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## Anti-Mullerian Hormone and Follicle Stimulating Hormone as Markers of Ovarian Aging in a Sample of Iraqi Women

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### Abstract

One-hundred and twenty Iraqi women (60 single women and 60 married women) with age ranges from (17-49) years have been involved in this study to estimate the levels of anti-mullerian hormone (AMH) and follicle stimulating hormone (FSH) as markers of ovarian aging. The descriptive data [age, body mass index (BMI), age at menarche, duration of menarche] have been recorded. Blood samples were collected from the studied women to determine the levels of AMH and FSH.

The results revealed non-significant ( $p>0.05$ ) differences in levels of AMH and FSH between single women and married women. A significant negative correlation was observed between AMH levels and age in single women ( $r=-0.519$ ,  $p<0.05$ ) and married women ( $r=-0.433$ ,  $p<0.05$ ). A non-significant correlation was found between AMH levels and BMI in single women, while a significant negative correlation was found in married women ( $r=-0.311$ ,  $p<0.05$ ). A non-significant correlation was observed between AMH levels and age of menarche in the two groups, while a significant negative correlation was observed between AMH levels and duration of menarche in single women ( $r=-0.520$ ,  $p<0.05$ ) and married women ( $r=-0.396$ ,  $p<0.05$ ). The results showed a significant positive correlation between FSH levels and age in single women ( $r=0.525$ ,  $p<0.05$ ) and married women ( $r=0.346$ ,  $p<0.05$ ), while a significant positive correlation was found with BMI ( $r=0.315$ ,  $p<0.05$ ) in single women only. Non-significant correlation was found with age at menarche in studied women, while a significant positive correlation was noticed with duration of menarche in single women ( $r=0.529$ ,  $p>0.05$ ) and married women ( $r=0.339$ ,  $p>0.05$ ). The correlation between AMH and FSH levels in studied women revealed a significant negative correlation in single women ( $r=-0.429$ ,  $p<0.05$ ), while a non-significant correlation was found in married women. In single and married women, a significant ( $p<0.05$ ) decrease was found in AMH levels; while a significant ( $p<0.05$ ) increase was found in FSH levels in ( $\geq 40$  years) age category compared with the other age categories. In single women, non-significant ( $p>0.05$ ) differences were found in AMH levels among the BMI categories, while a significant ( $p<0.05$ ) decrease was found in FSH levels in (18.5-24.9 kg/m<sup>2</sup>) BMI category compared with other BMI categories. In married women, non-significant ( $p>0.05$ ) differences were found in AMH and FSH levels between the BMI categories. In conclusion, this study demonstrated the importance of measurement of AMH and FSH as a good marker of ovarian aging in Iraqi women.

**Keywords:** Anti-mullerian hormone, Follicle stimulating hormone, Ovarian aging, Iraqi women.

الهرمون المضاد لمولر والهرمون المحفز للجريب كمؤشر لعمر المبيض في عينة من النساء العراقيات

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## الخلاصة

اجريت هذه الدراسة بهدف التعرف على مستويات التغير في الهرمون المضاد لمولر (AMH) والهرمون المحفز للجريب (FSH) كمؤشرات لعمر المبيض في عينة من النساء العراقيات.. شملت الدراسة مائة وعشرين امرأة عراقية (60 امرأة غير متزوجة و60 امرأة متزوجة) تراوحت اعمارهن بين 17-49 سنة. سجلت البيانات الوصفية (العمر، مؤشر كتلة الجسم، عمر بدء الاحاضة وفترةها). جمعت عينات الدم من جميع النساء المشتركات بالدراسة لقياس مستوى AMH و FSH .

