

Anti-Mullerian Hormone Serum Concentrations in Women with Polycystic Ovaries and Normoovulatory Women of Reproductive Age

Magdy A. Al-Tahhan⁽¹⁾, Selem S.El-Gendi⁽²⁾, Abdallah E. Diab⁽²⁾,
Medical Biochemistry⁽¹⁾ and Obstetric & Gynecology
⁽²⁾Departments, Faculty of Medicine, Zagazig, University.

ABSTRACT

Background: Anti-Mullerian hormone (AMH) is a member of the transforming growth factor- β superfamily. It may play an important role in the ovarian folliculogenesis. **Objective:** To study the relationship of AMH and some clinical, endocrinal and ultrasound parameters in anovulatory infertile women with polycystic ovary syndrome (PCOS) and in women with normal menstrual cycles. **Methods:** Sera were collected from 30 PCOS anovulatory women and from 15 normal women during the early follicular phase "day 3" of the menstrual cycle, stored frozen until assayed. **Results:** Serum AMH concentrations were significantly ($P < 0.001$) elevated in PCOS women ($9.55 \pm 3.39 \mu\text{g/liter}$) compared with controls ($2.33 \pm 1.8 \mu\text{g/liter}$). In PCOS patients, serum levels of AMH were correlated with features characteristics of PCOS such as LH concentrations ($r = 0.76$, $P < 0.001$), testosterone level ($r = 0.67$, $P < 0.001$), mean number of follicles ($r = 0.68$, $P < 0.001$) and ovarian volume ($r = 0.54$, $P < 0.001$). Also, AMH levels were correlated with age ($r = -0.51$, $P < 0.001$) and with cycle duration ($r = 0.46$, $P < 0.001$). The correlation between AMH levels and FSH, estradiol or BMI were found statistically non significant. **Conclusion:** The present study provides evidence that serum levels of AMH is elevated in PCOS patients. This may have a role in the disordered folliculogenesis characteristics of that disease. **Key words:** Anti-Mullerian hormone, polycystic ovary syndrome.

INTRODUCTION

Anti-Mullerian Hormone (AMH), also known as Mullerian-inhibiting substance, is a member of the transforming growth factor- β (TGF- β) superfamily that also includes the granulosa cell and theca cell-derived inhibins and activins as well as the oocyte-derived growth differentiation factor 9. In men, AMH is produced by Sertoli cells and it causes regression of the Mullerian ducts, which is a requirement for

normal male reproductive tract development⁽¹⁾. In females, AMH is produced only postnatal by granulosa cells from preantral and small antral follicles^(2,3). Its localization in granulosa cells of primary, preantral and small antral follicles, suggests an important role for AMH in human folliculogenesis⁽⁴⁾. Since AMH is secreted exclusively in the gonads, its serum concentrations in females are thought to reflect the size of the ovarian follicle pool⁽⁵⁾.

Recently serum AMH levels have been shown to decrease over time in young normoovulatory women, whereas other markers associated with ovarian aging did not change during that time interval⁽⁶⁾. AMH might represent a sensitive marker for ovarian aging⁽⁷⁾. Indeed, it has been shown that poor response during in vitro fertilization, indicative of a diminished ovarian reserve is associated with reduced baseline serum AMH concentrations⁽⁷⁾. Because AMH is predominantly expressed by small follicles in mice⁽⁸⁾ as well as in the human⁽⁷⁾, AMH serum concentrations may be increased in patients with polycystic ovaries (PCOS).

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, and is a leading cause of oligo/anovulatory infertility. Abnormalities of antral follicles in polycystic ovaries (PCO) have been well documented⁽⁹⁾. Recently it has been shown that there are also differences in the earliest stages of follicle development in polycystic ovaries when compared with normal ovaries⁽¹⁰⁾. Specifically, it was observed that an increased proportion of follicles had left the primordial pool and initiated growth. The causes of these differences are unclear, but recent studies of mice lacking the AMH gene have suggested that anti-Mullerian hormone (AMH) is important in regulating initiation of follicle growth and progression from the primordial (resting) stage to the primary stage of development⁽¹¹⁾. Indeed, in PCOS patients exhibiting the classical features of the syndrome,

AMH levels were found to be elevated compared with normal controls⁽¹²⁾. The aim of present study was to evaluate AMH as a clinically relevant marker for the extent of ovarian dysfunction in infertile anovulatory women, with polycystic ovaries (PCOS).

