Evaluation of Some Fertility Hormones in Patients Women of Polycystic Ovary Syndrome with Recurrent Miscarriages

Aseel Ehsan Mahmood[1], Rehab Jasim Mohammed[2], Hameedah Hadi AbdulWahid[3]

[1,2]Department of chemistry/College of Education for Pure Science/University of Karbala/Iraq
[3] Phd Gynaecologist and Obstetrician in College of Medicine/University of Karbala/Iraq
Email: aseel.angel.aa@gmail.com

Abstract

Hormonal changes related with menstruation problems, such as low and high hormone concentrations, have significant consequences on the body .PCOS (polycystic ovarian syndrome) is a prevalent endocrine condition that affects women of reproductive age. The prevalence of PCOS in women of reproductive age ranges from 5% to 18%, according to a systematic review and meta-analysis. Objective:- The purpose of this study were to evaluate the levels of FSH, LH, ESTROGEN, progesterone and testosterone hormones in the serum of women who suffer from recurrent miscarriage with PCOS and to compare it with women of the control group. Subjects and method: - A case and control study includes 90 women in reproductive age (15-45), 50 of them had polycystic ovarian syndrome diagnosed, and 40 female were control group healthy. Samples were taken from October 2021 to March 2022, and FSH, LH, ESTROGEN, progesterone and testosterone levels had analyzed by CL-900i from mindray company in china. Result: - The levels of FSH was insignificantly increased (P=0.02), LH level was insignificantly increased (P=0.01), progesterone level was insignificantly increased in patients women when compared with control group, ESTROGENS level was significantly increased (P=0.01) of patients, testosterone level was equal in patients when compared with control group. Conclusion: - The levels of FSH, LH, ESTROGEN and progesterone was increase in patients women with PCOS, while testosterone level was equal in patients and control group. BMI of FSH concentration was higher in patients in normal and obese when compared with overweight, but LH levels was highest BMI level in normal, overweight and obese when compared with control group, while the levels of ESTROGENS concentration in patients were the highest in BMI normal and overweight when compared with the obese women, the levels of testosterone and progesterone concentration in patients were the highest in BMI of normal weight from control group when compared with the women of pre obesity(over weight) and obese.

Keyword: Fertility hormones, polycystic ovary syndrome, recurrent miscarriages.

1. Introduction

The menstrual cycle is a natural phenomenon that marks the years of fertility in women's life. It is a sign of reproduction and menstruation, as well as a signal of Women's health is important. The majority of women at this point are in pain. Due to hormonal fluctuations, you may experience a variety of symptoms [1]. Hormonal changes related with menstruation problems, such as low and high hormone concentrations, have significant consequences on the body [2]. Luteinizing hormone (LH) is an important gonadotropin in the reproductive system's control. LH enhances progesterone secretion in the luteal phase and commences oocyte maturation by stimulating the generation of sex steroids [3]. LH is a pituitary hormone that stimulates the corpus lutein and causes ovulation. During the second half of the cycle, the ovary secretes progesterone and ESTROGEN (E2) cycle of menstruation [4]. The release of the hormones LH and FSH, which usually starts towards the start of the cycle, promotes a rise in E2 production from the ovaries between days 8 and 12 of the cycle (before ovulation). Around day 14, there is an increase in progesterone, and around day 18, there is a surge in E2 (after ovulation) [5]. FSH is a type of gonadotropin that is released by the anterior pituitary's basophilic cells and plays a key role in gonadal hormone synthesis and reproductive process regulation [6]. The ovarian follicle-stimulating hormone (FSH) increases the formation and development of ovarian follicles, where ova or egg cells develop and the ovaries secrete E2. The best predictor of FSH-based ovarian reserve is maximum FSH [7]. E2 along with progesterone are the major hormones released by the ovaries [8]. E2 regulates physiological and pathological processes in the reproductive, cardiovascular, skeletal, endocrine, neurological, and immunological systems in both women and men, mediating a variety of effects across the body. As a result, it is involved in a variety of disorders, including different malignancies, infertility, endometriosis, polycystic ovary syndrome [9, 10]. The main effects of E2 are the promotion of female secondary sex characteristics and the preparation of the uterus for ovulation and conception, It also has vascular benefits, such as increasing blood flow and forming new blood vessels, as well as endometrial and breast growth-promoting properties [11]. The hormone progesterone is a steroid hormone. This sex hormone is required for

doi.org/10.31838/hiv22.02.356 Received: 09.04.22, Revised: 09.05.22, Accepted: 17.08.22

