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ORIGINAL ARTICLE

THE EFFECT OF AGE ON ANTI-MÜLLERIAN HORMONE LEVEL IN OBESE VERSUS NON OBESE INFERTILE PATIENTS IN AL DIWANIYAH CITY

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ABSTRACT

Background: The fertility of women entirely depends upon their age. Fertility reaches peak levels in the early twenties and begins to diminish in the third and fourth decades of life, declining significantly beyond the age of 35. Aim of this study was to evaluate the Effect of Age on Anti-Müllerian Hormone levels in Obese.

Materials & Methods: This retrospective, single-centre study followed 400 patients who attended an Obstetrics and Gynecology clinic in AL Diwaniyah from 14/12/2022 to 29/08/2024. Age(≥35 years versus <35 years), Body mass index (BMI), and (AMH) test results were inclusion criteria that participants were required to meet.

Results: The infertile women's mean age was 32.53 ±7.73 years. In this study, infertile women were categorized into two groups based on age (≥35 years versus <35 years); the mean age in these groups was 41.09 ±3.92 years versus 27.49 ±4.14 years, consecutively (p <0.001). A comparison of mean body mass index (BMI) revealed that women ≥35 years had a mean BMI that was significantly greater than that of women <35 years. The comparison of serum AMH mean according to age and BMI shows that the difference was significant (p <0.001); the highest mean level was reported in the <35-Non-obese group, followed by <35-Obese group, then by ≥35-Non-obese and ≥35 –Obese groups.

Conclusion: The correlation between age of all enrolled infertile women and serum AMH level was significant and negative.

KEY WORDS: Age; Infertility; Obese; Hormone; Al diwaniyah.

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INTRODUCTION

Fertility describes the natural ability to conceive and is measured by the fertility rate, defined as the number of live births per 1,000 women. In demographic data or social and economic contexts, fertility is quantified using the total fertility rate index, which represents the entire number of offspring per woman throughout her lifetime. Women's fertility entirely depends upon their age. Fertility reaches peak levels in the early twenties and thereafter declines over the third and fourth decades of life, declining significantly beyond the age of 35.[1,2]

Obesity impacts all systems of the body, raising the danger of cardiovascular disease, stroke, type 2 diabetes, malignancy, and other diseases. In women, it is essential for addressing not just the overall health problems associated with obesity but additionally its effects on reproductive health.[3-5] Obesity is associated with reduced spontaneous conception rates and is linked with poor results in women seeking fertility treatments such as assisted reproduction. Women with greater body mass index (BMI) exhibit diminished responses to fertility drugs, a reduced number of oocytes retrieved, and poorer rates of pregnancy and live births compared to those with a normal BMI.[6-8]

In 2015, the Global Burden of Disease survey revealed that obesity affected 603.7 million adults, with a rising incidence globally, particularly among women compared to men. [9] The adverse impacts on reproductive function are believed to occur mainly

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through endocrine pathways.[10]

The influence of being obese on female reproductive health was recognized as multifactorial; numerous adverse reproductive outcomes may be associated with endocrine disturbances indicative of compromised ovarian function. [11] Infertility in obese women is typically linked to Ovulation dysfunction and menstrual cycle irregularity. Nonetheless, obese women who had their menstrual periods regular demonstrated an extended duration to achieve spontaneous conception and reduced rate of success of managed hyperstimulation of ovaries in comparison to their peers of average weight.[12-15]

The processes by which obesity negatively impacts AMH production remain unclear; nonetheless, it has been demonstrated that increased adiposity correlates with a reduction in AMH production per antral follicle. [16] A potential explanation pertains to modified metabolic control of the granulosa cells of the ovary. Obesity is frequently linked to systemic resistance to insulin and compensative hyperinsulinemia. Elevated Levels of insulin have been demonstrated to modify the receptivity of granulosa cells., hence affecting Anti-Müllerian hormone synthesis.[17] Similarly, Increased synthesis of leptin linked to obesity may directly inhibit AMH formation. This discovery stems from the inhibitory effects of leptin treatment on the Anti-Müllerian hormone and its receptor genes expression in cultured granulosa cells from individuals who have managed hyperstimulation of the ovary. [18] The concept that diminished levels of Anti-Müllerian hormone in obese patients may stem Because the hemodilution impact due to increased body size is more indirect. Another theory involves the influence of obesity on AMH catabolism and elimination. Obesity has been recognized as the modified elimination of various hormones, including FSH, progesterone and estradiol (Merhi et al., The precise mechanisms of Anti-Müllerian hormone elimination remain unidentified.[19] Finally, obesity may enhance the apoptotic impact at the ovary follicular level, a mechanism documented in models of animals.[20] This proposed mechanism may account for a diminished follicular reserve of the ovary and Anti-Müllerian hormone levels; nevertheless, it appears less plausible given the current findings indicating a delayed onset of ovarian senescence in obese women.

