University of Kerbala

College of Applied Medical Sciences

Assessment of Serum Sestrin 1 , Sestrin 2 and Beclin 1 Levels on the Regulation of Autophagy in Women with Polycystic Ovary Syndrome (PCOS)

A Thesis

Submitted to the council of the

College of Applied Medical Sciences – University of

Kerbala In Partial of Fulfillment of the Requirements

for the Master Degree in Clinical Laboratories

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2023 A.D   **1445 A.H**

بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

(وَلَمَّا بَلَغَ أَشُدَّهُ وَاسْتَوَىٰ ءَاتَيْنَـٰهُ حُكْمًا وَعِلْمًا ۚ
وَكَذَٰلِكَ نَجْزِى ٱلْمُحْسِنِينَ)

صدق الله العلي العظيم

سورة القصص
آية : 14

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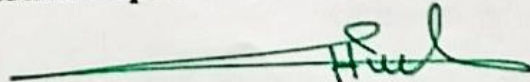*Lecture. Dr. Mawahib Basheer Jasim*

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\ 2 \ 16\ 2023

Dedication

To the one who lit the first candle for me, to the fragrance of my childhood, to the warmth of my life, to the one who endured every moment of pain in my life and turned it into moments of joy, to my beloved and the soul of my heart to my father .

To the one who supported me on the day of my weakness, to the one who shared my anxiety and sadness with me, to the one who shed tears for me, For who care about my smile, to my dear mother .

To pure hearts that still guide me safety to those whose heart still embraces me and guides me to giving, love and sacrifice to my dear husband.

To My beautiful jewel that gives me blessings and happiness in life to my daughter (Noor).

To those who supported me in my career, to the sparkling stars of my sky, and my support in life, to my brothers and sister.

Everyone who supported me and stood with me, even with a word or a sincere prayer in the back of the unseen.

Assalah

Acknowledgement

First and foremost, thanks to Merciful Allah for giving me, strength, health, patience, and making this academic requirement possible.

My deepest thanks, as well as my gratitude, go out to all of the patients who agreed to assist me in the completion of my work.

I'd like to give my deepest thanks to Prof.Dr. Ghasoun Ghanem and Dr. liqaa Hameed Hasan for them suggestion, support, advice, encouragement, and trust; without them thorough oversight, this thesis could never have been finished .

I would like to express my gratitude to M.S.C. Ehsan.K.Kadem for the help and support he provided during the process of completing this thesis .

We thank the University of Kerbala, the College of Applied Medical Sciences, and its esteemed Dean Dr. Jawdat Noori Gaaib and Head of Clinical Labs for their assistance.

I would like to express my gratitude to the medical staff who work in the labs of the "Gynecological and Obstetric Teaching Hospital" that are a part of the Kerbala health directorate. Without their invaluable assistance, it would not have been possible to carry out the study.

Assalah

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List of Abbreviations

Abbreviations	Description
AA	Adrenal androgen
AE	Androgen excess
AFC	Antral follicle cell
AMPK	5' adenosine monophosphate-activated protein kinase
ANOVA	Analysis of variance
AFC	Antral follicle cell
AKT	The AKR mouse strain that develops spontaneous thymic lymphomas
ATG	Autophagy-related genes
ATP	Adenosine triphosphate
AMP	Adenosine mono phosphate
BARA	β_α repeated autophagy
Bcl 2	B cell lymphoma 2
BECN1	Beclin1
BH3	The Bcl-2 homology domain 3
BMI	Body mass index
Bif 1	Bax-interacting factor 1
cAMP	Cyclic adenosine monophosphate
CVD	Cardiovascular Disease
DHEAS	Dehydroepiandrosterone sulfate
DMII	Diabetes mellitus Type 2
ESHRE/ASRM	The European Society of Human Reproduction and Embryology/ American Society for Reproductive Medicine

FAI	Free androgen index
FT	Free testosterone
FBG	Fasting Blood glucose
FSH	Follicular stimulating hormone
FOXO	Forkhead box protein O
GADD	Growth Arrest and DNA Damage
GAD	Gestational Diabetes Mellitus
GOD	Glucose oxidase
GnRH	Gonadotropin-releasing hormone
GTPases	Guanosine triphosphate enzymes
HA	Hyperandrogenemia
HCG	human chorionic gonadotropin
HOMA-IR	Homeostatic model assessment for insulin resistance
HRP	Horseradish peroxidase enzyme
IR	Insulin resistance
IRS-1	Insulin receptor substrate 1
Keap1	Kelch-like ECH-associated protein 1
LC3B	protein 1A/1B-light chain 3
LH	Luteinizing hormone
MTORC1	mammalian target of rapamycin complex 1
NIH	National institutes health
Nrf2	The nuclear factor erythroid 2–related factor 2
NAFLD	Nonalcoholic fatty liver disease
GnRH	Gonadotropin hormone-releasing hormone
OC	Ovary syndrome
OD	Optical density
P4	Progesterone
PCOM	Polycystic ovary morphology
PCOS	Polycystic ovary syndrome
POD	Peroxidase

PI3KC3	Phosphatidylinositol 3-kinase catalytic subunit type 3
r	Pearson's correlation coefficient
ROS	Reactive oxygen species
SESN1	Sestrin 1
SESN2	Sestrin2
SD	Standard deviation
SHBG	Sex hormone binding globulin
T2DM	Type 2 diabetes
TMB	Tetra methyl benzidine
TSH	Thyroid stimulating hormone
ULK1/2	Unc-51 Like Autophagy Activating Kinase 1
UVRAG	UV radiation resistance gene
VPS	Vacuolar protein sorting
PA26	P53-activated gene number 26

Summary

Many women worldwide of reproductive age are affected by the diverse endocrine condition known as polycystic ovary syndrome (PCOS). High testosterone levels, insulin resistance, and enlarged, dysfunctional ovaries are often associated with this syndrome.

Within the ovary, appropriate active autophagy is necessary for all stages of oocyte formation, follicle growth, and degeneration . It is necessary to maintain appropriate conditions for follicular atresia, follicular development and differentiation, oocyte production, and the reproductive cycle, follicular cells must participate in autophagy.

Several proteins, notably sestrins, control the complex process of autophagy.Sestrin2, a highly evolutionarily conserved protein, is thought to reduce insulin resistance through controlling lipid and glucose balance.

Beclin1, an evolutionarily conserved protein that plays a crucial part in the formation of autophagosomes, the double-membrane vesicles that contain degradation targets and merge with lysosomes, and is a widely acknowledged positive regulator of this process.

This study aimed to examine the association between Sestrin1 concentrations and Autophagy in PCOS and Find out the role of Sestrin2 in the pathogenesis of autophagy by assess the levels of Sestrin2 in PCOS patients' sera and compare them to those in the control group and observe the role of serum Beclin1 and its relationship with parameters of PCOS patients and evaluate the levels of LH, insulin resistance, and free testosterone were associated with, hirsutism, obesity, and hormonal alterations and to evaluate progesterone hormone in PCOS patient .

The study included 100 women with age ranged between (18 – 44) years involving 70 PCOS patients and 30 apparently healthy women as a control in reproductive age, at the reproductive fertility consultant of gynecological and obstetric teaching hospital, Kerbala health directorate Iraq and College of Applied Medical Sciences, University of Kerbala Iraq during the duration from October ., 2022 to April, 2023. The Rotterdam criterion-2003 was presumed to PCOS females. The biochemical parameters measured were serum Luteinizing hormone (LH), Follicular Stimulating Hormone (FSH), Estradiol hormone (E2), Progesterone Hormone, Free testosterone, Fasting Insulin Hormone, proteins of sestrin1 ,Sestrin 2, Beclin 1, Body mass index (BMI) These parameters were determined in the sera of both the PCOS patient and control groups. The result of our study revealed significant increase in LH levels ($p < 0.00$)   (M ±SD) (9.81±5.87) in women with PCOS compared with healthy women (4.7±1.66).

The results of this study showed decreased significant ($p < 0.00$) follicle stimulating hormone of patient PCOS (5.13± 1.29)   compared control group (6.42±1.99) and showed increased significant in Luteinizing hormone of patient PCOS compared control group. The results of our study showed increased significant free testosterone level ($p < 0.00$) of PCOS group (3.16±4.18) than control group (10.34±4.46) . The results of this research showed increased significant in insulin resistance($p < 0.00$) of PCOS patients (3.7±1.72) than controls group (2.12±0.40). while the study showed significantly reduced in Estradiol($p < 0.012$) of PCOS group (3.7±1.72) than control group (2.12±0.40). This research showed decreased significant progesterone of patient PCOS (3.16±4.18) than control group (10.34±4.46) . This research showed decreased significant Sestrin 1 of PCOS ($p < 0.00$)(4281.721±1296.25)   than control group (6238.511± 1416.13) .This research showed decreased significant Sestrin 2 of patient PCOS (147.4±31.1) than

control group(217.0±49.0). There is no significant change of beclin1 (p=0.89) of patient group (1310.28± 1018.08) than control group (1216.35 ±467.85) .

Finally, we conclude from this study that PCOS patients, elevated levels of LH, and Free testosterone were associated with, hirsutism, obesity, and hormonal alterations. PCOS patients have lower levels progesterone than healthy women. Our research shows that sestrin2, an autophagy marker, is dysregulated in PCOS and may serve as a new diagnostic for the condition.

Reduced serum Sestrin 2 levels may be has potential as a new PCOS diagnostic biomarker. Autophagy helps keep the human ovary healthy and works in the process of luteal decline. It also affects the ability of granulosa cells to live. And regulated indirect by Sestrins . According to the current findings, PCOS may have started as a result of Beclin 1 irregularities in the serum of PCOS patients .

Chapter One
Introduction

Chapter one

Introduction

1.1. Polycystic Ovary Syndrome

A prevalent reproductive endocrine condition called polycystic ovarian syndrome (PCOS) affects at least 10% of women of reproductive age. At least two of the three major characteristics of PCOS, including cystic ovaries, oligo- or anovulation, and hyperandrogenemia (high circulating androgen levels), are frequently present(Coutinho & Kauffman, 2019).Symptoms of (PCOS) involve menstrual cycles irregular, increase hair growth, infertility, and difficulties getting pregnant have all been related to PCOS (Louwers & Laven, 2020).In accordance with the European Society of Human Reproduction and Embryology/ American Society for Reproductive Medicine (ESHRE/ASRM-approved criteria, Rotterdam's criteria for diagnosing PCOS include the presence of the(Acne, hirsutism, and androgenetic alopecia are symptoms of these diseases, as are polycystic ovarian morphology (PCOM), which is identified by an ovary with at least 20 follicles and a diameter of 2 to 9 mm or a volume of at least 0.5 ml (Szeliga et al., 2022) .Sexual and reproductive health, as well as bone and heart health, and cancer risk, are all connected to the reproductive endocrine system, making menstruation an essential indicator of a woman's general health and quality of life(Li et al., 2020) .

The physiological process known as the menstrual cycle occurs when sex hormone fluctuations cause changes in the uterine lining and stimulate the development of eggs in the ovaries (Le et al., 2020). The endometrial cycle (which includes endometrial growth in order to acquire the implantation of the embryo, as well as maturation of the gland epithelium)occurs in conjunction with the ovarian cycle (Naftolin et al., 2019) .

A follicular stage of female's cycle of menstruation, estradiol is the major hormone, whereas the primary hormone during the luteal phase is progesterone. Menstruation marks the beginning of the follicular phase of the menstrual period, which continues until ovulation . It is the primary factor that determines the length of an individual's menstrual cycle, which can last anywhere 7 to 22 days, with 14 days on average (Itriyeva, 2022) .

In women with PCOS, anovulation is brought on by a variety of causes, such as problems with gonadotropin release, It is necessary to maintain appropriate conditions for follicular atresia, follicular development and differentiation, oocyte production, and the reproductive cycle, follicular cells must participate in autophagy (Kumariya et al., 2021).

The significance of autophagy in metabolic problems linked to PCOS is becoming apparent more frequently. For instance, autophagy is crucial for regulating inflammasome activity in response to metabolic stress(Pasha et al., 2017a).Several proteins, including sestrin2, control the complex process of autophagy(Saeedi et al., 2021) .

A protein called the sestrin molecule controls the effects of DNA damage, oxidative stress, and hypoxia. Insulin resistance has also been linked to the sestrin protein . Sestrin protein-induced autophagy regulates glucose metabolism and insulin sensitivity(Bestel et al., 2022).

Sestrins are a family of cytoplasmic proteins with a high degree of conservation involving sestrins (1, 2, 3) as well as a p53 gene product target. Sestrin 1 regulates cell growth and survival in particular when cells are subjected to a wide range of stresses, such as damage to DNA and cellular oxidative stress,Possible role

for Sestrin 1 as a regulator for a number illnesses, such as heart hypertrophy and retinal disorders . Sestrin 1's involvement in controlling PCOS (Xu et al., 2021) .

Since sestrin1was shown to be involved in mammalian target of rapamcin (mTOR) suppression, it is possible that p53 and Sestrin 1 regulate mTOR through the same methods ,mammalian target of rapamycin (mTOR) has been demonstrated to be an important inducer of protein synthesis, cell proliferation, and a negative regulator of autophagy (Xue et al., 2017) .

Beclin1 is yet another crucial autophagy regulator and is essential for the autophagic initiation process, which includes the development of the phagophore and the nucleation of the autophagic vesicle (Sahni et al., 2014).

ULK1/2 is one of the unc-51-like autophagy activating kinases the primary agents that activate the complex required for the production of autophagosomes (Holczer et al., 2020) .A Vps14/Beclin1/Atg14L complex uses their dephosphorylation to initiate the formation the precursory membrane component known as the phagophore that leads to the autophagic vesicle (Cordani et al., 2019) .

1.2 Aims of study

1. To evaluate the levels of LH, insulin resistance, and Free testosterone were associated with, hirsutism, obesity, and hormonal alterations. And evaluate progesterone hormone in PCOS patient .

2. Study the association between Sestrin1 concentrations and Autophagy in PCOS

3. Find out the role of Sestrin2 in the pathogenesis of autophagy by assess the levels of Sestrin2 in PCOS patients' sera and compare them to those in the control group.

4. Observe the role of serum Beclin1 and its relationship with parameters of PCOS patients.

Chapter Two
Literatures Review

Chapter two

Literatures Review

2.1. Polycystic Ovary Syndrome

PCOS is a long-term hormonal disease that impacts reproductive system 10 to 15% of women globally(Barrea et al., 2023). If at least two of the three criteria are met, a woman is confirmed with PCOS: elevated androgen activity, polycystic ovarian morphology, or oligo- or anovulation (Adone & Fulmali, 2023).

In order to preserve fertility, the ovary and the menstrual cycle must function normally, which is greatly influenced by hormones. A cyst can develop inside an ovary's sac if there is a persistent imbalance in a woman's hormone levels, which will affect how her ovaries operate. In contrast, androgen, a man hormone, is raised above normal in females who have PCOS(Ajmal et al., 2019).

In patients with polycystic ovarian syndrome (PCOS), levels of the hormones follicle-stimulating hormone (FSH), luteinizing hormone (LH), and gonadotropin-releasing hormone (GnRH) are consistently abnormal. which together cause ovulatory failure and increase androgen releases (Xu & Qiao, 2022).

Numerous metabolic issues, including hypertension, diabetes of type 2, lipid disorders, cardiovascular disease, and atherosclerosis, correlated with PCOS. PCOS, which affects 44–70% of adults, frequently manifests as resistant to insulin and hyperinsulinemia (Tefagh et al., 2022) .

2.1.1. Prevalence

The major factor contributing to infertility among Iraqi Arab females, according to the current study, is PCOS 46%(Saeed et al., 2021). Obesity, nutrition, and lifestyle changes all seem to be contributing to an increase in PCOS prevalence(Fattah et al., 2021) .Despite the fact that PCOS can develop at all ages

and begin with menstruation, People are most commonly affected by diseases when they are in their twenties and thirties, Worldwide, there are 1.55 million   PCOS-afflicted women of childbearing age(Singh et al., 2023) . In a December 2009 research done by Yildiz et al (2021) in a sample of 392 women ages 18-45 years of the same demographic. According to NIH standards, 6.1% to female can suffer from polycystic ovarian syndrome, so the occurrence of PCOS is rather common using society (AE-PCOS) criterion for Androgen Excess and PCOS was 15.3%, but the Rotterdam criteria showed a 19.9% prevalence of PCOS(Agard, 2023) .According to the researched population (in regards to race, age, and other characteristics) and applied standards. Between 4% and 26% of female may have PCOS(Kruszewska et al., 2022) .

2.1.2 . Diagnosis polycystic ovary syndrome

Three distinct collections of diagnostic standards have been offered for the diagnosis of PCOS in adults. Prior to making the definitive diagnosis of PCOS. It is necessary in all three sets to rule out any other endocrine conditions that may resemble it, including thyroid illness, non_classical congenital adrenal hyperplasia, and hyperprolactinemia(Mumusoglu & Yildiz, 2020) .

The first data were made available by the National Institutes of Health (NIH) for making diagnoses. These records list hyperandrogenism and monthly irregularities/ovulatory dysfunction as the two primary diagnosis criteria(Peña & Codner, 2022).although Polycystic ovary morphology (PCOM) was not considered to be a necessary function(Mumusoglu & Yildiz, 2020).

The second set of diagnostic criteria was put forth in Rotterdam in 2003. Three factors form the basis of the Rotterdam criteria. When two of them happen, PCOS may be   identified ,these are the three elements: three conditions: (1)oligo

menorrhea,(2)high levels of androgen (the male sex hormone), and (3) Polycystic ovary morphology (PCOM). An important technique for early PCOS prediction is the ultrasound imaging of the ovary ,this ovary image provides crucial details, including the number, size, and location of the follicles(Alamoudi et al., 2023) .

The Androgen Excess and PCOS Society (AE-PCOS) completed its most current round of reviews of diagnostic criteria for PCOS in 2009, PCOM, hyperandrogenism, and irregular periods are a few examples. A modified version that includes three criteria was created by combining the NIH and Rotterdam criteria. (1)Ovarian dysfunction includes conditions including PCOM, oligo-anovulation, and/or (2) hyperandrogenesis and (3) excluding out additional illnesses linked to androgen   excess(Fahs et al., 2023) .