a successful pregnancy. The chemical signal employed by the ovary to change the endometrial lining of the uterus into a highly secretory tissue that can maintain the fertilized egg is progesterone, which is released by cells of the luteal phase in a non-pregnant woman [12]. Progesterone is produced in the ovaries, adrenal glands, and the placenta during pregnancy. It is also kept in the adipose tissue. When it comes to women, during the pre - ovulatory phase of the menstrual cycle, progesterone levels are low. After ovulation, they rise and remain elevated during the luteal phase. Before ovulation, progesterone levels are normally less than 2ng/ml, and after ovulation, they are greater than 5ng/ml, about 7 days before menstruation [13, 14]. Although testosterone is commonly associated with men, women in the ovaries and adrenal glands also generate it. Women may have symptoms of what is now known as androgen deficiency when testosterone levels fall with age and menopause. Some experts call it "androgen insufficiency syndrome," and it includes the typical symptoms of menopause [15]. In women, testosterone has a biological role in follicular atresia and its present in the lowest concentration in the early stages. The luteal phase of the cycle begins with the follicular phase and progresses to the mid-cycle peak. Concentrations are higher in the late follicular phase than they are in the early follicular phase [16]. The main aberrant hormonal aspect of polycystic ovary syndrome is excessive ovarian androgen production [17]. One percent of couples attempting to get pregnant have recurrent miscarriage, which is defined as the loss of two or more successive pregnancies. One to two percent of first- and second-trimester pregnancies miscarry before the 24-week stage [18]. PCOS (polycystic ovarian syndrome) is a prevalent endocrine condition that affects women of reproductive age. The prevalence of PCOS in women of reproductive age ranges from 5% to 18%, according to a systematic review and meta-analysis [19]. Women with PCOS are at risk for fertility issues (menstrual cycle disorders, failure to ovulate, late menopause, endometrial cancer, and infertility), metabolic issues (insulin resistance, diabetes type 2, hypertension, and cardiovascular diseases), physical issues (central obesity, acne, hair loss, and baldness), and psychological issues (depression, stress and anxiety). Changes in the concentrations of luteinizing hormone (LH), E2, and serum androgens such as testosterone are often linked with PCOS. Many women with PCOS have an elevated LH/FSH ratio, according to hormonal measurements [20]. According to experts. Polycystic ovary syndrome disease, uterine septum, and uterine fibroid are just a few of the diseases, which are related to miscarriage [21].

2. Subject and Material

The chemical department of the College of Education for Pure Science at the University of Karbala conducted this study, which included 50 patients' women with PCOS and suffer from recurrent miscarriage, and 40 controls who appeared to be in good health. The participants' ages ranged from [16-18, 20-25] years. The study took place between October 2021 and March 2022. The Rotterdam criteria from 2003, which included polycystic ovaries (ovulation), ultrasonography, biochemical parameters, and clinical symptoms of high androgen level, were used to diagnose PCOS in women. Blood samples are taken from non-pregnant women from Gynecological and Obstetric Teaching Hospital and outpatient clinics for the purpose of medical tests for fertility hormones (FSH, LH, ESTROGEN, progesterone and testosterone). Five ml of blood was taken using a 5 ml medical syringe and the blood was placed in gelatine tubes free of anti-clotting material, as it contains a gelatinous substance that helps to increase the separation of serum formed after the centrifugation process. The samples were left for 15 minutes at room temperature, after which they were inside a centrifuged at a speed of 2500 (round / minute) for 10 minutes to obtain the serum that was stored at (-20)° C, unless it was used immediately.

3. Results

The study's data were examined using the SAS 2012 program's Statistical Package for the Statistical Analysis System (SAS). It was created with comparison and usage of notable distinctions in mind. If the mean ±SD was supplied with a p≤0.05, it was deemed significant (standard deviation). The parameters that were compared between the patient and control groups were compared using independent T-test statistics.

Table (1): Comparison of FSH, LH, ESTROGEN, progesterone and testosterone concentration between patients women and control group

P value	Mean± SD	subject	parameters
N. S	12.38 ± 64.32 12.64 ± 67.16	Control patients	Age (Years)
N. S	4.85 ± 25.70 4.60 ± 25.94	Control patients	BMI (kg/m^2)
0.02	1.64 ± 6.14 1.98 ± 7.01	Control patients	FSH (mIU/ml)
0.01	1.85 ±5.71 2.12± 7.52	Control patients	LH (mIU/ml)
N. S	0.17 ± 0.53 0.18 ± 0.54	Control patients	Progesterone (ng/ml)
0.01	13.45 ± 37.92 18.15± 51.82	Control patients	Estrogens (pg/ml)
N. S	0.20 ±0.60 0.25± 0.60	Control patients	Testosterone (ng/ml)

BMI: Body Mass Index; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; SD: Standard deviation; N.S: t-test p- value ≥ 0.05; No. of patients group=50; No. of control group=40

According to the presented data show mean of FSH level(7.01 ± 1.98mIU/ml) in patients was insignificantly increased (P=0.02) and (6.14 ± 1.64mIU/ml) in control, LH level(7.52 ±2.12mIU/ml) in patients was insignificantly increased (P=0.01) and (5.71±1.85mIU/ml) in control, progesterone level (0.54 ± 0.18ng/ml) was insignificantly increased in patients women when compared with control group

(0.53± 0.17ng/ml) , ESTROGENS level (51.82 ±18.15pg/ml) was significantly increased (P=0.01) of patients and (37.92 ± 13.45pg/ml) in control, testosterone level (0.60 ± 0.25ng/ml) was equal in patients when compared with control group (0.60± 0.20ng/ml).

