

Serum Anti-Müllerian Hormone (AMH) in Prediction of Oocyte Quality and in Vitro Fertilization (IVF) Outcome

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ABSTRACT:

BACKGROUND:

Growing evidence indicates that serum Anti-mullerian hormone (AMH) levels could be of great importance for understanding the relationship with oocyte quality for IVF. The aim of the present study was to investigate the role of serum level of AMH in differentiation of infertile female patients with good quality oocytes, who are good responder and ready to enter the IVF cycles.

OBJECTIVE:

This study included 25 infertile females classified into two groups: (15) females with Tubal obstruction and 10 females with polycystic ovary syndrome (PCOS). Also, nineteen fertile females were served as controls.

METHODS:

Investigation included serum measurements of AMH, Inhibin B, FSH, LH, E2, Prolactin and TSH on day 3 of previous menstrual cycle, serum measurement of progesterone (P4) on day 21 of previous menstrual cycle (before starting ovulation induction). Also AMH, Inhibin B and E2 were measured on day of hCG administration (after long ovulation protocol).

RESULTS:

The mean (\pm SD) value of serum AMH was significantly decreased in female patients after ovarian stimulation protocol compared to that before ovarian stimulation, ($P < 0.01$). Also, the mean (\pm SD) value of serum AMH of female patients with good quality oocyte was significantly higher than that of those with bad quality oocyte, ($P < 0.01$).

CONCLUSION:

This study revealed that measurement of serum AMH is good marker in prediction of good responder infertile females for IVF technique after ovarian stimulation protocol.

KEYWORDS: anti-mullerian hormone; female infertility; in vitro fertilization.

INTRODUCTION:

Infertility is inability of a couple to achieve a pregnancy after 12 months or more of regular contraceptive intercourse⁽¹⁾. Infertility is related to age, about 4% of couples in their early 20 years are infertile with the percentage rising to nearly 20% by their late 30 years. In about 35% of these couples the problem is with the female, 35% the male, 20% both partners whilst about 10% have unexplained infertility. Most of those couples are actually subfertile. Most causes of female infertility are due to underlying medical problems, these causes include: ovarian factors such Polycystic ovary Syndrome (PCOS), hypothalamic-pituitary factors, tubal (ectopic) /peritoneal factors, uterine fibroid and cervical factors. Also age, smoking, sexually transmitted

infections, and being over- weight or underweight can all affect⁽²⁾. Anti-Müllerian hormone (AMH) is a dimeric glycoprotein structurally related to inhibin and activin, and a member of the transforming growthfactor- β (TGF- β) superfamily⁽³⁾. It is also considered as a local growthfactor and a cellular differentiation factor⁽⁴⁾. Serum AMH concentrations during the early follicular phase of the menstrual cycle decrease consistently with age in women, and this change starts earlier than that of conventional markers such as follicle stimulating hormone (FSH), estradiol and inhibin B⁽⁵⁾. AMH is secreted by Sertoli cells of the testes during embryogenesis of the fetal male, it prevents the development of the mullerian ducts into the uterus and other mullerian structures⁽⁶⁾. AMH is produced in the ovary of female by the granulosa cells surrounding pre-antral and small antral follicles,

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it inhibits initial primordial follicles recruitment and decreases the sensitivity of pre-antral and small antral follicles to FSH. Hence, it is thought that serum AMH levels are a reflection of the size of the growing cohort of small follicles which in turn reflects the number of residual primordial follicles, or the ovarian reserve on FSH⁽⁷⁾. The highest level of AMH expression is present in granulosa cells of secondary, pre-antral, and small antral follicles < 12 mm in diameter, whereas in follicles growing into dominance this expression decline and then stops, there is almost no AMH made in follicles > 16 mm⁽⁸⁾. It has been shown that the basal AMH level is correlated with antral follicle count (AFC), total dose of gonadotrophins used, duration of controlled ovarian hyperstimulation (COH), estradiol level on hCG day, the number of mature follicles on hCG day and the number of oocytes retrieved⁽⁹⁾. It has been also suggested that the serum AMH level could predict poor response and ovarian hyperstimulation syndrome for IVF cycles (10)(11). This study aimed to investigate the role of serum level of AMH in differentiating of infertile female patients who are good responder for COS and ready to enter the IVF cycles.

