University of Kerbala

College of Applied Medical Sciences

Department of Clinical Laboratories

Evaluation of the Level of Dehydroepiandrosterone Sulfate (DHEA-S), Prolactin, Inhibin-B, and Thyroid Peroxidase Antibodies (TPO) in Females with Polycystic Ovarian Syndrome (PCOS) and Premature ovarian insufficiency (POI) in Karbala City

A thesis

Submitted to the Council of the College of Applied Medical Sciences - University of Kerbala as a Partial Fulfillment of the Requirements for the Degree of Master in Clinical Laboratories

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Dedication

Whoever says *" I am hers "*will get her. It was not a short journey, and the road was not easy, but I did, so thanks to my God, who facilitated beginnings and endings. Thanks to Almighty " *Allah* "always and forever for his bounty and kindness. First, I dedicate the fruit of this work to my great self, who endured all missteps and completed her story despite the difficulties. To who left me with his body but whose soul still flutters in the sky of my life, to the one whose name I carry with pride and love, my guardian angel, *" My Dad ".* To the woman who made me an ambitious girl who loved the challenges and difficulties, my first role model, who took the trouble of the road with me, and who supported and strengthened my *"My Beloved Mom".* To those who kept their hands in my hands in weak times, my steady ribs, "*My Brother and Sisters ".* To those who bet on my success and were always reminding me that I could do that "*Friends of the years* ".

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Summary

Infertility is defined as a failure to achieve pregnancy following one year of regular, repeat, consistent, and unprotected sexual intercourse.

Polycystic Ovarian Syndrome (PCOS) is a heterogeneous endocrine disorder distinguished by the manifestation of ovarian cysts, anovulation, and endocrine variation function. Premature Ovarian insufficiency (POI) is one of the ovulation problems and women's reproductive diseases and is among the leading causes of female infertility before 40 years of age.

The present study included 120 females with a range of age 20 to 45 years, collected from the infertility unit of Women Obstetrics & in Gynaecology Hospital during the period from November 2023 to April 2024, divided into two groups including group I 60 healthy apparent females used as controls and group II 60 infertile females with ovarian disorder used as patients, then divided to subcategories as follow: 47 infertile female with Polycystic Ovarian Syndrome, 13 infertile female with Premature Ovarian Insufficiency, individuals divided according to less and above 40 years. Parameters in this study measured include: infertility biomarkers (Inhibin-B, Anti-Müllerian hormone, Prolactin, Estrogen, Luteinizing hormone, and Follicle-stimulating hormone), androgen indices (Dehydroepiandrosterone Sulfate, and Testosterone), thyroid function indices (Thyroid-Stimulating Hormone, Triiodothyronine, and Thyroxine). Hormones levels were determined by using an enzyme-linked immunosorbent assay (ELISA) technique.

The present result found a significant increase ($P \leq 0.01$) in the level of Anti-TPO, DHEA-S, Inhibin-B, and AMH (16.34± 2.17), (2.79 ± 0.53), (266.97 ± 72.88), and (10.99 ± 2.37) in PCOS patients

respectively as compared to control groups (5.92 ± 0.96), (0.89 ± 0.12), (36.76 ± 10.62), and (3.11 ± 0.60), also found a significant decrease (P≤0.01) in the level of DHEA-S, Inhibin-B, and AMH (0.54 ± 0.23), (18.96 ± 1.21), and (0.60 ± 0.39) in POI patients respectively as compared to control groups (0.89 ± 0.12), (36.76 ± 10.62), and (3.11 ± 0.60), significant increase (P≤0.01) in the level of Anti-TPO (14.72 ± 1.19) in POI patients as compared to control (5.92 ± 0.96). The result showed a significant increase in BMI (P≤0.01) of PCOS and POI patients (30.93 ± 3.50), (31 ± 3.24) respectively as compared to the control group (27.90 ± 1.93). In PCOS according to age above and under (40 years), AMH showed a significant increase (P≤0.01) in both groups of years (>40), (<40) (10.89 ± 2.37), (11.45 ± 2.46) respectively, decrease in POI (<40) (0.60 ± 0.39), increase in Anti-TPO for both PCOS, POI (17.63±1.93), (17.63±1.93), increase (P≤0.01) in DHEA-S of PCOS (>40), (<40) (2.75 ± 0.55), (2.94 ± 0.46), decrease in POI (0.54 ± 0.23), Inhibin-B recorded a significant increase (P≤0.01) in PCOS (>40), (<40) (273.55 ±75.25), (234.91 ±52.43), decrease in POI (18.96 ±1.21). Prolactin, Testosterone, Estrogen, and LH revealed a significant increase (P≤0.01) in PCOS patients (29.24 ± 4.99), (58.53± 17.39), (186.25 ± 48.23), and (17.15± 5.81) respectively as compared to control groups (13.44 ± 3.30), (5.34 ± 0.90), (24.93 ± 7.23), and (2.93 ± 1.28), while FSH revealing a significant decrease (P≤0.01) levels in PCOS patients (5.83± 2.3) as compared to control (8.1± 2.15). The result found a significant increase (P≤0.01) in FSH, Prolactin, and LH in POI patients (33.51 ± 3.61), (26.06 ± 3.04), and (19.77 ± 2.88), a significant decrease (P≤0.01) in Estrogen and Testosterone (10.07 ± 1.43), and (3.24 ± 0.2) as compared to control groups (24.93 ± 7.23), and (5.34 ± 0.9) respectively.

TSH, T3, and T4 showed no any significant difference at (P>0.01) in PCOS patients than control. Also no significant difference at (P>0.01) in TSH, T3, and T4 in POI patients as compared to control groups.

In PCOS found a positive correlation found between DHEA-S with both AMH and Anti-TPO (r=0.729), (r=0.416). In POI patients found a positive correlation between DHEA-S and AMH (r=0.678) respectively. Also, in PCOS patients found a negative correlation between Inhibin-B and Anti-TPO (r= -0.520). With a negative correlation between AMH and Testosterone in POI patients (r= -0.578).

The result of receiver operating characteristic found Anti-TPO (95% CI: 0.907-1.000; P-value: 0.001; Cutoff Point: 9.306 AUC: 98.861%) with Sensitivity to Specificity 96.676%- 96.667%; and Accuracy: 97.000%, DHEA-S (95% CI: 0.992-1.0000; P-value: 0.001; Cutoff Point: 1.480 AUC: 99.694%) with Sensitivity to Specificity 98.333%-96.667%; and Accuracy: 97.500%, Inhibin-B (95% CI: 0.907-1.000; P-value: 0.001; Cutoff Point: 77.347; AUC: 95.417%) with Sensitivity to Specificity91.667%-98.333%; and Accuracy: 95.000%, AMH (95% CI: 0.918-1.0000; P-value: 0.001; Cutoff Point: 4.143; AUC: 96.306%) with Sensitivity to Specificity 96.667%-95.000%; and Accuracy: 95.830%.

In conclusion, Inhibin-B, Anti-TPO, Prolactin, AMH, and DHEA-S are more accurate biomarkers for assessing ovarian health and can be used as sensitive indicators for infertility and early diagnosis of PCOS and POI in women of reproductive age. The concentration of these parameters was higher in PCOS patients compared to the control, while AMH, DHEA-S, and Inhibin-B levels were significantly decreased in POI patients compared to the control. We showed that PCOS and POI were the most significant variables with weight gain and increased BMI.

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List of Abbreviations

Abbreviations	*Fill name*
AESG	Androgen Excess Society Guidelines
AFC	Antral Follicle Count
AMH	Anti-Müllerian Hormone
Anti-TPO	Anti-thyroid peroxidase
ASRM	American Society for Reproductive Medicine
AUC	Area Under Curve
BMI	Body Mass Index
BF %	Body Fat percentage
DHEA-S	Dehydroepiandrosterone-Sulfate
DOR	Diminished Ovarian Reserve
E2	Estradiol
ELISA	Enzyme-Linked Immunosorbent Assay
ESHRE	European Society of Human Reproduction and Embryology
FSH	Follicle-Stimulating Hormone
GnRH	Gonadotropin-releasing hormone
HRP	Horseradish Peroxidase
hPRL	Hyperprolactinemia
HPO-X	Hypothalamus-Pituitary-Ovary Axis
INHB-B	Inhibin-B
IR	Insulin resistance
LH	Luteinizing Hormone
MC	Menstrual Cycle
NIH	National Institute of Health

NICHD	National Institute of Child Health and Human Development
PCOS	Polycystic Ovarian Syndrome
PID	Pelvic Inflammatory Disease
PRL	Prolactin
POI	Premature Ovarian Insufficiency
POF	Premature Ovarian Failure
ROC	Receiver Operating Characteristic
SHBG	Sex Hormone-Binding Globulin
TG	Triglycerides
TGF-β	Transforming Growth Factor-β
SULT	Steroid Sulfotransferase
TMF	Total Mass Fat
TMB	Tetra-methylbenzidine
WHO	World Health Organization

Chapter One
Introduction

1. Introduction

Infertility is defined as a disorder of the reproductive system characterized by the inability to obtain a clinical pregnancy during 12 months or more of consistent unprotected sexual intercourse (Fathimunissa *et al*., 2022).

Infertility is a global health problem and is estimated to affect 12-30% of couples attempting to conceive, It may be attributed predominantly to the male (30%), the female (55%), or to a combination of both partners (40%). The most common causes of female infertility include ovulatory disorders (25%), tubal disorders (20%), and uterine/cervical factors (10%) (Koysombat *et al*., 2022). Ovulatory disorders are a serious clinical problem whose direct causes are still largely unknown, they appear as menstrual irregularities and are the cause of infertility in about 25% of couples who find it difficult to conceive and achieve it (Skowrońska *et al*., 2023). There are many causes of ovulation disorders such as polycystic ovarian syndrome (PCOS), premature ovarian insufficiency (POI), and diminished ovarian reserve (DOR), aging (obeagu *et al*., 2023).

Polycystic ovarian syndrome (PCOS) is currently viewed as one of the most common endocrine disorders in women of reproductive age (Sun *et al.*, 2020). There are some clinical characteristics of PCOs, the most prevalent of which are raised levels of androgens, hirsutism, an elevated ratio of luteinizing hormone to follicle-stimulating hormone (LH/FSH), with menstrual irregularity (Farhana, A. A., & Shi, J. 2024).

Premature ovarian insufficiency (POI) it is sometimes referred to as premature ovarian failure (POF) and is a mysterious and complicated disease, it is defined as a decrease in ovarian function under 40 years

(Mahmoud *et al*., 2020). Most women with POI have menstrual irregularities, 10% of them have amenorrhea (Alimovna e*t al.*, 2024).

Anti-thyroid peroxidase (Anti-TPO) is an enzyme found at the apical membrane of thyroid follicular cells (thyrocytes) involved in thyroid hormone biosynthesis (Akane *et al*., 2024). It is important in the thyroid hormone production operation because anti-TPO has two active positions and needs heme to function, its biostructure is complex (Godlewska *et al*., 2019). Anti-TPO is associated with infertility irrespective of thyroid hormone levels and can be used for screening as well as the marker for identifying the risk factor of infertility, thyroid autoantibodies may also be a component of more widespread autoimmune diseases and have a more universal impact on fertility and pregnancy (Konishi, S., & Mizuno, Y. 2022).

Dehydroepiandrosterone-Sulfate (DHEA-S) is a major adrenal androgen precursor and is considered the most abundant hormone in peripheral circulation (Naelitz *et al*., 2020). This androgen is synthesized from dehydroepiandrosterone (DHEA) by steroid sulfotransferase (SULT), DHEA and DHEA-S raised at a young age (Turcu *et al*., 2020). It is considered a primary hormone in the synthesis of testosterone and estradiol, as it is excreted by the zona reticularis of the adrenal gland, and from internal cells of the ovaries, brain, and gonads (Ozcil *et al.*, 2020). Decreased ovarian reserve, or a lower quantity and quality of ovum accessible for fertilization, has been linked in women with low DHEA-S levels. On the other hand, Women with elevated DHEA-S levels may be at risk for PCOS, which is the leading cause of female infertility (Zhang *et al.*, 2023)

Inhibin-B is a heterodimeric glycoprotein, non-steroidal widely recognized for its ability to inhibit the release of follicle-stimulating

hormone (FSH) (Yadav *et al*., 2022). In women, it enters into the formation of ovarian folliculogenesis and steroidogenesis and the regulation of the menstrual cycle, the high level of Inhibin-B means an increase in inhibition or decrease in the level of FSH and a low level in this hormone means a reduction in the quality and quantity of ovum resulting in problems with ovulation and causing infertility (Jankowska *et al.*, 2022).

Aims of the study

This study aimed to evaluate the relationship (Correlation) between some physiological and clinical parameters of fertile and infertile women undergone from different clinical statuses, this aim could be achieved by the following objectives:

1. The present study aims to estimate the level of Anti-TPO, Inhibin-B, DHEA-S, and AMH as biomarkers for infertility.
2. Study the association among biomarkers Anti-TPO, Inhibin-B, (DHEA-S), and AMH with body fat (BF%), body mass index (BMI), Total fat mass (TMF), age, and related with infertility in female patients.
3. Estimation of the level of hormone (FSH, Prolactin, LH, Testosterone, Estrogen, TSH, T3, and T4) and related infertility in female patients.
4. Find the correlation between diagnostic biomarkers with different hormonal parameters and related to infertility.

Chapter Two
Literature Review

2. Infertility

Infertility is defined as the inability to prove pregnant after 12 months or more of regular, repeats unprotected sexual contact (Skowrońska, M., 2023). It`s prevalent in a large proportion of the population. Globally The infertility rate is approximately 50-70 million couples affected by this syndrome, in some cases, infertility may be attributed to males, and female infertility may result from ovarian disorders such as insufficiency, diminished ovarian reserve (DOR), and fallopian tube obstruction, or maybe the cause of abnormal anatomy of the female reproductive system (Sirait *et al.*, 2023).

2.1 Etiology of the female infertility

1. Ovulatory causes
2. Tubal causes
3. Cervical causes
4. Uterine causes
5. Autoimmune diseases and uterine congenital defects affect conception and miscarriage (Sirait *et al.*, 2023).

2.2 Classification of Infertility

2.2.1 Primary infertility

This term is used to describe female infertility who have never been able to conceive a pregnancy, the most common causes of this type of infertility in women were the ovulatory factors (Magdum *et al.*, 2022). WHO estimated the overall prevalence of primary infertility lies between 3.8% and 16.8% (Purkayastha, N., & Sharma, H., 2021).

2.2.2 Secondary infertility

It is the inability to become pregnant after a prior pregnancy that occurs without the use of contraception, breastfeeding, or postpartum amenorrhea (Magdum *et al.*, 2022). The prevalence of secondary infertility is proportionally more than that of primary infertility, the main reasons for secondary infertility a pelvic inflammatory disease (PID) and tubal obstruction (Jabeen *et al.*, 2022).

2.3 Female infertility related to Ovarian causes

Ovarian disorders are the problems that affect ovaries and are considered a common cause of menopause, abnormal uterine bleeding, and infertility in females, and are a major cause of PCOS, there are many possible causes and contributions to ovarian dysfunction, and the therapeutic approach to ovulation dysfunction is likely to involve a range of psychological, medical, and procedural interventions (Munro *et al.*, 2022). This disorder causes infertility in about 25% of couples who find it difficult to conceive pregnant (Skowrońska *et al.*, 2023).

2.3.1 Causes of ovarian disorders

1. Aging
2. Endocrine disorder (Polycystic Ovarian Syndrome PCOS).
3. Primary Ovarian Insufficiency (POI) or Premature Ovarian Failure (POF)
4. Diminished Ovarian Reverse (DOR) (Abdelghani *et al.*, 2023).

2.4 Diagnostic of Infertility

Female infertility and associated diagnoses have overall health implications, beyond the treatment of patients' immediate reproductive needs, healthcare professionals must be aware of the broader health impact of specific causes of infertility to provide accurate counseling regarding long-term risk (Hanson *et al.*, 2017).

2.5 Treatment of infertility

Hormone replacement therapy is effective in some types of infertility, but there is substantial evidence from observational studies that such therapy increases the risk of breast cancer (Vermeulen *et al.*, 2019). Scientists have investigated other therapeutic measures, such as stem cell therapy, for infertility, stem cells are undifferentiated cells with the ability to renew themselves for long periods without significant changes in their general properties, they can differentiate into various specialized cell types under certain physiological or experimental conditions (Zhao *et al.*, 2019).

2.6 Ovaries

Ovaries are a pair of gonads intraperitoneal endocrine glands that are typically found in the lower left and right quadrants of the female abdomen, play a fundamental role in reproduction as well as the production of hormones (estrogen and progesterone), it also has a role in the fertility and cycling of reproductive activity in women, mainly by controlling the development of follicle (Tetkova *et al.*, 2020). The ovary structurally is made of an inner medulla, which contains vascular elements (blood and lymphatic vessels), and an outer cortex containing the ovarian follicles, the functional unit of the ovary (Dothard *et al.*, 2023). Figure (2-1).

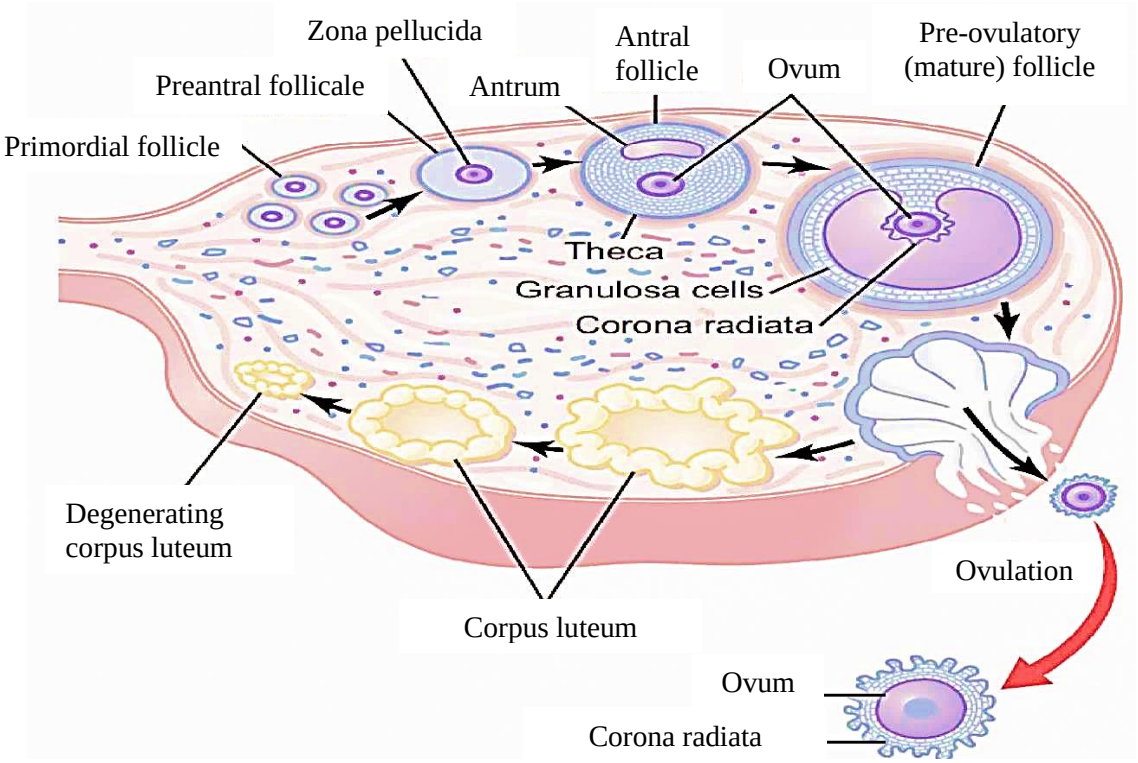


Figure (2-1) Ovarian structure and ovulation stages (Al-Deresawi *et al.*, 2006).

2.6.1 Ovum

In human physiology, the ovum is defined as a single cell released from the female genital organ (ovary), capable of evolving into a new organism when enriched with a sperm cell, during child-bearing years, 300 to 400 follicles mature and emit an ovum capable of being fertilized, the ovum has a large, centrally located nucleus which is covered by cytoplasm, this oocyte nucleus and nucleolus are termed germinal vesicle and germinal disc respectively, and is typically covered by 3 layers: inner thin vitelline membrane, middle zona pellucida, and outer corona radiata (Britannica, T., Siddharth, S. 2023) figure (2-2).

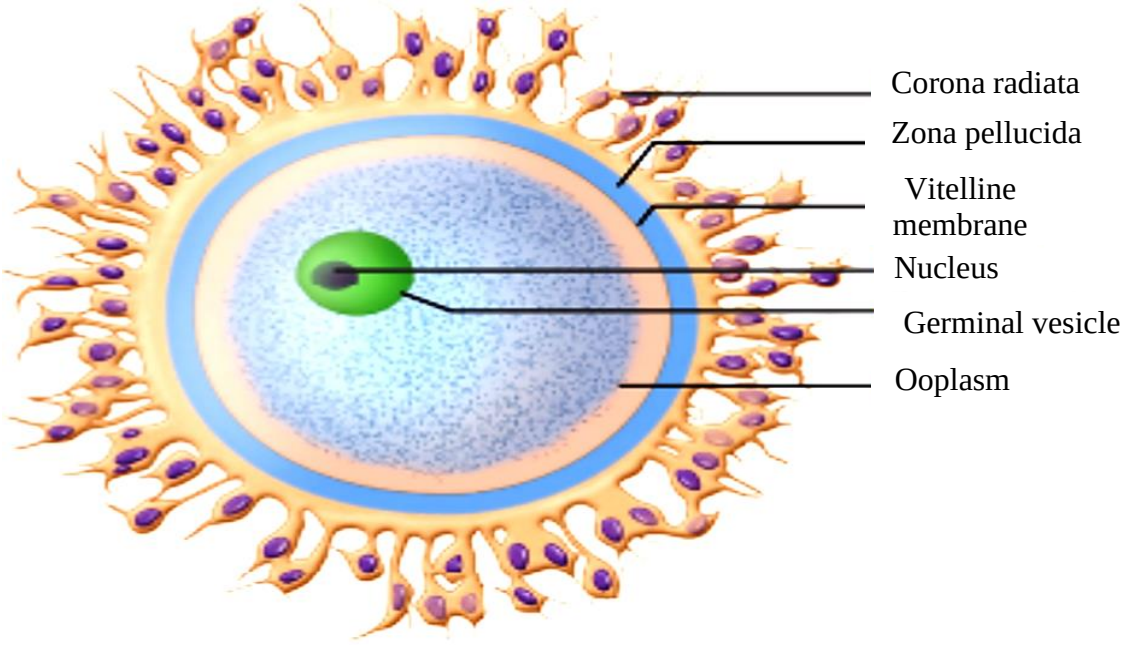


Figure (2-2) Structure of the human ovum (Britannica, T., Siddharth, S. 2023)

2.6.2 The Ovarian Hormones

2.6.2.1 Estrogen

Is a steroid hormone derived from cholesterol containing 18 carbon atoms, estrogens in females are produced by the ovaries, connected to the female reproductive system, and are in charge of the development of female sexual traits, it is also released from the yellow body after the ovum exits the vesicles and from the placenta (Lobo RA *et al.*, 2023). This hormone has a role in the ovum releases and has many forms:

1. Estrone (E1) is an estrogen that the body makes after menopause.
2. Estradiol (E2) is an estrogen produced in the body during reproductive years, it's a most potent form of estrogen.
3. Estriol (E3) is the main form of estrogen during pregnancy (Mahboobifard *et al.*, 2021).

2.6.2.2 Progesterone

Is the most abundant hormone produced by the gonads, adrenal cortex, and placenta in case of pregnancy, it is synthesized primarily by the

corpus luteum of the ovary, it`s also secreted by the ovarian corpus luteum during the first ten weeks of pregnancy, followed by the placenta in the later phase of pregnancy, the presence of this hormone is a prerequisite for embryo implantation, whereas its absence causes pregnancy loss (Kolatorova *et al.*, 2022). It has many other functions, such as preparing the endometrium, for gestation, and inhibiting lactation during pregnancy, it`s also plays a role in the menstrual cycle, causing capillary growth and development with the result of increased vascularization and blood flow (Bulletti *et al.*, 2022).

2.7 Menstrual Cycle (MC)

Is the periodic vaginal bleeding that occurs with the shedding of uterine mucus (menstruation), the length of the cycle is notoriously variable, but the average figure is 28 days from the start of one menstrual period to the start of the next (Rosner *et al.*, 2024).

2.7.1 Phases of Menstrual Cycle

2.7.1.1 Follicular or Proliferative phase

Is the first phase of the menstrual cycle it`s occurs from (1-14) days of the menstrual cycle, based on the average duration of 28 days, the main hormone during this phase is estrogen, that will provide negative feedback to the anterior pituitary (Herbison, A. E., 2020)

2.7.1.2 Ovulation phase

This phase it`s usually occurs 14 days before menses, and the estradiol provides positive feedback for FSH and LH production, that causes mature follicle breaks, and an oocyte is released (Soumpasis *et al.*, 2020).

2.7.1.3 Luteal or Secretory phase

The last phase of the menstrual cycle occurs from (14-28) days of the cycle, LH hormone stimulated to production of progesterone, that will be provide negative feedback to the anterior pituitary to decrease FSH and LH levels and, subsequently, reduce in the estradiol and progesterone levels (Guevarra *et al.*, 2023) figure (2-3).

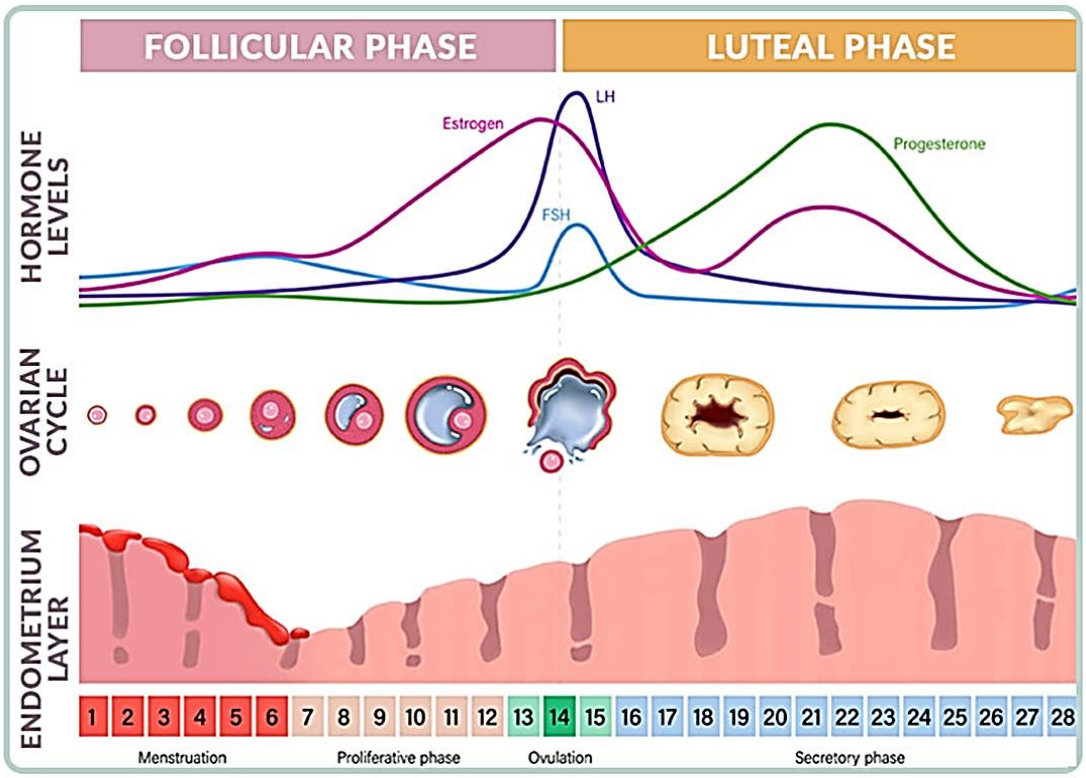


Figure (2-3) Diagram of the menstrual cycle and their phases (Chumduri, C., & Turco, M. Y. 2021).

2.8 Aging

The number and quality of the follicle are affected in an age-dependent manner, especially in women over 35 years of age follicular atresia accelerates sharply along with a lower pregnancy rate, higher abortion rate, and lower delivery rate (Chang *et al.*, 2022). When a woman is younger than 30, has an 85% chance to pregnant within 1 year, at the age of 30, there is a 75% chance to conceive in the first 12 months, this chance

declines to 66% at the age of 35 and 44% at the age of 40, this is due to the effect of aging on the ovary and ovum (Delbaere *et al.*, 2020). When a woman ages, the number and quality of ovum (ovarian reserve) decreases naturally and gradually from birth to menopause, and this decline gradually takes place until her early 30 years but accelerates rapidly after her mid-30 years, not only is it difficult to be pregnant, but abortion and chromosomal abnormalities in a child (such as Down's syndrome) are more common in older mothers, fibroids, endometriosis, and tubular diseases are more common and can affect fertility, older women are at risk of complications during pregnancy, such as pregnancy diabetes and preeclampsia (Chua *et al.*, 2020).

2.9 Endocrine disorders

These medical conditions occur in the endocrine system, which produces hormones, ultimately leading to a dysfunction of this system, and have several potential causes, such as tumors, genetic factors, or hormonal imbalances, and since the brain and peripheral glands, including the gonads, are vitally connected through the hypothalamus-pituitary axis, the hormones produced by this pathway can control several aspects of sexual activity, and defects in this hormonal pathway can affect many vital processes (Salvio *et al.,* 2021). The relation between endocrine disorders and fertility can be explained by the negative influence of hormonal disorders on ovarian function, and the increasing incidence of endocrine diseases with age may have further negative effects on the pregnancy rate (Herman *et al.*, 2023).

2.9.1 The Hypothalamus-Pituitary-Ovary Axis (HPO-X) disorders

This axis can be defined as a tightly regulated system controlling female reproduction and is a unit that cooperates to produce steroid and gonadotropic hormones on a cyclic basis to facilitate reproduction, this cycle is tightly regulated to select a dominant follicle for ovulation while priming the endometrium for implantation, and the ovary plays a pivotal role in the production of steroid hormones necessary for follicular development and oocyte maturation, this complex regulation can be negatively impacted when pathologies occur within any juncture of the HPO axis, the HPO axis constitutes 85% of ovulation disorders caused by PCOS, and endocrinopathies (Zhang *et al.*, 2020).

2.9.2 The physiological link of the HPO axis is explained as follows:

Gonadotropin-releasing hormone (GnRH) is the key regulator of the reproductive axis, which is generated by the hypothalamus gland, and stimulates the anterior pituitary gland to release the hormones LH and FSH, which in turn promote ovarian growth, FSH acts on granulosa cells and modulates the aromatase, while LH hormone stimulates theca cells, which produce estrogen, principally estradiol, in the ovary, the corpus luteum releases progesterone, these hormones (estrogen, estradiol, progesterone) can also inhibit the release of GnRH via negative feedback, to maintain the homeostasis of reproductive hormones (Ozawa H., 2022). Many studies have focused on the role of the hypothalamic-pituitary-ovarian (HPO) axis in the reproduction process, including follicle development and differentiation, ovulation, and follicular atresia, the HPO axis can regulate the ovulation cycle and ovum production directly or indirectly by mediating the levels of reproductive hormones secreted by the hypothalamus, pituitary, and ovaries (Li *et al.*, 2020). Figure (2-4)

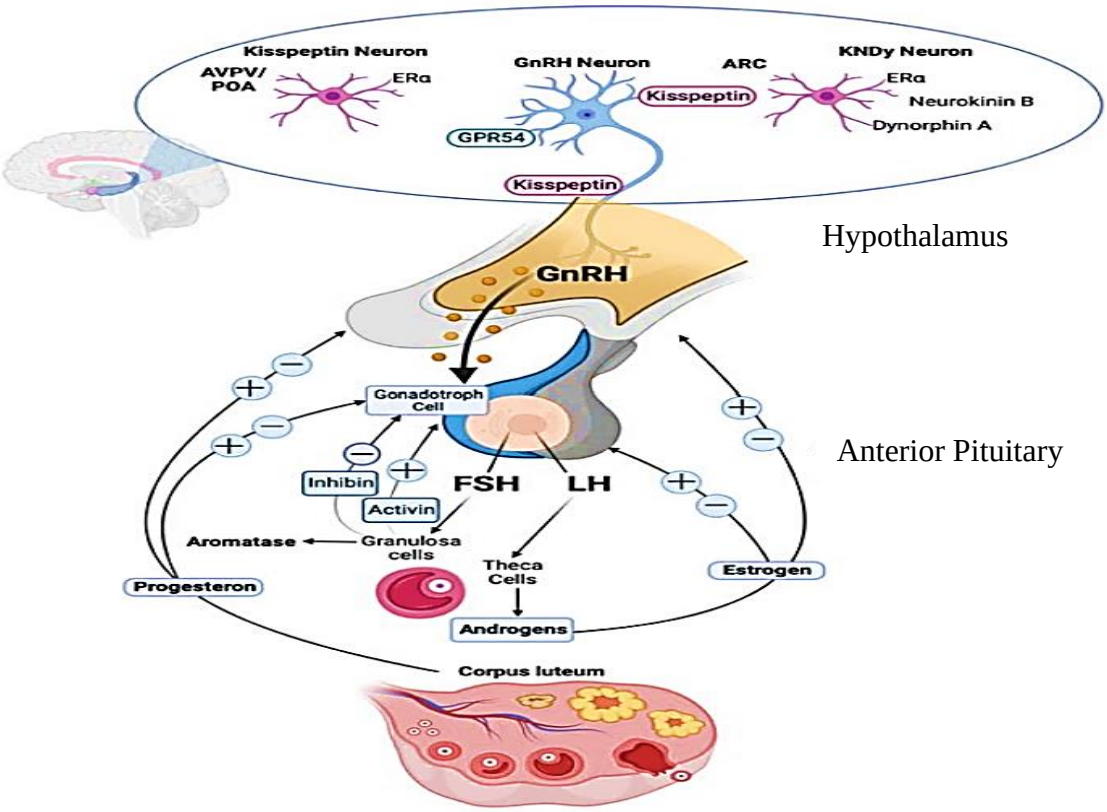


Figure (2-4) Female Hypothalamus-Pituitary-Ovary Axis (Tammasse IFU, Tamrin F., 2023)

2.9.3 Polycystic Ovarian Syndrome (PCOS)

Is the most common endocrine and metabolic disorder in reproductive women, with a prevalence affecting 5–20%, with up to 70% of affected women remaining undiagnosed (Pervin *et al*., 2022). It is characterized by hyperandrogenism, anovulatory cycles, the presence of multiple small follicles in one or both ovaries, infertility, and a higher risk of metabolic disorders such as; type 2 diabetes, liver fat disease, hypertension, clotting of blood vessels, possibly cardiovascular events, complications of childbirth, cancer, and possibly malignant tumors in the ovary and mood and psychological disorders (Xu, Y., & Qiao, J., 2022; Ali, S. S., & Jabeen, S., 2024).

2.9.4 History

In 1935 were recognized Leventhal and Stein as the main researchers of PCOS, while Vallisneri, an Italian physician, naturalist, and medical scientist, defined PCOS in 1721 when she observed many infertile women as enlarged size ovaries as pigeon ovum size with a shining white surface.

Formal diagnostic criteria were not proposed until the early 1990s at a National Institute of Health (NIH) sponsored conference on PCOS, and they were subsequently widely used (de Oliveira, N. M. G., 2020).

2.9.5 Etiology

Although the etiology of PCOS is unknown, evidence suggests that it is a complex condition in which interactions between environmental, genetic, endocrine, and metabolic factors work together to produce a shared outcome, it is unknown why these hormonal issues arise; some speculated that problem could stem from the ovaries, from a region of the brain that controls hormonal production, other glands in the body; also, insulin resistance could be to blame for these alterations (Siddiqui *et al.*, 2022). Figure (2-5)

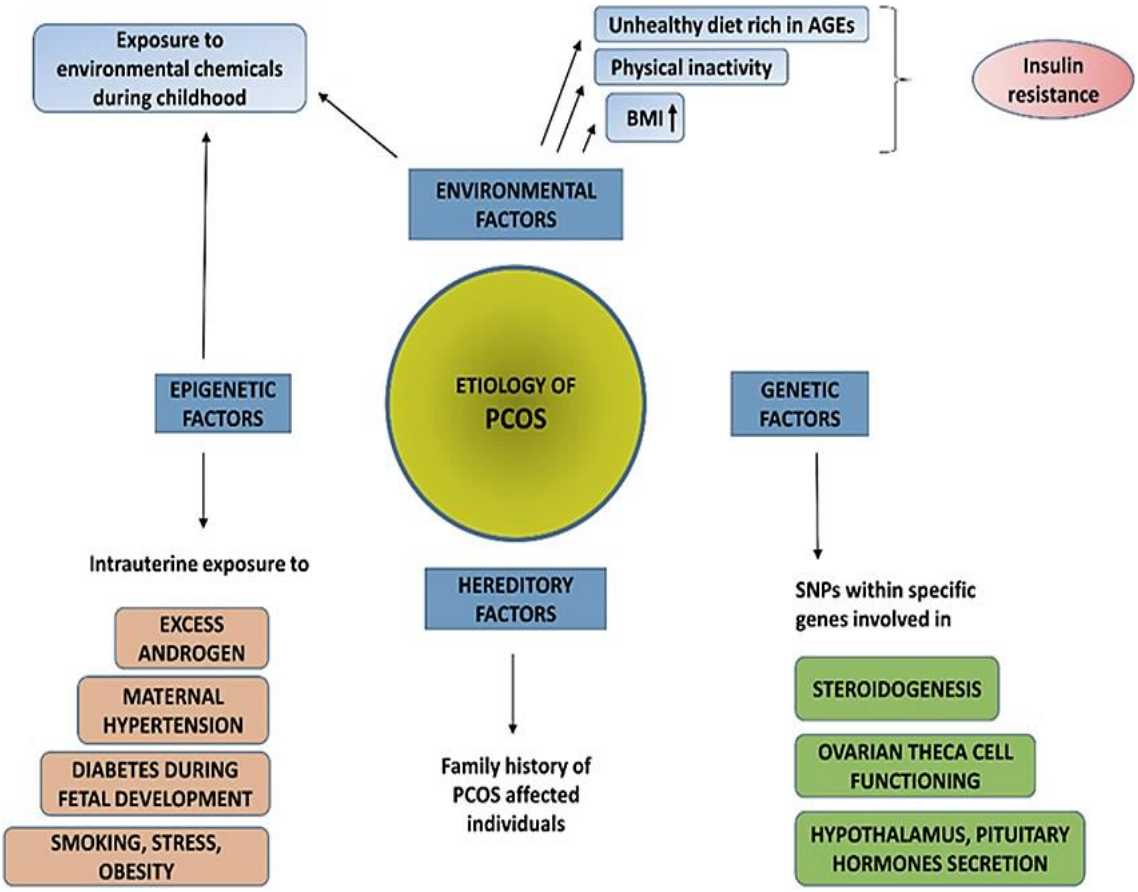


Figure (2-5) Etiology of PCOS (Siddiqui *et al.*, 2022).

2.9.6 Pathophysiology

Pathophysiology and neuroendocrine disruption of the hypothalamic-pituitary-gonadal axis in polycystic ovary syndrome can be explained by increased pulsatility of gonadotrophin-releasing hormone (GnRH) causes higher frequency of luteinizing hormone (LH) secretion and lower frequency of follicle-stimulating hormone secretion (FSH), consequent disrupted folliculogenesis, and increased production of ovarian androgens, adrenal androgens are also increased, excess levels of androgens stimulate deposition of abdominal adipose tissue which subsequently increases insulin resistance and hyperinsulinism (Dong, J., & Rees, D. A. 2023).

Hyperinsulinism causes increased androgen production from the ovaries and adrenal cortex, reduces the production of hepatic sex hormone binding globulin, and inhibits progesterone-mediated negative feedback onto GnRH neurons, worsening androgen excess in a vicious cycle (Safiri *et al.*, 2022). Figure (2-6)

So in women with polycystic ovary syndrome, raised levels of luteinizing hormone cause excess production of ovarian thecal androgens and Anti-Müllerian hormone (AMH), whereas relative deficiency of follicle-stimulating hormone (FSH) causes follicular arrest, polycystic ovarian morphology, and oligo-ovulation (Dong, J., & Rees, D. A., 2023).