Effects of BMI on the levels of FSH concentration in patients and control group

The measurement of the level of FSH concentration in patients shown the higher BMI in normal and obese when compared with overweight, while in control group shown higher BMI of FSH level in overweight than normal and obese weight, as shown in figure (1).

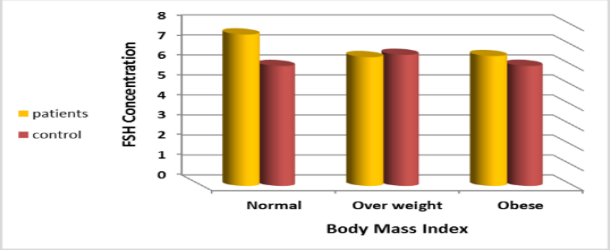

Figure (1): Levels of BMI with FSH concentration in patients and control group.

Effects of BMI on the levels of LH concentration in patients and control group

The measurement of the level of LH concentration in patient's women shown the highest BMI level in normal, overweight and obese when compared with control group, as shown in figure (2).

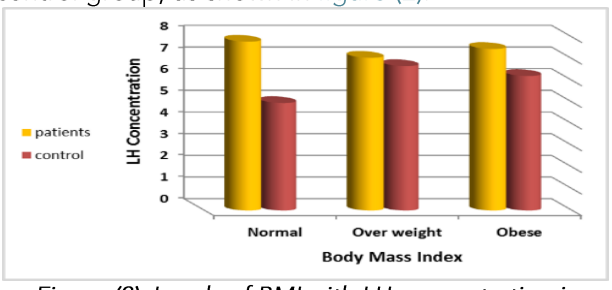

Figure (2): Levels of BMI with LH concentration in patients and control group.

Effects of BMI on the levels of ESTROGENS concentration in patients and control group

The study found that the levels of ESTROGENS concentration in patients were the highest in BMI normal and overweight when compared with the obese women, the control group in obese was the higher BMI from than patients when compared with the BMI of normal and overweight women, as shown in figure (3).

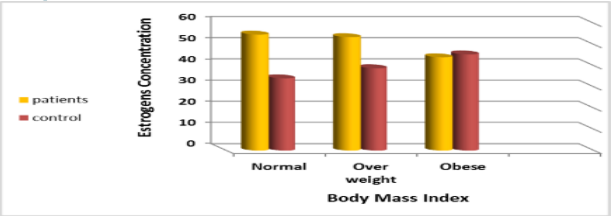

Figure (3): Levels of BMI with ESTROGENS concentration in patients and control group.

Effects of BMI on the levels of testosterone and progesterone concentration in patients and control group

The study found that the levels of testosterone and progesterone concentration in patients were the highest in Body Mass Index (BMI) of normal weight from control group when compared with the women of pre obesity (over weight) and obese, the level of BMI of control group in over weight and obese higher than patients women, as shown in figure (4), (5).

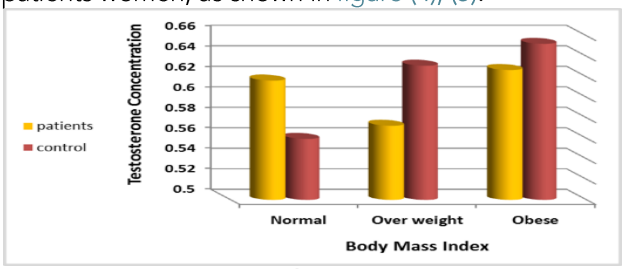

Figure (4): Levels of BMI with testosterone concentration in patients and control group.

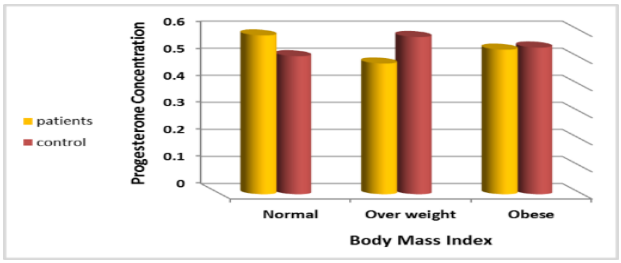

Figure (5): Levels of BMI with progesterone concentration in patients and control group.