SUBJECT AND METHODS:

SUBJECTS:

This study included 29 infertile women whom aged (20-40) years, their husbands were apparently normal (hormones and seminal fluid analysis). Those women were enrolled in Assisted Reproductive Technology (ART) programs to enter the first IVF cycle in Kamal AL-Samarai Hospital, center of fertility and IVF (Baghdad/ Iraq), during the period from December 2010 till the end of April 2011. Nineteen healthy women aged 20-40 years were clinically and hormonally normal, and served as control group. Biochemical investigations were carried out at Clinical Biochemistry Department, College of Medicine/ University of Baghdad. The 29 infertile women aged 20-40 years have primary infertility with duration between 2-20 years. The infertile women were divided into two groups according to the causes of infertility: group (1) 19 infertile women have had tubal obstruction (GI); group (2) 10 infertile women have had ovulatory factor such as polycystic ovary syndrome (PCOS) (GII). Infertile women with tubal obstruction were examined by a gynecologist. Their tubal patency

was previously checked by hysterosalpinography (HSG) and/ or laparoscopy under supervision of gynecologist⁽¹²⁾. Infertile women in the PCOS group were diagnosed according to the Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group criteria (13). The diagnosis was based on the association of at least two of three following criteria: [1] ovulatory disturbance (oligomenorrhea or amenorrhea); [2] hyperandrogenism, as defined either clinically by hirsutism, severe acne or seborrhea, or biochemically by total testosterone serum level greater than 0.6 ng/ml; and [3] more than 12 follicles in the 2- 9 mm range in each ovary on ultrasound examination and/or an ovarian volume greater than 10 ml.

Stimulation protocol:

The infertile women underwent ultrasound on day 13 of menstrual cycle before starting ovulation induction protocol to assess the number and size of ovarian follicles. Also, U/S examination on day 21 of previous menstrual cycle was performed to assess the endometrial thickness in order to exclude those females with ovarian cyst. All infertile patient groups enrolled in long ovulation induction protocol type for IVF/ ICSI cycle, which started on day 21 of previous menstrual cycle. Four women of group (1) were canceled due to ovarian hyperstimulation syndrome (OHSS). The women were down regulated (long luteal protocol) with gonadotropin releasing hormone agonist (GnRH-agonist) triptorelin (Decapeptyl 0.1 mg, Fering Co, Kiel, Germany) by daily Subcutaneous (SC.) injection and starting on day 21 of previous menstrual cycle. After 10-15 day and when the pituitary desensitization was completed by reaching the level of E2 < 50 pg/ml and endometrial thickness was ≤ 2-3 mm, the women received recombinant human Follicle Stimulating Hormone (rhFSH, Gonal-F®; Serono, Germany) containing 75 IU of FSH activity per ampoules by daily SC. injection (2-4) ampoules starting from day 2 of next menstrual cycle. Transvaginal ultrasound was performed on cycle day 5 and subsequent scan were done every 2-3 days as required. The doses of Gonal-F® and follicle growth were monitored by serum E2 level and transvaginal U/S after the day 6 of (Gonal-F®) injection and till the day of hCG administration (12-15 day of cycle). Then, ovulation was induced by administration of human chorionic gonadotropin

(hCG, 6500 IU, Ovitrelle; Serono, Germany) subcutaneously when at least 3 follicles > 16mm in diameter were detected on ultrasound examination, the leading follicle of the three reached 18-20 mm in diameter, serum E2 levels was >250⁽¹⁴⁾. Oocyte was evaluated for evidence of normal fertilization 2 distinct pronuclei (2PN) 18 hour after insemination. Embryo of day 2(4 cell stage) or day 3 (eight cell stage) of high quality were transferred. After transferring of embryos, all women received luteal support therapy for 2 weeks in form of (Duphastone) 10 mg orally and (Cyclogest) 400 mg. Pregnancy is documented by an elevated serum β -HCG level of ≥ 1500 mIU/ml on day 12-14 following eggs retrieval.

Blood sampling and hormones assays:

On day 3 of the menstrual cycle before starting control ovarian stimulation (COS), 5ml of blood samples for AMH, inhibin B, FSH, LH, E2, Prolactin and TSH assay were collected by venipuncture allowed to clot and centrifuged at 2500 rpm within 30 minute to separate the serum that stored in two aliquots at -20°C. One tube was used for the FSH, LH, E2, TSH and prolactin measurements by Mini-vidus technique, and the other was for inhibin B and AMH. Also a blood sample was collected on day 21 of previous menstrual cycle for progesterone measurement. Serum levels of AMH, inhibin B and progesterone were determined by using the enzyme immune assay (ELISA) technique. Another blood samples on the day of hCG (DhCG) administration were also collected to measure serum levels of AMH, inhibin B and E2 in the same way. Serum TSH and Prolactin were measured to exclude hyperthyroidism and hyperprolactinaemia.

Statistical Analysis:

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc, Chicago, IL, USA). When there were two independent groups, they were compared by Student's t-test or Mann Whitney U test, depending on the distributions of continuous data. The statistical importance of the differences among groups was evaluated by either one-way ANOVA, least significant differences (LSD) or Kruskal Wallis test. Nominal data were compared with either Chi-

square or Fisher's Exact test. The degree of association between continuous variables was calculated by Spearman's correlation coefficient (r). Values were expressed as mean \pm SD. A P-value < 0.05 was considered to be statistically significant, P. value <0.01 was considered to be statistically highly significant.

