ESTIMATION FOLLICULAR FLUID CONTENTS AND SERUM IN AGEING IRAQI WOMEN-DURING IVF/ICSI CYCLES

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ABSTRACT

This study was aimed to estimate the effects of women age on oocytes quantity and quality and to determine whether there is a correlation between serum and follicular hormone levels. The samples were collected in the higher institute for the diagnosis of infertility and assisted reproduction techniques, Baghdad, Iraq. The 60 participants were split into two age groups, G1involved women younger than 35, while G2 involved women older than 35 years of age. The levels of FSH, LH, AMH, and GH in the serum and follicular fluid (FF) could be measured using (ELISA). The results demonstrated differences in the serum levels of FSH, LH, AMH, and GH between the young and elderly groups of infertile women on the day of oocyte extraction. The results proved statistically highly significant (P≤0.01) drop in the mean levels of hormones in the aged women group (FSH 21.67±0.53; LH 62.12±0.54; AMH 1370.74±94.59; GH 9.76±0.32 mIU/ML) compared with the younger women group (FSH 33.86±0.72; LH 85.072±2.70; AMH 3310.92±83.87; GH 30.40±0.45 mIU/ML). in this study the level of studied hormones in FF were significantly decreased with increasing women age, the mean of (FSH 19.59 ±0.28; LH 63.34 ±0.27; AMH 1769.46 ±17.92; GH 7.86 ±027 mIU/ML) in comparison with those levels in the younger group (FSH 31.40 ±0.95; LH 95.07 ±2.58; AMH 2547.35 ±124.09; GH 23.33 ±0.67 mIU/ML).

Keywords: infertility, age, reproductive hormones, growth hormone, follicular fluid.
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مجلة العلوم الزراعية العراقية- 2024:55(2):868-874 طيبة وصالح

تقدير محتويات السائل الجريبي والمصل عند النساء العراقيات المتقدمات في السن– خلال دورات التلقيح الاصطناعي/الحقن المجهري

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المستخلص

تهدف الدراسة إلى تقدير تأثير عمر المرأة على نوعية واعداد البويضات في النساء العراقيات العقيمات ، وإثبات ما إذا كانت هناك علاقة بين مستوى هرمونات السائل الجريبي والمصل. من خلال مسح أجري في المعهد العالي لتقنيات الإنجاب المساعدة على تشخيص العقم، تم اختيار عينة من النساء المصابات بالعقم لإجراء هذا التحقيق في بغداد، العراق، في الفترة من أيلول 2022 لغاية شباط 2023. ومن المقرر أن تبدأ النساء المصابات بالعقم البالغ عددهن 60 امرأة اللاتي سجلن في هذه التجربة دورات IVF/ICSI الخاصة بهم. تم تقسيم المشاركين في الدراسة الستين الذين يعانون من العقم إلى فئتين عمريتين، G1 وG2. يتم تمثيل النساء الأصغر سنًا تحت 35 عامًا بواسطة G1، ويتم تمثيل النساء الأكبر سناً فوق 35 عامًا بواسطة G2. لقياس هرمونات FSH وLH وAMH وGH. تم استخدام تقنية ELISA، لتقييم (FSH، LH، AMH، و GH) في المصل و السائل الجريبي. اظهرت النتائج بشكل عالي المعنوية مستويات اقل من الهرمونات في مصل النساء المتقدمات في العمر (FSH21.67±0.53 وحدة عالمية/ مل)، LH (62.12±0.54 وحدة عالمية/ مل)، AMH(1370.74±94.59وحدة عالمية/ مل)، GH(9.76±0.32 وحدة عالمية/ مل) مقارنة مع الفئة الاصغر عمرا FSH (33.86±0.72 وحدة عالمية/ مل)، LH(85.072±2.70 وحدة عالمية/ مل)، AMH(3310.92±83.87 وحدة عالمية/ مل)، GH (30.40±0.45 وحدة عالمية/ مل)على التوالي. وكذلك اظهرت النتائج بشكل عالي المعنوية مستويات اقل من الهرمونات في السائل الجريبي للنساء المتقدمات بالعمر FSH(19.59±0.28وحدة عالمية/ مل)، LH(63.34±0.27 وحدة عالمية/ مل) ،AMH(1769.46±17.92)، و GH(7.86±0.27 وحدة عالمية/ مل) مقارنة مع الفئة الاصغر عمرا (31.40±0.95 وحدة عالمية/ مل)، (95.07±2.58 وحدة عالمية/ مل)، (2547.35±124.09 وحدة عالمية/ مل) و(23.33±0.67 وحدة عالمية/ مل) بالتتابع.