2.1.3. Clinical Features of Polycystic Ovary Syndrome

PCOS presents a broad spectrum of negative health consequences for women and wellbeing due to the fact that it is a complex and diversified condition(Ismayilova & Yaya, 2022) .The Menstrual cycle irregularities and hyperandrogenic characteristics (hirsutism, acne, and alopecia) are among the signs and symptoms of PCOS, which are also accompanied by obesity, psychological disorders, and fertility challenges(Niinuma et al., 2023). PCOS women are more prone to be insulin resistant, central obesity, and dyslipidemia, which might raise their chances of developing type II diabetes and cardiovascular illness (Ismayilova & Yaya, 2022).
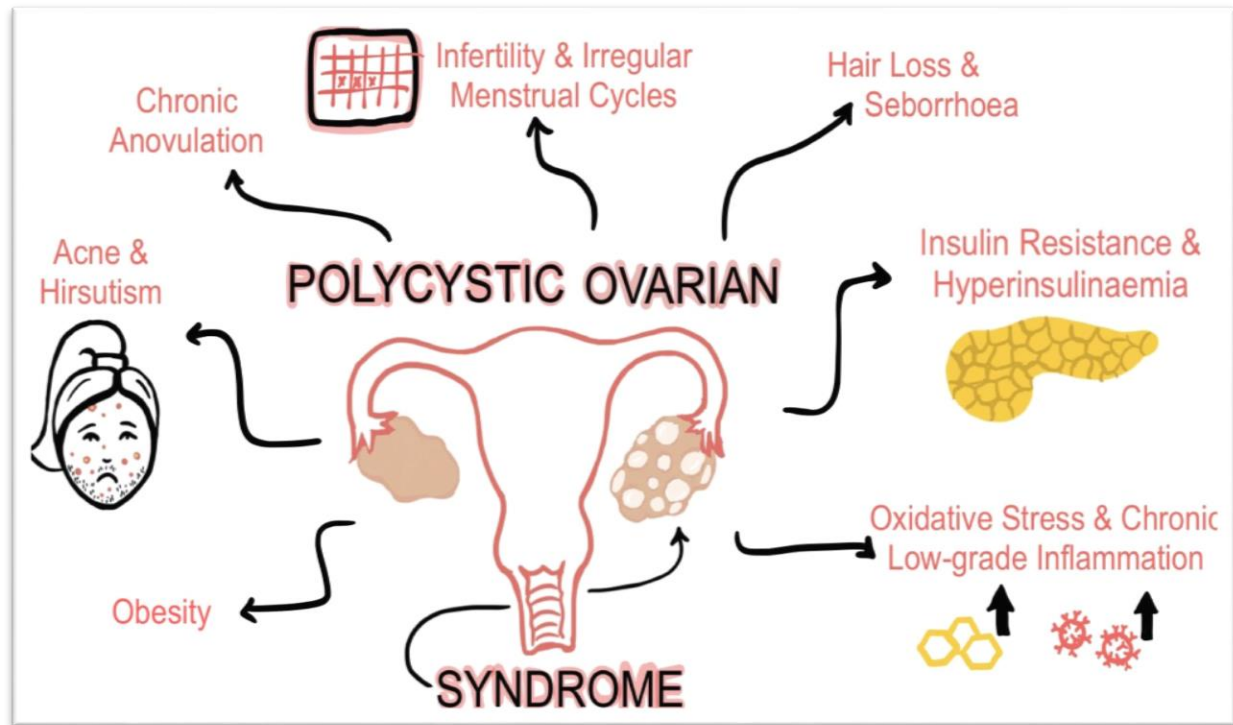
Figure 2.1: sign and symptom of polycystic ovary syndrome (Niinuma et al., 2023)

2.1.4. Pathophysiology of Polycystic Ovary Syndrome

PCOS pathogenesis is varied and complicated, which is the main reason why it is difficult to comprehend .The pathogenesis of PCOS has been linked to Ovulatory failure, elevated testosterone levels,and inadequate amounts of gonadotropin were produced, in addition to pulses of gonadotropin-releasing hormone (GnRH) that were unsuitable(McAllister et al., 2015) . In addition insulin resistance these variables interact and exacerbate one another ,PCOM is caused by ovulatory dysfunction, which is caused by the hypersecretion of androgens, which is linked to abnormal follicular growth(Harada, 2022) . The excessive secretion of gonadotropins, specifically an excess of LH, is a result of hyperandrogenism dysregulation of the pulsatile secretion of GnRH (Mccartney et al., 2022) . The

dysregulation of follicular development is made worse by high LH concentrations, which also lead to an imbalance in the LH/FSH ratio that causes thecal cells to secrete androgens in excess (Shabbir et al., 2023).

Obesity or overweight affects 60% to 70% of PCOS patients, and obesity is linked to the emergence of Diabetes and insulin resistance, Yet Several studies indicate that certain PCOS women exist who are not obese also have insulin resistance (Talib et al., 2022) .

Increased visceral as well as abdominal fat brought on by hyperandrogenism results in the emergence of insulin resistance. Possible compensatory hyperinsulinemia, which leads to an excess of androgens being secreted by ovaries and the adrenal glands(Aziz et al.,2018).

Anovulation and the appearance of infertility can be brought on by such a cycli pathogenetic between insulin resistance, hyperinsulinemia, hyperandrogenism, and hypothalamic- Abnormal GnRH pulsation pituitary dysfunction(Kh & Irgashev, 2022).   Increased carbohydrate consumption, the presence of Chronic low-level inflammation, hyperinsulinemia, and hyperandrogenemia which four major causes of PCOS pathophysiological alterations (figure2) (Singh et al., 2023).
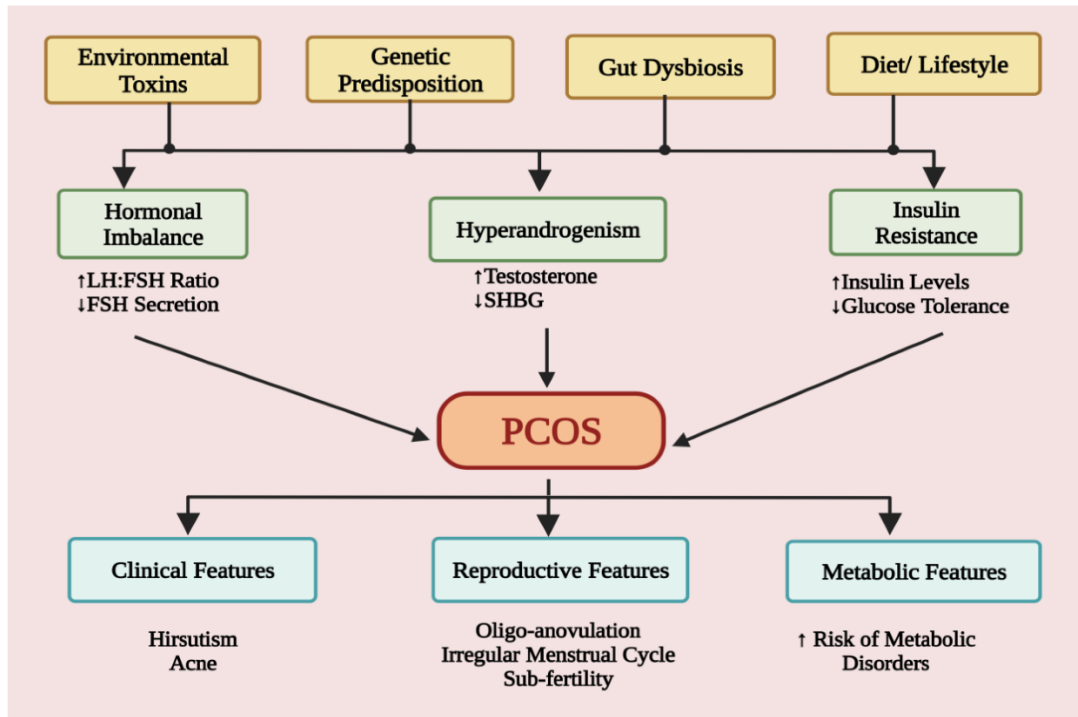
Figure 2.2 : The pathogenesis hypothesis and characteristics of PCOS are depicted in this schematic picture. Environmental contaminants, genetics, gut dysbiosis, and diet all take part in the cause of PCOS In addition to the increase the reproductive, metabolic, with clinical abnormalities in PCOS individuals (Singh et al., 2023)

2.1.5.Polycystic Ovary Syndrome and Obesity

The term "obesity" refers to a state characterized by an overabundance of fat which has negative health implications and makes an individual at risk for a number of disorders, comprised of a broad range of reproductive and endocrine abnormalities(Calcaterra et al., 2021).

Epidemiological studies, studies support the idea that PCOS and obesity are strongly related as they demonstrate that 38 %to 88 % of women with PCOS tend to be fat or overweight (Barber & Franks, 2021) .

Obesity exacerbates PCOS, particularly by developing insulin resistance (IR), Hyperinsulinemia, which is related to obesity, affects patients with PCOS lipid

profiles and intolerance to glucose. Inappropriate activation of the HPO axis has been associated to PCOS and obesity, hyperandrogenism results from obesity's increased androgen production, which activates LH (Singh et al., 2023) .

Compared to the overall female population, Overweight or obese individuals are more prone to have PCOS,In contrast, Women who are fat and overweight are more prone to develop PCOS as compared to the thin women (Orio et al., 2016) .

In addition to the possibility that PCOS itself contributes to obesity and rise in weight, excess fat appears to make the underlying hormonal imbalance worse. that is a bidirectional link between PCOS and excess adiposity(Bahri Khomami et al., 2022). The occurrence of overweight or obesity has been calculated to be 80% of PCOS females with greater body mass index (BMI) values (Yang et al., 2022) .

2.1.6. Polycystic Ovary Syndrome with Diabetes mellitus type2

Obesity may exacerbate a number of PCOS-related dysfunctions, such as anovulatory failure, hyperandrogenism, insulin resistance, and inflammation. This leads to adipogenesis to enhance and lipolysis to decrease when these dysfunctions intensify These processes affect follicular growth and could damage oocytes, and changes in how the ovary and obesity interact, particularly when lipids is high, make these procedures worse. Recent studies showed that most PCOS-afflicted women are impacted by IR and hyperinsulinemia(Layacha & Biswas, 2023).

PCOS-affected women often have problems with insulin sensitivity and beta-cell activity, Both of those conditions requirements for creating non-insulin-dependent type 2 diabetes, there may be some indication that type 2 diabetes (T2D) will develop in female with PCOS, it was discovered that a large proportion of PCOS patients had much increased proportions of IR than their BMI-matched healthy

counterparts, which is a crucial component in the illness' pathogenesis(Livadas et al., 2022).

Insulin resistance is a situation when, despite a rise in insulin concentrations, the hormone's action is insufficient to meet the metabolic needs of peripheral tissue(Livadas et al., 2022). Additionally, consuming foods derived from animals that are high in saturated fats is linked to decreased insulin sensitivity, which contributes to the malfunction and degeneration of pancreatic beta cells(Montaño &González, 2018).

2.1.7. Polycystic ovary syndrome and Pregnancy

An ectopic pregnancy is more likely to take place in female with PCOS(Bahri Khomami et al., 2022). Many risk indicators may assist in the elevated risk severe pregnancy difficulties in PCOS, given the heterogeneity of PCOS and confounding factors related with pregnancy complications. Obesity, IR, HA, and elevated oxidative stress may exacerbate PCOS severity and alter the frequency of problems during pregnancy and delivery(Bahri Khomami et al., 2019).

Pregnancy and birth issues, such as pre-eclampsia, hypertension during pregnancy, diabetes during pregnancy (GDM), induction of labor, cesarean sections, preterm birth, are generally more common in PCOS-affected women(Bahri Khomami et al., 2021). target audience, ethnic origin, and PCOS phenotype, personal or family records of reproductive, metabolic, and perhaps mental disorders either during or after pregnancy, as well as female lifestyle, all affect how PCOS is related to pregnancy and birth challenges (Bahri Khomami et al., 2021) . PCOS is linked to an elevated risk of negative perinatal outcomes even after correcting for these high-risk factors (fig. 3), although the impact of the various PCOS phenotypes on these negative outcomes is ambiguous(Valent & Barbour, 2021) .
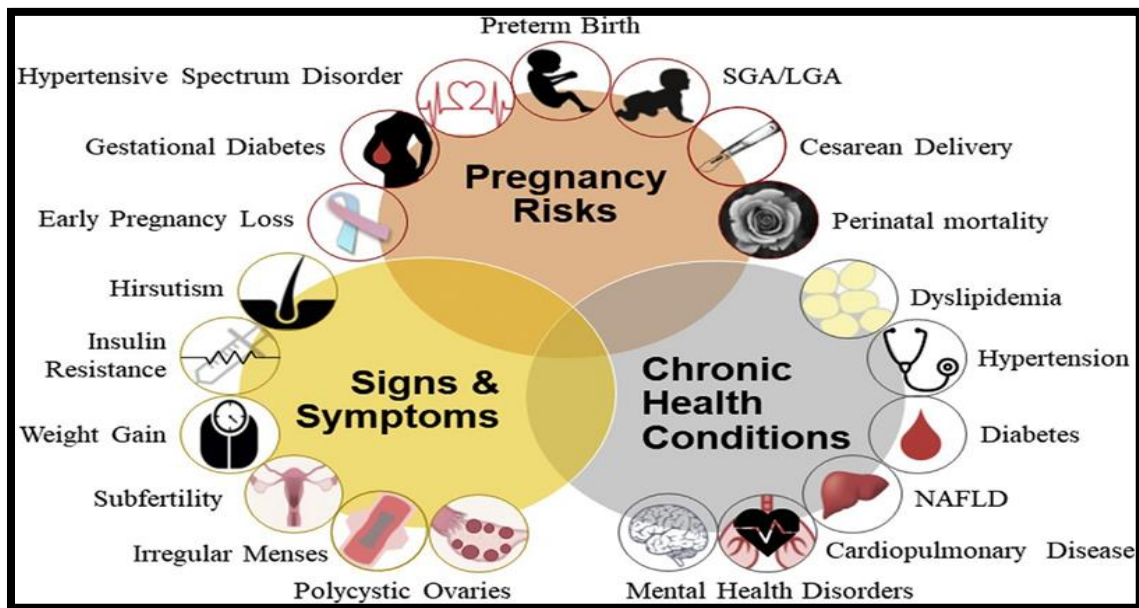
Figure 2.3: There are higher risks for prenatal problems and serious chronic health concerns in people with PCOS, a complicated illness. SGA/LGA stands for small-large-for-gestational-age; NAFLD is for non-alcoholic fatty liver (Valent & Barbour, 2021) .

2.1.8 . Hormonal disturbances in Polycystic Ovary Syndrome

2.1.8.1 Hypothalamus pituitary ovarian axis

The pituitary gland in the brain produces two hormones: follicle-stimulating hormone (FSH) and luteinizing hormone (LH).They essential for development of the gonadal follicles, when Hypothalamus-secreted gonadotropin-releasing hormone (GnRH) (Kitano et al., 2022).

The production of sexual steroids like progesterone, estrogen, and testosterone is controlled by these hormones after they bind to the FSH and LH receptors in reproductive gonadal cells (Kishi et al., 2018) .The ovary generates estrogens as part of the process of folliculogenesis, which modulates gonadotropin production via the hypothalamic-pituitary (H-P) axis (Lee et al., 2021).

Circulating estrogen causes an increase in LH mid-cycle ,Oocyte maturation and ovulation are induced when LH binds to the LH receptor, When the follicular phase is complete, estradiol levels peak. The increase in estradiol in the bloodstream causes LH surge in the pituitary(Arroyo et al., 2020).

During the luteal phase of a woman's menstrual cycle, a decrease in the pulse rate of gonadotropin-releasing hormone (GnRH) typically takes place. This decrease is caused by the hormone progesterone, which is responsible for this phenomenon. seems that is essential for proper persistent cycle functionality (Kim et al., 2022). By the mid-luteal phase after ovulation, LH pulse frequency has decreased to roughly every 4-6 hours, one pulse. This decrease is mostly due to progesterone's negative feed-back activities (Mccartney et al., 2022).

A large proportion of females and young people having PCOS that is hyperandrogenic show increased pulse frequency of LH and GnRH, Exogenous GnRH produced LH responses with increased amplitude and frequency. high levels of resistance to the effects of progesterone and estrogen negative feedback is also reflected by elevated LH pulse frequency in PCOS . Inadequate FSH and Excess LH of PCOS are significantly influenced by a continuously high GnRH pulse frequency(Kitano et al., 2022).

Due to an insufficient amount of FSH, which is needed to attract and drive the formation and maturation of ovarian follicles, this hormonal imbalance results in anovulatory cycles. Insufficient amounts of FSH can interfere with the process of selecting a dominant follicle, can result in an increase of pre-antral follicles in the ovaries that are between 2 and 9 millimeters in size. A minimum of 20 tiny, peripherally scattered follicles are present in ovaries with a volume greater than 10 cc, and also don't have the corpora luteal or the dominant follicle are indicative of polycystic ovarian morphology (PCOM) (Azziz, 2018). Moreover, elevated amounts

of LH will encourage release of androgen (hyperandrogenism), generated by theca cells in the ovary, is responsible for disorders including hirsutism, skin conditions such as acne and hair loss are present upon diagnosis in a high percentage patients who suffer from polycystic ovarian syndrome (Szeliga et al., 2022) .

2.1.8.2. Progesterone

connected to estrogens, a female sexual hormone, Progesterone is a steroid produced by both endocrine glands (adrenal and ovarian). During the menstrual cycle, progesterone and estrogen work together to control the accessory organs(Septadina, 2023). significantly contributes to maintaining pregnancy by regulating and stimulating ovulation. Progesterone has a crucial role in preparing the uterus for blastocyst implantation(Manocha et al., 2018). The dominant follicle's maturation marks the end of a woman's typical menstrual cycle's follicular phase. Through the anterior pituitary in reaction to an increase in the amount of estradiol secreted through the cells of the granulosa within the dominant follicle. Multiple events are spread by the LH surge(Mesen & Young, 2016) .

A rise in the production of estradiol-17 (E2) by the ovarian follicles causes a temporary shift in the hypothalamus and pituitary gland's response from negative to positive(Clay et al., 2021). Progesterone increases to reach its peak on day 21 of a 28-day cycle during the luteal phase, while estradiol decreases dramatically and then starts to rise again. In last stage of the luteal phase, just before menses, both hormone levels gradually decline(Le et al., 2020).

The ovarian steroid hormone synthesis is consequently altered by increased LH pulse frequency, increasing the production of androgens, but decreased FSH release impairs follicular growth. The syndrome's hyperandrogenaemia may also be

influenced by modifications to intrinsic ovarian steroidogenesis that favor androgen production(Moore, 2022).

2.1.8.3.Estrogen

The main female sex hormone, estrogen, is generated by the placenta and follicles in the ovaries and the corpus luteum. The three most common various estrogenic compounds and kinds are Estriol (E3), Estradiol (E2), and Estrone (E1, with estradiol being the most strong estrogen(Kelly et al., 2023) .The measurement of 17β estradiol, which is virtually entirely produced by the ovaries, is used to assess ovarian function .The hormones luteinizing (LH) and follicle stimulating (FSH)) come together to promote normal ovulation(Manocha et al., 2018).

The theca cells in the follicle of the ovary are stimulated by LH, which causes the creation of androgen. Some of them are connected to sex hormone-binding globulin (SHBG), as well as others, move to nearby granulosa cells (GCs), in which they are induced by FSH and converted into estrogen(Gargus et al., 2022). This causes an increase in estrogen hormone levels, which increases LH synthesis, resulting in an increase in LH and the beginning of ovulation. Following ovulation, the corpus luteum develops, secretes estrogen and progesterone, and subsequently increases the lining of the endometrial readiness for pregnancy(Xu et al., 2021).

In PCOS As a result of being stopped at the antral follicle stage, PCOS follicles do not release as much estrogen as dominant follicles do. aromatic enzyme expression and consequently Synthesis of E2 in big follicles antral are compromised in PCOS(Yang et al., 2021).

2.1.8.4. Luteinizing hormone and Follicle stimulating hormone

Glycoprotein hormones generated in the pituitary gland; members of the same family as, Human chorionic gonadotropin (HCG), Follicle stimulating hormone (FSH), Thyroid stimulating hormone (TSH) and, luteinizing hormone (LH), includes LH. Each one a molecule consists of two polypeptide subunits that are not chemically bound together(Gounden et al., 2021). Each of them have a beta subunit that is specific to a certain hormone as well as an identical alpha subunit. The human body has 121, 110, and 145 amino acids, LH, FSH, and HCG subunits, respectively, make up each of these molecules. A total of 92 amino acids make up the human common subunit (alpha subunit)(Arroyo et al., 2020) .

Through pulsating GnRH release, the hypothalamus triggers the ovulation process. This pulsatile release causes The anterior pituitary releases LH and FSH, which then encourage ovarian follicle. Theca cells, granulosa cells, and the oocyte make comprise this follicle's three primary cells. Androstenedione is produced by theca cells as a result of LH(Ruddenklau & Campbell, 2019). FSH stimulates the enzyme aromatase, which then transforms androstenedione to estradiol. As estrogen levels reach a crucial level, the normal negative feedback on LH that estrogen produces is turned off, and estrogen starts to have positive feedback on LH release, resulting in a "LH surge" that starts ovulation (El Sayed et al ., 2022).

