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Evaluation of Anti-Mullerian Hormone(AMH) Level and Relationship with Secondary infertility in Dhi-Qar in hospitals

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Abstract

Infertility is a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse. Infertility affects millions of people – and has an impact on their families and communities. Estimates suggest that approximately one in every six people of reproductive age worldwide experience infertility in their lifetime . The present study showed that distribution of AMH level in infertile patients across age groups, higher AMH level was observed in age group 18-29 with (Mean ± SD) of (1.99 ± 2.21.20 – 9.35) while lower AMH level was observed in age group 30-39 with (Mean ± SD) of (0.99 ± 1.60 ≤5.07) and lower AMH in age group (40-45) with (1.02 ± 1.385.31 – 5.66) .while the distribution of AMH level in control across age groups higher AMH level was observed in age group 18-29 with (Mean ± SD) of (2.99 ± 1.23) while lower AMH level was observed in age group 30-39 with (Mean ± SD) of (2.05±1.11) and lower AMH in age group (40-45) with (1.85 ±1.01). In conclusion, we evaluated serum AMH levels in Iraqi women and established age-specific AMH reference values based on a large representative sample. In this study, there is a relationship between aging, reproductive health, and the presence of genetic and immunological factors that affect hormone level). We address the application of AMH in clinical practice and the prediction of reproductive capacity and population health.

Keywords: AMH hormone, secondary infertility and Women Age

تقييم مستوى هرمون مضاد مولر (*AMH*) وعلاقته بالعقم الثانوي في مستشفيات محافظة ذي قار

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الخلاصة

العقم هو مرض يصيب الجهاز التناسلي الذكري أو الأنثوي، ويُعرَّف بالفشل في تحقيق الحمل بعد 12 شهرًا أو أكثر من ممارسة الجنس المنتظم دون وقاية. يؤثر العقم على ملايين الأشخاص - ويؤثر على أسرهم ومجتمعاتهم. تشير التقديرات إلى أن واحدًا من كل ستة أشخاص تقريبًا في سن الإنجاب في جميع أنحاء العالم يعانون من العقم في حياتهم. أظهرت الدراسة الحالية توزيع مستوى AMH في المرضى المصابين بالعقم عبر الفئات العمرية، لوحظ ارتفاع مستوى AMH في الفئة العمرية 18-29 مع (متوسط ± انحراف معياري) (1.99 ± 2.21.20 - 9.35) بينما لوحظ انخفاض مستوى AMH في الفئة العمرية 30-39 مع (متوسط ± انحراف معياري) (0.99 ± 1.60 ≥ 5.07) وانخفاض مستوى AMH في الفئة العمرية (40-45) مع (1.02 ± 1.385.31 - 5.66). في حين أن توزيع مستوى AMH في المجموعة السيطرة عبر الفئات العمرية لوحظ ارتفاع مستوى AMH في الفئة العمرية 18-29 مع (متوسط ± انحراف معياري) (2.99 ± 1.23) بينما لوحظ انخفاض مستوى AMH في الفئة العمرية 30-39 مع (متوسط ± انحراف معياري) (2.05±1.11) وانخفاض مستوى AMH في الفئة العمرية (40-45) بواقع (1.85±1.01). وفي الختام قمنا بتقييم مستويات AMH في مصل النساء العراقيات وتأسيس قيم مرجعية AMH خاصة بالعمر بناءً على عينة تمثيلية كبيرة. وفي هذه الدراسة هناك علاقة بين الشيخوخة والصحة الإنجابية ووجود عوامل وراثية ومناعية تؤثر على مستوى الهرمون). ونتناول تطبيق هرمون AMH في الممارسة السريرية والتنبؤ بالقدرة الإنجابية وصحة السكان .

1.Introduction

Infertility is the inability to become pregnant after one year of unprotected and regular sexual [1](1) There are many causes of infertility, including some that medical intervention can treat[2]. Estimates from 1997 suggest that worldwide about five percent of all heterosexual couples have an unresolved problem with infertility. Many more couples, however, experience involuntary childlessness for at least one year: estimates range from 12% to 28%. The main cause of infertility in humans is age, and an advanced maternal age can raise the probability of suffering a spontaneous abortion during pregnancy.Male infertility is responsible for 20–30% of infertility cases, while 20–35% are due to female infertility, and 25–40% are due to combined problems in both parts In 10–20% of cases [3].