اوضحت النتائج عدم وجود فروق معنوية في مستوى AMH و FSH بين النساء غير المتزوجات والمتزوجات. لوحظ وجود ترابط معنوي سالب بين مستوى AMH والعمر في النساء غير المتزوجات والمتزوجات، وجود ترابط معنوي بين مستوى AMH ومؤشر كتلة الجسم في النساء غير المتزوجات ؛ بينما هناك ترابط معنوي سالب في النساء المتزوجات. لوحظ عدم وجود ترابط معنوي بين مستوى AMH وعمر بدء الاحاضة في المجموعتين، بينما كان هناك ترابط معنوي سالب بين مستوى AMH وفترة بدء الاحاضة في النساء غير المتزوجات والمتزوجات. وظهرت النتائج وجود ترابط معنوي موجب بين مستوى FSH والعمر في النساء غير المتزوجات والمتزوجات ووجود ترابط معنوي موجب بين مستوى FSH ومؤشر كتلة الجسم في النساء غير المتزوجات فقط . لوحظ عدم وجود ترابط معنوي بين مستوى FSH وعمر بدء الاحاضة في المجموعتين ووجود ترابط معنوي موجب بين مستوى FSH وفترة بدء البلوغ في المجموعتين. اظهرت النتائج ان الترابط بين مستوى AMH ومستوى FSH هو ترابط معنوي سالب في النساء غير المتزوجات ، بينما لا يوجد ترابط في النساء المتزوجات. كان هناك نقصان معنوي في مستوى AMH وزيادة معنوية في مستوى FSH في الفئة العمرية ( $\leq 40$  سنة) مقارنة مع الفئات العمرية الاخرى لكل من النساء غير المتزوجات والمتزوجات . لا توجد فروق معنوية في مستوى AMH عند زيادة مؤشر كتلة الجسم في مجموعة النساء غير المتزوجات، بينما هناك نقصان معنوي في مستوى FSH عند الفئة (18.5 - 24.9 كغم/م<sup>2</sup>) من مؤشر كتلة الجسم مقارنة مع الفئات الاخرى. لا توجد فروق معنوية في مستويات AMH و FSH بين فئات مؤشر كتلة الجسم في مجموعة النساء المتزوجات. من الدراسة الحالية بالامكان الاستنتاج باهمية قياس الهرمون المضاد لمولر والهرمون المحفز للجريب كمؤشر لعمر المبيض في النساء العراقيات.

## Introduction

Ovarian reserve is a concept that refers to the quantity and quality of available oocytes [1]. The most suitable markers of ovarian reserve include early follicular phase determination of follicle stimulating hormone (FSH), estradiol (E2), inhibin-B, and noncyclic dependent estimation of anti-mullerian hormone (AMH) levels [2,3].

Anti-mullerian hormone, also known as mullerian inhibiting substance (MIS), is a member of the transforming growth factor-beta (TGF $\beta$ ) superfamily [4]. It is secreted by granulosa cells of follicles undergoing gonadotropin independent development. The main role of AMH is to inhibit follicular development from primordial to primary follicular stages [5]. Levels of AMH do not vary significantly through the menstrual cycle and it doesn't tend to vary from cycle to cycle like FSH levels [6]. It has been reported that AMH is a better marker of ovarian reserve compared with age alone or other markers such as basal FSH [5].

Follicle stimulating hormone is a hormone that is synthesized and secreted by gonadotropes in the anterior pituitary gland. It stimulates the growth of immature Graafian follicles in the ovary to reach maturation and plays a crucial role in the recruitment, selection, and dominance processes during the whole follicular phase [7]. The measurement of serum FSH levels at 2 or 3 days after the onset of full menstrual flow has been used as a marker of ovarian reserve since the 1980s, based on its association with reproductive outcome [8]. It has been reported that 81% of variation in ovarian reserve is due to age alone [3]. As a woman ages, the follicular supply decreases and AMH levels decrease. The AMH level is low in females at birth, rises rapidly during 0-3 months and in 4-8 years of age, and remains constant during 8-25 years of age, and is followed by a gradual decline after 25 years of age [9]. Elevated basal FSH concentration is a marker for decreased oocyte quality. As women and their follicles age, the amount of FSH secreted increases due to the lack of responsiveness of the ovary [10]. AMH predicts ovarian aging earlier than FSH [5].

The present study aims to estimate the levels of AMH and FSH as markers of ovarian aging in a sample of Iraqi women (single and married women); and to investigate the effect of some descriptive data [age, body mass index (BMI), age at menarche and duration of menarche] on the levels of these hormones.