الكلمات المفتاحية: العقم، العمر، الهرمونات التكاثرية، هرمون النمو، السائل الجريبي.
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INTRODUCTION

Infertility is defined as the inability to conceive within six months for women over 35 and the inability to become pregnant within twelve months for sexually women under this age the condition could be a significant emotional impact on the life of the individual afflicted (21). Infertility is a persistent challenge that impacts approximately 10 to 12% of couples globally (16). Globally, 48.5 million (15–20%) of couples who are of reproductive age are infertile, according to the World Health Organization (14). It is one of the most important social issues impacting developed nations, having an impact on families, communities, and both. It has an impact on more than 186 million people worldwide (3). Female infertility accounts for about one-third of infertility issues, while male infertility accounts for another third. In the other instances, both partners have infertility; however, it's unknown causes (2). The chance of being infertile rises gradually as a woman ages (1). Reproductive hormones are essential to sustain pregnancy, create gametes, and regulate the menstrual cycle (11). The primary regulators of puberty and fertility are hypothalamic neurons that release the gonadotropin-releasing hormone (GnRH). The gonadotropic cells in the hypothalamus release luteinizing hormone (LH) and follicle stimulating hormone (FSH) into the bloodstream. The synthesis and release of progesterone (P4) and 17-estradiol (E2) are subsequently induced by these hormones in the ovaries and testes (17). Age has an impact on all of these hormones in women (6). It is well-known that during adenohypophysis, cells secrete growth hormone (GH), which is composed of 191 amino acids. Follicle growth, steroidogenesis, oocyte maturation, ovulation, corpus luteum (CL) function, oocyte quality, and ovarian responsiveness to exogenous hormone treatment are among the numerous processes that are impacted by GH adjuvant therapy (19). This study was aimed to estimate the effects of women age on oocytes quantity and quality and to determine whether there is a correlation between serum and follicular hormone levels.

MATERIALS AND METHODS

A sample of infertile women was selected for this Investigation conducted at the High Institute for Diagnosis and Assisted Reproductive Technologies, Baghdad, Iraq, from October 2022 until February 2023. The sixty infertile women who joined this study were set to begin their IVF/ICSI cycles. Information was collected by means of questionnaire. The women's age ranged from 20 to 45 years. Every couple had the standard reproductive examinations conducted by the fertility center, which comprise a physical examination, ovulation detection, evaluation of the uterine cavity and tubal patency, and semen analysis. Based on their chronological ages, the participants divided into two age groups, namely G1 and G2. G1 represents women younger than 35, while G2 represents women older than 35years of age. Individuals with endometriosis, PCOs, and long-term metabolic disorders did not eligible. Every participant provided a comprehensive medical history document that included information on menstrual history, biographical background, and state of infertility. Each patient willingly provided written consent after learning about the trial. The Biology Department, College of Science, University of Baghdad's research ethics committee and science committee both authorized the study's design (Ref. NO: CSEC/0922/0081). Using a disposable syringe (Becton Dickinson, USA), five milliliters of blood were drawn from each infertile woman's median cubital vein. These samples were utilized to determine the hormone levels of GH, AMH, LH, and FSH. The blood samples were placed in a plain or gel tube, allowed to clot for thirty minutes, and then centrifuged for 10 minutes at 3000 rpm to separate the serum. Using a sterile micropipette, 1.5 ml of serum aliquots was transferred into sterile Eppendorf tubes. which were stored at -20 °C in a refrigerator until analysis. Follicular fluid was extracted from the first follicle that was aspirated from each patient. In order to minimize the collection of samples contaminated with blood or media, a midstream aspirate was obtained from every patient. After centrifuging FF samples for ten minutes at 3000 rpm, the supernatant was removed and stored for further analysis at -20

°C or lower. Based on the biotin double antibody sandwich technique, the ELISA was used to assess the levels of FSH, LH, AMH, and GH in serum and FF samples. The Statistical Analysis was conducted SAS (2018) program and mean compared using t-test (P<0.01). The correlation between two group variants was investigated using the correlation coefficient ® (20).