Effect of the number of miscarriages on the levels of FSH, ESTROGEN, progesterone and testosterone concentration in patients

In this study, it was found that the concentration of these hormones with the number of miscarriages for patient's women, that women with two recurrent miscarriages have a higher percentage than other women with the number of miscarriages of three or more, as shown in figure (6), (7), (8), and (9).

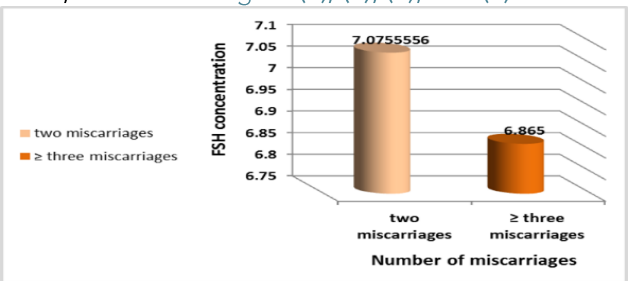

Figure (6): The relation of number of miscarriages with FSH concentration.

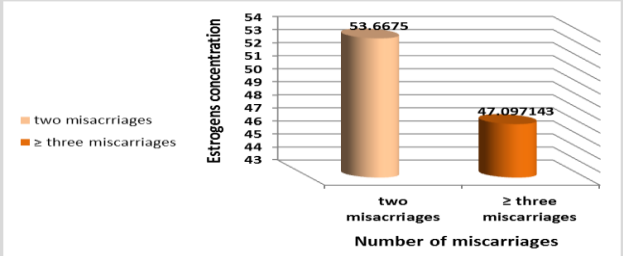

Figure (7): The relation of number of miscarriages with ESTROGENS concentration.

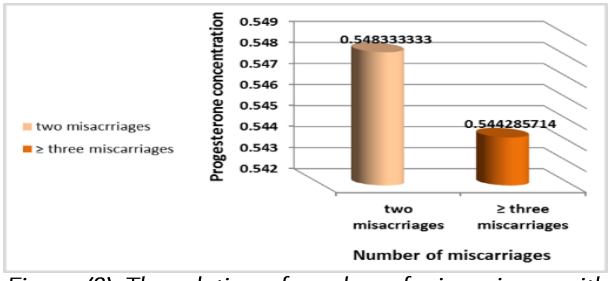

Figure (8): The relation of number of miscarriages with progesterone concentration.

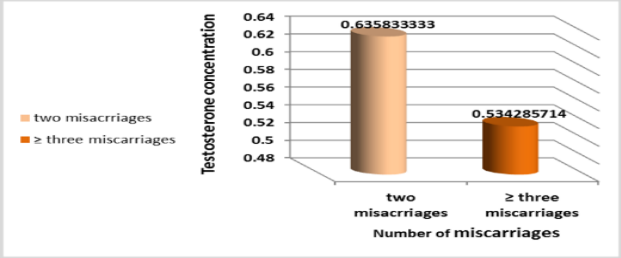

Figure (9): The relation of number of miscarriages with testosterone concentration.

Effect of the number of miscarriages on the level of LH concentration in patients

The measurement of the concentration of LH hormone with the number of miscarriages, it was found that the level of hormone in women with three miscarriages or more is higher than in women with two recurrent miscarriages, as shown in figure (10).

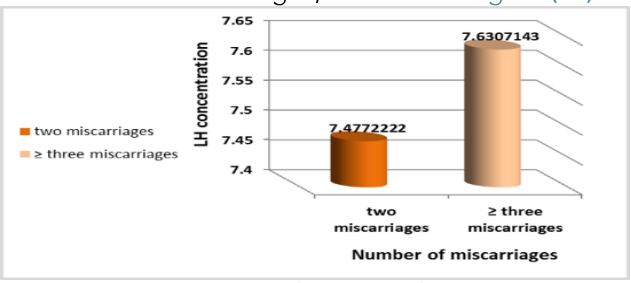

Figure (10): The relation of number of miscarriages with LH concentration.

4. Discussion

According to the presented data show mean of FSH level(7.01 ± 1.98mlU/ml) in patients was insignificantly increased (P=0.02) and (6.14 ± 1.64mlU/ml) in control, LH level(7.52 ±2.12mlU/ml) in patients was insignificantly increased (P=0.01) and (5.71±1.85mlU/ml) in control, progesterone level (0.54 ± 0.18ng/ml) was insignificantly increased in patients women when compared with control group (0.53± 0.17ng/ml) , ESTROGENS level (51.82 ±18.15pg/ml) was significantly increased (P=0.01) of patients and (37.92 ± 13.45pg/ml) in control, testosterone level (0.60 ± 0.25ng/ml) was equal in patients when compared with control group (0.60± 0.20ng/ml).