AMH levels are demonstrated to be dependent on age. The decline in Anti-Müllerian hormone levels that is associated with advancing age may precede alterations in other factors related to age, indicating that serum Anti-Müllerian hormone levels could serve as a most reliable indicator of ovarian ageing. It is essential to acknowledge that AMH natural levels exhibit significant inter-individual variability by age, reflecting a broad spectrum of ovarian reserve within the healthy population.[21,22]

Anti-Müllerian Hormone (AMH), also known as Mullerian-inhibiting substance (MIS), is a homodimeric, disulfide-linked glycoprotein that is related to the transforming growth factor-beta (TGF-β) class. Anti-Müllerian Hormone (AMH) serves as an effective indicator of ovarian reserve (OR) since it maintains stability throughout cycles and has a strong connection with ultrasonographic antral follicular count. Obesity can impact reproductive health. Nonetheless, prior research concerning the influence of body mass index (BMI) on AMH levels is inconsistent.[23,24]

Aim of this study was to evaluate the Effect of Age on Anti-Müllerian Hormone levels in Obese.

MATERIALS AND METHODS

2.1 Study design and Patient selection

From 04/11/2022-12/07/2024, 400 patients were managed at Al Diwaniyah Obstetrics and Gynecology Clinic; this retrospective study involved the purposive sample division and evaluation of 200 obese women in addition to 200 non-obese women aged 18-45 years into two cohorts (≥ 35 years versus < 35 years) furthermore each group subdivided into <35-Non-obese group, <35-Obese group, ≥35-Non-obese and ≥35 Obese groups with confidence level 95% and margin of error 5%. An academic statistical consultant in the relevant committee approved the size of the sample. Obese, in addition to non-obese women, were chosen from records of private clinics. The PCOS diagnosis had been established according to the Rotterdam Criteria. [25] The exclusion criteria were individuals with diabetes or systemic diseases, galactorrhea, any endocrine disorders related to 17α-hydroxy progesterone, prolactin, or thyroid stimulating hormone (TSH) levels; the medications used that influencing the hypothalamus-pituitary-ovarian axis or insulin-sensitizing agents like metformin in the preceding three months; and the contraceptives usage within the past four weeks. Additionally, women who were engaged in regular exercise during the study period were eliminated.

Venipuncture was carried out to collect five millilitres of blood. The MINI VIDAS /VIDAS 3 /BIOMERIEUS AMH immunoassay was utilized to measure AMH levels in accordance with the manufacturer's guidelines. A standard procedure was utilized for the collection of serum samples, and the samples were left at room temperature for 5-10 minutes to facilitate complete clotting. The samples undergo centrifugation for 5 minutes at 2000-2500 revolutions per minute (rpm) to isolate the serum from cells and other blood constituents. The serum had been added to a labelled plastic screw-cap container. Vidas AMH immunoassay utilizes two AMH–specific antibodies in a sandwich design to determine hormone levels in the serum.

2.2 Variables collected

The data collected consisted of the patient's age, weight, and height for the calculation of body

mass index (BMI) from BMI = weight/height2 kg/m2 (Stensland and Margolis 1990). The AMH level, which is measured by the MINI VIDAS /VIDAS 3 /BIOMERIEUS, and the type of infertility, whether primary or secondary, were also taken into account. Additionally, women were categorized into two categories: PCOS or not based on the Rotterdam criterion.

2.3 Statistical analysis

The SPSS (version 26) software and Microsoft Office Excel 2010 were used to perform statistical analysis. Descriptive statistics included minimum, maximum, standard deviation and mean. The statistical tools used in this study included chi-square, independent samples *t*-test, and Pearson correlation. The *p*-value of ≤0.05 was set as the level of significance.

RESULTS

The infertile women's Demographic characteristics included in the present research are shown in Table 1. The infertile females' mean age was 32.53 ±7.73 years. In this study, infertile patients were divided into two categories based on age (≥ 35 years versus < 35 years); the mean age in these groups was 41.09 ±3.92 years versus 27.49 ±4.14 years, consecutively ($p < 0.001$).

Comparison body mass index (BMI) means revealed that women ≥ 35 years had a mean BMI that was significantly greater than that of women < 35 years, 31.74 ±5.61 kg/m^2 versus 29.61 ±5.79 kg/m^2, consecutively ($p < 0.001$). Additionally, the proportion of obese women was more significant in women ≥ 35 years compared to women < 35 years, 63.5 % versus 42.1 %, respectively ($p < 0.001$).

The type of infertility and proportions of women with PCOS are shown in Table 2. The proportion of women with PCOS was lower significantly in women ≥ 35 years in comparison with women < 35 years, 18.9 % versus 68.3 %, respectively ($p < 0.001$). The proportion of women with primary infertility was lower significantly in women ≥ 35 years in comparison with women < 35 years, 43.2 % versus 55.6 %, respectively ($p = 0.017$).