RESULTS:

This study included 29 infertile female patients and 19 fertile female controls. The infertile females were classified into two groups: GI tubal factor (n=19) and GII) PCOS (n=10). Four of 19 infertile females of GI were cancelled because of OHSS (serum E2 > 3000 pg/ml). The remainder 25 infertile female patients were underwent their first IVF treatment. Table (1) showed clinical characteristics of studied groups. The percentage of female patients with tubal factor was 60% (n=15) and females with PCOS was 40% (n=10). ANOVA test revealed no significant differences in age mean (\pm SD) values among GI (33.00 \pm 3.81 year), GII (32.70 \pm 6.30 year) and controls group (30.74 \pm 5.11) (P>0.05). Also, ANOVA test revealed no significant differences in BMI values among GI (23.66 \pm 6.52 kg/m²), GII (27.86 \pm 2.17 kg/m²) and controls group (27.03 \pm 5.10 kg/m²), table (1). Table (1) also revealed the mean value of number of follicles of GI at hCG triggering day (FN-dhCG) was significantly increased in comparison to that of the same group before COS (FN-d3); P< 0.01. Also, the mean value of (FN- dhCG) GII was significantly increased compared to FN-d3 of the same group, P< 0.01. t-test revealed significant difference between GI and GII in (FN-d3) and in (FN-dhCG), P< 0.01. The mean values of oocyte number for GI and GII are shown in table (1). There was significant difference in the mean value of oocyte number between GI and GII, P<0.05. Quality of oocyte was divided into two types: Bad and good according to its morphology that defined by embryologist. Three out of 15 females of GI had bad quality oocyte (20 %), while 80 % (12 out of 15) of GI had good quality oocyte. In GII, 50% (5 out of 10) have had bad quality oocyte and the same percentage of this group had good quality oocyte 50%, table 2.

IN VITRO FERTILIZATION

Table 1: Demographic and ultrasonographic characteristics of tubal obstruction patients, PCOS patients and controls.

Parameters	Tubal Group I	PCOS Group II	Controls	P-value
No.	15 (60%)	10 (40%)	19	
Age (years)	33.00±3.81	32.70±6.30	30.74±5.11	NS
BMI (kg/m ²)	23.66 ± 6.52	27.86 ± 2.17	27.03 ± 5.10	NS
Duration of infertility (years)	9.63 ± 4.36	8.20 ± 2.65		NS
FN-d3	2.84±1.66	6.20 ±2.93		< 0.01
FN-dhCG	12.13±5.69	19.10 ±9.86		< 0.01
No of retrieved oocytes	7.86±4.14	4.83±2.31		< 0.05

Value are presented as mean±SD.NS=not significant; FN-d3=follicles number on day 3 of menstrual cycle; FN-dhCG=follicles number on hCG administration(after COS) .

Table 2: Percentage of oocyte quality of GI and GII.

groups	No. of patients with good quality oocyte	No. of patients with bad quality oocyte
GI n=15	12 (80%)	3 (20%)
GII n=10	5 (50%)	5 (50%)

Table (3) showed the mean values of basal serum AMH (AMH-d3) for GI and GII were significantly higher compared to that of control (2708.40±860.99 pg/ml, 2850.51±902.08 pg/ml, 759.918±599.89 pg/ml, respectively; P< 0.01). t-test revealed that the mean value of serum AMH-d3 of GII was significantly higher than that of GI (P <0.01). The mean value of serum AMH after COS (AMH-dhCG) of GI(851.10±422.71 pg/ml) was significantly decreased compared to serum AMH before COS (AMH-d3) of the same group (2708.40±860.99 pg/ml; P<0.01), as well as the mean value of serum AMH-dhCG of GII was significantly decreased compared to serum AMH-d3 of the same group (982.91±540.06 pg/ml, 2850.51±902.08 pg/ml, respectively; P<0.01). The mean value of serum AMH-dhCG of GI was significantly lower than that of serum (AMH-dhCG) of GII (851.10±422.71 pg/ml, 982.91±540.06 pg/ml, respectively; p< 0.01) table (3). The mean value of serum AMH-d3 of tubal obstruction patients (GI) with good quality oocyte was significantly higher than that of those with bad quality oocyte for the same group (2636.89±659.06pg/ml,645.77±121.53pg/ml;