## Materials and Methods

### Subjects

One hundred and twenty Iraqi women have been involved in this study, divided into two groups; the first group involved 60 single women and the second group involved 60 married women. All the women have regular menstrual cycle.

The participants were handed questionnaire asking for descriptive data (age, weight, height, age at menarche and duration of menarche). The BMI was calculated as weight (kg) divided by the square of height (m<sup>2</sup>) [11].

### Collection of Blood Samples

Blood samples were collected from the participants during the early follicular phase (cycle day 2-4). Blood samples were left at room temperature for about half an hour then the sera were separated by centrifugation for 15 minutes at 3000 rpm and stored at -20°C until used.

### Hormonal Assay

Serum concentrations of AMH and FSH were measured by using enzyme-linked immunosorbent assay (ELISA) according to [12,13]. Kit of AMH provided from diagnostic systems (CUSABIO, China), and kit of FSH provided from Human company/Germany.

### Statistical Analysis

The Statistical analysis has been done using statistical package of social sciences (SPSS), version 18, and computer software. The results were expressed as mean  $\pm$  standard deviation (SD). The statistical analysis included Student's t-test to examine the differences between the single and married women groups. [14]. Differences between the studied age and BMI categories were analyzed using analysis of variance (ANOVA). The differences were considered significant at  $P < 0.05$ . Pearson correlation coefficient (r) was calculated to study the relationship between variables

### Results

Descriptive data were recorded for the participants as shown in Table-1. Non-significant ( $p > 0.05$ ) differences were found in mean value of age between single women ( $30.10 \pm 10.09$  years) and married women ( $31.03 \pm 9.73$  years). The mean value of BMI in single women ( $25.92 \pm 4.12$  kg/m<sup>2</sup>) was significantly ( $p < 0.05$ ) lower than that in married women ( $28.15 \pm 4.96$  kg/m<sup>2</sup>). Non-significant ( $p > 0.05$ ) differences were observed in mean value of age at menarche between single women ( $12.66 \pm 1.05$  years) and married women ( $12.76 \pm 1.14$  years). Also, non-significant ( $p > 0.05$ ) differences were found in mean value of duration of menarche between single women ( $17.43 \pm 10.11$  years) and married women ( $17.75 \pm 9.76$  years).

**Table 1-**Descriptive data of the studied groups (single and married women)

Groups Parameters	Single women (No. 60)	Married women (No. 60)
Age (years)	30.10 <sup>a</sup> $\pm$ 10.09	31.03 <sup>a</sup> $\pm$ 9.73
BMI (kg/m <sup>2</sup> )	25.92 <sup>a</sup> $\pm$ 4.12	28.15 <sup>b</sup> $\pm$ 4.96
Age at menarche (years)	12.66 <sup>a</sup> $\pm$ 1.05	12.76 <sup>a</sup> $\pm$ 1.14
Duration of menarche (years)	17.43 <sup>a</sup> $\pm$ 10.11	17.75 <sup>a</sup> $\pm$ 9.76

►Values are means  $\pm$  standard deviation.

►Means in rows carrying similar small letters indicate a non-significant difference ( $p > 0.05$ ).

►Means in rows carrying different small letters indicate a significant difference ( $p < 0.05$ ).

Table-2 shows levels of AMH and FSH in the studied women. Non-significant ( $p > 0.05$ ) differences were found in AMH levels between single women ( $1.610 \pm 1.515$  ng/ml) and married women ( $1.647 \pm 1.722$  ng/ml). Also, non-significant ( $p > 0.05$ ) differences were found in FSH levels between single women ( $8.617 \pm 4.398$  IU/l) and married women ( $7.973 \pm 3.404$  IU/l).

**Table 2-** Levels of AMH and FSH in the studied groups (single and married women)

Groups Hormone	Single women (No. 60)	Married women (No. 60)
	AMH (ng/ml)	1.610 <sup>a</sup> ± 1.515
FSH (IU/l)	8.617 <sup>a</sup> ± 4.398	7.973 <sup>a</sup> ± 3.404

►Values are means ± standard deviation.

►Means in rows carrying similar small letters indicate a non-significant difference ( $p>0.05$ ).