In the pathogenesis of polycystic ovarian disease, abnormality of the hypothalamic-pituitary-ovarian or adrenal axis has been suggested disturbance in the excretion type of the gonadotropin-releasing hormone results in the relative increase in LH - FSH release [22]. Ovulation does not occur in polycystic ovarian disease patients due to a high LH/FSH ratio [23]. Cook et al. [24] Found that the women with PCOS showed greater serum LH levels than women without the condition [24]. On the other hand Cho et al. [25] found that the LH/FSH ratio is of limited help in identifying polycystic ovarian syndrome since the median LH/FSH ratio did not differ substantially between the PCOS and non-affected groups [25]. Infertility and spontaneous abortion are most commonly caused by a lack of luteal phase progesterone production and activity, the reason of progesterone deficiency in PCOS patients during the luteal phase is unknown. Women with polycystic ovary syndrome (PCOS) need higher levels of progesterone to slow the frequency of gonadotropin releasing hormone pulse secretion [26]. Women who have had a history of miscarriage and are experiencing early pregnancy bleeding may benefit from vaginal progesterone therapy. Treatment with 400 mg of vaginal micronized progesterone twice day was linked to a higher incidence of live births [27]. In patients with PCOS, there are certain anomalies in progesterone synthesis that may be linked to a high risk of miscarriages [28]. Found that ESTROGENS are released in considerable numbers exclusively by the ovaries in non-pregnant women, however small amounts are also secreted by the adrenal cortices. The placenta also secretes large amounts of ESTROGENS during pregnancy. High ESTROGEN levels, can develop in women with polycystic ovarian syndrome (PCOS). This disorder is characterized by a hormonal imbalance that can result in irregular periods, unwelcome hair growth, and acne [29]. The ESTROGEN dominance found in women with PCOS is caused by hormone imbalance. Environmental variables might also influence the outcome [30]. The levels of total testosterone and free testosterone were linked to the risk of PCOS [31]. In PCOS, testosterone levels may be normal. Oral contraceptives reduce testosterone levels [32].

In this study, we found that the results of age (15-45) years, levels was increased, and the body mass index level almost equal in control and patients women with PCOS. Table (1). Because a woman's reproductive ability reduces with age due to decreased ovarian reserve, oocyte quality, and the increased occurrence of embryonic aneuploidy, age is a key factor impacting female fertility [33]. PCOS is the most commonly hormonal condition among women of reproductive age [34]. Malizia et al. [35] In general, female fertility decreases as she gets older [35]. Alsamarai et al. [36] PCOS was associated with an increase in ovarian volume and follicle number from the age of 15 until menopause [36]. Each person's body mass index (BMI) was calculated using the formula weight (kg)/height (m^2), and the results were assessed to determine their obesity status. Not all PCOS women are obese, and not all PCOS women have an abnormal LH/FSH ratio, nor do they all have the hormonal and biochemical abnormalities associated with the illness. According to another studies, a higher BMI always associated with a higher

LH/FSH ratio and a higher risk of menstrual disorders. Therefore, compared to obese female, non-obese women with PCOS had significantly higher blood LH levels [37].

They were classified into three groups, those with a BMI. Normal weight were classified as group A (18.5-24.9 Kg/m²), overweight were classified as group B (25-29.9 Kg/m²), while those over (>30 Kg/m²) obese as group C. The normal BMI and obese with concentration of FSH hormone was higher in women with PCOS than the control group, and the results of levels of BMI with LH concentration in patients women higher than control, as shown in figure (1),(2). Some studies have found a higher of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in PCOS patients with normal weight than in obese PCOS patients ; however, this result has not been found in other research [38-40]. Obesity is more common in women who have PCOS. Although increasing research shows that BMI adds significantly to the severity of many problems, like the risk of miscarriage, the relative impact of PCOS diagnosis and obesity in this group of women is still unknown [41].

In this study, it was found that the body mass index of ESTROGEN hormone in normal-weight and overweight women with PCOS was higher than in obese women compared to the control group, as shown in figure (3). Randolph Jr et al. [42] when compared to non-obese women, obese women had lower premenopausal ESTROGEN levels, which are measured on days 2–5 of a spontaneous menstrual cycle, but greater postmenopausal ESTROGEN levels [42].

When the women with PCOS and control group were subdivided according to their BMI, the concentration of testosterone and progesterone in normal-weight women with PCOS were higher than in the control group, and healthy women overweight and obese women were higher compared to the patients, as shown in figure (4),(5). A study found that a little but significant percentage of women with PCOS have a normal or low BMI which may or may not even have symptoms like irregular menstruation [43]. A Study similar by Bellver, J., and Rodríguez-Varela in (2022) found that the patients who were overweight or obese had significantly lower serum progesterone levels than those who were underweight or normal weight [44]. When compared to obese women with PCOS, non-obese females with PCOS showed higher levels of testosterone [40]. Another study, on the other hand, indicated that testosterone levels are higher in overweight and obese women, and that disagreement with our study [45].

