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Investigate the Correlation of Growth Hormone with the Level of Some Reproductive Hormones and Certain Parameters in a Number of Iraqi-Aging and Younger Women During IVF/ICSI Cycles.

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Abstract

An estimated 12–20% of couples struggle with infertility, It is described as not becoming pregnant after a year of trying. About one-third of these couples are infertile because of female factors, and another third are infertile because of male factors. A combination of the two may be experienced by couples in the remaining one-third. Its distributed into two groups, primary and secondary. A total of 60 participants, 30 of whom were older women (>35) and 30 of whom were younger women (<35). In this study the results were showed statistically highly significant positive correlation-r between GH and both serum and follicular fluid (FF) FSH (0.84, 0.81 mIU/ML), LH (0.76, 0.74 mIU/ML) and AMH (0.89, 0.86 mIU/ML), and there was highly significant positive correlation between GH and number of retrieval Oocyte (0.47, 0.46 mIU/ML), negatively significant correlation was found between GH and number of abortion (-0.10, -0.14 mIU/ML). Notably, the vast majority of the assessments GH hormone levels in serum and follicular fluid revealed a statistically significant positive correlation with FSH, LH, AMH, the frequency of miscarriages, the retrieval of oocytes and infertility duration. These findings imply a strong positive correlation between GH and female fertility. As a result, much research is required to assess, estimate the importance of GH levels on women's fertility, and to get an early hormone level assessment before beginning a GH-containing therapy program.

1. Introduction:

Infertility is a failure to conceive after 12 months of trying affects approximately 12-20% of couples. Approximately one-third of these couples are infertile because of female causes, another third are infertile because of male factors, and the other one-third of couples may be infertile due to a mix of the two [1]. Infertility among the younger generation is ranked by the World Health Organization (WHO) as the fifth most severe handicap worldwide. The Maternal Health Task Force (2010) estimates that 50 million couples worldwide are infertile [2].

Infertility can be caused by both male and female factors, or it can be classified as unexplained when no cause can be determined during baseline infertility studies [3]. A multitude of factors, such as social pressure, delayed marriage and motherhood, have contributed to the sharp rise in female infertility. Conventional treatments, such as hormone therapy, in vitro fertilization (IVF), and embryo transfer, have serious side effects and poor obstetric outcomes [4]. Primary and secondary infertility are the two categories of infertility, women who have never previously become pregnant are considered

primary infertile. When a conception happens at least once but does not repeat, it is referred to as secondary infertility [5]. There are several factors can affect female fertility including, environmental factors, life style, smoking, alcohol drinking, weight factors, stress and emotional status [6]. Ovarian hormonal factors, age factors [7]. Women's age is a very important factor that affects fertility, female fertility peaks between the ages of 18 and 24, while it starts to decline after age 27 and declines more significantly after age 35 so, the women's ability to become pregnant declines gradually with decreasing fertility beyond age 35, this declines yearly until menopause [8].

Aging of the ovaries starts significantly earlier than normal aging, a notable decline in the quantity and quality of oocytes is the most visible marker of ovarian age [9]. Anovulatory cycle and female infertility result from abnormalities in axis' chemical communication, which is regulated by the gonadotropin-releasing hormone secreted from the hypothalamus and pituitary gland hormones, which in turn regulate the release of most body hormones either directly or indirectly [10].

In addition to working together to regulate animal reproduction under physiological conditions, luteinizing hormone and follicle stimulating hormone have a particular biological effect on the hypothalamus, pituitary, ovary, testis, and other target tissues. They can also regulate the synthesis of steroid hormones, cell metabolism and growth, and other physiological activities [11].

Anti-Müllerian hormone (AMH) the homodimeric glycoprotein, also referred to as Müllerian Inhibiting Substance (MIS), is a member of the TGF-superfamily [12]. It is released by the ovarian granulosa cells of the pre-antral and antral follicles in women starting in the 36th week of pregnancy [13]. The anterior pituitary secretes the 191-amino acid.

Protein known as human growth hormone (hGH). Growth hormone-releasing hormone (GHRH) and somatostatin alternately secrete in a pulsatile, rhythmic pattern, primarily every two hours. Pulsatile release is produced by the anterior pituitary's somatotroph cells [14].