PCOS women have been observed to possess considerably higher serum LH concentrations . FSH levels, however, have been noticeably reduced in PCOS-affected individuals. As a result, increased LH/FSH ratios are typically noted (Emanuel et al., 2022)**.** LH:FSH ratios increased in oligo/anovulatory patients with PCOS compared to ovulatory PCOS patient(Tosi et al., 2021) .Moreover, increased levels testosterone, both total as well as free are more prevalent in PCOS-affected women. Due to elevated LH levels' contribution to enhanced ovarian theca cell

androgen synthesis.Excessive androgen impairs sex-steroids' ability to provide negative feedback, allowing GnRH pulsatility to be unchecked and raising levels of LH and then androgen in a vicious cycle(Garg et al., 2022) .

2.1.8.5. Testosterone

The important female hormone known as testosterone. In addition to being a necessary precursor for the synthesis of estradiol, it also functions directly as an androgen(Alemany, 2022).

Examples of pre-androgens are androstenedione and dehydroepiandrosterone, often known as DHEA. These pre-androgens are created in the adrenal glands and the ovarian glands, are peripherally converted to testosterone in women's bodies, where it is also created(Davis & Jacobsen, 2015) .

When testosterone concentrations reach their peak in the middle of the menstrual cycle as well as remain elevated through the luteal stage, ovulation signals the start of the ovaries' cyclical synthesis of testosterone. The majority of circulating testosterone is protein-conjugated; more than 66 % of It is connected to a protein called sex hormone-binding globulin (SHBG). 30 % of that is joined with albumin(Labrie et al., 2017). And just 2-4% is testosterone that is unbound. This is the presumed active form. DHT and testosterone have substantial androgen receptor affinity and strong androgenic characteristics despite their extremely very low levels in plasma in women(Ali et al., 2023). If the free testosterone level is greater than two times the typical upper limit, hirsutism may occur. (Kanbour & Dobs, 2022).

2.1.8.6. Insulin resistance

Insulin, a hormone necessary for cellular growth, metabolic processes, and glucose regulation, is composed of 51 amino acids (Lewis & Brubaker, 2021). The

pancreatic beta cells secrete the peptide hormone insulin, one to two million pancreatic islets are found in the human pancreas (Wendt & Eliasson, 2020).

By both increasing Adipose tissue, skeletal muscle, liver, as well as heart are among the insulin-sensitive tissues that take up glucose and decreasing hepatic glucose synthesis(Armanini et al., 2022). Insulin serves as a regulator of glucose homeostasis. Insulin's effect on hepatic glucose production may be mediated through its ability to reduce lipolysis, resulting in lower amounts of free fatty acids(Lewis et al., 2021) .

Many chronic diseases have insulin resistance as the main cause which includes metabolic syndrome, and is characterized as a physiological state defined by insulin-targeting tissue resistance to high physiological insulin (Lee et al., 2022)Insulin resistance has been shown to affects up to 50–80% of women with PCOS (Sharif & Alwakeel, 2019)

Hyperinsulinemia reducing the liver's production of sex hormone binding globulin (SHBG), which raises the levels of free and bioactive androgens in the blood (Polak et al., 2017a). As well as potentiating the luteinizing hormone (LH)-dependent effect on ovarian cells which increases androgen synthesis, The progression of metabolic problems is further exacerbated in females by hyperandrogenemia (Kruszewska et al., 2022).

Hyperinsulinemia is brought on by compromised pancreatic metabolic processes that are a result of increased androgen production that is linked to islets of Langerhans dysfunction(Xu & Qiao, 2022).

2.1.8.7. Etiology Insulin resistance

Insulin resistance and obesity are connected to increased amounts of the hormone endogenous insulin (Henstridge et al., 2019).

IR is characterized as a deficit in insulin's capacity to mediate its metabolic effects. Because of the altered insulin action, more insulin must be produced and released from the pancreatic β cells in order to achieve the same metabolic effects. This is why compensatory hyperinsulinemia IR is common(Sanchez &Sempere, 2020).

In ovarian theca cells, insulin has the ability to work in concert with luteinizing hormone to operate as a co-gonadotrophin by increasing androgen synthesis. Additionally ,the hormone insulin regulates follicular growth, encouraging the stoppage of development of pre-antral follicles in hyperinsulinemia circumstances (Barber et al., 2016) and (Armanini et al., 2022).

Increased free testosterone is achieved by lowering the luteinizing hormone's pulse's amplitude, a rise in adrenal releasing androgen and a decline in hepatic sex hormone binding globulin synthesis, other insulin-related systemic effects have an impact on PCOS patients' clinical presentations(Herman et al., 2023).

PCOS which is worse by obesity and associated with T2D, in addition to reproductive, metabolic, and mental health problems(Hiam et al., 2019).

Most recent research came to the conclusion that overweight is the main cause of insulin resistance of people with PCOS. Previous studies have revealed that a sizable proportion of women with PCOS had diabetes type 2 and decreased glucose tolerance (Xu & Qiao, 2022).

Insulin resistance is thought to have an essential part in PCOS raises symptoms of hyperandrogenism and has clinical consequences(Bestel et al., 2022).

PCOS can cause irregularities in steroidogenesis and ovulation due to cells in the granulosa are resistant to insulin. It has been demonstrated that IR can be found in cells of granulosa as well as other peripheral cells of female who have (PCOS), which results from abnormalities in the insulin post-receptor signaling system,

certain irregularities in insulin receptor substrate 1/2 (IRS1/2). Protein kinase B is prevalent(commonly known as Akt) phosphorylation, among others(Zhang et al., 2020) .Insulin-sensitive cells produce more ATP under the influence of extra power, typical people who are overweight, regardless of the energy needed to function, which causes mitochondria to overheat. Through a variety of processes, including as AMPK suppression, ATP overproduction, which is a feedback regulator of energy excess, the stimulation of Mammalian target of rapamycin, and mitochondrial dysfunction may all contribute to IR(Ye, 2021).Additionally, beta cell hypersecretion of insulin and alpha cell secretion of glucagon are also potentially influenced by excessive ATP synthesis(Herman et al., 2023).

2.2. Sestrins

A protein known as the Sestrin molecule protects cells from DNA deterioration, oxidative stress, and hypoxia. This protein has two essential domains for its dual purpose of regulating the mammalian target of rapamycin complex1 (mTORC1) and decreasing reactive oxygen species (ROS)(Fay, 2022).Additionally, Sestrin proteins play crucial roles in redox function, effects of aging & metabolic diseases on glucose, lipid, & insulin metabolism (Ro et al., 2020).

Sestrin1, Sestrin2, and Sestrin3 are the three isoforms of this protein, Sestrins expression is minimal in healthy human cells, but it is increased and performs multiple activities in response to injury, stress, other diseases, or immunological response(Sun et al., 2020).

Sestrin were dubbed Sestrin after a human genetics course held in Sestri Levante, a tiny town on the Ligurian coast of Italy, where researchers identified the amino acid sequence homology between the three proteins (Cordani et al., 2019).

As Sestrin1 is controlled by the tumor-suppressor protein (p53), it gene 26 that p53 activates, or PA26. An etiological factor in damage to DNA and growth arrest (GADD)(Pasha et al., 2017b) **.**

In addition to DNA deterioration, endoplasmic reticulum stresses, and oxidative stress, malnutrition, and a diet heavy in fat, Sestrin2, often referred to represents the gene 95 for hypoxia, is increased when there is low oxygen levels in cells. Moreover, it has been discovered to be an essential leucine sensor for mTORC1 pathway(Chen et al., 2022).The tumor-suppressor protein p53 regulates sestrin1 and sestrin2, But FoxO transcription factors primarily activate sestrin3 (Huang et al., 2020) .

Sestrins are associated with conditions like osteoarthritis, epilepsy, neuropathic pain, sleep apnea, and obesity because they regulate cellular homeostasis(Lovisari et al., 2021) . According to study, PCOS patients' endothelium and mitochondrial oxidative   metabolism has been disturbed, which raises the risk of oxidative stress (Bestel et al., 2022). Oxidative stress may be a significant factor in PCOS that encourages

hyperandrogenemia by suppressing the expression of a variety of proteins (Sun et al., 2021).The Sestrin protein has also been linked to insulin resistance. Insulin sensitivity and glucose metabolism are preserved by sestrin-induced autophagy(Li et al., 2017) .

2.2.1. Sestrin1 and Polycystic Ovary Syndrome

Sestrin 1, a cytoplasmic protein belonging to a highly conserved family also known as PA26 gene relatives involved in growth arrest and DNA repair, Sestrin1,is responsible for responding to DNA damage. All human tissues contain Sestrin 1, but it's particularly abundant in the skeletal muscle, the heart, the liver, and the brain

(Chen et al., 2022) . Sestrin1 controls cellular survival and proliferation via various kinds of cell stress conditions, such as damage to DNA, oxidative stress, and others (Xue et al., 2017).

Due of sestrin 1's involvement in mTOR suppression, p53 and sestrin 1 may both regulate mTOR through the same methods . It has been determined that mTOR is a critical inducer of protein synthesis and cell proliferation as well as a depressor of autophagy (Xue et al., 2017) .By regulation complexes 1 and 2 of the mammalian target of rapamycin kinases, Sestrins regulate cellular homeostasis(Ding et al., 2019).

Sestrin deficiency was found to speed up a number of processes, including mTORC1 activation, redox dysfunction, aging, fat storage, insulin resistance, muscle deterioration, cardiac failure, mitochondrial diseases, and cancer(Kim et al., 2021). Sestrin 1's function in controlling PCOS, however, is still not well studied (Xu et al., 2021) .

2.2.2. Sestrin2 and Polycystic Ovary Syndrome

Antioxidant protein Sestrin 2 (SESN2) that is produced in response to a variety of pressures, such as oxidative and energetic strains, and it shields cells from harm in these circumstances. Numerous disorders of metabolism, including IR, mitochondrial malfunction, oxidative stress and weight gain, may result from the loss of endogenous sestrins (Nourbakhsh et al., 2017).

SESN2 regulates metabolism in response to stress, primarily through processes Involving AMPK, which stands for adenosine monophosphate-dependent protein kinase, and mTORC1, which stands for the mammalian target of rapamycin complex 1, are two proteins that work together(Chung et al., 2018) .

containing two structurally similar but functionally subdomains that are homologous but not identical (SESN-A and SESN-C), human SESN2 has a peculiar internal symmetry in its structure, A key function of while SESN-A is an alkylhydroperoxide reductase, and SESN-C acts as mTOR Complex 1 inhibitor, Sestrin can completely inhibit both ROS and mTORC1 through these two separate domains(Kim et al., 2015) .

More recently, it has been discovered that SESN2 is by oxidative stress . via activating the nuclear factor erythroid 2 (Nrf2) (Cordani et al., 2019) .sestrins may directly physically associate with AMPK or indirectly regulate transcription to activate it. In addition to their effects mediated by AMPK, Rag GTPases are required for mTORC1 action, and they can be inhibited by sestrins . Sestrins inhibit mTORC1 by these coordinated effects, which limit protein production across the UPR, protecting cells from damage caused by ER stress(Pasha et al., 2017b) .

Multiple critical cellular processes, including as proliferation, metabolism, differentiation, and autophagy, are under the control of mTORC1, It is sensitive to different stimuli in the environment, like growth hormones and nutrition . Recent research has demonstrated that mTOR-regulated mechanisms are critical for steroidogenesis, ovarian somatic cell proliferation, oocyte meiotic maturation, and folliculogenesis (Guo & Yu, 2019) .The control of gonadotropin synthesis, puberty onset, with a reduction in the hormone LH secretion in female rats following rapamycin treatment have all been linked to mTOR signaling .We have previously shown that the new mitotic survival checkpoint mTOR controls follicle development in vivo (Yaba & Demir, 2012) . Insufficiency of SESN2 accelerated the process of development severity of Obesity-related hepatosteatosis is characterized by insulin resistance, same as diabetes (Chen et al., 2022). PCOS-related metabolic abnormalities are another potential relationship between the condition and

mTORC1 signaling. Sestrin2 deficiency may result in dysregulated mTORC1 signaling, which may then cause a variety of reproductive and metabolic diseases connected to PCOS(Saeedi et al., 2021) .

The regulator of autophagy with the most known name is mTORC1. Removal of damaged cellular components requires a process called autophagy, which is activated by inhibition of mTORC1(Song et al., 2018).

2.3. Beclin 1 and Polycystic Ovary Syndrome

Beclin1, a regulator protein from members of the B-cell lymphoma 2 family, that react with either phosphoinositide 3-kinase (PI3K) or B-cell lymphoma 2 to control cell autophagy and death(Lu et al., 2023) .

With three main functional domains, the amino acid sequence of human Beclin1 is 450. The coiled-coil domain (aa175-264), the Bcl-2-homology-3 domain (aa105-130), and the evolutionarily conserved domain (aa248-450) are its three conserved structural domains(Xu & Qin, 2020) .

In order to interact with other Bcl -2 proteins, It has a BH3 domain, which is shared with Bcl -2 proteins, a coiled-coil (CC) domain, which react with each of the CC domains of UV radiation resistance associated gene(UVRAG) or autophagy related gene (ATG14) and an autophagy-specific (BARA)domain that binds to membranes(Chang et al., 2019). Evolutionarily Conserved Domain (ECD), which comprises a portion of the area shared by the BARA domain and the C-terminus of the CC domain, is frequently confused with domain of BARA due to the substantial overlap between these sections Additionally, residues 141–171 within the BH3 and CC domains, also known as the Flexible Helix Domain, were discovered to adopt helical conformations(Tran et al., 2021) .

Beclin 1 interacts with a number of other proteins to carry out its membrane trafficking and autophagy processes, these interactions are most frequently seen with vacuolar protein sorting(VPS15, VPS34, UVRAG, and ATG14, which is a component of autophagy. Complex 1 (C1) and Complex 2 (C2), two separate Class III PI3K complexes, are created when they are combined. For these two complexes, Beclin1 acts as a scaffolding protein(Nishimura & Tooze, 2020). Beclin-1 expression has been documented, generally in the cytoplasm and organelles of mammalian cells like the Endoplasmic reticulum, trans-Golgi network, and mitochondria Additionally, Beclin-1 has been linked to the pathophysiology of Nervous system diseases (including Alzheimer's illness and Parkinson's illness), viral infections (Sahni et al., 2014) .

2.4. Autophagy

Through the process of autophagy, cells recycle nutrients from damaged organelles and proteins to preserve cellular homeostasis, often referred to as macroautophagy, a lysosomal breakdown catabolic process that has been conserved over evolutionary time. Autophagy, for instance, is physiologically distinct from apoptosis and necrosis in terms of programmed cell death . A former is a proactive process of cell killing that is carried out through blebbing of the plasma membrane without changes to the organelles found within the cytoplasm, whereas the latter involves a ruptured plasma membrane ,the loss of intracellular content, and ultimately death of cells (Zhou et al., 2019) . The three unique type of autophagy chaperone-mediated autophagy, microautophagy, and macroautophagy (Hubert et al., 2022).

The macroautophagy process is the one that has garnered the most interest among the many autophagy processes. as well as is broken down into two successive stages: The first stage representation of the formation structure consisting of two

membranes that is known as the phagophore, that's engulfs cargo and develops into ultimately reaching the form an enclosed vesicle with two membranes that is known as an autophagosome, the next step is lysosomes and autophagosomes joining together to create autolysosomes, which are in charge of digesting and recycling contained(Peters et al., 2019) .

Disorders of metabolism for instance, insulin resistance, obesity, and diabetes type 2,and atherosclerosis that are linked to problems in autophagy homeostasis(Zhang et al., 2018).The ovary needs adequate, effective autophagy for the follicular development, the origin of eggs, and their degradation(Zhang et al., 2020) .

2.4.1. Autophagy in Polycystic Ovary Syndrome

Under normal conditions, autophagy is required for follicular cells to maintain follicular atresia, oocyte improvements, follicular growth, and the cycle of reproduction(Zhou et al., 2019) .Ovarian follicular cells undergo Insufficient autophagy results in poor oocyte quality, which cause female infertility(Li et al., 2019). From oocyte differentiation through placental physiology maintenance after birth, recently, the significance of autophagy in female reproduction at every stage has been demonstrated in studies ,Autophagy is crucial for the survival of developing oocytes and germ cells, According to an animal study, the absence of important autophagic molecules, including Beclin1 and Autophagy related gene (ATG7), increases the likelihood of oocyte maturation defects. And deformed germ cells(Cao et al., 2017).

The process of macroautophagy has also been identified as an important stimulator of oocyte and follicle maturation. Therefore, it has been demonstrated that the mTOR (mammalian target of rapamycin) inhibitor rapamycin, promotes

primordial follicle development and oocyte survival when administered to murine ovaries. This effect is due to an increase in the upregulation of LC3B and Beclin 1 (BECN1), two autophagy markers(Sun et al., 2018) . Mammalian target of rapamycin complex1 (mTORC1) in the ovary as well as Mammalian target of rapamycin complex2 (mTORC2) Expression in Mouse was found to be increased in a PCOS model induced by DHEA .This disordered ovarian physiology originates from the ovary's MTOR signaling pathway With PCOS, which interferes with the ovary's natural method of choosing a dominant follicle and induces abnormal proliferation of follicles (Kumariya et al., 2021) .

ULK1/2, also known as Activating kinase for autophagy (like unc-51), one of the major inducers of the complex necessary to create autophagosomes, and it is regulated by both mTORC1 and AMPK(Holczer et al., 2020). Studies have shown that metabolic abnormalities linked to PCOS are significantly impacted by autophagy. In obesity, autophagy is critical for controlling systemic insulin sensitivity and is linked to adipose dysfunction (Kang et al., 2016) .Previous research indicates that as hyperandrogenemia (HA) is a defining hallmark of PCOS, modulation of autophagy in response to androgen may be important in PCOS pathogenesis(Kumariya et al., 2021).

2.4.2. Autophagy and sestrins 1,2

Sestrin1 and Sestrin2 are two of the p53 target genes that have recently been discovered to inhibit mTOR Via indirect processes that require activating AMPK(Marniche, 2023). SESN2's protective functions may be explained by at least two different chemical pathways. If there are too many reactive oxygen species, SESN2's may initially operate as an antioxidant. In addition, the p53-produced SESN2 may cause AMPK to be   activated, which then inhibits mTOR and eventually induces autophagy in response to stress(Kim et al., 2015) .

Protein kinase mTORC1 recognizes the availability of nutrients and regulates cellular metabolism , It is an evolutionarily conserved enzyme. The persistent stimulation of mTORC1 that can result from a surplus of nutrients promotes the synthesis of proteins and lipids while limiting the catabolism of autophagic cells(Lee et al., 2012).Numerous disorders, comprising ovarian cancer (OC) and polycystic ovary syndrome (PCOS),are thought to be influenced by the mTOR pathway becoming overactive (Liu et al., 2016). And diabetes, obesity, cardiovascular conditions,cancer, and autoimmune disorders are also associated with persistent mTOR activation(Pasha et al., 2017b) .

Loss of Sestrin 2 causes a variety of age- and obesity-related diseases, including fat buildup and heart dysfunction, all of which are triggered by mTORC1 activation (Kim et al., 2021) . Multiple signaling pathways have been identified that collaborated with regard to autophagy control, and AMPK, a key metabolic sensor, is crucial in controlling energy homeostasis and metabolic stress(Li et al., 2017). AMPK directly interacts with phosphorylates ULK1 to promote autophagy, according to several studies, however mTORC1 affects this connection(Dossou & Basu, 2019) . ULK1/2 is one of the unc-51-like autophagy activating kinases the primary agents that activate the complex required for the production of autophagosomes (Holczer et al., 2020) . A Vps14/Beclin1/Atg14L complex uses their dephosphorylation to initiate the formation the precursory membrane component known as the phagophore that leads to the autophagic vesicle (Cordani et al., 2019).

2.4.3. Autophagy and Beclin1

The start of the autophagy process with the development of the autophagosome, afterwards, the autophagosomal membrane initiates nucleation, elongation, sealing, and closure (Chen et al., 2021) . Beclin 1 performs a crucial

Impact on the beginning stages of autophagy, which involves the phagophore's creation and the nucleation of the autophagic vesicle (Kaur & Changotra, 2020) .