SUBJECTS & METHODS

The present work had been conducted in the Departments of Obstetrics & Gynecology and Medical Biochemistry, Faculty of Medicine, Zagazig University, between February 1st and July 31, 2008. Fifteen healthy women and thirty women previously diagnosed as PCOS were counseled to participate in the study and their consent was taken. All healthy women had regular cycles (28-35 days) and normal appearing ovaries on transvaginal ultrasonography. Diagnosis of PCOS was based on clinical manifestations (oligomenorrhea, hirsutism, and obesity) and standard ultrasound criteria. The ovaries were classified as normal or polycystic according to ultrasonographic criteria established by **Jonard et al.**⁽¹³⁾ based on transvaginal probe. To classify ovaries as normal or polycystic, we counted the total number of follicles 2 to 9 mm in diameter in both ovaries, and then set the threshold at 12, defining polycystic ovaries (PCOS) by the presence of 12 or more follicles (mean of both ovaries). If the mean follicle number was <12, the ovaries were categorized normal. The ovarian volume was considered to be increased if above 10ml.

Normal and women with PCOS were otherwise healthy and were under no medication "including oral contraceptive pills" during the last three months.

Sampling:

Blood samples were collected on cycle day 3 in all patients. In PCOS patients, the last menstrual period was either spontaneous or induced by the administration of micronised progesterone (200 mg/d for 7 d). Sera were separated and stored at -70°C till the time of analysis.

Methods:

The levels of AMH were measured by using an ultra-sensitive enzyme-linked immunosorbent assay "Immunotech-Coulter, Marseilles, France" as described by Long et al.⁽¹⁴⁾.

Serum LH and FSH were assayed by using an ELISA kit (Cat No. KIF 4057 and 4023, respectively/Hedix Biotech Inc., USA with a sensitivity of <0.5 mIU/mL. Testosterone was measured by using an ELISA kit (Orion Diagnostica, Finland) with a sensitivity of 0.02 - 0.05 ng/ml. Estradiol was measured using solid-phase RIA I¹²⁵ kit; Diagnostic Products Corp., Los Angeles, CA) with a sensitivity of 6 pg/ml.

Statistical analysis:

Data were coded, checked, entered and analyzed by using EPI-INFO software computer package. Data were expressed as mean ± standard deviation. Correlation, t-test and Mann-Whitney test were used, as appropriate. P<0.05 was considered significant.

RESULTS

The obtained data and their statistical analysis were listed in tables (from 1 to 3) and illustrated in figures (from 1 to 6). The mean values of cycle duration and body mass index were significantly higher in PCOS compared with controls.

Serum levels of LH as well as, testosterone were significantly higher in PCOS patients in comparison to control group. Also, the mean number of follicles and the ovarian volume was increased significantly in patients than in healthy controls.

Regarding to AMH, its level was significantly higher in PCOS women compared with controls.

A significant negative correlation was observed between AMH and age in PCOS women. Also, in that group, AMH was significantly correlated with cycle duration, LH, testosterone, mean number of follicles and ovarian volume.

Table-1: Clinical, endocrine and ultrasound parameters among studied groups "mean \pm SD"

	Control n = 15	PCOS n = 30	P
Clinical parameters			
Age (yr)	26.6 \pm 3.7	28 \pm 3.6	0.23
BMI (k/m ²)	24.7 \pm 2.7	27.4 \pm 3.4	0.01
Cycle duration (d)	28 \pm 1.95	79.8 \pm 30.8	0.001
Endocrine parameters			
LH (IU/L)	3.17 \pm 1.98	6.86 \pm 5.2	0.01
FSH (IU/L)	6.16 \pm 2.75	5.2 \pm 2.43	0.2
Estradiol (pg/ml)	43.9 \pm 28.02	62.7 \pm 45.33	0.14
Testosterone (nmol/L)	1.39 \pm 0.87	2.68 \pm 1.33	0.01
Ultrasound parameters			
Mean number of follicles ovaries)	6.8 \pm 3.1	22.9 \pm 5.1	<0.001
Ovarian volume (per ovary)	7.2 \pm 2.2	12.83 \pm 2.7	<0.001

Table-2: AMH in studied groups (Mean \pm SD).

