

Evaluation of the Concentrations of Some Hormones and Vitamins in Infertile Women in Dhi Qar Governorate

Azhar Shallal Ma'araj^{1,*}, Ali M. Hussein²

¹ Department of Science, College of Basic Education, University of Sumer, Dhi Qar, Iraq

² Department of Pathological Analysis, College of Science, University of Sumer, Dhi Qar, Iraq

* Email address of the Corresponding Author: azharshallal@hs.uos.edu.iq

Phone number: 07829367988

Abstract

Objective: To evaluate the association between female infertility and the concentrations of estrogen, progesterone, testosterone, prolactin, and vitamin C, E, and D. **Methodology:** The study initially included 230 women with infertility. Using stratified random sampling, 30 women were selected for laboratory analysis: 15 with primary infertility and 15 with secondary infertility. Ten apparently healthy women served as controls. Approximately 7 mL of venous blood was collected from each participant during the first week of the menstrual cycle and used to measure all study parameters. Estrogen, progesterone, testosterone, prolactin, and vitamin D were measured using a Cobas e 411 analyzer based on electrochemiluminescence technology, whereas vitamin C and E were measured by ELISA using USCNK kits (China). **Results:** One-way analysis of variance showed significantly lower estrogen and progesterone concentrations and significantly higher prolactin concentrations in both infertility groups than in the control group ($P \leq 0.05$). Estrogen was also significantly lower in the primary infertility group than in the secondary infertility group. Testosterone did not differ significantly among the control group, whereas vitamins C and E showed no significant group differences. **Conclusions:** Female infertility was associated with altered estrogen, progesterone, prolactin, and vitamin D concentrations. These findings should be interpreted with considerations of body mass index, dietary intake, sun exposure, supplement use, and lifestyle factors.

Keywords: Female infertility, primary infertility, secondary infertility, hormones, vitamins

تقييم تركيزات بعض الهرمونات والفيتامينات لدى النساء المصابات بالعقم في محافظة ذي قار

ازهار شلال معارج^{1,*}, علي مانع حسين²

القسم العلوم، كلية التربية الأساسية، جامعة سومر، محافظة ذي قار، العراق

قسم التحليلات المرضية، كلية العلوم، جامعة سومر، محافظة ذي قار، العراق

الخلاصة

الهدف: تقييم العلاقة بين العقم عند النساء وتركيزات هرمون الإستروجين، والبروجسترون، والتستوستيرون، والبرولاكتين، وفيتامينات C، E، D. **الطريقة:** شملت الدراسة في البداية 230 امرأة يعانين من العقم. وباستخدام أسلوب المعاينة العشوائية الطبقية، تم اختيار 30 امرأة لإجراء التحاليل المخبرية: 15 امرأة يعانين من العقم الأولي و15 امرأة يعانين من العقم الثانوي. كما تم اختيار عشر نساء يتمتعن بصحة جيدة كمجموعة ضابطة. تم جمع حوالي 7 مل من الدم الوريدي من كل مشاركة خلال الأسبوع الأول من الدورة الشهرية، واستخدمت هذه العينات لقياس جميع معايير الدراسة. تم قياس هرمون الإستروجين والبروجسترون والتستوستيرون والبرولاكتين وفيتامين د باستخدام جهاز تحليل Cobas e 411 بتقنية التآلق الكيميائي الكهربائي (electrochemiluminescence technology)، بينما تم قياس فيتامين C وفيتامين E باستخدام تقنية ELISA باستخدام الأدوات (الكثات) التشخيصية المجهزة من شركة (الصين) USCNK. **النتائج:** أظهر تحليل التباين أحادي الاتجاه وجود انخفاضاً ملحوظاً في تركيز هرموني الإستروجين والبروجسترون، وارتفاعاً ملحوظاً في تركيز هرمون البرولاكتين في كلتا مجموعتي العقم مقارنةً بالمجموعة الضابطة ($P \leq 0.05$). كما كان مستوى الإستروجين أقل بشكل ملحوظ في مجموعة العقم الأولي مقارنةً بمجموعة العقم الثانوي. لم يختلف مستوى التستوستيرون بشكل ملحوظ في المجموعة الضابطة، بينما لم تظهر فيتامينات C و E أي اختلافات ملحوظة بين المجموعات. **الاستنتاجات:** ارتبط العقم عند النساء بتغيرات في مستويات هرمونات الإستروجين والبروجسترون والبرولاكتين وفيتامين D. ينبغي تفسير هذه النتائج مع مراعاة مؤشر كتلة الجسم، والنظام الغذائي، والتعرض لأشعة الشمس، واستخدام المكملات الغذائية، وعوامل نمط الحياة.