After stress, Bcl 2 releases Beclin1, which promotes autophagy ,thereafter Class III phosphatidylinositol 3-kinase and Beclin1 link, The Beclin1-PIK3C3 complex is formed when PIK3C3/Vps34 is in conjunction with the beneficial cofactors UVRAG in addition to ATG14/Barkor and the cofactors that are negative, which include Rubicon .The development of autophagosomes as well as the transport of autophagic proteins to the place where phagophores are assembled rely on the presence of this complex(Liu et al., 2020) .

Increased autophagic initiation is the outcome of UVRAG's interaction with Beclin-1, which in turn increases PI3KC3/Vps34 kinase activity. UVRAG is also involved in the activation of PI3KC3/Vps34 through its interaction with Bif and Beclin-1 figure (2.4) (Sahni et al., 2014) .All types of Beclin 1 can combine with PI3K to form complexes, while around Fifty percent of PI3K is unbound, it cannot form a complex with Beclin1 (Xu & Qin, 2020) . Autophagy dysfunction is factors that are well-known to have a role in PCOS's etiology .An earlier investigation revealed that the Patients with PCOS their granulosa cells had lower concentrations of DNA damage regulated autophagy modulator 2, a protein involved in autophagy (Dai et al., 2012) .

It has been demonstrated that encouraging autophagy may reduce insulin resistance in nonalcoholic steatohepatitis revealing a direct link between autophagy regulation and resistance insulin Additionally, When PCOS develops pathologically, mechanistic target of rapamycin kinase (mTOR), a critical autophagy regulating protein, can be overexpressed (Peng et al., 2020) .
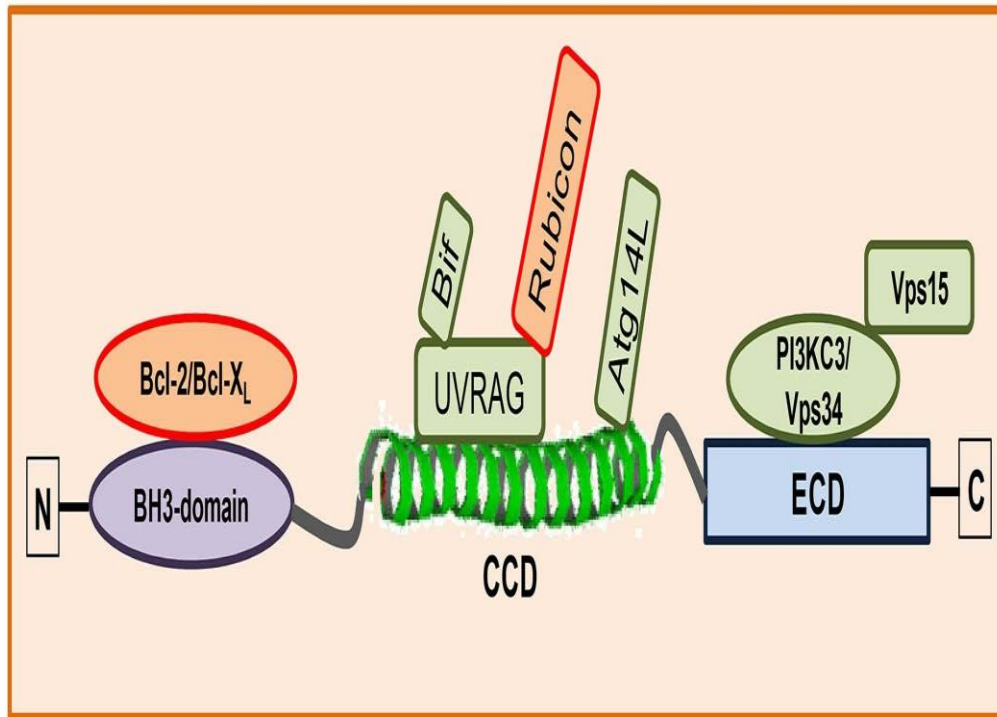
Figure 2.4: shows how distinct protein domains interact with partners for Beclin-1. Beclin-1 has three domains: the ECD, CCD, and BH3 domains are three evolutionarily conserved domains. Protein-binding partners are involved in either trigger the start of autophagy (UV radiation resistance-associated gene (UVRAG), atg14L, and vps34/vps15, and Bif) or Stop it (Rubicon, Bcl-2, and Bcl) are two competing hypotheses. UVRAG stands for UV radiation resistance-associated gene (Sahni et al., 2014).

Chapter Three
Materials and Methods

Chapter Three
Materials and Methods
3.1. Subject
3.1.1. Study Design

A case control study with 100 women was performs from October 2022 to April 2023. The University of Kerbala's College of Applied Medical Sciences and Karbala Health Directorate gave their permission for the study's ethical conduct. The administration of the gynecological and obstetric teaching hospital and every patient granted their agreement after being informed of the study's objectives, as shown in figure (2.1).
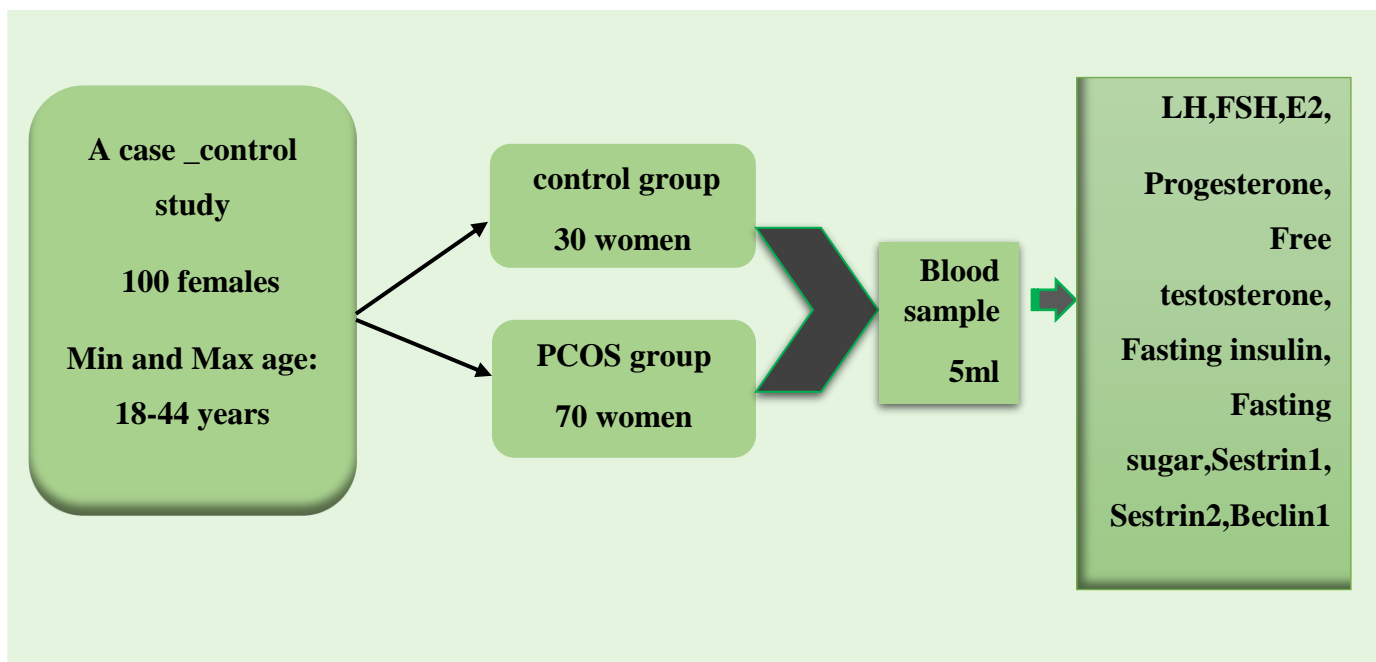
Figure (3.1) Study Design

3.1.2. Patients

Samples of whole blood from 70 PCOS women Age of childbearing (between the ages of 18 and 44) The information was collected in reproductive infertility specialist of the Kerbala health directorate of Iraq's teaching gynecology and obstetrics hospital. There was extensive questioning on the subject, as well as laboratory tests. In order to learn more about the patients, a questionnaire was designed, such as age, weight, height circumference, efficacy fertility , hirsutism ,menstrual regularity, and. Body mass index (BMI) and other physiological measurements were taken in these investigations.

3.1.3. Control

Thirty Women who appeared to be in healthy condition and whose ages ranged from (18 to 44 years). They have a normal menstrual cycle & have healthy ovaries, according to gynecologists' observations, weight, age, height measurements, hirsutism, together with regular menstrual cycles and fertility were all questioned for the control group. Not smokers, those without a history of renal, liver, cancer, stroke, autoimmune illness, any acute or chronic disease, diabetes, type 1 or type 2 or any sort of cerebrovascular accident.

Inclusion criteria

As a control group, 30 reproductive-age women from the age of 18 until the age of 44 which appeared healthy with had regular cycles were chosen. Each of them had a history of consistent menstrual cycles of 26 to 32 days, symptoms of hyperandrogenism, such as hirsutism, and thyroid dysfunction are absent. 70 PCOS-positive women were chosen as patients (all of whom had recently received Rotterdam criteria-based PCOS diagnoses from gynecologists).Every participant in the study gave their consent. And each participant got a thorough medical

examination to determine their body mass index (BMI), as well as their history of various diseases, gynecological conditions, and infertility ,as well features including coarse body and facial hair, acne, irregular menstruation, and other disorder .

Exclusion criteria

None of the participants in this study was smokers. Patient with congenital adrenal hyperplasia ,hyperprolactinemia, Cushing's syndrome, diabetes mellitus ,thyroid disorders, hypertension, medication for preventing pregnancy, or the absence of polycystic ovary syndrome were also excluded from the trial, as were those with cancer.

3.1.4. Collection Blood Samples

For blood collection, Needles and syringes 5ml were disposable .In day two of thecycle, vein punctures were used to collect blood samples from PCOS patients and control groups. at room temperature the samples of blood were allowed to coagulate for10–15 minutes. After dividing the blood into four equal pieces, we centrifuged it at 2000 x g for 10 to 15 minutes to separate the serum. luteinizing hormone (LH),Free testosterone(FT), follicle stimulating hormone (FSH),fasting blood insulin as well as sugars levels that all assessed promptly by validated assays of respective serum hormones. The rest were stored in a deep freezer at a temperature of -80 degrees ℃. until levels of Sestrin 1 and Sestrin 2 and Beclin 1 are examined by (ELISA) kits .

3.2. Kits and Chemicals

Table **(3.1)** provides a summary of the study's kit usage.

Table (3.1): Lists the kits and the compounds used in this study

NO	Kits and Chemicals	Company and Country
1	Estradiol kit	Roche\Germany
2	Follicular Stimulating Hormone reagent kit	Roche \Germany
3	Human Beclin 1 kit	Pars Biochem\ China
4	Human Sestrin 1 kit	Pars Biochem\ China
5	Human Sestrin 2 kit	Pars Biochem\ China
6	Insulin Kit	Cobas e 411\ Germany
7	Luteinizing Hormone reagent kit	Roche\Germany
8	Maglum Free Testosterone (CLIA) Kit	Nanshan,Shenzhen\CHINA
9	Progesterone III	Roche\Germany

3.3. Instruments and lab tools

Table (3.2) provides a summarized of the equipment and lab supplies utilized in this study.

Table (3.2) Lists the devices and equipment that used in this research

NO.	Devices &Tools	Companies	Origins
1	Centrifuge	Kokusan	Japan
2	Deep freeze	ALS	Italy
3	EL x 800 Auto Reader	BIOTEK	U.S.A
4	ELx50 Auto strip washer	EASY MED	China
5	Eppendorf tubes	Carl ROTH	Switzerland

6	Micropipettes	Micropipettes	Germany
7	Refrigerator	LG	South Korea
8	spectrophotometer	Apel	Japan
9	Syringe, 10 ml, single-use	BIOTEK	U.S.A
10	Syringe, 5 ml, single-use	ULTRA HEALTH	China
11	Tourniquet	Voltaren	China
12	Vortex	Scientific Industries	Korea

3.4. The Methods

3.4.1. The body's mass index calculation

(The BMI, or body mass index), This calculated by applying the equations was used to categorize obesity(Keys et al., 1972).

Weight (kg) / Height (m^2) equals BMI (Kg/m^2).

Table 3-3: Weight status was categorized using their body mass index

Weight Condition	(BMI)(kg/m²) WHO
Under weight	Lower than 18.50
Normal	18.50 to 24.90
Overweight	25 to 29.90
Obese	30 or higher

3.4.2. The measurement of Follicle Stimulating, Luteinizing , as well as Progesterone hormone

The Cobas e 411 (Roche Diagnostic, Germany) chemiluminescent automatic immunoassay system (ECL) was used to test luteinizing hormone (LH) as well as follicle stimulating hormone (FSH).

3.4.2.1. The measurement Luteinizing Hormone Level

A . Two specialized monoclonal antibodies detect human LH are used in a sandwich formation in the Elecsys LH level test.

B_The reagents:

The LH label can be found on the reagent rackpack.

Table (3.4) : Component of Luteinizing Hormone kit

Reagents	Contents	Concentration
M	Microparticles coated with streptavidin (clear cap), 0.72 mg/mL, 1 container (6.5 mL), preservation	6.5 mL.
R1	Biotinylated mouse monoclonal anti-LH antibody, 8.0-pH TRIS buffer, and a preservative make up Gray-capped vial of R1 Anti-LH-Ab-Biotin.	10 mL
R2	Anti-LH-AbRu(bpy) in a black-capped bottle, ruthenium complex with Monoclonal anti-mouse LH antibody,0.3 mg/ml; pH 8.0,TRIS buffer 50 mmol/L,; preservatives agent	10 mL

3.4.2.2 The measurement Follicular Stimulating Hormone Level

A_principle of Test

Two specialized monoclonal antibodies detect human FSH are used in a sandwich formation in the Elecsys FSH level test .

B_ working solution and Reagent

There is an FSH label on the rackpack of chemicals.

Table (3.5) : Component of Follicular Stimulating Hormone kit

Reagents	Contents	Concentration
M	Microparticles coated with streptavidin (clear cap), 0.72 mg/mL, 1 container (6.5 mL), preserving agent	6.5 mL.
R1	One bottle of biotinylated monoclonal anti-FSH antibody	10 mL

	(mouse); one vial of anti-FSH-Abbiotin (gray cap);buffer, pH 6.0,50 mmol/L,; preservatives;	
R 2	monoclonal anti-FSH antibody from mice with ruthenium compound 0.8 mg/L anti-FSH-Ab-Ru(bpy) in a black-capped bottle ,pH 6.0 of MES buffer 50 mmol/L; preservatives	10 mL

3.4.2.3. Determination Progesterone Hormone Level

❖ **Testing principles**

The Elecsys Progesterone level assay uses sandwich-principle technology with two monoclonal antibodies against human Progesterone.

working solutions_ Reagents.

Table (3.6) Show component of Progesterone Hormone kit

Reagents	Contents	Concentration
M.	Microparticles with streptavidin coating (clear top): Microparticles coated with streptavidin; preserving agent concentration of 0.72 mg/mL.	6.5 mL
R1.	1 bottle of Biotin, an anti-progesterone medication (gray cap): Anti-progesterone antibody, sheep recombinant, biotinylated monoclonal pH 7.0, phosphate buffer 25 mmol/L, concentration 30 ng/mL, preserving agent.	10 mL
R2	Progesterone-peptide, 1 bottleProgesterone (of vegetable origin) coupled to a synthetic peptide containing ruthenium complex at 2 ng/mL; phosphate buffer 25 mmol/L, pH 7.0; preserving agent.	9 mL

3.4.2.4 .Determination of free testosterone Levels

A. Test principle

Label FITC with a pure TEST antigen and ABEI with a monoclonal antibody against the TEST. Following thorough mixing and incubation at 37°C to form antibody-antigen complexes, calibrator or control are labeled with ABEI, the sample FITC ,the displacing reagent and magnetic microbeads coated with anti-FITC. Following The supernatant is decanted after magnetic field-induced sedimentation, after which the

sample undergoes one cycle washing. A flash chemiluminescent reaction is then started when the beginning reagents have been applied. A photomultiplier measures the light signal as Relative Light Units within 3 seconds, and Relative Light Units the value is proportionate to the concentration of f-TEST in the specimens.

B. The ingredients

Table (3.7) Show ingredients of free Testosterone kit

Integral of the Reagents Used in 100 tests	
Nano magnetic microbeads coated with a polyclonal antibody against FITC from sheep (1.2% (W/V) TRIS, 0.2% (NaN3) NaN3).	2.5 ml
Bovine serum with 0.2 percent sodium nitrite is used as a low-precision calibrator.	2.5 ml
Bovine serum with 0.2% sodium nitrite is used as a high-precision calibrator.	2.5 ml
Bovine serum albumin, 0.2% sodium nitrite, FITC-labeled TEST antigen, and 0.2% sodium nitrite	6.5ml
Bovine serum albumin, 0.2% sodium nitrite, anti-TEST monoclonal antibody with an ABEI label.	6.5ml
All kinds of reagents are supplied and ready to be used.	

3.4.2.5. Determination of serum Estradiol (E2) levels

The hormone Estradiol measured by ELISA. In order to bind to the finite number of 17-estradiol-specific antibodies coated on the microplate (solid phase) of the Estradiol ELISA Kit, There is a competition between the antigen of 17-estradiol in the sample and the 17-estradiol antigen coupled to (HRP).

After incubation, unbound and bound fractions are separated by simple solid-phase washing. The enzyme HRP, a bound-fraction, reacts with the substrate 35 (H_2O_2) and TMB Substrate when the stop solution (H_2SO_4) is added to create a blue color that eventually becomes yellow. The concentration of 17- estradiol in the sample has an adverse relationship with the color's intensity.

A concentration the 17-estradiol in a sample is determined using a calibration curve.

3.4.2.6. Human Sestrin-1（SESN1）Level Determination

Principle of test

The quantity of Human SESN1 in the sample is determined, and solid-phase antibody is produced by coating microtiter plate wells with Purified Human SESN1 antibody, introduces SESN1 into well,as well as Human SESN1 antibody is conjugated to horseradish peroxidase (HRP) to create an antigen-antibody-enzyme complex. Finally, After being washed thoroughly, the substrate will turn blue when TMB substrate solution is introduced. To inhibit the HRP enzyme-catalyzed reaction, sulphuric acid solution is add , furthermore, the shift in color is detected using spectrophotometry 450 nm in wavelength. By comparing the specimens O.D. to standardized curve, the quantity of SESN1 found in the samples is then determined.

❖ **The components of the kit**

Table（3.8) The components of the kit that are included Sestrin 1

Contents of the Kit	Determinations 48	Determinations 96	The storage
cover for the closing plate	**(2)**	**(2)**	
Micro well plate	**(1)**	**(1)**	2-8 ℃
Standard	(0.5ml×1 bottle)	(0.5ml×1 bottle)	2-8 ℃
4500 ng/l of Standard diluent	(1.5ml×1 bottle)	(1.5ml×1 bottle)	2-8 ℃
Reagent conjugated to (HRP)	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
A Dilute Sample	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
Solution A for chromogens	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
solution B for chromogens	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
Solution to Stop	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
washing solution	(20ml×20 fold) ×1bottle	(20ml×30 fold) ×1bottle	2-8 ℃

Calculate

Draw a graph using the optical density (OD) value on the vertical and the standard density (SD) on the horizontal to create the standard curve. Sample density may be calculated by entering the observed OD value into the standard curve's straight line regression equation, and then multiplying the result by the factor of dilution, To determine the density of the samples from the OD value.