Women who are fertile experience a period of fertility before and during ovulation, and are infertile for the rest of the menstrual cycle. Fertility awareness methods are used to discern when these changes occur by tracking changes in cervical mucus or basal body temperature. An important global health issue, infertility affects a couple's social, psychological, economic, and sexual well-being. When a couple is unable to conceive within a year of getting married despite having frequent, unrestricted sex, they are said to be infertile[4] .also , Infertility is a condition of the human reproductive system that is one of the most frequent health concerns all over the world. The inability of a man or woman to produce children, whether due to psychological or biological factors. According to the data, between 10% and 15% of couples between the ages of 18 and 45 are infertile, and as a result of certain changes in lifestyle and environmental changes, the prevalence of infertility has risen rapidly, becoming the third most hazardous disease after cancer and heart disease [5]

Anti-müllerian hormone (AMH) is a member of the transforming growth factor beta family that has derived its name from its role during male sex differentiation by inducing the regression of the müllerian ducts. To date, AMH is best known as a serum marker for ovarian function, with assessment of AMH levels at both ends of the spectrum, that is, ovarian reserve and polycystic ovarian syndrome. In the ovary, AMH is expressed by granulosa cells of growing follicles from the primary up to the small a natural stage. After follicle-stimulating hormone (FSH)-dependent selection, AMH expression disappears, although some expression remains in cumulus cells of pre ovulatory follicles. Also, in atretic follicles and corpora lute a, AMH expression is lost [6].

Recently, hormonal disturbances have been considered of great importance in the knowledge of causes and diagnosis of female infertility . An increase in FSH in women may indicate a reduction in the production of good quality eggs and embryos for fertilization. a woman’s chances for pregnancy may be lower than expected for her age. However, it does not mean she has no chance of conceiving. She may have more difficulty conceiving and may require infertility treatment. [7]. LH is a hormone that is produced in the pituitary gland in both men and women. In women, LH is an important part of the menstrual cycle. It works in conjunction with follicle-stimulating hormone (FSH). The rise in estrogen tells the pituitary gland to stop producing FSH and to start making more LH. The shift to LH causes the egg to be released from the ovary, a process called ovulation .general, higher than normal levels of LH in a woman may mean the ovaries are absent or not functioning. In a young woman, high levels may mean that puberty is early Low levels of LH in the blood may indicate anorexia, an issue in the pituitary gland, stress, or damage to the hypothalamus in both men and women. Mary [8]

To achieve this goal, the present a study evaluating the AMH hormone level in cases of secondary infertility and finding out the reasons for the AMH hormone decrease and detection the relationship between the age and level AMH hormone

2. Study Design

The present study is a case control study that was based on shown in figure (2-1), blood Samples had been collected from patients hospitals in Dhi-Qar Health Department for women who suffer from secondary infertility after diagnosis by repeated visits and Infertile women between the age range of (18 to 45) years who agreed to participate in the study.

About 3 ml of venous blood sample was collected aseptically from the ante-cubital fossa of each participant using sterile syringe and needle, into a vacutainer tube gel activator. The sample was allowed to clot and then centrifuged at 3000rpm for 5 minutes. The separated serum was transferred into pre-labelled serum for examination to conduct tests AMH Hormones . This study was done in agreement with clinical ethical concern of hospitals in Dhi-Qar Health Department and verbal informed consent were obtained from all the participants.

2.1 Inclusion Criteria

Infertile women between the age range of (18 to 45) years who agreed to participate in the study. Infertile women who have no underlying disease condition known to affect FSH, AMH and LH such as cancer, systemic lupus erythematous, pelvic infections, endometriosis and tubal diseases.

2.2 Exclusion Criteria

Patients on contraceptives. Patients who were known to have hysterectomy

3. Materials and Methods

3.1 Serological Kit

The Serological Kits specifications that were used in this study with their companies and countries of origin shown in table 1.

Table 1- The Serological Kits specifications that were used in this study with their companies and countries of origin

NO	Serological Kit	Company	Country
1	*AMH kit*	*A FIAS*	*China*

3.2 Sample collection and processing

The sample type for AFIAS AMH is human serum/plasma. It is recommended to test the sample within 24 hours after collection. The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood. The samples (serum, plasma) may be stored for a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples should be frozen at -20 °C. The samples (serum, plasma) stored frozen at -20 °C for 2 months showed no performance difference. As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

3.3 Statistical analysis

The data were collected, analyzed and presented using statistical package for social sciences (SPSS) and Microsoft Office Excel 2010. used to compare parameters between the total control number and patients based on occupancy at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. The level of significance was considered at P-value of equal or less than 0.05. The level of high significance was considered at P-value of equal or less than 0.01 [9].(T test and one - way a nova) (Prabhaker Mishra et al 2019) .