RESULTS AND DISCUSSION

Table 1. Comparison between young and aged women groups based on the levels of serum hormones

	Mean ± SE			
Age group	**FSH (mIU/ML)**	**LH (mIU/ML)**	**AMH (mIU/ML)**	**GH (mIU/ML)**
Young (<35 yr.)	**33.86 ±0.72**	**85.07 ±2.70**	**3310.92 ±83.87**	**30.40 ±0.45**
Aged (>35 yr.)	**21.67 ±0.53**	**62.12 ±0.54**	**1370.74 ±94.59**	**9.76 ±0.32**
T-test	**1.798 ****	**5.521 ****	**253.08 ****	**1.103 ****
P-value	**0.0001**	**0.0001**	**0.0001**	**0.0001**

**** (P≤0.01).**

Table 1, shows the results of the hormone level of FSH, LH, AMH, and GH in the serum samples on oocyte day retrieval, in the infertile women between young and age groups. The results revealed highly significant (P≤0.01) decreases in the levels of all tested hormones in aged women group (FSH 21.67±0.53; LH 62.12±0.54; AMH 1370.74±94.59; GH 9.76±0.32 mIU/ML) compared with those levels in the younger women group (FSH 33.86±0.72; LH 85.072±2.70; AMH 3310.92±83.87; GH 30.40±0.45 mIU/ML). Tehraninezhad, *et al.,* (24). After the age of forty, a woman's natural ability to conceive reduced due to diminished ovarian reserve It was obtained that as female age, their FSH levels increase because their ovaries become less receptive to stimulation, resulting in the characteristic gross elevation of FSH and LH that characterizes ovarian aging. This result did not agree with our results of this study (26). Additionally, as women aged in the current study, their AMH levels decreased; this finding is consistent with that of an earlier study that demonstrated a decrease in AMH levels with advancing female age (22). This study finding also reveal that GH levels dramatically decrease as a function of age. The process of aging is linked to several modifications in the endocrine system, including the age-related decreases in hormone levels in the blood, such as GH and estradiol 17 beta (5). GH replacement therapy can successfully induce unassisted pregnancies in previously infertile women with GH deficit, and GH deficient women have lower fertility, indicating that GH is an essential component of high fertility in women (27). This study carried out according to sample size after stimulation protocol, so the results could be change if sample change.

Table 2. Comparison between young and aged groups in terms of hormone levels in the follicular fluid

	Mean ± SE			
Age group	**FSH (mIU/ML)**	**LH (mIU/ML)**	**AMH (mIU/ML)**	**GH (mIU/ML)**
Young (<35 yr.)	**31.40 ±0.95**	**95.07 ±2.58**	**2547.35 ±124.09**	**23.33 ±0.67**
Aged (>35 yr.)	**19.59 ±0.28**	**63.34 ±0.27**	**1769.46 ±17.92**	**7.86 ±027**
T-test	**1.985 ****	**5.211 ****	**250.97 ****	**1.466 ****
P-value	**0.0001**	**0.0001**	**0.0001**	**0.0001**

**** (P≤0.01).**

This current study was found that as women age, the levels of the tested hormones in the FF dramatically dropp. This is evident by comparing the mean values of FSH (19.59 ±0.28), LH (63.34 ±0.27), AMH (1769.46 ±17.92) and GH (7.86 ±027 mIU/ML) in the aged women group with those levels in the young age group (31.40 ±0.95, 95.07 ±2.58, 2547.35 ±124.09 and 23.33 ±0.67, respectively) respectively. Table (2) shows a drop in the hormone profile. A number of proteins that are essential to oocyte maturation and follicle growth make up the FF, which was formed during the process of folliculogenesis. The general consensus was that age-related infertility could be addressed with assisted reproductive technology. Numerous studies interpreted the presence of gonadotropins and steroids in the FF as markers of oocyte maturation. The intricated and ever-changing

hormonal composition of follicular fluid undergoes significant changes during follicle growth and in a woman's lifetime based on her age, health, and fertility (13). this research shows that the concentrations of the hormones FSH, LH, and AMH decrease as age increases during the IVF/ICSI cycles of the participated women. However, it was established by several studies that lower AMH and higher FSH levels are linked to lower oocyte quality in FF from older women (9). A previous study shows that during the reproductive window, the levels of LH in the follicular fluid are unexpectedly steady. Numerous studies revealed non variation in the amounts of LH follicular fluid between young and older women (10). Age-related changes in FF AMH levels in follicular fluid also indicated a higher likelihood of clinical pregnancy in older women (12). The current findings reveal that when women's age increase, FF GH concentration decrease, and eventually achieved a reduction in GH concentration states that compared to younger women, women over 40 have lower GH concentrations in their follicular fluid (25). Good shape of cleaving embryo, rapid cleavage, and outstanding potential for embryo implantation have all been linked to elevated GH concentrations in follicular fluid. In addition, GH improved the implantation rate and blastocyst availability for older IVF patients. GH showed the greatest stable correlation with different embryo quality measures (18).