There was a mean age of 32.53 ±7.73 years among infertile women. The study divided infertile women into two groups, one for those aged 35 and above and another for those aged less than 35. The average age in the first group was 27.49 ±4.14 years, whereas, in the second group, it was 41.09 ±3.92 years (p < 0.001). The mean body mass index (BMI) of women aged 35 and above was 31.74 ±5.61 kg/m2 compared to 29.61 ±5.79 kg/m2 for women aged less than 35 (p < 0.001). Moreover, the proportion of obese women aged 35 and above was significantly higher than that of women aged less than 35 (63.5 % versus 42.1%, respectively) (p < 0.001).

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Table 1: The infertile women Demographic characteristics included in present research

Characteristic	Total n=400	≥35 years n=148	<35 years n=252	P value
Age (years)				
Mean ±SD	32.53 ±7.73	41.09 ±3.92	27.49 ±4.14	<0.001 I ***
Range	19 -51	35 -51	19 -34	
BMI (kg/m^2)				
Mean ±SD	30.40 ±5.81	31.74 ±5.61	29.61 ±5.79	<0.001 I ***
Range	17.1 -49.1	20.9 -44.4	17.1 -49.1	
Obesity				
Yes, n (%)	200 (50.0%)	94 (63.5%)	106 (42.1%)	<0.001 C ***
No, n (%)	200 (50.0%)	54 (36.5%)	146 (57.9%)	

*BMI: body mass index; SD: standard deviation; I: independent samples t-test; C: chi-square test; ***: significant at p ≤ 0.001*

Table 2: The infertility type in addition to the proportions of women with PCOS

Characteristic	Total n=400	≥ 35 years n=148	< 35 years n=252	p
PCOS				
Yes, n (%)	200 (50.0 %)	28 (18.9 %)	172 (68.3 %)	<0.001 C ***
No, n (%)	200 (50.0 %)	120 (81.1 %)	80 (31.7 %)	
Infertility				
Primary, n (%)	204 (51.0 %)	64 (43.2 %)	140 (55.6 %)	0.017 C *
Secondary, n (%)	196 (49.0 %)	84 (56.8 %)	112 (44.4 %)	

*PCOS: polycystic ovarian syndrome; C: chi-square test; *: significant at p ≤ 0.05; ***: significant at p ≤ 0.001*

Table 3: A comparison of mean serum anti-mullerian hormone (AMH) between young and old infertile women

Characteristic	Total n=400	≥ 35 years n=148	< 35 years n=252	p
AMH (ng/ml)				
Mean ±SD	7.90 ±2.22	6.37 ±2.01	8.79 ±1.82	<0.001 I ***
Range	1.45 -13.34	1.45 -9.73	5.34 -13.34	

AMH: *anti-mullerian hormone;* ***SD****: standard deviation;* ***I****: independent samples t-test; ***: significant at p ≤ 0.001*

Table 4: Comparison of mean serum AMH according to age and BMI

Sub-group	n	Mean AMH (ng/ml)	SD	Minimum	Maximum	p
<35-Non-obese	146	9.02 [a]	1.86	5.35	13.34	<0.001 O ***
≥35-Non-obese	54	6.32 [c]	2.18	1.69	9.69	
<35-Obese	106	8.48 [b]	1.72	5.34	12.2	
≥ 35 -Obese	94	6.39 [c]	1.91	1.45	9.73	

AMH*: anti-mullerian hormone;* ***SD****: standard deviation;* ***O****: one-way ANOVA; ***: significant at p ≤ 0.001; small letters a, b and c are the results of post-hoc LSD test; similar letters indicated no significant difference; different letters indicated no significant difference; the highest mean value was labelled using a letter*

women who have polycystic ovary syndrome. In women aged 35 and above, the percentage of PCOS was significantly lower than in women aged less than 35 (18.9% vs. 68.3%, respectively) (p < 0.001).

A comparison of serum Anti-Mullerian Hormone (AMH) mean between young and old infertile women is shown in Table 3 and Figure 1. Mean serum AMH was lower significantly in women ≥ 35 years in comparison with women < 35 years, 6.37 ±2.01 ng/ml versus 8.79 ±1.82 ng/ml, respectively (p <0.001). The correlation between the age of infertile patients and serum AMH level in all enrolled women was demonstrated in Figure 2. The correlation was significant and negative (p < 0.001; r = - 0.360).

A comparison of serum AMH's mean based on age and BMI is shown in Table 4. The differences in mean AMH were significant (p < 0.001); the highest mean level was reported in the <35 Years-Non-obese groups, followed by < 35 Years-Obese then by both ≥35 Years-Non-obese and ≥ 35years –Obese.