	Control group n = 15	PCOS group n = 30	P
AMH (μ g/L)	2.33 \pm 1.8	9.55 \pm 3.39	< 0.001

Table-3: Correlation between AMH and other parameters in anovulatory infertile women with PCOS.

	R	P
Age (yr)	-0.51	<0.001
BMI (k/m ²)	0.08	>0.05
Cycle duration (d)	0.46	<0.01
LH (IU/L)	0.76	<0.001
FSH (IU/L)	0.18	>0.05
Testosterone (nmol/L)	0.67	<0.001
Estradiol (pg/ml)	0.03	>0.05
Mean number of follicles "both ovaries"	0.68	<0.001
Ovarian volume (ml)/ ovary	0.54	<0.001

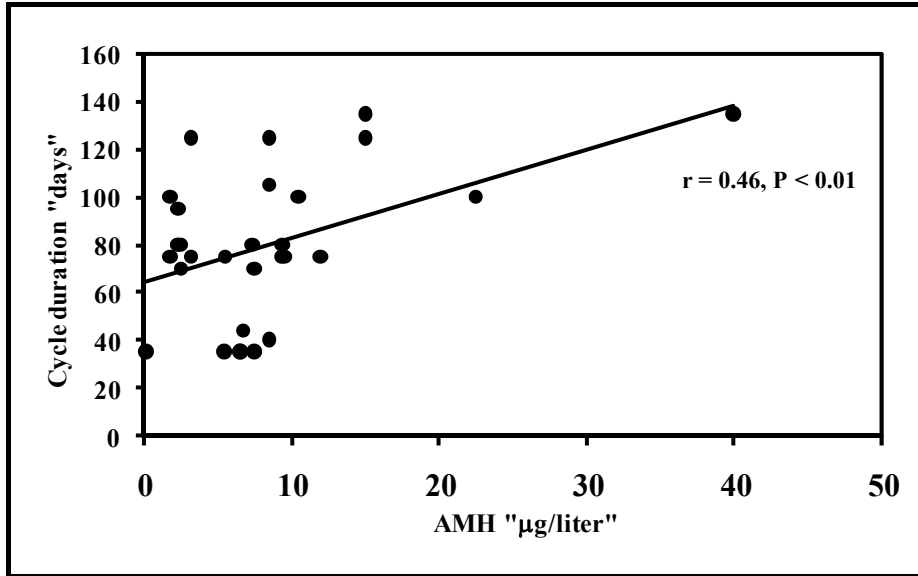


Fig (1): Correlation between AMH and cycle duration in anovulatory infertile women