respectively; P<0.01). In contrast, there was no significant difference in the mean value of serum AMH-d3 of GII with good quality oocyte compared to those with bad quality oocyte for the same group (2602.60±501.02 pg/ml, 2556.11±490.14 pg/ml, respectively). t- test showed no significant difference in mean value of serum Inhb-d3 and Inhb-dhCG of GI (417.48±112.68 pg/ml, 473.88±130.45 pg/ml, respectively), as well as of GII (451.89±129.73 pg/ml, 469.27±178.39 pg/ml, respectively). There was no significant differences in the mean values of serum Inhb-d3 among GI, GII and controls, P>0.05. Also there was no significant difference in the mean values of serum Inhb-d3 of both GI and GII with good quality oocytes compared to those with bad quality oocytes for the same group. There was significant negative correlation between serum AMH-d3 and age in GI & GII, (figure 1). Also there was significant positive correlation between serum AMH-d3 and number of follicles before starting COS in both GI&GII (figure 2), as well as between serum AMH-d3 and number of retrieved oocytes in both GI & GII (figure 3).

Table 3: The Biochemical parameters Of GI ,GII and control .

Parameters	Tubal Group I	PCOS Group II	Controls
Serum AMH-d3 * * (pg/ml)	2708.40±860.99	2850.51±902.08	759.918±599.89
Serum AMH-dhCG * (pg/ml)	851.10±422.71	982.91±540.06	
Serum inhibinB-d3 (pg/ml)	417.48±112.68	451.89±129.73	444.79±148.19
Serum inhibinB-dhCG (pg/ml)	473.88±130.45	469.27±178.39	
Serum E2-d3 (pg/ml)	30.43±12.69	37.02±10.37	34.48±9.30
Serum E2-dhCG * (pg/ml)	1479.17±1028.08	2715.62±1786.00	
FSH mIU/ml	5.01 ± 1.99	6.95 ± 3.46	6.05 ± 2.04
LH mIU/ml	3.00 ± 1.63	7.03 ± 2.80 §	3.61 ± 1.01
P4. ng/ml	2.88 ± 1.06	2.00 ± 0.99	4.96 ± 2.15
PRL ng/ml	12.93 ± 4.05	12.20 ± 5.57	13.06 ± 8.55
TSH (mmol/L)	1.31 ± 0.59	3.69 ± 2.13	1.59 ± 0.88
* * . ANOVA test revealed significant differences among GI,GII and controls before COS, P<0.01. * . t-test revealed significant difference between GI and GII after COS, P< 0.01. §. LSD test revealed significant differences among GI,GII and controls before COS, P<0.01.			

Table 4: The Mean (±SD) Values Of Serum (AMH, InhbB and E2) of GI and GII with Good and Bad Quality Oocyte.

Parameters	GI n= 15	GII n=10
Serum AMH-d3 with good quality oocyte (pg/ml)	2636.89±559.06 *	2815.06±908.02
Serum AMH-d3 with bad quality oocyte (pg/ml)	645.77±121.53	2756.81±890.14
Serum InhbB-d3 with good quality oocyte (pg/ml)	450.42±101.43	502.17±288.02
Serum InhbB-d3 with bad quality oocyte (pg/ml)	395.41±110.42	409.06±279.33
Serum E2-d3 with good quality oocyte (pg/ml)	36.18±14.82	41.69±15.05
Serum E2-d3 with bad quality oocyte (pg/ml)	34.10±12.65	38.06±14.89
* . t-test revealed significant differences in serum AMH -d3 between patients with good and bad quality oocyte in GI , P<0.01.		

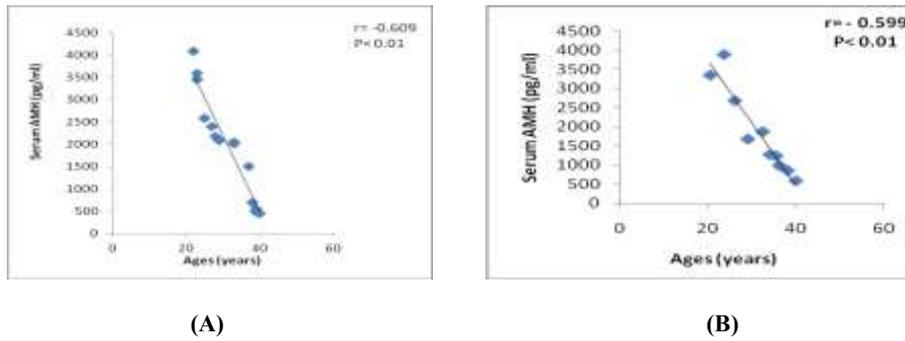


Figure 1 : (A) correlation of serum AMH-d3 with age ($r = -0.609$, $p < 0.01$) in tubal obstruction patients.
(B) : correlation of serum AMH-d3 with age ($r = -0.599$, $P < 0.01$) in PCOS patients.