Table 3. Correlation coefficient values among the levels of hormones in the serum and the follicular fluid of participants

Serum	FF	Correlation coefficient-r
FSH	FSH	0.75 **
LH	LH	0.57 **
AMH	AMH	0.67 **
GH	GH	0.92 **

** (P≤0.01).

The results of this investigation also demonstrate a statistically significant positive correlation between the levels of GH, AMH, FSH, and LH in the serum and those in the FF of infertile women (r= 0.75, 0.57, 0.67, 0.92) respectively, (Table 3). According to this study, there was perfect positive correlation between levels of hormones in serum and FF. This result is in agreement with that of Ciepiela *et al*., (8), they believe there was on the day of oocyte retrieval, there was a moderate positive correlation between FF AMH and serum AMH and a high positive correlation between FF FSH and serum FSH. Studies showed a positive correlation between serum and follicular-fluid AMH concentrations imply that individual AMH generation capability is a factor that influences peripheral AMH concentrations in addition to follicle count (7). According to a different study, follicular-fluid AMH is a significant predictor of oocyte quality and, in turn, of the success of reproduction. Moreover, follicular-fluid concentrations of AMH are higher in patients with higher rates of embryo fertilization, suggesting that this chemical could be crucial for oocyte growth and fertilization. This study was indicating that follicles with a diameter of 5–8 mm, which produced approximately 60% of serum AMH, are the main factor influencing the circulating concentration of AMH. Additionally, there was a positive association found between serum and FF AMH (15). The results of this study indicated that there is a high positive correlation between the level of the mentioned reproductive hormones and growth hormone in both serum and follicular fluid, the results were relied upon exclusively due to the lack of sufficient references that match the current study.

Table 4. Distribution of study samples according to the number of retrieved oocytes in the two age groups.

Number of retrieved Oocyte	Young (<35 yr.) (No=30)	Aged (>35 yr.) (No= 30)	P-value
≤10	15 (50.00%)	29 (96.67%)	0.0348 *
11-20	15 (50.00%)	1 (3.33%)	0.0005 **
P-value	1.00 NS	0.0001 **	---

* (P≤0.05), ** (P≤0.01) , NS: Non-Significant.

Furthermore, we demonstrate significant differences in the number of retrieved oocytes between the two age groups, since the percentage of younger women with of the oocyte number lower than 10 was (50.00%), whereas the percentage was (96.67%) in the older women. Also, highly significant differences were also found between the two age groups in terms of percentage of women with oocyte number higher than 10, which was (50.00%) in the younger group and (3.33%), in the elder one. In addition, numbers of oocytes showed non-significant differences within the younger group, whereas statistically significant differences (P< 0.001) were observed within the older group, (Table 4). The decline in the amount and quality of oocytes is most likely the cause of the age-related decline in female fertility, females whom their age more than 35 years produced significantly lower oocytes number with a lower fertilized oocyte When compared to females under 35 years of age. The maternal age is one of the most important predictor of ICSI outcomes, this results agreed with Zahir, *et al.*, (28). since, they prove advanced females age has a negative impact on ICSI outcomes and females older than 35 years usually produced lower number of oocytes during oocytes' retrieval with a lower fertilization rate. Embryos produces from those females usually of bad quality and exhibited a lower implantation potential. Several study showed there was a strong association between the numbers of oocytes retrieved and the clinical miscarriage rate where the possibility of success is largely determined by ovarian response, numbers of retrieved oocyte, and numbers of good-quality embryos when infertile women carried out the cycles of IVF-ET/ICSI (23). The two most prominent indicators of cumulative live birth rate are the age of the female and the number of oocytes (4).

CONCLUSSION

The results of the current study demonstrated that a women aged, their levels of growth hormone and reproductive hormones (FSH, LH, and AMH) decrease in both serum and follicular fluid. Therefore, women age affects quantity and quality of oocytes. Also, there was a perfect positive association between the hormones evels in serum and follicular fluid.

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