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Figure 3.2:standard curve for sestrin 1

3.4.2.7. Human Sestrin-2（SESN2）Level Determination

Principle ❖

SESN2 concentration is determined by this assay kit., coats the wells of microtiter plate with Purified Human SESN2 antibody to create solid-phase antibody, adds SESN2 to existing well, as well as combines HRP labeling with Human SESN2 antibody to result.an antigen-antibody-enzyme-antibody complex. Following a thorough wash.When TMB substrate solution is added, the substrate takes on a bluish color Reactions catalyzed by the HRP enzyme are inhibited when a stop solution is introduced., also The alteration in color where is detected using spectrophotometry 450 nm in wavelength. By comparing the specimens O.D. to standardized curve, The quantity of SESN2 found in the samples is then determined.

❖ **The components of the kit**

Table（3.9) The components of the kit that are included Sestrin 2

Contents of the Kit	Determinations 48	Determinations 96	The storage
cover for the closing plate	**(2)**	**(2)**	
Micro well plate	**(1)**	**(1)**	2-8 ℃
Standard	(0.5ml×1 bottle)	(0.5ml×1 bottle)	2-8 ℃
4500 ng/l of Standard diluent	(1.5ml×1 bottle)	(1.5ml×1 bottle)	2-8 ℃
Reagent conjugated to (HRP)	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
A Dilute Sample	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
Solution A for chromogens	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
solution B for chromogens	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
Solution to Stop	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
washing solution	(20ml×20 fold）×1bottle	（20ml×30 fold） ×1bottle	2-8 ℃

Calculate

Draw a graph using the optical density (OD) value on the vertical and the standard density (SD) on the horizontal to create the standard curve. Sample density may be calculated by entering the observed OD value into the standard curve's straight line regression equation, and then multiplying the result by the factor of dilution, To determine the density of the samples from the OD value.

Figure 3.3 :standard curve for sestrin 2

3.4.2.8. Human Beclin 1 Level Determination

Test principle

The quantity of Human Beclin 1 in the sample is determined, and solid-phase antibody is produced by coating microtiter plate wells with Purified Human beclin1 antibody, introduces beclin 1 into well,as well as Human beclin1 antibody is conjugated to horseradish peroxidase (HRP) to create an antigen-antibody-enzyme complex. Finally, after being washed thoroughly, the substrate will turn blue when TMB substrate solution is introduced. To inhibit the HRP enzyme-catalyzed reaction, sulphuric acid solution is add, furthermore, the shift in color is detected using spectrophotometry 450 nm in wavelength. By comparing the specimens O.D. to standardized curve, The quantity of SESN1 found in the samples is then determined**.**

❖ **The components of the kit**

Table（3.10) The components of the kit that are included Beclin 2

Contents of the Kit	Determinations 48	Determinations 96	The storage
cover for the closing plate	**(2)**	**(2)**	
Micro well plate	**(1)**	**(1)**	2-8 ℃
Standard	(0.5ml×1 bottle)	(0.5ml×1 bottle)	2-8 ℃
4500 ng/l of Standard diluent	(1.5ml×1 bottle)	(1.5ml×1 bottle)	2-8 ℃
Reagent conjugated to (HRP)	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
A Dilute Sample	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
Solution A for chromogens	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
solution B for chromogens	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
Solution to Stop	(3ml×1 bottle)	(6ml×1 bottle)	2-8 ℃
washing solution	（20ml×20 fold） ×1bottle	（20ml×30 fold） ×1bottle	2-8 ℃

Calculate

Draw a graph using the optical density (OD) value on the vertical and the standard density (SD) on the horizontal to create the standard curve. Sample density may be calculated by entering the observed OD value into the standard curve's straight line regression equation, and then multiplying the result by the factor of dilution, To determine the density of the samples from the OD value.
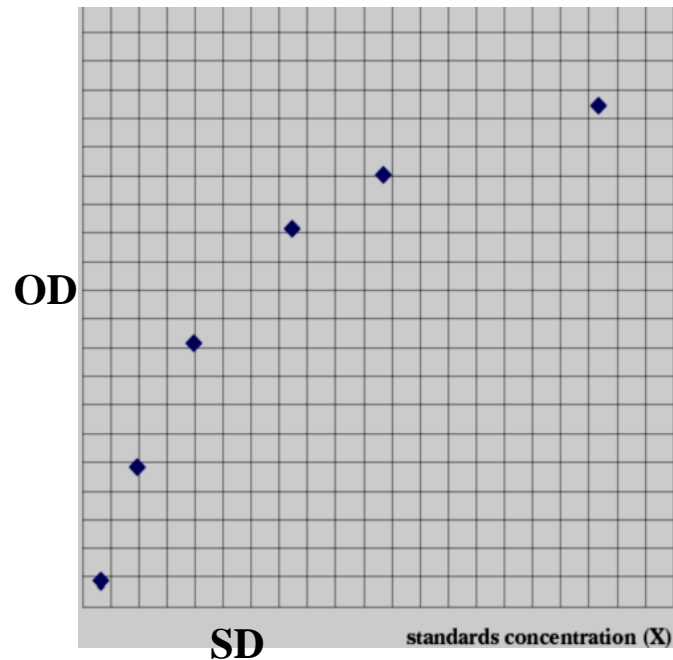
Figure 3.4 :standard curve for Beclin2

3.4.2.9. Determination of Insulin levels

Reagents - working solutions

Table （3.11）: The components of the Insulin hormone kit

Reagents	Contents	Concentration
M	Streptavidin-coated microparticles 0.72 mg/mL ,Streptavidin-coated microparticles (transparent cap), 1 bottle,; preservative agent	6.5 mL.
R1	(MESb) buffer 50 mmol/L, pH 6.0 Anti-insulin-Abbiotin (gray cap),biotinylated monoclonal anti-insulin antibody (mouse), 1 mg/L	10mL
R2	Anti-insulin-Ab-Ru(bpy) (black cap), monoclonal anti-insulin antibody from a mouse that has been tagged with ruthenium complex at a concentration of 1.75 mg/L; MES buffer with a pH of 6.0 and a preservative.	10 mL

Test principle

Cobas e 411 fully automated the process**.**

The duration of the examination will be 18 minutes in duration.

 Insulin from a sample of 20µl, During the first phase of the incubation process, a sandwich complex is created when two monoclonal insulin-specific antibodies one that has been biotinylated and the other that has been ruthenium complex-labeled combine.

Streptavidin-coated micro particles are added during the second incubation phase, biotin and streptavidin interact in such a way that the complex then forms bonds with the solid.

On the electrode, the micro particles are magnetically captured after being dragged into the measuring cell by the reaction mixture. To get rid of the chemicals that aren't bound, the ProCell/Porcell M system is employed following that, an electrode is given a voltage to start a chemiluminescent reaction. A photomultiplier detects this radiation. The results of an analysis are determined with the help of a master curve provided by either the reagent barcode or the e barcode and an instrument-specific calibration curve generated using a two-point calibration.

3.4.2.10 . Determination of Fasting blood glucose levels

Principle of test

The enzyme glucose oxidase (GOD) oxidizes glucose, creating D-gluconate and hydrogen peroxide in the process.. Phenol and 4-aminoantipyrine (4-AA) are oxidized by hydrogen peroxide to create a red quinoneimine dye in the presence of peroxidase (POD). The concentration of glucose in the sample is directly correlated to the dye's brightness.

O2+ H2O + β-D-Glucose → Phenol + H2O2 4-AA + D-Gluconate → H2OReagent composition + Quinone mine

Reagent Composition of Blood Glucose kit

Necessary Components –

Colorimeter or Photometer with the ability to detect absorbance at 500 nm 20 nm; stable temperature incubator with the temperature set to 37 degrees Celsius; pipettes for measuring reagent and sample amounts

Table （3.12) :The components of the blood glucose kit

Tube	Sample	Blank	Cal.
CAL. Standard	-	-	$10\,\mu l$
Sample	$10\,\mu l$	-	-
R1 Mono reagent	1 ml	1 ml	1 ml

3.4.2.11Homeostatic model assessment for insulin resistance(HOMA-IR)

Fasting blood glucose and insulin concentration are used in the following equation to determine insulin resistance(Polak et al., 2017).

HOMA-IR =insulin mU/ml × glucose (mmol/l) \ 22.5

Or HOMA-IR = Insulin (µU / ml) × Glucose (mg / dl) \405

Estimated Value when a subject is fasting. The HOMA-IR standards indicate "HOMA-IR lower than 2.60 consider the normal range and HOMA-IR between 2.60 to 3.80 consider borderline high," but that doing so would not define these individuals as having insulin resistance.

Insulin resistance is strongly correlated with a HOMA-IR score of 3.80 (Qu et al., 2011)**.**

By dividing by 18, the fasting blood sugar was converted to mmol/L(SALGADO et al., 2010).

3.5- Statistic Analysis

Case-control study best describes this investigation. ANOVA table with least significant difference for data measurement were used in statistical assessment using SPSS (Statistical Package for the Social Sciences which is version twenty-four)The information is shown as a mean accompanied by a standard deviation (SD).Statistical significance was assumed to exist at the (p0.05) level. The P value (i.e., the least significant difference) was obtained for the comparison between the groups ((*Armitage*, 2008)

Chapter Four
Results and Discussion

Chapter four

4. Results and Discussion

4.1. Clinical study

4.1.1. A comparison of patients and controls with regard to their BMI and age.

The correlation between age and BMI and PCOS was estimated by analyzing the sera of 70 PCOS female and 30 healthy female employed as a control group. According to our results in table (4.1)and figure (4.1A) and figure (4.1B).There was statistically non-significant changes in age mean values between the control group (27.50±6.93) and the patients groups of PCOS (26.17±5.91) ,while there are significant differences in the BMI of the PCOS group (29.24±5.28)than in the control group (25.53±2.78) .

Table (4.1A):The average and standard deviation of age and body mass index for women with and without PCOS .

	Sample	Mean	Std. Deviation	P Value
Age (Years)	Control	27.50	6.93	0.331
	Patients	26.17	5.91	
BMI Kg/ m2	Control	25.53	2.78	0.00
	Patients	29.24	5.28	

The data represented as mean ± SD

Table (4.1B):Show distribution of polycystic PCOS according to age groups

Parameters	Age distribution			P value
	(18_25) year N=12 Mean±SD	(25.5_30) year N= 30 Mean±SD	More than 30 year N= 28 Mean±SD	
LH	9.48±4.72	10.42±7.6	9.3±4.01	0.75
FSH	4.77±1.18	5.40±1.21	4.99±1.40	0.28
Free testosterone	2.32±1.31	2.63±1.02	2.27±1.29	0.73
Insulin resistance	3.29±1.33	3.33±1.27	4.44±2.10	0.3
Progesterone	4.22±5.34	2.48 ±3.30	3.43±4.48	0.44

The data represented as mean ± SD

The age of PCOS was divided into three groups in the current study. The first age group is 18-25 years, n=12/70. The second age group, 25.5-30 years, n=30/70 , and this group of PCOS patients was most predominant. The third age group is more than 30 years, n=28/70 .

According to results in table (4.1 B) There was statistically non-significant changes in age mean values between the normal control group and the patients groups of PCOS and this result agreed with Joshi et al., (2017) study, In which PCOS patients from Nepal had a mean age of 24. In the Bazarganipour et al. (2014) study, 300 PCOS patients were included, with a mean (SD) age of 26.5 years. There was no statistically significant difference between the patients and control groups(Hussein & Alalaf, 2013).
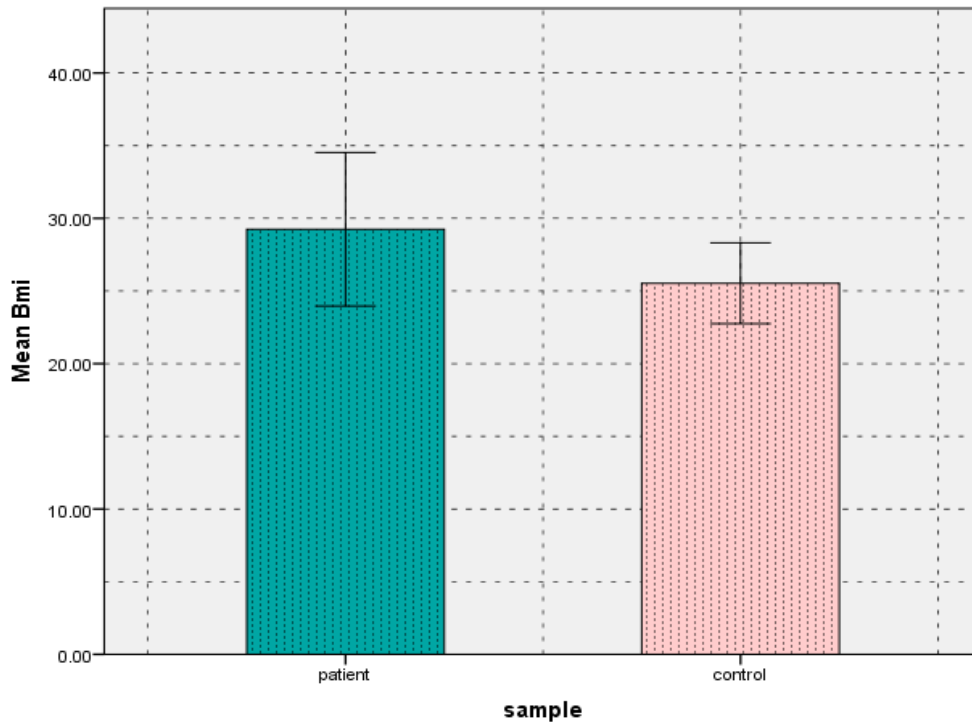
Figure (4.1A) : Mean of level BMI in PCOS group and control group
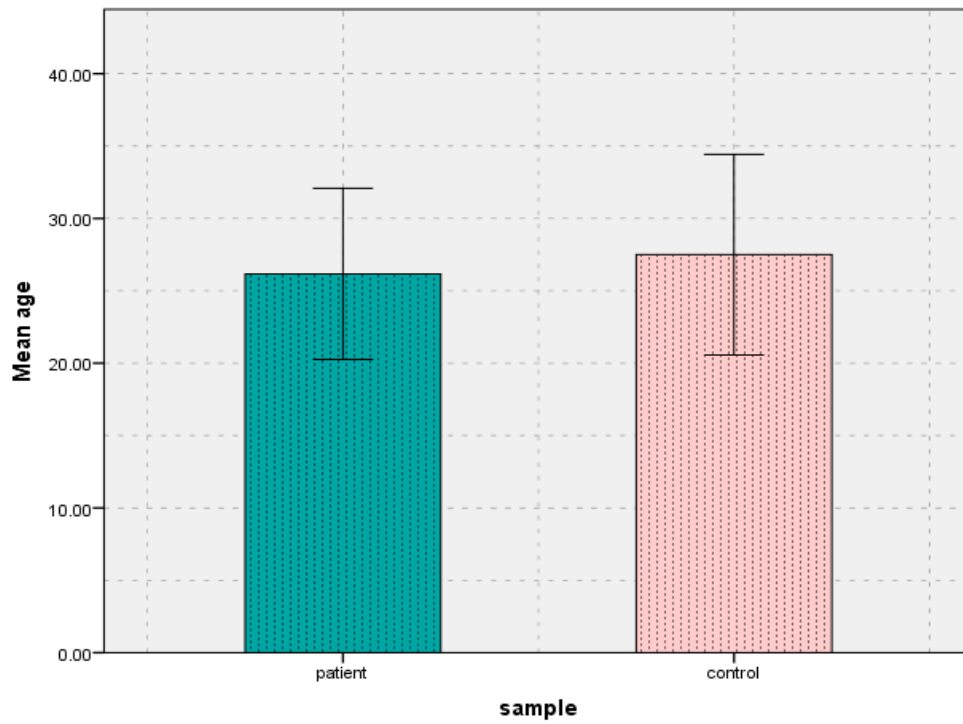
Figure (4.1B) : Mean of level age in PCOS group and control group

Obesity considerably enhances the prevalence and severity of insulin resistance, even though PCOS is linked to it independently of obesity As a result, PCOS also raises the possibility of developing Diabetes type 2(Barber & Franks, 2021).

Since the body cannot properly use insulin, approximately 70 % of women who have PCOS have insulin resistance. It should be noted that obesity is the primary cause of insulin resistance, and that both of these conditions increase

the risk of developing diabetes(Ali, 2021) .

When insulin is stimulated and used incorrectly, it prolongs hyperglycemic situations and puts more stress on the beta cells of the pancreas. It similarly raises the risk of diabetes mellitus II development in PCOS patients. Additionally, because non-oxidative glucose metabolism is inhibited by metabolic inflexibility, it raises the risk of weight gain and obesity(Mann, 2021) .

4.1.2. The biochemical Parameters of Patients and Control Group

4.1.2.1. Determination of LH and FSH in study groups

The result showed in table (4.2) and figure (4.2A) ,(4.2B)a significant increase in levels of LH (9.81±5.87) in women with PCOS compared with healthy women (4.7±1.66),whereas significantly lower level of FSH in PCOS women (6.70±6.70) and healthy women(6.421±1.98).

Table (4.2): showed the LH and FSH concentration of both the control groups and the PCOS patients

Parameters	Control group N=30 Mean	SD	Patient group N=70 Mean	SD	P value
LH (m.IU/mL)	4.8	1.66	9.82	5.87	0.000
FSH (m.IU/mL)	6.421	1.98	5.13	1.29	0.000

The data represented as mean ± SD

The pituitary gland is normally stimulated to release gonadotrophins, such as LH and FSH, by the hypothalamus's pulsatile release of the gonadotropin-releasing hormone. Luteinizing hormone stimulates the generation of androgens by acting predominantly on ovarian theca cells that have LH receptors(Ashraf et al., 2019) . FSH actions on the ovarian granulosa cells and alters the androgens produced in the theca cells into estrogens, primarily estradiol, which is essential to the formation of follicles(Liu et al., 2021) .

Patients with PCOS demonstrate an LH levels are higher, which encourages ovarian theca cells to secrete androgens(Szeliga et al., 2022) .

An imbalance in gonadotropin-releasing hormone (GnRH) secretion causes a greater proportion LH to follicle-stimulating hormone (FSH) . The aberrant feedback process that led to an increase in LH release was triggered on by ovarian estrogen (Saadia, 2020) Meiotic maturation to the oocyst a critical step in progression of the Oocyte , the LH surge during this procedure causes oocytes to resume meiosis and complete the first meiotic division by releasing them from meiotic prophase arrest

(Arroyo et al., 2020).In a normal ovarian cycle, the dominant follicle is the only one that responds to the peak surge of LH. Early terminal differentiation was seen in tiny follicles that are antral from a set of female with PCOS-related anovulation in response to increased LH levels (Szeliga et al., 2022) .

Ovarian theca cells expand (hyperplasia) as a consequence of elevated amounts of the luteinizing hormone (LH). As a result of this process, a string of pearl-like cystic structures called follicles forms along the border of the ovary. The theca cells in the ovaries are responsible for this condition, which occurs naturally(Ashraf et al., 2019). This condition is characterized by abnormally active theca follicles, especially through pre antral as well as an Antral stages of a woman's menstrual cycle. Because of an increase in the number of follicles as well as the release of essential enzymes involved in the process of androgen production, an oversupply of androgens results(Singh et al., 2023).