Furthermore, it triggers tissue-specific catabolic and anabolic processes, suppresses the action of insulin (diabetogenic impact), and raises the expression of insulin growth factor-1 (IGF-1) in the liver and other target tissues [15]. Growth hormone is suppressed by the peripheral target hormone IGF-1 through negative feedback and regulation of paracrine growth hormone receptor trafficking [16]. Aim of the current study was to estimate the effects of growth hormone in serum and follicular fluid on some reproductive hormones like (FSH, LH, and AMH), the amount of oocytes harvested, and the incidence of abortion during IVF/ICSI cycles.

2. Methodology:

1. The enzyme-linked immunosorbent assay (ELISA), which is based on the biotin double antibody sandwich technique, was used to manually examine each value in order to assess FSH, LH, AMH, and GH. By used Human HS Reader device.

2. A sample of infertile women was chosen for this study through a survey at the High Institute for Infertility Diagnosis Assisted Reproductive Technologies. From October 2022 to February 2023 in Baghdad, Iraq.

3. The 60 infertile women who signed up for this trial are about to start. The age range of the women was 20 to 45.

4. The study's 60 infertile participants were distributed into two age groups, G1 and G2, according on their chronological ages. G1 represents younger women under 35, and G2 represents older women over 35. Patients with PCOs, endometriosis, and chronic metabolic diseases are disqualified.

5. Each infertile woman had five milliliters of blood drawn from the median cubital vein using a disposable syringe made by the American business Becton Dickinson in order to assess the hormones FSH, LH, AMH, and GH. The blood samples were put into a plain or gel tube and allowed to coagulate for 30 minutes before the serum was separated by centrifuging them at 3000 rpm for 10 minutes. Using a sterile micropipette, 1.5 ml of the serum sample was transferred into sterile Eppendorf tubes in preparation for the subsequent test. After that, these tubes were stored at -20 °C in a refrigerator until analysis.

6. Follicular fluid was extracted from the first follicle that was aspirated from each patient. A midstream aspirate was taken from each patient to reduce the acquisition of blood- or media-contaminated samples. FF samples were centrifuged for 10 minutes at 3000 rpm, and the supernatant was collected and kept in storage at or below -20 degrees Celsius for later analysis [17].

7. 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).

8. The Statistical Analysis System (2018). SAS Institute Inc. Cary, North Carolina, USA, Version 9.6th ed. A program was utilized to determine how the patient and control groups' effects on the study's parameters. The information was presented as mean SE. A P- value of

($P<0.01$)* or higher was considered significant for statistical comparisons between groups when using the t-test, and in this study, the correlation between two group variants was examined using the correlation coefficient-r. [18].

3. Results and Discussion:

Total number of 60 infertile women (Table 1) were selected in this cross sectional comparative study were diagnosed, patients with unexplained cause was (26.67%) in women age under 35 yr. while it was (23.33%) in women age older 35 yr. there was Non-significant differences between two age groups, patients with male factor was (60.00%) in women age under 35 yr. while it was (26.67%) in women age older 35 yr. there was significant differences between two age groups, patient with female factor was (6.67%) in women age under 35 while it was (40.00%) in women age older 35 yr. there was highly significant differences between two age groups, patient with mixed factor (6.67%) in women age under 35 yr. while it was (10.00%) in women age older 35 yr. there was Non-significant differences between two age groups. This distribution was highly significant statistically($P\leq0.01$) in the same group.

In 50% of infertility cases, the male component is the key cause [19]. And up to 2% of males will have sperm parameters that are below optimum. Low sperm concentration, slow sperm movement, or aberrant morphology could all be contributing factors, It is well established that variables affecting male infertility lower generation of sperms with normal morphology and progressive motility [20]. Male fertility peaks at roughly 35 years old and dramatically drops after age 45, making the age-related decline in fecundity for men more contentious. Advanced male age has been linked to significantly lower pregnancy rates and longer pregnancy wait times [21]. One of the most significant elements that affects human fertility is female aging, female fertility declines gradually and steadily in women between the ages of 30 and 35, it accelerates after that age [22]. Reduced oocyte quantity and quality are directly related to the age-related decline in female fertility [23]. Primordial follicles, which range from 500,000 to 1,000,000 at birth, continue to decline as a woman ages, mostly due to atresia and apoptosis [24]. The current study indicated that the age of women had no significant impact, which was contrary to what had been discovered in earlier research.