4. Results

The distribution of the patients and the control subjects according to age in shown in table 2 and figure 1 the number of patient Infertility (18-29) years 4 with lower percentage (%16.6) , (30-39) years 12 with higher percentage (% 50) and 40-45 years 8 with lower percentage (%33.4) ,Also the numbers of the control group that consist of the (8) in each three groups age (18-29),(30-39) and (40-45) .

Table 2- Demographic characteristics of patients Infertility and control group

Characteristic	Control N (24)	Secondary Infertility (N)	frequency N (%)	p
Age (years)				
18-29	*8*	*4 c*	*16.6%*	*HS*
30-39	*8*	*12 a*	*50 %*	*HS*
40-45	*8*	*8 b*	*33.4%*	*HS*
Total	*24*	*24*	*100%*	*(p < 0.05)*

N number of cases; SD: standard deviation; O: one way ANOVA; C: chi-square test; HS: highly significant at p 0.01; NS: not significant at p > 0.05; Capital letters A and B were used to indicate the level of significance following post-hoc LSD multiple comparison test so that similar letters indicate no significant difference (p > 0.05), whereas, different letters indicate significant difference (p < 0.05); letter (A) takes the highest mean value.

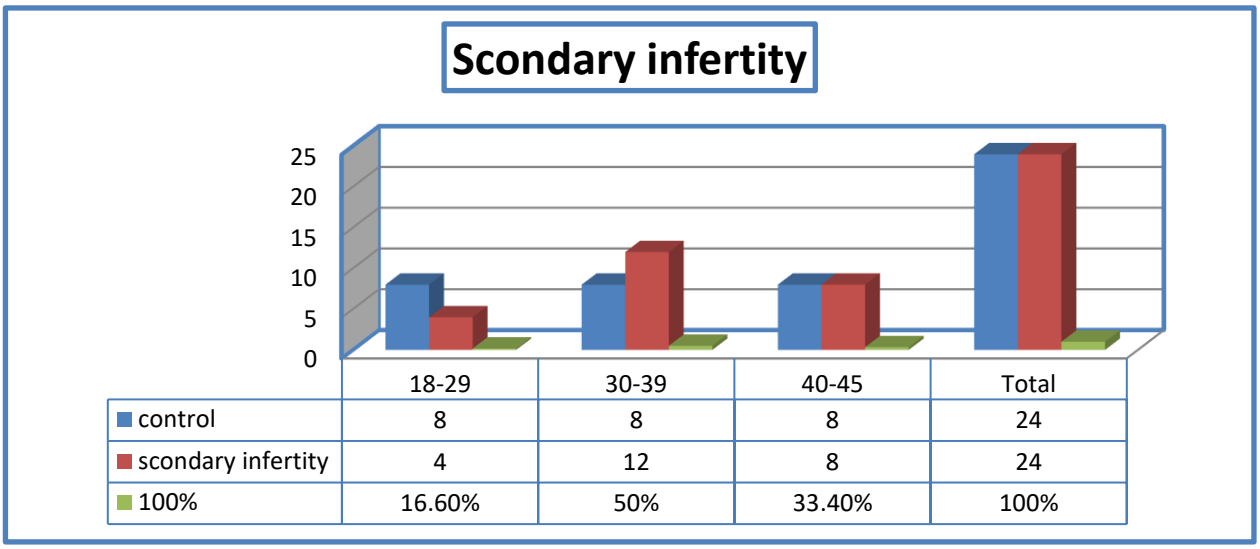

Figure -1 The frequency distribution of patients infertity and control according to age

The distribution of AMH level in infertile patients across age groups, higher AMH level was observed in age group 18-29 with (Mean ± SD) of (1.99 ± 2.21.20 – 9.35) while lower AMH level was observed in age group 30-39 with (Mean ± SD) of (0.99 ± 1.60 ≤5.07) and lower AMH in age group (40-45) with (1.02 ± 1.385.31 – 5.66) Table 3 .