الكلمات المفتاحية: العقم عند النساء، العقم الأولي، العقم الثانوي، الهرمونات، الفيتامينات.

1. Introduction

Infertility is defined as the inability to conceive after at least 12 months of regular, unprotected sexual intercourse. It affects approximately 10–15% of couples worldwide and represents an important medical, psychological, and social challenges [1], [2], [3]. Female infertility is commonly classified as primary or secondary. Primary infertility refers to the absence of a previous conception, whereas secondary infertility refers to failure to conceive after at least one previous conception [4]. Unexplained infertility is diagnosed when standard evaluation of both partners does not identify a clear cause despite continued failure to conceive [5], [6]. The condition may involve physiology, hormonal, genetic, metabolic or immunological abnormalities [7], [8].

Sex hormones are central to follicular development, ovulation, endometrial preparation, implantation, and maintenance of pregnancy. Estrogen and progesterone are produced primarily by the ovaries, whereas prolactin and the gonadotropins contribute to regulation of ovulation and reproductive function [9], [10], [11], [12], [13]. Female infertility may therefore be associated with disturbances of the hypothalamic-pituitary-ovarian axis, ovulatory dysfunction, oxidative stress, and chronic inflammation [14]. Vitamins also contribute to reproductive health: vitamins C and E act as non-enzymatic antioxidants, whereas vitamin D participates in ovarian and endometrial regulation [15]. Age, body mass index, diet, physical activity, psychological stress, metabolic disorders, and other lifestyle factors may also influence fertility and circulating hormone and vitamin concentrations [16].

2. Materials and Methods

2.1. Study Design

The study initially enrolled 230 women with infertility in Dhi Qar Governorate. For the hormonal and vitamin analyses, 30 women were selected by stratified random sampling, with 15 women randomly selected from the primary infertility group and 15 from the secondary infertility group. Their ages ranged from 18 to 40 years. The control group consisted of 10 apparently healthy women aged 18-38 years who had no known infertility or menstrual-cycle disorder. The prevalence of polycystic ovary syndrome was assessed in the full cohort of 230 women, whereas laboratory comparisons were performed in the 30 selected women and 10 controls.

2.2. Measurement of Hormone and Vitamin Concentrations

Blood samples were collected during the first week of the menstrual cycle. Approximately 7 mL of venous blood was collected from each participant and used to measure all hormonal and vitamin parameters included in the study. Estrogen, progesterone, prolactin, testosterone, and vitamin D concentrations were measured using a Cobas e 411 electrochemiluminescence (ECL) analyzer with Roche reagents

(Germany). Vitamins C and E were measured using an ELISA analyzer and USCNK kit (China), according to the manufacturer's instructions.

2.3. Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 27. Results are presented as mean $\pm$ standard deviation (SD). Differences among the primary infertility, secondary infertility, and control groups were assessed using one-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) post hoc test for pairwise comparisons. A P Value ≤ 0.05 was considered statistically significant.

2.4. Ethical Approval

The study was approved by the Research Ethics Committee of the College of Basic Education, University of Summer (approval no. 2025CBE13) and was conducted in accordance with the principles of the Declaration of Helsinki.