In this research, patients group were categorized according to the number of miscarriages into women with two, three or more miscarriages and its relation with the level of fertility hormones to find out the difference between the hormones. Several research have investigated the correlation between PCOS and recurrent miscarriages in recent years. Women with recurrent miscarriages have been found to have higher rates of PCOS [46]. Several previous studies have found that women with lower levels of serum progesterone can lead to miscarriage [47]. However, increased ESTROGEN levels might cause miscarriage too [39]. It was discovered that use vaginal progesterone enhanced the live birth rate and decreased the miscarriage rate for women who had experienced recurrent miscarriages. The group of women with a history of three or more miscarriages had a greater live birth rate than the group of women with a history of two or more losses [48]. In comparison to fertile controls, ovarian reserve was evaluated in women who experience recurrent miscarriages. Women who often miscarried had higher FSH levels [49]. While high levels of luteinizing hormone in the early to mid-follicular phase have been linked to a higher risk of miscarriages in women with and without PCOS, as well as increased testosterone levels are linked to a decrease in uterine growth during the luteal phase and are thought to be responsible in recurrent miscarriages. Women who suffer from recurrent miscarriages had greater testosterone levels than fertile controls [50].

References

1. Whitney EN, Cataldo CB, DeBruyne LK, et al. Nutrition for health and health care. Wadsworth/Thomson Learning, 2001. Available from: https://www.cabdirect.org/cabdirect/abstract/20013018218

2. Al-Hamdany WAS, Gaffar MK. A Comparison Effect of Dysmenorrhea and Secondary Amenorrhea in the Concentration of Some Biochemical and Some Hormones in Women in Tikrit City. Tikrit Journal of Pure Science. 2019;24(2):37-42. Available from: http://tjps.tu.edu.iq/index.php/j/article/view/798

3. Baskind NE, Balen AH. Hypothalamic–pituitary, ovarian and adrenal contributions to polycystic ovary syndrome. Best Practice & Research Clinical Obstetrics & Gynaecology. 2016;37:80-97. https://doi.org/10.1016/j.bpobgyn.2016.03.005

4. Escobar-Morreale H, Carmina E, Dewailly D, et al. Epidemiology, diagnosis and management of hirsutism: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome Society. Human reproduction update. 2012;18(2):146-70. https://doi.org/10.1093/humupd/dmr042

5. Protopopescu X, Butler T, Pan H, et al. Hippocampal structural changes across the menstrual cycle. Hippocampus. 2008;18(10):985-8. https://doi.org/10.1002/hipo.20468

6. Coss D, Jacobs SB, Bender CE, et al. A novel AP-1 site is critical for maximal induction of the follicle-stimulating hormone β gene by gonadotropin-releasing hormone. Journal of Biological Chemistry. 2004;279(1):152-62. https://doi.org/10.1074/jbc.M304697200

7. Gingold JA, Lee JA, Whitehouse MC, et al. Maximum basal FSH predicts reproductive outcome better than cycle-specific basal FSH levels: waiting for a "better" month conveys limited retrieval benefits.