The correlation between AMH levels and the values of other studied parameters is shown in Table-3. This table indicated that a significant negative correlation was found between AMH levels and age in single ( $r= -0.519$ ,  $p<0.05$ ) and married women ( $r= -0.433$ ,  $p<0.05$ ). A non-significant correlation was found between the AMH levels and the value of BMI in single women ( $r= -0.259$ ,  $p>0.05$ ); while a significant negative correlation was found in married women ( $r= -0.311$ ,  $p<0.05$ ). A non-significant correlation ( $p>0.05$ ) was found between AMH levels and age of menarche in the two groups of the present study. A significant negative correlation was found between AMH levels and duration of menarche in single women ( $r= -0.520$ ,  $p<0.05$ ) and married women ( $r= -0.396$ ,  $p<0.05$ ).

**Table 3-** Correlation coefficient between AMH levels and other parameters in the studied groups (single and married women).

Groups Parameters	Correlation coefficient (r)	
	Single women (No. 60)	Married women (No. 60)
Age (years)	-0.519 *	-0.433 *
BMI (kg/m <sup>2</sup> )	-0.259 NS	-0.311 *
Age of menarche (years)	0.027 NS	0.113 NS
Duration of menarche (years)	-0.520 *	-0.396 *

\*Significant differences ( $p<0.05$ ).

NS: Non-significant.

Table-4 shows the correlation between FSH levels and the values of other studied parameters. This table indicated that a significant positive correlation was found between FSH levels and age in single women ( $r= 0.525$ ,  $p<0.05$ ) and in married women ( $r= 0.346$ ,  $p<0.05$ ). A significant positive correlation was noticed between the FSH levels and the value of BMI in single women ( $r= 0.315$ ,  $p<0.05$ ); while a non-significant correlation was found in married women ( $r= 0.072$ ,  $p>0.05$ ). A non-significant correlation ( $p>0.05$ ) was found between FSH levels and age of menarche in the two groups of the current study. A significant positive correlation was found between FSH level and duration of menarche in single women ( $r= 0.529$ ,  $p<0.05$ ) and in married women ( $r= 0.339$ ,  $p<0.05$ ).

**Table 4-** Correlation coefficient between FSH levels and other parameters in the studied groups (single and married women).

Groups Parameters	Correlation coefficient (r)	
	Single women (No. 60)	Married women (No. 60)
Age (years)	0.525 *	0.346 *
BMI (kg/m <sup>2</sup> )	0.315 *	0.072 NS
Age of menarche (years)	-0.050 NS	-0.139 NS
Duration of menarche (years)	0.529 *	0.339 *

\*Significant differences ( $p<0.05$ ).

NS: Non-significant.

The correlation between AMH levels and FSH levels in studied groups is shown in Table-5. A significant negative correlation was found between AMH levels and FSH levels in single women ( $r= -0.429$ ,  $p<0.05$ ); while a non-significant correlation was found in married women ( $r= -0.223$ ,  $p>0.05$ ).

**Table 5-** Correlation coefficient between AMH levels and FSH levels in the studied groups (single and married women).

Groups	Correlation coefficient (r)
Single women (No. 60)	-0.429 *
Married women (No. 60)	-0.223 NS

\*Significant differences ( $p < 0.05$ ).

NS: Non-significant.

The effect of the Age on AMH levels is shown in Table-6. AMH levels in single women demonstrated an age-related decline. However, the statistical analysis showed a significant ( $p < 0.05$ ) decrease in AMH level ( $0.315 \pm 0.157$  ng/ml) only in ( $\geq 40$  years) age category compared with ( $2.340 \pm 1.775$  ng/ml,  $2.246 \pm 1.508$  ng/ml and  $1.559 \pm 1.279$  ng/ml) in the other age categories (<20 years, 20-29 years and 30-39 years, respectively). Also, the serum levels of AMH decreased with increasing age of the married women. However, the statistical analysis showed a significant ( $p < 0.05$ ) decrease in AMH levels ( $0.521 \pm 0.354$  ng/ml) only in ( $\geq 40$  years) age category compared with ( $2.521 \pm 2.822$  ng/ml and  $2.312 \pm 1.601$  ng/ml) in the age categories (<20 and 20-29 years, respectively); while a non-significant ( $p > 0.05$ ) decrease was reported compared with ( $1.479 \pm 1.197$  ng/ml) in the (30-39 years) age category.