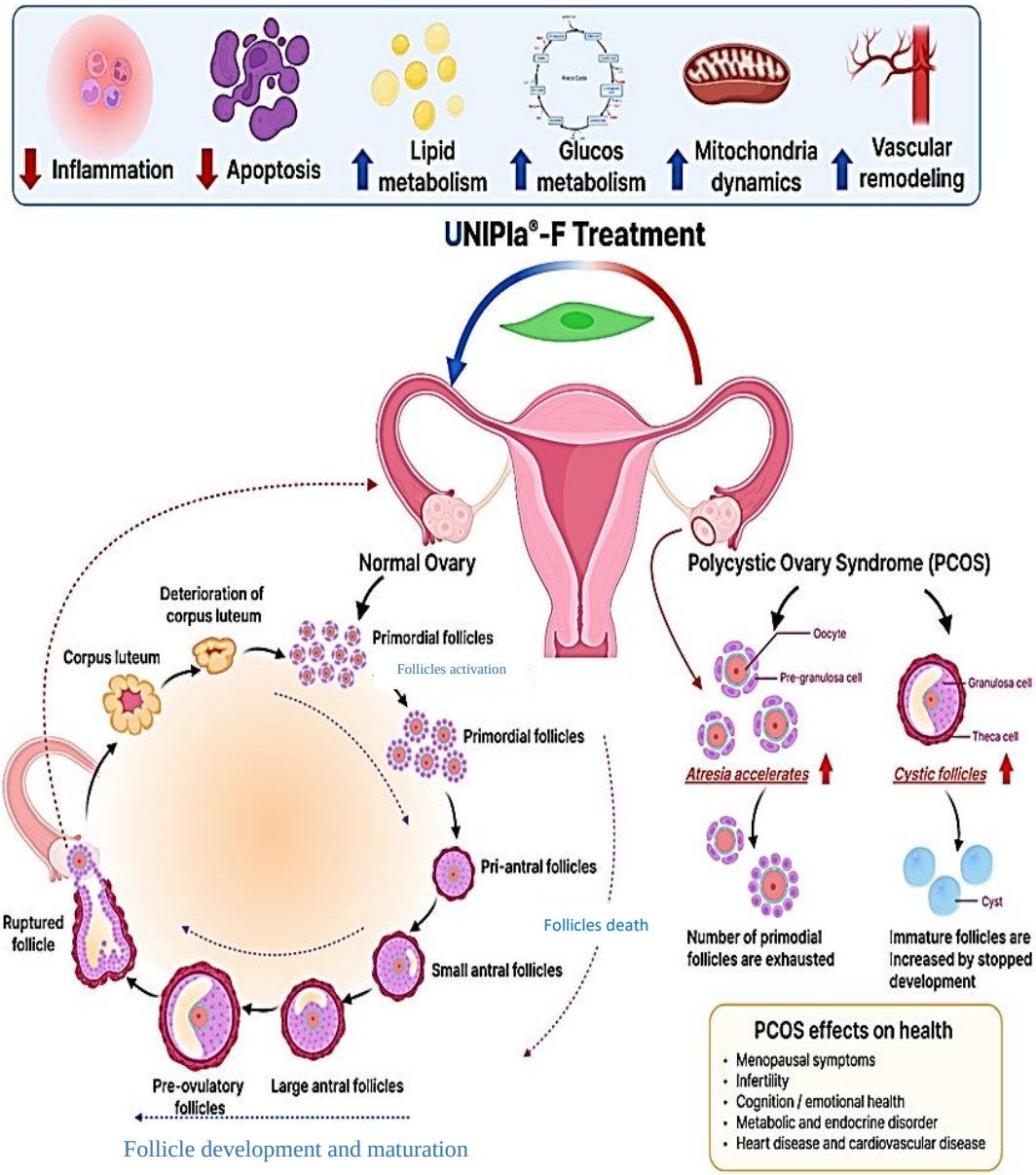


Figure (2-6) The pathophysiology of PCOS and the various clinical features that arise as a result (Bodepudi *et al.*, 2023).

2.9.7 Common Symptoms

The most common symptoms found for PCOS

1. Missed or irregular menstrual cycle
2. Hirsutism, Acne
3. Obesity or weight gain

4. Insulin resistance (IR)
5. Polycystic ovarian morphology (Karkera *et al.*, 2023).

2.9.8 Hyperandrogemia

Hyperandrogemia is a key diagnostic feature of PCOS, approximately 75% to 80% of patients with PCOS, androgen hyperactivation leads to ovulation disorder, menstrual disorder, hair, and acne formation, suggesting that hyperandrogenism is a clinical characteristic of PCOS and an important risk factor (Ye *et al.*, 2021). The persistent elevation of androgens in people with PCOS affects the synthesis of multiple proteins, particularly a decrease in the amount of sex hormone-binding globulin (SHBG), that allows for higher levels of free testosterone which disrupt ovarian theca cell function. Anovulation is progress as a result of androgen excess that developed because of the follicular arrest, there is a significant association between decreased maturation rates and developmental competency of ovum with androgen excess, further exacerbating the cycle of anovulation (Francone *et al.*, 2023).

2.9.9 Hyperprolactinemia

Hyperprolactinemia (hPRL) is defined as an increase of prolactin in blood above the normal range, is also common in women of reproductive age, and mimics the clinical phenotype of PCOS, the prevalence of hPRL is hard to accurately assess, however, it could be made about 8-17% of women with reproductive disorders (Rodier *et al.*, 2022). The hypothalamic is responsible for controlling the prolactin secretion process, in women with polycystic ovarian syndrome the defect in the HPO-axis effects the production of prolactin, High prolactin levels interfere with the normal production of other hormones, such as estrogen and progesterone, these can change or stop ovulation (the release of an ovum from the

ovaries), hyperprolactinemia is frequently reported with polycystic ovary syndrome (PCOS), however, there is a controversy about whether they share a common mechanism have a cause–result relationship, or just are coincidental (Hussein *et al.*, 2020). This defect causes hypogonadism, irregular menstruation, or menopause in women, it`s usually appears in the form of an ovulation disorder and is often associated with amenorrhea and ovulatory infertility (Gierach *et al.*, 2022).

2.9.10 Diagnostic Criteria

PCOS can be diagnosed in any female when at least two of these three features: are clinical hyperandrogenic, ovulation abnormalities, and an ultrasound image showing cysts in the ovaries, this type of diagnosis is termed a Rotterdam Criteria for the diagnosis of PCOS (Kolhe *et al.*, 2022). For the diagnosis of PCOS in premenopausal women, there are three sets of diagnostic criteria demonstrated in Figure (2-7).

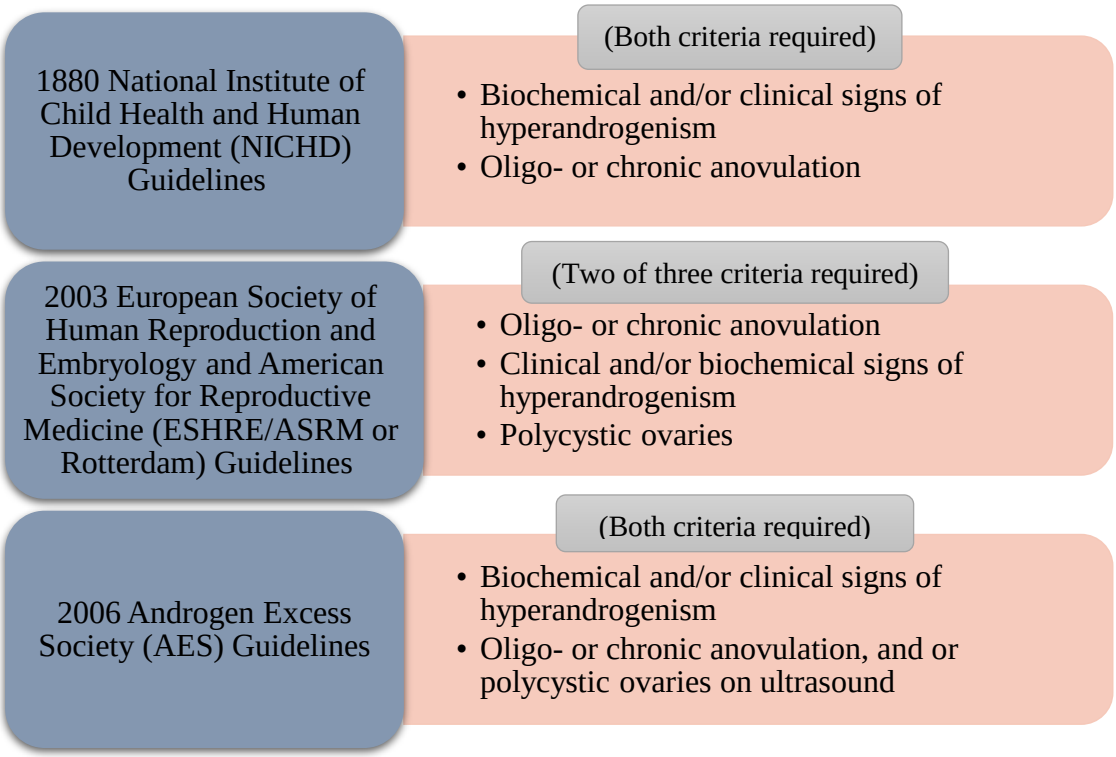


Figure (2-7) List of criteria for diagnosis of PCOS (Crespo *et al.*, 2018).

2.10 Premature Ovarian Insufficiency (POI)

Premature ovarian insufficiency (POI) currently the preferred term for premature ovarian failure (POF), is defined as a clinical disorder manifested with menstrual disturbance (oligomenorrhea or amenorrhea) in the female, or the intermittent or permanent gonadal insufficiency before the age of 40 years, the European Society of Human Reproduction and Embryology (ESHRE) defines POI as amenorrhoea for at least 4 months and a high, postmenopausal level of FSH in at least two samples at least 4 weeks apart (Silvén *et al.*, 2022). POI affects approximately 1% of women globally, year by year the incidence rate of POI is rising due to various influences from the environment and diseases (Yuk *et al.*, 2021).

The symptoms of this gynecological endocrine disease include a decrease in ovarian function in women before the age of 40, followed by amenorrhoea, an increase in luteinizing hormone (LH), follicle-stimulating hormone (FSH), and a decrease in estrogen, POI may cause loss of normal endocrine function, which could lead to early depletion of the primordial follicle pool (Han *et al.*, 2023).

POI not only affects the reproductive woman's potential but is also related to an increased risk of hypoestrogenism-related problems such as heart disease, osteoporosis, and early mortality, these women may also suffer from other autoimmune problems such as autoimmune hypothyroidism or adrenal insufficiency, in addition to other co-morbidities such as depression and anxiety, therefore, could be considered a substantial health issue, not only for the affected person but also for her family and the general public health (Ge, L., & Tan, J. 2020).

2.10.1 History

In 1842, American endocrinologist Fuller Albright was the first to prescribe "primary" ovarian insufficiency, as a loss of ovarian activity that leads to a chronic hypo-estrogenic state in women under the age of 40 years, research suggests a lot of terminologies that have been used to describe this disorder, such as premature ovarian failure, premature menopause, and hypergonadotropic hypogonadism, however, the most commonly used term in Europe is premature ovarian insufficiency, which encompasses both primary POI, and secondary POI which resulting from iatrogenic interventions including chemotherapy, radiotherapy, and surgery (Rahman *et al.*, 2021).

2.10.2 Etiology

The etiology of POI is complex and has not yet been known and elucidated, but the genetic factors, there are an increasing number of studies have revealed that epigenetic changes play an important role in the occurrence and development of POI, also causes of POI could include a reduction in the primordial follicle pool via accelerated follicular atresia or destruction, or problems in the support, recruitment, or maturation of primordial or growing follicles (Demayo *et al.*, 2018).

Most occurrences of spontaneous POI are idiopathic, this failure has a wide range of recognized causes, including infections, chromosomal abnormalities, autoimmune diseases, radiation therapy, chemotherapy, genetics, and disorders related to metabolism and storage (Elhddad, A. S., 2020) figure (2-8).

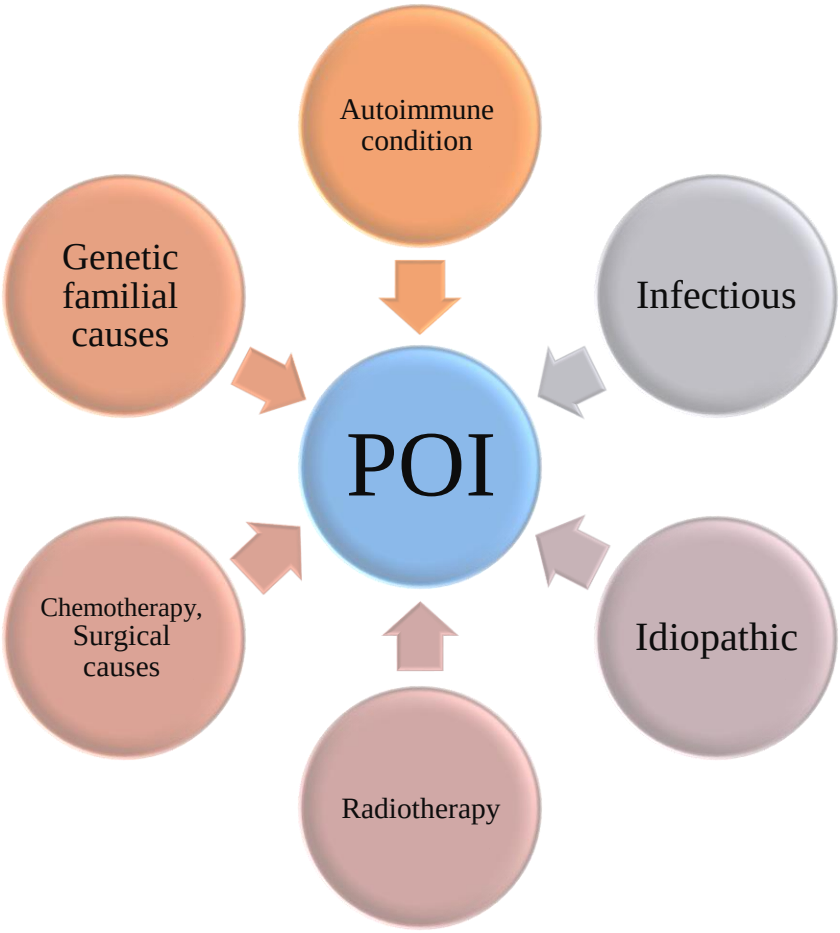


Figure (2-8) General causes of premature ovarian insufficiency (Wang *et al.*, 2023).

2.10.3 Pathophysiology

Women are born with 700,000–1 million oocytes within primordial follicles, POI occurs due to follicle dysfunction and follicle depletion, subsequent cause infertility finally loss of ovarian estrogen production, it`s a rare disorder have elevated levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) despite normal Anti-Mullerian hormone (AMH) and antral follicle count (AFC), the ovaries are unresponsive to endogenous and exogenous FSH due to genetic or immunological inactivation of the FSH or LH receptor (Ford EA *et al.*, 2020). Also possible that spontaneous POI may occur as part of an aging syndrome in some women, there is increasing evidence that epigenetic aging can begin

as early as a few weeks post-conception, this is a major public health issue given the well-recognized long-term sequelae of POI, such as osteoporosis, cardiovascular disease, and dementia, the pathophysiological mechanisms of POI, including the phenomenon of epigenetic aging in developing organs such as the ovaries (Hellstrom *et al.*, 2021). figure (2-9).

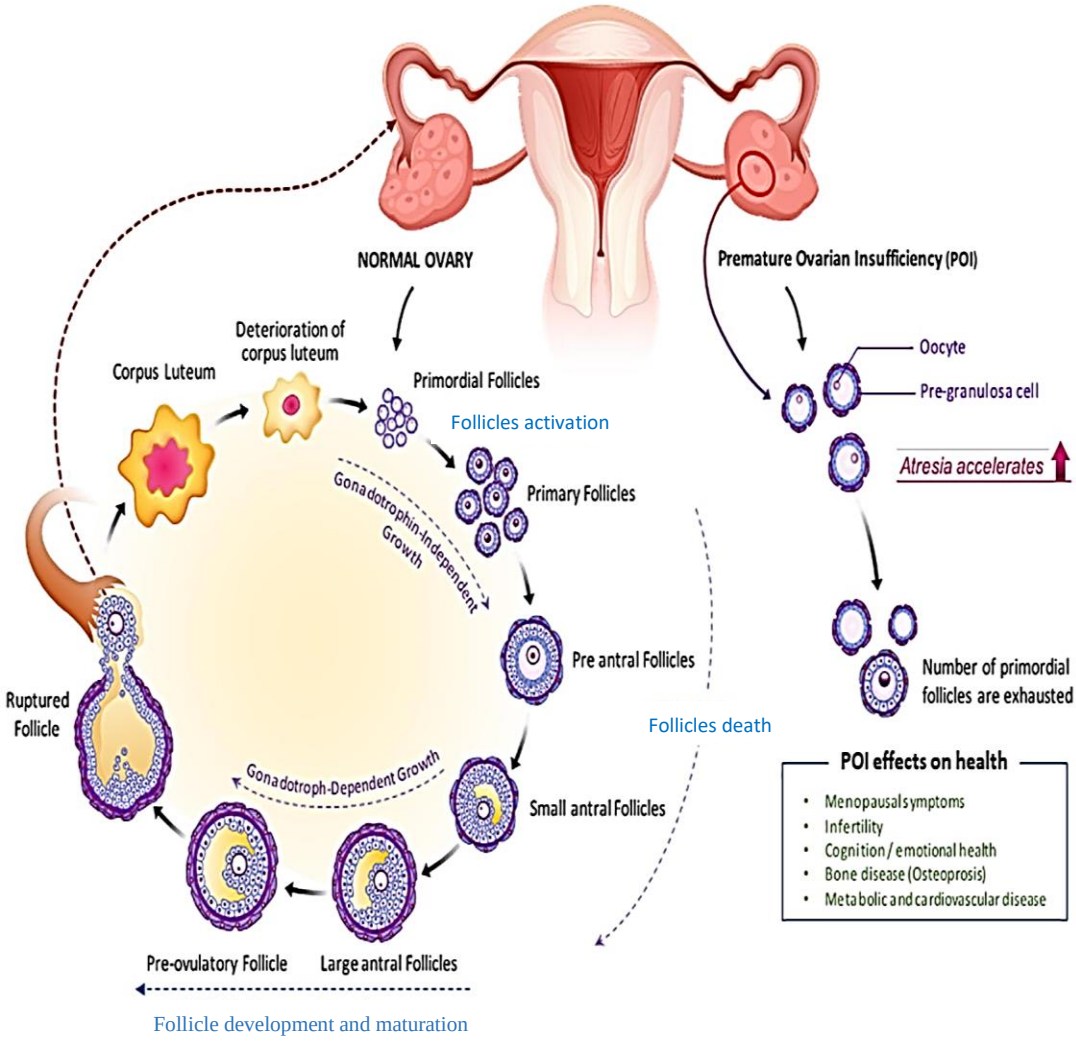


Figure (2-9) Pathophysiology of Premature ovarian insufficiency (Chon *et al.*, 2021).

2.10.4 Diagnostic criteria

POI is diagnosed when occurring amenorrhea in women, with elevated pituitary gonadotropin follicle-stimulating hormone (FSH), and low levels of estradiol (E2), serum levels of FSH and E2 are measured at

least two times in more than 4 weeks, and patients that present with continuously elevated FSH levels are diagnosed with POI (Wesevich *et al.*, 2020).

Therefore, figure (2-10) shows the criteria for the diagnosis of POI in women before the age of 40, when these traits are present in patients

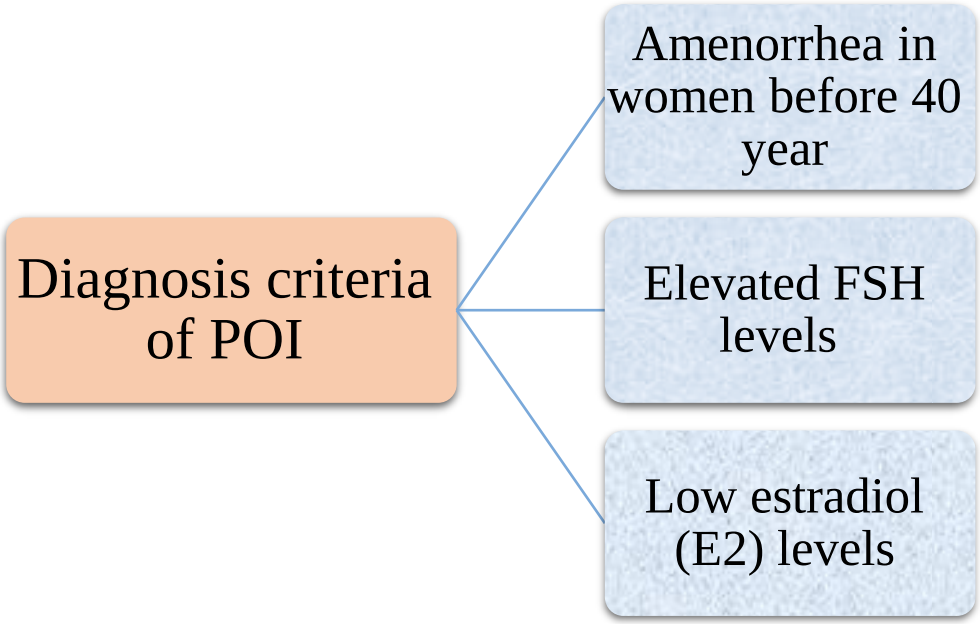


Figure (2-١0) Diagnostic criteria of POI (Wesevich *et al.*, 2020)

2.11 Obesity

Obesity's influence on female infertility is a topic of significant concern in reproductive medicine, these changes can affect ovarian function, and disrupt the hormonal balance necessary for ovulation, there are several research studies into the intricate relationship between obesity and female infertility a linear decline in spontaneous pregnancy rates was observed with each increase in body mass index (BMI) (Ennab, F., & Atiomo, W. 2023).

2.11.1 Obesity and related to PCOS

Epidemiological evidence supports the tight relationship between obesity and PCOS, showing that 38% to 88% of women with PCOS are

overweight or obese (Barber, T. M., & Franks, S. 2021). Obesity is considered a significant risk factor for PCOS, the association between obesity and PCOS may be explained by the connection between obesity and PCOS could be attributed to the potential of excess weight to induce insulin resistance, a condition in which the body's cells do not respond appropriately to the hormone insulin, which regulates blood sugar levels, elevated blood sugar levels resulting from insulin resistance may lead to increased production of androgens and male hormones, which can exacerbate PCOS symptoms, so people who are overweight or obese are significantly more likely to develop PCOS than those who maintain a healthy weight, suggesting that genes and fat intake may interact to increase the risk of PCOS (Sekar *et al.*, 2024).

2.11.2 Obesity and related to POI

Infertility is recurrently observed in obese women of reproductive age, who usually present menstrual disorders and anovulatory cycles, lower implantation and pregnancy rates, as well as failed assisted reproductive interventions, infertility is, therefore, a prominent co-morbidity of obesity, the etiology of which remains largely understudied. The impairment of reproductive function in obese females occurs at both central and peripheric levels and can affect either the ovaries or the endometrium (Sharma, Y., & Galvão, A. M. 2023).

2.12 Thyroid disorders and related to infertility

As of the present, several questions remain unresolved; new studies still continue to study and investigate the correlation between thyroid dysfunction and female infertility (Poppe, K., 2021).

Thyroid hormones have a crucial role in several facets of female reproductive function, including folliculogenesis and placentation.

Thyroid function, delicately controlled by the hypothalamic-pituitary axis, can significantly impact reproductive processes when altered (Concepción-Zavaleta *et al.*, 2023).

2.7.1 Thyroid disorders and related to PCOS

Thyroid abnormalities can lead to menstruation irregularities, infertility, and metabolic issues, and are more prevalent among females (Palomba *et al.*, 2023). Despite the limited and ambiguous data demonstrating the relationship between thyroid dysfunction with PCOS, increasing evidence indicates a possible association between these diseases (Hu *et al.*, 2022). Genetic predisposition to the development of both conditions may serve as a potential link between thyroid illness and PCOS; however, a shared genetic profile has yet to be identified, moreover, it was postulated that a disturbed estrogen/androgen equilibrium in women with PCOS may predispose them to hypothyroidism; nevertheless, the evidence remains equivocal, further extensive studies in these fields are needed to assess which mechanisms cause this correlation (Palomba *et al.*, 2023).

2.8.2 Thyroid disorders and related to POI

Autoimmune-related illnesses are believed to account for 4–30% of instances of Primary Ovarian Insufficiency (POI). The most strongly associated group is that of thyroid-related disturbances such as hypothyroidism, Hashimoto thyroiditis, and Grave's disease, the second most prevalent category of autoimmune illnesses, behind thyroid diseases, pertains to the adrenals (Colella *et al.*, 2020). Literature indicates that 10–20% of people with Addison's illness will develop primary ovarian insufficiency (POI), women with diabetes mellitus face an elevated chance of having primary ovarian insufficiency, with an estimated incidence of 2.5%, POI has been linked to several other conditions, including

rheumatoid arthritis, Crohn’s disease, myasthenia gravis, systemic lupus erythematosus, and multiple sclerosis (Szeliga *et al.*, 2021).

2.13 Biomarkers related to infertility

2.13.1 Anti-thyroid peroxides (Anti-TPO)

Are the thyroid-specific antibodies, a membrane-bound protein located on thyrocytes, moreover, it acts as the key enzyme in the synthesis of thyroid hormones (Esfandiari *et al.*, 2018). And functions as a defense against thyroglobulin, or anti-TG, the substance that thyroid hormones are synthesized and stored in opposition to, these antibodies' interaction with TPO and TG results in a lymphocytic response, which in turn leads to fibrosis of the thyroid gland and decreased thyroid hormone synthesis, thyroid autoantibodies may also be a component of more widespread autoimmune disease and may have a more universal impact on fertility and pregnancy (Poppe K., 2021). Anti-TPO can be used for screening and as a marker to identify the infertility risk factor because it is highly linked to infertility regardless of thyroid hormone levels, additionally, the identification of anti-TPO expression in the endometrium and placenta may explain the higher incidence of abortion and infertility in patients with thyroid autoimmunity (Rahnama *et al.*, 2021). The mechanism is clarified by the Anti-TPO may cross through the blood follicular barrier and create a cytotoxic environment that damages the maturing oocyte during the maturation period, thus, it is proven that the rise in these antibodies can lead to impaired fertility and miscarriage and sometimes lead to premature birth (Silva *et al.*, 2022).

2.13.2 Anti-Müllerian hormone (AMH)

AMH is classified as a dimeric glycoprotein within the superfamily of transforming growth factor β (TGF β), and is considered as a predictor of ovarian response to gonadotropin stimulation (Vijay *et al.*, 2022). Is expressed in females by the ovaries, from granulosa cells of small, large preantral, and small antral follicles, where it regulates folliculogenesis (Dilbaz, B., & Mert, Ş. A. 2022).

The production of AMH initiates when activation of primordial follicles occurs, with maximal secretions from preantral and small antral follicles, and there is a subsequent decrease in secretion from follicles that develop to the antral stage, regardless of AMH biochemical and biological properties, this hormone has been recognised to be associated with antral follicle count (Akbarinejad *et al.*, 2020).

Many researchers have found that as people age, their serum AMH levels decrease, which is indicative of fewer developing follicles that are accessible for recruitment (Chen C, Kattera S., 2020). AMH is also used as a quantitative function for ovarian aging as a sign of ovarian cancer and as a sign of ovum quality, and is used to assess fertility in women (Umarsingh *et al.*, 2020). This hormone is notably increased in women with polycystic ovary syndrome (PCOS), the main cause of infertility in women (Di Clemente, N., 2021) figure (2-11).

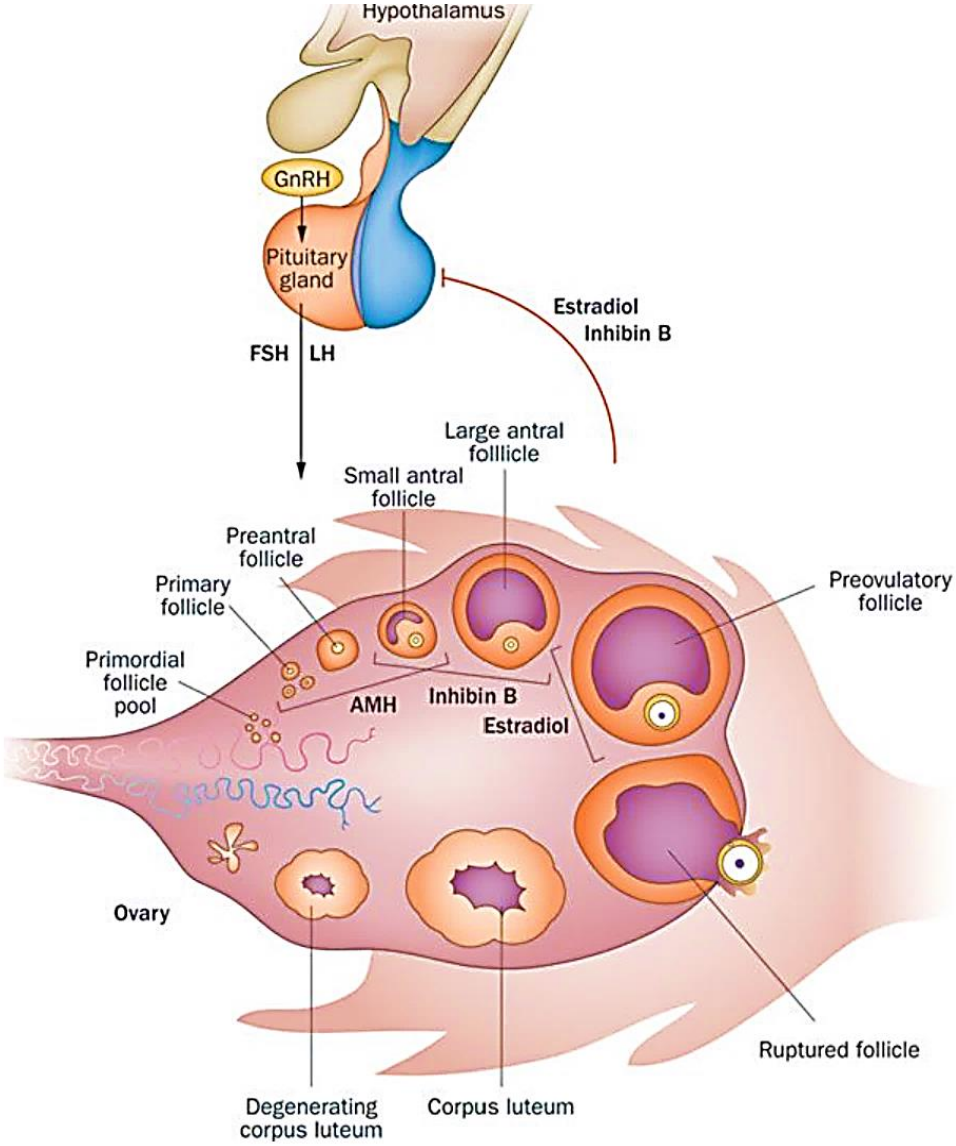


Figure (2-11) Anti-Müllerian hormone: an ovarian reserve marker (Visser *et al.*, 2012).

2.13.3 Inhibin-B

Is a heterodimeric glycoprotein that comprises an alpha subunit linked to a beta-β subunit, and belongs to the superfamily of transforming growth factor-β, this non-steroidal hormone, which is secreted by the granulosa cells of developing follicles, is widely recognized for its ability to inhibit the release of follicle-stimulating hormone (FSH), the pituitary gland has a direct negative feedback loop when there is an elevated level of Inhibin-B in the serum, which causes the FSH to drop, thus, one of the key elements

in maintaining a low level of serum FSH is the increased amount of Inhibin-B in women of reproductive age, but as they become older, their ovarian follicles become fewer in number and quality, their blood levels of Inhibin-B gradually drop, and their ability to block FSH is diminished, this is another significant factor contributing to the steady rise in their serum FSH levels (Wen *et al.*, 2021). Figure (2-12).

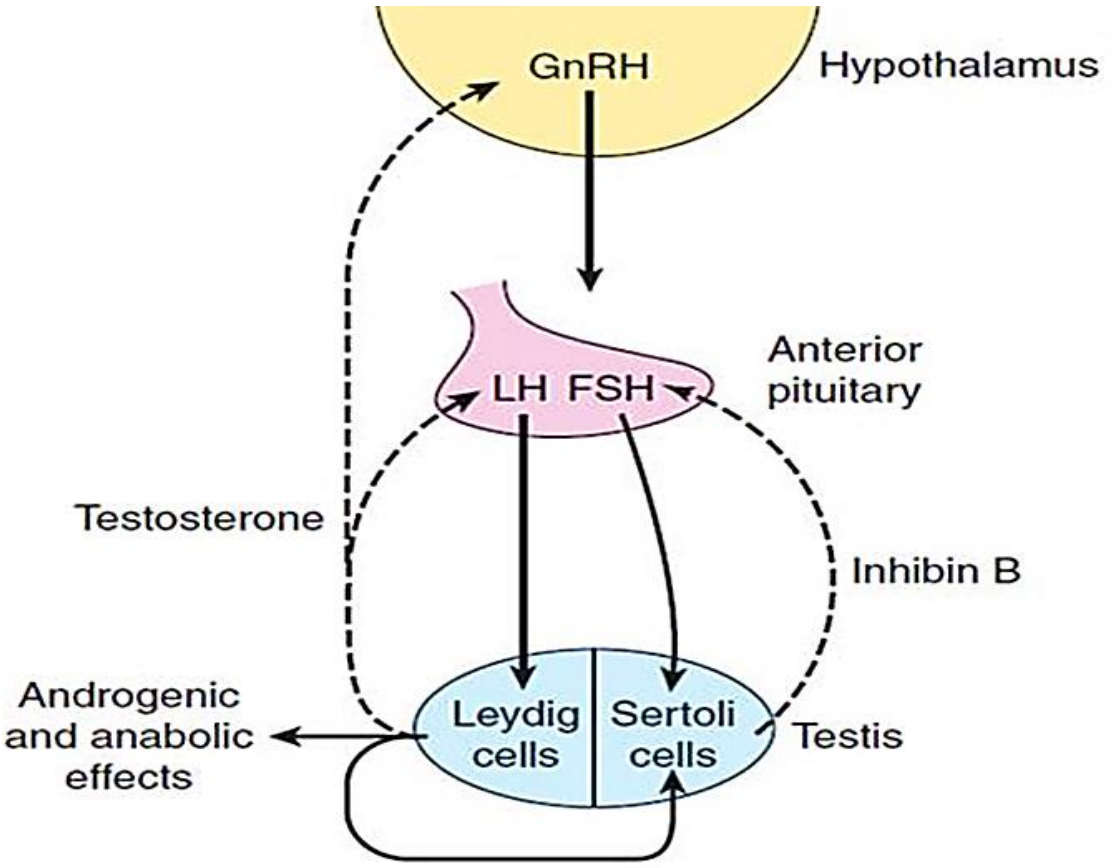


Figure (2-12) Mechanism of Inhibin B that secretion from granulosa cell and inhibits FSH secretion (Chen, C., & Kattera, S. 2020).

2.13.4 Dehydroepiandrosterone Sulfate (DHEA-S)

Is the most abundant steroid in human blood circulation, an essential substrate for the synthesis of steroid hormones with many potential effects, and is mainly produced in the adrenal cortex (produce about 80%) and ovarian cells of women (produce about 20%) (Li, C. J., 2021). And the epidemiological evidence suggests that serum DHEA levels in women decrease with age, previous studies have shown that in the ovary, DHEA

increases the sensitivity of granulosa cells to gonadotropin production such as FSH, promotes follicle recruitment, and increases the number of follicles that can be recruited in the ovary (Xie *et al.*, 2020). It is generally the DHEA and DHEA-S that inter-convert freely retrospectively and continuously by hydroxysteroid sulfotransferases and steroid sulfatase, and the DHEA primary hormone in the synthesis of sex steroid hormones (Estradiol, Testosterone, and Esteron) by the zona reticularis of the adrenal gland, and from internal cells of the ovaries, brain, and gonads (Ozcil, M. D., 2020). Decreased ovarian reserve, or a lower quantity and quality of ovum accessible for fertilization, has been linked in women with low DHEA-S levels, on the other hand, women with elevated DHEA-S levels may be at risk for PCOS, which is the leading cause of female infertility figure (2-13) (Zhang *et al.*, 2023).

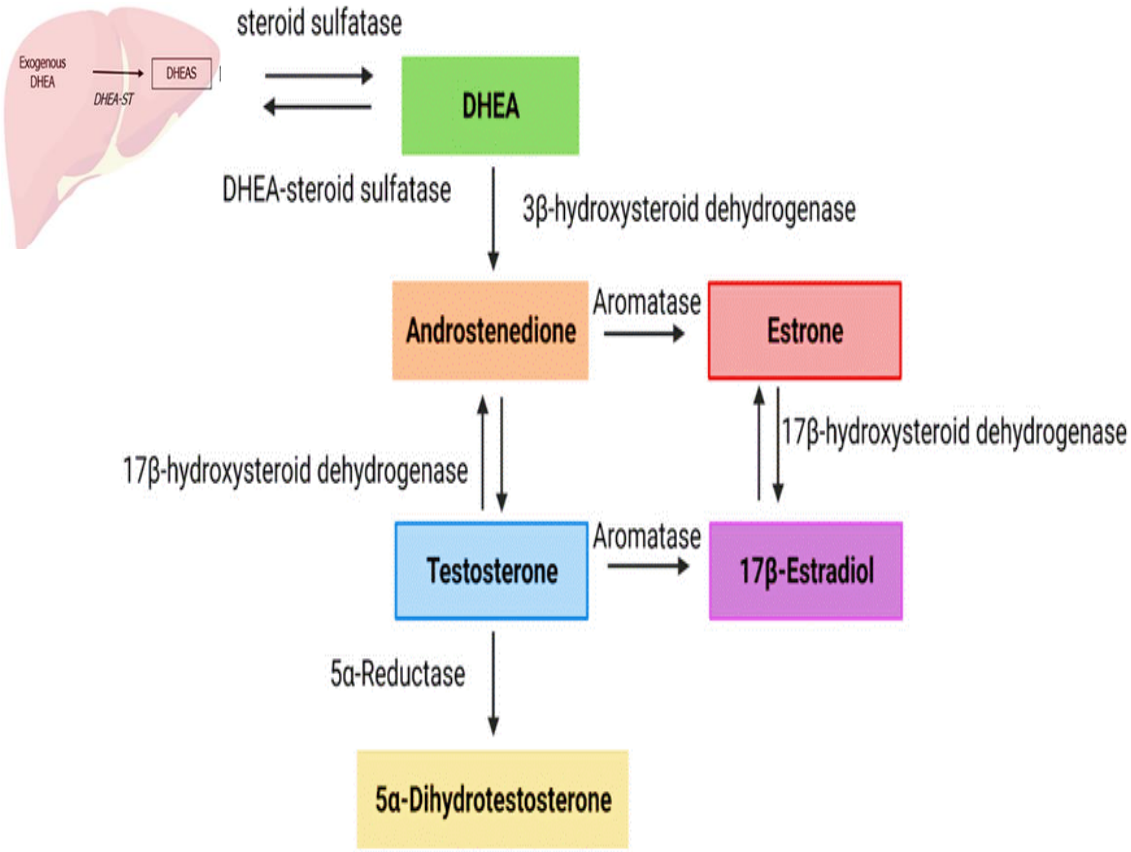


Figure (2-13) Metabolism of exogenous dehydroepiandrosterone (Jethwani *et al.*, 2023).

Chapter Three
Material and Methods

3.1 Instruments and Laboratory Equipment used in the present study

The main instruments and apparatus used in the present study were found in table (3-1)

Table (3-1): List of the instruments with their company and origin.

No	Instrument Name	Company	Origin
1.	Centrifuge	Universal 16 A	Sweden
2.	Cotton	Dunya cotton	Iraq
3.	Disposable syringes 5ml	SUGAMA	China
4.	Deep freeze	Concord	Lebanon
5.	Enzyme-linked immune sorbent assay (ELISA) system	Human	Germany
6.	Eppendorf tube	Fisher Scientific	China
7.	Gel tube	Biozek	China
8.	Gloves	TGS	China
9.	Human Reader HS	Human	Germany
10.	Micropipettes	Huma pette	Germany
11.	Multichannel Micropipettes, Fixed with different sizes	Slamed	Germany
12.	Plain tube	Livcare	India
13.	Refrigerator	Concord	Lebanon
14.	Spectrophotometer	Human	Germany
15.	Tourniquet	Guangjing Medical	China
16.	Tip (micropipette yellow, blue)	Expondo	Germany

3.2 Kits have been used in the research

The important diagnostic kits used in this study were found in table (3-2):

Table (3-2) List of Kits used in the present study:

No	Kits	Company	Origin
1.	Anti-Müllerian hormone (AMH)	Cloud-Clone Crop	USA
2.	Anti-thyroid peroxidase (anti-TPO)	Cloud-Clone Crop	USA
3.	Dehydroepiandrosterone sulfate (DHEA-S)	Elabscience	USA
4.	Estrogen (E2)	Could-Clone Crop	USA
5.	Follicle-stimulating hormone (FSH)	Could-Clone Crop	USA
6.	Inhibin-B	Could-Clone Crop	USA
7.	Luteinizing hormone (LH)	Could-Clone Crop	USA
8.	Prolactin hormone	Cloud-Clone Crop	USA
9.	Thyroid-stimulating hormone (TSH)	Cloud-Clone Crop	USA
10.	Triiodothyronine (T3	Could-Clone Crop	USA
11.	Thyroxine (T4)	Could-Clone Crop	USA

3.3 Study Design

The case-control study design is demonstrated in Figure (3-1).

In this study was thorough 120 females were enrolled, ages 20-45 years. And samples were collected from Women Obstetrics & Gynecology Hospital and private clinics. The time frame for conducting the study was between October 2023 and May 2024. And based on the study`s experimental design and data formation for all participants. The 120 volunteers in the current study were divided into two groups as follows:

Group A: (60) infertile females suffering from ovarian disorders are used as patients (Kabodmehri., 2022). Then subdivided into the following groups:

Group I: 47 infertile females with Polycystic Ovarian Syndrome

Group II: 13 infertile females with Premature Ovarian Insufficiency

Group III: Infertile females divided according to less and above (40 years) (Haapakoski., 2024).

Group B: 60 healthy apparent females used as control

This group includes 60 healthy females who appeared to be without symptoms or chronic disease at the time of collection of samples. They were age-matched to the patient group.