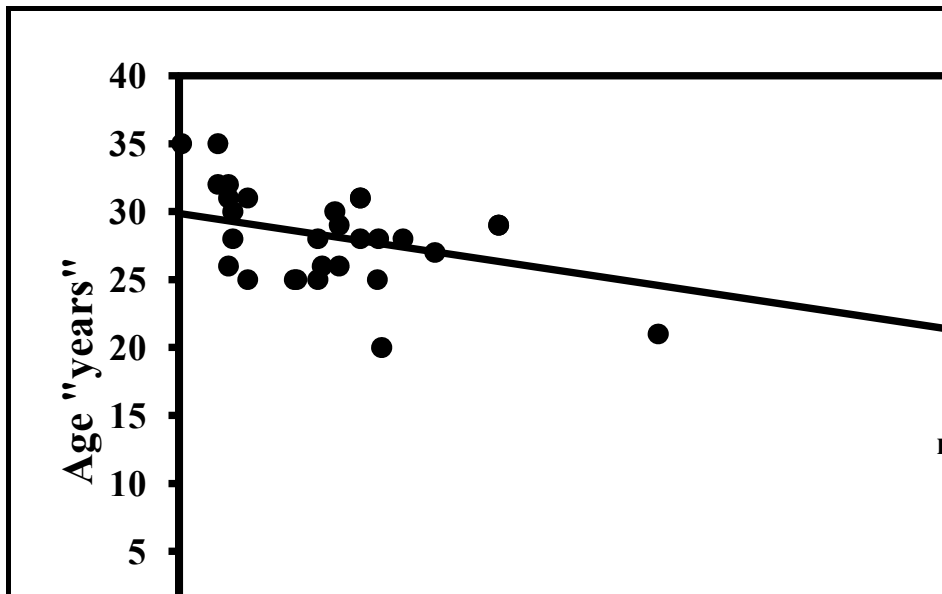


Fig (2): Correlation between AMH and age in anovulatory infertile women

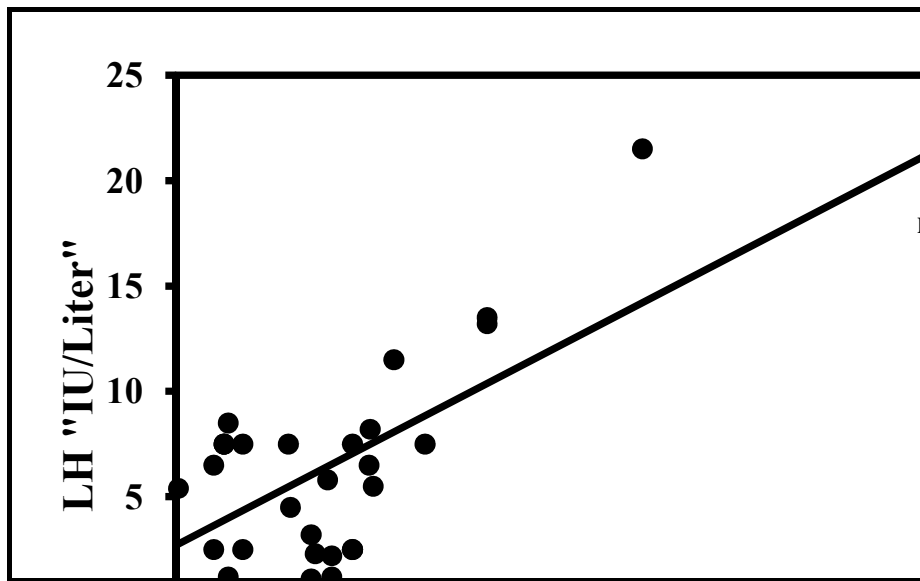


Fig (3): Correlation between AMH and LH in anovulatory infertile women

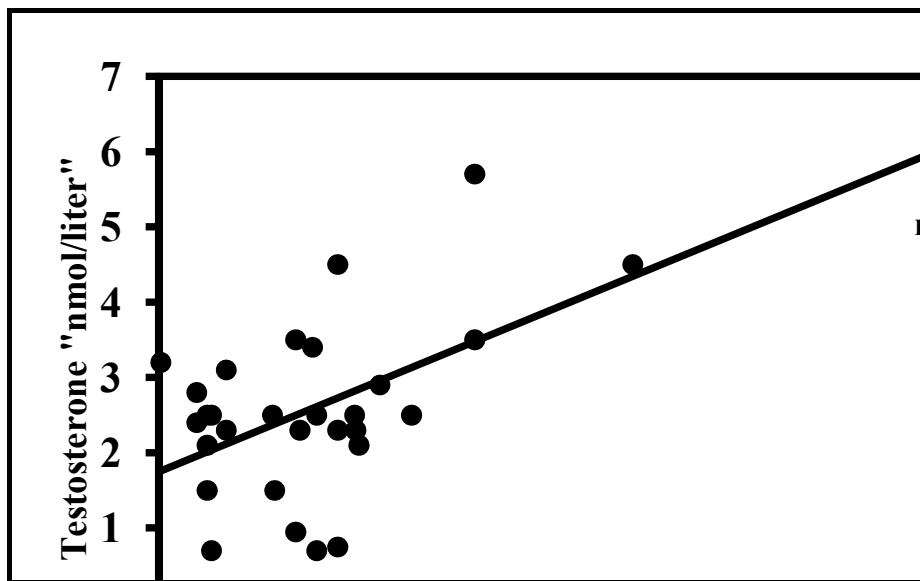


Fig (4): Correlation between AMH and Testosterone in anovulatory infertile women

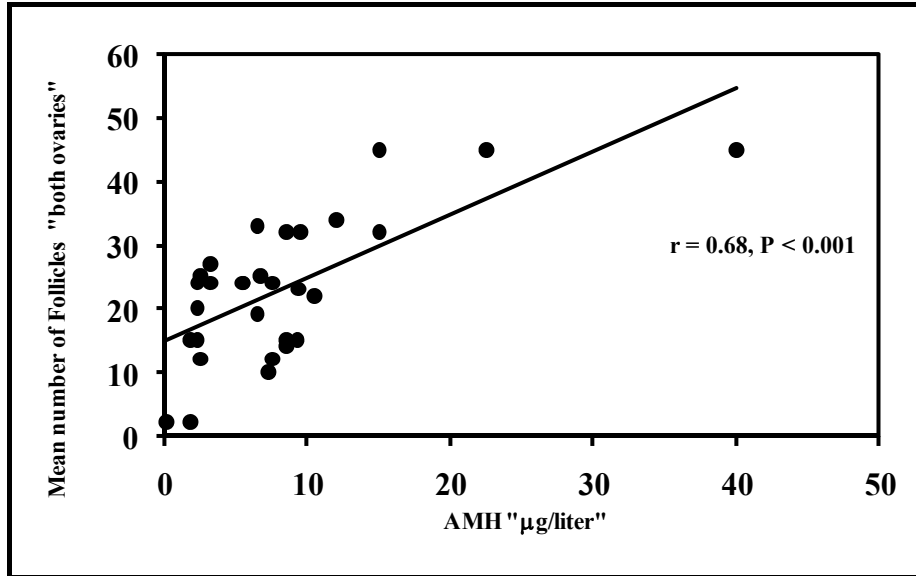


Fig (5): Correlation between AMH and Mean number of follicles in anovulatory infertile women.

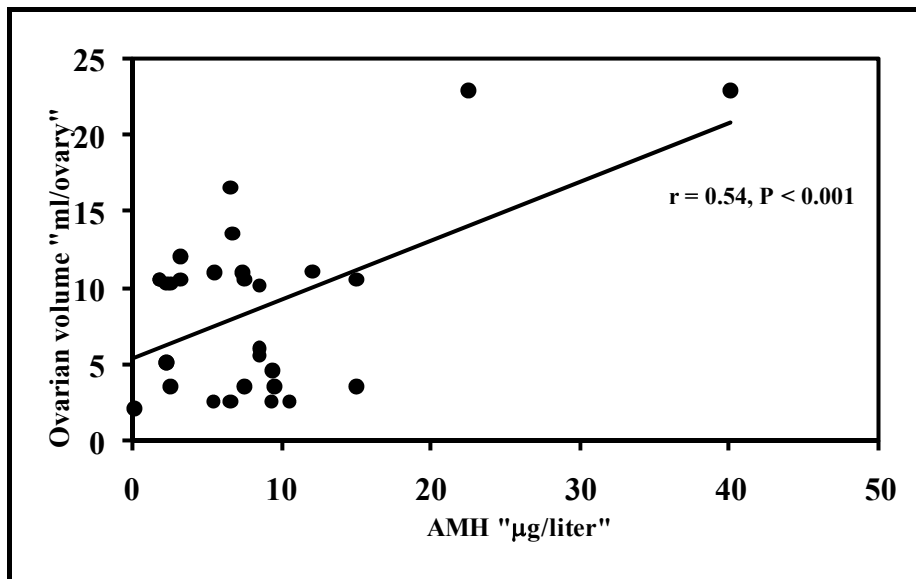


Fig (6): Correlation between AMH and Ovarian volume in anovulatory infertile women.

DISCUSSION

Polycystic ovary syndrome (PCOS) is the most common endocrinological disorder in women of fertile age. It is characterized by menstrual irregularity, androgen excess, polycystic ovaries (PCO) and disturbances in glucose metabolism⁽¹⁵⁾. 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⁽¹⁶⁾, 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.

The present study clearly shows that AMH levels are increased in women with PCOS compared with that in controls. Our results are in agreement with **Seifer et al.**⁽¹⁷⁾ who reported that, AMH levels are 2 to 3 folds higher in women with PCOS compared with healthy women and, also, demonstrated that such difference remains until late reproductive age. Increased serum AMH concentrations in PCOS have been explained by the increased number of small ovarian follicles responsible for AMH secretions. It appears that follicle development is arrested in PCOS at the stage in which dominant follicle selection occurs under normal conditions. Follicle maturation arrest during later stages of development may lead to a build up of many immature follicles, which in itself could explain increased AMH levels^(2,12).