Unexplained infertility is more likely to manifest in women over 35 than younger women [25]. Male and female partners acting alone are responsible for 20–30% of incidents, although they are also responsible for 50–60% of cases overall [26]. We must have mentioned that the distribution was done according to the sample volume of this study, so it may differ when the other samples may change.

Table 2, summarize the results about duration of infertility according to women age groups.

In this study the duration of infertility less than 10 years was (73.33%) in the women group younger than 35 years and it was (33.33%) in women group older than 35 years, there was significant differences between two age groups.

While the duration more than 10 years was (26.67%) in women group younger than 35 years and it was (66.67%) in women group older than 35 years, also there was significant differences between two age groups. In the same time the results showed a highly significant differences ($P< 0.001$) in the two durations of infertility in the young age group.

Less attention has been paid to the duration of infertility, which is correlated to the severity of infertility. Longer-term infertile women are more likely to be older and have fewer pregnancies as they get older Women who had been infertile for more than four to eight years had a lower success rate with IVF [27].

Table 3, summarized the results of types of infertility according to women age groups. In this study the primary infertility was (90%) in women group younger than 35years and it was (70%) in women group older than 35years, there was non-significant differences between two age groups in the primary infertility. While the secondary infertility was higher percentage in the aged group (30.00%) compared with young group (10.00%) with non-significant differences between them. But there were statistically highly significant differences ($P\leq0.01$) between primary and secondary type of infertility in younger women group, but only significant differences ($P\leq0.05$) in older women between the two types of infertility were studied group.

About two percent of infertile women have primary infertility [28]. Primary infertility among women was more common than secondary infertility [29].

Table 4, summarize the results of type of infertility treatment according women age groups. In the present study and according to the sample volume the results showed that the IVF programmes were more used in the aged group (60.00%) compared with young group, while the ICSI programmes were more common choice in the younger women (38.33%) than aged women (40.00%). With highly significant differences ($P< 0.001$) between all groups mentioned above. And there were statistically highly significant differences between two cycle in the younger women group, but there were non-significant differences in the older women group.

The introduction of ICSI a few years after the first use of conventional IVF has expanded the use of ART [30]. Although it was created to treat male infertility because the pregnancy rate of IVF is still under 40%, ICSI is now more

Table 1. Distribution of sample study according to Cause of Infertility with difference age groups.

Cause of Infertility	Young (<35 yr.) (No=30)	Aged (>35 yr.) (No= 30)	P-value
Unexplained	8 (26.67%)	7 (23.33%)	0.796 NS
Male factor	18 (60.00%)	8 (26.67%)	0.0499*
Female factor	2 (6.67%)	12 (40.00%)	0.0075**
Mixed	2 (6.67%)	3 (10.00%)	0.654 NS
P-value	0.0001**	0.0001**	—
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.			

Table 2. Distribution of sample study according to the duration of infertility with different age groups.

Duration of infertility	Young (<35 yr.) (No=30)	Aged (>35 yr.) (No= 30)	P-value
10 yr.	22 (73.33%)	10 (33.33%)	0.0339*
11-20 yr.	8 (26.67%)	20 (66.67%)	0.0233*
P-value	0.0001**	0.0679 NS	—
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.			

frequently utilized for non-male factor reasons. For infertile couples, the development of ART was encouraging, IVF success, however, is still less than 100% and unsatisfactory [31]. So, according to the present results, the younger women may be having a big chance to choose the ICSI protocol or may be a decision of the doctor according to the health state or some clinical status or may to ensure the success the fertilization with less attempts.

Table 5, summarize the results of GH correlations with other FSH, LH,AMH, numbers of abortion and retrieval oocytes number in serum and follicular fluid during IVF/ICSI cycles. In this study the correlation-r was statistically highly significant positive between GH and both FF and serum FSH (0.88, 0.81), LH (0.76, 0.74) and AMH (0.89, 0.86), and there was highly significant positive correlation between GH and number of retrieval Oocyte (0.47, 0.46), non-significant correlation was found between GH and number of abortion (-0.10, -0.14).