**Table 6-** Effect of the age on levels of AMH in the studied groups (single and married women).

Age categories (years)	AMH (ng/ml)	
	Single women (No. 60)	Married women (No. 60)
< 20	$2.340^a \pm 1.775$	$2.521^a \pm 2.822$
20-29	$2.246^a \pm 1.508$	$2.312^a \pm 1.601$
30-39	$1.559^a \pm 1.279$	$1.479^{a,b} \pm 1.197$
$\geq 40$	$0.315^b \pm 0.157$	$0.521^b \pm 0.354$

► Values are means  $\pm$  standard deviation.► Means in column carrying similar small letters indicate a non-significant difference ( $p > 0.05$ ).► Means in column carrying different small letters indicate a significant difference ( $p < 0.05$ ).

Regarding the effect of the age on FSH levels Table-7, The results of the single women showed a significant ( $p < 0.05$ ) increase in FSH level ( $13.147 \pm 2.612$  IU/l) only in ( $\geq 40$  years) age category compared with ( $6.733 \pm 1.722$  IU/l,  $6.824 \pm 1.533$  IU/l and  $7.747 \pm 1.627$  IU/l) in the other age categories (<20 years, 20-29 years and 30-39 years, respectively). Also, the results of the married women revealed a significant ( $p < 0.05$ ) increase in FSH level ( $10.324 \pm 2.447$  IU/l) only in the ( $\geq 40$  years) age category compared with the other age categories (<20 years, 20-29 years and 30-39 years) ( $7.545 \pm 2.097$  IU/l,  $6.999 \pm 1.691$  IU/l and  $6.986 \pm 2.075$  IU/l, respectively).

**Table 7-** Effect of the age on levels of FSH in the studied women (single and married women).

Age categories (years)	FSH (IU/l)	
	Single women (No. 60)	Married women (No. 60)
< 20	$6.733^a \pm 1.722$	$7.545^a \pm 2.097$
20-29	$6.824^a \pm 1.533$	$6.999^a \pm 1.691$
30-39	$7.747^a \pm 1.627$	$6.986^a \pm 2.075$
$\geq 40$	$13.147^b \pm 2.612$	$10.324^b \pm 2.447$

► Values are means  $\pm$  standard deviation.► Means in column carrying similar small letters indicate a non-significant difference ( $p > 0.05$ ).► Means in column carrying different small letters indicate a significant difference ( $p < 0.05$ ).

Body mass index categories according to the value of European society of human reproduction and embryology are four groups ( $\leq 18.5$  kg/m<sup>2</sup>, 18.5-24.9 kg/m<sup>2</sup>, 25-29.9 kg/m<sup>2</sup> and  $\geq 30$  kg/m<sup>2</sup>). In the current study, the category of  $\leq 18.5$  kg/m<sup>2</sup> is not included because the BMI of the studied women was more than 18.5 kg/m<sup>2</sup>. The effect of the BMI on AMH levels in the studied groups is shown in Table-

8, the results showed that there were non-significant ( $p > 0.05$ ) differences in the levels of AMH in the single women ( $1.962 \pm 1.547$  ng/ml;  $1.447 \pm 1.484$  ng/ml and  $1.373 \pm 0.991$  ng/ml) between the three BMI categories ( $18.5-24.9$  kg/m<sup>2</sup>;  $25-29.9$  kg/m<sup>2</sup> and  $\geq 30$  kg/m<sup>2</sup> respectively). Also, the findings revealed that there were non-significant ( $p > 0.05$ ) differences in the levels of AMH in the married women ( $1.649 \pm 1.545$  ng/ml,  $1.985 \pm 1.917$  ng/ml and  $1.275 \pm 0.947$  ng/ml) between the three BMI categories ( $18.5-24.9$  kg/m<sup>2</sup>,  $25-29.9$  kg/m<sup>2</sup> and  $\geq 30$  kg/m<sup>2</sup> respectively).