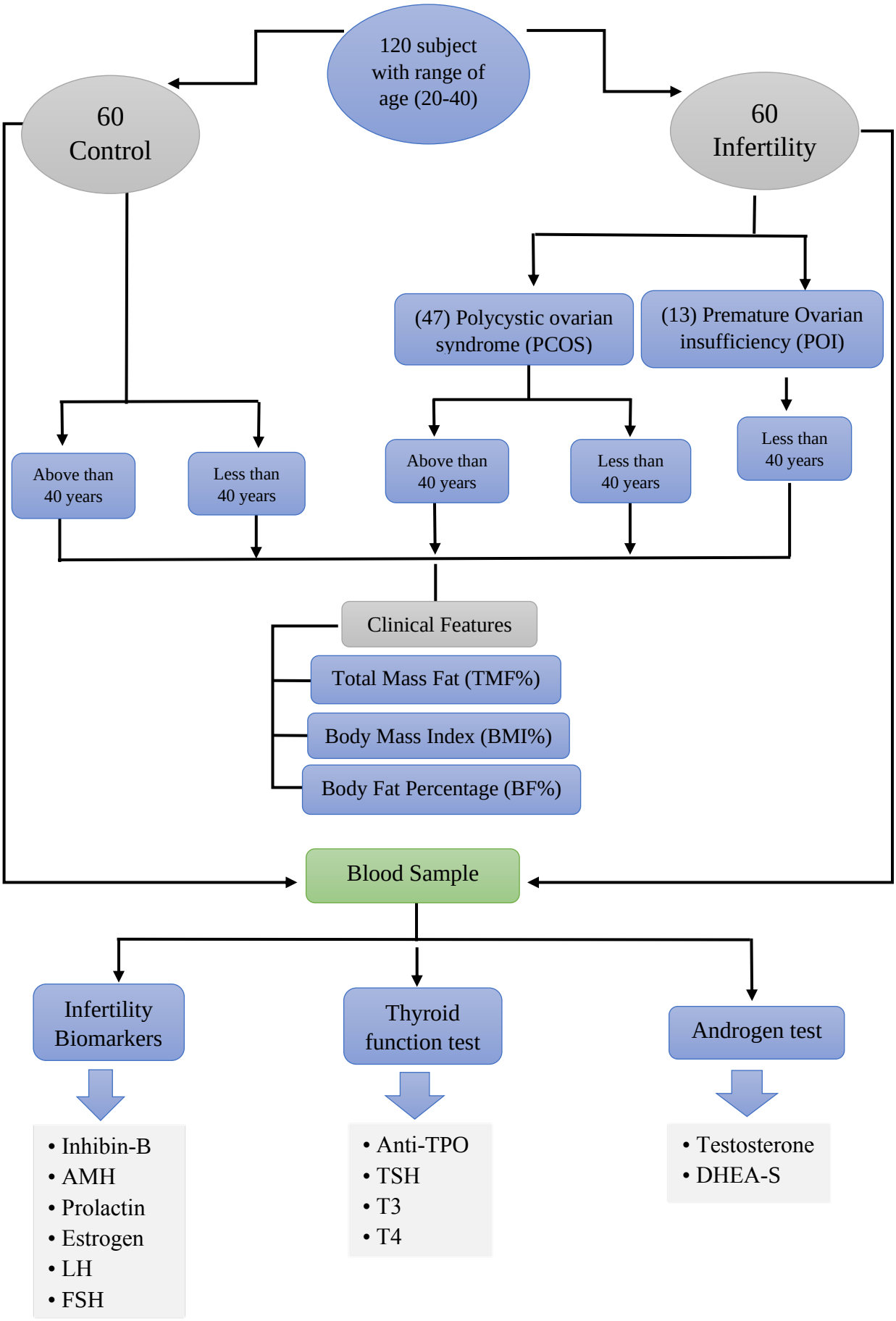


Figure (3-1) Diagram of case- control study design

3.4 Data Collection

Patients who had signs and symptoms were diagnosed with infertility by the specialist physician who diagnosed patients by Rotterdam standards or ultrasound and was managing the patients and sent them to the laboratory for investigations. The questionnaire shown in table (3-3) contains descriptive information about each patient including Name, Age, Job, Infertility type, lab test, and whether patients have any other diseases or not.

Table (3-3) Questioner sheet for the subject of the study

CodeNumber					
Age	20-24	25-29	30-34	35-39	40-45
Job	Housewife	Student	Employee	Teacher	None
Education level	Illiterate				
	Primary School				
	Secondary School				
	Institute				
	University				
Address	A rural		Urban		
Duration of marriage	Month		Years		
Date of last delivery					
Type of delivery	Cesarean delivery		Natural delivery		
No. Of Gravida	No. of Para		No, oF abortion (if she has)		
Menstrual Cycle	Regular		Irregular		
Duration of infertility	Month		Years		
Type Of Infertility	Primary		Secondary		
Causes of infertility	Husband		wife		
Drug History	Received drug? Type …….		Not received		
Medical Disease	D.M				
	Obesity				
	Liver disease				

	Heart Disease Kidney Disease			
The type of problem				
Ovulation causes	Aging	Polycystic ovarian syndrome	Premature ovarian failure	Ovarian reverse

Biochemical test	
1.	Dehydroepiandrosterone sulfate (DHEA-S)
2.	Prolactin hormone
3.	Inhibin-B
4.	Anti-thyroid peroxidase (Anti-TPO)
5.	Anti-Müllerian hormone (AMH)
6.	Follicle-stimulating hormone (FSH)
7.	Testosterone
8.	Luteinizing hormone (LH)
9.	Estrogen
10.	Prolactin hormone
11.	Thyroid-stimulating hormone (TSH)
12.	Triiodothyronine (T3)
13.	Thyroxine (T4)

3.5 Inclusion Criteria

- Only female with fertile age
- Infertility female patients

3.6 Exclusion Criteria

- The females with any chronic disease such as heart disease, Kidney disease, and liver disease
- Infertile result from male factors
- Females with any cancer
- Female infertility results from husband causes

3.7 Blood Sample Collection

5 ml of venous blood samples withdrawn were collected from all participant via vein puncture by sterile syringe from sterilizing the area, and all samples were identified by their specific numbers (ID) generated by the laboratory information system. Blood was put in a gel tube to let clot (to get the serum) and then, centrifuged at (4000rpm) for five minutes (Ashton., 2021). The serum was separated from blood contents and was conserved in sterile Eppendorf tubes, labeled with the sample name, and kept in deep freezing (-18 °C) to be used for LH, FSH, AMH, Testosterone, Estrogen, T3, T4, TSH. Subsequently, the remainder serum was used for DHEA-S, Inhibin-B, Prolactin, and Anti-TPO.

3.8 Ethical statement

This study protocol was accepted after its review by the Medical Ethics Committee of the Faculty of Applied Medical Sciences of Karbala University **Appendix (1).**

3.9 Methods

3.9.1 Body mass index (BMI)

BMI was calculated by using an electronic balance and height device to measure weight and length and then applying the equation below (Goacher *et al.*, 2012).

$$\textbf{BMI = Weight (kg) / Height (m}^2\textbf{)}$$

3.9.2 Body Fat percentage (BF%)

BF was calculated according to deurenberg formula (Deurenberg *et al.*, 1991)

$$\textbf{BF \% = (1.20 × BMI) + (0.23 × Age) – (10.8 × Gender) – 5.4 × 100\%}$$

3.9.3 Total Mass Fat (TMF)

The body fat mass is the weight of fat in the body. It was calculated according to the heitmann formula (Heitmann, B. L. 1990).

$$\textbf{TMF = (0.988×BMI) +(0.344×Wight) +(0.094×Age)-30}$$

3.10 Measurement of Biochemical Markers

3.10.1 Determination of Anti-thyroid peroxidase (Anti-TPO)

This kit is a sandwich enzyme-linked immunosorbent assay (ELISA) for in vitro quantitative measurement of anti-TPO in human tissue homogenates, cell lysates, and other biological fluids. It is a specific test for diagnosing the immune response to defects in patients. *Anti-TPO, No.SEA557Hu, Could-Clone Crop, USA.* **Appendix (2).**

3.10.2 Determination of Dehydroepiandrosterone sulfate (DHEA-S)

This kit is a Competitive-enzyme-linked immunosorbent assay (ELISA) that applies to the in vitro quantitative determination concentrations of DHEA-S in serum, plasma, and other biological fluids, and is a specific test for diagnosing patients with infertility. *DHEA-S, No.E-EL-0115, Elabscience, USA.* **Appendix (3)**

3.10.3 Determination of serum Inhibin-B

This kit is a Competitive inhibition enzyme-linked immunosorbent assay (ELISA) technique for the in-vitro quantitative measurement of INHB-B in human serum, plasma, tissue homogenates, cell lysates, cell culture supernates, and other biological fluids. *Inhibin-B, No.CEA760Hu, Could-Clone Croup, USA.* **Appendix (4).**

3.10.4 Determination of Anti-Müllerian hormone (AMH)

This kit is a competitive inhibition enzyme-linked immunosorbent assay (ELISA) technique for the in vitro quantitative measurement of AMH in human serum, plasma, tissue homogenates, cell lysates, cell culture supernates, and other biological fluids. *AMH, No.CEA228Hu, Could-Clone Croup, USA.* **Appendix (5).**

3.10.5 Determination of Follicle-stimulating hormone (FSH)

This kit is a sandwich enzyme-linked immunosorbent assay (ELISA) technique in vitro quantitative measurement of FSH in human serum, plasma, tissue homogenates, cell lysates, cell culture supernates, and other biological fluids. *FSH, No.SEA830Hu, Could-Clone Croup, USA.* **Appendix (6).**

3.10.6 Determination of Luteinizing hormone (LH)

This kit is a competitive enzyme-linked immunosorbent assay (ELISA) technique in vitro quantitative measurement of luteinizing hormone in human serum, plasma, and other biological fluids. *LH, No.CEA441Hu, Could-Clone Croup, USA.* **Appendix (7).**

3.10.7 Determination of Estrogen (E2)

This kit is a competitive enzyme-linked immunosorbent assay (ELISA) technique in vitro quantitative measurement of E2 in serum, plasma, tissue homogenates, cell lysates, cell culture supernates, and other biological fluids. *Estrogen, No.CEA461Ge, Could-Clone Croup, USA.* **Appendix (8).**

3.10.8 Determination of Testosterone

This kit is a competitive enzyme-linked immunosorbent assay (ELISA) technique for the in vitro quantitative measurement of testosterone in serum, plasma, and other biological fluids. *Testosterone, No.HEA458Ge, Could-Clone Croup, USA.* **Appendix (9).**

3.10.9 Determination Prolactin hormone (PRL)

This kit is a sandwich enzyme-linked immunosorbent assay (ELISA) technique for in vitro quantitative measurement of PRL in human serum, plasma, tissue homogenates, cell lysates, cell culture supernates, and other biological fluids. *PRL, No.SEA846Hu, Could-Clone Croup, USA*. **Appendix (10).**

3.11 Estimation of Thyroid Hormone Tests

3.11.1 Determination of Thyroid-stimulating hormone (TSH)

This kit is a sandwich enzyme-linked immunosorbent assay (ELISA) technique for in vitro quantitative measurement of TSH in human serum. *TSH, No.SEA463Hu, Could-Clone Croup, USA.* **Appendix (11).**

3.11.2 Determination of Triiodothyronine (T3)

This kit is a competitive enzyme-linked immunosorbent assay (ELISA) for the in vitro quantitative measurement of T3 in serum, plasma, and other biological fluids. *T3, No.CEA453Ge, Could-Clone Croup, USA.* **Appendix (12).**

3.11.3 Determination of Thyroxine (T4)

This kit is a competitive enzyme-linked immunosorbent assay (ELISA) for the in vitro quantitative measurement of T4 in serum, plasma, and other biological fluids. *T4, No.CEA452Ge, Could-Clone Croup, USA.* **Appendix (13).**

3.12 Statistical Analysis

Data analysis has been done statistically by employing software IBM SPSS statistical packages version 23. The analysis results have been summarized using descriptive statistics. In addition, Mean and Standard Deviation have been calculated in order to assess the statistical significance of the experimental results, a p-value of 0.01 probability threshold was utilized (Amrhein *et al.*, 2019). Furthermore, the normality of the data was verified using the Shapiro-Wilk test, while the homogeneity of variance was examined with the Levene test. Nevertheless, in order to investigate the association between categorical and numerical variables, chi-square and Pearson's correlation analyses were conducted. Besides, multiple

comparisons between groups were performed using analysis of variance (ANOVA), followed by Scheffe's and Duncan post-hoc tests for multiple comparisons within groups. Moreover, to find out cut-off points of the research parameters for critical patients, receiver operating characteristic (ROC) analyses were administrated. AUC was used for prediction strength, and optimum cut-off points were chosen using Youden’s index. Asterisks indicate data having a P value below 0.05 (Wasserstein *et al.*, 2019). Finally, all graphs were generated using GraphPad Prism 9.

Chapter Four
Results & Discussion

4.1 The Demographic Study

Table (4-1) Distribution and Characteristics of Patients and Control According to the study subjects.

Parameters		Level	Control		Patients		Total		P-value
			NO	%	No	%	No	%	
Age Group		20-25	24	40.00%	20	33.33%	44	36.67%	0.811
		26-30	19	31.67%	21	35.00%	40	33.33%	
		31-35	9	15.00%	12	20.00%	21	17.50%	
		36-40	8	13.33%	7	11.67%	15	12.50%	
BMI Group		P-Overweight	0	0.00%	26	43.33%	26	21.67%	0.003**
		P-Obese	0	0.00%	34	56.67%	34	28.33%	
		C-Overweight	60	100.00%	0	0.00%	60	50.00%	
Type of cause of infertility	Classification A	Endocrine disorder (PCOS)	0	0.00%	47	78.33%	47	39.17%	0.008**
		Premature ovarian insufficiency(POI)	0	0.00%	13	21.67%	13	10.83%	
		Control	60	100.00%	0	0.00%	60	50.00%	
	Classification B	P-PCOS(<40)	0	0.0%	39	65.0%	39	32.5%	0.005**
		P-PCOS(>40)	0	0.0%	8	13.3%	8	6.7%	
		P-POI(<40)	0	0.0%	13	21.7%	13	10.8%	
		C-Age(<40)	49	81.7%	0	0.0%	49	40.8%	
		C-Age(>40)	11	18.3%	0	0.0%	11	9.2%	
	Medical Disease	Diabetes mellitus	0	0.00%	1	1.67%	1	0.83%	0.005**
		Obesity	5	8.33%	34	56.67%	39	32.50%	
		Hypertension	4	6.67%	7	11.67%	11	9.17%	
		Heart disease	1	1.67%	0	0.00%	1	0.83%	
		None	50	83.33%	18	30.00%	68	56.67%	
Drug History		Not receiving	60	100.00%	60	100.00%	120	100.00%	0.001**
		Receiving	0	0.00%	0	0.00%	0	0.00%	
Type of infertility		Primary	0	0.00%	25	41.67%	25	20.83%	0.005**
		Secondary	0	0.00%	35	58.33%	35	29.17%	
		N/A	60	100.00%	0	0.00%	60	50.00%	
Menstrual Cycle		Regular	49	81.67%	12	20.00%	61	50.83%	0.001**
		Irregular	11	18.33%	48	80.00%	59	49.17%	
Job		House Wife	47	78.33%	53	88.33%	100	83.33%	0.001**
		Student	2	3.33%	7	11.67%	9	7.50%	
		Employee	11	18.33%	0	0.00%	11	9.17%	
Education level		Illiterate	14	23.33%	3	5.00%	17	14.17%	0.001**
		Primary schools	24	40.00%	17	28.33%	41	34.17%	
		Secondary school	6	10.00%	17	28.33%	23	19.17%	
		Institute	8	13.33%	2	3.33%	10	8.33%	
		University	8	13.33%	21	35.00%	29	24.17%	
Address		Rural	14	23.33%	20	33.33%	34	28.33%	0.224
		Urban	46	76.67%	40	66.67%	86	71.67%	
Type of delivery		Cesarean delivery	32	53.33%	37	61.70%	69	57.50%	0.356
		Natural delivery	28	46.67%	23	38.30%	51	42.50%	

**. Association is significant at p≤ 0.01 level.

P- Patients, C- Control

4.2 AMH, Anti-TPO, DHEA-S levels in PCOS patients and control according to the BMI

In Figure (4-1) the results demonstrated a high significant increase ($p \leq 0.01$) in the AMH level for both overweight and obese categories (10.83 ± 2.18), (11.10 ± 2.54) in PCOS patients, as compared to the overweight control group (3.11 ± 0.60). Also a high significant increase ($p \leq 0.01$) in the Anti-TPO level for both overweight and obese (16.20 ± 2.48), (16.20 ± 1.95) PCOS patients, as compared to the overweight control group (5.92 ± 0.96), also a high significant increase ($p \leq 0.01$) in DHEA-S level for overweight, obese (2.57 ± 0.36), (2.94 ± 0.59) PCOS patients, as compared to the overweight control group (0.89 ± 0.12).

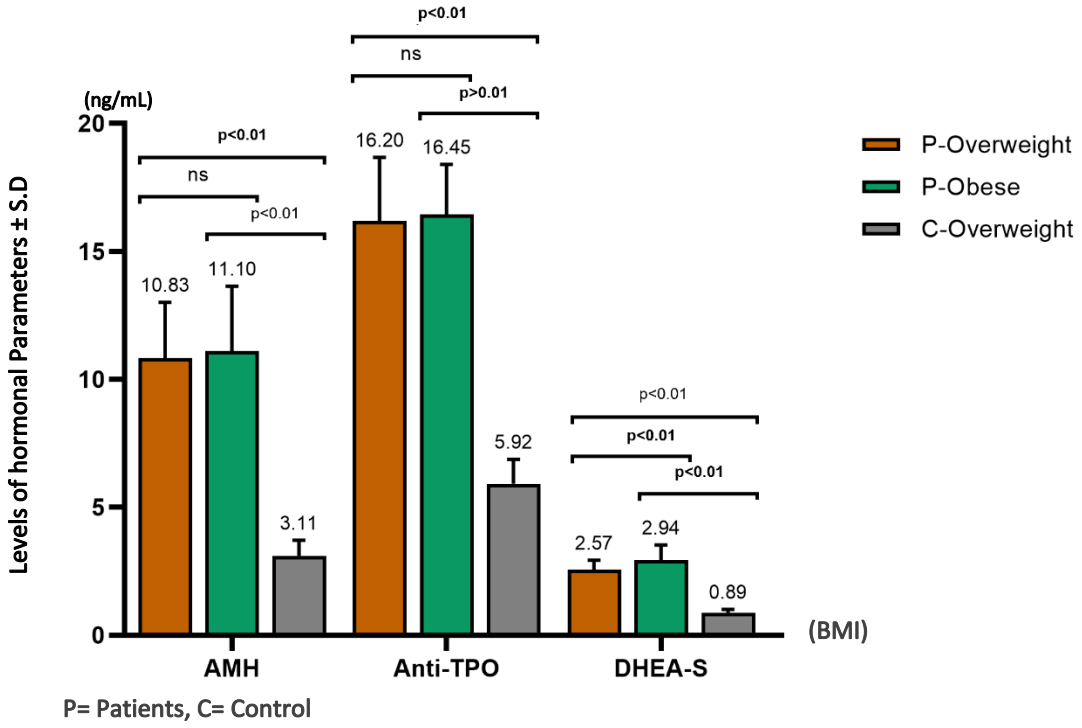


Figure (4-1) Comparison of the level of Parameters in PCOS patients and control at different classes of BMI

In the PCOS patients there is an increase in the number of follicles that did not develop until their full maturity, which means that the ovum did not reach to a mature stage, indicating increased levels of AMH produced from

ovaries, and that one of the reasons for PCOS was increase in BMI, that agrees with (Luo *et al.*, 2021), disagrees with (Kloos *et al.*, 2023). Anti-TPO levels were also found to be elevated in PCOS individuals who were overweight or obese, PCOS is associated with some of the clinical signs and problems of thyroid dysfunctions i.e., irregular menstrual cycles, subfertility, insulin resistance, obesity, and dyslipidemia, according to researches, thyroid autoimmunity is linked to infertility and pregnancy complications like premature labor and loss due to the autoimmune response of the thyroid gland, which elevated in response to Anti-TPO levels, that agrees with (Gawron *et al.*, 2022). DHEA-S levels are elevated in both overweight and obese PCOS patients, because it's considered androgens and PCOS will cause changes in the pattern or metabolism of androgen and its actions at the target tissue level, especially fatty tissue and that agreement with (Yasar *et al.*, 2022)

4.2.1 levels of Inhibin-B biomarker in PCOS patients and control according to the BMI

The result in Figure (4-2) recorded a significant increase ($p \leq 0.01$) in Inhibin-B levels of both overweight and obese (265.52 ± 69.00), (268.05 ± 76.91) PCOS patients, as compared to the overweight control group (36.76 ± 10.62).

Inhibin-B levels showed an increase in both PCOS patients with overweight and obesity because a hormonal imbalance in these cases disrupts experimental development and increases Inhibin-B levels due to an increased number of granular cells (Ibrahim *et al.*, 2022).

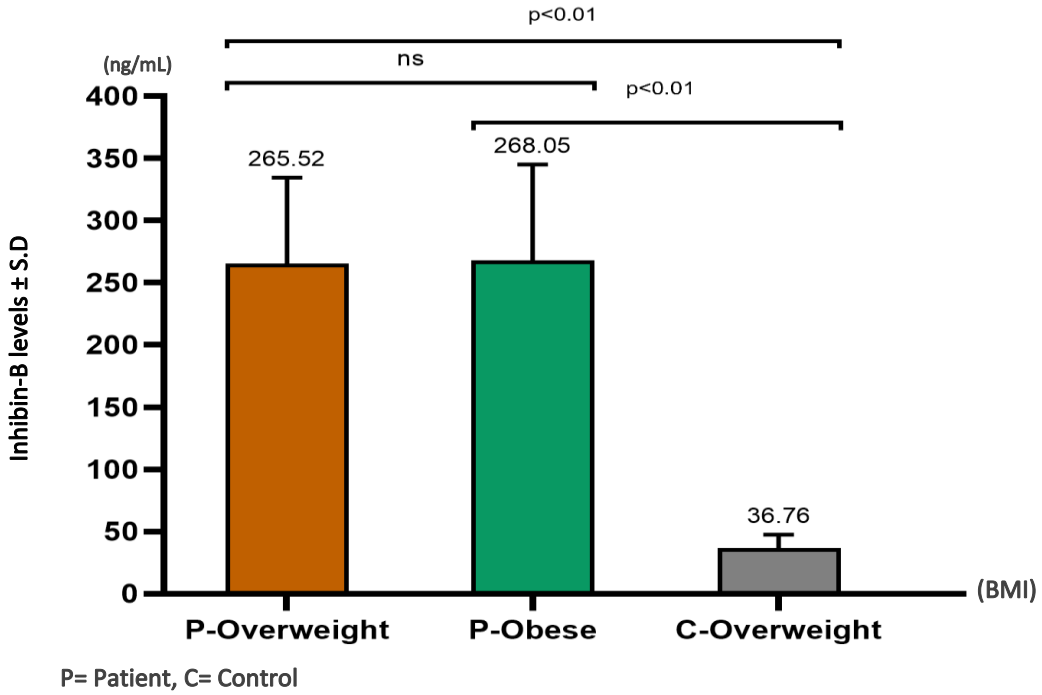


Figure (4-2) Comparison of the level of Inhibin-B in PCOS patients and control at different classes of BMI

4.2.2 AMH, Anti-TPO, DHEA-S levels in Premature Ovarian Insufficiency (POI) Patients and control according to BMI

The result in figure (4-3) demonstrated a significant decrease ($p \leq 0.01$) in the AMH level for both overweight and obese (0.46 ± 0.15), (0.72 ± 0.50) POI patients, as compared to the overweight control group (3.11 ± 0.60). There was a significant increase ($p \leq 0.01$) in the Anti-TPO level for both overweight and obese (14.42 ± 1.39), (14.98 ± 1.03) POI patients, as compared to the overweight control group (5.92 ± 0.96). There as a significant decrease ($p \leq 0.01$) in DHEA-S level for overweight and obese (0.57 ± 0.21), (0.51 ± 0.25) POI patients, as compared to the overweight control group (0.89 ± 0.12).

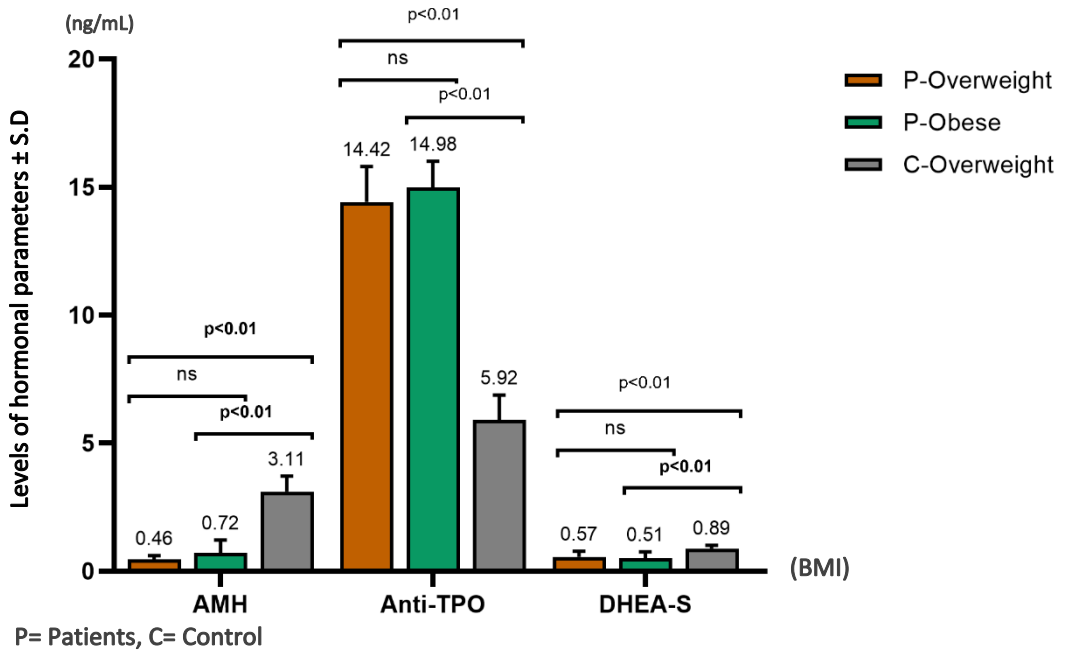


Figure (4-3) Comparison of the level of research parameters in POI patients and control at different classes of BMI

This decrease in AMH levels are explained by failure in ovary function and reduced ovarian reserve of the ovum and that agreement with (Makolle *et al.*, 2021). Anti-TPO levels it showed an increase in both groups of patients due to the increase in the autoimmunity of the thyroid gland in cases of ovarian failure is increasing the excretion of Anti-TPO antibodies, and that agreement with (Serin *et al.*, 2021). Low levels of DHEA-S in both overweight and obese POI patients, that may be related to that DHEA-S is an important source of estrogen in the body, it provides about 75% pre-menopausal estrogen and 100% post-menopausal estrogen in the body, so its decline leads to lower levels of androgens in POI patients, that agrees with (Wang *et al.*, 2023).

4.2.3 Levels of Inhibin-B biomarkers in POI patients and control according to the BMI

The result showed a high significant decrease ($p \leq 0.01$) in Inhibin-B levels of both overweight and obese (18.97 ± 0.97), (18.95 ± 1.46) POI patients, as compared to the overweight control group (36.76 ± 10.62) fig (4-4).

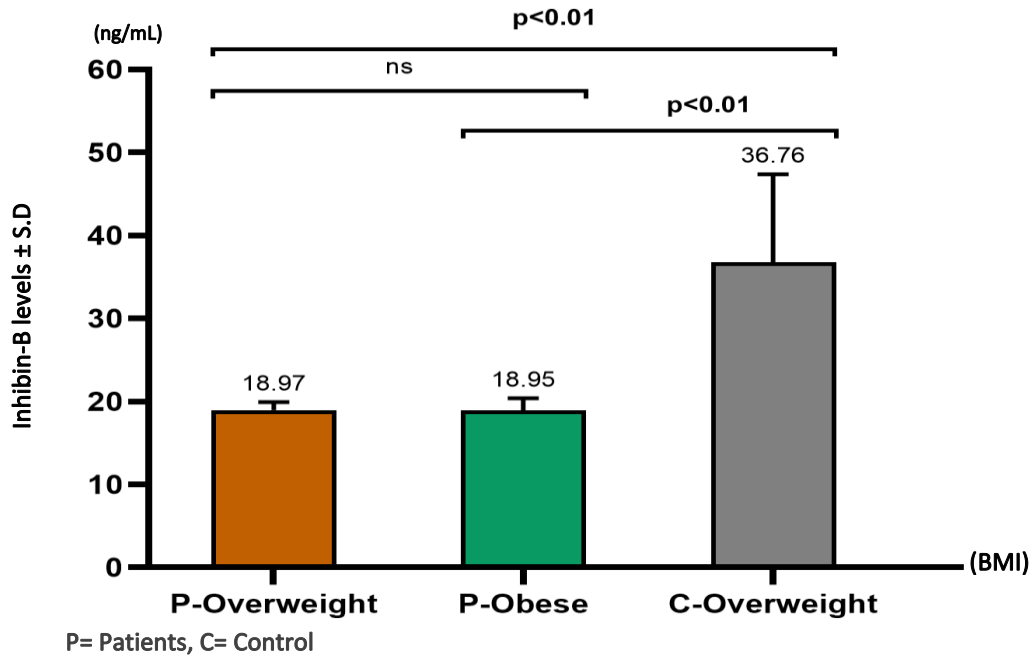


Figure (4-4) Comparison of the level of Inhibin-B in POI patients and control at different classes of BMI

The reduced in levels of Inhibin-B for both overweight and obese POI patients. Inhibin-B is associated with the maturation of follicles in the ovaries, and it reduced in POI patients due to the failure of ovarian cells responsible for its production (Hussein *et al.*, 2022).

4.2.4 AMH, Anti-TPO, DHEA-S in patients and control according to infertility causes (PCOS, POI)

In Figure (4-5), the result revealed a high significant increase ($p \leq 0.01$) in the AMH level of PCOS patients (10.99 ± 2.37), while a high significant decrease ($p \leq 0.01$) in POI patients (0.60 ± 0.39), as compared to the control group (3.11 ± 0.60). Also a high significant increase ($p \leq 0.01$) in Anti-TPO levels of both (16.34 ± 2.17) PCOS, (14.72 ± 1.19) POI patients, as compared to the control group (5.92 ± 0.96). High significant increase ($p \leq 0.01$) in DHEA-S level of PCOS patients (2.79 ± 0.53), while a significant decrease ($p \leq 0.01$) in POI patients (0.54 ± 0.23) as compared to the control group (0.89 ± 0.12).

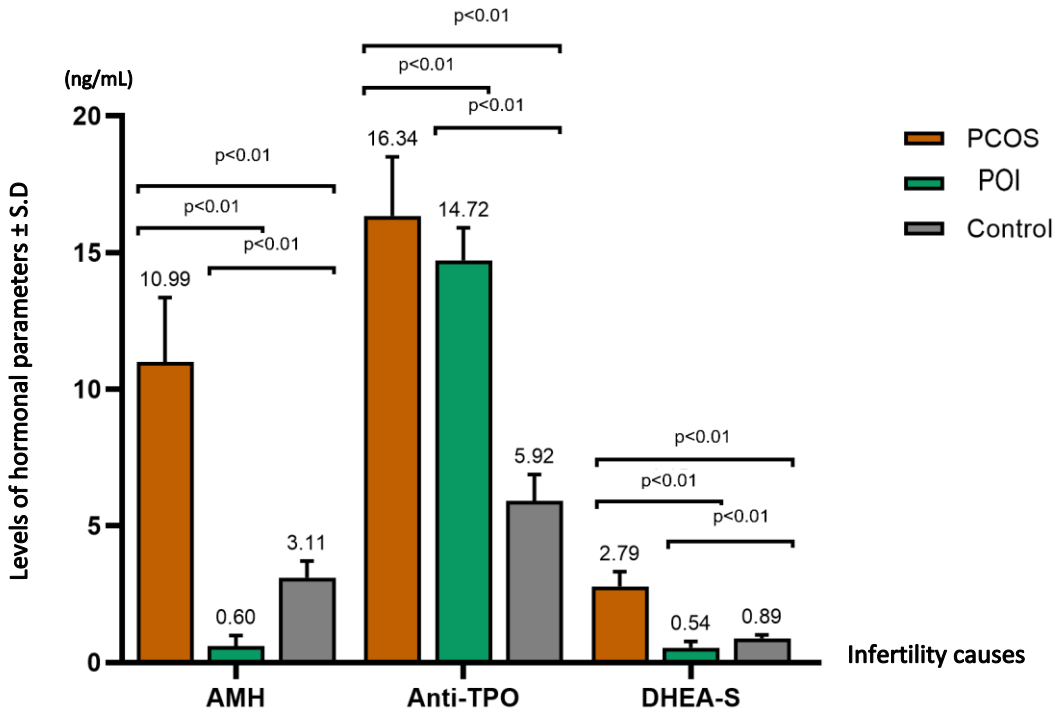


Figure (4-5) Comparison of the level of Biomarkers in PCOS and POI infertile patients as compared to control

AMH levels in PCOS are correlated with ovarian reserve, and it increased due to increase its synthesis and secretion by preantral and small antral follicles, the level of AMH increases with antral follicles count (AFC) at a consistent rate of 0.2 ng/ml per follicle (Muharam *et al.*, 2022). Premature

ovarian failure is characterized by decreasing gradually of AMH, and this decline is a better marker for evaluating ovarian function than Inhibin-B or antral follicle count (AFC) in females with increased FSH levels and can be used as a sensitive index for early diagnosis of POI (Cai *et al.*, 2022).

In PCOS patients, the increase in the levels of serum Anti-TPO due to the pathophysiological mechanism of these antibodies could be related to the fact that Anti-TPO is likely to pass through the blood-follicle barrier during the maturation period and leads to a cytotoxic environment that damages the maturing oocyte; thus, it is proven that the rise in these antibodies can lead to impaired fertility, this agrees with (Van der Ham *et al.*, 2023), disagrees with (Kim *et al.*, 2022). In POI patients increase in the concentration of Anti-TPO in follicular fluid is about half that of serum Anti-thyroid antibodies and ultimately leads to ovarian dysfunction, this agreement with (Wang *et al.*, 2024).

The elevated DHEA-S concentrations for PCOS as a response to acute stress affect metabolic, endocrine, and ovarian functions (Stogowska *et al.*, 2022). DHEA-S levels were decreased in POI patients, this decrease due to a reduction in the ovarian reserve, this hormone has a critical role in the synthesis of estrogen important in the building tissue of women's reproductive system, which agrees with (Soman *et al.*, 2020).

4.2.5 Levels of Inhibin-B in patients and control according to infertility causes (PCOS, POI)

Figure (4-6) demonstrated a significant increase ($p \leq 0.01$) in the Inhibin-B level of PCOS patients (266.97 ± 72.88), while a high significant decrease ($p \leq 0.05$) in POI patients (18.96 ± 1.21), as compared to the control group (36.76 ± 10.62).

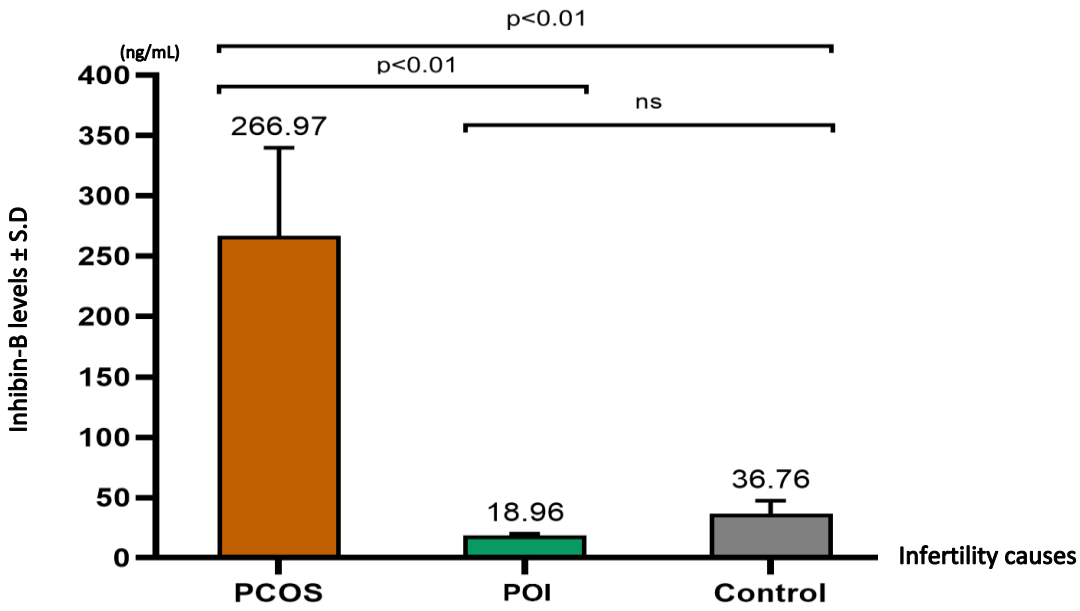


Figure (4-6) Comparison of the level of Inhibin-B in PCOS and POI infertile patients as compared to control

Elevated in the levels of Inhibin-B in PCOS patients are due to the increase in ovarian reserve, so the elevated concentration means over activity in the ovaries, which agrees with (Sadig *et al.*, 2023). While in POI patients the reduced level of Inhibin-B indicates ovarian inefficiency, and this agreement with (Yang, X., & Yang, L. 2023).

4.2.6 The AMH, Inhibin-B, Anti-TPO, DHEA-S levels in PCOS, POI patients, and control according to Age.

The result in the table (4-2) found a very high significant increase ($p \leq 0.01$) in the AMH level for age (<40) years (10.89 ± 2.37), (>40) years (11.45 ± 2.46) PCOS patients, while a high significant decrease at ($p \leq 0.01$) in POI patients (<40) years (0.60 ± 0.39), as compared to both control age (<٤٠) years (3.13 ± 0.59), (>٤٠) years (3.06 ± 0.67) respectively, also a high significant increase at ($p \leq 0.01$) in Inhibin-B level of age (<٤٠) years (273.55 ± 75.25), (>٤٠) years (234.91 ± 52.43) PCOS patients, while decrease in the Inhibin-B level of POI patients (<٤٠) years (18.96 ± 1.21), as compared to both group of control age (<٤٠) years (35.58 ± 10.48),

(42.04 ± 10.04). Also a high significant increase at (p≤0.01) in Anti-TPO levels for age (<٤٠) years (16.08 ± 2.14), (>٤٠) years (17.63± 1.93) PCOS patients, (<٤٠) years (14.72 ± 1.19) POI patients, as compared to both control age (<٤٠) years (5.92 ± 0.99), (>٤٠) years (5.93 ± 0.84), high significant increase at (p≤0.01) in the DHEA-S level for both (<٤٠) years (2.75 ± 0.55), (>٤٠) years (2.94 ± 0.46) PCOS patients, while a high significant decrease at (p≤0.01) in POI patients (<٤٠) years (0.54 ± 0.23), as compared to both control age (<٤٠) years (0.88 ± 0.11), (>٤٠) years (0.89 ± 0.13).

ماذا تحتاج الكائنات الحية كي تعيش ؟

ما هي الكائنات الحية ؟	**انسان - حيوان - نبات .**		
ما هي الأشياء الغير **حية ؟**	الحجر - الباب - **الكتاب - القلم .**		
بماذا **تختلف الكائنات الحية عن الأشياء الغير حية ؟**	الكائنات الحية : **تأكل وتشرب وتتنفس وتتحرك وتنمو . الأشياء غير الحية : لا تأكل ولا تشرب ولا تتنفس ولا تتحرك ولا تنمو**		
ما **الفرق بين الطائرة والطائر ؟**	الطائرة غير حية **والطيار كائن حي .**		
ما **هي النباتات ؟**	كائنات حية مثل **الاعشاب والشجر ولا تنتقل من مكان لآخر .**		
ماذا **تحتاج النباتات لكي تعيش ؟**	ماء - هواء - **ضوء الشمس .**		
ما هي الحيوانات ؟	كائنات حية مثل **الأبقار - الدجاج - الأغنام - الأسود - النمور - الصقور .**		
ماذا **تحتاج الحيوانات كي تعيش ؟**	ماء - هواء - **غذاء . وتنتقل من مكان لآخر .**		
ما هي الحيوانات التي تتغذى على النباتات ؟	الأغنام - الابقار - **الخيول - الماعز**		
ما هي الحيوانات التي تتغذى على لحوم حيوانات أخرى ؟	الأسود - النمور - **الصقور - الذئاب**		

هل السيارة كائن حي ؟ ولماذا ؟	غير حي . **لأنها لا تتغذى ولا تشرب ولا تنمو ولا تتنفس**		
ما معنى النمو ؟	يعني ان الكائن **الحي يكبر ويتغير .**		
بماذا يتكاثر الانسان ؟ وكيف ؟ ما هي مراحل نمو الانسان ؟	بالولادة . تلد **الام طفلا صغيرا وترضعه الحليب ثم ينمو ويتعلم الحبو والجلوس والوقوف ثم المشي ثم يتناول الطعام بنفسه ثم ينمو ويذهب الى الحضانة ثم الروضة والمدرسة .**		
ما **معنى التكاثر ؟**	زيادة أعداد الكائنات **الحية**		
ما طرق تكاثر الحيوانات ؟	الولادة : كالأرانب **والبقر والغنم الخيول القطط الكلاب .**		
اذكر **ثلاث حيوانات تتكاثر بالولادة وثلاث حيوانات تتكاثر بالبيض .**	البيض : الطيور - **الدجاج - الأسماك. ,الابقار - الاغنام - الخيول الطيور - الدجاج - الأسماك.**		
ما طرق **تكاثر النباتات ؟**	البذور - الفسائل - **اخذ جزء منها وزراعته .**		
مما يتكون النبات ؟	الجذر الساق - **الأوراق - الزهرة - الثمرة - البذرة .**		
ما هو الجذر وأين ينمو ؟	الجذر جزء من **النبات - ينمو داخل التربة .**		
ما وظيفة الجذر ؟	امتصاص الماء من **التربة - تثبيت النبات في الأرض .**		
يأكل الانسان جذور بعض النبات ما هي ؟	الجزر - الفجل - **الشلغم .**		

الأسئلة

ما هو الساق وأين ينمو ؟

ما وظيفة الساق ؟

Generally, the AMH serum is at its highest levels at a young age and then gradually declines after 35 years in healthy women, it is thought that the decrease in AMH with age was attributed to an overall decrease in the

number of antral follicles, the AMH levels increase in PCOS is due to their high levels of follicles, so AMH is suggested as the strongest diagnostic marker in patients with PCOS compared to the LH/FSH ratio (Evliyaoglu *et al.*, 2020), while in POI patients AMH levels are very low or negligible, and this decreased in serum AMH levels are a reflection of decreased ovarian reserve and suggests ovarian failure (Wafa *et al.*, 2020).