Our research demonstrates that GH levels affect female fertility since they were synergistically correlated with gonadotropin levels, AMH levels, number of retrieved oocytes, and duration of infertility, as indicated in Table (5) above.

The current results showed that there was a highly significant but less significant link with LH and a perfect highly significant correlation between serum and FF GH and FSH. Additionally, there was a highly significant association between AMH and the quantity of recovered oocytes; however, there was no significant difference in the number of abortions, and there was a negative correlation with the length of infertility. This result, which is in line with other research, implies that the GH might be essential for human reproduction. GH was first provided to individuals whose ovarian response to conventional ovarian stimulation regimens for IVF had been subpar thirty years ago [32], [33]. GH and gonadotropic cycles are closely associated throughout life, starting with the regulation of the onset of puberty. Research indicates that GH and IGF1 stimulate the hypothalamo-gonadotropic cycles on all levels. Gonadotropins are secreted as a result of GH. Furthermore, GH has direct and IGF1-mediated effects on the ovary, influencing oocyte development and granulosa cell production of estradiol.

The positive effects of GH supplementation on ovarian stimulation in women with GH deficit (GHD) have not been extensively studied [34]. It was shown through IVF that GH enhanced the activity of low dosages of FSH, hence boosting the content of LH receptors. It reduced the quantity of gonadotropin required to induce ovulation and enhanced its effects on the ovary. The idea that GH was a co-gonadotropin that worked with FSH and LH to stimulate ovarian activity was supported by the substantial evidence showing that it might affect ovarian function [35]. By encouraging the development of IGF1, growth hormone (GH) may improve conception success in individuals over 35. A number of studies provide a unique treatment approach for infertile patients who need individualized care and show different ways for younger and older patients to use GH in ART [36].

According to certain studies, GH modifies the ovarian steroidogenic response to gonadotropins in people undergoing in vitro fertilization (IVF), which may contribute to the regulation of human ovarian function [37]. Studies have shown that GH promotes follicular development, stimulates follicular maturation, regulates ovarian function, and increases the ovary's sensitivity to FSH. It also raises the proliferation of

Table 3. Distribution of sample study according to Type of Infertility with different age groups.

Type of Infertility	Young (<35 yr.) (No=30)	Aged (>35 yr.) (No= 30)	P-value
Primary	27 (90.00%)	21 (70.00%)	0.386 NS
Secondary	3 (10.00%)	9 (30.00%)	0.0833 NS
P-value	0.0001**	0.0285 NS	—
* (P≤0.05), ** (P≤0.01), NS: Non-Significant.			

Table 4. Type of Treatment with different age groups.

Type of Treatment	Young (<35 yr.) (No=30)	Aged (>35 yr.) (No= 30)	P-value
IVF	5 (16.67%)	18 (60.00%)	0.0001**
ICSI	25 (83.33%)	12 (40.00%)	0.0001**
P-value	0.0001**	0.273 NS	—
**(P0.01), NS: Non-Significant.			

Table 5. Type of Treatment with different age groups.

	Correlation coefficient –r with GH	
Parameters	Serum	FF
FSH	0.84**	0.81**
LH	0.76**	0.74**
AMH	0.89**	0.86**
No of Oocyte	0.47**	0.46**
Duration of infertility	-0.48**	-0.51**
**(P0.01). NS: non-significant		

the granulosa and thecal cells. In addition, GH may raise the number of retrieved oocytes, the live birth rate, and the clinical pregnancy rate for individuals having IVF or ICSI who have impaired ovarian function.

Furthermore, GH decreased the rate of cancelled cycles and the dosage of gonadotropin in women with poor ovarian response [38], [39]. GH has been demonstrated in numerous studies to enhance endometrial quality, raise oocyte quality by lowering oxidative stress, boost ovarian responsiveness by activating the IGF system, and encourage GH receptor expression in granulosa cells [40].

Furthermore, when GH levels rose, there was a discernible rise in the quantity of retrieved oocytes [32]. The age-related decline in growth hormone levels may be the cause of women with advanced maternal age having insufficient reproductive capability. By lowering apoptosis, GH treatment is a useful tactic to reverse the aging-related reduction in ovarian reserve and the deterioration in oocyte quality [41]. Studies conducted in vivo suggest that GH could improve nuclear maturation and, in turn, the quality of oocytes, as gonadotropin does not seem to be necessary for the activation of primordial follicles or their development into late preantral follicles; rather, a range of locally active growth factors are involved. Additionally, GH expedites the retrieval and fertilization of human oocytes [42].