**Table 8-** Effect of the BMI on levels of AMH in the studied groups (single and married women).

BMI categories (kg/m <sup>2</sup> )	AMH (ng/ml)	
	Single women (No. 60)	Married women (No. 60)
18.5-24.9	$1.962^a \pm 1.547$	$1.649^a \pm 1.545$
25-29.9	$1.447^a \pm 1.484$	$1.985^a \pm 1.917$
$\geq 30$	$1.373^a \pm 0.991$	$1.275^a \pm 0.947$

► Values are means  $\pm$  standard deviation.

► Means in column carrying similar small letters indicate a non-significant difference ( $p > 0.05$ ).

Concerning the effect of the BMI on FSH levels in the studied groups Table-9, the results showed a significant ( $p < 0.05$ ) increase in FSH levels of the single women ( $10.108 \pm 2.241$  IU/l) in ( $25-29.9$  kg/m<sup>2</sup>) BMI category compared with ( $7.083 \pm 1.715$  IU/l) in ( $18.5-24.9$  kg/m<sup>2</sup>) BMI category, while non-significant ( $p > 0.05$ ) differences were found in FSH levels ( $9.241 \pm 2.461$  IU/l) in ( $\geq 30$  kg/m<sup>2</sup>) BMI category compared with ( $7.083 \pm 1.715$  IU/l and  $10.108 \pm 2.241$  IU/l) in BMI categories ( $18.5-24.9$  kg/m<sup>2</sup> and  $25-29.9$  kg/m<sup>2</sup>, respectively). In contrast, the results of the married women revealed that there were non-significant ( $p > 0.05$ ) differences in the levels of FSH ( $7.519 \pm 1.537$  IU/l,  $7.845 \pm 2.627$  IU/l and  $8.661 \pm 2.207$  IU/l) between the three BMI categories ( $18.5-24.9$  kg/m<sup>2</sup>,  $25-29.9$  kg/m<sup>2</sup> and  $\geq 30$  kg/m<sup>2</sup>, respectively).

**Table 9 -** Effect of the BMI on levels of FSH in the studied groups (single and married women).

BMI categories (kg/m <sup>2</sup> )	FSH (IU/l)	
	Single women (No. 60)	Married women (No. 60)
18.5-24.9	$7.083^a \pm 1.715$	$7.519^a \pm 1.537$
25-29.9	$10.108^b \pm 2.241$	$7.845^a \pm 2.627$
$\geq 30$	$9.241^{a,b} \pm 2.461$	$8.661^a \pm 2.207$

► Values are means  $\pm$  standard deviation of means.

► Means in column carrying similar small letters indicate a non-significant difference ( $p > 0.05$ ).

► Means in column carrying different small letters indicate a significant difference ( $p < 0.05$ ).

## Discussion

The results of the current study revealed a significant increase in mean of BMI in married women when compared with single women. These findings indicated that the Iraqi married women have BMI higher than the single women. This may be due to hormonal changes occurring in married.

The most common methods of checking the status of the ovarian reserve is to perform a blood test on day 3 of the menstrual cycle to measure serum FSH level, alternatively a blood test to measure the serum AMH level can give similar information. The results of the current study showed that the AMH levels were nearly same in single women and married women. This may be due to the fact that both groups were healthy. Although the levels of AMH were in normal values but it was slightly more in married women compared to single women. However, non-significant differences were found between them. Serum AMH was recently added as a marker for menopausal staging because it declines much earlier than other signs of menopause such as increasing serum FSH or irregular menses [15]. The results showed that the FSH levels in single women were slightly higher than in married women, but the differences were non-significant. It has been reported that the women with normal AMH and FSH levels produced high number of oocytes, whereas women with normal FSH but decreasing AMH produced a significantly lower number of oocytes [16]. This also indicates that AMH levels are more important predictors of ovarian aging than FSH levels.