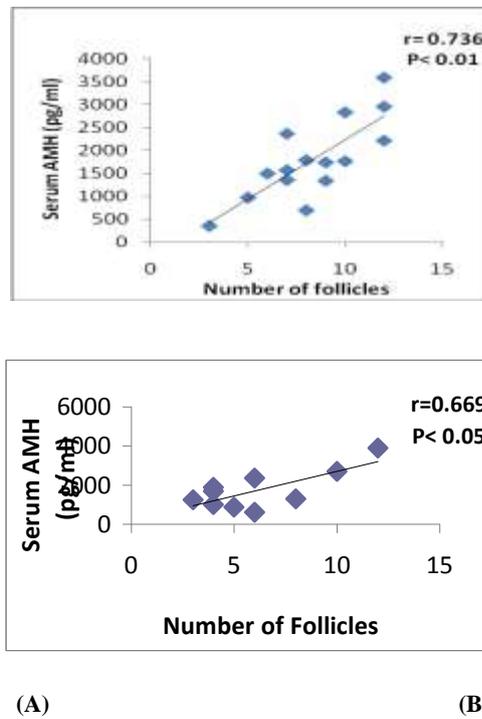


Figure 2: (A) correlation of serum AMH-d3 with FN-d3 ($r = 0.736$, $p < 0.01$) in tubal obstruction patients group.
(B) correlation of serum AMH-d3 with FN-d3 ($r = 0.669$, $p < 0.05$) in PCOS patients.

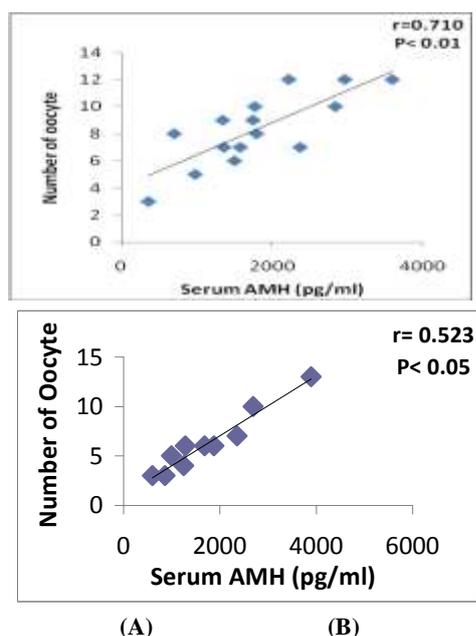


Figure 3: (A) correlation of serum AMH-d3 with number of oocytes ($r =0.710$, $P<0.01$) in tubal obstruction patients group.
 (B) correlation of serum AMH-d3 with number of oocytes ($r=0.523$, $p<0.05$) in PCOS patients.

DISCUSSION:

The results of the present study revealed that both infertile woman with tubal obstruction (GI) and PCOS (GII) had significantly higher mean values of serum AMH than that of fertile controls (table 3), these results confirmed that of (Pigny et al., 2003). In agreement with other studies^(15,11,16) the mean value of serum AMH of GII was significantly higher than that of GI (table 3). This is thought to be the result of increased synthesis by granulosa cells (GC) and secretion of AMH in the polycystic ovaries⁽¹⁷⁾. Women with PCOS show increased development of antral follicles compared with normal women⁽¹⁸⁾. On histological examination, polycystic ovaries (PCO) exhibit a normal number of primordial follicles, whereas the number of developing follicles is double compared with normal ovaries⁽¹⁹⁾. It appears that follicle development is arrested in PCOS at the stage in which dominant follicle selection occurs under normal conditions, through a negative interaction between AMH and FSH. Indeed, higher levels of AMH in previous studies of (Cook et al.,2002) and (Pigny et al.,2003) were associated with lower values of FSH and lower E2 levels, it is therefore tempting to speculate that excess of AMH is involved in the lack of FSH-induced aromatase activity, which characterizes the follicular arrest of PCOS.

Follicle maturation arrest during later stages of development may lead to a buildup of many immature follicles, which in itself could explain increased AMH levels. Hence, it might be anticipated that the increased number of follicles, which are generally present in PCOS, are the source of increased AMH production. Accordingly circulating AMH levels in women with PCOS are two to three times higher than healthy controls^(21,22,16). The mean values of serum AMH of GI and GII were significantly decreased after (COS) protocol compared to those before protocol (table 3). These results confirm those of previous studies that demonstrated a gradual decrease in serum AMH level during gonadotrophin administration as a part of (COS) till the day of hCG triggering and showed its minimum level on the hCG day^(23,24,25,26). There are several possible causes for the gradual decrease of serum AMH levels during the follicular phase of the COH cycle. First, FSH could have a negative role on AMH secretion. It have been reported that FSH may suppress the expression of AMH and AMH type II receptor (*AMHRII*) ovaries⁽²⁵⁾. In addition, other investigators have shown that FSH treatment significantly reduces AMH expression in cultured GC⁽²⁷⁾. However, the mechanism of