Inhibin-B levels of reproductive-age women decrease with age. In PCOS patients, Inhibin-B levels increase due to the increased number of small growing follicles that are characteristic of PCOS, also excess in Inhibin-B production inhibits the FSH levels production, contributing to the hormonal irregularities seen in PCOS (Sadig *et al.*, 2023). In POI patients a decrease in levels of Inhibin-B is considered one of the predictive factors for ovarian function in POI patients, recently, studies have shown a significantly continuous decline in Inhibin-B accompanying the progress of POI, so when the level of Inhibin-B decreases, the excretion of the hormone FSH will increase and this hormonal imbalance indicates a failure of ovarian function (Zhu *et al.*, 2021).

At every age, women were twice as likely to have increase TPO antibodies as men. The elevated Anti-TPO levels in both PCOS and POI adversely affect fertility and reproductive outcomes by creating a cytotoxic environment that damages the maturing oocyte reducing its quality and fertilization chances (Hsieh, Y. T., & Ho, J. Y. 2021; Akdulum *et al.*, 2022).

The variation in levels of androgens is significantly influenced by age in healthy women, as well as in those with PCOS and other reproductive problems. The rise of DHEA-S in individuals with PCOS is attributed to

the stimulation of adrenal steroidogenesis associated with the condition (Carmina, E., & Longo, R. A. 2022).

Whereas POI patients have a decline in DHEA-S levels due to a deficiency of estrogens, they may also have a loss of ovarian androgens because of the atrophy of the ovarian cortex, DHEA-S is thought to be one of the fundamental prerequisites for a healthy woman, the lack of androgens such as DHEA-S may lead to symptoms of sexual dysfunction, such as decreased libido, loss of sexual responsiveness, or decreased sexual arousal, other clinical manifestations are a diminished sense of well-being, dysphoric mood, cognitive dysfunction, and persistent, and unexplained loss of energy (Soman *et al.*, 2020).

4.3 Hormonal parameters in patients and control.

4.3.1 Hormonal levels in PCOS patients and control according to BMI.

In Figure (4-7) the result found a highly significant decrease ($p \leq 0.01$) in the FSH level for both overweight and obese (5.65 ± 2.50), (5.97 ± 2.18) PCOS patients, as compared to the overweight control group (8.10 ± 2.15), also a high significant increase ($p \leq 0.01$) in the prolactin level for both overweight and obese (28.49 ± 5.32), (29.79 ± 4.75) PCOS patients, as compared to the overweight control group (13.44 ± 3.30). Found a high significant increase ($p \leq 0.01$) in the Testosterone level for both overweight and obese (55.64 ± 17.23), (60.67 ± 17.52) PCOS patients, as compared to the overweight control group (5.34 ± 0.90). In addition a high significant increase ($p \leq 0.01$) in the LH level for both overweight and obese (16.09 ± 4.62), (17.94 ± 6.53) PCOS patients, as compared to the overweight control group (2.93 ± 1.28), (16.09 ± 4.62), (17.94 ± 6.53) PCOS patients, as compared to the overweight control group (2.93 ± 1.28).

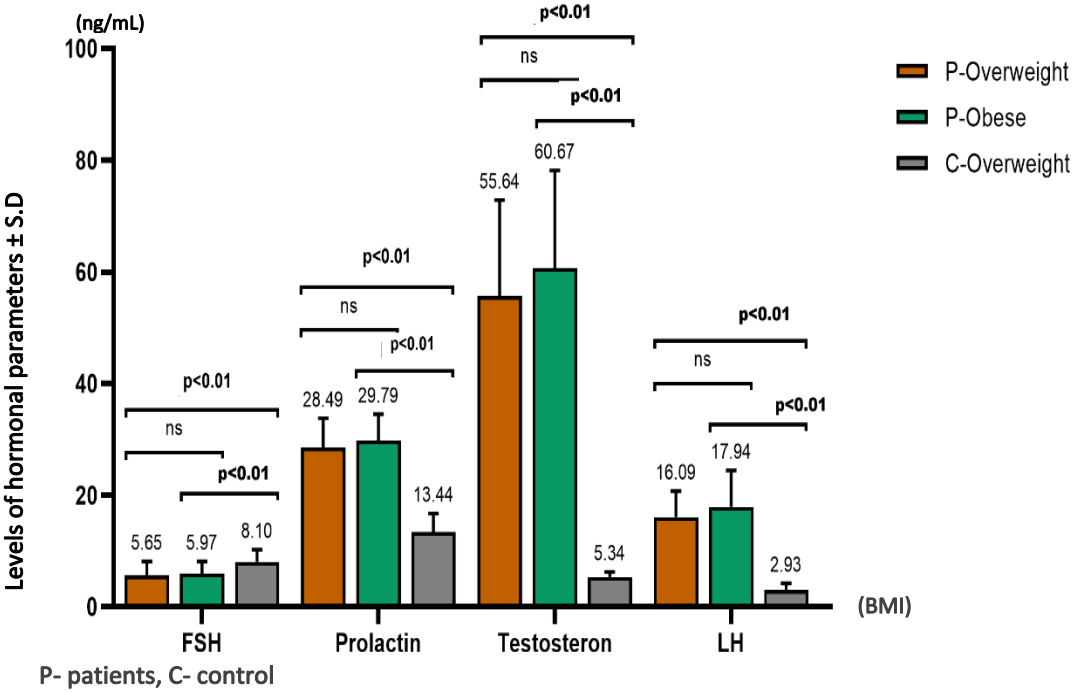


Figure (4-7) Comparison of hormonal Parameters in PCOS patients and control according to the BMI Group.

The primary abnormalities in PCOS is the abnormal release of GnRH. Instead of being released in a regular cyclic manner, GnRH is released rapidly, this disrupts the release of the LH and FSH in a way that LH is increased but FSH is decreased into the peripheral circulation (Saadia, Z., 2020). Higher levels of prolactin in overweight and obese PCOS patients were due to insulin resistance phenomenon (Mastnak *et al.*, 2023). Levels of testosterone is elevated in overweight and obese PCOS due to the high levels of LH but also because of increased levels of insulin that are usually seen with PCOS (Sudhakaran *et al.*, 2023).

4.3.2 Estrogen levels in PCOS patients and control according to the BMI

In figure (4-8) a result found high significant increase ($p \leq 0.01$) in the Estrogen levels of both overweight and obese (200.03 ± 39.65), (176.04 ± 52.08) PCOS patients, as compared to the overweight control group (24.93 ± 7.23).

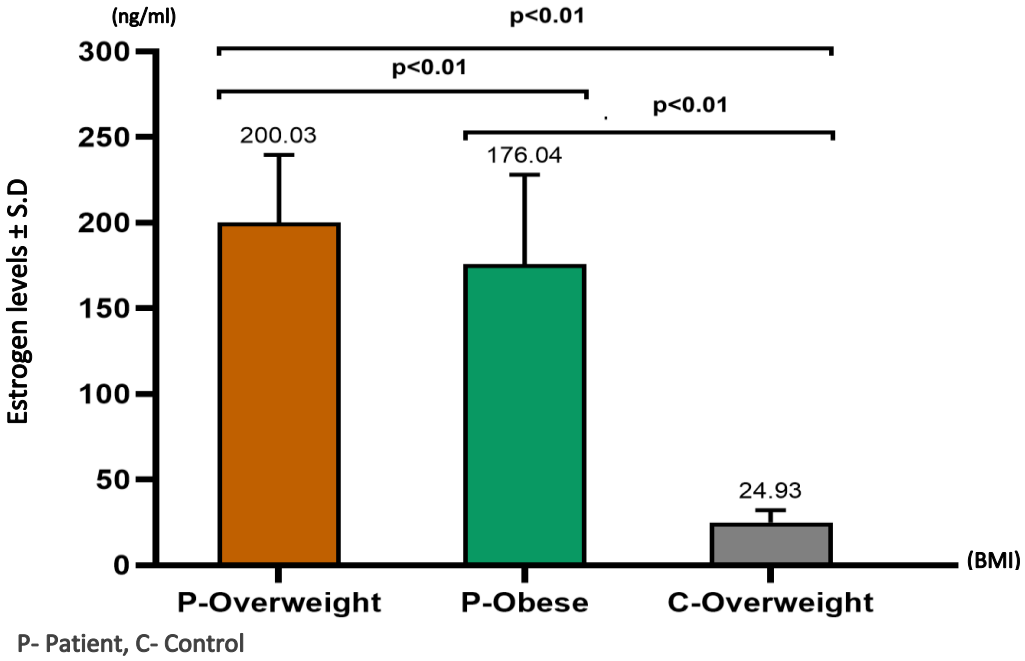


Figure (4-8) Comparison of Estrogen in PCOS patients and control according to the BMI Group

The elevation in the Estrogen levels in both overweight and obese PCOS patients due to having a high percentage of body fat which in turn affect the production of estrogen from the ovaries and peripheral conversion of fat to estrogen (Khmil *et al.*, 2020).

4.3.3 Hormonal levels in POI patients and control according to BMI

The result of the present study found a high significant increase ($p \leq 0.0$) i1n the FSH level for both overweight and obese (35.51 ± 1.63), (31.80 ± 4.06) POI patients, as compared to the overweight control group (8.10 ± 2.15), also a high significant increase ($p \leq 0.01$) in the prolactin level for both

overweight and obese (23.69 ± 2.13), (28.09 ± 2.06) POI patients, as compared to the overweight control group (13.44 ± 3.30). Results found no significant ($p \leq 0.01$) in the Testosterone level of obese POI patients (6.19 ± 2.02), a significant increase ($p \leq 0.01$) in overweight patients POI (8.47 ± 1.19), as compared to the overweight control group (5.34 ± 0.90). Additionally, a high significant increase ($p \leq 0.01$) in the LH level for both overweight and obese (19.42 ± 4.01), (20.07 ± 1.73) POI patients, as compared to the overweight control group (2.93 ± 1.28) fig (4-9).

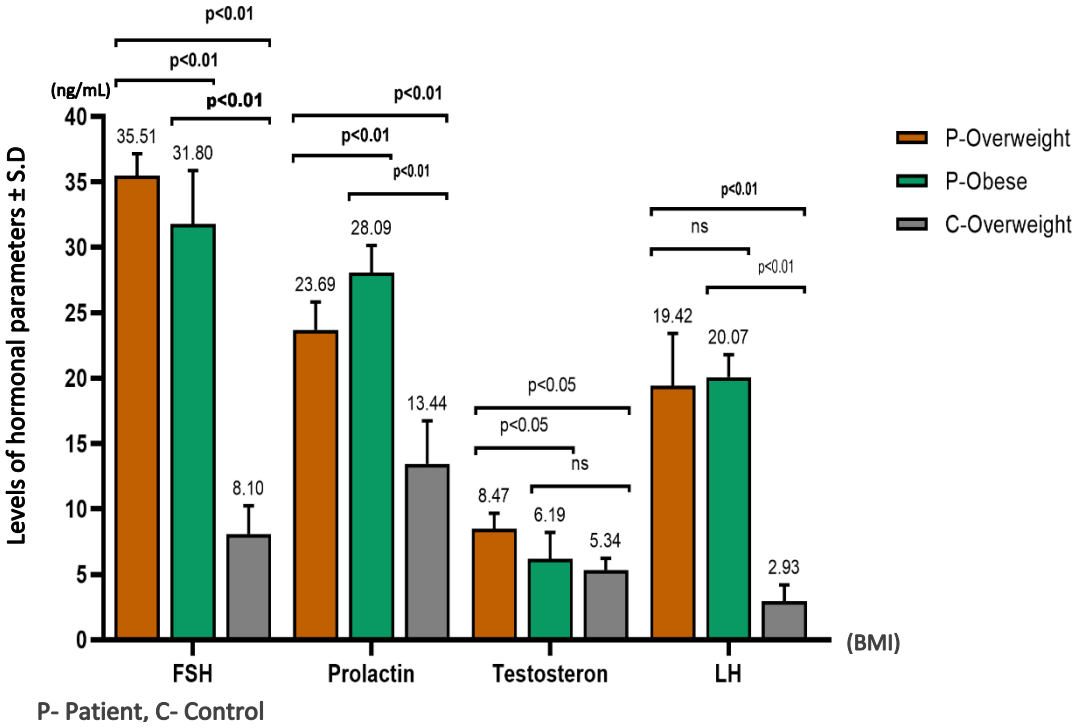


Figure (4-9) Comparison of hormonal Parameters in POI patients and control according to the BMI Group.

This raised in FSH for POI patients is related to the ovary does not produce enough estrogen, that agrees with (Baheti *et al.*, 2020). The levels of prolactin are elevated in all POI because of the insulin resistance phenomenon caused as a result of hypothalamic-pituitary abnormality (Du *et al.*, 2024). Testosterone mildly increase in patients may be due to the high levels of LH (Fatima *et al.*, 2020). High levels of LH can signify that

there are no enough steroid hormones needed for reproductive process due to failure of ovarian ability to produce estrogen and testosterone (Hong *et al.*, 2022).

4.3.4 Levels of Estrogen in POI patients and control according to the BMI.

The result in figure (4-10) demonstrated a significant decrease ($p \leq 0.01$) in the Estrogen level for both overweight and obese (10.07 ± 1.55), (10.07 ± 1.45) POI patients, as compared to the overweight control group (24.93 ± 7.23).

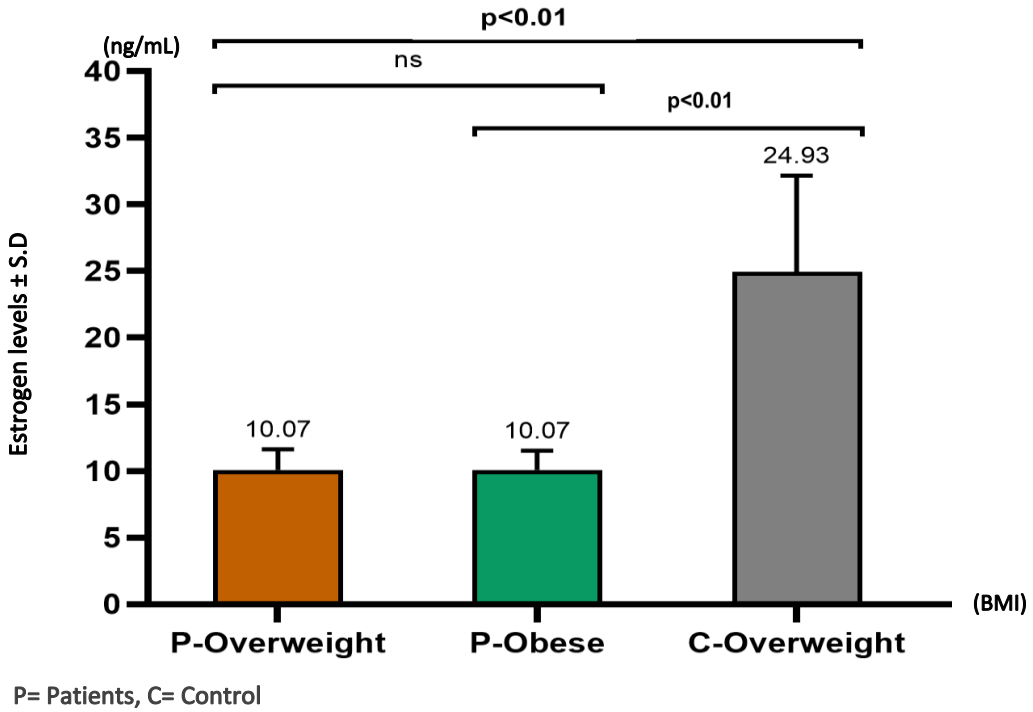


Figure (4-10) Comparison of Estrogen levels in POI patients and control according to the BMI Group.

The decrease in levels of estrogen in both overweight and obese POI patients because inability of the ovarian cell to produce estrogen (Ghahremani-Nasab *et al.*, 2020).

4.3.5 The level of hormonal parameters in patients and control according to infertility causes (PCOS, POI)

The result of the present study in figure (4-11) found a high significant decrease ($p \leq 0.01$) in the FSH level of PCOS patients (5.83 ± 2.30), while a high significant increase ($p \leq 0.01$) in POI patients (33.51 ± 3.61), as compared to the control group (8.10 ± 2.15), also a high significant increase ($p \leq 0.01$) in the Prolactin level in both PCOS and POI patients (29.24 ± 4.99), (26.06 ± 3.04), as compared to the control group (13.44 ± 3.30). Also, a high significant increase ($p \leq 0.01$) in the Testosterone levels of PCOS patients (58.53 ± 17.39), but no any significant in POI patients (7.24 ± 2.01), as compared to the control group (5.34 ± 0.90). Also, there was a highly significant increase ($p \leq 0.01$) in the LH level of both PCOS, and POI patients (17.15 ± 5.81), (19.77 ±2.88), as compared to the control group (2.93 ± 1.28).

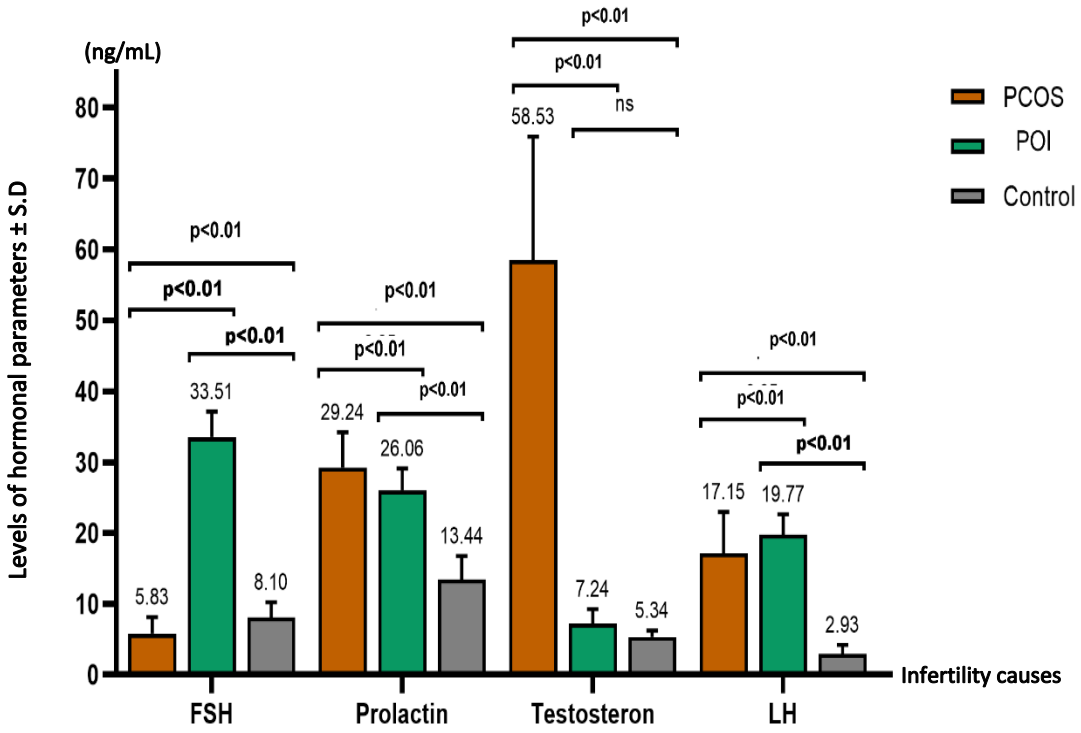


Figure (4-11) Comparison of the level of Parameters in PCOS, POI infertile patients as compared to the control.

This increase in the levels of FSH in POI patients this rise is due to the low E2 levels production from ovaries. E2 levels is needed for the negative feedback mechanism of the pituitary gland to secrete gonadotropic hormone (high FSH levels) (Karaer, I., & Tuncay, G. 2020).

As for the PCOS patients, the levels of FSH are relatively decreased. The primary abnormality in PCOS is the abnormal release of GnRH; instead of being released in a regular cyclic manner, GnRH is released in large amounts throughout the day, this defect in the release process of the LH and FSH in the way that LH is increased but FSH is decreased in the peripheral circulation (Xu *et al.*, 2022).

The levels of prolactin are elevated in both PCOS and POI. In PCOS prolactin could inhibit ovulation and lead to polycystic ovarian morphology. In POI, high prolactin levels interfere with the normal production of other hormones, such as estrogen and progesterone, this can change or stop ovulation (the release of an ovum from the ovary), it can also lead to irregular or missed menstrual cycles but it wasn't as high as in PCOS (Mahmoud *et al.*, 2020; Kamrul-Hasan, A. B. M., & Aalpona, F. T. Z. 2024).

In PCOS patient androgen excretion increases, and since testosterone is considered an androgen family member, its production increases either because of the high levels of LH or because of increased levels of insulin that are usually seen with PCOS (Valdimarsdottir *et al.*, 2021). While there was no difference between normal levels at controls and testosterone levels in POI patients.

High levels of LH recorded in patients with polycystic ovaries this increase due it is speculated that hyperandrogenic conditions in PCOS may reduce hypothalamic sensitivity to negative feedback from estradiol and

progesterone, leading to increased LH secretion and decreased FSH secretion from the pituitary, regarding POI LH also increase due to same mechanism of negative feedback of estradiol and progesterone, leading to increased LH secretion and decreased FSH secretion, that agrees with (Wang *et al.*, 2022).

4.3.6 Levels of Estrogen in patients and control according to infertility causes (PCOS, POI)

the results in figure (4-12) demonstrated a high significant increase ($p \leq 0.01$) in the Estrogen level of PCOS patients (186.25 ± 48.23), decrease ($p \leq 0.01$) in POI patients (10.07 ± 1.43) as compared to the control group (24.93 ± 7.23).

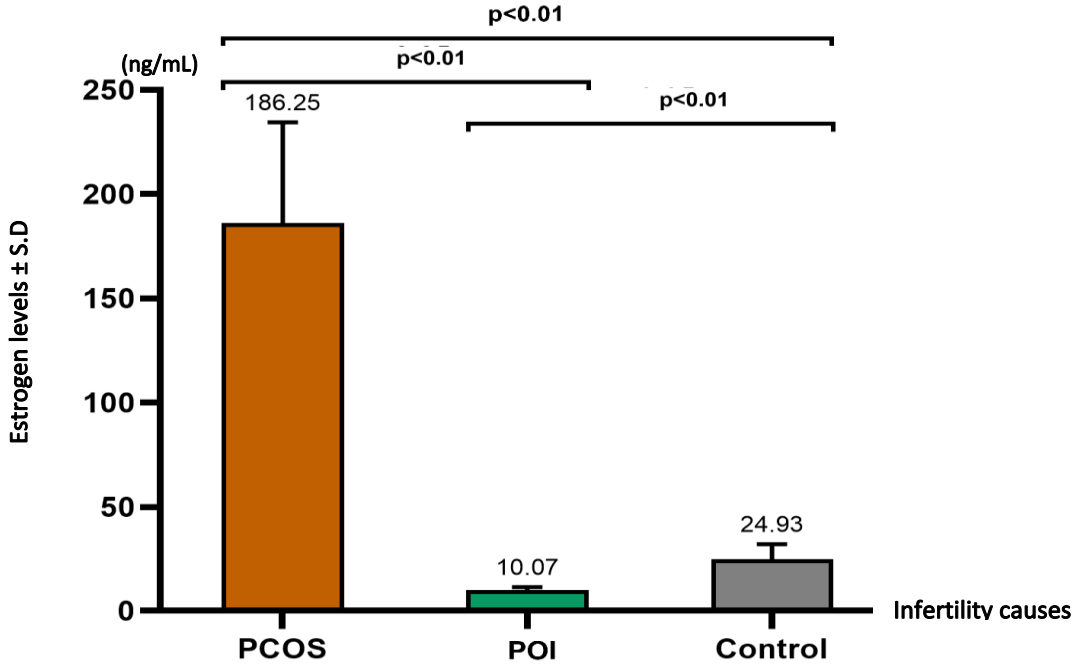


Figure (4-12) Comparison of the level of Estrogen in PCOS, POI infertile patients as compared to the control

The raised in levels of LH in PCOS cause excess production of ovarian thecal estrogen, whereas relative deficiency of FSH causes follicular arrest, polycystic ovarian morphology, and oligo-ovulation, and that agreement with (Liu *et al.*, 2022) and, disagreement with (Yang, J., & Chen, C. 2024).

While in POI decrease in estrogen occurs when the defect in the ovaries leads causes anovulation and produces less estrogen due to dysfunction in the cells responsible for its production and that agree with (Zhang, C., 2020).

4.3.7 Hormonal levels in an infertility patients and control according to Age

The result in table (4-3) demonstrated high significant decrease ($p \leq 0.01$) in the FSH level of (<40) (5.63 ± 2.22), (>40) (6.81 ± 2.59) PCOS patients, while a high significant increase ($p \leq 0.01$) in POI patients(<40) (33.51 ± 3.61), as compared to both control age (<40) (8.10 ± 2.23), (>40) (8.10 ± 1.85), also a high significant increase ($p \leq 0.01$) in the Prolactin level of both (<40 years) (29.14 ± 4.87), (>40 years) (29.69 ± 5.88)PCOS, (<40 years) (26.06 ± 3.04) POI, as compared to both control age (<40 years) (13.28 ± 3.31), (>40 years) (14.17 ± 3.32). Also found a high significant increase ($p \leq 0.01$) in Testosterone level of both age (<40 years) (57.64 ± 17.56), (>40 years) (62.83± 16.99) PCOS, and no any significant difference in POI (<40 years) (7.24 ± 2.01), as compared to both control age (<40 years) (5.31 ± 0.86), (>40 years) (5.43 ± 1.10). Estrogen showed high significant increase ($p \leq 0.01$) in (<40 years) (183.26 ± 49.46), (>40 years) (200.81 ± 41.37) PCOS, while no significant difference in POI (<40 years) (10.07 ± 1.43), as compared to both control age (<40 years) (24.01 ± 6.93), (>40 years) (29.05 ± 7.42). Also a very high significant increase ($p \leq 0.01$) in the LH level in both (<40 years) (17.54 ± 6.07), (>40 years) (15.27 ± 4.17), and POI (<40 years) (19.77 ± 2.88), as compared to both control age (<40 years) (2.96 ± 1.36), (>40 years) (2.75 ± 0.92).

In table (4-3) the rising levels of serum FSH with age is at least in part due to declining Inhibin-B secretion, so reduced Inhibin-B production by a decreasing growing follicle population (as the ovarian reserve is depleted

with age) may elevate FSH secretion, and this toxic to ovulatory oocytes (Dursun, P., 2024). Prolactin levels are elevated in women under 50 years due to the insulin resistance phenomenon, that occurs in both PCOS and POI (Geng *et al.*, 2023). Testosterone levels are at their highest during adolescence and early adulthood and may start to decline between ages 45 and 55 years in females, in PCOS high levels of insulin causes the ovaries to produce too much testosterone, which interferes with the development of the follicles and prevents normal ovulation (Zhao *et al.*, 2020). Aging is associated with a loss of sex hormones (androgens and estrogens), and with age and access to menopause, LH levels will increase as your levels of estrogen and progesterone decrease (Negrón, A. L., & Radovick, S. 2020).

4.3.8 Levels of thyroid hormone in PCOS patients and control according to BMI

The result in Table (4-4) demonstrated no significant difference ($p \leq 0.01$) in the TSH level of overweight and obese (1.99 ± 0.78), (2.10 ± 0.99) PCOS patients, as compared to the overweight control group (2.03 ± 1.31), Also, a no any significant difference ($p \leq 0.01$) in T3 level of overweight and obese (1.00 ± 0.25), (0.97 ± 0.24) PCOS patients, as compared to the overweight control group (1.05 ± 0.40). And no significant difference ($p \leq 0.01$) in the T4 level of overweight and obese (9.73 ± 1.27), (9.68 ± 2.09) PCOS patients, as compared to the overweight control group (10.46 ± 2.39).

Table (4-4): Comparison of the level of thyroid hormone in PCOS patients and control according to BMI

Parameters	Level	Mean ± Std. D	P. value
TSH(ulU/ml)	P-Overweight	2.06 ± 0.78	0.934
	P-Obese	2.10 ± 0.99	
	C-Overweight	2.02 ± 1.31	
T3(ng/mL)	P-Overweight	1.00 ± 0.25	0.586
	P-Obese	0.97 ± 0.24	
	C-Overweight	1.05 ± 0.4	
T4(ng/L)	P-Overweight	9.73 ± 1.27	0.202
	P-Obese	9.68 ± 2.09	
	C-Overweight	10.46 ± 2.39	

**. The mean difference is non-significant
P- Patient, C- Control

According to studies small increases in TSH levels may lead weight gain, If the thyroid is over or underactive is causes abnormal T3 and T4 production, which can affect ovulation regulation and reduce fertility (Zia, B., Nisar, S., & Ansari, S. Z. 2023).

4.3.9 Levels of thyroid hormone in POI patients and control according to the BMI

In Table (4-5) the result showed no significant difference ($p \leq 0.01$) in the TSH level of overweight and obese (2.03 ± 1.08), (2.11 ± 1.09) POI patients, as compared to the overweight control group (2.02 ± 1.31), Also, a no any significant difference ($p \leq 0.01$) in T3 level of overweight and obese (1.07 ± 0.26), (1.04 ± 0.29) POI patients, as compared to the overweight control group (1.05 ± 0.40). And found no significant difference ($p \leq 0.01$) in the T4 level of overweight and obese (9.31 ± 1.27), (9.42 ± 1.94) POI patients, as compared to the overweight control group (10.46 ± 2.39).

Table (4-5): Comparison of the level of thyroid hormone in POI patients and control according to BMI

Parameters	Level	Mean ± Std. D	P. value
TSH (ulU/ml)	P-Overweight	2.03 ± 1.08	0.984
	P-Obese	2.11 ± 1.09	
	C-Overweight	2.02 ± 1.31	
T3 (ng/mL)	P-Overweight	1.07 ± 0.26	0.987
	P-Obese	1.04 ± 0.29	
	C-Overweight	1.05 ± 0.4	
T4 (ng/L)	P-Overweight	9.31 ± 1.27	0.305
	P-Obese	9.42 ± 1.94	
	C-Overweight	10.46 ± 2.39	

**. The mean difference is non-significant
P- Patient, C- Control

Hyperthyroidism can lead to elevated levels of the protein SHBG and prolactin hormone, which can prevent the ovaries from releasing ovum, this can lead to infertility and other reproduction-related problems, including loss of pregnancy, menstrual disruptions (Poppe, K., 2021).

While between 2 to 4% of people of reproductive age have hypothyroidism, which is characterized by an unusually high TSH cause decrease in T3, T4 levels, and Low of thyroid hormone levels can interfere

with the release of an ovum from ovaries (ovulation), which impairs fertility (Koyyada, A., & Orsu, P., 2020).

4.3.10 Levels of thyroid hormone in patients, and control according to infertility causes (PCOS, POI)

The result in table (4-6) demonstrated no any significant difference ($p \leq 0.01$) in the TSH level of PCOS patients (2.05 ± 0.90), and POI patients (2.07 ± 1.04), as compared to the control group (2.02 ± 1.31), also no any significant difference ($p \leq 0.01$) in T3 level of PCOS patients (0.98 ± 0.24), and POI patients (1.05 ± 0.27), as compared to the control group (1.05 ± 0.40). Also no significant difference ($p \leq 0.01$) in T4 levels of both patients group (9.71 ± 1.77) PCOS, (9.37 ± 1.60) POI, as compared to the control group (10.46 ± 2.39).

Table (4-6): Comparison of the thyroid hormone in patients and control according to Infertility causes

Parameters	Level	Mean ± Std. D	P. Value
TSH (ulU/ml)	PCOS	2.05 ± 0.9	0.9812
	POI	2.07 ± 1.04	
	Control	2.02 ± 1.31	
T3(ng/mL)	PCOS	0.98 ± 0.24	0.5629
	POI	1.05 ± 0.27	
	Control	1.05 ± 0.4	
T4(ng/L)	PCOS	9.71 ± 1.77	0.0888
	POI	9.37 ± 1.6	
	Control	10.46 ± 2.39	

**. The mean difference is non-significant

Since both hypothyroidism and hyperthyroidism interfere with the ovulation process, they affect the fertility status of the woman and the abillity to conceive by preventing the release of an ovum once a month during menstruation or by not releasing one at all (Bari *et al.*, 2020).

Thyroid abnormalities may exacerbate reproductive problems and have an impact on fertility, abnormal thyroid-stimulating hormone levels may also be linked to many metabolic disorders and are considered as one of PCOS risks (Kirkegaard *et al.*, 2024).

The most prevalent type of autoimmune disease linked to POI is thyroid disease, with 12 to 33% of POI patients presenting with varying degrees of autoimmune thyroid disease (AITD), in particular, hypothyroidism brought on by Hashimoto's thyroiditis can upset the delicate hormonal balance necessary for normal ovarian function, which may lead to ovarian dysfunction and POI (Li *et al.*, 2022).

4.3.11 Levels of thyroid hormone in PCOS and POI patients and control according to the Age

The result in table (4-7) showed no any significant difference ($p \leq 0.01$) in the TSH level of (<40 years) (2.04 ± 0.91), (>40 years) (2.13 ± 0.91) PCOS patients, POI (<40 years) (2.07 ± 1.04), as compared to both control age (1.89 ± 1.19), (2.58 ± 1.71), no any significant difference ($p \leq 0.01$) in T3 level of (<40 years) (0.99 ± 0.24), (>40 years) (0.95 ± 0.23) PCOS patients, POI (<40 years) (1.05 ± 0.27) as compared to both control age (1.06 ± 0.40), (1.03 ± 0.45). Additionally, no significant difference ($p \leq 0.01$) in T4 level of (<40 years) (9.85 ± 1.64), (>40 years) (9.03 ± 2.31) PCOS patients, POI (<40 years) (9.37 ± 1.60) as compared to both group of control (10.73 ± 2.29), (9.28 ± 2.60).

Recent data from observational studies suggest that serum TSH levels increase in older people and cause decreases in T3 and T4 hormones, also, very mild TSH elevations in older individuals may not reflect subclinical thyroid dysfunction but rather be a normal consequence of aging (Mele *et al.*, 2022).

Table (4-7): Comparison of hormonal Parameters in patients and control according to Age

Parameters	Aging	Mean ± Std. D	P.value
TSH(ulU/ml)	P-PCOS(<40)	2.04 ± 0.91	0.5064
	P-PCOS(>40)	2.13 ± 0.91	
	P-POI(<40)	2.07 ± 1.04	
	C-Age(<40)	1.89 ± 1.19	
	C-Age(>40)	2.58 ± 1.71	
T3(ng/mL)	P-PCOS(<40)	0.99 ± 0.24	0.8642
	P-PCOS(>40)	0.95 ± 0.23	
	P-POI(<40)	1.05 ± 0.27	
	C-Age(<40)	1.06 ± 0.4	
	C-Age(>40)	1.03 ± 0.45	
T4(ng/L)	P-PCOS(<40)	9.85 ± 1.64	0.0377*
	P-PCOS(>40)	9.03 ± 2.31	
	P-POI(<40)	9.37 ± 1.6	
	C-Age(<40)	10.73 ± 2.29	
	C-Age(>40)	9.28 ± 2.6	

**. The mean difference is non-significant
P- Patient, C- Control

4.4 Correlation among studied groups

4.4.1 Correlation Coefficient of different parameters according to the PCOS patients.

In table (4-8) shows a significant positive correlation ($p \leq 0.01$) between DHEA-S with BMI (r=0.228) and TBF% (r=0.293), also found a strong positive correlation ($p \leq 0.01$) with prolactin, testosterone, and estrogen (r=0.484), (r=0.43), and (r=0.36) respectively. Also a strong negative correlation ($p \leq 0.01$) between FSH with both prolactin and testosterone (r=-401), (r=-334) respectively. In additionally found a significant positive correlation ($p \leq 0.01$) between Anti-TPO with both prolactin and testosterone (r=0.585), (0.445) respectively, showed positive correlation ($p \leq 0.01$) between AMH with both testosterone and estrogen (r=0.32) and (r=0.309). Also a strong positive correlation ($p \leq 0.01$) between LH and testosterone (r=0.344), and a negative correlation ($p \leq 0.01$) between Estrogen and testosterone (r=-0.39). Also, found a negative correlation

(p≤0.01) between Testosterone and FSH (r=-0.334), and a positive correlation (p≤0.01) with prolactin (r=0.806). The table demonstrated a strong negative correlation (p≤0.01) between prolactin and FSH (r=-0.401).

Table (4-8): Correlation Coefficient Among Research Parameters in PCOS Patients

Parameters	Value	TBF%	BF%	FSH (mlU/ml)	Prolactin (ng/ml)	Testosterone (pg/ml)	Esrtrogen (pg/mL)	LH (mlU/mL)	AMH (ng/mL)	Anti-TPO (ng/ml)	DHEA-S (ul)
BMI(kg/m2)	R	0.987**	0.965**	0.009	0.072	0.026	-0.261	0.012	0.055	0.049	0.288*
	P	0	0	0.95	0.632	0.861	0.077	0.934	0.714	0.745	0.049
TBF%	R	1	0.986**	0.041	0.071	0.027	-0.22	-0.001	0.06	0.085	0.293*
	P		`0.0001	0.782	0.636	0.857	0.138	0.996	0.69	0.571	0.045
BF%	R		1	0.054	0.065	0.015	-0.171	-0.082	0.079	0.104	0.314*
	P			0.717	0.664	0.922	0.25	0.582	0.599	0.487	0.031
FSH(mlU/ml)	R			1	`-0.401**	`-0.334*	0.251	-0.044	0.092	-0.226	-0.2
	P				0.005	0.022	0.089	0.767	0.54	0.127	0.178
Prolactin(ng/ml)	R				1	0.806**	-0.263	0.206	-0.25	0.585**	0.484**
	P					0	0.074	0.164	0.09	0	0.001
Testosterone(pg/ml)	R					1	`-0.391**	0.344*	`-0.320*	0.445**	0.430**
	P						0.007	0.018	0.028	0.002	0.003
Esrtrogen(pg/mL)	R						1	-0.165	.309*	0.097	-0.36
	P							0.267	0.034	0.516	0.013
LH(mlU/mL)	R							1	-0.07	0.093	0.004
	P								0.64	0.534	0.98
AMH(ng/mL)	R								1	-0.239	-0.372
	P									0.106	0.01
Anti-TPO(ng/ml)	R									1	`0.416**
	P										0.004
DHEA-S(ul)	R										1
	P										

-**. Correlation is significant at the 0.01 level.

-*. Correlation is significant at the 0.05 level.

High levels of BMI correlated to high levels of DHEA-S which causes symptoms of hyperandrogen, an essential symptom of PCOS, and that agrees with (Aghayeva *et al.*, 2023). Prolactin directly affects fertility, through its inhibition of FSH hormone and GnRH, these hormones are directly responsible for ovulation, so higher prolactin affects the ability to ovulate and leads to delayed pregnancy, that agrees with (Yang *et al.*, 2021). Higher levels of FSH are often a sign of a condition in the reproductive glands that prevents them from making normal levels of sex hormones and that explains the negative correlation between FSH and testosterone, that agrees with (Morshed *et al.*, 2021). The increase in

prolactin levels in plasma is associated with high concentrations of DHEA-S, that agree with (Krysiak *et al.*, 2023). In PCOS limiting the conversion of testosterone to estrogen, thereby leading to increased endogenous testosterone and decreased estrogen (Li *et al.*, 2020). And in PCOS, the pituitary gland secrets high amounts of hormone (LH) and ovaries also release high amounts of testosterone, which delays or misses the menstrual cycle and leads to difficulty in pregnancy, as well as increased hair in the face and body and the appearance of acne, that agree with (Moshfegh *et al.*, 2022). Testosterone is able to promote the upregulation and secretion of AMH in granulosa cells, so a high level of testosterone leads increase in AMH, that agrees with (Alhassan *et al.*, 2023). Positive correlation between testosterone and Anti-TPO in PCOS patients, that agree with (Sharma *et al.*, 2022). Hyperandrogenism is common in PCOS, often as elevated testosterone and DHEA-S levels in PCOS patients, that agree with (Boucher *et al.*, 2024). The positive correlation between estrogen and AMH, that agrees with (Capuzzo, M., & La Marca, A. 2021). The body uses DHEA-S to make androgens and estrogens hormones so in PCOS patients the reason for the high estrogen is due to the high DHEA-S, that agree with (Rababa'h *et al.*, 2020). In PCOS patients the higher serum DHEA-S levels correlated with higher serum AMH levels in infertile women, that agrees with (Lin, L. T., & Tsui, K. H. 2021). With a positive correlation between Anti-TPO and DHEA-S, that agrees with (Fawzy Mohamed Abo-Tahoon *et al.*, 2023).

