiation (1, 2). More recently, AMH has been studied for its role in ovarian folliculogenesis and as a potential marker of ovarian reserve. A number of studies (3, 4, 5) have suggested that the measurement of serum AMH is a superior test of ovarian reserve because it is highly associated with the number of antral follicles, has little cycle variability, and declines throughout the reproductive lifespan. There is also evidence supporting the ability of AMH levels to predict age of menopause (6).

Since women with PCOS are known to have an excessive amount of small antral follicles in the ovaries and at the same time increased serum AMH levels (7, 8, 9, 10), it is possible that AMH may indeed play a role in PCOS, being one of the factors that cause reflect functional or morphological features, typical of the syndrome (9).

Based on these considerations, the aim of the present study was to evaluate in infertile women with different clinical characteristics, whether serum Anti-müllerian hormone measurement, predict ovarian follicular status and whether the assessment of AMH would improve the diagnosis and the management of infertility.

2 Materials and Methods:

This prospective study included a total of 97 women (aged 22–40) consulting for fertility problems at the department of gynecology and obstetrics, Hedi Chaker Hospital of Sfax between May 2011 and September 2013. All of the participants in this research were asked for their consent. As a control group, we also studied 30 fertile women aged (20-35) and who had regular menstrual cycles of 21–35 days duration. The study was approved by the University of Sfax (Histo-Embryology Anatomy Unit (99 /UR/08-60), Faculty of Medicine of Sfax, Tunisia).

The 97 infertile women were divided into four groups :(G1; n=29) with regular menstrual cycle (cycle lengths ranged from 21 to 35 days), (G2; n=30) with irregular menstruation, (G3; n=21) with secondary amenorrhea (absence of vaginal bleeding for at least 6 months unrelated to PCOS), and a last group (G4; n=17) with PCOS. According to the Rotterdam criteria [7], the diagnosis of PCOS was based on the association of at least two of the three following criteria: 1) oligo-and/or anovulation (OA), hyperandrogenism (either clinical or biochemical) (HA), 3) more than 12 follicles in the 2- to 9-mm range in each ovary at U/S and/or an ovarian volume higher than 10 ml.

The body mass index (BMI) of study participants ranged from 24.5 to 26.5 kg/m2. Women volunteers were recruited from the University of Medicine of Sfax. They were healthy, fertile and normal cycling (21–35 days) and were without a history of hyperandrogenism and without diseases including polycystic ovary syndrome, chronic disease such as genetic syndromes, thyroid, renal, liver, or cardiac disease. Women who had been pregnant during the previous 6 months, used sex steroids or contraceptive preparations or who smoked were not recruited. Protocols were approved by the Habib Bourguiba Hospital re-

search ethical committee, and written informed consent was obtained from each subject before participation.

Serum aliquots were immediately separated from blood samples, and then frozen at -80 C° till assay. The concentrations of serum AMH were measured by ELISA assay using a second generation enzyme immunoassay (AMH Gen II ELISA, A73818 Beckman Coulter, Inc.). The method used was in accordance with the manufacturer's instructions. The limit of detection with this assay is 0.01ng/mL. Intra-assay and interassay coefficients of variation (CV) were ≥12.3% and≥14.2%, respectively. Each sample was run in duplicate within a single assay.

On Day 3–5 of a spontaneous cycle patients underwent transvaginal ultrasound examination to count the number of antral follicles (AFC).

For statistical analysis, comparison between groups was assessed by Student's t test. The correlation between continuous variables was assessed by Spearman regression analysis. Statistical analysis was performed using the Statistical Package for Social Science software (SPSS 18.0 Inc., Chicago). A P value of <0.05 was considered statistically significant.

The lowest detection limit and the intra-assay and inter-assay coefficients of variation for FSH were 0.37 IU/L, <5%, and <5%, respectively.

3 Results:

The clinical characteristics of patients and controls are shown in (Table 1). Age and BMI were similar between the groups (p>0.05). The average serum levels of AMH, were 1.02±1.23ng/ml in infertile women (G1, G2 and G3) and 3.4±1.3ng/ml in fertile women (p=0.009) and 9.3±4.6 ng/ml in PCOS group. AMH levels were higher in fertile group than in G1, G2 and G3 groups of infertile women (3.4±1.3vs 1.5±1.2 in G1 vs 0.88±1.0 in G2 and vs 0.22±0.3 ng/ml in G3, respectively), but it was approximately three-fold higher in serum from women with PCOS (G4) than in fertile women (9.3±4.6 vs 3.4±1.3) (figure1). The average levels of FSH in infertile women (G1, G2 and G3) was (21.9±23.40) the mean FSH level within each group was (13.06±10.2 in G1 vs 16.3±0.1 in G2 and vs 41.5±27.9 UI//ml in G3 respectively) (p=0.002). In PCOS group the levels of FSH were (7.08±3.43 UI//ml) (Table1), (figure2) and in fertile group FSH levels were (9.2±3.0 UI//ml). In agreement with this, serum levels of AMH in the infertile group were negatively correlated with age (r = -0.40, p =0.00) (figure 3) and with FSH level (r=-0.32; p=0.003) but not in PCOS group (p>0.05). Finally, There was a significantly elevated correlation between levels of AMH and antral follicle count AFC (r=0.6, p=0.00) (figure 4) and moderate negative correlation between Age and CFA (r=-0.44, p=0.00).