3. Results and Discussion

3.1. The Relationship between Infertility and the Concentration of Some Hormones

As shown in Table (1), estrogen and progesterone concentrations were significantly lower, whereas prolactin concentrations were significantly higher, in both infertility groups than in the control group ($P \leq 0.05$). Estrogen was also significantly lower in women with primary infertility than in those with secondary infertility. Testosterone concentrations did not differ significantly among the three groups.

Table 1- Hormone concentrations in the study groups (Mean $\pm$ SD)

Values are presented as mean ± SD					
Standards Groups	Estrogen (pg/mL)	Progesterone (ng/mL)	Testosterone (ng/mL)	Prolactin (ng/mL)	
	Primary Infertility(n=15)	57.395 ± 7.770 ^c	0.877 ± 2.114 ^b	0.337 ± 0.219 ^a	18.775 ± 3.428 ^a
	Secondary Infertility(n=15)	87.833 ± 7.802 ^b	0.425 ± 0.229 ^b	0.391 ± 0.132 ^a	20.675 ± 1.189 ^a
	Control (n=10)	152.837 ± 19.186 ^a	2.141 ± 0.834 ^a	0.378 ± 0.109 ^a	15.429 ± 2.862 ^b
	P. Value (ANOVA)	0.001 <	0.014	0.658	0.001<

The reproductive process and the health of the reproductive system in both sexes, especially in women, depend directly on hormones, particularly sex hormones such as estrogen and progesterone which are essential for the growth and development of the reproductive system, in addition to their role in libido, the maturation and development of eggs in the ovary, ovulation, implantation, and the maintenance and protection of pregnancy [17]. Therefore, any disruption in the secretion of these hormones is a likely cause of infertility in women, as it is the direct and primary cause of infertility. This disruption results directly from either a malfunction in the sites where these hormones are secreted or in the nature of the stimulating factor for secretion in the hypothalamus or pituitary gland via the hypothalamic-pituitary-ovarian axis. Thus, any disruption in any of these three sites leads to a decrease in the secretion of these hormones, whether the cause is hormonal or tissue-related, and consequently, infertility, whether primary or secondary. [16] indicated that estrogen plays a pivotal role in the maturation of ovarian follicles and the preparation of the uterine lining, and that a decrease in Estrogen concentration is a result of hypothalamic dysfunction, and any disturbances in the secretion of gonadotropin-releasing hormone (GnRH) negatively affect the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), thus impairing ovarian function and lowering estrogen levels. On the other hand, the reason for the decrease in sex hormones (estrogen and progesterone) may be due to the high concentration of prolactin, which exerts negative feedback on the secretion of these hormones. It was indicated by [18] that the inverse relationship between the concentration of prolactin and these hormones is limited by the negative feedback mechanism, and that increased prolactin production causes inhibition of the secretion of sex hormones as a result of the effect on the secretion of gonadal nutrients. This was proven by the results of this study, which showed high prolactin concentration in women with infertility.

The low concentration of progesterone in infertile female patients compared to healthy women may be attributed to the link between this hormone and the ovulation process and the formation of the corpus luteum, which is formed from the remnants of the follicle from which ovulation occurred. The corpus luteum is the site of secretion of this hormone. Therefore, any disruption in the ovulation process is a direct cause of a decrease in the concentration of this hormone due to the failure of the corpus luteum to form. [19] indicated that ovulation disorder leads to a decrease in progesterone concentration, which is one of the causes of infertility in women due to the failure to prepare the uterine lining for the implantation of the fertilized egg. Several studies have confirmed that low progesterone is a clear indicator of weak ovulation and irregular ovarian cycle.

On the other hand, the results indicated a non-significant increase in testosterone concentration in the first and second groups when compared with the control group. This may be due to polycystic ovary syndrome, which is a direct cause of infertility. The results of our current study proved this, showing that 47% of the study sample had polycystic ovaries. [20] indicated an increase in testosterone concentration in women with infertility, while [21], [22] indicated that the effect of testosterone on fertility

depends on the severity of the change in its concentration, as a severe increase, as in the case of polycystic ovary syndrome, is associated with ovulation disorders.