4.4.2 Correlation Coefficient of biomarkers according to the POI patients.

In Table (4-9) the result demonstrated a significant positive correlation ($p \leq 0.01$) between FSH, testosterone, and prolactin ($r=0.744$), ($r=0.619$), and ($r=0.653$) respectively with BMI. AMH revealed a significant positive

correlation (p≤0.01) with testosterone (r=0.578), found a strong positive correlation (p≤0.01) between DHEA-S and AMH (r=0.678).

Table (4-9): Correlation Coefficient Among Research parameters in POI patients

Parameters	Value	TBF%	BF%	T3 (ng/mL)	FSH (mlU/ml)	Prolactin (ng/ml)	Testosterone (pg/ml)	AMH (ng/mL)	Anti-TPO (ng/ml)	DHEA-S (ul)
BMI(kg/m2)	R	`0.672*	`0.805**	-0.142	`0.744**	`0.653*	`0.619*	0.171	0.392	-0.167
	P	0.012	0.001	0.643	0.004	0.015	0.024	0.575	0.185	0.584
TBF%	R	1	0.451	-0.262	-.633*	0.443	-0.205	0.088	-0.027	-0.495
	P		0.122	0.387	0.02	0.13	0.502	0.775	0.93	0.086
BF%	R		1	-0.288	`0.643*	0.358	`0.680*	0.174	0.247	-0.081
	P			0.34	0.018	0.229	0.011	0.569	0.415	0.791
T3(ng/mL)	R			1	0.321	0.038	-0.308	`0.608*	`0.629*	`0.733**
	P				0.285	0.901	0.305	0.028	0.021	0.004
FSH(mlU/ml)	R				1	-0.169	0.489	-0.184	-0.127	0.121
	P					0.582	0.09	0.548	0.679	0.694
Prolactin(ng/ml)	R					1	-0.279	0.308	0.222	-0.123
	P						0.356	0.306	0.465	0.688
Testosterone(pg/ml)	R						1	`-0.578*	-0.463	-0.378
	P							0.039	0.111	0.203
AMH(ng/mL)	R							1	0.436	`0.678*
	P								0.137	0.011
Anti-TPO(ng/ml)	R								1	0.541
	P									0.056
DHEA-S(ul)	R									1
	P									

**. Correlation is significant at p≤ 0.01 level.

*. Correlation is significant at p≤ 0.05 level.

Increase FSH levels in POI patients, could be responsible for weight gain due are causes imbalance in form of fat storage, that agree with (Huang *et al.*, 2021). And elevated in prolactin is associated with increased food intake and weight and that agreement with (Pirchio *et al.*, 2022). The obesity effect on ovaries when the loss of ovarian activity comes a decrease in levels of testosterone, as well as estrogen and progesterone. and that agreement with (Soman *et al.*, 2020). Since testosterone is able to promote the upregulation and secretion of AMH in granulosa cells in POI patients when lower in testosterone is decrease in AMH levels and that agree with (Shrikhande *et al.*, 2020; Wang *et al.*, 2023).

4.4.3 Correlation coefficient between AMH and DHEA-S concentration in PCOS Patients.

According to figure (4-13) the result found a strong positive correlation ($p \leq 0.01$) between AMH and DHEA-S in PCOS, that related to the DHEA-S hormone which converts into androgens (mainly testosterone) in females, which is essential in the production and development of a healthy ovum. When elevated DHEA-S levels the ovum production rate increases, and so AMH levels increase and that agree with (Gleicher *et al.*, 2022), while disagreement with (Lin, L. T., & Tsui, K. H. 2021).

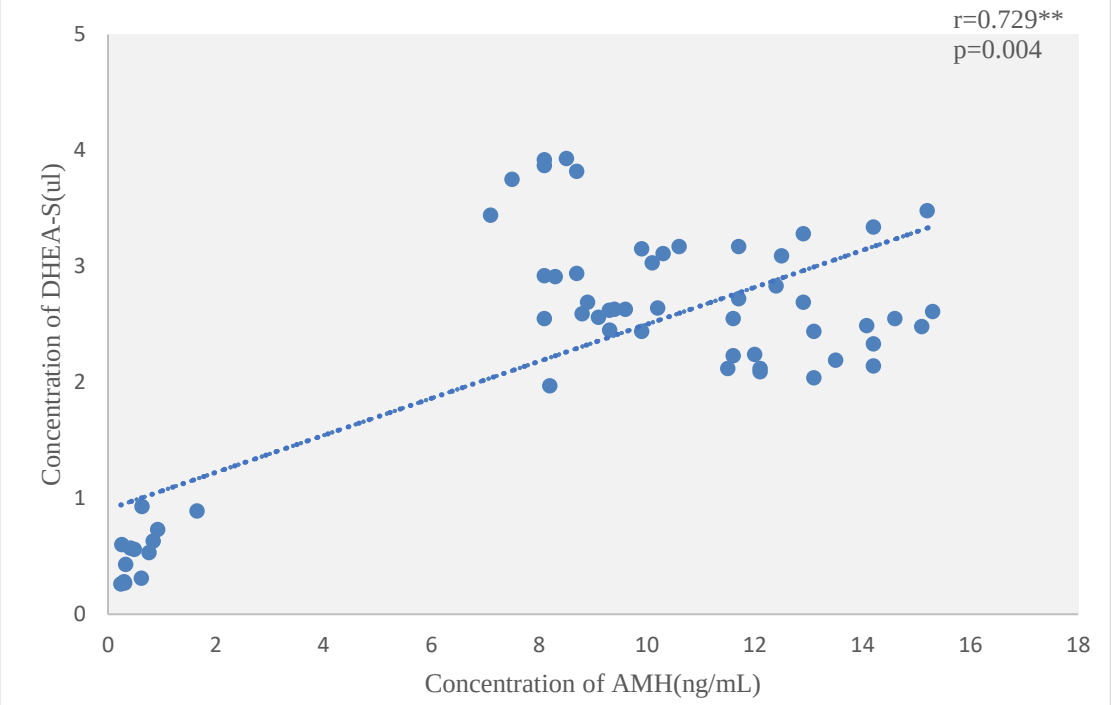


Figure (4-13): Correlation coefficient between AMH and DHEA-S level in PCOS patients.

4.4.4 Correlation coefficient between Inhibin-B and Anti-TPO concentration in PCOS Patients

The result in figure (4-14), demonstrated that highly significant a negative correlation ($p \leq 0.01$) between Inhibin-B and Anti-TPO in PCOS patients. When occurs abnormal processes the thyroid gland releases Anti-TPO as an immune response to these defects, the increase in these autoantibodies

is related to the fact that anti-TPO is likely to pass through the blood–follicle barrier during the maturation period and lead to a cytotoxic environment that damages the maturing oocyte, and that effect on ovaries, lead to decrease in Inhibin-B levels, thus, it is proven that the rise in these antibodies can lead to impaired fertility and miscarriage (Silva *et al.*, 2022).

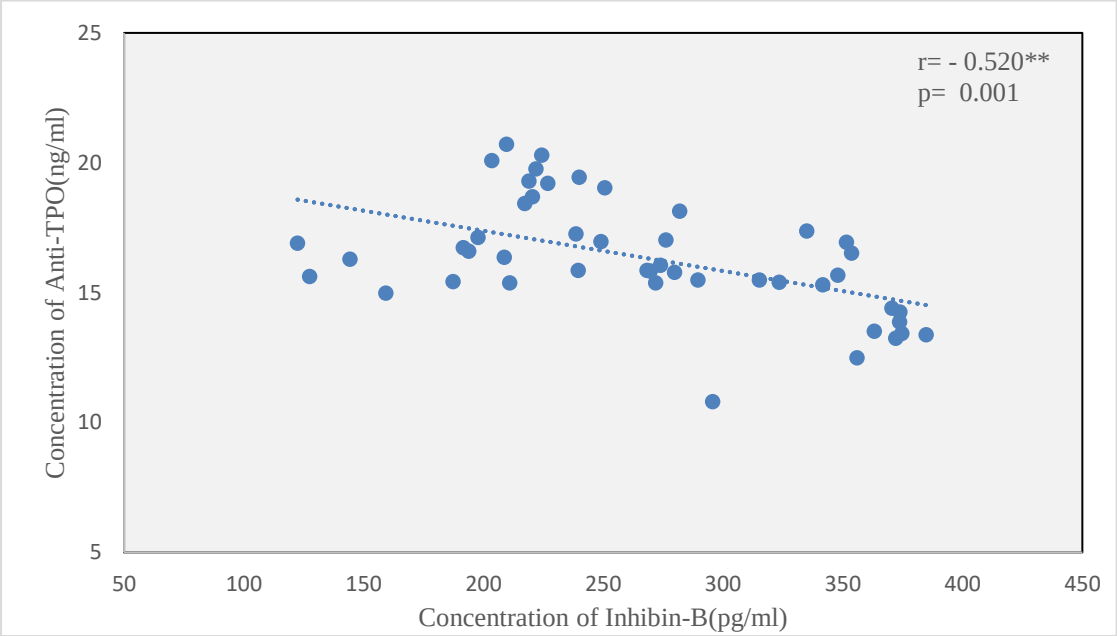


Figure (4-14) Correlation coefficient between Inhibin-B and Anti-TPO level in PCOS patients.

4.4.5 Correlation coefficient between Anti-TPO and DHEA-S concentration in PCOS Patients

According to figure (4-15), showed a positive correlation ($p \leq 0.01$) between DHEA-S and Anti-TPO in PCOS. In PCOS patients DHEA-S elevation is due to stimulation of adrenal steroidogenesis and the high levels of LH in this disease, in general, the DHEA-S is converted to testosterone, which interferes with the development of the follicles and prevents normal ovulation, the physiological amount of testosterone regulates both the secretion of thyroid-stimulating hormone and the amount of its receptor; however, in higher amounts of testosterone, this function would be

reversed and may lead increase in the rate of release of Anti-TPO (Sharma *et al.*, 2022).

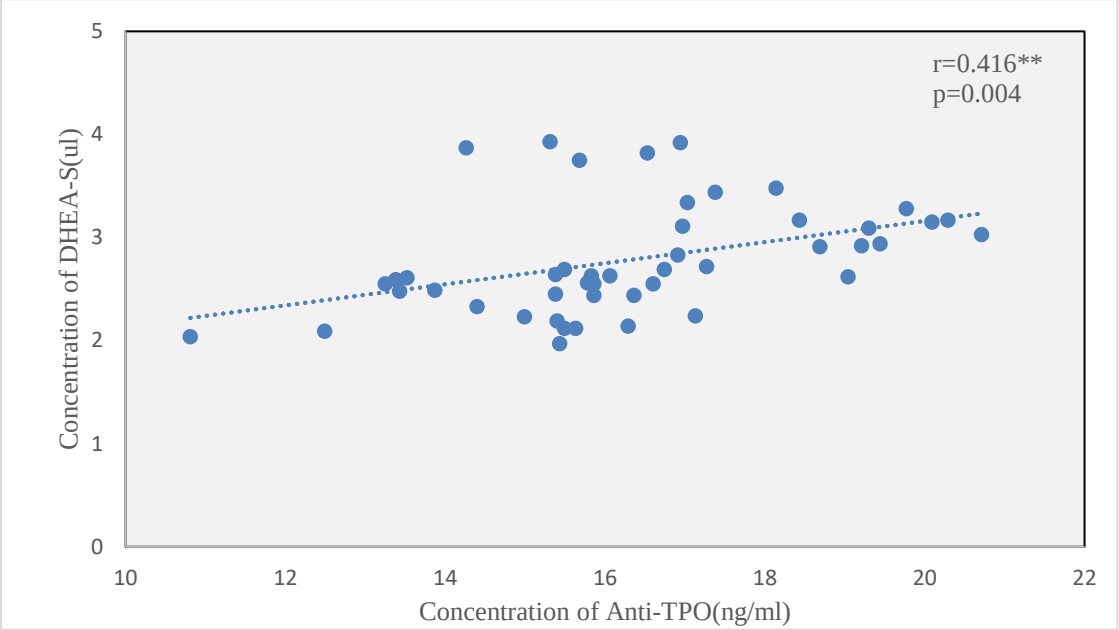


Figure (4-15) Correlation coefficient between Anti-TPO and DHEA-S level in PCOS patients

4.4.6 Correlation coefficient between AMH and DHEA-S concentration in POI Patients.

In figure (4-16) the result revealed that a strong positive correlation ($p \leq 0.01$) between AMH and DHEA-S in POI patients. POI patients suffer from low ovarian reserve and failure to function normally; one of the hallmarks of this imbalance can be considered to be decreased or absent AMH levels. In addition, ovaries do not manufacture sufficient quantities of DHEA-S and androgens as their primary source, so there is a marked decrease in DHEA-S levels as well (Wang *et al.*, 2023).

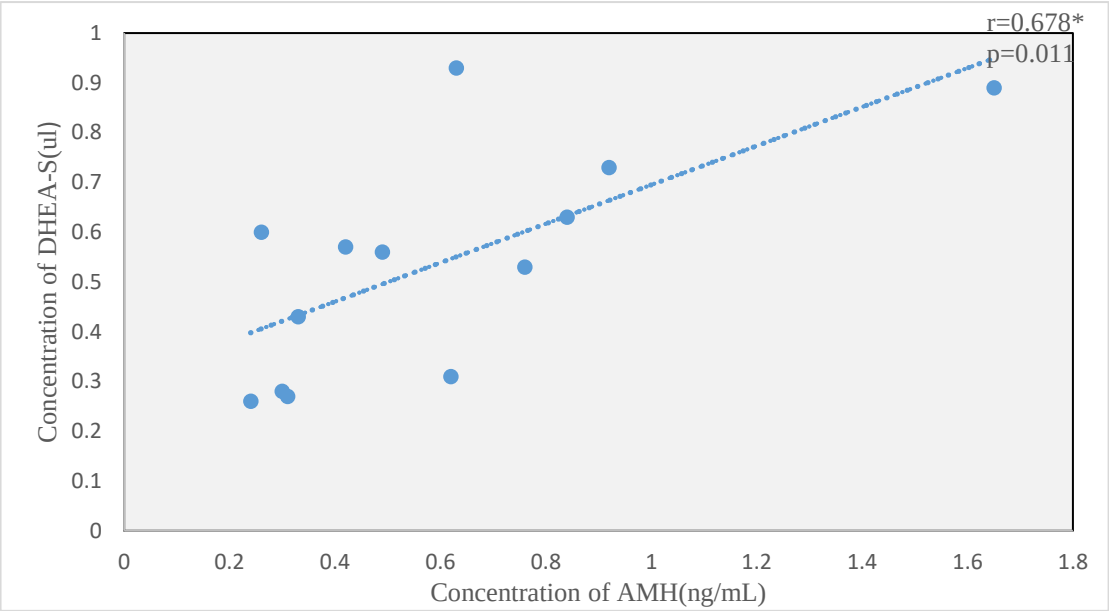


Figure (4-16) Correlation coefficient between AMH and DHEA-S levels in POI patients.

4.4.7 Correlation coefficient of Testosterone and AMH concentration in POI Patients

In figure (4-17) results found a strong negative correlation ($p \leq 0.01$) between AMH and Testosterone in POI patients. The testosterone that output from DHEA-S is able to promote the upregulation and secretion of AMH in granulosa cells, that means testosterone able to affect in recruitment and development of follicle, so in POI patients when decrease in DHEA-S that effect on AMH levels, poor AMH value results in a low in ovarian reserve, indicating poor follicle growth, thus reducing the number of oocytes produced (Shrikhande *et al.*, 2020).

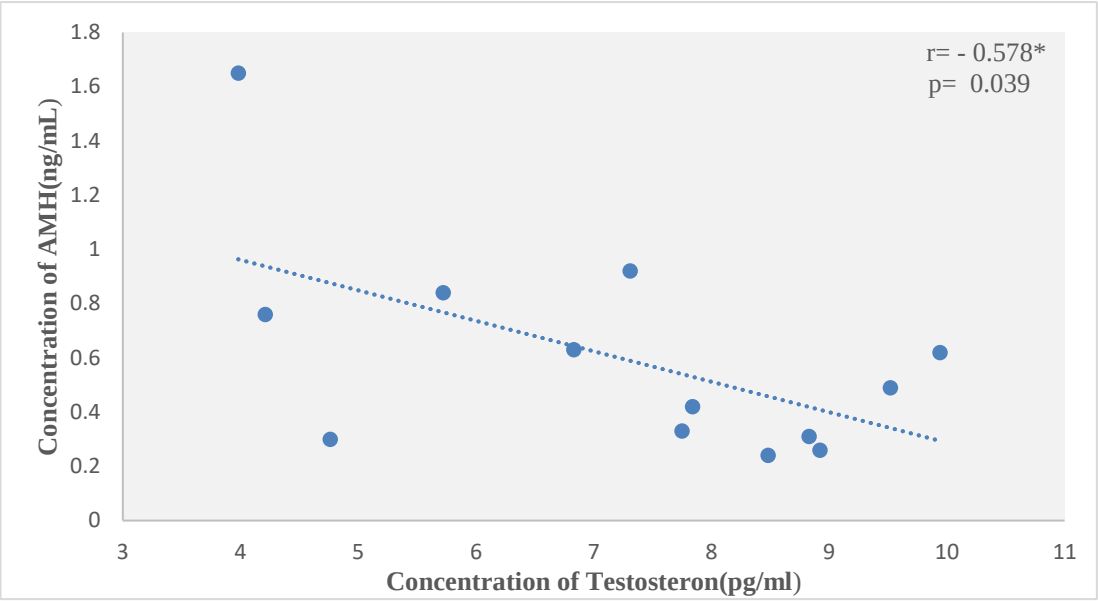


Figure (4-17) Correlation coefficient between Testosterone and AMH levels in POI patients

4.5 Receiver Operative Characteristic Curve (ROC)

4.5.1 ROC Curve according to research Parameters

The result in table (4-10) and figure (4-18) suggested that AMH (95% CI: 0.918-1.000; P-value: 0.001; Cutoff Point: 4.143; AUC: 96.306%) with Sensitivity to Specificity 96.667%-95.000%; and Accuracy: 95.830%. Inhibin-B (95% CI: 0.907-1.000; P-value: 0.001; Cutoff Point: 77.347; AUC: 95.417%) with Sensitivity to Specificity 91.667%-98.333%; and Accuracy: 95.000%. And for Anti-TPO (95% CI: 0.970-1.000; P-value: 0.001; Cutoff Point: 9.306 AUC: 98.861%) with Sensitivity to Specificity 96.676%- 96.667%; and Accuracy: 97.000%. And for DHEA-S (95% CI: 0.992-1.000; P-value: 0.001; Cutoff Point: 1.480 AUC: 99.694%) with Sensitivity to Specificity 98.333%-96.667%; and Accuracy: 97.500% in identifying infertility.

Table (4-10): Receiver Operative Characteristic (ROC) for analysis

Metrics		AMH (ng/mL)	Inhibin-B (pg/ml)	Anti-TPO (ng/ml)	DHEA-S (ul)
Std. Error		0.023	0.024	0.010	0.003
Asymptotic Sig.		0.002	0.009	0.002	0.006
Asymptotic 95% Confidence Interval	Lower Bound	0.918	0.907	0.970	0.992
	Upper Bound	1.000	1.000	1.000	1.000
Cutoff Point		4.143	77.347	9.306	1.480
Area Under Curve (AUC)		96.306%	95.417%	98.861%	99.694%
Sensitivity		96.667%	91.667%	96.676%	98.333%
Specificity		95.000%	98.333%	96.667%	96.667%
Accuracy		95.830%	95.000%	97.000%	97.500%
Positive Predictive Value		95.080%	98.210%	96.671%	96.720%
Negative Predictive Value		96.610%	92.190%	96.670%	98.310%

Comparable results were documented in previous studies that showed that the AUC, sensitivity, and specificity for AMH are (91.6%, 100%, 100%) (Sun *et al.*, 2022). Another previous study showed the sensitivity and specificity for AMH (88.6% and 84.6%) (De Loos *et al.*, 2021). Studies were that showed that the AUC, sensitivity, and specificity of both Inhibin-B were (82.84%, 36.8 %, 86.6 %) (AL-Azawea, B. R., & Mossa, H. A. 2021). and Anti-TPO (88.45%, 91.7%, 87.1%) (Rashad *et al.*, 2024). Another previous study showed sensitivity and specificity for Inhibin-B (95%, 86%) (Al-Ezairjawi *et al.*, 2020). And (95.7%, 88.5%) (He *et al.*, 2020). And DHEA-S (4.0%, 95%, 85.1%) (Wang, Z., 2023), another previous study showed the sensitivity and specificity for DHEA-S (72.0% and 82.93%) (Chen *et al.*, 2021).

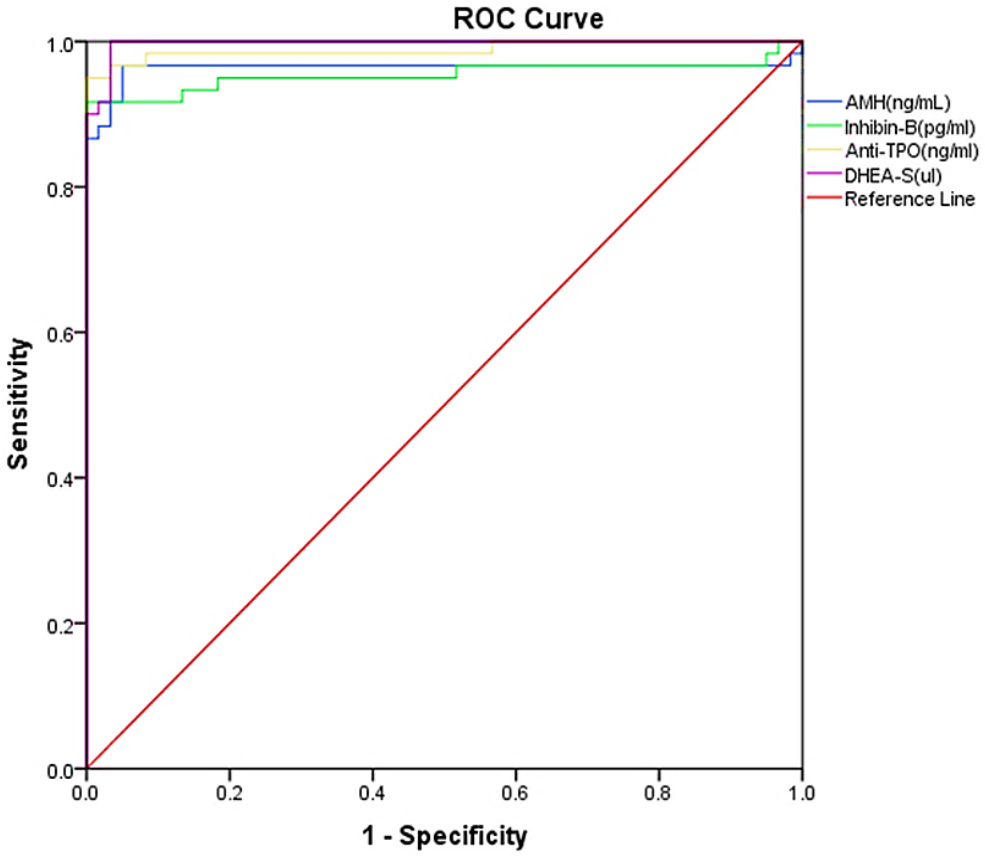


Figure (4-18) ROC Curve demonstrating the sensitivity and specificity values for research biomarkers

4.5.2 ROC Curve according to research hormonal parameters

The results in table (4-11) and figure (4-19) suggest that prolactin (95% CI: 0.907-0.982; P-value: 0.001; Cutoff Point: 19.373; AUC: 94.472%) with Sensitivity to Specificity 93.333%-85.000%; and Accuracy: 89.167%. And for Testosterone (95% CI: 0.900-0.997; P-value: 0.001; Cutoff Point: 7.254; AUC: 94.875%) with Sensitivity to Specificity 88.333%-98.333%; and Accuracy: 93.330%. Also for Estrogen (95% CI: 0.668-0.871; P-value: 0.001; Cutoff Point: 56.945; AUC: 76.917%) with Sensitivity to Specificity76.667%-93.333%; and Accuracy: 85.000%. And for LH (95% CI: 0.891-0.985; P-value: 0.001; Cutoff Point: 11.325; AUC: 93.778%) with Sensitivity to Specificity 83.333%-96.667%; and Accuracy: 90.000% in identifying infertility.

Table (4-11): Receiver Operative Characteristic (ROC) according to research analysis

Metrics		Prolactin (ng/ml)	Testosterone (pg/ml)	Estrogen (pg/mL)	LH (mlU/mL)
Std. Error		0.019	0.025	0.052	0.024
Asymptotic Sig.		0.004	0.002	0.003	0.001
Asymptotic 95% Confidence Interval	Lower Bound	0.907	0.900	0.668	0.891
	Upper Bound	0.982	0.997	0.871	0.985
Cutoff Point		19.373	7.254	56.945	11.325
Area Under Curve (AUC)		94.472%	94.875%	76.917%	93.778%
Sensitivity		93.333%	88.333%	76.667%	83.333%
Specificity		85.000%	98.333%	93.333%	96.667%
Accuracy		89.167%	93.330%	85.000%	90.000%
Positive Predictive Value		86.154%	98.150%	92.000%	96.150%
Negative Predictive Value		92.727%	89.390%	80.000%	85.290%

Comparable results were documented in previous studies that showed that the AUC, sensitivity, and specificity of Prolactin were (83.2%, 77%, 88%,) (Pedachenko *et al.*, 2021). And Testosterone (88.9%, 92.6%, 85.4%) (Wang *et al.*, 2020). As the Estrogen (76.6%; 92.1%, 55.8%) (Deng *et al.*, 2022). And LH (93.2%; 80%, 93%) (Weghofer, A et al., 2020). Another studies that showed that the AUC, sensitivity, and specificity of Prolactin were (85.3%, 76.9%, 86.1%) (Kim *et al.*, 2023). And LH (93.2%; 86.3%: 95%) (Alhassan *et al.*, 2023).

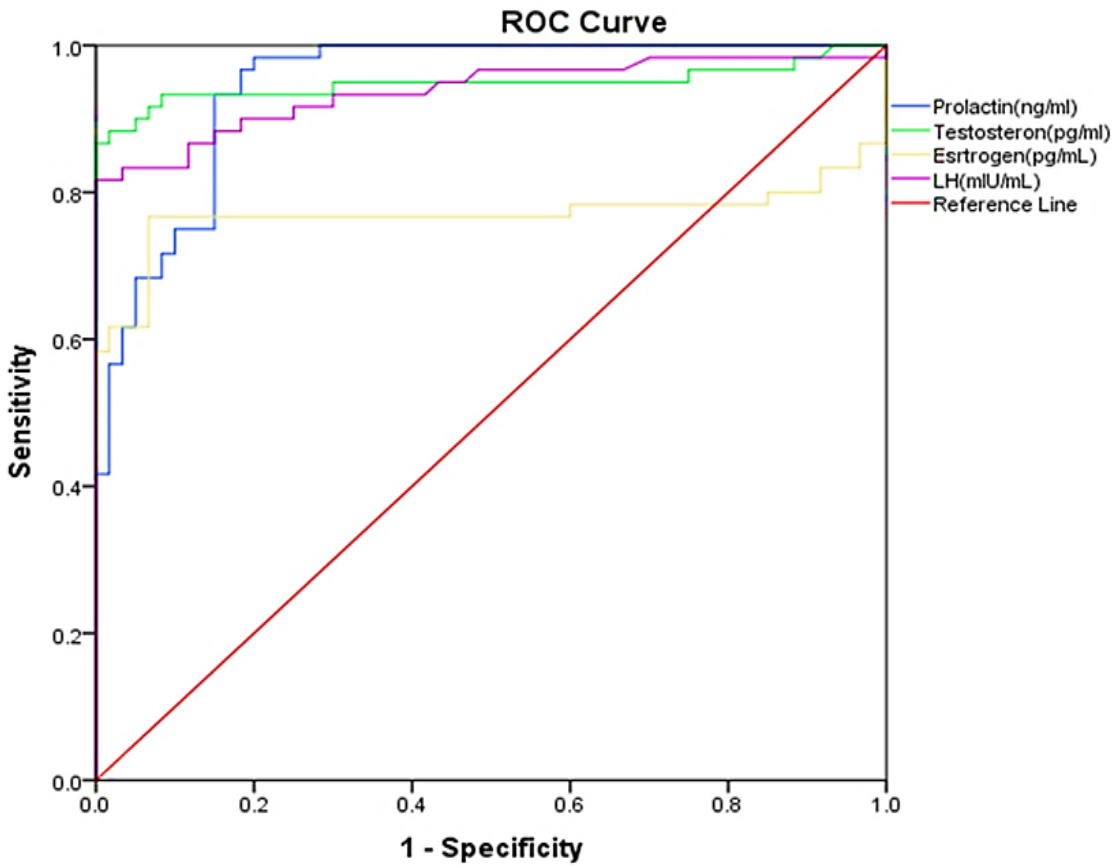


Figure (4-19) ROC Curve demonstrated the sensitivity and specificity values for research parameters

Conclusions and Recommendations

Conclusions

Based on the results obtained in this study, we can conclude the following:

1. Anti-TPO, DHEA-S, prolactin, and Inhibin-B are good parameters to evaluate and prognosis for PCOS and POI in an infertile female.
2. The levels of anti-TPO, Prolactin, and LH biomarkers increased in all types of infertility patients; which can be used as early detection of infertility, and intervention is considered crucial to decreasing the damage caused by these changes which subsequently affect the patient future fertility.
3. Females with a high body mass index (BMI) are more susceptible to the development of polycystic ovarian syndrome (PCOS).
4. In PCOS, and POI cases the concentrations of AMH, Anti-TPO, Inhibin-B, DHEA-S biomarkers, and androgen hormones are affected by age, BMI, and weight.
5. There was a significant positive correlation between AMH, DHEA-S, and between DHEA-S, Anti-TPO in PCOS patients, with a positive correlation between AMH and DHEA-S in POI patients. Meanwhile, there was a significant negative correlation between Inhibin-B, Anti-TPO in PCOS patients, with a negative correlation between AMH and DHEA-S in POI patients.
6. No significant changes were found in the thyroid hormones among PCOS and POI patients when compared with the control.

Recommendations

In future studies, the following points are recommended:

1. Recommended to increase the study population for POI patients in order to reach to the important knowledge about pathogenesis, and metabolic pathway for this disease.
2. Recommended to study the other novel biomarkers such as (Leptin, adiponectin, MMP-9, and S100A8) in PCOS and POI patients
3. Increasing future studies to validate the sensitivity and specificity of plasma Anti-TPO, Inhibin-B, DHEA-S, and prolactin as diagnostic biomarkers associated with infertility could well improve knowledge.
4. Recommended to study the relationship of other parameters in the occurrence of infertility in obese and non-obese females.
5. Recommended to expand the use of Prolactin, Inhibin-B, FSH, LH, and androgen as biomarkers for the diagnosis or prediction of PCOS, and POI.
6. Recommended to study the genetic factors and family history to predict and detect infertility.

References

1. Abdelghani, A. M. A., Abu Hashem, M. F., & Aemmaar, W. A. E. (2023). Epidemiology, Risk Factors, Pathogenesis and Etiology of Polycystic Ovarian Syndrome. *The Egyptian Journal of Hospital Medicine*, *81*(1), 4878-4883. https://dx.doi.org/10.21608/ejhm.2023.301776
2. Akane, H., Toyoda, T., Matsushita, K., Morikawa, T., Kosaka, T., Tajima, H., ... & Ogawa, K. (2024). Comparison of the sensitivity of histopathological and immunohistochemical analyses and blood hormone levels for early detection of antithyroid effects in rats treated with thyroid peroxidase inhibitors. *Journal of Applied Toxicology*. https://doi.org/10.1002/jat.4604
3. Aghayeva, S., Sonmezer, M., Şükür, Y. E., & Jafarzade, A. (2023). The role of thyroid hormones, vitamins, and microelements in female infertility. *Revista Brasileira de Ginecologia e Obstetrícia/RBGO Gynecology and Obstetrics*, *45*(11), e683-e688. https://do.org/10.1055/s-0043-1772478
4. Akbarinejad, V., Gharagozlou, F., Vojgani, M., & Ranji, A. (2020). Evidence for quadratic association between serum anti-Müllerian hormone (AMH) concentration and fertility in dairy cows. *Animal reproduction science*, *218*, 106457. https://doi.org/10.1016/j.anireprosci.2020.106457
5. Akdulum, M. F. C., Erdem, M., Barut, G., Demirdag, E., İyidir, Ö. T., Guler, I., & Erdem, A. (2022). The relationship between thyroid autoimmunity and poor response to ovarian stimulation in in vitro fertilization women with infertility. *Endokrynologia Polska*, 73(4), 699-705. https://doi.org/10.5603/EP.a2022.0061
6. Alimovna, K. F., Srajitdinovna, F. S., & Sadikovich, A. B. (2024). The Pathogenetic Meaning (Pathogenetic Significance) of Inhibin in the

Development of Premature Ovarian Failure. *Annals of the Romanian Society for Cell Biology*, 6268-6278. https://doi.org/docview/2563221336

7. Al-Deresawi, M. S. G. (2006). *Hormonal-Genetical Study on Women with Polycystic Ovary Syndrome and Thyroid Disorders* (Doctoral dissertation, Baghdad University). https://www.researchgate.net/publication/323239721
8. Alhassan, S., Elmugadam, A., Elfadil, G. A., Abubaker, N., Elfaki, E. M., Hamza, A., ... & Karar, T. (2023). Diagnostic performance of anti Müllerian hormone, luteinizing hormone to follicle-stimulating hormone ratio, testosterone, and prolactin to predict polycystic ovary syndrome among Sudanese women. *International Journal of Women's Health*, 837-843. https://doi.org/full/10.2147/IJWH.S403347
9. AL-Azawea, B. R., & Mossa, H. A. (2021). Evaluation of Inhibin-B Levels as a predictive marker in a sample of Iraqi Women Undergoing ICSI. *British Journal of Medical & Health Sciences (BJMHS)*, *3*(7). https://doi.org/354341989
10. Al-Ezairjawi, R., Risan, F. A., & Al-Shareef, D. (2020). Determination of inhibin B levels in hypothyroidism infertile Iraqi women. *journal of the college of basic education*, 26(109), 459-468. https://doi.org/3ed2830c5cb5a599
11. Amrhein, V., Greenland, S., & McShane, B. (2019). Scientists rise up against statistical significance. *Nature*, 567(7748), 305-307. https://doi.org/articles.d41586-019-00857-9
12. Ashton, N. J., Janelidze, S., Al Khleifat, A., Leuzy, A., van der Ende, E. L., Karikari, T. K., ... & Hansson, O. (2021). A multicentre validation study of the diagnostic value of plasma neurofilament light. *Nature*

communications, *12*(1), 3400. https://doi.org/10.1038/s41467-021-23620-z

13. Baheti, S., Verma, A., & Sharma, M. (2020). Premature ovarian failure incidence, risk factors and its relation to BMI and infertility. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, *8*(3), 947-951. http://www.ijrcog.org/index.php
14. Barber, T. M., & Franks, S. (2021). Obesity and polycystic ovary syndrome. *Clinical endocrinology*, *95*(4), 531-541. https://doi.org/10.1111/cen.14421
15. Bari, S., Begum, R., & Akter, Q. S. (2020). Hypothyroidism and hyperprolactinemia in women with primary and secondary infertility. *IMC Journal of Medical Science*, 14(1), 41-46. https://d1wqtxts1xzle7.cloudfront.net/107478130/34321
16. Bulletti, C., Bulletti, F. M., Sciorio, R., & Guido, M. (2022). Progesterone: The key factor of the beginning of life. *International Journal of Molecular Sciences*, *23*(22), 14138. https://doi.org/10.3390/ijms232214138
17. Britannica, T., Siddharth, S. (2023). Stem Cell Biology: An Overview. *Biotechnological Advances for Microbiology, Molecular Biology, and Nanotechnology*, 297-310. https://doi.or/10.1201/9781003161158-14
18. Bodepudi, Ranita & Seher, Saniya & Khan, Shenel & Emmanuel, Sonya & Shantha Kumar, Vivig & Nerella, Resheek & Ameen, Basim & John, Jabez & Khan, Safeera. (2023). Myoinositol Versus Metformin in the Treatment of Polycystic Ovarian Syndrome: *A Systematic Review*. *Cureus*. 15. 10.7758/cureus.41748. https://doi.org/10.7759/cureus.41748

19. Boucher, H., Robin, G., Ribière, L., Martin, C., Espiard, S., & Catteau Jonard, S. (2024, April). Is it useful to measure DHEAS levels in PCOs?. In *Annales d'Endocrinologie* (Vol. 85, No. 2, pp. 95-99). Elsevier Masson. https://doi.org/10.1016/j.ando.2024.01.011
20. Cai, Y., Zhang, Q., Yu, K., & Wang, Q. (2022). [Retracted] Study of Serum Anti-Mullerian Hormone in the Diagnosis of Ovarian Reserve Function in Patients with Premature Ovarian Insufficiency. *BioMed Research International*, *2022*(1), 3878359. https://doi.org/10.1155/2022/3878359
21. Carmina, E., & Longo, R. A. (2022). Increased prevalence of Elevated DHEAS in PCOs Women with non-classic (B or C) phenotypes: a retrospective analysis in patients aged 20 to 29 years. *Cells*, 11(20), 3255. https://doi.org/10.3390/cells11203255
22. Capuzzo, M., & La Marca, A. (2021). Use of AMH in the differential diagnosis of anovulatory disorders including PCOs. *Frontiers in Endocrinology*, 11, 616766. https://doi.org/10.3389/fendo.2020.616766
23. Chen, C., & Kattera, S. (2020). Comparison of pronuclear zygote morphology and early cleavage status of zygotes as additional criteria in the selection of day 3 embryos: a randomized study. *Fertil Steril*, *85*(2), 347-352. https://doi.org/10.1016/j.fertnstert.2005.07.1319
24. Colella, M., Cuomo, D., Giacco, A., Mallardo, M., De Felice, M., & Ambrosino, C. (2020). Thyroid hormones and functional ovarian reserve: systemic vs. peripheral dysfunctions. *Journal of clinical medicine*, *9*(6), 1679. https://doi.org/10.3390/jcm9061679
25. Concepción-Zavaleta, M. J., Coronado-Arroyo, J. C., Quiroz-Aldave, J. E., Concepción-Urteaga, L. A., & Paz-Ibarra, J. (2023). Thyroid dysfunction and female infertility. A comprehensive review. *Diabetes*

& Metabolic Syndrome: Clinical Research & Reviews, 17(11), 102876. https://doi.org/10.1016/j.dsx.2023.102876

26. Chon, S. J., Umair, Z., & Yoon, M. S. (2021). Premature ovarian insufficiency: past, present, and future. *Frontiers in cell and developmental biology*, 8, 672880. https://doi.org/10.3389/fcell.2021.672890
27. Crespo, Raiane & Bachega, Tânia & Mendonca, Berenice & Gomes, Larissa. (2018). An update of genetic basis of PCOS pathogenesis. *Archives of Endocrinology and Metabolism*. 62. 352-361. https://doi.org/10.20945/2359-3997000000049
28. Chua, S. J., Danhof, N. A., Mochtar, M. H., Van Wely, M., McLernon, D. J., Custers, I., ... & Van Eekelen, R. (2020). Age-related natural fertility outcomes in women over 35 years: a systematic review and individual participant data meta-analysis. *Human Reproduction*, *35*(8), 1808-1820. https://doi.org/10.1093/humrep/deaa129
29. Chang, H., Li, J., Wu, Q., Fu, X., Zhu, M., Ge, H., Shi, B., Shao, X., & Han, Y. (2022). The Effect of Age on IVF-ET Outcome in Infertile Patients with PCOS Based on Tiangui Theory in Chinese Medicine. *Evidence-based complementary and alternative medicine* : eCAM, 2022, 2877636. https://doi.org/10.1155/2022/2877636.
30. Chen, F., Chen, M., Zhang, W., Yin, H., Chen, G., Huang, Q., ... & Yin, G. (2021). Comparison of the efficacy of different androgens measured by LC-MS/MS in representing hyperandrogenemia and an evaluation of adrenal-origin androgens with a dexamethasone suppression test in patients with PCOs. *Journal of Ovarian Research*, *14*, 1-9. https://doi.org/10.1186/s13048-021-00781-5

31. Chen, L., Lin, C., & Yin, G. (2021). Fu Chen1, Minjie Chen2, 3, Weichun Zhang2, Huihuang Yin2, Guishan Chen2, Qingxia Huang2, Xiaoping Yang2. https://doi.org/10.1186/s13048-021-00781-5

32. Chumduri, C., & Turco, M. Y. (2021). Organoids of the female reproductive tract. *Journal of molecular medicine*, *88*(4), 531-553. https://doi.org/10.1007/s00109-020-02028-0

33. Delbaere, I., Verbiest, S., & Tydén, T. (2020). Knowledge about the impact of age on fertility: a brief review. *Upsala journal of medical sciences*, *125*(2), 167-174. https://doi.org/10.1080/03009734.2019.1707913