4 Discussion:

AMH is a product of granulosa cells of primordial follicles that have undergone initial recruitment and is thought to reflect the size and quality of the ovarian reserve (3, 4, 5, 8, 11, 12). Added, there is particular interest in using measures of AMH as a biomarker because it is thought to have minimal within-menstrual cycle variation as compared to inhibin B or the more classically assessed follicle-stimulating hormone (FSH). In females Serum AMH level decreases progressively until it becomes undetectable at around menopause age, disorders can be indicator of ovarian damage in young women (13, 14, 15)

In our study we found that serum levels of AMH, were higher in fertile than in infertile groups and they varied widely between women with secondary amenorrhea (0.22±0.3 ng/ml) and those with PCOS (9.3±4.6 ng/ml) confirming the earlier suggestions that AMH may vary according to the cause of subfertility (16) and that AMH is correlated to the number of small antral follicles in ovaries. We also demonstrated that in infertile women, the average levels of serum AMH were higher in women with regular cycle than in those with irregular cycle and than those with secondary amenorrhea (1.5±1.2 vs 0.88±1.0 ng/ml vs 0.22±0.3 ng/ml respectively)

In our study a strong correlation of serum AMH levels with AFC was observed. This positive correlation was also confirmed by Fanchin et al. (12), who showed a stronger correlation between serum AMH levels and follicle count than between AMH and serum levels of inhibin B, FSH, and E2 on cycle day 3. All these findings confirm those of previous studies (17, 18, 19, 20, 21)indicating that AMH would be a new measure of ovarian reserves.

The results of the current study showed that AMH levels were higher in fertile women (normal cycling) than in infertile women with regular menstrual cycle (G1) and women with prolonged or irregular cycles (G2). It has been hypothesized that AMH measurement could provide additional information during the diagnostic evaluation of the reproductive capacity in infertile women with regular menstrual cycle. Longitudinal studies have demonstrated that AMH measurement can be used as a reliable and an early marker of ovarian damage, and that a decrease in AMH precedes alterations in other markers such as FSH, age, inhibin B (22). It is interesting to note that reduced AMH levels in the majority of women with regular menstrual cycle were associated with reduced antral follicle count (AFC), suggesting that AMH measurement could provide earlier information on ovarian reserve even in the setting of continued regular menstrual have infertility problem and this may explain why AMH is considered one of the earliest markers for ovarian reserve (22, 23).

In the current study, levels of AMH in PCOS were always 2 to 3 fold higher than in healthy women with

normal ovaries (9.3±4.6 vs 3.4±1.3) and there are more follicles in PCOS with high (AFC), compared with fertile group. This suggest that serum AMH could be used as a diagnostic tool for PCOS which affects in general 5–20%
of women of reproductive age and is the primary cause of anovulatory infertility (24,25). The presence of elevated serum AMH concentrations in PCOS women, at a time when other hormonal markers of ovarian function such as FSH still normal (7.08±3.43), confirm that AMH measurement could provide information in ovarian reserve when other markers did not change and serum AMH levels may also be used in diagnosis of PCOS . Finally, it has been shown that metformin administration in women affected by PCOS is associated with a reduction in both AMH serum levels and antral follicles number, suggesting that the measurement of AMH could be used to evaluate treatment efficacy (25).

Consistent with the literature data indicating that AMH declines throughout the reproductive lifespan and that it might be used as a marker for ovarian ageing (26, 27, 28, 29). In our study we found a negative and significant correlation between AMH and age (r = -0. 40, p =0.00), in infertile groups; a positive and strong correlation was also found between AMH and AFC (r=0.6, p=0. 00). These results might give us the ability to demonstrate the additional value of AMH levels in explaining the decline in the reproductive capacity of women with increasing age and in the assessment for infertility problem related to ovarian reserve and to early loss of ovarian function. Recently, it has been reported that AMH can also predict the outcome of pregnancy in assisted reproduction (30,31).