Reproductive Biology and Endocrinology. 2015;13(1):1-14. https://doi.org/10.1186/s12958-015-0078-0
8. Holt EH, Peery HE. Basic Medical Endocrinology. Elsevier Science, 2010. Available from: https://books.google.com.pk/books?id=gjpi2MYVKGAC
9. Callard GV, Tarrant AM, Novillo A, et al. Evolutionary origins of the estrogen signaling system: insights from amphioxus. The Journal of steroid biochemistry and molecular biology. 2011;127(3-5):176-88. https://doi.org/10.1016/j.jsbmb.2011.03.022
10. Jeswani R, Patil Y, Patil S. Intravenous magnesium sulfate and isoxsuprine for arrest of preterm labor: A comparative study. J Nat Sci Biol Med. 2021;12(2):135-9. https://doi.org/10.4103/jnsbm.JNSBM_147_20
11. Coad J, Pedley K, Dunstall M. Anatomy and Physiology for Midwives E-Book. Elsevier Health Sciences, 2019. Available from: https://books.google.com.pk/books?id=YnetDwAAQBAJ
12. Wilcox AJ. Fertility and Pregnancy: An Epidemiologic Perspective. Oxford University Press, USA, 2010. Available from: https://books.google.com.pk/books?id=URkTDAAAQBAJ
13. Egbagba Ao. Assessment Of Serum, Follicle Stimulating Hormone, Luteinizing Hormone, Oestradiol, Progesterone, Prolactin, And Urinary Follicle Stimulating Hormone And Luteinizing Hormone In Women Presenting With Infertility In Benin City. Faculty of Pathology. 2016.
14. Singh S, Sukkala R. Evaluation and comparison of performance of low-dose 128-slice CT scanner with different mAs values: A phantom study. J Carcinog. 2021;20:13. https://doi.org/10.4103/jcar.jcar_25_20
15. Huang G, Basaria S. Do anabolic-androgenic steroids have performance-enhancing effects in female athletes? Molecular and cellular endocrinology. 2018;464:56-64. https://doi.org/10.1016/j.mce.2017.07.010
16. Jassim TS, AlFaisal AHM. Large Deletion of Sex Hormones Binding Globulin-SHBG Gene in Iraqi Women with Polycystic Ovary Syndrome-PCOS. Iraqi journal of biotechnology. 2020;3(19). Available from: https://jige.uobaghdad.edu.iq/index.php/IJB/article/view/402
17. Haddad N, Nori E, Hamza SA. Correlations of Serum Chemerin and Visfatin with other Biochemical Parameters in Iraqi Individuals with Metabolic Syndrome and Type Two Diabetes Mellitus. Jordan J Biol Sci. 2018;11(4):4369. Available from: https://jjbs.hu.edu.jo/files/v11n4/Binder11n4.pdf
18. Ismail AM, Abbas AM, Ali MK, et al. Peri-conceptional progesterone treatment in women with unexplained recurrent miscarriage: a randomized double-blind placebo-controlled trial. The Journal of Maternal-Fetal & Neonatal Medicine. 2018;31(3):388-94. https://doi.org/10.1080/14767058.2017.1286315
19. Ding T, Hardiman PJ, Petersen I, et al. The prevalence of polycystic ovary syndrome in reproductive-aged women of different ethnicity: a systematic review and meta-analysis. Oncotarget. 2017;8(56):96351. https://doi.org/10.18632%2Foncotarget.19180
20. Aali B, Naderi T. Evaluation of clinical, ultrasound and laboratory features of PCOS in Kerman in 1381. Iranian Journal of Endocrinology and Metabolism. 2004;6:153-61.
21. Cocksedge KA, Li T-C, Saravelos SH, et al. A reappraisal of the role of polycystic ovary syndrome in recurrent miscarriage. Reproductive biomedicine online. 2008;17(1):151-60. https://doi.org/10.1016/S1472-6483(10)60304-5
22. Revathi R, Julius A. Effect of LH/FSH Ratio and its Correlation with Insulin Resistance in PCOS Obese woman of Reproductive age Group. Research Journal of Pharmacy and Technology. 2018;11(6):2217-9. https://doi.org/10.5958/0974-360X.2018.00410.9
23. Wu Y, Robinson N, Hardiman PJ, et al. Acupuncture for treating polycystic ovary syndrome: guidance for future randomized controlled trials. Journal of Zhejiang University-Science B. 2016;17(3):169-80. https://doi.org/10.1631/jzus.B1500301
24. Cook CL, Siow Y, Brenner AG, et al. Relationship between serum müllerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovary syndrome and normal women. Fertility and sterility. 2002;77(1):141-6. https://doi.org/10.1016/S0015-0282(01)02944-2
25. Cho LW, Jayagopal V, Kilpatrick ES, et al. The LH/FSH ratio has little use in diagnosing polycystic ovarian syndrome. Annals of clinical biochemistry. 2006;43(3):217-9. https://doi.org/10.1258/000456306776865188
26. Keen MA, Shah IH, Sheikh G. Cutaneous manifestations of polycystic ovary syndrome: A cross-sectional clinical study. Indian dermatology online journal. 2017;8(2):104. https://doi.org/10.4103%2F2229-5178.202275
27. Coomarasamy A, Devall AJ, Brosens JJ, et al. Micronized vaginal progesterone to prevent miscarriage: a critical evaluation of randomized evidence. American journal of obstetrics and gynecology. 2020;223(2):167-76. https://doi.org/10.1016/j.ajog.2019.12.006
28. De Leo V, Musacchio M, Cappelli V, et al. Genetic, hormonal and metabolic aspects of PCOS: an update. Reproductive Biology and Endocrinology. 2016;14(1):1-17. https://doi.org/10.1186/s12958-016-0173-x
29. Mohan V, Mehreen T, Ranjani H, et al. Prevalence of polycystic ovarian syndrome among adolescents and young women in India. Journal of Diabetology. 2021;12(3):319-25. https://doi.org/10.4103/jod.jod_105_20
30. Krusko O, Rashidova M, Brichagina A, et al. Features of the Functional State of the Hypophysis-Ovarian System and Processes of Lipid Peroxidation–Antioxidant Protection in Women with Hyperandrogenism of Ovary Genesis in the Early Reproductive Period. Acta Biomedica Scientifica. 2021;5(6):20-6. https://doi.org/10.29413/ABS.2020-5.6.2
31. Ruth KS, Day FR, Tyrrell J, et al. Using human genetics to understand the disease impacts of testosterone in men and women. Nature medicine. 2020;26(2):252-8. https://doi.org/10.1038/s41591-020-0751-5
32. Aljenaee K, Ali S, Cheah SK, et al. Marked hyperandrogenicity in a 60-year-old woman. Endocrinology, Diabetes & Metabolism Case