34. Deng, W., Sun, R., Du, J., Wu, X., Ma, L., Wang, M., & Lv, Q. (2022). Prediction of miscarriage in first trimester by serum estradiol, progesterone and β-human chorionic gonadotropin within 9 weeks of gestation. *BMC Pregnancy and Childbirth*, *22*(1), 112. https://doi.org/10.1186/s12884-021-04158-w

35. Deurenberg P, Weststrate JA, Seidell JC: Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. Br J Nutr. 1991, 65 (2): 105-114. 10.1079/BJN19910073). https://doi.org/10.1079/BJN19910073

36. De Loos, A. D., Hund, M., Buck, K., Meun, C., Sillman, J., & Laven, J. S. (2021). Antimüllerian hormone to determine polycystic ovarian morphology. *Fertility and sterility*, *116*(4), 1149-1157. https://doi.org/10.1016/j.fertnstert.2021.05.094

37. De Oliveira, N. M. G. (2020). The efficiency of a complementary approach to Stein-Leventhal Syndrome (Polycystic Ovary Syndrome)-a comprehensive review. *PQDT-Global*. https://doi.oeg/openview/f0b184775ad6a29cf514ea0d3e76cc1

38. Demayo, S., Giannone, L., Monastero, A., Nolting, M., Landeau, M. P., Lana, M. B. P., ... & Sternberg, K. (2019). Reality of premature ovarian failure in Argentina. *Revista da Associação Médica Brasileira*, *65*, 419-423. https://doi.org/10.1590/1806-9282.65.3.419

39. Dilbaz, B., & Mert, Ş. A. (2022). Evaluation and interpretation of AMH in female infertility. *Duzce Medical Journal*, *24* (Special Issue), 82-85.https://doi.org/10.18678/dtfd.1186131

40. Di Clemente, N., Racine, C., Pierre, A., & Taieb, J. (2021). Anti-Müllerian hormone in female reproduction. *Endocrine reviews*, *42*(6), 753-782. https://doi.org/10.1210/endrev/bnab012

41. Dong, J., & Rees, D. A. (2023). Polycystic ovary syndrome: pathophysiology and therapeutic opportunities. *BMJ medicine*, *2*(1). https://doi.org/10.1136/bmjmed-2023-000548

42. Dothard, M. I., Allard, S. M., & Gilbert, J. A. (2023). The effects of hormone replacement therapy on the microbiomes of postmenopausal women. *Climacteric*, *26*(3), 182-182. https://doi.org/10.1080/13697137.2023.2173568

43. Du, M., Peng, L., Zhang, R., & Bao, S. (2024). Successful Pregnancy and Delivery after Premature Ovarian Insufficiency Combined with Undifferentiated Connective Tissue Disease: A Case Report. *Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders)*, *24*(7), 840-844. https://doi.org/10.2174/0118715303248685231023114212

44. Dursun, P. (2024). Correlation between high FSH levels and increased risk of aneuploidy: the origin of the hypothesis. *Molecular Human Reproduction*, *30*(8), gaae028. https://doi.org/10.1093/molehr/gaae028

45. Ennab, F., & Atiomo, W. (2023). Obesity and female infertility. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *88*, 102336. https://doi.org/10.1016/j.bpobgyn.2023.102336

46. Esfandiari, N. H., Reyes-Gastelum, D., Hawley, S. T., Haymart, M. R., & Papaleontiou, M. (2018). Patient requests for tests and treatments impact physician management of hypothyroidism. *Thyroid*, *28*(11), 1536-1544. https://doi.org/10.1089/thy.2019.0383

47. Evliyaoglu, O., Imöhl, M., Weiskirchen, R., & van Helden, J. (2020). Age-specific reference values improve the diagnostic performance of AMH in polycystic ovary syndrome. *Clinical Chemistry and Laboratory Medicine (CCLM)*, 58(8), https://doi.org/10.1515/cclm-2019-1059 91 1291-1301

48. Elhddad, A. S. (2020). The Prevalence of Premature Ovarian Failure among Subfertile Patient in Albayda Fertility Centre/A Public Center in Libya. https://doi.org/10.36348/sijap.2020.v03i05.002

49. Fathimunissa, M. A., Natarajan, P., & Pandiyan, R. (2022). Infertility−What do we mean?. *Fertility Science and Research*, *9*(2), 77-79. https://doi.org/10.4103/fsr.fsr_25_22

50. Fatima, S., Liaqat, S., Aamir, M., Haroon, Z. H., Chaudhry, N., & Khadim, M. T. (2020). Biochemical prevalence of polycystic ovarian diseases, premature ovarian failure and thyroid dysfunction in women of Rawalpindi cohort. *Pakistan Journal of Pathology*, *31*(4), 114-117. https://doi.org/19.234/article/561

51. Fawzy Mohamed Abo-Tahoon, W., El-Omda, A. E. A., Taha Abd El Fattah, A., & Abd Elatif Hashish, M. (2023). Thyroiditis as a risk factor in polycystic ovarian syndrome women. *Al-Azhar Medical Journal*, 52(2), 601-616. https://dx.doi.org/10.21608/amj.2023.291652

52. Farhana, A. A., & Shi, J. (2024). Systematic Review on the Various Clinical Features of Polycystic Ovary Syndrome (PCOS) Patients. *J Life Sci*, *10*(2), 5159-5166. https://doi.org/10.21276/SSR-IIJLS.2024.10.2.22

53. Francone, N. O., Ramirez, T., & Boots, C. E. (2023). Contemporary Management of the Patient with Polycystic Ovary Syndrome. *Obstetrics and Gynecology Clinics*, *50*(4), 685-705. https://doi.org/10.1016/j.ogc.2023.08.003

54. Ford EA, Beckett EL., Roman SD., McLaughlin EA, Sutherland JM (2020). Advances in human primordial follicle activation and premature ovarian insufficiency. *Reproduction*, R15, R28,10. https://doi.org/10.1530/REP-19-0201

55. Gawron, I. M., Baran, R., Derbisz, K., & Jach, R. (2022). Association of subclinical hypothyroidism with present and absent anti-thyroid antibodies with PCOs phenotypes and metabolic profile. *Journal of Clinical Medicine*, *11*(6), 1547. https://doi.org/10.3390/jcm11061547

56. Geng, X., He, Z., Bao, Z., Di, W., & Gu, Z. (2023). Aberrant HPO axis alterations and autoimmune abnormalities in PCOs patients with DOR: a retrospective analysis. *Journal of Clinical Medicine*, *12*(16), 5212. https://doi.org/10.3390/jcm12165212

57. Ge, L., & Tan, J. (2020). Development of three-dimensional effects and stretch for polymeric optical fiber (POI) textiles with double weave structure containing spandex. *The Journal of The Textile Institute*, *112*(3), 388–405. https://doi.org/10.1080/00405000.2020.1761678

58. Ghahremani-Nasab, M., Ghanbari, E., Jahanbani, Y., Mehdizadeh, A., & Yousefi, M. (2020). Premature ovarian failure and tissue

engineering. *Journal of cellular physiology*, *235*(5), 4217-4226. https://doi.org/10.1002/jcp.29376

59. Gierach, M., Bruska-Sikorska, M., Rojek, M., & Junik, R. (2022). Hyperprolactinemia and insulin resistance. *Endokrynologia Polska*, *73*(6), 858-867. https://doi.org/10.5603/EP.a2022.0075

60. Godlewska, M., & Banga, P. J. (2019). Thyroid peroxidase as a dual active site enzyme: Focus on biosynthesis, hormonogenesis and thyroid disorders of autoimmunity and cancer. *Biochimie*, *160*, 34–45. https://doi.org/10.1016/j.biochi.2019.02.003

61. Goacher, P. J., Lambert, R., & Moffatt, P. G. (2012). Can weight-related health risk be more accurately assessed by BMI, or by gender specific calculations of Percentage Body Fatness?. *Medical hypotheses*, *79*(5), 656-662. https://doi.org/10.1016/j.mehy.2012.08.003

62. Guevarra, D. A., Louis, C. C., Gloe, L. M., Block, S. R., Kashy, D. A., Klump, K. L., & Moser, J. S. (2023). Examining a window of vulnerability for affective symptoms in the mid-luteal phase of the menstrual cycle. *Psychoneuroendocrinology*, *147*, 105858. https://doi.org/10.1111/cen.14704

63. Hanson, B., Johnstone, E., Dorais, J., Silver, B., Peterson, C. M., & Hotaling, J. (2017). Female infertility, infertility-associated diagnoses, and comorbidities: a review. *Journal of assisted reproduction and genetics*, *34*, 167-177. https://doi.org/10.1007/s10815-016-0836-8

64. Han, S., Li, H., Lu, R., Feng, J., Tang, K., Li, S., & Lin, J. (2023). Effect and mechanism of pearl on ovarian function with premature ovarian failure induced by tripterygium glycosides. *Journal of Traditional and Complementary Medicine*, *13*(4), 368-378. https://doi.org/10.1016/j.jtcme.2023.02.004

65. Herbison, A. E. (2020). A simple model of estrous cycle negative and positive feedback regulation of GnRH secretion. *Frontiers in neuroendocrinology*, *57*, 100837. https://doi.org/10.1016/j.yfrne.2020.100837

66. Herman, T., Csehely, S., Orosz, M., Bhattoa, H. P., Deli, T., Torok, P., ... & Jakab, A. (2023). Impact of endocrine disorders on IVF outcomes: results from a large, single-centre, prospective study. *Reproductive Sciences*, *30*(6), 1878-1880. https://doi.org/10.1007/s43032-022-01137-0

67. Hellstrom, E. A., Ziegler, A. L., & Blikslager, A. T. (2021). Postoperative ileus: comparative pathophysiology and future therapies. *Frontiers in Veterinary Science*, *8*, 714800. https://doi.org/10.3389/fvets.2021.714800

68. Heitmann, B. L. (1990). Evaluation of body fat estimated from body mass index, skinfolds and impedance. A comparative study. *European journal of clinical nutrition*, *44*(11), 831-837. https://www.altmetric.com/details/170784867

69. Hu, X., Chen, Y., Shen, Y., Zhou, S., Fei, W., Yang, Y., & Que, H. (2022). Correlation between Hashimoto's thyroiditis and polycystic ovary syndrome: A systematic review and meta-analysis. *Frontiers in endocrinology*, *13*, 1025267. https://doi.org/10.3389/fendo.2022.1025267

70. Hong, Z., Zhou, L., Ji, H., Xu, C., Yang, D., Yang, X., & Wang, H. (2022). Growth hormone protects oocytes from premature ovarian failure by alleviating apoptosis (GH protects oocytes from POI by alleviating apoptosis). https://doi.org/10.21203/rs.3.rs-2041717/v1

71. Hsieh, Y. T., & Ho, J. Y. (2021). Thyroid autoimmunity is associated with higher risk of premature ovarian insufficiency—a nationwide

health insurance research database study. *Human Reproduction*, 36(6), 1621-1629. https://doi.org/10.1093/humrep/deab025

72. Hussein, W., Mustafa, H., & Khalil, A. (2020). Hyperprolactinemia and polycystic ovary syndrome: The debate continues. *Ibnosina Journal of Medicine and Biomedical Sciences*, *12*(03), 233-234. https://doi.org/10.4103/ijmbs.ijmbs_87_20

73. Hussein, R. A., Kredy, H. M., & Fahad, N. S. (2022). Correlation between oxidative stress and oxytocin hormone in women with premature ovarian insufficiency. *Biochemical & Cellular Archives*, *22*(1). https://connectjournals.com/03896.2022.22.119

74. Huang, Y., Lv, Y., Qi, T., Luo, Z., Meng, X., Ying, Q., ... & Zhou, J. (2021). Metabolic profile of women with premature ovarian insufficiency compared with that of age-matched healthy controls. *Maturitas*, 148, 33-39. https://doi.org/10.1016/j.maturitas.2021.04.003

75. Jankowska, K., Suszczewicz, N., Rabijewski, M., Dudek, P., Zgliczyński, W., & Maksym, R. B. (2022). Inhibin-b and FSH are good indicators but not the best indicators of fertility. *Life*, *12*(4), 511. https://doi.org/10.3390/life12040511

76. Jabeen, F., Khadija, S., & Daud, S. (2022). Prevalence of primary and secondary infertility. *Saudi J Med*, *7*(1), 22-8. https://doi.org/10.36348/sjm.2022.v07i01.004

77. Jethwani, Parth & Rastogi, Ashu & Shukla, Ravindra. (2023). Dehydroepiandrosterone sulfate supplementation in health and diseases. *World Journal of Endocrine Surgery*. 78-133. 10.13105/wjma.v11.i4.102. http://dx.doi.org/10.13105/wjma.v11.i4.102

78. Karaer, I., & Tuncay, G. (2020). The effect of premature ovarian failure on inner ear function. *Journal of Obstetrics and Gynaecology*, *40*(2), 247-251. https://doi.org/10.1080/01443615.2019.1621815

79. Karkera, S., Agard, E., & Sankova, L. (2023). The clinical manifestations of polycystic ovary syndrome (PCOS) and the treatment options. *European Journal of Biology and Medical Science Research*, *11*(1), 57-81. https://tudr.org/id/eprint/1451

80. Kamrul-Hasan, A. B. M., & Aalpona, F. T. Z. (2024). Metabolic association of serum prolactin in polycystic ovary syndrome: A retrospective analysis of 840 patients in Bangladesh. *Endocrine and Metabolic Science*, *14*, 100153. https://doi.org/10.1016/j.endmts.2023.100153

81. Khmil, M., Khmil, S., & Marushchak, M. (2020). Hormone imbalance in women with infertility caused by polycystic ovary syndrome: is there a connection with body mass index?. *Open Access Macedonian Journal of Medical Sciences*, *8*(B), 731-737. https://doi.org/10.3889/oamjms.2020.4569

82. Kirkegaard, S., Torp, N. M. U., Andersen, S., & Andersen, S. L. (2024). Endometriosis, polycystic ovary syndrome, and the thyroid: a review. *Endocrine Connections*, 13(2). https://doi.org/10.1530/EC-23 0431

83. Kim, S. I., Yoon, J. H., Park, D. C., Yang, S. H., & Kim, Y. I. (2023). What is the optimal prolactin cutoff for predicting the presence of a pituitary adenoma in patients with polycystic ovary syndrome?. *International Journal of Medical Sciences*, *20*(4), 463. https://doi.org/10.7150/ijms.80891

84. Krysiak, R., Kowalcze, K., Szkróbka, W., & Okopień, B. (2023). Impaired Prolactin-Lowering Effects of Metformin in Women with

Polycystic Ovary Syndrome. *Journal of Clinical Medicine*, 12(17), 5474. https://doi.org/10.3390/jcm12175474

85. Kim, S. I., Yoon, J. H., Park, D. C., Yang, S. H., & Kim, Y. I. (2023). What is the optimal prolactin cutoff for predicting the presence of a pituitary adenoma in patients with polycystic ovary syndrome?. *International Journal of Medical Sciences*, 20(4), 463. https://doi.org/10.7150/ijms.80891

86. Kloos, J., Perez, J., & Weinerman, R. (2023). Increased body mass index (BMI) is associated with a decrease in ovarian reserve as measured by anti-mullerian hormone (AMH) in patients with polycystic ovarian syndrome (PCOs). *Fertility and Sterility*, *120*(1), e32. https://doi.org/10.1016/j.fertnstert.2023.05.065

87. Koyyada, A., & Orsu, P. (2020). Role of hypothyroidism and associated pathways in pregnancy and infertility: Clinical insights. *Tzu chi medical journal*, *32*(4), 312-317. https://doi.org/10.4103/tcmj.tcmj_255_19

88. Kolhe, J. V., Chhipa, A. S., Butani, S., Chavda, V., & Patel, S. S. (2022). PCOS and depression: common links and potential targets. *Reproductive Sciences*, 1-18. https://doi.org/10.1007/s43032-021-00765-2

89. Kolatorova, L., Vitku, J., Suchopar, J., Hill, M., & Parizek, A. (2022). Progesterone: a steroid with wide range of effects in physiology as well as human medicine. *International Journal of Molecular Sciences*, *23*(14), 7888. https://doi.org/10.3390/ijms23147989

90. Konishi, S., & Mizuno, Y. (2022). Pre-conceptional anti-thyroid antibodies and thyroid function in association with natural conception rates. *International Journal of Environmental Research and Public Health*, *19*(20), 13177. https://doi.org/10.3390/ijerph192013177

91. Koysombat, K., Abbara, A., & Dhillo, W. S. (2023). Current pharmacotherapy and future directions for neuroendocrine causes of female infertility. *Expert Opinion on Pharmacotherapy*, *24*(1), 37-47. https://doi.org/10.1080/14656566.2022.2064217

92. Li, F., Lu, H., Huang, Y., Wang, X., Zhang, Q., Li, X., ... & Yang, Q. (2022). A systematic review and meta-analysis of the association between Hashimoto's thyroiditis and ovarian reserve. *International Immunopharmacology*, *108*, 108670. https://doi.org/10.1016/j.intimp.2022.108670

93. Li, T., Zhang, T., Cui, T., Yang, Y., Liu, R., Chen, Y., & Yin, C. (2020). Involvement of endogenous testosterone in hepatic steatosis in women with polycystic ovarian syndrome. *The Journal of Steroid Biochemistry and Molecular Biology*, *204*, 105752. https://doi.org/10.1016/j.jsbmb.2020.105752

94. Li, C. J., Lin, L. T., & Tsui, K. H. (2021). Dehydroepiandrosterone shifts energy metabolism to increase mitochondrial biogenesis in female fertility with advancing age. *Nutrients*, *13*(7), 2448. https://doi.org/10.3390/nu13072449

95. Liu, F., Tian, L., Tan, J., Li, Z., Qin, H., Xu, D., ... & Zou, Y. (2022). Identification of a novel ESR1 mutation in a Chinese PCOs woman with estrogen insensitivity in IVF treatment. *Reproductive Biology and Endocrinology*, 20(1), https://doi.org/10.1186/s12958-022-01029-7

96. Lin, L. T., & Tsui, K. H. (2021). The relationships between serum DHEA-S and AMH levels in infertile women: a retrospective cross-sectional study. *Journal of Clinical Medicine*, 10(6), 1211. https://doi.org/10.3390/jcm10061211

97. Lobo RA, Pickar JH, Stevenson JC, Mack WJ, Hodis HN. (2023). Back to the future: Hormone replacement therapy as part of a prevention

strategy for women at the onset of menopause. *Atherosclerosis*. Nov;254:282-280. https://doi.org/10.1016/j.atherosclerosis.2016.10.005

98. Luo, E., Zhang, J., Song, J., Feng, D., Meng, Y., Jiang, H., ... & Fang, Y. (2021). Serum anti-Müllerian hormone levels were negatively associated with body fat percentage in PCOs patients. *Frontiers in endocrinology*, *12*, 659717. https://doi.org/10.3389/fendo.2021.659717

99. Mahboobifard, F., Dargahi, L., Jorjani, M., Tehrani, F. R., & Pourgholami, M. H. (2021). The role of ERα36 in cell type-specific functions of estrogen and cancer development. *Pharmacological Research*, *163*, 105307. https://doi.org/10.1016/j.phrs.2020.105307

100. Mahmoud, S. T. M., Alkazaz, A. K. A., & Al-Musawi, B. J. (2020). The Impact of LHR Gene Polymorphism Rs12470652 in Women with POF and Nihh, A Case-Control Study. *Iraqi Journal of Science*, 508-516. https://doi.org/10.24996/ijs.2020.61.3.6

101. Magdum, M., Chowdhury, M. A. T., Begum, N., & Riya, S. (2022). Types of infertility and its risk factors among infertile women: a prospective study in Dhaka city. *Journal of Biosciences and Medicines*, *10*(4), 158-168. https://doi.org/10.4236/jbm.2022.104014

102. Makolle, S., Catteau-Jonard, S., Robin, G., & Dewailly, D. (2021). Revisiting the serum level of anti-Müllerian hormone in patients with functional hypothalamic anovulation. *Human Reproduction*, *36*(4), 1043-1051. https://doi.org/10.1093/humrep/deab024

103. Mastnak, L., Herman, R., Ferjan, S., Janež, A., & Jensterle, M. (2023). Prolactin in polycystic ovary syndrome: metabolic effects and therapeutic prospects. *Life*, *13*(11), 2124. https://doi.org/10.3390/life13112124

104. Mele, C., Mai, S., Cena, T., Pagano, L., Scacchi, M., Biondi, B., ... & Marzullo, P. (2022). The pattern of TSH and fT4 levels across different BMI ranges in a large cohort of euthyroid patients with obesity. *Frontiers in Endocrinology*, *13*, 1029376. https://doi.org/10.3389/fendo.2022.1029376

105. Morshed, M. S., Banu, H., Akhtar, N., Sultana, T., Begum, A., Zamilla, M., ... & Hasanat, M. A. (2021). Luteinizing hormone to follicle-stimulating hormone ratio significantly correlates with androgen level and manifestations are more frequent with hyperandrogenemia in women with polycystic ovary syndrome. *Journal of Endocrinology and Metabolism*, 11(1), 14-21. https://doi.org/10.14740/jem716

106. Moshfegh, F., Balanejad, S. Z., Shahrokhabady, K., & Attaranzadeh, A. (2022). Crocus sativus (saffron) petals extract and its active ingredient, anthocyanin improves ovarian dysfunction, regulation of inflammatory genes and antioxidant factors in testosterone-induced PCOs mice. *Journal of ethnopharmacology*, 282, 114594. https://doi.org/10.1016/j.jep.2021.114594

107. Muharam, R., Prasetyo, Y. D., Prabowo, K. A., Putri, Y. I., Maidarti, M., & Hestiantoro, A. (2022). IVF outcome with a high level of AMH: a focus on PCOs versus non-PCOs. *BMC Women's Health*, 22(1), 172. https://doi.org/10.1186/s12905-022-01756-4

108. Munro, M. G., Balen, A. H., Cho, S., Critchley, H. O., Díaz, I., Ferriani, R., ... & van der Spuy, Z. M. (2022). The FIGO ovulatory disorders classification system. *Human Reproduction*, *37*(10), 2446-2464. https://doi.org/10.1093/humrep/deac180

109. Naelitz, B. D., & Sharifi, N. (2020). Through the looking-glass: reevaluating DHEA metabolism through HSD3B1

genetics. *Trends in Endocrinology & Metabolism*, *31*(9), 680-690. https://doi.org/10.1016/j.tem.2020.05.006

110. Negrón, A. L., & Radovick, S. (2020). High-fat diet alters LH secretion and pulse frequency in female mice in an estrous cycle dependent manner. *Endocrinology*, 161(10), https://doi.org/10.343876179

111. Obeagu, E. I., Njar, V. E., & Obeagu, G. U. (2023). Infertility: Prevalence and consequences. *Int. J. Curr. Res. Chem. Pharm. Sci*, *10*(7), 43-50. https://doi.org/10.22192/ijcrcps

112. Ozcil, M. D. (2020). Dehydroepiandrosterone supplementation improves ovarian reserve and pregnancy rates in poor responders. *European Review for Medical & Pharmacological Sciences*, *24*(17). https://doi.org/uploads/9104-9111

113. Ozawa H. (2022) Kisspeptin neurons as an integration center of reproductive regulation: Observation of reproductive function based on a new concept of reproductive regulatory nervous system. *Reprod Med Biol* 21: e12418. https://doi.org/10.1002/rmb2.12419

114. Palomba, S., Colombo, C., Busnelli, A., Caserta, D., & Vitale, G. (2023). Polycystic ovary syndrome and thyroid disorder: a comprehensive narrative review of the literature. *Frontiers in Endocrinology*, 14, 1251866. https://doi.org/10.3389/fendo.2023.1251866

115. Pedachenko, N., Anagnostis, P., Shemelko, T., Tukhtarian, R., & Alabbas, L. (2021). Serum anti-Mullerian hormone, prolactin and estradiol concentrations in infertile women with endometriosis. *Gynecological Endocrinology*, *37*(2), 162-165. https://doi.org/10.1080/09513590.2020.1855634

116. Pirchio, R., Graziadio, C., Colao, A., Pivonello, R., & Auriemma, R. S. (2022). Metabolic effects of prolactin. *Frontiers in endocrinology*, 13, 1015520. https://doi.org/10.3389/fendo.2022.1015520

117. Poppe, K. (2021). Management of endocrine disease: thyroid and female infertility: more questions than answers?!. *European journal of endocrinology*, *184*(4), R123-R135. https://doi.org/10.1530/EJE-20-1284

118. Purkayastha, N., & Sharma, H. (2021). Prevalence and potential determinants of primary infertility in India: Evidence from Indian demographic health survey. *Clinical Epidemiology and Global Health*, *8*, 162-170. https://doi.org/10.1016/j.cegh.2020.08.008

119. Rababa'h, A. M., Matani, B. R., & Ababneh, M. A. (2020). The ameliorative effects of marjoram in dehydroepiandrosterone induced polycystic ovary syndrome in rats. *Life sciences*, 261, 118353. https://doi.org/10.1016/j.lfs.2020.118353

120. Rahnama, R., Mahmoudi, A. R., Kazemnejad, S., Salehi, M., Ghahiri, A., Soltanghoraee, H., ... & Zarnani, A. H. (2021). Thyroid peroxidase in human endometrium and placenta: a potential target for anti-TPO antibodies. *Clinical and Experimental Medicine*, *21*, 78-88. https://doi.org/10.1007/s10238-020-00663-y

121. Rashad, N., Elnagar, W. M., Hassan, T. A., Mohy, N., Atef, R., & Issa, D. (2024). Altered expression patterns of circular RNAs Hsa-circ-0089172 as a predicting and Promising Biomarker of Unexplained Infertility in Female Patients Suffering from Hashimoto's Thyroiditis. *Zagazig University Medical Journal*, *30*(4), 1372-1380. https://dx.doi.org/10.21608/zumj.2024.290954.3406

122. Rodier, C., Courbiere, B., Fernandes, S., Vermalle, M., Florence, B., Resseguier, N., ... & Cuny, T. (2022). Metoclopramide Test in Hyperprolactinemic Women with polycystic ovarian syndrome: old wine into new bottles?. *Frontiers in Endocrinology*, *13*, 832361. https://doi.org/10.3389/fendo.2022.832361

123. Rosner, J., Samardzic, T., & Sarao, M. S. (2024). *Physiology, Female Reproduction*. https://www.ncbi.nlm.nih.gov/sites/books/NBK537132/

124. Soumpasis, I., Grace, B., & Johnson, S. (2020). Real-life insights on menstrual cycles and ovulation using big data. *Human Reproduction Open*, *2020*(2), hoaa011. https://doi.org/10.1093/hropen/hoaa011

125. Sadig., Fazil, G. J., H. A., Tofig, M. N., & Ali, I. J. (2023). The levels of inhibin A and inhibin B in PCOs patients. *GSC Biological and Pharmaceutical Sciences*, *24*(1), 346-349. https://doi.org/10.30574/gscbps.2023.24.1.0302

126. Saadia, Z. (2020). Follicle stimulating hormone (LH: FSH) ratio in polycystic ovary syndrome (PCOs)-obese vs. non-obese women. *Medical Archives*, *74*(4), 289. https://doi.org/10.5455/medarh.2020.74.289-293

127. Silva, D. F., Carvalho, T., Gomes, L., Paiva, I., Cortesao, P., & Santos, T. A. (2022). Functional ovarian reserve in women with infertility and euthyroidism: what is the role of thyroid autoimmunity?. *Journal of Gynecology & Reproductive Medicine*, *6*(2), 96-99. https://dx.doi.org/10.33140/JGRM

128. Safiri, S., Noori, M., Nejadghaderi, S. A., Karamzad, N., Carson-Chahhoud, K., Sullman, M. J., ... & Avery, J. (2022). Prevalence, incidence and years lived with disability due to polycystic

ovary syndrome in 204 countries and territories, 1880–2018. *Human Reproduction*, *37*(8), 1818-1831. https://doi.org/10.1093/humrep/deac091

129. Salvio, G., Martino, M., Giancola, G., Arnaldi, G., & Balercia, G. (2021). Hypothalamic–pituitary diseases and erectile dysfunction. *Journal of Clinical Medicine*, *10*(12), 2551. https://doi.org/10.3390/jcm10122551

130. Serin, A. N., Birge, Ö., Uysal, A., Görar, S., & Tekeli, F. (2021). Hashimoto's thyroiditis worsens ovaries in polycystic ovary syndrome patients compared to Anti-Müllerian hormone levels. *BMC Endocrine Disorders*, *21*, 1-8. https://doi.org/10.1186/s12902-021-00706-9

131. Sekar, P. K. C., & Veerabathiran, R. (2024). Genes linked to obesity-related infertility: bridging the knowledge gap. *Reproductive and Developmental Medicine*, *8*(02), 121-129. https://doi.org/10.1097/RD9.0000000000000096

132. Sharma, Y., & Galvão, A. M. (2023). Maternal obesity and ovarian failure: is leptin the culprit?. *Animal Reproduction*, *19*, e20230007. https://doi.org/10.1590/1984-3143-AR2023-0007

133. Sharma, M., Modi, A., Goyal, M., Sharma, P., & Purohit, P. (2022). Anti-thyroid antibodies and the gonadotrophins profile (LH/FSH) in euthyroid polycystic ovarian syndrome women. *Acta Endocrinologica (Bucharest)*, *18*(1), 79. https://doi.org/10.4183/aeb.2022.79

134. Shrikhande, L., Shrikhande, B., & Shrikhande, A. (2020). AMH and its clinical implications. *The Journal of Obstetrics and Gynecology of India*, 70(5), 337-341. https://doi.org/10.1007/s13224020-01362-0

135. Silvén, H., Savukoski, S. M., Pesonen, P., Pukkala, E., Gissler, M., Suvanto, E., & Niinimäki, M. (2022). Incidence and familial risk of premature ovarian insufficiency in the Finnish female population. *Human Reproduction*, *37*(5), 1030-1036. https://doi.org/10.1093/humrep/deac014

136. Siddiqui, S., Mateen, S., Ahmad, R., & Moin, S. (2022). A brief insight into the etiology, genetics, and immunology of polycystic ovarian syndrome (PCOS). *Journal of assisted reproduction and genetics*, *38*(11), 2438-2473. https://doi.org/10.1007/s10815-022-02625-7

137. Sirait, B. I., Reviani, N., & Udjung, G. I. (2023). Factors Affecting Infertility in Women of Reproductive Age in the IVF Programme. *International Journal of TROPICAL DISEASE & Health*, *44*(1), 65-75. http://repository.uki.ac.id/id/eprint/10351

138. Soman, M., Huang, L. C., Cai, W. H., Xu, J. B., Chen, J. Y., He, R. K., ... & Zhu, X. M. (2020). Serum androgen profiles in women with premature ovarian insufficiency: a systematic review and meta-analysis. *Menopause*, *26*(1), 78-93. https://doi.org/10.1097/GME.0000000000001161

139. Skowrońska, M., Pawłowski, M., & Milewski, R. (2023). A literature review and a proposed classification of the relationships between ovulatory infertility and lifestyle factors based on the three groups of ovulation disorders classified by WHO. *Journal of Clinical Medicine*, *12*(19), 6275. https://doi.org/10.3390/jcm12196275

140. Stogowska, E., Krentowska, A., Adamska, A., Lebkowska, A., & Kowalska, I. (2022, May). Serum concentrations of 17-hydroxyprogesterone and dehydroepiandrosterone sulfate in women with polycystic ovary syndrome and their relation to the parameters of

glucose metabolism. In *Endocrine Abstracts* (Vol. 81). Bioscientifica. https://doi.org/10.1530/endoabs.81.P714

141. Sudhakaran, G., Rajesh, R., Murugan, R., Velayutham, M., Guru, A., Boopathi, S., ... & Arockiaraj, J. (2023). Nimbin analog N2 alleviates high testosterone induced oxidative stress in CHO cells and alters the expression of Tox3 and Dennd1a signal transduction pathway involved in the PCOs zebrafish. *Phytotherapy Research*, *37*(4), 1449-1461. https://doi.org/10.1002/ptr.7685

142. Sun, C., Rong, X., Cai, Y., Qiu, S., & Farzaneh, M. (2020). Mini review: The FDA-approved prescription drugs that induce ovulation in women with ovulatory problems. *Drug Development Research*, *81*(7), 815-822. https://doi.org/10.1002/ddr.21687

143. Sun, X., Xiong, W., Liu, L., Xiong, J., Liao, C., Lan, Y., ... & Mao, X. (2022). Comparison of the predictive capability of antral follicle count vs. the anti-Müllerian hormone for ovarian response in infertile women. *Frontiers in Endocrinology*, *13*, 862733. https://doi.org/10.3389/fendo.2022.862733

144. Szeliga, A., Calik-Ksepka, A., Maciejewska-Jeske, M., Grymowicz, M., Smolarczyk, K., Kostrzak, A., ... & Meczekalski, B. (2021). Autoimmune diseases in patients with premature ovarian insufficiency—our current state of knowledge. *International Journal of Molecular Sciences*, *22*(5), 2594. https://doi.org/10.3390/ijms22052594

145. Tammasse IFU., Tamrin F. (2023) Different of Hypothalamic-Pituitary-Gonadal Axis in Male and Female *Reprod Med Biol* 6:023. https://doi.org/10.23937/2643-4555/1710023

146. Tetkova, A., Susor, A., Kubelka, M., Nemcova, L., Jansova, D., Dvoran, M., ... & Kalous, J. (2020). Follicle-stimulating hormone

administration affects amino acid metabolism in mammalian oocytes. *Biology of reproduction*, 101(4), 718-732. https://doi.org/10.1093/biolre/ioz117

147. Turcu, A. F., Rege, J., Auchus, R. J., & Rainey, W. E. (2020). 11-Oxygenated androgens in health and disease. *Nature Reviews Endocrinology*, *16*(5), 284-296. https://doi.org/10.1038/s41574-020-0336-x

148. Umarsingh, S., Adam, J. K., & Krishna, S. B. N. (2020). The relationship between anti-Müllerian hormone (AMH) levels and pregnancy outcomes in patients undergoing assisted reproductive techniques (ART). *PeerJ*, *8*, e10390. https://doi.org/10.7717/peerj.10390

149. Valdimarsdottir, R., Wikström, A. K., Kallak, T. K., Elenis, E., Axelsson, O., Preissl, H., ... & Poromaa, I. S. (2021). Pregnancy outcome in women with polycystic ovary syndrome in relation to second-trimester testosterone levels. *Reproductive BioMedicine Online*, *42*(1), 217-225. https://doi.org/10.1016/j.rbmo.2020.09.019

150. Van der Ham, K., Stekelenburg, K. J., Louwers, Y. V., van Dorp, W., Schreurs, M. W., van der Wal, R., ... & Laven, J. S. (2023). The prevalence of thyroid dysfunction and hyperprolactinemia in women with PCOs. *Frontiers in Endocrinology*, *14*, 1245106. https://doi.org/10.3389/fendo.2023.1245106

151. Vermeulen, R. F. M., Korse, C. M., Kenter, G. G., Brood-van Zanten, M. M. A., & Beurden, M. van. (2019). Safety of hormone replacement therapy following risk-reducing salpingo-oophorectomy: systematic review of literature and guidelines. *Climacteric*, *22*(4), 352–360. https://doi.org/10.1080/13697137.2019.1582622

152. Visser, J. A., Schipper, I., Laven, J. S., & Themmen, A. P. (2012). Anti-Müllerian hormone: an ovarian reserve marker in primary ovarian insufficiency. *Nature Reviews Endocrinology*, *8*(6), 331-341. https://doi.org/10.1038/nrendo.2011.224

153. Vijay, A. S., Gopireddy, M. M. R., Fyzullah, S., Gollapalli, P., Maheswari, M., Rani, U., & Rajesh, S. (2022). *Association Between AMH Levels and Fertility/Reproductive Outcomes Among Women Undergoing IVF*: A Retrospective Study. Journal of reproduction & infertility, 23(1), 54–60. https://doi.org/10.18502/jri.v23i1.8453

154. Wafa, A. E. S., Mohammad Taha, E. S., Nar, A. E. R. A., El-Raouf, A., & Ahmad Ateia, A. (2020). Correlation between plasma lead and serum anti mullerian hormone levels in women with premature ovarian failure. *Al-Azhar Medical Journal*, *49*(2), 469-480. https://doi.org/10.21608/amj.2020.82566

155. Wasserstein, R. L., Schirm, A. L., & Lazar, N. A. (2019). Moving to a world beyond "p< 0.05". *The American Statistician*, *73*(sup1), 1-19. https://doi.org/10.1080/00031305.2019.1583913

156. Wang, J., Sun, X., Yang, Z., Li, S., Wang, Y., Ren, R., ... & Yu, D. (2023). Epigenetic regulation in premature ovarian failure: A literature review. *Frontiers in physiology*, *13*, 888424. https://doi.org/10.3389/fphys.2022.998424

157. Wang, R., Lv, Y., Dou, T., Yang, Q., Yu, C., & Guan, Q. (2024). Autoimmune thyroid disease and ovarian hypofunction: a review of literature. *Journal of Ovarian Research*, *17*(1), 125. https://doi.org/10.1186/s13048-024-01451-y

158. Wang, J., Ding, J., Qu, B., Zhang, Y., & Zhou, Q. (2022). Does serum LH level influence IVF outcomes in women with PCOs

undergoing GnRH-antagonist stimulation: a novel indicator. *Journal of Clinical Medicine*, *11*(16), 4670. https://doi.org/10.3390/jcm11164670

159. Wang, Z. (2023). Discriminatory value of steroid hormones on PCOs diagnosis in women with obesity and infertility and clustering of biochemical hyperandrogenism and metabolic factors. *Lifestyle intervention in women with obesity and infertility*, 123. https://doi.org/652142991

160. Wang, J., Sun, X., Yang, Z., Li, S., Wang, Y., Ren, R., ... & Yu, D. (2023). Epigenetic regulation in premature ovarian failure: A literature review. *Frontiers in physiology*, *13*, 888424. https://doi.org/10.3389/fphys.2022.998424

161. Wesevich, V., Kellen, A. N., & Pal, L. (2020). Recent advances in understanding primary ovarian insufficiency. *F1000Research*, *9*. https://doi.org/10.12688/f1000research.26423.1

162. Wen, J., Huang, K., Du, X., Zhang, H., Ding, T., Zhang, C., Ma, W., Zhong, Y., Qu, W., Liu, Y., Li, Z., Deng, S., Luo, A., Jin, Y., Zhang, J., & Wang, S. (2021). *Can Inhibin B Reflect Ovarian Reserve of Healthy Reproductive Age Women Effectively?*. Frontiers in endocrinology, 12, 626534. https://doi.org/10.3388/fendo.2021.626534

163. Weghofer, A., Barad, D. H., Darmon, S. K., Kushnir, V. A., Albertini, D. F., & Gleicher, N. (2020). The ovarian sensitivity index is predictive of live birth chances after IVF in infertile patients. *Human Reproduction Open*, *2020*(4), hoaa049. https://doi.org/10.1093/hropen/hoaa049

164. Xie, M., Zhong, Y., Xue, Q., Wu, M., Deng, X., Santos, H. O., ... & Jiao, P. (2020). Impact of dehydroepianrosterone (DHEA) supplementation on serum levels of insulin-like growth factor 1 (IGF-

1): A dose-response meta-analysis of randomized controlled trials. *Experimental Gerontology*, *136*, 110848. https://doi.org/10.1016/j.exger.2020.110949

165. Xu, Y., & Qiao, J. (2022). Association of insulin resistance and elevated androgen levels with polycystic ovarian syndrome (PCOs): a review of literature. *Journal of healthcare engineering*, *2022*(1), 9240569. https://doi.org/10.1155/2022/9240569

166. Xu, S., Zhang, Y., Qiang, C., & Zhang, C. (2022). Effect of TSH on oocyte maturation of PCOs patients with normal thyroid function in IVF. *Reproductive Biology and Endocrinology*, *20*(1), 133. https://doi.org/10.1186/s12958-022-01005-1

167. Yasar, H. Y., Demirpence, M., Colak, A., Zeytinli, M., Yasar, E., & Taylan, A. (2022). Serum neudesin levels in patients with polycystic ovary syndrome. *Ginekologia Polska*, *93*(7), 525-530. https://doi.org/10.5603/GP.a2021.0139

168. Yadav, G., Aggarwal, I., Nalwa, A., & Sureka, B. (2022). Inhibin B Secreting Ovarian Fibroma. *The Journal of Obstetrics and Gynecology of India*, *72*(4), 356-359. https://doi.org/10.1007/s13224-021-01601-y

169. Yang, X., & Yang, L. (2023). Current understanding of the genomic abnormities in premature ovarian failure: chance for early diagnosis and management. *Frontiers in Medicine*, *10*, 1194865. https://doi.org/10.3389/fmed.2023.1194865

170. Yang, H., Lin, J., Li, H., Liu, Z., Chen, X., & Chen, Q. (2021). Prolactin is associated with insulin resistance and beta-cell dysfunction in infertile women with polycystic ovary syndrome. *Frontiers in Endocrinology*, *12*, 571229. https://doi.org/10.3389/fendo.2021.571229

171. Ye, W., Xie, T., Song, Y., and Zhou, L. (2021). The role of androgen and its related signals in PCOS. *Journal of cellular and molecular medicine*, 25(4), 1825-1837. https://doi.org/10.1111/jcmm.16205

172. Yuk, J. S., & Kim, M. (2021). Incidence and prevalence of primary ovarian insufficiency in South Korea: a population-based study. *Archives of Gynecology and Obstetrics*, 1-8. https://doi.org/10.1007/s00404-021-05962-7

173. Zia, B., Nisar, S., & Ansari, S. Z. (2023). PCOs and Obesity: The Role of TSH on Fat Metabolism and Its Dysfunctional Repercussions. *Obstetrics and Gynecology Advances*, 81. https://doi.org/378963093

174. Zhang, C. (2020). The roles of different stem cells in premature ovarian failure. *Current stem cell research & therapy*, 15(6), 473-481. https://doi.org/10.2174/1574888X14666190314123006

175. Zhang, J., Jia, H., Diao, F., Ma, X., Liu, J., & Cui, Y. (2023). *Efficacy of dehydroepiandrosterone priming in women with poor ovarian response undergoing IVF/ICSI:* a meta-analysis. Frontiers in endocrinology, 14, 1156280. https://doi.org/10.3388/fendo.2023.1156280

176. Zhang, R., Linpeng, S., Li, Z., Cao, Y., Tan, H., Liang, D., & Wu, L. (2020). Deficiency in GnRH receptor trafficking due to a novel homozygous mutation causes idiopathic hypogonadotropic hypogonadism in three prepubertal siblings. *Gene*, *668*, 42-46. https://doi.org/10.1016/j.gene.2018.05.050

177. Zhang, J., Jia, H., Diao, F., Ma, X., Liu, J., & Cui, Y. (2023). *Efficacy of dehydroepiandrosterone priming in women with poor ovarian response undergoing IVF/ICSI:* a meta-analysis. Frontiers in

endocrinology, 14, 1156280. https://doi.org/10.3388/fendo.2023.1156280

178. Zhao, Y. X., Chen, S. R., Su, P. P., Huang, F. H., Shi, Y. C., Shi, Q. Y., & Lin, S. (2019). Using mesenchymal stem cells to treat female infertility: an update on female reproductive diseases. *Stem cells international*, *2019*(1), 9071720. https://doi.org/10.1155/2019/9071720

179. Zhu, C., Luo, W., Li, Z., Zhang, X., Hu, J., Zhao, S., ... & Qin, Y. (2021). New theca-cell marker insulin-like factor 3 is associated with premature ovarian insufficiency. *Fertility and Sterility*, *115*(2), 455-462. https://doi.org/10.1016/j.fertnstert.2020.08.005

Appendices

Appendix (1) Compliance & Ethical Statement

Ethical statement

This study protocol was accepted after its review by the Medical Ethics Committee of the Faculty of Applied Medical Sciences of Karbala University. The research ethics of this study also identify authorization at the Women's and Obstetrics Hospital in Karbala, as well as some community health centers or laboratories. The objectives of the study were described to all participants, and oral approvals were obtained from them. Before being included in the study, they were made aware of any possible risks in accordance with the Declaration of Helsinki principles. Sample processing and laboratory examinations of the examined parameters were conducted in the hospital's laboratories and infertility unit. Reviewed and approved the study protocol, subject data, and permission form by the local ethics committee. This was done by document number IQ.UOK.CAMS.DCL.REC.4.