We also found that in infertile group AMH levels were negatively correlated with FSH concentrations (r=-0.32; p=0.003) supporting the hypothesis that FSH may behave as a negative regulator of AMH synthesis in ovary (32). This correlation has been observed in several studies (32, 33, 34). The last correlation between AMH and FSH was not observed in women with PCOS, which can be explained by increased levels of AMH which retards follicle growth and reduces sensitivity to FSH unchanged serum FSH levels until late reproductive age (34).

Compared to other ovarian tests, AMH seems to be the best marker compared to FSH, regarding its non significant variations during the menstrual cycles, that make its measurement more advantageous, both for patients and clinicians than FSH which can only be determined during the early follicle phase. AMH measurement will ultimately replace FSH in the assessment of ovarian reserve, especially as an initial test of ovarian reserve (35, 36, 37). Recently Kucera et al have suggested that AMH is a good predictor of ovarian reserve damage during radio- and chemotherapy (37).

5 Conclusion :

AMH is a hormone made by small follicles as they grow in the ovaries. This test is more convenient and less expensive than alternative tests like Egg Check, because it uses a simple blood test rather than ultrasound scanning, and can be done at any time in the menstrual cycle. It is available from all Fertility Associates clinics. An AMH test can pick up who might lose their fertility more quickly but it does not show who is more fertile than average.

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Competing interests

The authors declare that they have no competing interests.

References:

[1] Sinisi AA, Pasquali D, Notaro A, Bellastella A. Sexual differentiation j endocrinol invest 2003, (26) 23-28.

[2] Mishina Y, Rey R, Finegold MJ, Matzuk MM, Josso N, Cate RL, Behringer RR. Genetic analysis of the Müllerian-inhibiting substance signal transduction pathway in mammalian sexual differentiation. Genes Dev. 1996; 10(20):2577-87.

[3] 3.van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, Themmen AP. Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve. Hum Reprod. 2002 (12):3065-71.

[4] Gnoth C, Schuring AN, Friol K, Tigges J, P Mallmann, Godehardt E: Relevance of anti-Mullerian hormone measurement in a routine IVF program. Hum Reprod 2008, 23, (6): 1359–1365.

[5] La Marca A, Volpe A : Anti-Müllerian hormone (AMH) in Female Reproduction: is Measurement of Circulating AMH a Useful tool? Clin Endocrinol 2006, 64:603–610. (Review article).

[6] Sowers MR, Eyvazzadeh AD, McConnell D, Yosef M, Jannausch ML, Zhang D, Harlow S, Randolph JF Jr : Anti-Mullerian Hormone and Inhibin B in the Definition of Ovarian Aging and the Menopause Transition. J Clin Endocrinol Metab 2008, 93(9):3478–3483.

[7] The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group 2004Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19:41–47

[8] Pigny P , E Merlen , Robert Y , Cortet-Rudelli C , C Decanter , Jonard S , D Dewailly : Elevated Serum Level of Anti-Mullerian Hormone in Patients with Polycystic Ovary Syndrome : Relationship to the Ovarian Follicle Excess and to the Follicular Arrest. The J Clin Endocrinol Metab 2003, 88(12):5957–5962.

[9] Codner E, Iñíguez G, Villarroel C, Lopez P, Soto N, Sir-Petermann T, Cassorla F, Rey RA: Hormonal Profile in Women with Polycystic Ovarian Syndrome with or without Type 1 Diabetes Mellitus. The J Clin Endocrinol Metab 2007, 92(12):4742–4746.

[10] Pellatt L , S riz , Mason HD : Anti-Mullerian hormone and polycystic ovary syndrome: a mountain too high? Reproduction 2010,139; 825–833 (Review).

[11] Gruijters MJ , JA Visser , Durlinger AL , Themmen AP: Anti-Müllerian Hormone and its Role in Ovarian Function. Mol Cell Endocrinol 2003, 211: 85–90.

[12] Fanchin R , Schonauer LM , Righini C , Guibourdenche J , Frydman R , Taieb J : Serum anti-Muellerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3 . Hum Reprod 2003, 18, (2): 323-327.

[13] Piltonen T , Morin-Papunen L , R Koivunen , Perheentupa A , Ruokonen A , Tapanainen JS: Serum anti-Mullerian hormone levels remain high until late reproductive age and decrease during metformin therapy in women with polycystic ovary syndrome. Hum Reprod 2005, 20, (7): 1820–1826.

[14] Milewicz T , Krzysiek J , Blakała B , Krzyczkowska-Sendrakowska M , Radowicki S , Kacalska O , Zabinska M : Clomiphene citrate challenge test and serum anti-Müllerian hormone levels in women with menstrual irregularities and/or infertility. Fol Histoch Cytochem 2007, 45: 119- 121.

[15] Laven JS , Mulders AG , JA Visser , Themmen AP , De Jong FH , Fauser BC : Anti-Mullerian Hormone Serum Concentrations in Normoovulatory and Anovulatory Women of Reproductive Age. The J Clin Endocrinol Metab 2004, 89(1):318–323.