Table 3- Distribution of AMH level in infertile patients across age group

Characteristic Age (years)	Secondary Infertility (N : 24)	AMH (Mean ±SD)
18-29	*4c(16.6%)*	*1.99 ± 2.21.20 – 9.35*
30-39	*12a (50%)*	*0.99 ± 1.60 ≤5.07*
40-45	*8b(33.4%)*	*1.02 ± 1.385.31 – 5.66*
		(P< 0.05)

N number of cases; SD: standard deviation; O: one way ANOVA; C: chi-square test; HS: highly significant at p 0.01; NS: not significant at p > 0.05; Capital letters A and B were used to indicate the level of significance following post-hoc LSD multiple comparison test so that similar letters indicate no significant difference (p > 0.05), whereas, different letters indicate significant difference (p < 0.05); letter (A) takes the highest mean value .

The distribution of AMH level in control across age groups higher AMH level was observed in age group 18-29 with (Mean ± SD) of (2.99 ± 1.23) while lower AMH level was observed in age group 30-39 with (Mean ± SD) of (2.05±1.11) and lower AMH in age group (40-45) with (1.85 ±1.01), table 4.

Table 4- Distribution of AMH level in control across age groups

Characteristic Age (years)	Control (N : 24)	AMH (Mean ±SD)
18-29	*8*	*2.99 ± 1.23*
30-39	*8*	*2.05 ± 1.11*
40-45	*8*	*1.85 ± 1.01*

5. Discussion

Anti-Müllerian hormone (AMH), is a protein hormone generated by granulosa cells in the ovarian follicle that influences female fertility [10] . higher levels indicate a high number of eggs in the body [11] and its highest levels are usually recorded when a woman reaches about 25 years old, and then its levels begin to decline after reaching the age of thirty, and studies have indicated that the AMH hormone can be analyzed on any day of the menstrual cycle .The present study came in agreement with previous studies mentioned the mean distribution of AMH is slightly lower in infertile group than in control group. older women are more susceptible to deficiency AMH due to decreased ovarian production of eggs with age and decreased fertility, especially after the age of 35 due to a decrease in the number and quality of eggs, as an egg is sent from the ovary to the female's uterus every month from the time of puberty to menopause [12].

The current study results showed when comparing the level of AMH hormone among infertile women with different age groups, a positive significant correlation in the level of AMH hormone for the age

groups (18-29), (30-39) and (40-45), as the younger the age, the higher the AMH level. The evaluated the levels of these markers in a group of women without fertility problems and looked for an association between the endocrine profile, ovarian aging, and fecund ability with measurements of FSH, estradiol, AMH, and in hibin B in serum and FSH and estrogen in urine of women. This study included a group of women aged between 30 and 45 years, which had a higher risk of developing ovarian aging. They have found that among all markers tested, only AMH was significantly associated with natural fertility, as measured daily for the probability of pregnancy in women.

All other markers were not significantly associated with female fertility. It was concluded that AMH is a good predictor of age-related reduction of fecund ability in women. Also Serum AMH shows negative correlation with age and a positive correlation with follicle count at ultrasound and to a lesser extent negative correlation with plasma levels of FSH this a green with present study . The decrease AMH in this a study relationship with some factors ,one of them , the natural aging process in women decreases or causes a loss of number and features her oocyte and follicular lagoon. This process is considered a physiological outcome of menstrual cycle non-uniformity and the eventual termination of periodical bleeding [13]. After that, women beyond 35 years old and onward have their attempts to conceive delayed [14] .Relatively older females' granulosa cells produce AMH and inhibin B to a lesser extent than younger females because of decreased oocyte and follicular pool [15].

Multiple studies reported that those women who have infertility concurrently had psychological stress, e.g., anxiety, depression, psychological trauma [16].Additionally, several research reported that poor nutritional status of women promotes infertility. In conclusion, we evaluated serum AMH levels in Iraqi women and established age-specific AMH reference values based on a large representative sample. In this study, there is a relationship between aging, reproductive health, and the presence of genetic and immunological factors that affect hormone level . We address the application of AMH in clinical practice and the prediction of reproductive capacity and population health. In conclusion, we evaluated serum AMH levels in Iraqi women and established age-specific AMH reference values based on a large representative sample. In this study, there is a relationship between aging, reproductive health, and the presence of genetic and immunological factors that affect hormone level).We address the application of AMH in clinical practice and the prediction of reproductive capacity and population health.

6. Conclusions

The present a study indicated that AMH, which is considered to be of high predictive value in evaluating ovarian , AMH is considered an important hormone in the egg process. It is the hormone that gives the reserve percentage of eggs inside the ovary, and through it we can determine the number of remaining eggs through the percentage and numbers. On the other hand, by measuring the level of the hormone AMH , we can determine the type of treatment, strategies, or techniques that help in treating Secondary infertility.

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