how FSH affects AMH secretion remains unclear⁽²⁵⁾. Second, increased serum estradiol levels during COH could have a negative effect on AMH secretion⁽²⁸⁾. Although the causality of this relationship between estradiol increase and AMH decrease is not clear, a previous experimental study showed that estradiol could inhibit the expression of AMH and AMHRII in pre-antral and small antral follicles, supporting a causal relationship between these two hormones⁽²⁹⁾. Third, the decrease in AMH levels during COH could be explained by the decrease in small antral follicles. A study⁽³⁰⁾ showed that serum AMH is significantly positively correlated with the decrease in the number of small antral follicles. This study revealed significant negative correlation between serum AMH-d3 and the values of age of GI and GII (figure 1). This present study revealed strong positive correlation between serum AMH-d3 levels and number of follicles before (COS) in GI (figure 3-1) and GII (figure 2), in agreement with other studies who have also showed significant positive correlation between serum AMH changes and the number of small follicles with diameters 2-5 mm^(16,26). Also, serum levels of AMH-d3 (before starting COS) were significantly positively correlated with the number of retrieved oocyte in both GI and GII (figure 3). These findings are in agreement with those reported previously^(24,26). Also, there was a significant positive correlation between serum AMH-dhCG (at ovulation triggering day) and the number of retrieved oocyte in GI, which supported the findings of^(8,25,4). More recently, it has been found that AMH correlates better than inhibin B, FSH, LH and E2 with the number of retrieved oocytes^(26,31). Therefore, basal serum AMH levels may reflect the size of antral follicle pool and provide a marker associated with the number of retrieved oocytes after controlled ovarian hyperstimulation (COH). This study also revealed significant positive correlation between serum AMH and serum inhibin B before COS in GI and GII, that confirms the finding of^(15,32) and⁽²⁶⁾. Also this study demonstrated significant positive correlation between serum AMH and Inhibin B after COS only in GII, supporting that of⁽¹⁵⁾. The results also showed significant negative correlation between serum AMH-d3 and serum E2-d3 in GI and GII. Also, serum E2-dhCG revealed significant positive correlation with serum AMH-dhCG in GI. These results are in

agreement with other studies^(33,8,34,25). This study demonstrated significant negative correlation between serum AMH and serum FSH before COH in GI, which is in agreement with that reported^(23,26). Also this study demonstrated significant negative correlation between serum FSH and follicles number before COH in GI and GII which confirm previous studies of^(23,26). The mean values of serum inhibin B did not differ significantly between each of GI and GII and controls, as well as between GI and GII themselves (table 3). With regard to COS, the mean values of serum inhibin B after stimulation were insignificantly increased compared to those before stimulation protocol in GI and GII (table 3).

Study of (Jee et al., 2008) and (Kunt et al., 2011) elucidated that mean (\pm SD) values of serum inhibin B of PCOS and tubal obstruction patients were lower before starting COS then increased after COS. Serum levels of inhibin B increased during COS, reached a peak on the hCG day and abruptly decreased after hCG injection. The increased serum inhibin B level during stimulation may reflect follicle growth in response to exogenous FSH administration⁽³⁵⁾. It is well known that ovarian inhibin B production is partly FSH-driven and could be assumed that the differences in inhibin B concentration during (COS) may be due to high doses of administered FSH⁽³⁶⁾. After hCG injection, this stimulatory effect from FSH disappeared, and the serum level of Inhibin B decreased. Inhibin B has been presented as a potential predictive marker of ovarian response to COH⁽³⁷⁾. This study showed that the mean value of serum AMH-d3 of GI was significantly higher in patients with good quality oocyte compared to those with bad quality oocytes (table 4). While, in PCOS (GII) there was no significant difference in mean value of serum AMH-d3 level between female patients with good quality oocyte and those with bad quality oocyte (table 4). Also, this study demonstrated that the mean value of serum AMH-dhCG of GI was significantly decreased in female patients with good quality oocyte compared to those with bad quality oocyte (table 4). For tubal factor patients, the serum level of AMH-d3 (\geq 2000 pg/ml) was proposed in this study as a cutoff value for predicting of those female patients with good oocytes quality, which was similar to previous studies of^(38,39). A study of (4) suggested that AMH levels correlated with the number of good quality oocyte. Furthermore, it has been suggested that serum