[16] La Marca A , Broekmans FJ , Volpe A , Fauser BC , Macklon NS : Anti-Mullerian hormone (AMH): what do we still need to know? Hum Reprod 2009, 24, (9): 2264–2275.

[17] van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, Themmen AP: Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. Hum Reprod 2002, 17, (12): 3065–3071.

[18] Fiçicioglu C , T Kutlu , Baglam E , Bakacak Z : Early follicular antimüllerian hormone as an indicator of ovarian reserve. Fertil Steril 2006, (85); 592– 596.

[19] Visser JA, de Jong FH, Laven JS, Themmen AP: Anti-Mullerian hormone: a new marker for ovarian function. Reproduction 2006, 131 –139 (Review).

[20] van Houten EL, Themmen AP, Visser JA: Anti-Müllerian hormone (AMH): Regulator and marker of ovarian function. Annales d'Endocrinologie 2010, 71:191–197.

[21] Anderson RA, Nelson SM, Wallace WH: Measuring anti-Müllerian hormone for the assessment of ovarian reserve: when and for whom is it indicated? 2012, 71(1):28-33.

[22] de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC.Antimüllerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril.2002 .77(2):357-62.

[23] Tremellen KP, Kolo M, Gilmore A, Lekamge DN. Anti-mullerian hormone as a marker of ovarian reserve. Aust N Z J Obstet Gynaecol. 2005 45(1):20-4.

[24] Adams JM, Taylor AE, Crowley WF Jr, Hall JE: Polycystic Ovarian Morphology with Regular Ovulatory Cycles: Insights into the Pathophysiology of Polycystic Ovarian Syndrome. J Clin Endocrinol Metab 2004, 89(9):4343–4350.

[25] Falbo A, Rocca M, Russo T, D'Ettore A, Tolino A, Zullo F, Orio F, Palomba S: Serum and follicular anti-Mullerian hormone levels in women with polycystic ovary syndrome (PCOS) under metformin. J Ovarian Res 2010, 16:1757-2215.

[26] Sowers M, McConnell D, Gast K, Zheng H, Nan B, McCarthy JD, Randolph JF: Anti-Mullerian hormone and inhibin B variability during normal menstrual cycles. Fertil Steril 2009, 94(4): 1482–1486.

[27] Wunder DM, Bersinger NA, Yared M, Kretschmer R, Birkhäuser MH: Statistically significant changes of anti-mullerian hormone and inhibin levels during the physiologic menstrual cycle in reproductive age women. Fertil Steril 2008, 89, (4): 927-933.

[28] Gougeon A, Ecochard R, Thalabard JC: Age-related changes of the population of human ovarian follicles: increase in the disappearance rate of non-growing and early-growing follicles in aging women. Biol Reprod 1994, 50:653-663.

[29] te Velde ER, Pearson PL: The variability of female reproductive ageing. Hum Reprod 2002, 8:141-154.

[30] Fréour T , Mirallié S , Colombel A , Bach-Ngohou K , Masson D , P Barrière : Anti-mullerian hormone: clinical relevance in assisted reproductive therapy

[31] Annales d'Endocrinologie 2006, 67, (6): 567-574(Review).

[32] Mazen R. Fouany, Fady I. Sharara: The role of anti-Mullerian hormone in assisted reproductive medicine. Middle East Fertility Society Journal 2011, 16 :103–106.

[33] Durlinger AL, Visser JA, Themmen AP: Regulation of ovarian function: the role of anti-Mullerian hormone. Reproduction 2002, 124:601–609

[34] Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, Kramer P, Fauser BC, Themmen AP : Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod 2004, 10, (2): 77-83.

[35] Singer T, Barad DH, Weghofer A, Gleicher N: Correlation of antimullerian hormone and baseline follicle-stimulating hormone levels. Fertil Steril 2009, 91: 2616–2619.

[36] Jee BC, Ku SY, Suh CS, Kim KC, Lee WD, Kim SH: Serum Anti-Mullerian Hormone and Inhibin B Levels at OvulationTriggering Day Can Predict the Number of Immature Oocytes Retrieved in In Vitro Fertilization Cycles. J Korean Med Sci 2008, 23: 657-660.

[37]

[38] Sezai S , Meral C, Pelin O, Semih K, Hulya S, Fatih B and Tulay I :Serum anti-Müllerian hormone level as a predictor of poor ovarian response in vitro fertilization patients. Reprod Med Biol 2011, (10), 1 ; 9-14.

[39] Kučera R, Topolčan O, Rumpíková T, Rumpík D, Dostál J: Determination of anti-Müllerian hormone in women. cezska gynecol 2013, 78(3):282-288.