Republic of Iraq
Ministry of Higher Education and
Scientific Research
University of Karbala
College of Applied Medical Sciences
Department of Clinical Laboratories

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية العلوم الطبية التطبيقية
قسم التحليلات المرضية

Approval Number: IQ.UOK.CAMS.DCL.REC.4

Date: 1-9-2024

Research Ethics Committee

The Research Ethics Committee has considered the application for research ethics approval for the following research:

Correlation of the level of Thyroid peroxidase Antibodies (TPO), Dehyroepiandrosterone Sulfate (DHEA-S), prolactin and Inhibin-B Between fertile and infertile female in Holy City Kerbala

The study involves data collection through on population of human or animal experiments were included.

University / College / Center of principle investigator:

University of Kerbala / College of Applied Medical Sciences / Department of Clinical Laboratories

Name of principal investigator: **Zahraa Abd Alamir Jalil**

Please tick appropriate box:

1.The research ethics committee gives ethics approval for the research project. ☑

Please note it the research protocol laid down in the application must not be changed without the approval of research ethics committee.

2.The research ethics committee gives ethics approval for the research project after completed the following actions. ☐

3.The research ethics committee cannot give ethics approval for the research project. ☐

Assist Prof. Dr.
Linda H Al-Ghazali

Prof. Dr.
Ghusoon G AL-Janabi

Assist Prof. Dr.
Hussein A. Al-Ghanimi

Appendix (2): Determination of Anti-thyroid peroxidase (Anti-TPO)

Anti-thyroid peroxidase (Anti-TPO) ELISA Kit

A specific kit for measuring human Anti-TPO concentration in serum was supplied by USA- Could-Clone Crop. No.SEA557Hu

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120μL	Assay Diluent A	1×12mL
Detection Reagent B	1×120μL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

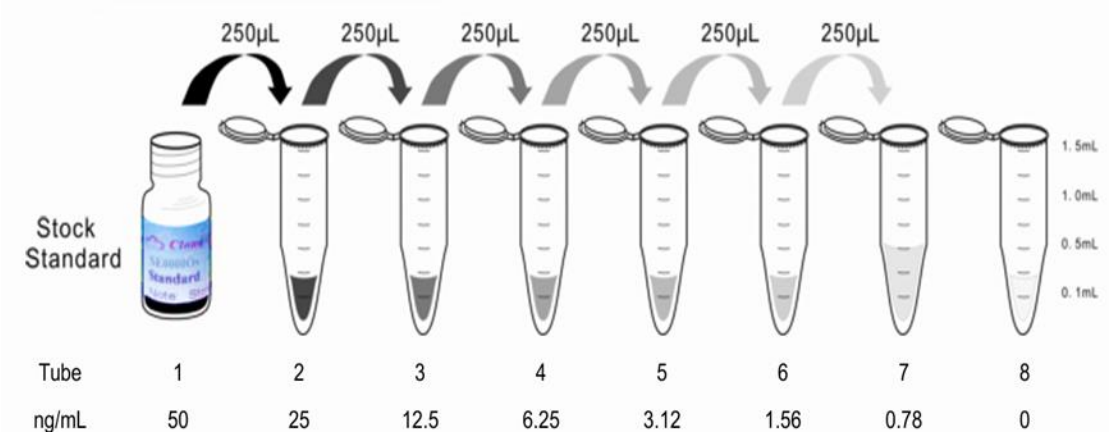
Principle

The microplate has been pre-coated with an antibody specific to TPO. Anti-TPO in the samples is added and binds to the antibody coat on the wells that is specific to TPO, and the biotinylated Anti-TPO is added and binds to Anti TPO in the sample then Avidin conjugated to Horseradish Peroxidase (HRP) and binds to biotinylated Anti-TPO. After incubation, unbound Avidin-HRP\ is washed. The TMB substrate solution is added and color changes proportionately to the amount of human Anti-TPO. The reaction is terminated by the addition of sulphuric acidic solution (Stop solution) and absorbance is measured at 450nm.

Reagent Preparation

1. All reagents should bring to room temperature (18-25 °C) before use.
2. Standard- Reconstitute the Standard with 0.5mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 50ng/mL. Please prepare 7 tubes containing 0.25mL Standard Diluent and

produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 50ng/mL, 25ng/mL, 12.5ng/mL, 6.25ng/mL, 3.12ng/mL, 1.56ng/mL, 0.78ng/mL, and the last EP tubes with Standard Diluent is the blank as 0ng/mL

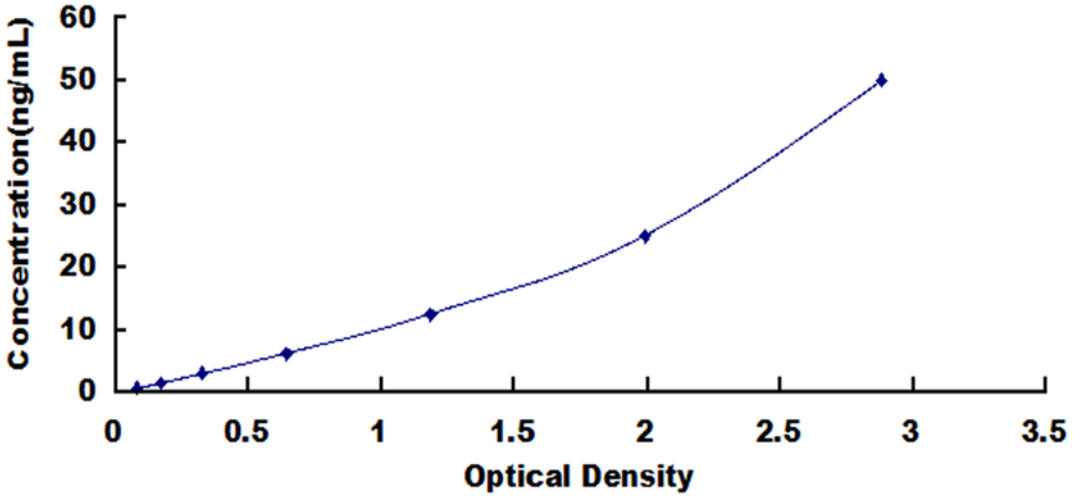


3. Detection Reagent A and Detection Reagent B- Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with Assay Diluent A and B, respectively.
4. Wash Solution- Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).
5. TMB substrate- Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Assay Procedure

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 1 hour at 37 °C.

2. Remove the liquid of each well, don’t wash.
3. Add 100μL of **Detection Reagent A** working solution to each well, cover the wells with the plate sealer and incubate for 1 hour at 37 °C.
4. Aspirate the solution and wash with 350μL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
5. Add 100μL of **Detection Reagent B** working solution to each well, cover the wells with the plate sealer and incubate for 30 minutes at 37 °C.
6. Repeat the aspiration/wash process for total 5 times as conducted in step 4.
7. Add 90μL of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate for 10- 20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
8. Add 50μL of **Stop Solution** to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.



Standard curve of Anti-thyroid peroxidase (Anti-TPO)

Appendix (3): Determination of Dehydroepiandrosterone sulfate (DHEA-S)

Dehydroepiandrosterone sulfate (DHEA-S) ELISA Kit

A specific kit for measuring human DHEA-S concentration in serum was supplied by USA- Elabscience. No.E-EL-0115

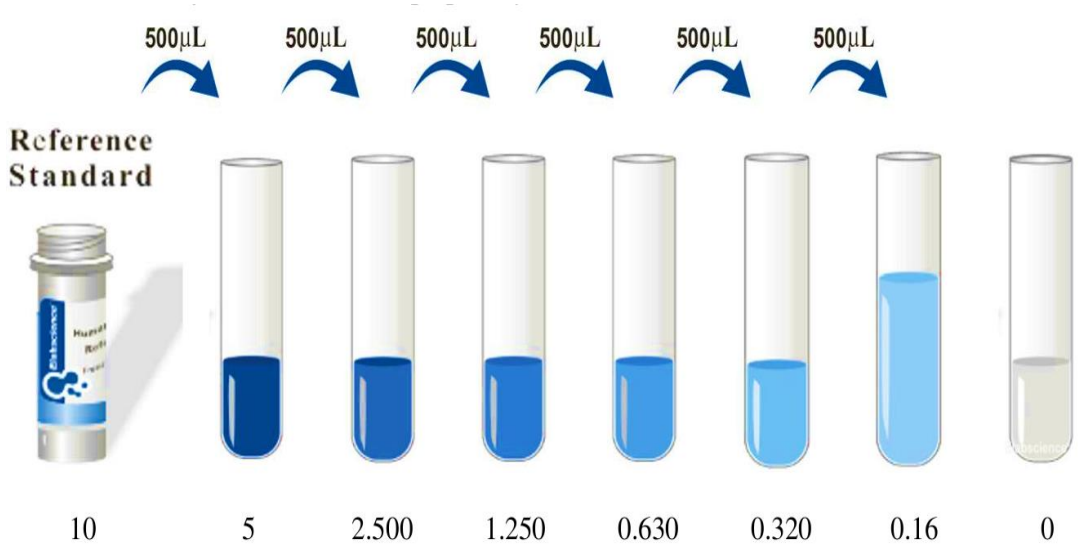
Principle

This kit includes a microplate that has been pre-coated with DHEA-S. DHEA-S present in samples is bound with a specific DHEA-S antibody on the solid phase and then a Biotinylated DHEA-S antibody is added and bound to DHEA-S in the sample. Avidin conjugated to Horseradish Peroxidase (HRP) and binds to the Biotinylated DHEA-S antibody. After incubation, unbound Avidin-HRP is washed. The TMB substrate solution is added and color changes proportionately to the amount of human DHEA-S. The reaction is terminated by the addition of sulphuric acidic solution (Stop solution) and absorbance is measured at 450nm.

Reagent Preparation

1. All reagents should to room temperature (18-25 ℃) before use.
2. **Wash Buffer:** Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40 ℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard & Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a

working solution of 10 ng/mL (or add 1 mL of Reference Standard &Sample Diluent, let it stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles generated during vortex could be removed by centrifuging at a relatively low speed). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 10, 5, 2.500, 1.250, 0.630, 0.320, 0.16, 0 ng/mL. Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 10 ng/mL working solution to the first tube and mix up to produce a 5 ng/mL working solution. Pipette 500uL of the solution from the former tube into the latter one according to this step. The illustration below is for reference.



Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube. Gradient diluted standard working solution should be prepared just before use.

4. **Biotinylated Detection Ab working solution**: Calculate the required amount before the experiment (50 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 min, then dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with

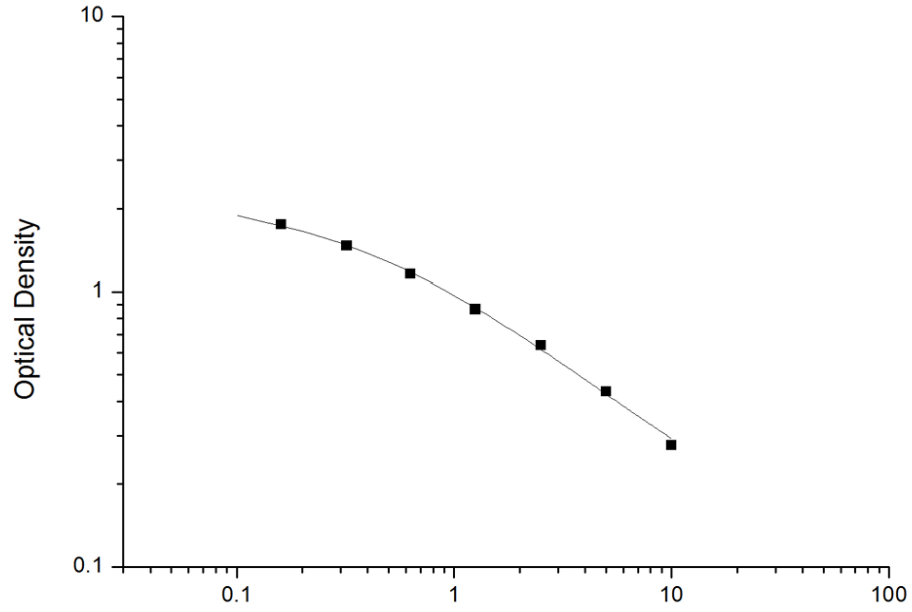
Biotinylated Detection Ab Diluent (Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1: 99). The working solution should be prepared just before use.

5. **Concentrated HRP Conjugate working solution**: HRP Conjugate is HRP conjugated avidin. Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent(Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99). The working solution should be prepared just before use.

Assay procedure

1. Determine wells for **diluted standard, blank,** and **sample**. Add 50 μL each dilution of standard, blank, and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. It is recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations). Immediately add 50 μL of **Biotinylated Detection Ab** working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37 ℃. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Decant the solution from each well, and add 350 μL of **wash buffer** to each well. Soak for 1 minute and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

3. Add 100 μL of **HRP Conjugate working solution** to each well. Cover the plate with a new sealer. Incubate for 30 min at 37 °C.
4. Decant the solution from each well, repeat the wash process 5 times as conducted in step 2.
5. Add 90 μL of **Substrate Reagent** to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37 °C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.
6. Add 50 μL of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.



Standard curve of Dehydroepiandrosterone sulfate (DHEA-S) ELISA Kit

Appendix (4) Determination of serum Inhibin-B

Inhibin-B ELISA kit

A specific kit for measuring human Inhibin-B concentration in serum was supplied by USA- Could-Clone Croup. No.CEA760Hu

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1	Assay Diluent A	1×12mL
Detection Reagent B	1×120μL	Assay Diluent B	1×12mL
Reagent Diluent	1×300μL	Stop Solution	1×6mL
TMB Substrate	1×9mL	Instruction manual	1
Wash Buffer (30 × concentrate)	1×20mL		

Principle

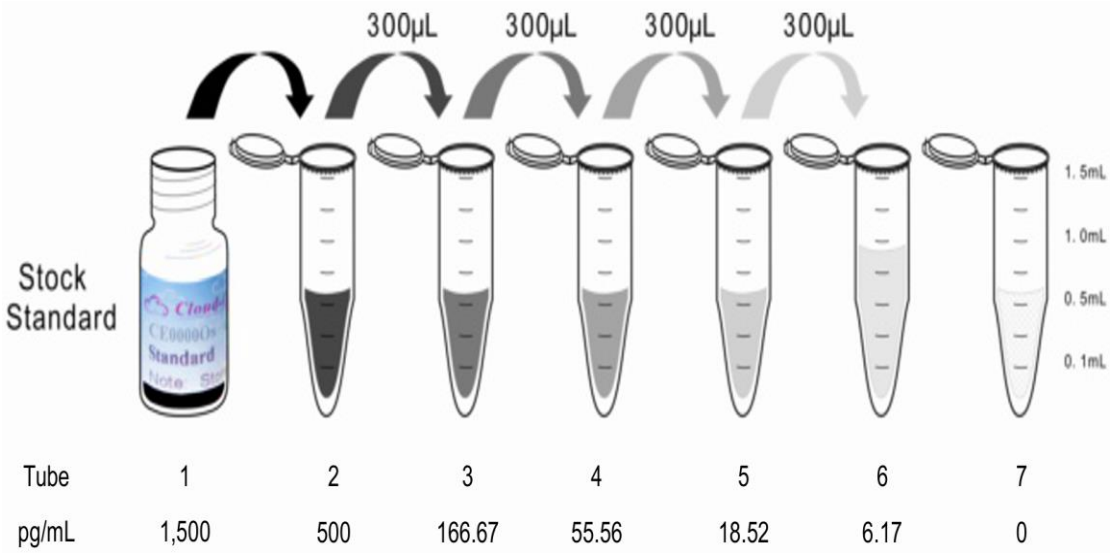
On a microplate, an INHB-B specific to monoclonal antibodies has been pre-coated. Using the pre-coated INHB-specific antibody, a competitive inhibition process is initiated between biotin-labeled and unlabeled INHB-B (Standards or samples). After incubation, unbound Avidin-HRP is washed. The TMB substrate solution is added and color changes proportionately to the amount of human INHB-B. The reaction is terminated by the addition of sulphuric acidic solution (Stop solution) and absorbance is measured at 450nm.

Reagent Preparation

1. All reagents should brought to room temperature (18-25 °C) before use
2. Standard- Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 1,500pg/mL. Please firstly dilute the stock solution to 500pg/mL and the diluted

standard serves as the highest standard (500pg/mL). Then prepare 5 tubes containing 0.6mL Standard Diluent and produce a triple dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 500pg/mL, 166.67pg/mL, 55.56pg/mL, 18.52pg/mL, 6.17pg/mL, and the last EP tubes with Standard Diluent is the blank as 0pg/mL.

3. **Detection Reagent A**- Reconstitute the **Detection Reagent A** with 150µL of **Reagent Diluent**, kept for 10 minutes at room temperature,



shake gently (not to foam). Dilute to the working concentration with **Assay Diluent A** (1:100).

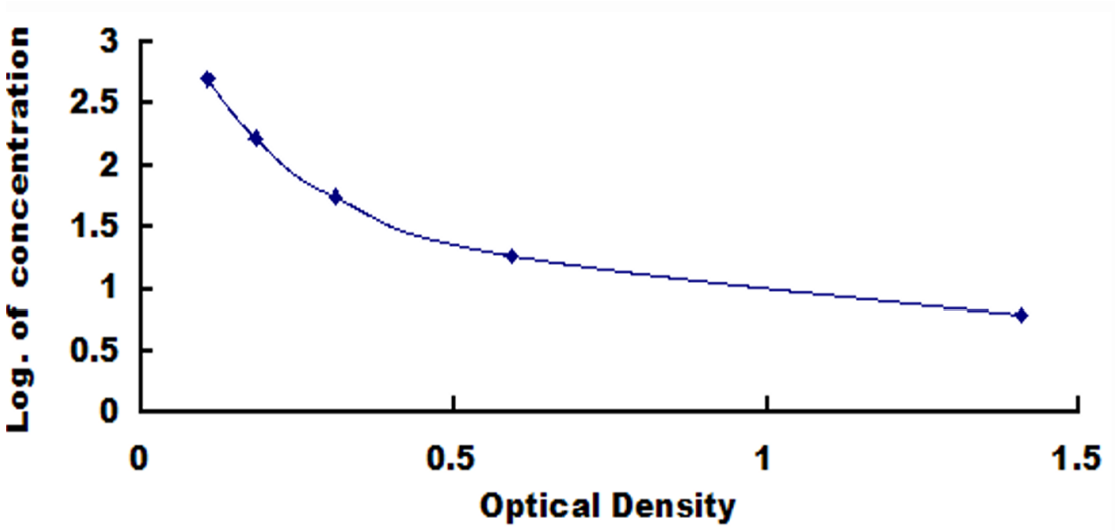
4. **Detection Reagent B**- Briefly spin or centrifuge the stock Detection B before use. Dilute to the working concentration with **Assay Diluent B** (1:100).
5. **Wash Solution**- Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).
6. **TMB substrate**- Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Assay procedure

1. Determine wells for diluted standard, blank and sample. Prepare 5 wells for standard points, 1 well for blank. Add 50μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50μL of Detection Reagent A to each well immediately. Shake the plate gently (using a microplate shaker is recommended). Cover with a Plate sealer. Incubate for 1 hour at 37 °C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
2. Aspirate the solution and wash with 350μL of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
3. Add 100μL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37 °C after covering it with the Plate sealer.
4. Repeat the aspiration/wash process for total 5 times as conducted in step 2.
5. Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10- 20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
6. Add 50μL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.

This standard curve is only for demonstrated purposes. A standard curve should be generated with each assay.



Standard curve of Inhibin-B ELISA Kit

Appendix (5) Determination of serum Anti-Müllerian hormone (AMH)

Anti-Müllerian hormone (AMH) ELISA kit

A specific kit for measuring human AMH concentration in serum was supplied by USA- Could-Clone Croup. No.CEA228Hu

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120μL	Assay Diluent A	1×12mL
Detection Reagent B	1×120μL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

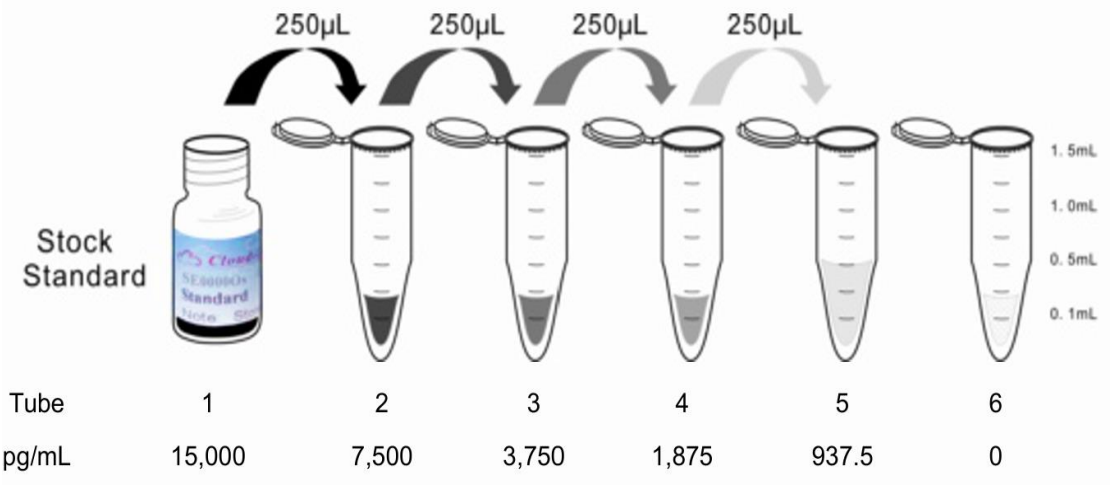
Principle

The monoclonal antibody specific to AMH is pre-coated onto a microplate. After the incubation, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of AMH in the sample. The TMB substrate solution is added and color changes proportionately to the amount of human AMH. The reaction is terminated by the addition of sulphuric acidic solution (Stop solution) and absorbance is measured at 450nm.

Reagent Preparation

1. All reagents should bring to room temperature (18-25 °C) before use.
2. **Standard-** Reconstitute the Standard with 0.5mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 15,000pg/mL. Please prepare 5 tubes containing 0.25ml Standard Diluent and produce a double dilution series according to the picture shown below. Mix each

tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 15,000pg/mL, 7,500pg/mL, 3,750pg/mL, 1,875pg/mL, 937.5pg/mL, and the last EP tubes with Standard Diluent is the blank as 0pg/mL.



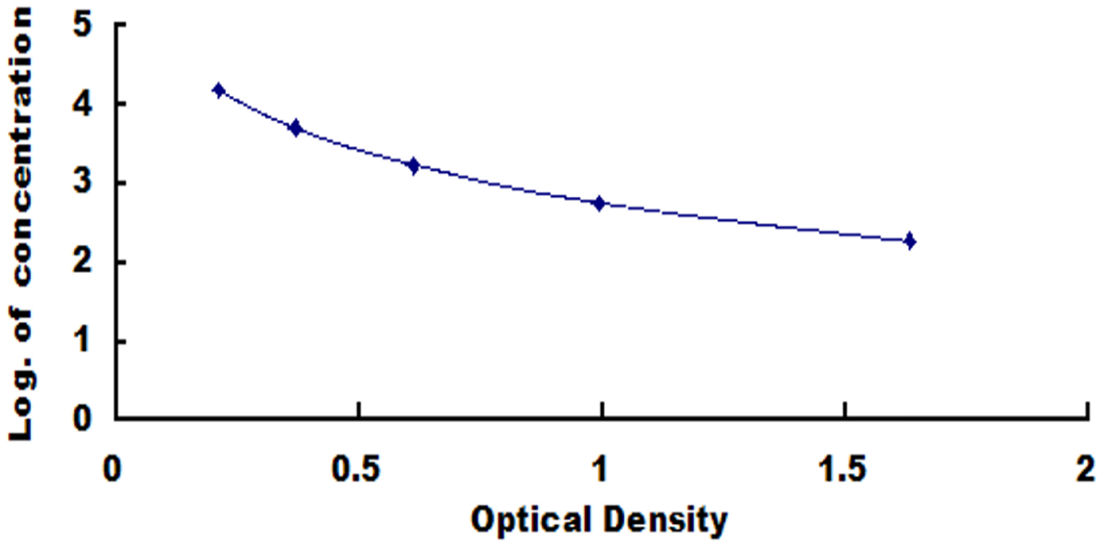
3. **Detection Reagent A and Detection Reagent B**- Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with **Assay Diluent A and B**, respectively.
4. **Wash Solution**- Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).
5. **TMB substrate**- Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Assay procedure

1. Determine wells for diluted standard, blank and sample. Prepare 5 wells for standard points, 1 well for blank. Add 50μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50μL of Detection Reagent A to each well immediately. Shake the plate gently (using a microplate shaker is recommended). Cover with a Plate sealer. Incubate

for 1 hour at 37 °C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

2. Aspirate the solution and wash with 350µL of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
3. Add 100µL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37 °C after covering it with the Plate sealer.
4. Repeat the aspiration/wash process for total 5 times as conducted in step 2.
5. Add 90µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10- 20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
6. Add 50µL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.



Standard curve of Anti-Müllerian hormone (AMH) ELISA Kit

Appendix (6) Determination of Follicle-stimulating hormone (FSH)

Follicle-stimulating hormone (FSH) ELISA kit

A specific kit for measuring human FSH concentration in serum was supplied by USA- Could-Clone Croup. No.SEA830Hu

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120μL	Assay Diluent A	1×12mL
Detection Reagent B	1×120μL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

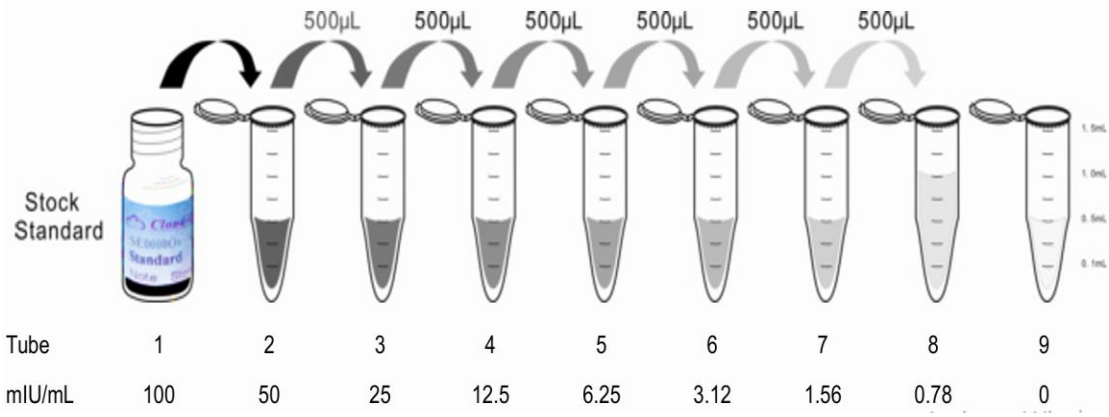
Principle

An antibody specific to FSH has already been pre-coated on the microplate. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After the TMB substrate solution is added, biotin-conjugated antibody, and enzyme-conjugated Avidin will exhibit a color change. By adding a solution of sulfuric acid (Stop solution), the enzyme-substrate reaction is stopped, and terminated the reaction and absorbance is measured at 450nm

Reagent Preparation

1. All reagents should bring to room temperature (18-25 °C) before use.
2. Standard- Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 100mIU/mL. Please firstly dilute the stock solution to 50mIU/mL and the diluted

standard serves as the highest standard (50mIU/mL). Then prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 50mIU/mL, 25mIU/mL, 12.5mIU/mL, 6.25mIU/mL, 3.12mIU/mL, 1.56mIU/mL, 0.78mIU/mL, and the last EP tubes with Standard Diluent is the blank as 0mIU/mL.



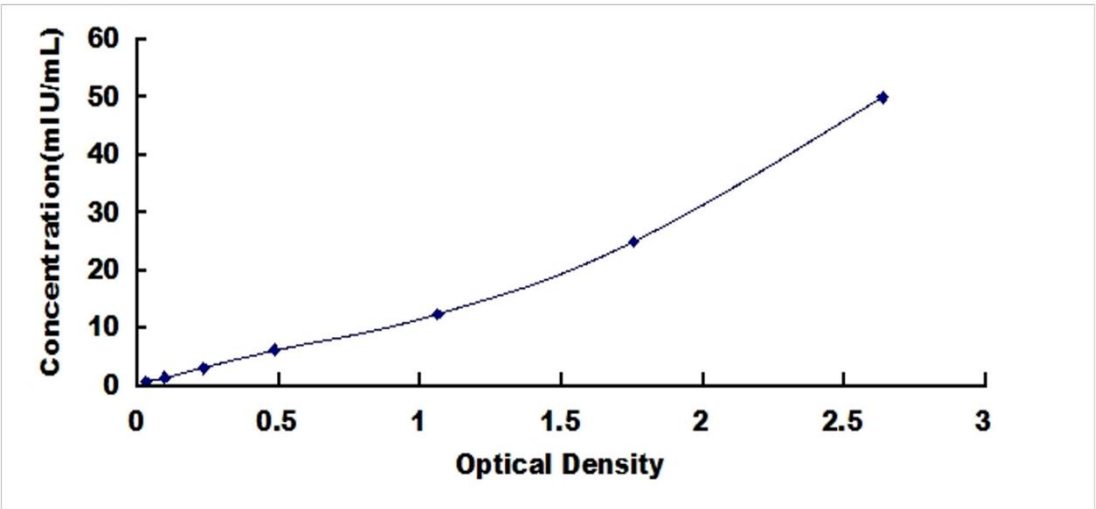
3. **Detection Reagent A and Detection Reagent B**- Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with **Assay Diluent A and B,** respectively

4. **Wash Solution**- Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).
5. **TMB substrate**- Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Assay Procedure

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 1 hour at 37 °C.

2. Remove the liquid of each well, don’t wash.
3. Add 100μL of **Detection Reagent A** working solution to each well, cover the wells with the plate sealer and incubate for 1 hour at 37 °C.
4. Aspirate the solution and wash with 350μL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
5. Add 100μL of **Detection Reagent B** working solution to each well, cover the wells with the plate sealer and incubate for 30 minutes at 37 °C.
6. Repeat the aspiration/wash process for total 5 times as conducted in step 4.
7. Add 90μL of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate for 10- 20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
8. Add 50μL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.



Standard curve of Follicle-stimulating hormone (FSH) ELISA kit

Appendix (7) Determination of serum Luteinizing hormone (LH)

Luteinizing hormone (LH) ELISA kit

A specific kit for measuring human LH concentration in serum was supplied by USA- Could-Clone Croup. No.CEA441Hu

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120µL	Assay Diluent A	1×12mL
Detection Reagent B	1×120µL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

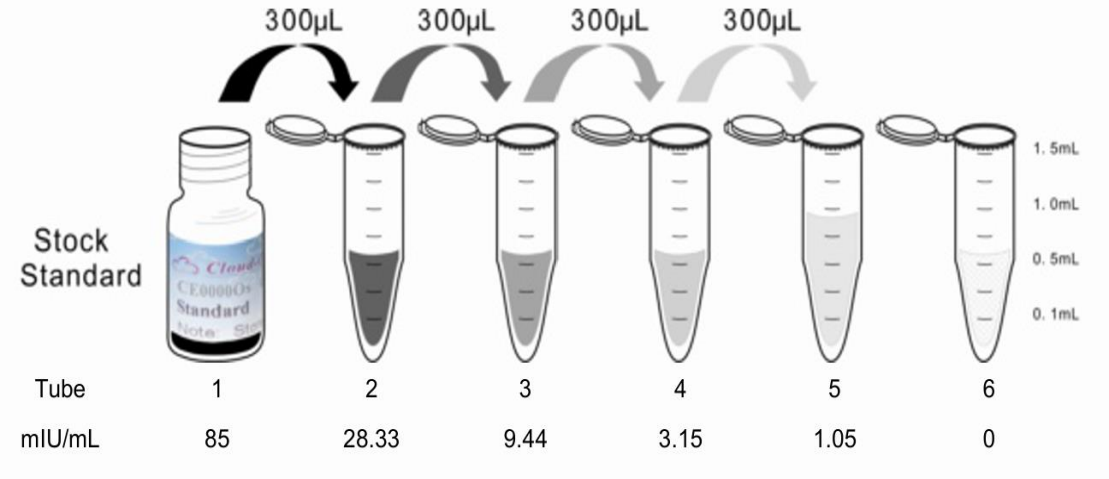
Principle

An anti-luteinizing hormone antibody has been pre-coated on a microplate, this antibody is specific to the luteinizing hormone, and a competitive inhibitory reaction is initiated between the unlabeled luteinizing hormone and the biotin-labeled luteinizing hormone. The unbound conjugate is removed following incubation by washing. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added, the amount of bound HRP conjugate is reverse proportional to the concentration of luteinizing hormone in the sample. By adding a solution of sulfuric acid (Stop solution), the enzyme-substrate reaction is stopped, and the reaction and absorbance are measured at 450nm.

Reagent Preparation

1. All reagents should bring to room temperature (18-25 °C) before use.
2. **Standard**- Reconstitute the **Standard** with 0.4mL of **Standard Diluent**, kept for 10 minutes at room temperature, shake gently (not to

foam). The concentration of the standard in the stock solution is 85mIU/mL. Please prepare 5 tubes containing 0.6mL Standard Diluent and produce a triple dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 85mIU/mL, 28.33mIU/mL, 9.44mIU/mL, 3.15mIU/mL, 1.05mIU/mL, and the last EP tubes with **Standard Diluent** is the blank as 0mIU/mL.



3. **Detection Reagent A and Detection Reagent B**- Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with **Assay Diluent A** and **B**, respectively.

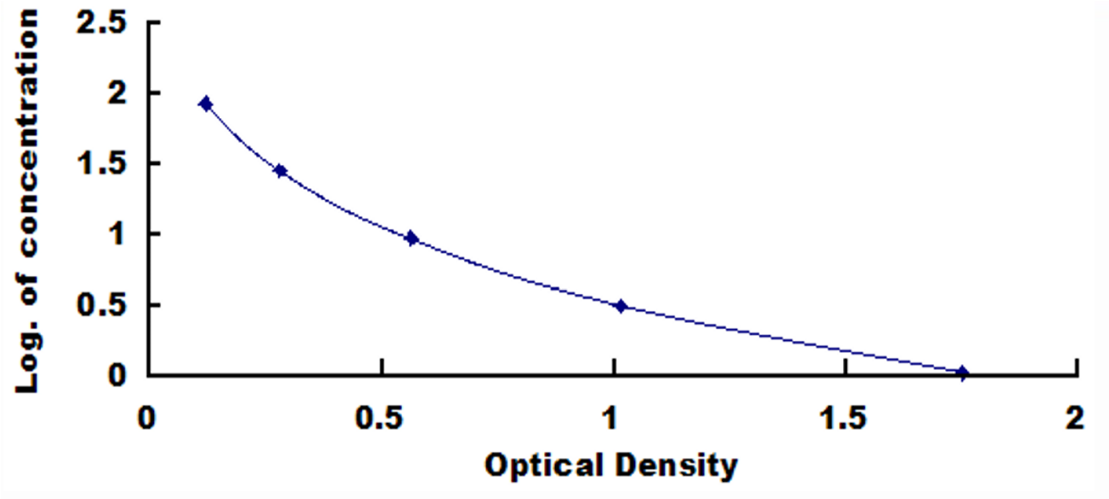
4. **Wash Solution**- Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).

5. **TMB substrate**- Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Assay Procedure

1. Determine wells for diluted standard, blank and sample. Prepare 5 wells for standard points, 1 well for blank. Add 50μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50μL of Detection Reagent A to each well immediately. Shake the plate gently (using a microplate shaker is recommended). Cover with a Plate sealer. Incubate for 1 hour at 37 °C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
2. Aspirate the solution and wash with 350μL of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
3. Add 100μL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37 °C after covering it with the Plate sealer.
4. Repeat the aspiration/wash process for total 5 times as conducted in step 2.
5. Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10- 20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
6. Add 50μL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.



Standard curve of luteinizing Hormone (LH) ELISA kit

Appendix (8) Determination of serum Estrogen (E2)

Estrogen (E2) ELISA kit

A specific kit for measuring human Estrogen (E2) concentration in serum was supplied by USA- Could-Clone Croup. No.CEA461Ge.

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120μL	Assay Diluent A	1×12mL
Detection Reagent B	1×120μL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

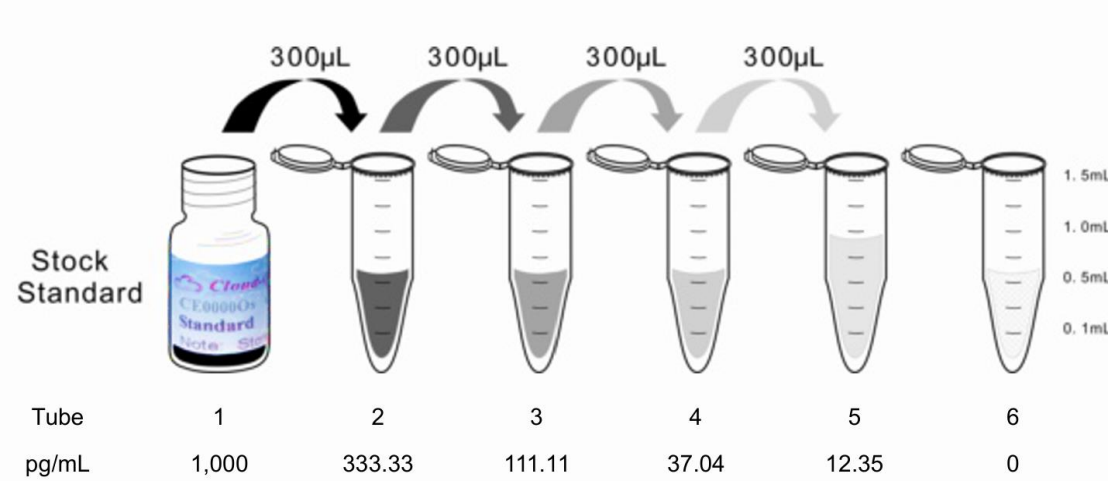
Principle

On a microplate, a monoclonal antibody specific to E2 has been pre-coated. A competitive inhibitory process is initiated between biotin-labeled E2 and unlabeled E2. After incubation, the unbound conjugate is washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of E2 in the sample. By adding a solution of sulfuric acid (Stop solution), the enzyme-substrate reaction is stopped, and terminated the reaction and absorbance is measured at 450nm.

Reagent Preparation

1. All reagents should bring to room temperature (18-25 °C) before use.
2. **Standard**- Reconstitute the **Standard** with 2.0mL of **Standard Diluent**, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is

1,000pg/mL. Please prepare 5 tubes containing 0.6mL Standard Diluent and produce a triple dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 1,000pg/mL, 333.33pg/mL, 111.11pg/mL, 37.04pg/mL, 12.35pg/mL, and the last EP tubes with **Standard Diluent** is the blank as 0pg/mL.