Reports. 2017;2017(1). Available from: https://edm.bioscientifica.com/view/journals/edm/2017/1/EDM17-0075.xml
33. Harper J, Boivin J, O'Neill HC, et al. The need to improve fertility awareness. Reproductive Biomedicine & Society Online. 2017;4:18-20. https://doi.org/10.1016/j.rbms.2017.03.002
34. Bazarganipour F, Taghavi SA, Montazeri A, et al. The impact of polycystic ovary syndrome on the health-related quality of life: A systematic review and meta-analysis. Iranian journal of reproductive medicine. 2015;13(2):61. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4426142/
35. Malizia BA, Hacker MR, Penzias AS. Cumulative live-birth rates after in vitro fertilization. New England Journal of Medicine. 2009;360(3):236-43. https://doi.org/10.1056/NEJMoa0803072
36. Alsamarai S, Adams JM, Murphy M, et al. Criteria for polycystic ovarian morphology in polycystic ovary syndrome as a function of age. The Journal of Clinical Endocrinology & Metabolism. 2009;94(12):4961-70. https://doi.org/10.1210/jc.2009-0839
37. Fazli D, Moghaddasi SP. Correlations Between Gonadotropin Hormones and the Manifestations of Polycystic Ovary Syndrome. Thrita. 2021(In Press). https://doi.org/10.5812/thrita-117396
38. Dale PO, Tanbo T, Vaaler S, et al. Body weight, hyperinsulinemia, and gonadotropin levels in the polycystic ovarian syndrome: evidence of two distinct populations. Fertility and sterility. 1992;58(3):487-91. https://doi.org/10.1016/S0015-0282(16)55249-2
39. Norwitz ER, Robinson JN, Challis JR. The control of labor. New England Journal of Medicine. 1999;341(9):660-6. https://doi.org/10.1056/NEJM199908263410906
40. Moran C, Arriaga M, Arechavaleta-Velasco F, et al. Adrenal androgen excess and body mass index in polycystic ovary syndrome. The Journal of Clinical Endocrinology & Metabolism. 2015;100(3):942-50. https://doi.org/10.1210/jc.2014-2569
41. Wang JX, Davies MJ, Norman RJ. Obesity increases the risk of spontaneous abortion during infertility treatment. Obesity research. 2002;10(6):551-4. https://doi.org/10.1038/oby.2002.74
42. Randolph Jr JF, Zheng H, Sowers MR, et al. Change in follicle-stimulating hormone and estradiol across the menopausal transition: effect of age at the final menstrual period. The Journal of Clinical Endocrinology & Metabolism. 2011;96(3):746-54. https://doi.org/10.1210/jc.2010-1746
43. Williams RM, Ong KK, Dunger DB. Polycystic ovarian syndrome during puberty and adolescence. Molecular and Cellular Endocrinology. 2013;373(1-2):61-7. https://doi.org/10.1016/j.mce.2013.01.005
44. Bellver J, Rodríguez-Varela C, Brandão P, et al. Obese women exhibit reduced serum progesterone levels on the day of embryo transfer in artificially prepared cycles involving administration of vaginal progesterone. Reproductive BioMedicine Online. 2022. https://doi.org/10.1016/j.rbmo.2022.05.022
45. Lim S, Norman RJ, Davies M, et al. The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. Obesity Reviews. 2013;14(2):95-109. https://doi.org/10.1111/j.1467-789X.2012.01053.x
46. Dimitriadis E, Menkhorst E, Saito S, et al. Recurrent pregnancy loss. Nature reviews disease primers. 2020;6(1):1-19. https://doi.org/10.1038/s41572-020-00228-z
47. Hudić I, Fatušić Z. Progesterone–induced blocking factor (PIBF) and Th1/Th2 cytokine in women with threatened spontaneous abortion. 2009. https://doi.org/10.1515/JPM.2009.061
48. Coomarasamy A, Williams H, Truchanowicz E, et al. A randomized trial of progesterone in women with recurrent miscarriages. New England Journal of Medicine. 2015;373(22):2141-8. https://doi.org/10.1056/NEJMoa1504927
49. Pils S, Promberger R, Springer S, et al. Decreased ovarian reserve predicts inexplicability of recurrent miscarriage? A retrospective analysis. PLoS One. 2016;11(9):e0161606. https://doi.org/10.1371/journal.pone.0161606
50. Sultana S, Nallari P, Ananthapur V. Recurrent pregnancy loss (RPL): an overview. Journal of Womens Health and Development. 2020;3(3):302-15. Available from: https://fortuneonline.org/articles/recurrent-pregnancy-loss-rpl-an-overview.html