The results also showed a significant increase in prolactin levels in infertile women compared to healthy women. This hormone is secreted by the anterior pituitary gland under the stimulation of the hypothalamus and plays important roles in the body, particularly in preparing the mammary glands and producing milk. Elevated prolactin levels may be explained by activity in either of the two glands, or by hypothyroidism or increased oxidative stress in women. Therefore, it may be a logical cause of infertility. It is well-established that hyperprolactinemia inhibits fertility by affecting the secretion of hormones that stimulate the ovaries to produce sex hormones. [23] indicated that elevated prolactin leads to inhibition of GnRH secretion from the hypothalamus, and consequently, a decrease in LH and FSH secretion from the pituitary gland. This results in ovulation disturbances and a decrease in estrogen and progesterone levels. Recent studies have confirmed that hyperprolactinemia is one of the common causes of infertility in women and is linked to menstrual cycle disorders and anovulation.

3.2. The Relationship between Infertility and the Concentration of Some Vitamins

As shown in Table (2), vitamin D concentrations were significantly lower in both infertility groups than in the control group ($P \leq 0.05$). In contrast, vitamin C and vitamin E concentrations did not differ significantly among the primary infertility, secondary infertility, and control groups.

Table 2- Vitamin concentrations in the study groups (Mean $\pm$ SD)

Values are presented as mean $\pm$ SD			
Standards Groups	Vitamin (C) (ng/mL)	Vitamin (E) (ng/mL)	Vitamin (D) (ng/mL)
primary infertility(n=15)	2086.363 $\pm$ 2249.659 ^a	0.524 $\pm$ 0.238 ^a	22.867 $\pm$ 2.588 ^b
secondary infertility(n=15)	1405.812 $\pm$ 726.790 ^a	0.928 $\pm$ 0.935 ^a	20.600 $\pm$ 5.152 ^b
Control (n=10)	1585.306 $\pm$ 1014.580 ^a	0.677 $\pm$ 0.725 ^a	34.800 $\pm$ 2.201 ^a
P. Value(ANOVA)	0.468	0.286	<0.001

The lack of significant differences in vitamin C and vitamin E concentrations may be attributed to adequate dietary intake of these vitamins or a balance of antioxidants within the body. [24] indicated that these vitamins function within an integrated system of enzymes, such as superoxide dismutase and glutathione peroxidase, enabling functional compensation that maintains their concentration within normal limits despite the presence of pathological disorders. [25], [26], [27], [28] indicated that the body's complex antioxidant system is affected by the intake of antioxidant vitamins and minerals

in the diet, such as vitamin C, vitamin E, selenium, zinc, taurine, hypochlorite, glutathione, beta-carotene, and carotene. [29] indicated that vitamin C acts as an antioxidant, interrupting the chain reaction and halting the spread of oxidation. In addition, vitamin C helps to recycle oxidized vitamin E and glutathione. [30] indicated that the lack of significant differences in non-enzymatic antioxidants may occur in cases where oxidative stress does not reach severe levels or is compensated for by other internal mechanisms.

These results can also be explained by the fact that vitamins C and E act primarily as direct antioxidants, and therefore their effect is related to the degree of oxidative stress more than to its direct relationship to the hormonal regulation of fertility. [31] indicated that if the intensity of oxidative stress is limited or equal among individuals, no clear statistical differences may appear between the groups.

On the other hand, the results of this study showed a significant decrease in the concentration of vitamin D in female with infertility compared to healthy women. It is known that this vitamin has a role in reproductive function, as [32], [33] indicated that vitamin D is one of the vitamins similar to steroid hormones that affect the ovary through its association with its receptors (VDR) found in granulosa cells, where it contributes to regulating follicle growth and the secretion of sex hormones. Therefore, its deficiency may lead to weak ovulation and menstrual cycle disorders. As for the causes of low vitamin D, it may be due to polycystic ovary syndrome and obesity. [34], [35] indicated that the decrease in vitamin D is because it is a fat-soluble vitamin, so it remains within the fatty tissues of obese females, which leads to a decrease in its level in the blood, and this is common among females with infertility. The decrease in vitamin D may be attributed to a thyroid disorder. [36] indicated that thyroid hormones regulate metabolism and absorption processes, and that any disruption in these hormones affects the absorption process, including the absorption of vitamin D, leading to a decrease in its level in the blood.