The levels of FSH could be increased, decrease lower than their typical levels, or stay stable in patients of condition (PCOS)(Mohammed & Qasim, 2021).An imbalance along the hypothalamic-pituitary-ovarian (HPO) pathway is thought to be able to arise, according to the beliefs of several experts because of neuroendocrine systeminstability(Walters et al., 2018). leading to gonadotropin overproduction. As GnRH   levels rise, LH production is favored over FSH production, causing the proportion of LH to FSH. to rise dramatically(Bergo et al., 2023). Patients with polycystic ovarian disease do not ovulate because of a high LH/FSH ratio (Saadia, 2020).The ratio of LH, to FSH. ranges from 1 to 2 in women who are normally healthy. The ratio is reversed of women suffering from PCOS and in certain instances, the number can even approach 2 or 3(Abdelghany et al., 2023).
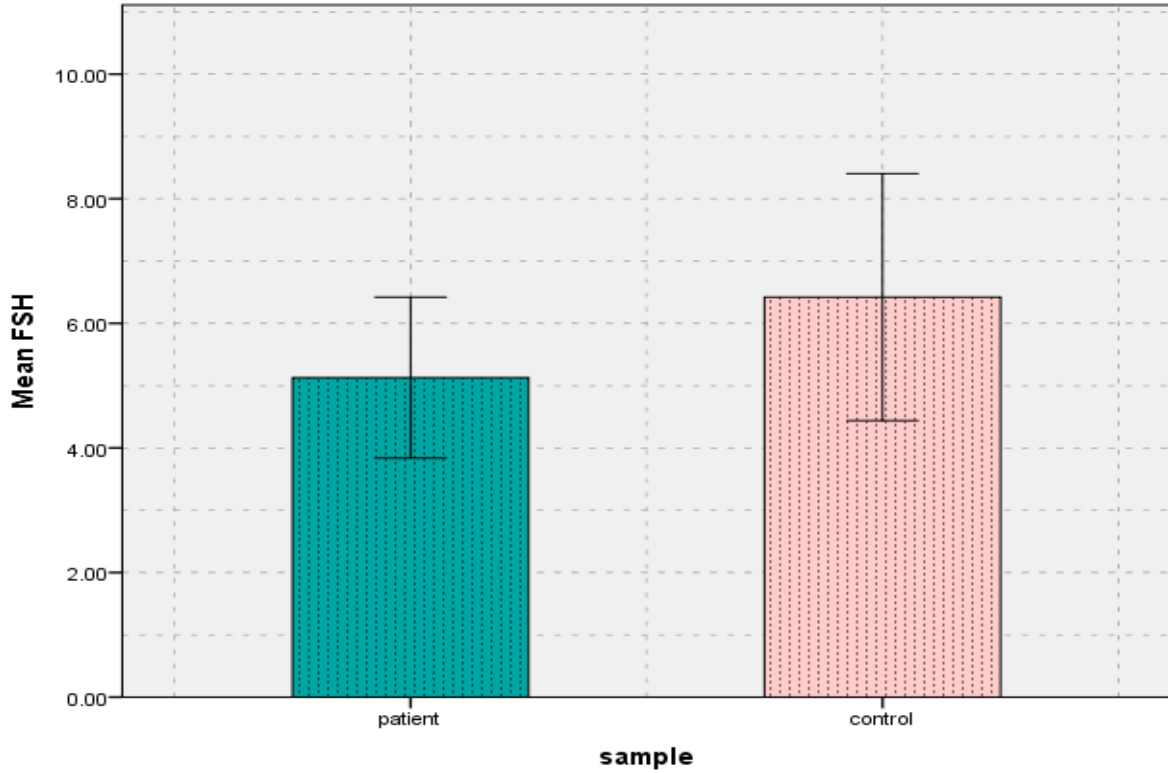
Figure (4.2A) : Mean of level FSH in control group and PCOS group
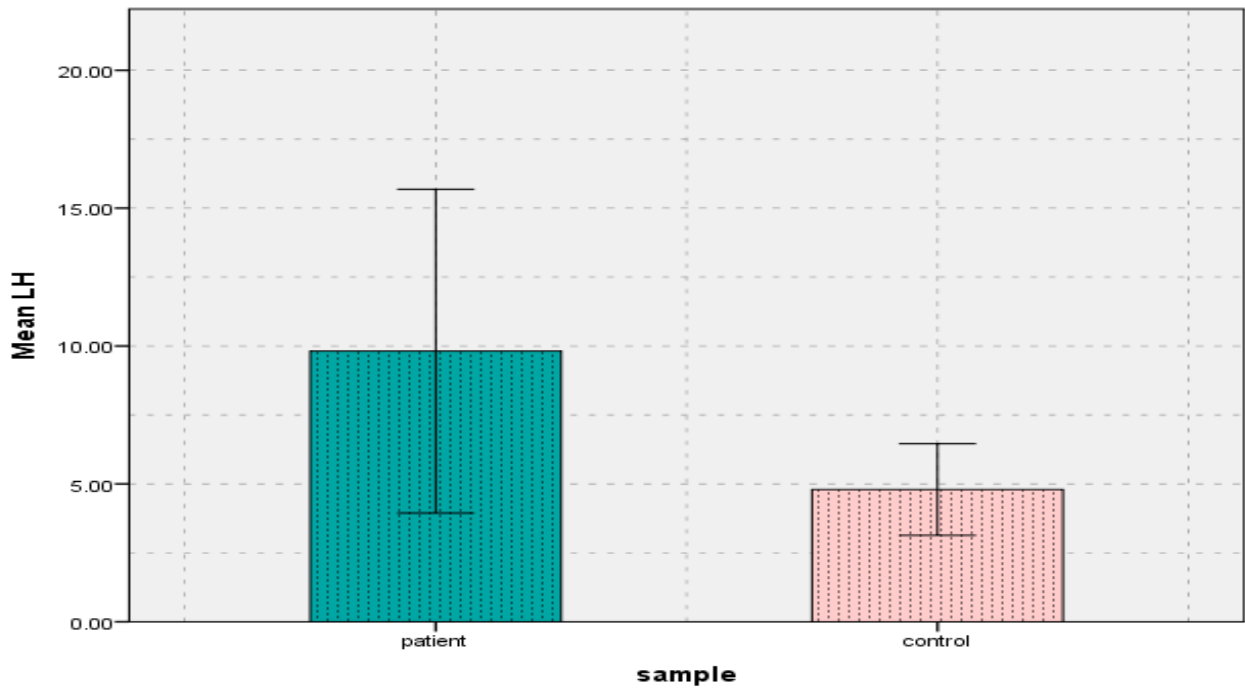
Figure (4.2B) : Mean of level LH in control group and PCOS group

4.1.2. 2. Progesterone and Free testosterone in PCOS Patients and Healthy Control Groups

The presented data showed in the table(4.3) and the significantly decreased in progesterone levels of patients group of PCOS(3.16 ± 4.18) in contrast to control group (10.34 ± 4.46) as in figure(4.3),while there was increase significantly in the levels of free testosterone of PCOS group(3.16 ± 4.18) than control group (10.34 ± 4.46) as showed in figure (4.4) .

Table (4.3):Progesterone and Free testosterone in control and PCOS groups

Parameters	Control group N=30 Mean	SD	Patient group N=70 Mean	SD	P value
Progesterone	10.34	4.46	3.16	4.18	0.00
Free testosterone (pg/mL)	1.52	0.72	2.55	1.17	0.00

The data represented as mean ± SD

Recurrent, cyclical lowering of LH (GnRH) pulsatility is brought on by cyclic (luteal) rises in progesterone in ovulatory women(Billhaq & Lee, 2023). PCOS is characterized by the presence of persistent oligoovulation or anovulation cycles, women who have PCOS almost never experience the post-ovulatory surge in progesterone which is typical of women who have regular menstrual(Solorzano et al., 2010). In fact, the steadily increasing GnRH pulse rate, which is a hallmark of PCOS in adult women may be caused by decreased levels of progesterone that are related with anovulation. In line with previous results, elevated LH levels also suppressed progesterone levels in our investigation(Malini & George, 2017) .

Women with PCOS frequently present with an irregular menstrual cycle and anovulation, which leads to reduced or absent P4 levels production, as shown by the thicker endometrium when comparing PCOS patients to non-PCOS controls (Li et al., 2014).

Progesterone has been shown to inhibit gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) are both released in a pulsatile manner(Eftekhar, 2019) .

Unlike the normal endometrium, the endometrium from PCOS patients receives consistent and prolonged estrogen stimulation, but little to no P4 activation (Li et al., 2014).

After ovulation, the corpus luteum develops and progesterone is produced with the help of luteinizing hormone (LH), Ovarian functions can also be controlled by progesterone, and have an impact on target organs down the line, including placenta, oviduct, and uterine(Kolatorova et al., 2022). PCOS female produce low progesterone levels that prevent the development of the corpus luteum, which interferes with ovulation(Zheng et al., 2018) .

 after ovulation, as well as in the early stages of pregnancy until placental function is established, ovarian granulosa cells production of progesterone is crucial for maintaining pregnancy(Qi et al., 2020) .
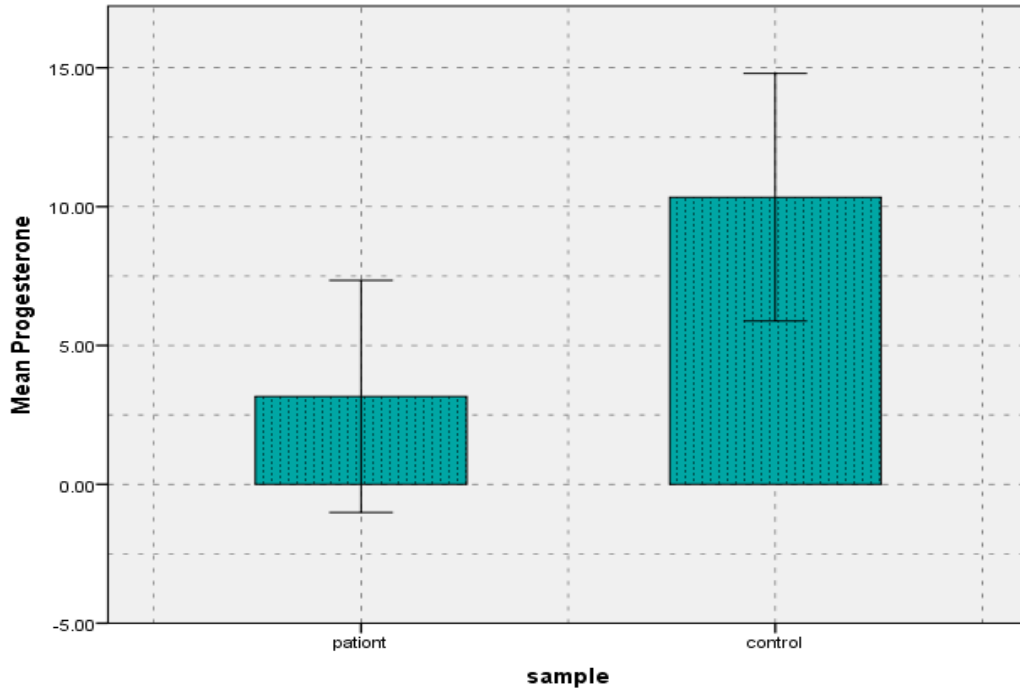
Figure (4.3) : Mean of level progesterone control group and PCOS group
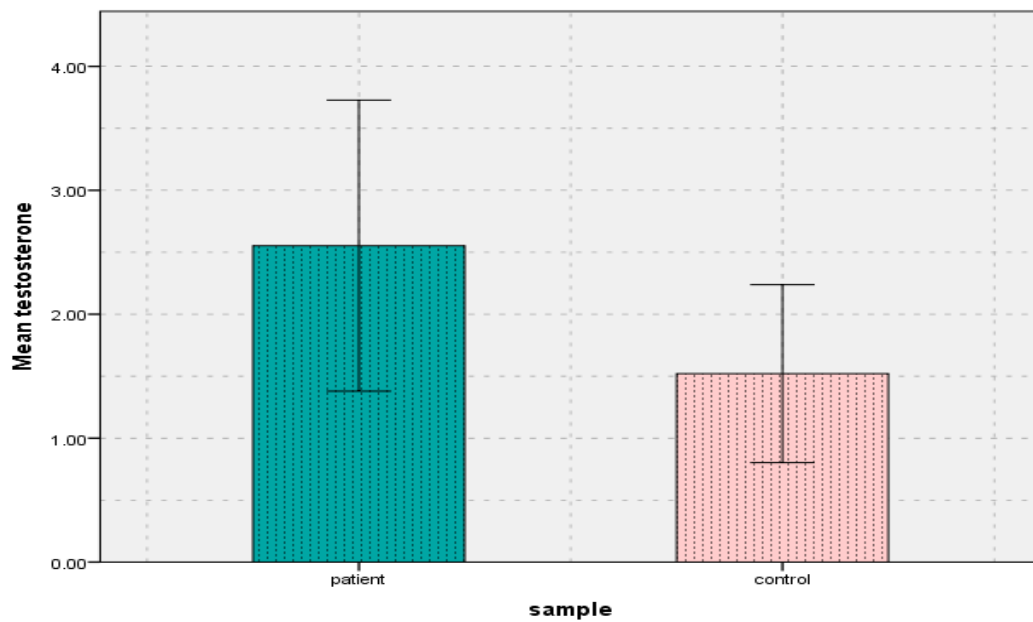
Figure (4.4) : Mean of Free testosterone in control group and PCOS group

Most people who have PCOS display high concentrations in free testosterone in the form of androgen excess(Shea et al., 2014).

Free Testosterone is a type of androgen that is biologically active. It is a chemical that is secreted, and its origins may be linked to both the ovary and the adrenal zona fasciculate (Y. Sun et al., 2021).Only 1% unbound amounts of testosterone are present, with most of it was connected to albumin and sex hormone-binding globulin (about 33% and 66%, respectively)(Njoroge et al., 2022). It is interesting to note testosterone that is unbound is the only type of the hormone with biological activity, while testosterone bound to proteins is inactive form(Zamir et al., 2021) **.**PCOS patients who have elevated the amount of free testosterone as well as the free androgen index (FAI) been diagnosed with the condition known as hyperandrogenism (Maksym et al., 2019) .

An enzyme called 17-reductase is found within the cells of the ovary known as the theca, where The conversion of androstenedione to testosterone is the key factor in hyperandrogenism is considered to be the most significant clinical feature of PCOS(Abinaya et al., 2019). PCOS affects around 80 percent of all females, with high levels of androgenic symptoms or indications, for example, hirsutism, skin conditions such as acne or hair loss, according to estimates(garrido&sempere, 2020).Hyperandrogenism observed in individuals having polycystic ovary syndrome (PCOS) is almost always generated by a rise in the amount of androgen. That has worsened by GnRH-dependent LH secretion, which increases androgen synthesis in theca cells(Ibáñez et al., 2017). while suppressing follicular maturation, leading to an abundance of small antral follicles and anovulation. Although the exact cause of follicle arrest is unclear, an excessive amount of androgens, LH, and insulin are probably secreted(victorin, 2022).

In order to determine the severity of PCOS, The duration of the monthly menstruation cycle as well as the amount of AFC present in the ovaries are the two

most important indicators. There is a positive link between serum testosterone levels and the number of Antral Follicle cell that are present as well as the length of the menstrual period (Song et al., 2019) . Importantly, only clinical symptoms of androgen excess are present in 20-40% of PCOS women whose total testosterone readings are within the standard range. Most total testosterone assays are inaccurate and insensitive at the low quantities normally encountered in women. Which is likely why this is the case (Shea et al., 2014). The amount of ovarian and adrenal testosterone synthesis as well as the percentage of testosterone bound to sex hormone binding globulin(SHBG) are both reflected in FT circulating levels(Livadas &Kandarakis, 2012)

4.1.2. 3. Insulin resistance and Estradiol in PCOS Patients and Healthy Control Groups

In our result the presented data showed in the table (4.4) and (figure 4.5) demonstrated that insulin resistance levels significantly Increased in patients group of PCOS than controls group (3.7±1.72) (2.12±0.40) respectively. Furthermore, there was decrease significantly in the levels of estradiol of PCOS group (3.7±1.72) than control group (2.12±0.40) as showed in figure (4.6) .

Table (4.4). Insulin resistance and Estradiol in PCOS and control groups

Parameters	Control group N=30 Mean	SD	Patient group N=70 Mean	SD	P value
Insulin resistance	2.12	0.40	3.7	1.72	0.00
Estradiol	94.1	28.65	54.46	16.69	0.01

The data represented as mean ± SD

The fact that the human follicular membrane contains distinct, highly-affinity insulin receptors means that insulin can have major physiological effects by mediating the thecal cells directly(Cadagan et al., 2016). Androstenedione production in thecal cells can be stimulated directly by insulin(Zeng et al., 2020). Recently, mitochondrial damage and decreased glucose uptake due to mammalian target of rapamycin complex 1-autophagy pathway dysregulation were found, which in turn causes skeletal muscle insulin resistance brought on by hyperandrogenism(Zeng et al., 2020).

As a result, there are various mechanisms by which PCOS patients who have insulin resistance and hyperinsulinemia may be a greater likelihood to occur hyper androgenism(Malini & George, 2017).

Hyperinsulinemia causes metabolic and cardiovascular problems , as well as reducing the liver's produce SHBG (sex hormone binding globulin), which elevates a amounts of free and bioactive androgens in the blood(Baranova et al., 2011). Also enhancing the ovarian cells' response to luteinizing hormone (LH), which is dependent on it, which increases androgen synthesis. The progression of metabolic problems is further exacerbated in females by hyperandrogenemia(Ye et al., 2021). Normal blood sugar levels are the result of insulin resistance, while high insulin levels are the opposite, the pancreas needs to produce progressively more insulin. Weight gain and inflammation are side effects of excessive insulin use(mohamed et al., 2023).

High insulin levels can prevent ovulation and cause the ovaries to produce more testosterone. Hyperinsulinemia also stimulates LH receptors in theca cells and prevents   follicular maturation and development, exacerbating androgen-dependent anovulation(Victorin, 2021).
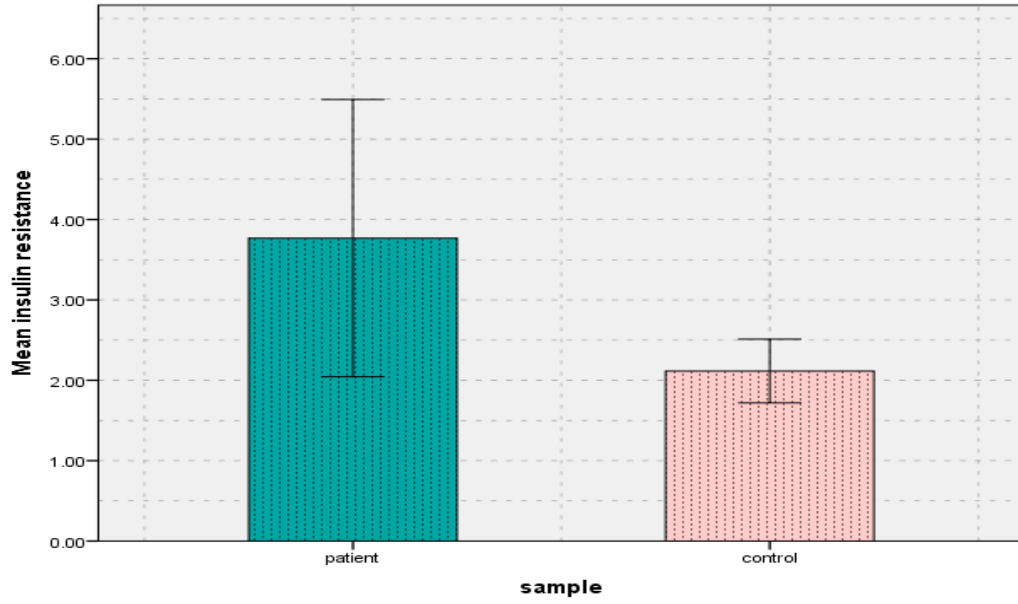
Figure (4.5): Insulin resistance in control and PCOS groups
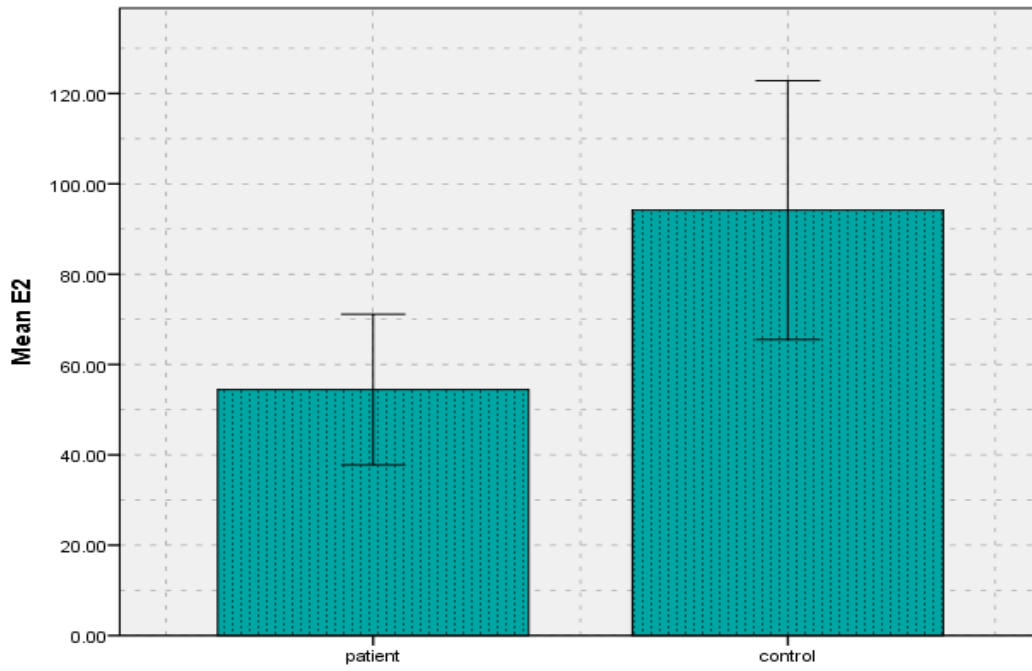
Figure (4.6). Estradiol in control and PCOS groups

The results of the investigation demonstrate the PCOS group's serum estradiol levels were extremely reduced compared to the normal group.