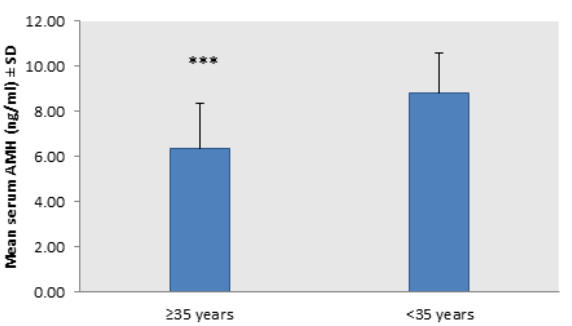

Figure 1: Bar chart showing a comparison of mean serum anti-mullerian hormone (AMH) between young and old infertile women

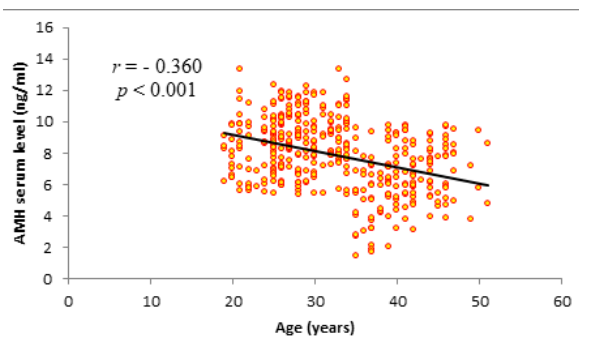

Figure 2: Scatter plot showing the correlation between age of infertile women and serum AMH level

DISCUSSION

Willes et al. found a substantial association between age, BMI, obesity, and AMH, and our results agree with that. Additionally, research carried out by Kloos et al. indicates that the average age of the patients was 35.2 ±5.5 years, the average body mass index was 30.6 ±8.1 kg/m2, and the average amount of AMH was 4.2 ±5.4 ng/mL. In comparison to women without polycystic ovary syndrome (PCOS), those having PCOS exhibited a greater median body mass index (BMI) (35.8 ±9.6 vs 30.2 ±7.8 kg/m2, p = .001), a greater prevalence of obesity (48.8% vs 3.7%, p <.001). In their retrospective investigation of infertile women's ovarian reserve and ART results, Li et al. [26] found that AMH levels were considerably lower in the overweight and obese groups compared to the standard weight group (P <0.001). As body mass index (BMI) increased, AMH levels declined across all age categories. Also, Oldfield et al. [27] in there study noticed either markedly reduced AMH levels in obese individuals relative to non-obese counterparts and/or an inverse correlation

between AMH and BMI. The investigations conducted by Chiofalo et al. [27] and Olszanecka-Glinianowicz et al. [28] demonstrated that AMH levels were 9.7 per cent ($p < 0.0001$) and 23.5 per cent ($p < 0.01$) lower in obese women compared to non-obese women, respectively. In addition, the research conducted by Olszanecka-Glinianowicz et al. demonstrated a negative relationship ($r = -0.30$, $p < 0001$) between AMH levels and BMI. In the context of their interventional trial incorporating surgery to treat obesity, Chiofalo et al. assessed AMH levels. Consequently, the women in their obese group had a mean body mass index (BMI) of 46 kg/m2. Olszanecka-Glinianowicz et al., on the other hand, looked at AMH levels in relation to mostly Class 1 obesity. Taking everything into consideration, the results indicate that AMH may be negatively affected by obesity across the board and that the dose effect is non-linear.

As demonstrated by the current study and previous research worldwide, female fertility is still strongly correlated with age, according to an Assessment of Ovarian Reserve study by Islam et al. [29] As women age, their ovarian pool diminishes and the incidence of secondary infertility increases. Furthermore, a survey by Dragos Albu and Alice Albu, [30] which examined the relationship between BMI and serum AMH levels in subgroups of patients, demonstrated a positive correlation between the two variables in women who were ≤ 35 years old (< 0.05), of average weight ($p < 0.05$), and with normal ovarian reserve ($p < 0.05$) after controlling for age. BMI ≥ 25 kg/m2 was substantially linked to higher AMH readings than normal weight patients, even after adjusting for age.

CONCLUSION

The Anti-Müllerian hormone (AMH) is the most effective endocrine marker for assessing age-related decline of the reserve of the ovary in healthy women. AMH and BMI are favourably connected in infertile patients, particularly in those under 35 who are of average weight and have a normal ovarian reserve. From our study, it can be suggested that variations in serum AMH in young women (<35 years) are correlated significantly with mean BMI. Still, such correlation in older women (>35 years) will be lost because of the marked reduction of AMH by this age. Our findings suggest that the correlation between the age of all enrolled infertile women and serum AMH level was significant and negative.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
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