In IVF/ICSI, high growth hormone (GH) levels during ovarian stimulation have been demonstrated to improve oocyte quality, pregnancy rate, and live birth outcomes [43]. GH could encourage more live births, particularly in younger women. Furthermore, it seems that embryos created with a sufficient amount of GH have a better chance of implanting [44]. It has been shown that GH raises the chance of implantation and live birth in women over 40 who have had in vitro fertilization (IVF). According to additional research, GH can also increase uterine receptivity for embryo implantation. Initially, this result was explained by GH's effect on oocyte quality [45]. GH has been shown in numerous studies to assist older women in producing more mature oocytes, high-quality embryos, and retrieved oocytes [46]. The current findings demonstrated that the GH has a significant impact in fertility and the success of ART cycles.

4. Conclusions:

The amount of GH in serum and follicular fluid revealed a statistically significant positive link with FSH, LH, AMH, the frequency of miscarriages, the retrieval of oocytes and infertility duration. These findings imply a strong positive correlation between GH and female fertility. As a result, much research is required to assess, estimate the importance of GH levels on women's fertility, and to get an early hormone level assessment before beginning a GH-containing therapy program.

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دراسة علاقة هرمون النمو بمستوى بعض الهرمونات التكاثرية وبعض المؤشرات لدى النساء العراقيات خلال دورات التلقيح الاصطناعي / الحقن المجهري

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الخلاصة

يعاني ما يقدر بـ 20%−12 من الأزواج من العقم، ويوصف بأنه عدم القدرة على الحمل بعد عام من المحاولة. حوالي ثلث هؤلاء الأزواج يعانون من العقم بسبب العوامل الأنثوية، وثلث آخر يعانون من العقم بسبب العوامل الذكورية. قد يختبر الأزواج مزيجًا من الاثنين في الثلث المتبقي. وتنقسم إلى مجموعتين، العقم الاولي والثانوي. بلغ عدد المشاركين 60 مشاركًا، 30 منهم من النساء الأكبر سناً (< 35 عامًا) و 30 منهم من النساء الأصغر سنًا (أقل من 35 عامًا). في هذه الدراسة أظهرت النتائج وجود علاقة إيجابية ذات دلالة إحصائية عالية بين هرمون النمو وكل من المصل والسائل الجريبي (*FF*) . *FSH* (0.84 ، 0.81 (*mIU/ML* ، *LH* 0.76) ، 0.74 (*AMH and mIU/ML* 0.89) ، 0.86 (*mIU/ML* ،وكان هناك ارتباط إيجابي عالي المعنوية بين هرمون النمو وعدد البويضات المستخرجة (*mIU/ML* 0.47) ، (0.46 ،تم العثور على علاقة غير ربط سلبية بين هرمون النمو وعدد حالات الإجهاض *mIU/ML*(0.10− ، 0.14−) .والجدير بالذكر أن الغالبية العظمى من تقييمات مستويات هرمون النمو في المصل والسائل الجريبي كشفت عن وجود علاقة إيجابية ذات دلالة إحصائية مع *FSH* ، *LH* ، *AMH* ، وتواتر حالات الإجهاض، واسترجاع البويضات ومدة العقم. تشير هذه النتائج إلى وجود علاقة إيجابية قوية بين هرمون النمو وخصوبة الإناث. ونتيجة لذلك، هناك حاجة إلى الكثير من الأبحاث لتقييم وتقدير أهمية مستويات هرمون النمو على خصوبة المرأة، والحصول على تقييم مبكر لمستوى الهرمون قبل البدء في برنامج علاجي يحتوي على هرمون النمو.

الكلمات الدالة: العقم ، الهرمون المحفز للجريبات؛ هرمون اللوتين؛ هرمون ضد موليريان؛ هرمون النمو.

التمويل: لايوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: لم يتم نشر المخطوطة أو تقديمها لمجلة أخرى، كما أنها ليست قيد المراجعة.