The negative feedback loop between estradiol and progesterone and the pituitary gland appears to be disrupted by androgen excess, leading to a rise in the intensity and frequency of LH secretion surges (Bongrani et al., 2022) .

Estradiol (or 17-estradiol) is sometimes referred to as "estrogen" due to its physiological importance and prevalence throughout reproductive years. The corpora luteal and the ovarian follicles' granulosa cells are the principal secretors of estradiol, the main circulating estrogen in humans(Fuentes et al., 2019) .

In PCOS, the granulosa cells don't have the ability to develop into follicles, therefore they don't make much estrogen. The inability to ovulate may be the primary due to infertility in women with PCOS (Khattak et al., 2022).

Due to defective GnRH signaling, Luteinizing Hormone (LH) levels are elevated in PCOS. A high quantity of LH subsequently increases androgen production(Coyle & Campbell, 2019). A drop in follicle-stimulating hormone (FSH) may indirectly contribute because to the decreased testosterone to estrogen conversion, which has worsened the increased androgen in the ovaries when the levels of luteinizing hormone (LH) rise. The negative feedback is a decrease in FSH when LH levels rise(Khan et al., 2022).

The buildup of androstenedione in PCOS is caused by decreasing FSH levels and predominance of LH activity. This androstenedione encourages the development of the follicles until the antral stage(Witchel et al., 2019). At which point the continued presence of high amounts of androstenedione damages the developing follicles and causes them to stop developing altogether. due to , an ovary with PCOS has a lot of vesicular follicles (Khattak et al., 2022).

due to lowered aromatase activity, there may be a reduction in estradiol production and an increase in androgen production(Amato et al., 2011).

There is some evidence to support the theory that one of the processes causing follicular development stop and the subsequent anovulatory condition in PCOS is a decrease in aromatase activity(Shabbir et al., 2023).

4.1.3 Sestrin 1 Level in PCOS Patients and Healthy Control Group

The table (4.5) and the figure (4.7) compare the levels of sestrin 1 in each of the research groups. According to the results, PCOS women' levels of sestrin1 are significantly lower than those of control group.

The Table (4.5) Comparison of serum Sestrin 1 levels in study groups

Parameter	Control group Mean ± SD	Patients group Mean ± SD	P- value
Sestrin 1	6238.51± 704.95	4267.44±713.40	0.000

The data represented as mean ± SD
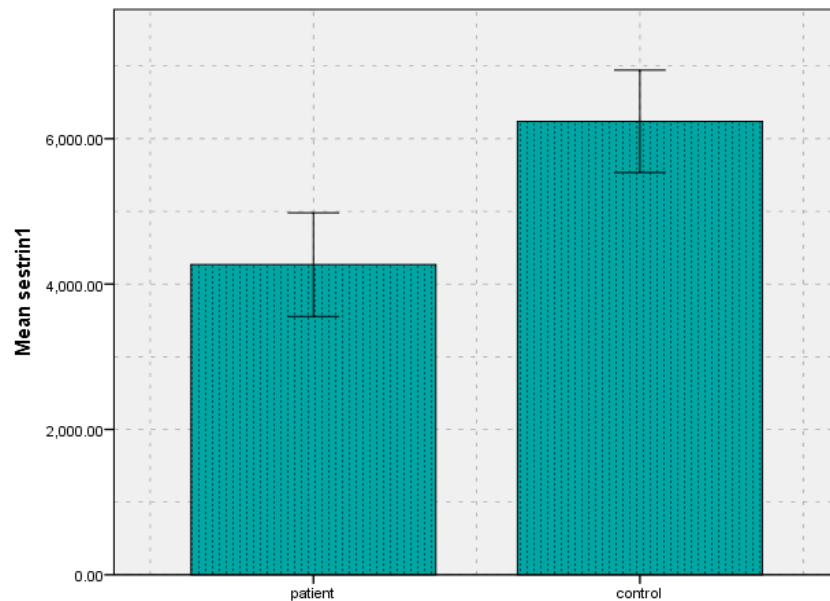
Figure (4.7) : The mean levels of Sestrin 1 found in women who have PCOS compared to a control group of women

the current finding demonstrated that individuals who had PCOS had significantly lower levels of sestrin1in contrast to female without PCOS that were in good health .

Autophagy was inhibited when Sestrin 1 was knocked down, which led to an increase in reactive oxygen species and the death of cells. This modifications may have prevented the onset and progression of PCOS. Sestrins antioxidant properties and processes have been studied(Rhee & Bae, 2015). There are two principal mechanisms through which sestrins exert their antioxidant action. In the initial stage, sestrins promote Keap1's p62-dependent autophagy, which in turn increases Nrf2 signaling and, as a result, the production of antioxidant enzymes (Xu et al., 2021). Sestrins in the second inhibited the buildup of reactive oxygen species by blunting mTORC1 activation. Insulin resistance, a key pathophysiological component of PCOS, can be caused by overexpression of the mTOR pathway(Liu et al., 2018). Another study supports that Sestrin1 levels were significantly lower in people with obesity(Nascimento et al., 2013). By modulating AMPK/mTORC1, Sestrins have been shown to play a crucial function in metabolic control and glucose homeostasis Metabolic disorders like diabetes and obesity are marked by changes in the main nutrient-sensing systems, AMPK and mTOR(Chen et al., 2022).

4.1.4 . Sestrin 2 levels in Women with PCOS & a Healthy Control Group

The current study demonstrated that considerably lower concentration of sestrin 2 in female with PCOS(147.4±31.1) than healthy female group (217.0 ±49.0 showed in table (4.6) and figure (4.8).

Table (4.6) :The differences of the levels of serum sestrin2 found in PCOS patients and in the control group.

Parameter	Control group Mean ± SD	Patients group Mean ± SD	P- value
Sestrin 2	217.0 ±49.0	147.4±31.1	0.000

The data represented as mean ± SD
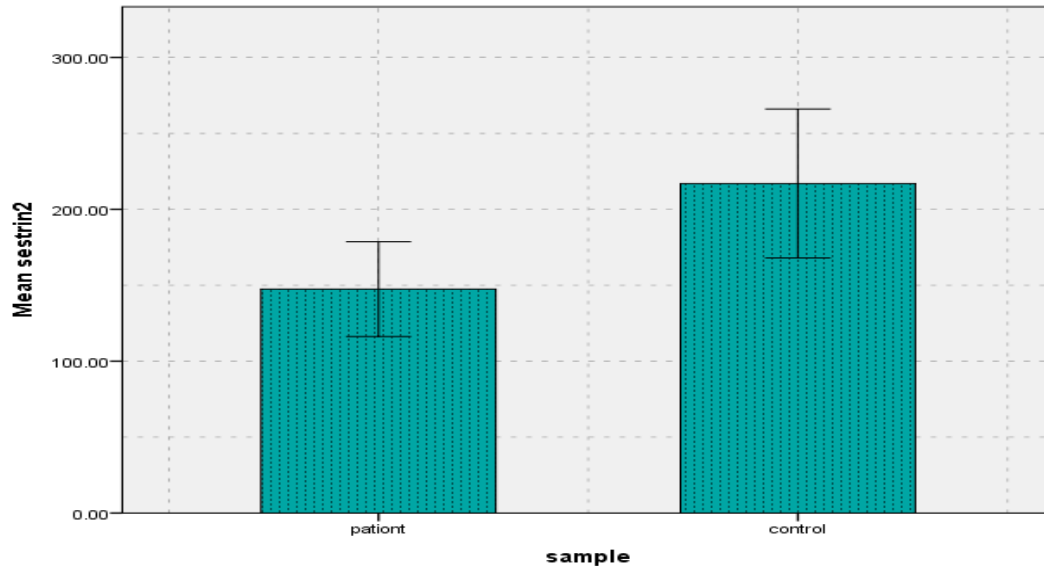
Figure (4.8) :Demonstrates the difference in mean levels of Sestrin 2 between PCOS patients and a healthy control group.

New research shows Sestrin2 is regulated within protective ways. by several mechanisms, including nuclear factor erythroid 2 (Nrf2) and p53) for encouraging autophagy as well as reducing oxidative damage and ER stress(Che et al., 2021). At this time, widely accepted the level of oxidative stress played a significant effect in the etiology of a wide range of diseases, including PCOS(Y. Sun et al., 2021). Sestrin2 deficiency may therefore be one of the elements that makes PCOS patients more vulnerable to harmful stimuli, particularly oxidative stress. Additionally, by activating the important energy sensor 5' adenosine monophosphate-activated protein kinase and inhibiting mammalian target of rapamycin complex 1, sestrin2 plays a significant role in metabolic homeostasis(Cordani et al., 2019).

Mammalian target of rapamycin signaling is involved in a number of ovarian processes, including ovarian age, reserve, follicle growth, and meiotic oocyte development, as well as ovarian somatic cell growth and steroid production, among others(Guo & Yu, 2019).

It's probable that low levels of sestrin2 cause dysregulated mTORC1 signaling, which in turn causes a variety of PCOS-related metabolic and

reproductive diseases. Sestrin2 levels inside cells can drop, and this can have a variety of negative effects, including oxidative stress, mitochondrial malfunction, and insulin resistance. Insulin resistance can result from long-term mTOR-C1 activity by preventing the phosphorylation of substrates for the insulin receptor(Mohany & Al Rugaie, 2020).

while the earlier studies on cancer, neurological disorders, and diseases of the heart and lungs that discovered elevated levels of circulating sesrin2 in the patient According to some theories, these disorders' activation of sestrin2 shields cells against injury brought on by various forms of stress(Rai & Dey, 2020).

The dynamic expression of sesrin2 and its function as a compensating factor in various clinical situations may take up a part in the discrepancy between results of the research and those of earlier investigations, however this is not entirely obvious. Sesrin2's plasma concentrations may fluctuate as a result of a number of putative upstream regulators and downstream pathways(Wang et al., 2017).Patients with PCOS have shown oxidative stress brought on by hyperglycemia, insulin resistance, and persistent inflammation (Deba et al., 2017).

The anti-oxidation mechanism is induced and stimulated by the Sestrin 2 protein, which it is believed to have an essential role for the adaptation process to stress situations(Liu et al., 2021) . Sestrin2 transcriptional activity is a well-known negative feedback mechanism that helps cells avoid the detrimental consequences of prolonged mTORC1 activation, When activated, Sestrin2 increases autophagy via activating AMPK signaling, which in turn inhibits mTORC1 activation(Pasha et al., 2017a) .Diabetes, obesity-induced insulin resistance, and the severity of obesity-related hepatosteatosis were all exacerbated in the absence of Sestrin 2 (Lee et al., 2012).

4.1.5.Beclin 1 levels in Women with PCOS Compared to a Healthy Control Group

At table (4.7) and figure (4.9) We found no statistically significant differences between the study groups.in which the beclin 1 in PCOS female (1216.35 ±467.85)

in compared to control group .

Table 4.7: The difference in mean levels of Beclin 1 between PCOS patients and control group.

Parameter	Control group Mean ± SD	Patients group Mean ± SD	P- value
Beclin 1	(6238.51±290.15)	(1310.28± 348.48)	0.570

The data represented as mean ± SD
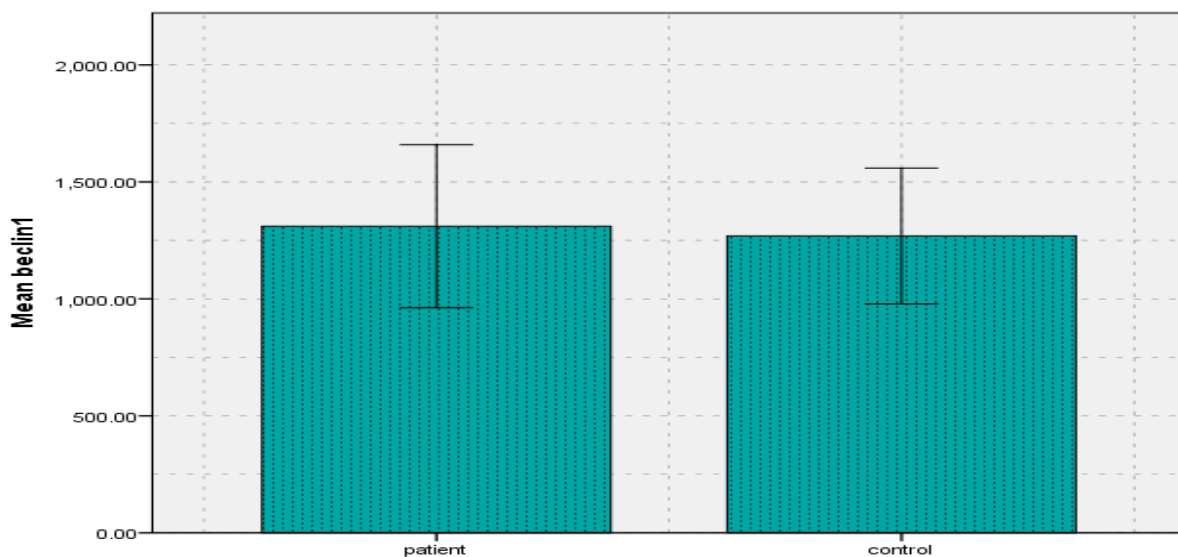
Figure (4.9): Difference in mean levels of Beclin 1 between PCOS patients with healthy control group

Beclin-1 has been shown to take crucial part of the development of autophagosomes(Xing et al., 2017). Within the framework of autophagy and the sorting of proteins, Beclin 1 has an important role . It serves as a scaffold for the formation of a multi protein assembly during the initial stages of autophagy(Kaur & Changotra, 2020).

The ovary needs adequate, effective autophagy for the development of follicles, the origin of oocytes, and their degradation. the progress that has been made in understanding the significance of autophagy in relation to PCOS-related metabolic diseases. The process of Autophagy is essential part of the regulation of the activation of inflammasomes as a result of metabolic stress.(Li et al., 2017).

Autophagy that is not functioning properly, on the other hand, might lead to cell dysfunction. as well as even death. Despite the fact that maintaining insulin sensitivity requires optimal autophagy. In peripheral tissues, unchecked autophagy reduces insulin sensitivity. It has also been acknowledged that atresia and follicular development in the ovary depend on optimal autophagy. Despite the fact that increased in autophagy observed in PCOS patient women's ovarian tissue It is possible that this is one of the causes that contributes to the progression of IR in the ovary of ladies with PCOS (Zhang et al., 2020).

On the other hand, excessive autophagic self-digestion, the degradation of vital cellular components, and the induction of apoptosis can all contribute to the promotion of cell death(Dehlaghi et al., 2023) .

4.2.1 Correlation of Sestrins (1,2) levels and parameters

Table 4.8 :The correlation between sestrin1 ,sestrin2 and Parameters of groups.

Parameters	Sestrrin1	Sestrin2
	r	r
BMI (K/m^2)	-0.155	-0.183
LH (mIU/ml)	-0.040	0.122
FSH (mIU/ml)	-0.016	0.014
Testosterone	-0.034	0.178
Progesterone	0.063	-0.030
IR	0.164	0.067
E2	-0.020	-0.156

NS: t-test p- value ≥ 0.05 ; S: p-value ≤0.05 ; HS: p- value ≤0.01

   *--significant correlation p≤0.05   ,r: Pearson's correlation coefficient

4.2.1 A. Correlation of Sestrins1 level and biochemical parameters

   The results concerning sestrin1 showed a negative correlation with BMI this result agreed with study of in which appear sestrin1 a negative correlation with BMI(Sundararajan et al., 2021).And a negative correlation with LH,FSH ,testosterone, estradiol while appear sestrin1 a positive correlation with IR, progesterone.

Metabolic disorders caused by obesity, including issues with insulin sensitivity, high blood sugar, and type 2 diabetes are all made worse by hypernutrition (Lee et al., 2013) . In a recent study, obese mice lacking exhibited glucose intolerance, insulin

resistance, and hepatosteatosis, all of which were exacerbated by the activation of mTORC1 in response to nutrient availability (Chen et al., 2022) . Numerous metabolic diseases, such as fat accumulation, mitochondrial dysfunction, and oxidative damage, may result from the loss of endogenous sestrins (Nourbakhsh et al., 2017) . In people with dyslipidemia and diabetes with dyslipidemia, but not in people with only diabetes, sestrin1 mRNA expression was significantly reduced(Sundararajan et al., 2021).

4.2.1 B .Correlation of Sestrins2 level and biochemical parameters

Sestrins2 protein level correlation with biochemical parameters in PCOS patients group was shown in Table (4.7) The result demonstrates that there are a negative correlations between serum Sestrins2 level and BMI , Progesterone, Estradiol while positive correlations with LH, FSH, testosterone .

Affected AMPK and mTOR are the main nutrient-sensing systems in diseases including diabetes and obesity. In cells, long-term mTORC1 activation during over nutrition promotes protein and fat synthesis while suppressing autophagic catabolism(Pasha et al., 2017b).

The transcriptional activation of Sestrin2 is one of the key negative feedback mechanisms to avoid the deleterious effects of prolonged mTORC1 activation(Pasha et al., 2017b).However, the study revealed that the expression of Sesn2 was downregulated in the livers of mice fed a high-fat diet, although Sesn1 expression was unaffected(Jin et al., 2013) .

4.2.2. Correlation of Beclin1 levels and biochemical parameters

The results concerning beclin1 showed a negative correlation with BMI , LH ,FSH, IR this result while appear beclin1 had positive correlation with testosterone, progesterone, estradiol.

Table 4.9: The correlation beclin1and Parameters of groups.

Parameters	Beclin1	
	r	
BMI (K/m^2)	-0.271*	
LH (mIU/ml)	-0.071	
FSH (mIU/ml)	-0.027	
Free testosterone	0.158	
Progesterone	0.233	
Insulin resistance	-0.241*	
Estradiol	0.029	

NS: t-test p- value ≥ 0.05 ; S: p-value ≤0.05 ; HS: p- value ≤0.01

*--significant correlation p≤0.05   , r: Pearson's correlation coefficient

In obesity, there is an imbalance between excessive food intake and insufficient energy expenditure, which causes autophagy to be suppressed as a result of elevated mTOR signaling(Castañeda et al., 2019).