The results of this study showed a significant negative correlation between AMH level and the age in single women and married women. This finding may be due to the fact that increase of age leading

to decrease of follicles so the AMH levels decrease and also leading to ovarian impairment. This result is in agreement with [6, 17] who reported a linear decline of AMH levels over time, which may be attributed to a decreasing number of follicles in the primordial pool. In agreement with previous studies [18,19], the present study revealed non-significant correlation between levels of AMH and values of BMI in single women; while in married women, a significant negative correlation was observed. This result seems to agree with the finding of [20,21]. All these findings suggested that the aging women tend to have a higher BMI; therefore, AMH levels tend to decline as a result of aging and not owing to the increase in BMI [22]. The correlation between AMH and age of menarche in the two studied groups was non-significant. This result disagree with results of [23] who showed a strong negative correlation between the years since menarche and AMH in normo-ovulatory women, indicating that the ovarian follicle pool decreases with the increase in the gynecological age. Also, [24] reported that lower AMH levels were associated with a higher age at menarche. A significant negative correlation was found between the levels of AMH and the duration of menarche in single and married women. These findings are logical, since increasing of duration of menarche is associated with increasing of the age of these women which had high levels of AMH.

Concerning the correlation between FSH levels and the other studied parameters, the results showed a significant positive correlation between FSH and the age in single and married women; these results are in agreement with previous studies [25,26] who reported a positive correlation of age with serum FSH levels, and disagree with result of [27] who reported that FSH had a negative correlation with age. Serum FSH levels indirectly reflect a woman's residual ovarian function [28]. Elevated basal FSH concentrations are a marker for decreased oocyte quality. As women and their follicles age, the amount of FSH secreted increases due to the lack of responsiveness of the ovary [10]. The relatively lower slopes of increasing FSH in older age have made FSH a late predictor of ovarian reserves [29]. The correlation between FSH and BMI was significantly positive in single women. This result is in agreement with [26] who reported that the BMI had a moderate positive correlation with FSH. While non-significant correlation was found in married women. These findings disagree with [30] who reported that FSH levels were significantly decreased in overweight women. The results showed non-significant correlation between FSH and age of menarche in the two studied groups. These findings disagree with the result of [31] who reported a significant positive correlation between FSH and age of menarche. The correlation between FSH and duration of menarche was significantly positive in single and married women; this mean that the increase of duration of menarche reflect the increase of age of women so when the women ages the level of FSH increase therefore the correlation was significantly positive.

The correlation between AMH and FSH in this study was significantly negative in single women and non-significant in married women; these results are in agreement with the finding of [32] who confirmed the negative linear relationship between AMH and FSH. The negative relationship between AMH and FSH made using the combination of both FSH and AMH to improve the evaluation of ovarian reserve [33].

Regarding the effect of age on AMH levels in single and married women, the findings revealed that the levels of AMH decreased gradually in the two groups with the progress of age. However, a significant decline was observed only in the  $\geq 40$  years age category. These results are in agreement with several previous studies [33,34] who reported that the AMH level decline with the age increase. When a comparison was done between single women and married women, the results showed that the AMH levels was less in the single women regarding the  $\geq 40$  years age category compared with the married women. This finding may be explained on the ground that, married women have a chance to be pregnant so they preserve more follicles than single women; therefore the levels of AMH are higher in this group.

Concerning the effect of age on FSH levels in single and married women, the results revealed a significant increase of this hormone with the increase of age and it is obvious that it was higher in the  $\geq 40$  years age category in these two groups. A similar results were reported by other researchers [35,36] who reported elevated FSH when the age increase and reported that the elevation is associated with shorter follicular phase and cycle length in aging women (late 30 to mid-40) before the menopause transition, coinciding with the marked decline in fecundity. Although the results of the present study revealed that the AMH levels in single and married women decrease when the BMI increase, the statistical analysis revealed that this effect was non-significant.

This finding suggests that the aging women tend to have a higher BMI. Therefore, AMH levels tend to decline as a result of aging and not owing to the increase in BMI [22]. Also, the results indicated that the BMI didn't affect the levels of FSH in the two studied groups. These findings are in agreement with other studies [37, 38].

In conclusion, this study demonstrated the importance of measurement of AMH and FSH as a good marker of ovarian aging in Iraqi women.

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