We found that AMH levels correlate with the extent of ovarian dysfunction in PCOS women, as represented by elevated LH, testosterone and an increased follicle number and ovarian volume as established on ultrasound. The mean number of follicles was significantly increased and correlated with AMH in patients with PCOS. These results are in agreement with those of **Jonard et al.**⁽¹³⁾ who reported that, the AMH represents the size of the resting pool of follicles.

Normal or decreased serum estradiol levels⁽¹⁹⁾ and a negative correlation between AMH and estradiol concentrations⁽²⁰⁾ have been shown in women with PCOS. The relationship between estradiol and increased AMH levels could be explained by an inhibitory effect of AMH on aromatase activity⁽²¹⁾ that could cause the well known increase in androgen levels and unchanged/decreased levels of estradiol in PCOS subjects. So, AMH may have a role in the pathogenesis of PCOS. However, the present study and a previous one⁽²²⁾ do not support that concept since no correlation between AMH and estradiol concentrations was observed. This may be explained by the possibility, that aromatase activity is not maximal at early follicular phase when our measurements were performed, and this could have affected the results.

Because AMH constitutes a marker for the number of small follicles, its correlation with ovarian volume and the number of follicles present in the ovary is not surprising. PCOS constitute a sensitive marker

for the extent of ovarian dysfunction in anovulatory women as well as for ovulation induction outcome⁽²³⁾. AS AMH is solely synthesized by granulosa cells of preantral and small antral follicles⁽²⁴⁾, small follicles, which are not readily detected on ultrasound, may significantly contribute to serum AMH levels. Therefore, AMH might even constitute a more sensitive marker of ovarian dysfunction in PCOS patients. In conclusion, serum AMH concentrations are elevated in PCOS patients; which appears to be related to the increased number of small preantral and early antral follicles. Because AMH concentration correlated well with other clinical, endocrine, and ultrasound parameters indicative of ovarian dysfunction in these patients, AMH may constitute a novel marker for the extent of the disease.

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تركيز الهرمون المضاد لقناة موليريان بمصل السيدات اللاتي تعانين من متلازمة تكيس المبايض وفي السيدات ذوات التبويض الطبيعي في سن الإنجاب

مجدى احمد الطحان* سليم صفوت الجندى** عبد الله دياب**
أقسام الكيمياء الحيوية الطبية*، النساء والتوليد** – كلية الطب البشرى – جامعة الزقازيق

أجرى هذا البحث فى قسمى النساء والتوليد والكيمياء الحيوية الطبية بكلية الطب- جامعة الزقازيق فى الفترة من أول فبراير وحتى اخر يوليو ٢٠٠٨ .
الغرض من البحث: هو دراسة العلاقة بين الهرمون المضاد لقناة موليريان وبعض المتغيرات الإكلينيكية والهرمونية وناتج الفحص بالموجات فوق الصوتية فى السيدات اللاتي تعانين من العقم نتيجة عدم التبويض مع متلازمة تكيس المبايض وكذلك فى السيدات ذوات الطمث المنتظم.
الطريقة: تم جمع عينات مصل الدم من ٣٠ سيدة تعانين من عدم التبويض مع متلازمة تكيس المبايض ومن ١٥ سيدة طبيعية (مجموعة ضابطة) فى اليوم الثالث من بداية الطمث وتم تجميد العينات عند ٧٠ درجة مئوية تحت الصفر حتى قياس الهرمونات.
النتائج: وُجد أن تركيز الهرمون المضاد لقناة موليريان أعلى بدرجة ذات دلالة إحصائية فى السيدات اللاتي تعانين من متلازمة تكيس المبايض مقارنة بالمجموعة الضابطة. كما وجد أن تركيز الهرمون المضاد لقناة موليريان تناسب طردياً مع هرمون تنشيط الجسم الأصفر وهرمون الذكورة ومتوسط عدد الجريبات بالمبيض وكذلك مع حجم المبيض وطول فترة الدورة الشهرية، وتناسب عكسياً مع عمر السيدات.
الاستنتاج: تم استنتاج أن تركيز الهرمون المضاد لقناة موليريان يكون مرتفعاً فى مصل السيدات اللاتي تعانين من متلازمة تكيس المبايض وربما يكون هذا له دور فى اختلال نمو البويضات المميز لهذا المرض.