3.3. The relationship between infertility rates and polycystic ovary syndrome (PCOS)

In the full cohort of 230 women with infertility, 47% had polycystic ovary syndrome, whereas 53% did not (Figure 1).

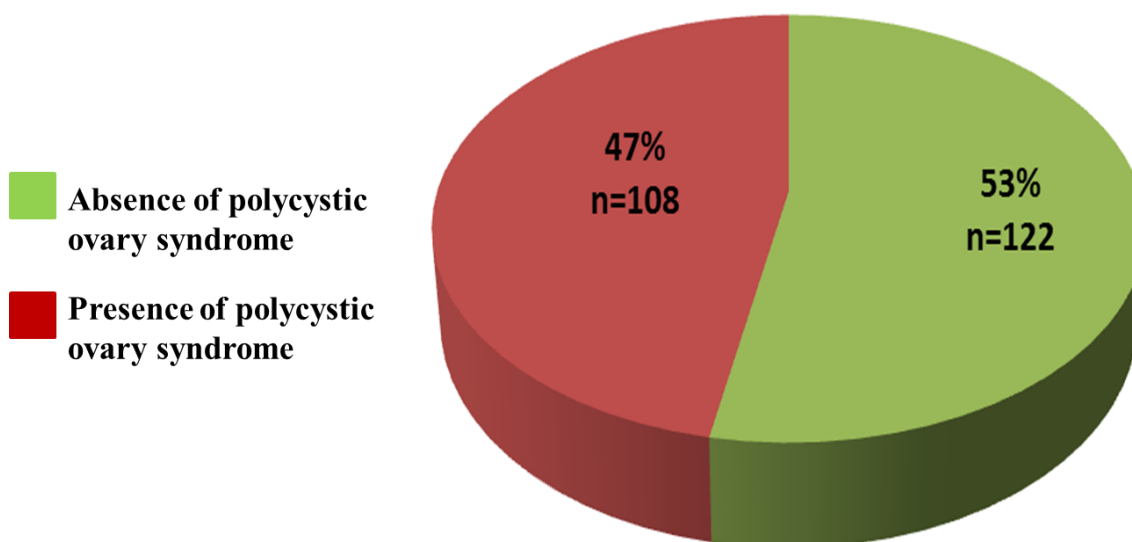


Figure 1- Shows the relationship between the incidence of infertility and the incidence of polycystic ovary syndrome.

A large percentage of females suffer from polycystic ovary syndrome, which is associated with reproductive disorders such as hyperandrogenism, menstrual irregularities, infertility, and anovulation. [37], [38] indicated that the prevalence of polycystic ovaries among females with infertility varies significantly according to age, reaching about 30% in younger females and up to 59% in older females. It is characterized by high androgens (high male hormones), ovulation disorders (irregular menstrual cycles), cysts around the ovary, and hirsutism, as confirmed by [39].

[40], [41] also showed that the high rate of polycystic ovaries among females with infertility is closely related to increased insulin resistance and obesity. It was observed that females with a high body mass index are more prone to polycystic ovaries and infertility compared to others, which may explain the high rate in some societies that are experiencing lifestyle changes.

[42], [43], [44] indicates that polycystic ovary syndrome is the most common cause of ovulation hormone disorders, representing approximately 70-80% of infertility cases resulting from ovulation dysfunction, this reinforces the explanation of the high percentage recorded in the current study, and confirms that the presence of (47%) of causes related to polycystic ovaries is a logical indicator in light of the physiological role of the syndrome in inhibiting follicle maturation and ovulation.

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