AMH-d3 level is predictive of oocyte quantity and quality in patients with tubal obstruction, in evident by the higher number of oocyte with good quality in tubal obstruction patients rather than PCOS patients ^(16,31) demonstrated that serum AMH levels were highly correlated with the number of antral follicles, and the oocyte quality and embryo development. Also, they observed that AMH has significant positive correlation with PCOS. A lower day 3 serum AMH concentration preceding or during COS was strongly associated with reduced oocyte yield and low oocyte quality. This might be expected if day 3 serum AMH concentrations produced by the small follicles indirectly reflect the remaining follicle pool. Ironically, the opposite appears to be true for successful treatment of infertility in women with polycystic ovary syndrome (PCOS), in that those who had the highest concentrations of AMH seemed to respond less well ⁽³⁴⁾. This study revealed that there were no significant differences in mean (\pm SD) values of serum Inhibin B-d3 between patients with good quality oocyte compared to those with bad quality oocyte in both GI and GII (table 4). Also this study showed no significant differences in mean values of serum inhibin B in GI and GII after COS between patients with good quality oocyte compared to those with bad quality oocyte (table 4).

CONCLUSION:

Measurement of basal serum AMH(AMHd3) level is a good predictor of oocyte quality after COS in patients with tubal obstruction and so for selection of those infertile females who may benefit from IVF technique. A serum level of AMH-d3 of (≥ 2000 pg/ml, ≥ 2.0 ng/ml) was proposed in this study as a cutoff value for prediction of those infertile women with good quality oocytes, who are good responder to COS & IVF technique. In contrast to basal serum AMH level, serum inhibin B level is poor indicator of oocyte quality and a successful IVF in infertile females.

REFERENCES:

1. Shevell T, Malone FD, Vidaver J, et al. Assisted reproductive technology and pregnancy outcome. *Obstet Gynecol.* 2005;106:1039-45.
2. Makar RS and Toth T. "The evaluation of infertility". *Am J Clin Pathol.*2002;117 Suppl :S95–103.

3. La Marca A, Broekmans FJ, Volpe A, et al. Anti-Müllerian hormone (AMH): what do we still need to know? ESHRE Special Interest Group for Reproductive Endocrinology – AMH Round Table. *Hum Reprod.* 2009;24:2264-75.
4. Majumder K, Gelbaya TA, Laing I, et al. The use of anti-Müllerian hormone and antral follicle count to predict the potential of oocytes and embryos. *Euro Jof Obst& Gyne and Reprod Biology.*2010;150:166-70.
5. DeVet A, Laven JS, DeJong FH, et al. Anti-mullerian hormone serum levels: a putative marker of ovarian aging. *Fertil Steril.* 2002;77:357–62.
6. Nelson RJ. *An Introduction to Behavioral Endocrinology.female reproductive behaviore* 3rd ed. Sinauer Associates Inc., Sunderland MA.2005:319-85.
7. Skalba P, Cygal A, Dabkowska-Huc ´ A . The influence of anti-Mullerian hormone on folliculogenesis. *Ginekol Pol.* 2008;79:137–40.
8. Jee BC, Ku SY, Suh CS, et al. Serum anti-Müllerian hormone and inhibin B levels at ovulation triggering day can predict the number of immature oocytes retrieved in in vitro fertilization cycles. *J Korean Med Sci* 2008;23:657-61.
9. Muttukrishna S, Suharjono H, McGarrigle H, et al. Inhibin B and anti-Mullerian hormone: markers of ovarian response in IVF/ICSI patients? *BJOG.* 2004;111:1248-53.
10. Nakhuda GS, Chu MC, Wang JG, Sauer MV, Lobo RA. Elevated serum müllerian-inhibiting substance may be a marker for ovarian hyperstimulation syndrome in normal women undergoing in vitro fertilization. *Fertil Steril* 2006;85:1541–43.
11. Gnoth C, Schuring AN, Friol K, et al. Relevance of anti-Mullerian hormone measurement in a routine IVF program. *Hum Reprod* 2008;23:1359–65.
12. Kiyokawa K, Masuda H, Fuyuki T, et al. The use of three dimensional hysterosalpingo-contrast sonography (3D-HyCoSy) as a routine outpatient procedure for evaluating infertile women. *Ultrasound in Obstetrics and Gynecol.*2000;16:648-54.

13. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Hum Reprod* 2004;19:41-47.
14. Rienzi L, Vajta G, Ubaldi F. Predictive value of oocyte morphology in human IVF: a systematic review of the literature. *Hum. Reprod.*2011;17:34–45.
15. Pigny P, Merlen E, Robert Y, et al. Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. *J Clin Endocrinol Metab.*2003;88:5957–62.
16. Arabzadeh S, Hossein G, Rashidi BH, et al. Comparing serum basal and follicular fluid levels of anti-Müllerian hormone as a predictor of in vitro fertilization outcomes in patients with and without polycystic ovary syndrome. *Ann Saudi Med* 2010;3:442-47.
17. Mulders AG, Laven JS, Eijkemans MJ, et al. Changes in anti-Mullerian hormone serum concentrations over time suggest delayed ovarian ageing in normogonadotrophic anovulatory infertility. *Hum Reprod* 2004;19:2036–42.
18. Pigny P, Jonard S, Robert Y, et al. Serum Antimullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006;91:941-45.
19. Webber LJ, Stubbs S, Stark J, et al. Formation and early development of follicles in the polycystic ovary. *Lancet.* 2003;362:1017–21.
20. Cook CL, Siow Y, Brenner AG, et al. Relationship between serum mullerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovary syndrome and normal women. *Fertil Steril* 2002;77:141–46.
21. Piltonen T, Morin-Papunen L, Koivunen R, et al. 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;20:1820–26.
22. Wachs DS, Coffler MS, Malcom PJ, et al. Serum anti-mullerian hormone concentrations are not altered by acute administration of follicle stimulating hormone in polycystic ovary syndrome and normal women. *J Clin Endocrinol Metab* 2007;92:1871–74.
23. Wu C.-H, Chen Y.-C, Wu H.-H, et al. Serum anti-Müllerian hormone predicts ovarian response and cycle outcome in IVF patients. *J Assist Reprod Genet.* 2009;26:383–89.
24. La Marca A, Sighinolfi G, Radi D, et al. Anti-Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). *Hum Reprod.* 2010;16:113–30.
25. Lee JR, Kim SH, Kim SM, et al. Anti-Müllerian hormone dynamics during controlled ovarian hyperstimulation and optimal timing of measurement for outcome prediction. *Human Reproduction* .2010;25:2597–04.
26. Kunt C, Ozaksit G, Kurt RK, 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.
27. Pellatt L, Hanna L, Brincat M, et al. Granulosa cell production of anti-Müllerian hormone is increased in polycystic ovaries. *J Clin Endocrinol Metab* 2007;92:240–45.
28. Andersen CY and Byskov AG. Estradiol and regulation of anti-Müllerian hormone, inhibin-A, and inhibin-B secretion: analysis of small antral and preovulatory human follicles' fluid. *J Clin Endocrinol Metab* 2006;91:4064–69.
29. Baarends WM, Uilenbroek JT, Kramer P, et al. Anti-mullerian hormone and anti-mullerian hormone type II receptor messenger ribonucleic acid expression in rat ovaries during postnatal development, the estrous cycle, and gonadotropin-induced follicle growth. *Endocrinology* 1995;136:4951–62.
30. Fanchin R, Schonauer LM, Righini C, et al. Serum anti-Mullerian hormone dynamics during controlled ovarian hyperstimulation. *Hum Reprod* 2003;18:328–32.
31. Irez T, Ocal P, Guralp O, et al. Different serum anti-Müllerian hormone concentrations are associated with oocyte quality, embryo development parameters and IVF-ICSI outcomes. *Arch Gynecol Obstet.* 2011;12. [Epub ahead of print].
32. Dehghani-Firouzabadi R, Tayebi N, Asgharnia M. et al. Serum Level of Anti-Mullerian Hormone in Early Follicular Phase as a Predictor of Ovarian Reserve and Pregnancy Outcome in Assisted Reproductive Technology Cycles. *Arch Iranian Med* 2008;11:371-76.

33. Laven JS, Mulders AG, Visser JA, Themmen APN, De Jong FH & Fauser BC. Anti-Mullerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. *Journal of Clinical Endocrinology and Metabolism* 2004;89:318–323.
34. Pellatt L, Rice S, Mason H. Anti-Mu'llerian hormone and polycystic ovary syndrome: a mountain too high? *Reproduction* .2010;139:825–33.
35. Welt CK, Smith ZA, Pauler DK, et al. Differential regulation of inhibin A and inhibin B by luteinizing hormone, follicle-stimulating hormone, and stage of follicle development. *J Clin Endocrinol Metab.* 2001;86:2531–37.
36. Enskog A, Nilsson L, Brännström M, et al. Peripheral blood concentrations of inhibin B are elevated during gonadotrophin stimulation in patients who later develop ovarian OHSS and inhibin A concentrations are elevated after OHSS onset. *Hum Reprod.* 200;15:532-38.
37. Urbancsek J, Hauzman E, Klinga K, et al. Use of serum inhibin B levels at the start of ovarian stimulation and at oocyte pickup in the prediction of assisted reproduction treatment outcome. *Fertil Steril* 2005;83:341–48.
38. Ebner T, Sommergruber M, Moser M, et al. Basal level of anti-Mu'llerian hormone is associated with oocyte quality in stimulated cycles. *Human Reprod* 2006;21:2022–26.
39. McIlveen M, Skull JD, Ledger WL. Evaluation of the utility of multiple endocrine and ultrasound measures of ovarian reserve in the prediction of cycle cancellation in a high-risk IVF population. *Hum.Reprod* 2007;22:778–85.