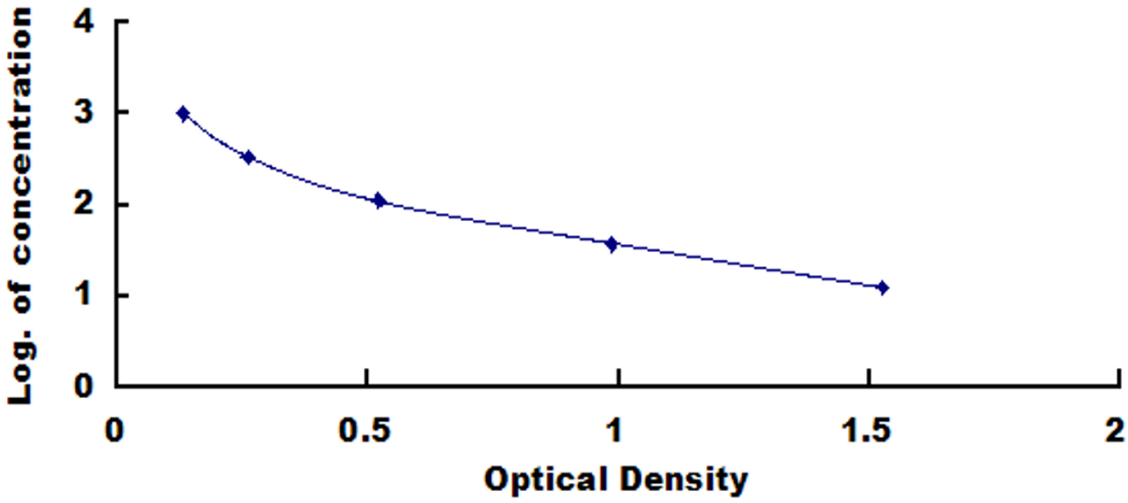
3. **Detection Reagent A and Detection Reagent B**- Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with **Assay Diluent A** and **B**, respectively.
4. **Wash Solution**- Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).
5. **TMB substrate**- Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Assay Procedure

1. Determine wells for diluted standard, blank and sample. Prepare 5 wells for standard points, 1 well for blank. Add 50μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50μL of Detection Reagent A to each well immediately. Shake the plate gently (using a

microplate shaker is recommended). Cover with a Plate sealer. Incubate for 1 hour at 37 °C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

2. Aspirate the solution and wash with 350µL of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
3. Add 100µL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37oC after covering it with the Plate sealer.
4. Repeat the aspiration/wash process for 5 times as conducted in step 2.
5. Add 90µL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10- 20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
6. Add 50µL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.



Standard curve of Estrogen ELISA Kit

Appendix (9) Determination of serum Testosterone

Testosterone ELISA kit

A specific kit for measuring human Testosterone concentration in serum was supplied by USA- Could-Clone Croup. No.HEA458Ge

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120μL	Assay Diluent A	1×12mL
Detection Reagent B	1×120μL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

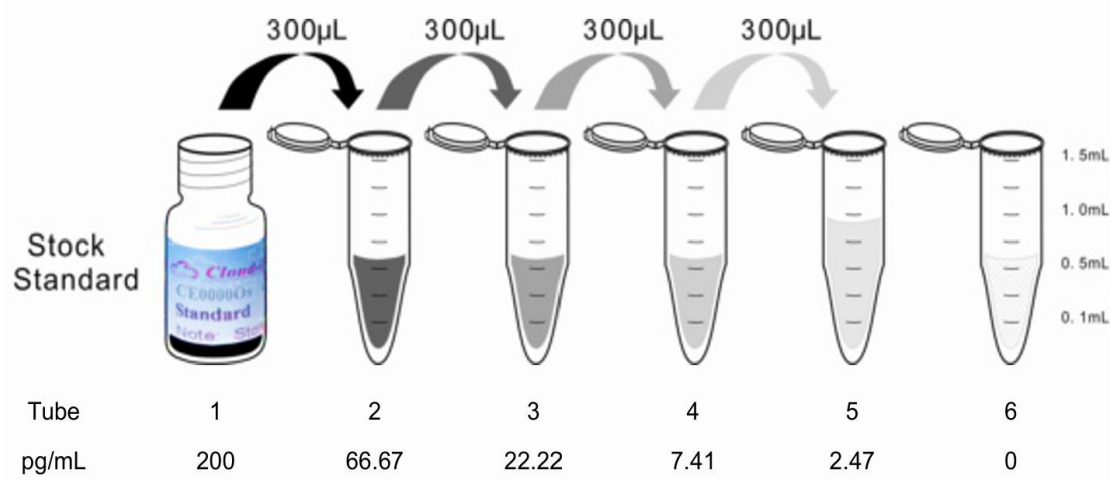
Principle

A microplate that contains of pre-coated antibody that is specific to testosterone. A competitive inhibitory response is initiated between unlabeled testosterone and biotin-labeled testosterone. Washing out the unbound conjugate occurs after incubation. After that, add Avidin conjugated horseradish peroxidase (HRP), and the mixture is then incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of testosterone in the sample. By adding a solution of sulfuric acid (Stop solution), the enzyme-substrate reaction is stopped and determined the reaction and absorbance are measured at 450nm

Reagent Preparation

1. All reagents should bring to room temperature (18-25 °C) before use.
2. **Standard**- Reconstitute the **Standard** with 0.5mL of **Standard Diluent**, kept for 10 minutes at room temperature, shake gently (not to

foam). The concentration of the standard in the stock solution is 200pg/mL. Please prepare 5 tubes containing 0.6mL Standard Diluent and produce a triple dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 200pg/mL, 66.67pg/mL, 22.22pg/mL, 7.41pg/mL, 2.47pg/mL, and the last EP tubes with **Standard Diluent** is the blank as 0pg/mL.



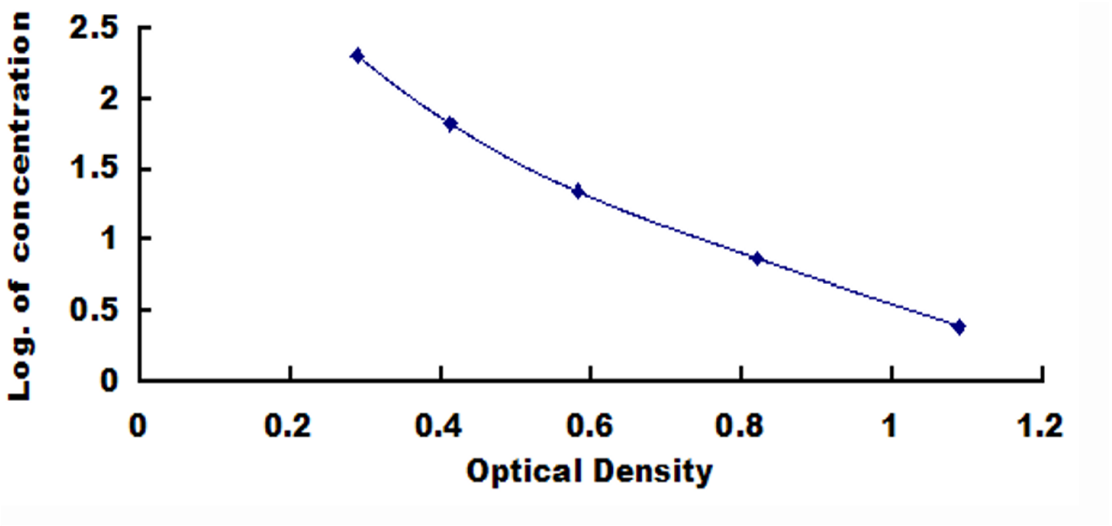
3. **Detection Reagent A and Detection Reagent B**- Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with Assay Diluent A and B, respectively.
4. **Wash Solution**- Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).
5. **TMB substrate**- Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Assay Procedure

1. Determine wells for diluted standard, blank and sample. Prepare 5 wells for standard points, 1 well for blank. Add 50μL each of dilutions of standard (read Reagent Preparation), blank and samples into the

appropriate wells, respectively. And then add 50μL of Detection Reagent A to each well immediately. Shake the plate gently (using a microplate shaker is recommended). Cover with a Plate sealer. Incubate for 1 hour at 37 °C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

2. Aspirate the solution and wash with 350μL of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
3. Add 100μL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37 °C after covering it with the Plate sealer.
4. Repeat the aspiration/wash process for total 5 times as conducted in step 2.
5. Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10- 20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
6. Add 50μL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.



Standard curve of Testosterone ELISA Kit

Appendix (10) Determination of serum Prolactin hormone (PRL)

Prolactin hormone (PRL) ELISA kit

A specific kit for measuring human Prolactin hormone (PRL) concentration in serum was supplied by USA- Could-Clone Croup. No.SEA846Hu

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120µL	Assay Diluent A	1×12mL
Detection Reagent B	1×120µL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

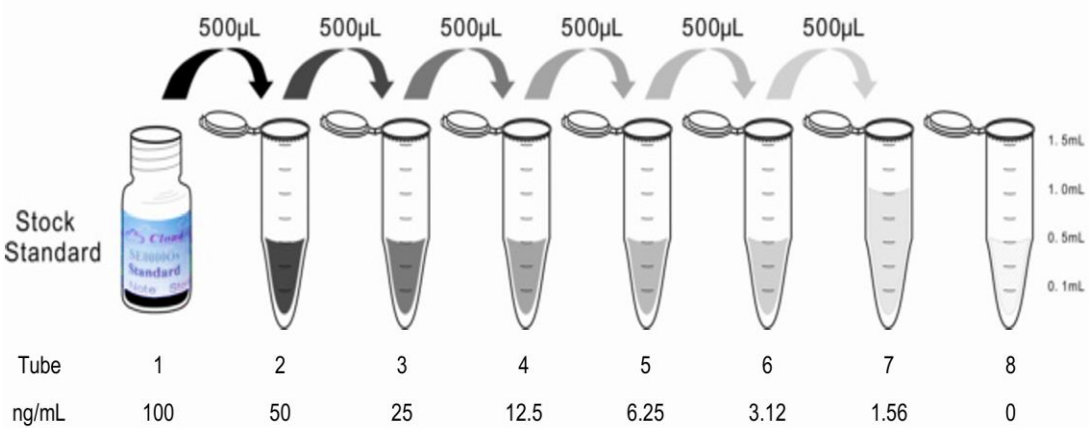
Principle

An antibody specific to PRL has been pre-coated on the microplate. Next, standards or samples are put with a biotin-conjugated antibody that is specific to PRL into the corresponding microplate wells. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added. After incubation, add Avidin conjugated horseradish peroxidase (HRP), and the mixture is then incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of prolactin in the sample. By adding a solution of sulfuric acid (Stop solution), the enzyme-substrate reaction is stopped and determined the reaction and absorbance are measured at 450nm.

Reagent Preparation

1. All reagents should bring to room temperature (18-25 °C) before use.
2. **Standard**- Reconstitute the **Standard** with 1.0mL of **Standard Diluent**, kept for 10 minutes at room temperature, shake gently (not to

foam). The concentration of the standard in the stock solution is 100ng/mL. Please prepare 7 tubes containing 0.5mL Standard Diluent and produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 100ng/mL, 50ng/mL, 25ng/mL, 12.5ng/mL, 6.25ng/mL, 3.12ng/mL, 1.56ng/mL, and the last EP tube with **Standard Diluent** is the blank as 0ng/mL.



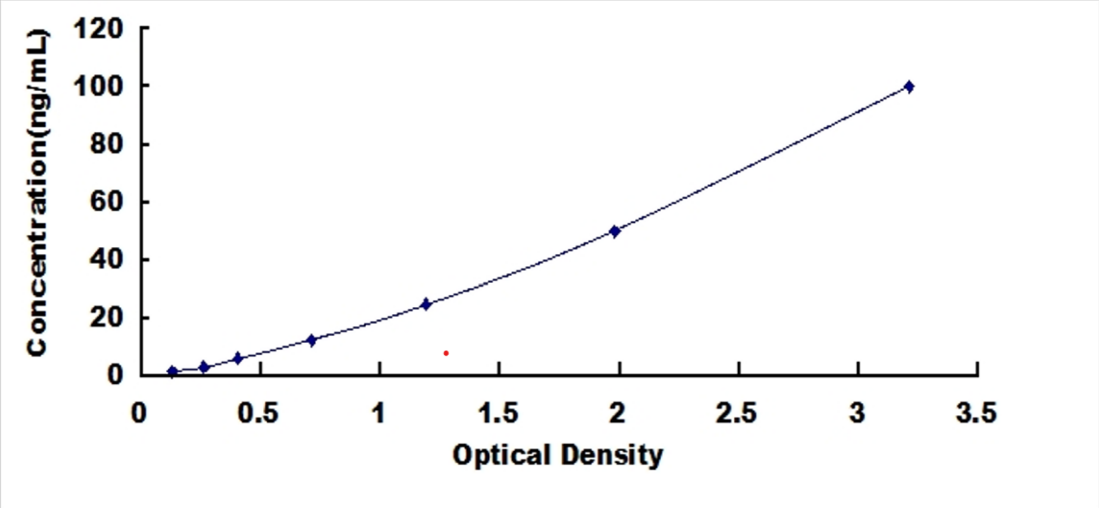
3. **Detection Reagent A and Detection Reagent B**- Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with **Assay Diluent A** and **B**, respectively.
4. **Wash Solution**- Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).
5. **TMB substrate**- Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Assay Procedure

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100μL each of dilutions of standard

(read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 1 hour at 37 °C.

2. Remove the liquid of each well, don't wash.
3. Add 100μL of **Detection Reagent A** working solution to each well, cover the wells with the plate sealer and incubate for 1 hour at 37 °C.
4. Aspirate the solution and wash with 350μL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
5. Add 100μL of **Detection Reagent B** working solution to each well, cover the wells with the plate sealer and incubate for 30 minutes at 37 °C.
6. Repeat the aspiration/wash process for total 5 times as conducted in step 4.
7. Add 90μL of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate for 10- 20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
8. Add 50μL of **Stop Solution** to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.



Standard curve of Prolactin (PRL) ELISA Kit

Appendix (11) Determination of Thyroid-stimulating hormone (TSH)

Thyroid-stimulating hormone (TSH) ELISA kit

A specific kit for measuring human Thyroid-stimulating hormone (TSH) concentration in serum was supplied by USA- Could-Clone Croup. No.SEA463Hu

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard -1,2,3,4,5,6	6×0.5mL	Wash Buffer (20×concentrate)	1×15mL
Detection Reagent A (red)	1×6mL	Stop Solution	1×6mL
TMB Substrate	1×9mL	Instruction manual	1

Principle

A monoclonal antibody specific to TSH has been pre-coated on the microplate. Next, standards or samples are added with another HRP conjugated monoclonal antibody specific for TSH to the corresponding microtiter plate wells. Addition of TMB substrate solution after washing. The only wells that will change color are those that contain HRP conjugated monoclonal antibodies and TSH. By adding a solution of sulfuric acid (Stop solution), the enzyme-substrate reaction is stopped and determined the reaction and absorbance are measured at 450nm.

Reagent Preparation

1. All reagents should bring to room temperature (18-25 °C) before use.
2. **Standard**- The concentration of the standard is as following: 12.0mIU/L, 6.0mIU/L, 3.0mIU/L, 1.5mIU/L, 0.6mIU/L, 0.3mIU/L.

3. **Wash Solution**- Dilute 15mL of Wash Solution concentrate (20×) with 285mL of deionized or distilled water to prepare 300 mL of Wash Solution (1×).
4. **TMB substrate**- Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

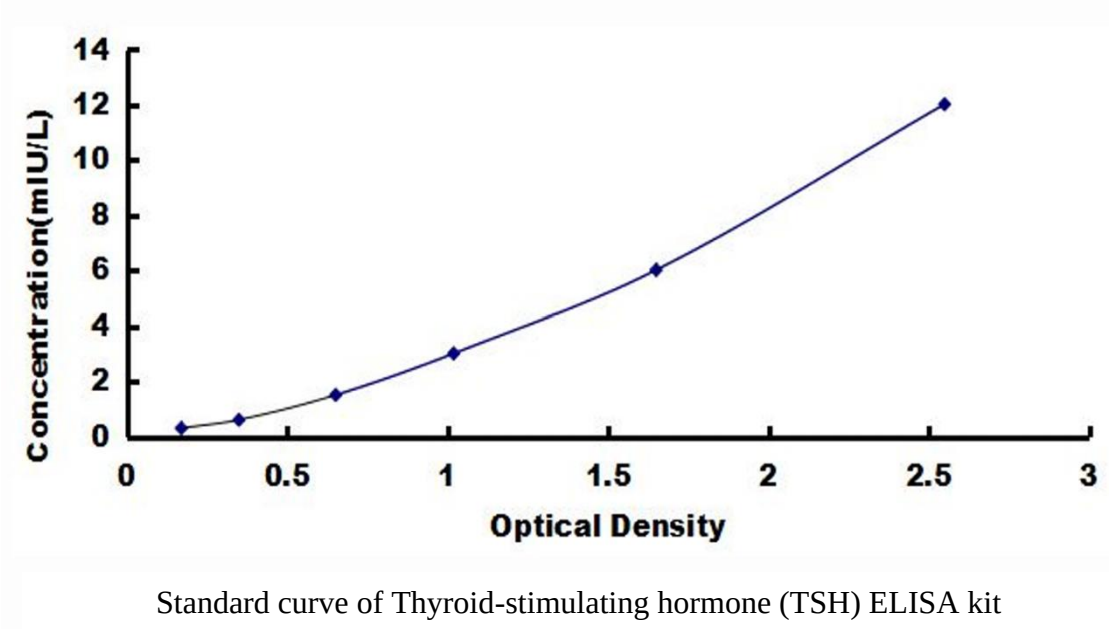
Note: If crystals have formed in the Wash Solution concentrate (20×), warm to room temperature and mix gently until the crystals are completely dissolved.

Assay Procedure

1. Determine wells for standard and sample. Add 100μL each of different concentration of standard (read Reagent Preparation) into the appropriate wells. Add 100μL sample into the other well.
2. Add 50μL of **Detection Reagent A** to each well, mix thoroughly. Incubate for 2 hours at 37 °C after covering it with the Plate sealer.
3. Aspirate the solution and wash with 350μL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
4. Add 90μL of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate for 15- 20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
5. Add 50μL of **Stop Solution** to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the

plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

6. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.



Standard curve of Thyroid-stimulating hormone (TSH) ELISA kit

Appendix (12) Determination of Triiodothyronine (T3)

Triiodothyronine (T3) ELISA kit

A specific kit for measuring human Triiodothyronine (T3) concentration in serum was supplied by USA- Could-Clone Croup. No.CEA453Ge

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120µL	Assay Diluent A	1×12mL
Detection Reagent B	1×120µL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

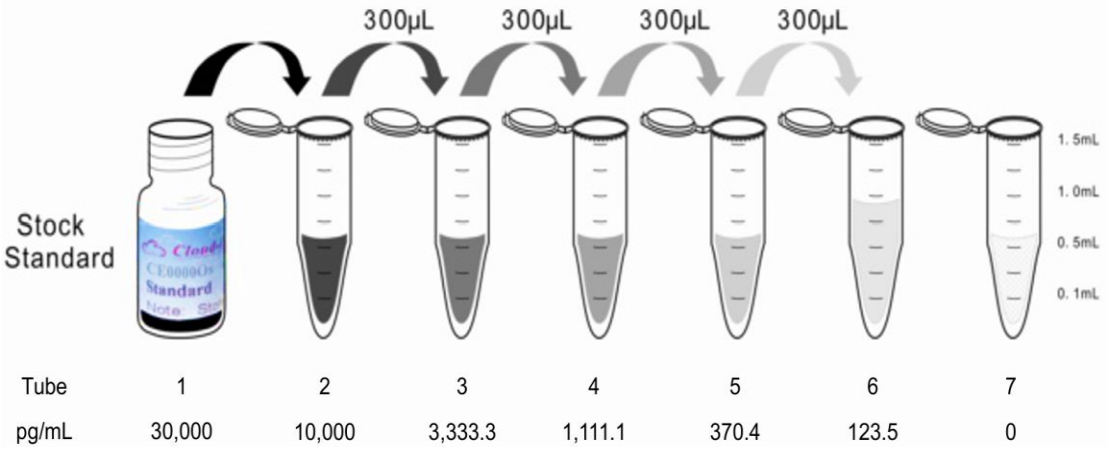
Principle

On a microplate, a monoclonal antibody specific to T3 has been pre-coated. A competitive inhibitory process is initiated between biotin labeled T3 and unlabeled T3 (Standards or samples). Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to microplate well The amount of bound HRP conjugate is reverse proportional to the concentration of T3 in the sample. By adding a solution of sulfuric acid (Stop solution), the enzyme-substrate reaction is stopped and determined the reaction and absorbance are measured at 450nm.

Reagent Preparation

1. All reagents should bring to room temperature (18-25 °C) before use.
2. **Standard**- Reconstitute the **Standard** with 1.0mL of **Standard Diluent**, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 30,000pg/mL. Please firstly dilute the stock solution to 10,000pg/mL

and the diluted standard serves as the highest standard (10,000pg/mL). Then prepare 5 tubes containing 0.6mL Standard Diluent and produce a triple dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 10,000pg/mL, 3,333.3pg/mL, 1,111.1pg/mL, 370.4pg/mL,123.5pg/mL, and the last EP tubes with **Standard Diluent** is the blank as 0pg/mL.

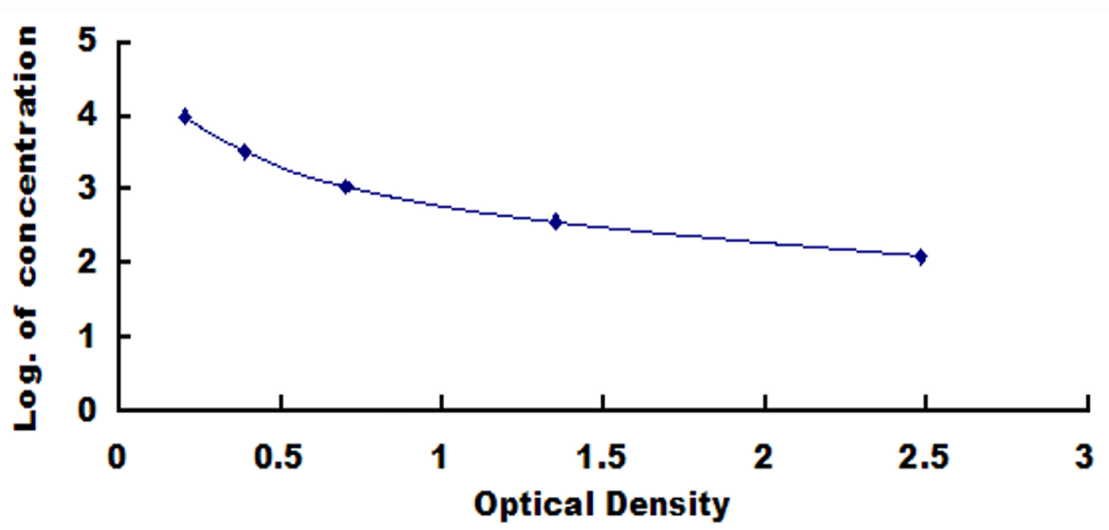


3. **Detection Reagent A and Detection Reagent B**- Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with **Assay Diluent A** and **B**, respectively.
4. **Wash Solution**- Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).
5. **TMB substrate**- Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Assay Procedure

1. Determine wells for diluted standard, blank, and sample. Prepare 5 wells for standard points, 1 well for blank. Add 50μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50μL of Detection Reagent A to each well immediately. Shake the plate gently (using a microplate shaker is recommended). Cover with a Plate sealer. Incubate for 1 hour at 37 °C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
2. Aspirate the solution and wash with 350μL of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
3. Add 100μL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37 °C after covering it with the Plate sealer.
4. Repeat the aspiration/wash process for total 5 times as conducted in step 2.
5. Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10- 20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
6. Add 50μL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.



Standard curve of Triiodothyronine (T3) ELISA kit

Appendix (13) Determination of Thyroxine (T4)

Thyroxine (T4) ELISA kit

A specific kit for measuring human Thyroxine (T4) concentration in serum was supplied by USA- Could-Clone Croup. No.CEA452Ge

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1	Assay Diluent A	1×12mL
Detection Reagent B	1×120μL	Assay Diluent B	1×12mL
Reagent Diluent	1×300μL	Stop Solution	1×6mL
TMB Substrate	1×9mL	Instruction manual	1
Wash Buffer (30 × concentrate)	1×20mL		

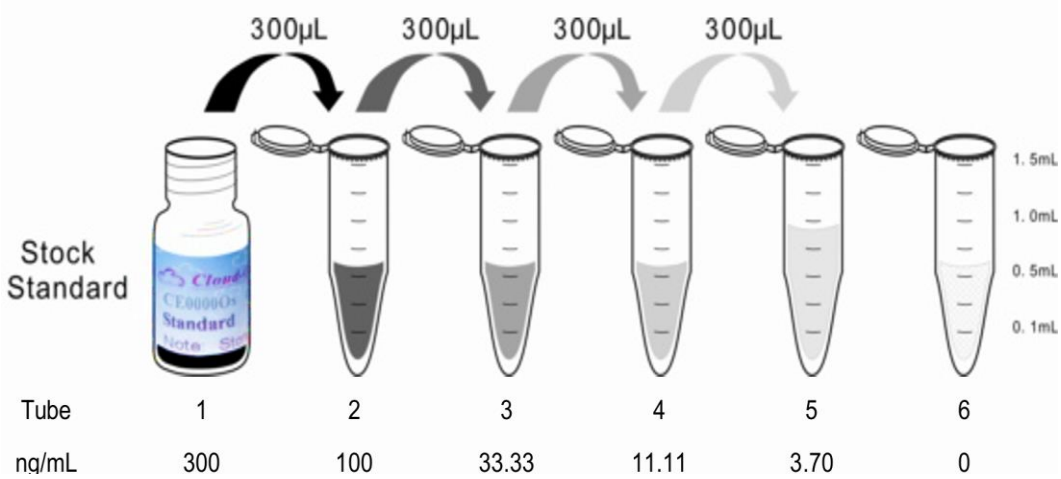
Principle

The microplate has been pre-coated with a monoclonal antibody that is specific to T4. T4-specific antibody initiates a competitive inhibitory response between biotin-labeled T4 and unlabeled T4. The unbound conjugate is removed. After incubation, add Avidin conjugated horseradish peroxidase (HRP), and the mixture is then incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of prolactin in the sample. By adding a solution of sulfuric acid (Stop solution), the enzyme-substrate reaction is stopped, and determined the reaction and absorbance are measured at 450nm.

Reagent Preparation

1. All reagents should bring to room temperature (18-25 °C) before use.
2. **Standard**- Reconstitute the **Standard** with 0.5mL of **Standard Diluent**, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is

300ng/mL. Please prepare 5 tubes containing 0.6mL Standard Diluent and produce a triple dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 300ng/mL, 100ng/mL, 33.33ng/mL, 11.11ng/mL, 3.70ng/mL, and the last EP tubes with **Standard Diluent** is the blank as 0ng/mL.

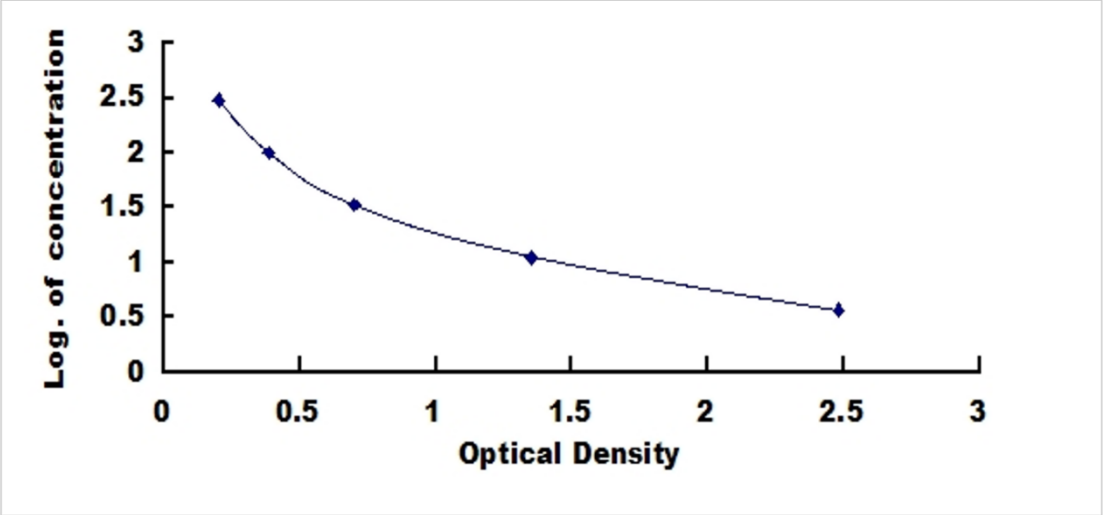


3. **Detection Reagent A**- Reconstitute the **Detection Reagent A** with 150μL of **Reagent Diluent**, kept for 10 minutes at room temperature, shake gently (not to foam). Dilute to the working concentration with **Assay Diluent A** (1:100).
4. **Detection Reagent B**- Briefly spin or centrifuge the stock Detection B before use. Dilute to the working concentration with **Assay Diluent B** (1:100).
5. **Wash Solution**- Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).
6. **TMB substrate**- Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Assay procedure

1. Determine wells for diluted standard, blank and sample. Prepare 5 wells for standard points, 1 well for blank. Add 50μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50μL of Detection Reagent A to each well immediately. Shake the plate gently (using a microplate shaker is recommended). Cover with a Plate sealer. Incubate for 1 hour at 37 °C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
2. Aspirate the solution and wash with 350μL of 1X Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
3. Add 100μL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37 °C after covering it with the Plate sealer.
4. Repeat the aspiration/wash process for total 5 times as conducted in step 2.
5. Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10-20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
6. Add 50μL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.



Standard curve of Thyroxine (T4) ELISA Kit

الخُلاصة

يُعرَّف العقم بأنه الفشل في تحقيق الحمل بعد عام واحد من الجماع المنتظم والمتكرر والمستمر وغير المحمي. متلازمة تكيس المبايض (PCOS) هي اضطراب غير متجانس في الغدد الصماء يتميز بظهور أكياس المبيض، وعدم حدوث الإباضة، تباين في وظائف الغدد الصماء. قصور المبيض المبكر (POI) هو أحد مشاكل الإباضة والأمراض الإنجابية للمرأة، وهو احد الأسباب الرئيسية لعقم الإناث قبل سن ٤٠ عامًا.

شملت الدراسة الحالية ١٢٠ أنثى تتراوح أعمارهن بين ٢٠ و ٤٥ عاماً تم جمعها من وحدة العقم في مستشفى امراض النسائية والتوليد خلال الفترة من نوفمبر ٢٠٢٣ إلى أبريل ٢٠٢٤، و قُسمت عينات الدراسة إلى مجموعتين ٦٠ انثى سليمة استخدمت كمجموعة ضابطه ٦٠ انثى مصابة بالعقم المصابات بالعقم كمجموعة مرضى، حيث قسمت الأخيرة الى مجموعتين ثانوية على النحو التالي: ٤٧ أنثى عقيمة مصابة بمتلازمة تكيس المبايض، ١٣ أنثى عقيمة مصابة بفشل المبيض الاولي، ثم قُسمت جميع الإناث المشاركة في الدراسة حسب العمر اما اكثر او اقل من ٤٠ عام. شَملت المعايير التي تم قياسها في هذه الدراسة: المؤشرات الحيوية للعقم (Inhibin-B, -Anti Müllerian hormone, Prolactin, Estrogen, Luteinizing hormone و -Follicle stimulating hormone) ومؤشرات الأندروجينات (-Dehydroepiandrosterone Sulfate و Testosterone) ومؤشرات وظائف الغدة الدرقية (Thyroid-Stimulating Hormone, Triiodothyronine, Thyroxine). ثم حُددت مستويات الهرمونات باستخدام فحص المرض المناعي المرتبط بالإنزيم ELISA.

أظهرت نتائج الدراسة الحالية إرتفاعاً معنوياً (P≤0.0001) في مستوى الأجسام المضادة لأنزيم TPO , DHEA-S , Inhibin-B وAMH (16.34 ± 2.17) , (٢,٧٩ ± ٠,٥٣) , (٢٦٦,٩٧ ± ٧٢,٨٨) , (١٠,٩٩ ± ٢,٣٧) لمرضى متلازمة تكيس المبايض على التوالي مقارنة بالمجموعة الضابطة (٥,٩٢ ± ٠,٩٦) و (٠,٨٩ ± ٠,١٢)، (٣٦,٧٦ ± ١٠,٦٢)، و (٣,١١ ± ٠,٦٠)، كما وجد أيضًا انخفاضًا معنوياً (P≤0.0001) في مستويات DHEA-S و -Inhibin B وAMH و (0.23 ± 0.54)، (١٨,٩٦ ± ١,٢١)، و (٠,٦٠ ± ٠,٣٩) لمرضى فشل المبيض الأولي على التوالي مقارنة بالمجموعة الضابطة (٠,٨٩ ± ٠,١٢)، (٣٦,٧٦ ± ١٠,٦٢)، و (٣,١١ ± ٠,٦٠)، كذلك زيادة معنوية (P≤0.0001) في مستويات الأجسام المضادة لأنزيم TPO (1.19± 14.72) في مرضى فشل المبيض الولي مقارنة بالمجموعة الضابطة (٥,٩٢ ±

٠,٩٦). أظهرت النتائج زيادة معنوية كبيرة (P≤0.01) في مؤشر كتلة الجسم لمرضى متلازمة تكيس المبايض (30.93 ± 3.50)، و فشل المبيض الأولي (3.24± ٣١) على التوالي مقارنة بالمجموعة الضابطة (1.92 ± ٢٧,٩٠). أظهرت مستويات هرمون AMH ارتفاعاً معنوياً كبيراً (P≤0.0001) في مرضى تكيس المبايض للفئات العمرية (> ٤٠)، (< ٤٠) (١٠,٨٩ ± ٢,٣٧)، (١١,٤٥ ± ٢,٤٦) على التوالي، بينما أظهرت انخفاضاً معنوياً (P≤0.01) بمستويات AMH في مرضى فشل المبيض الأولي (40 >) (٠,٦٠ ± ٠,٣٩). ايضاً أظهرت النتائج زيادة معنوية في مستويات الأجسام المضادة لأنزيم TPO لكل من مرضى تكيس المبايض (17.63 ± 1.93)، ومرضى فشل المبيض الأولي (١٧,٦٣ ± ١,٩٣)، ايضاً كانت هناك زيادة معنوية (P≤0.01) بمستويات DHEA-S في مرضى تكيس المبايض للفئة العمرية (40 > ± 2.75) (0.55، (< ٤٠) (٢,٩٤ ± ٠,٤٦)، وانخفضت مستويات DHEA-S في مرضى فشل المبيض الأولي (0.23 ± 0.54)، سجلت الدراسة زيادة معنوية كبيرة في مستويات Inhibin-B (P≤0.0001) في متلازمة تكيس المبايض (> ٤٠) (٢٧٣,٥٥ ± ٧٥,٢٥)،(< ٤٠) (٢٣٤,٩١ ± ٥٢,٤٣)، انخفضت مستويات Inhibin-B في مرضى فشل المبيض الأولي (١٨,٩٦ ± ١,٢١). كشف البرولاكتين والتيستوستيرون والإستروجين و الهرمون اللوتيني عن زيادة معنوية كبيرة (P≤0.01) في مرضى متلازمة تكيس المبايض (٢٩,٢٤ ± ٤,٩٩)، (٥٨,٥٣ ± ١٧,٣٩)، (١٨٦,٢٥±٤٨,٢٣)، و (١٧,١٥ ± ٥,٨١) على التوالي مقارنة بمجموعة المراقبة (١٣,٤٤ ±٣,٣٠)، و (٥,٣٤ ± ٠,٩٠)، و (٢٤,٩٣ ± ٧,٢٣)، و (٢,٩٣ ± ١,٢٨)، في حين كشفت عن انخفاض معنوي كبير(P≤0.01) في مستويات FSH مرضى متلازمة تكيس المبايض (٥,٨٣ ± ٢,٣) مقارنة بالمجموعة الضابطة (٢,١٥ ± ٨,١). وجدت النتائج زيادة معنوية كبيرة (P≤0.01) في مستويات FSH, Prolactin , LH في مرضى فشل المبيض الأولي (3.61 ± 33.51), (٢٦,٠٦±٣,٠٤) , (١٩,٧٧ ± ٢,٨٨)، بانخفاض كبير (P≤0.0001) في مستويات هرمون الاستروجين والتستوستيرون (١٠,٠٧ ± ١,٤٣) على التوالي، (٣,٢٤± ٠,٢ عند مقارنتها بالمجموعة الضابطة (٢٤,٩٣ ± ٧,٢٣), (0.9 ± 5.34)

لم تظهر مستويات TSH و T3 و T4 أي فرق معنوي في (P > 0.01) في مرضى PCOS مقارنةً بالمجموعة الضابطة. أيضًا لا يوجد فرق معنوي كبير عند (P > 0.01) في مستويات TSH و T3 و T4 في مرضى POI مقارنة بالمجموعة الضابطة.

أظهرت الدراسة وجود ارتباط معنوي موجب بين DHEA-S و كل من AMH و Anti-TPO في مرضى تكيس المبايض (r = 0.729) , (r = 0.416). اما في مرضى فشل المبيض الأولي اظهرت علاقة معنوية إيجابية بين DHEA-S وAMH (r = 0.678) على التوالي. أيضًا، اوجدت النتائج علاقة سلبية بين Inhibin-B وAnti-TPO في مرضى تكيس المبايض (r = -0.520). وكان هناك ارتباط معنوي سلبي بين AMH و DHEA-S في مرضى فشل المبيض الأولي (r = -0.578).

كانت نتيجة منحني Roc لتقييم الأداء التشخيصي الإجمالي لاختبار ما لمستوى مضادات انزيم TPO ;P-value: 0.001 ;95% CI: 0.907-1.000, نقطة قطع: 9.306 ,والمسافة تحت المنحني: 98.861% مع حساسية وخصوصية: 96.667% -96.676% ومستوى الدقة (97.000%,

اما بالنسبة ل DHEA-S

;P-value: 0.001 ;95% CI: 0.992-1.0000, نقطة القطع: 1.480 مع مسافة تحت المنحني : 99.694% وحساسية وخصوصية: 96.667%-98.333% مستوى الثقة: 97.500%).

وسجلت النتائج لمؤشر Inhibin-B 95% CI: 0.907-1.000 ؛ P-value: 0.001؛ , ونقطة القطع: ٧٧,٣٤٧ ؛ مع مسافة تحت المنحني 95.417 % مع حساسية وخصوصية ٩١,٦٦٧٪ -٩٨,٣٣٣٪ ؛ ومستوى الثقة : ٩٥,٠٠٠٪

نستنتج من الدراسة الحالية أن الأجسام المضادة لأنزيم TPO و Inhibin-B و AMH و DHEA-S تعد من اكثر المؤشرات الحيوية تنبؤًا بالعقم، وتقييم صحة المبيض، ويمكن استخدامها كمؤشرات حساسة للتشخيص المبكر لـتكيس المبايض و فشل المبيض الأولي عند النساء في سن الإنجاب. كانت تراكيز مستويات الأجسام المضادة لأنزيم TPO و Inhibin-B و AMH و DHEA-S مرتفعًا في متلازمة تكيس المبايض مقارنة بالمجموعة الضابطة، بينما انخفضت مستويات AMH و DHEA-S و Inhibin-B بشكل كبير في مرضى POI مقارنة بالمجموعة الضابطة. لقد وجدنا أن تكيس المبايض وفشل المبيض الأولي تتأثر بزيادة الوزن وزيادة مؤشر كتلة الجسم.

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿ لِّلَّهِ مُلْكُ ٱلسَّمَٰوَٰتِ وَٱلْأَرْضِ ۚ يَخْلُقُ مَا يَشَآءُ ۚ يَهَبُ لِمَن يَشَآءُ إِنَٰثًا وَيَهَبُ لِمَن يَشَآءُ ٱلذُّكُورَ ۝ أَوْ يُزَوِّجُهُمْ ذُكْرَانًا وَإِنَٰثًا ۖ وَيَجْعَلُ مَن يَشَآءُ عَقِيمًا ۚ إِنَّهُۥ عَلِيمٌ قَدِيرٌ ۝ ﴾

صَدَقَ اللهُ العَلِيُّ العَظِيمْ

سورة الشورى - الآية (٤٩- ٥٠)

جامعة كربلاء

كلية العلوم الطبية التطبيقية

قسم التحليلات المرضية

تقييم مستوى كبريتات ديهيدروابيندروستيرون (DHEA-S) ، البرولاكتين، إنهيبين- ب, والأجسام المضادة لبيروكسايديز الدرقية (TPO) في الإناث المصابات بمتلازمة تكيس المبايض (PCOS) وقصور المبيض المبكر (POI) في مدينة كربلاء

رسالة

مقدمة إلى مجلس كلية العلوم الطبية التطبيقية - جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية

كتبت بواسطة

زهراء عبد الأمير جليل ماميثة

بكلوريوس تحليلات مرضية (٢٠٢١)

بإشراف

ا. د. عبير جياد يوسف الفتلاوي **ا.م. د. منال ناصح أحمد التميمي**

هـ/ ١٤٤٦ **م/ ٢٠٢٥**