In experimental models and in obese people, activation of mTOR due to increased growth factor and insulin signaling, as well as increased branched-chain amino acids consumption, is frequent. This is thought to be the primary mechanism behind nutrition-triggered suppression of autophagy(Zhang et al., 2018). Autophagy is sensitive to alterations in the status of nutrients, especially high-fat and/or excessive calorie diets(Isouza et al., 2014) .Furthermore, alterations in autophagy may negatively affect metabolism either locally (in a metabolically active organ) or globally, promoting metabolic dysregulation locally or systemically via endocrine

pathways (Zhang et al., 2018). The primary cell signaling pathways that are often activated in response to changes in nutrition availability are AMP-activated protein kinase (AMPK), phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT), and JUN N-terminal kinase (JNK),Low-grade inflammation caused by JNK activation in obese individuals causes Bcl-2 to be phosphorylated in order to reverse Bcl-2s suppression of the autophagy start molecule beclin 1(Zhang et al., 2018) .

The beclin-1 complex is activated while the ULK1 complex is situated in the membrane, facilitating the development of the autophagosome (Castañeda et al., 2019). Mechanistic target of rapamycin complex 1 (mTORC1) activation inhibits the ULK1 complex (consisting of ULK1-ATG13-ATG17), which is necessary for the induction of autophagy, hence suppressing autophagy(Hodson et al., 2021).

Conclusions and Recommendations

Conclusions and Recommendations

Conclusions

Current study concludes the following:

1.In PCOS patient elevated levels of LH , and Free testosterone were associated with, hirsutism, obesity, and hormonal alterations. And   have lower levels progesterone

2. Our research shows that sestrin2, an autophagy marker, is dysregulated in PCOS and may serve as a new diagnostic for the condition

3. Reduced serum sestrin 2 levels may be has potential as a new PCOS diagnostic biomarker.

4. Autophagy helps keep the human ovary healthy and works in the process of luteal decline. It also affects the ability of granulosa cells to live. And regulated indirect by sestrins .

5. According to the current findings, PCOS may have started as a result of beclin 1 irregularities in the serum of PCOS patients.

Conclusions and Recommendations

Recommendations

1_Additional studies can be done for Sestrin 2 dynamic expression and compensatory role in various clinical circumstances.

2_Studies of beclin_1 gene expression of serum in polycystic ovary syndrome female.

3_Studies of sestrin expression in people with diabetes combined with an elevated lipid.

4_Studying the role of insulin resistance in PD_1 genes in pathogenesis of PCOS .

5_Using more recent diagnostic methods for diagnosis of PCOS in PCR in the further studies .

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Appendices

Appendix
Appendix 1:

Questioner for participants

Name: address: height:

Age: duration:

                              weight:Phone Number:

❖ Clinical features

(1) hirtisum

(2) acne

(3) alopecia

  ❖ Menstrual irregulaties

(A) Regular menstruation

(B) Oligomenstrual

  ❖ Ultrasound image

   (1) normal ovary

   (2) Pcos ovary

  ❖ Diseases

  A)DM   (B) Hypertension   (C) Kidney

  (D) Liver   (E) Thyroid

  ❖ Drug history

  ❖ Hormones 1. LH   2. FSH   3. Prolactin

   4.free testosterone   5. Progesterone   6. Estradiol

  ❖ Fasting blood sugar   fasting blood insulin

  ❖ Smoker   non smoker

Appendix 2:

Procedure to determination Luteinizing Hormone Level
Procedure

It took 18 minutes to finish the assay.

1. First incubation was performed with twenty microliter of the material, monoclonal antibodies against LH (both biotinylated and ruthenium complex-labeled) make a sandwich complex

2. When streptavidin-coated microparticles were introduced during the second incubation, a biotin-streptavidin reaction formed, which covalently linked the complex to the solid phase.

3. The measuring cell is filled with the reaction mixture, which is subsequently drawn by magnetism to the surface of the electrode, with utilized Pro Cell/Pro Cell M to get rid of any loose particles.

When a voltage is given to an electrode, chemiluminescent emission is produced.

4. The results of an analysis are determined with the help of a master curve provided by either the reagent barcode or the e barcode and an instrument-specific calibration curve generated using a two-point calibration..

Estimation Analyte concentration (in IU/L or mIU/mL) is calculated by the analyzer for each sample.

Appendices

Appendix 3:
Procedure to determination Follicular Stimulating Hormone Level procedure

1. Sandwiching 40µl of sample between biotinylated and ruthenium complex-tagged monoclonal FSH-specific antibodies was the initial step in the incubation process.

 2. When streptavidin-coated microparticles were introduced during the second incubation, a biotin-streptavidin reaction formed, which covalently linked the complex to the solid phase

 3. The measuring cell is filled with the reaction mixture, which is subsequently drawn by magnetism to the surface of the electrode After that, we utilized Pro Cell/Pro Cell M to get rid of any loose particles. When a voltage is given to an electrode, chemiluminescent emission is produced.

Appendices

Appendix 4:

Procedure to determination Progesterone Hormone Level

Assay time in total: 18 minutes.

▪ First incubation: The number of immunocomplexes produced by incubating the sample (20 µl) with a progesterone-specific biotinylated antibody is based on the amount of the analyte in the specimen.

▪ 2st incubation, an antibody hapten complex is formed between the biotinylated antibodies and the streptavidin-coated microparticles and ruthenium-labeled progesterone derivative. Biotin and streptavidin bind to one another, attaching the whole complex with the solid surface.

▪ Aspiration delivers the reaction mixture to the measurement cell, where it is subsequently followed by magnetic attraction of the microparticles to the surface of the electrode.. ProCell /Pro Cell M is then utilized to dissolve any unbound materials. The electrode is then applied a voltage, which causes chemiluminescent emission that a photomultiplier may subsequently measure.

▪ Results are obtained by the master curve given by the reagent e-barcode or barcode and the calibration curve that is instrument-specifically created via two-point calibration.

Appendix 5:

Procedure to determination free testosterone Levels
C. Test procedure

Use the Fully-Auto Chemiluminescence Immunoassay (CLIA) Analyzer MAGLUM in accordance with the manufacturer's instructions for best results. The Reagent Integral has an RFID tag for each test parameter.

Dilution

 This reagent kit does not allow for analyzer-based sample dilution. It is possible to manually dilute samples whose concentrations are too high for the measuring range. Multiply the outcome of the manual dilution by the dilution factor. Before any manual dilution is carried out, it is imperative that appropriate diluents be selected.

MAGLUM Analyzer User's Guide,

Maglum Analyzer User's Guide,

20μl +40μl +40μl 40μl	Nano magnetic ABEI Label Sample calibrator FITC Label
400μl	Cycle washing
15 Min	Incubation
3 s	Measurement

The results

The free Testosterone concentration in each sample is determined mechanically by the analyzer using a calibration curve prepared using a master curve calibration method based on two points of data. The values were reported within units of pg\ml. The MAGLUM Fully-Automatic Chemiluminescence Immunoassay Analyzer User's Guide can provide additional details

Appendices

Appendix 6:
Procedure to Determination of serum Estradiol (E2) levels

Procedure

1. (C0...C5) Calibrator Preparation The calibrators are set up and contain the following levels 17-Estradiol:

	C0	C1	C 2	C 3	C4	C5
Pg /m	0	20	120	300	600	2000

2.the Conjugate Process

It is now possible to use the conjugate. Using a rotating mixer, mix gently for five minutes. Six months after being opened, it remains steady at 28 °C.

3. Sample preparation

It is possible to measure the amounts of 17-Estradiol in blood plasma or serum. Keep the sample at – 20 °C when the examination isn't done the same day it was taken. Samples shouldn't be kept froze & thaw continually..Before usage, stir with a rotating mixer for five minutes.

4.Making a wash solution

Conc. Wash Solution 10X should be diluted with distilled water to a final volume of 500 mL before use. To dilute smaller quantities, use a 1:10 dilution ratio.

The diluted wash solution is stable for 30 days between 2 and 8 °C. Dilution of the entire bottle of concentrated wash solution to 500 mL will increase accuracy, being careful to transfer crystals, and then mix until crystals are completely dissolved at room temperature.

The procedure

All reagents should be allowed to reach room temperature (22–28 °C).

Appendices

Unused coated microwell strips should be stored at 2 to 8 degrees C in a foil bag with desiccant.

To avoid chemical and/or microbiological contamination, used reagents should never be put again into the same vials.

Make two wells for every step of the curve's calibration to improve the precision of the test results.. This is because the determination must be made in triplicate.

Reagent	Sample/Control	Calibrator	Blank
CalibratorC0_C5		25 µL	
Conjugates	200 µL	200 µL	
Sample_Control	25 µL		

2 hours at 37 °C incubation

Clean the wells three times with 300µL of diluted wash solution after removing each well's contents.

Reagent	Sample/Control	Calibrator	Blank
TM B Substrates	100 µL	100 µL	100 µL

30 minutes should be spent incubating in the dark at ambient temperature (22 to 28°C).

Reagent	Sample/Control	Calibrator	Blank
Stop solution	100µL	100 µL	100 µL

Compare the absorbance to the blank after a brief shake of the microplate at 450 nm.

Appendices

Appendix 7:

Procedure to Determination of serum Human Sestrin-1（SESN1）Level Determination
Procedure of assay

1.Sample dilution and addition to Standard: placing ten Standard wells on micro Elisa plates that coated , adding Standard 100 µl to wells of first and second & 50 µl Standard dilution to wells of first and second , respectively & mix. Separately to the 3rd and 4th wells add 100µl from each of the 1st and 2nd wells, then, add 50µl of standard dilution to the 3rd and 4th wells and mix. Then, remove 50µl from the 3rd and 4th wells and discard them away. add 50µ l to the 5th and 6th well, add 50µl of Standard dilution to the same wells as well as mix . from each of the 5th and 6^{th}wells remove 50µl and put them into the wells of 7th and 8th . added Standard dilution 50 µl to the 7th and 8th ,mix . from the 7th and 8th wells remove 50 µl then place them into the wells of 9th and 10th , to the 9th and 10th wells add 50µl of Standard dilution and stir. remove 50µl from the 9th and 10th wells and throw them away. After Diluting, add 50µl of Sample to each well (Intensity (ng/L): 3000, 2000, 1000, 500, and 250)

2. Sample addition : Prepare the blank wells separately. (comparison in the blank wells do not add HRP Conjugate reagent or samples ; all other steps are identical). Well of test sample, add 40µl sample dilution to testing well of sample then add sample 10µl (end dilution of sample is 5-time avoiding the wall of the well as much as possible, , add the sample to wells, and stir

3. Cover micro Elisa plate with the Cover plate membrane and put it in an incubator at a temperature of 37 degrees Celsius for thirty minutes..

4. Formation fluid: Make a wash solution dilution of 30 or 20 times the original strength in distill water and save it

5.The wash：Remove membrane from Closure plate, pour out fluid , dry pat, wash wells in the washing buffer, wait 30 seconds, then drain, repeat 5 times.

6.Add enzyme：In each well, except the blank well, add 50µl of the HRP-Conjugate reagent.

7. Work with three to incubate.

8. work with five to wash.

9.The Color: To each well, add 50µl each of Chromogen A Solution and Chromogen B Solution, . With it, spend 15 minutes at 37°C without lighting.

10. Stopping the reaction：to pause the reaction (the blue color changing to yellow), add to every well50µl of Stop Solution

11.Assay：consider blank as the number zero, After adding solution to stop reaction , examine absorbance in 15 minutes at 450 nm .

Appendices

Appendix 8:

Procedure to determination of serum Human Sestrin2(SESN2)Level

Procedure of test

1. Sample dilution and addition to Standard: placing ten Standard wells on microelisa plates that coated , adding Standard 100 µl to wells of first and second & 50 µl Standard dilution to wells of first and second , respectively & mix. Separately to the 3rd and 4th wells add 100µ l from each of the 1st and 2nd wells Then, add 50µ l of standard dilution to the 3rd and 4th wells and mix. Then, remove 50µ l from the 3rd and 4th wells and discard them away,add 50µ l to the 5th and 6th well, add 50µ l of Standard dilution to the same wells as well as mix . from each of the 5th and 6thwells remove 50µl and put them into the wells of 7th and 8th . added Standard dilution 50 µl to the 7th and 8th ,mix . from the 7th and 8th wells remove 50 µl then place them into the wells of 9th and 10th , to the 9th and 10th wells add 50µl of Standard dilution and stir. remove 50µl from the 9th and 10th wells and throw them away. After Diluting, add 50µl of Sample to each well (Intensity (ng/L): 3000, 2000, 1000, 500, and 250)

2. Sample addition : Prepare the blank wells separately. (comparison in the blank wells do not add HRP Conjugate reagent or samples ; all other steps are identical). Well of test sample, add 40µl sample dilution to testing well of sample then add sample 10µl (end dilution of sample is 5-time avoiding the wall of the well as much as possible, , add the sample to wells, and stir

3. Cover microelisa plate with the Cover plate membrane and put it in an incubator at a temperature of 37 degrees Celsius for thirty minutes..

4. Formation fluid: Make a wash solution dilution of 30 or 20 times the original strength in distilled water and save it

 5.The wash： Remove membrane from Closure plate, pour out fluid , dry pat, wash wells in the washing buffer, wait 30 seconds, then drain, repeat 5 times.

6.Add enzyme： In each well, except the blank well, add 50μ l of the HRP-Conjugate reagent.

7. Work with three to incubate. 8. work with five to wash.

 9.The   Color: To each well, add 50 µl each of Chromogen A Solution and Chromogen B Solution, . With it, spend 15 minutes at 37°C without lighting.

10. Stopping the reaction： to pause the reaction (the blue color changing to yellow), add to every well50μ l of Stop Solution .

11.Assay： consider blank as the number zero, After adding solution to stop reaction , examine absorbance in 15 minutes at 450 nm

Appendix 9:
Procedure to Determination of serum Human Human Beclin 1 Level Determination

Procedure of assay

1. Sample dilution and addition to Standard: placing ten Standard wells on microelisa plates that coated , adding Standard 100 µl to wells of 1st and 2nd & 50 µl Standard dilution to wells of 1st and 2nd , respectively & mix.Separately to the 3rd and 4th wells add 100µ l from each of the 1st and 2nd wells. Then, add 50µl of standard dilution to the 3rd and 4th wells and mix. Then, remove 50µl from the 3rd and 4th wells and discard them away. add 50µl to the 5th and 6th well, add 50µ l of Standard dilution to the same wells as well as mix . from each of the 5th and 6th wells remove 50µl and put them into the wells of 7th and 8th . added Standard dilution 50 µl to the 7th and 8th ,mix . from the 7th and 8th wells remove 50 µl then place them into the wells of 9th and 10th , to the 9th and 10th wells add 50µ l of Standard dilution and stir. remove 50µl from the 9th and 10th wells and throw them away. After Diluting, add 50µ l of Sample to each well (Intensity(pg/ML): , 900, 600, 300, 150, 75)

2. Sample addition : Prepare the blank wells separately. (comparison in the blank wells do not add HRP Conjugate reagent or samples ; all other steps are identical). Well of test sample, add to testing sample well 40µl sample dilution then add testing sample 10µ l (sample end dilution is 5-time avoiding the wall of the well as much as possible, , add the sample to wells, and stir

3. Cover micro Elisa plate with the Cover plate membrane and put it in an incubator at a temperature of 37 degrees Celsius for thirty minutes..

4. Formation fluid: Make a wash solution dilution of 30 or 20 times the original strength in distilled water and save it

5.The wash :   Remove membrane from Closure plate, pour out fluid , dry pat, wash wells in the washing buffer, wait 30 seconds, then drain, repeat 5 times.

6.Add enzyme : in each well, except the blank well, add 50µ l of the HRP-Conjugate reagent.

7. Work with three to incubate. 8. work with five to wash.

9.The   Color: To each well, add 50µl each of Chromogen A Solution and Chromogen B Solution, . With it, spend 15 minutes at 37°C without lighting.

10.Stopping the reaction : to pause the reaction (the blue color changing to yellow), add to every well50µ l of Stop Solution .

11.Assay : consider blank as the number zero, After adding solution to stop reaction , examine absorbance at 450 nm in 15 minutes .

Appendix 10:

Procedure to determination of Fasting blood glucose levels

The reaction mixtures and specimens had been brought reaching room temperature. These things have been written down and placed in appropriately labeled tubes:

Table (3.12) :The components of the blood glucose kit

Tube	Sample	Blank	Cal.
CAL. Standard	-	-	10 μ l
Sample	10 μ l	-	-
R1 Mono reagent	1 ml	1 ml	1 ml

3. In combination, the tubes have been used, &then they were left to stand for either five minutes at 37 degrees Celsius or ten min at room temperature.

4. At a 500 nm wavelength, the absorbance, denoted by "A," of both the standard and the samples was measured and compared to the blank value of the reagent.

5. The color remains pink for around two hours when light can't get to it.

Calculation:

$$\frac{\text{Ab of sample}}{\text{sample Ab}} \text{ x Concentration of Standard} = \text{mg/dl glucose of the standard}$$

Normal significance in adult ranges from 70 to 105 mg/dl (3.89 to 5.83 mmol/l).

الخلاصة

متلازمة المبيض المتعدد الكيسات (PCOS) هي حالة تتميز بخلل في بنية ووظيفة المبيض (ضعف التبويض) وإنتاج الأندروجين. في المراحل الأولى من سن الإنجاب

تتأثر العديد من النساء في جميع أنحاء العالم في سن الإنجاب بحالة الغدد الصماء المتنوعة المعروفة باسم متلازمة تكيس المبايض (PCOS). غالبًا ما ترتبط مستويات هرمون التستوستيرون المرتفعة ومقاومة الأنسولين وتضخم المبايض بهذه المتلازمة.

تم تحديد كلا من (الإستراديول ، هرمون البروجسترون ، هرمون التستوستيرون الغير مرتبط ، هرمون الأنسولين ، السيسترين 1 ، السيسترين 2 ، البيكلين 1 ، مؤشر كتلة الجسم (BMI) في مصل كل من مجموعة مرضى متلازمة تكيس المبايض ومجموعة الاصحاء. كشفت نتيجة دراستنا عن زيادة معنوية في مستويات الهرمون اللوتيني (0.00> p) لدى النساء المصابات بالـ PCOS (9.81±5.87) مقارنة بالنساء الأصحاء (4.7±1.66) .

أظهرت نتائج هذه الدراسة زيادة معنوية في مستوى هرمون التستوستيرون الحر (0.00 > P) لمجموعة PCOS (4.18 ± 3.16) مقارنة بمجموعة الاصحاء (10.34±4.46), زيادة معنوية في مقاومة الأنسولين ((0.00> p) لمرضى متلازمة تكيس المبايض (3.7 ± 1.72) مقارنة بمجموعة الاصحاء (0.40 ±2.12). أظهر هذا البحث انخفاضًا ملحوظًا في الهرمون المنبه للجريب(FSH) عند مرضى تكيس المبايض(5.13±1.29) من مجموعة الاصحاء (6.42±1. 99) بينما أظهرت الدراسة انخفاضًا كبيرًا (0.012> p)في الاستراديول من مجموعة PCOS (3.7±1.72) مقارنة بمجموعة الاصحاء (40. 0± 2.12) , وانخفاضًا معنويًا في هرمون البروجسترون في مرضى متلازمة تكيس المبايض(3.16±4.18) مقارنة بمجموعة الاصحاء(10.34±4.46), وانخفاضًا معنويًا في السيسترين 1 من متلازمة تكيس المبايض(1296.25 ± 4281.721) (P<0.00) مقارنة بمجموعة الاصحاء (6238.511±1416.13).وشهدت الدراسة ايضا انخفاض في السيسترين 2 من متلازمة تكيس المبايض(147.4±31.1) مقارنة بمجموعة الاصحاء(217.0±49.0) .لم تشهد هذه الدراسة تغير معنوي في مستويات البكلين 1 في كلا من مجموعة متلازمة تكيس المبايض(1310.28±1018.08) ومجموعة الاصحاء(1216.35±467.87). وقد وجدنا في هذه الدراسة ان متلازمة تكيس المبايض قد ارتبطت بالمستويات المرتفعة من LH والتستوستيرون الحر بالشعرانية والسمنة والتغيرات الهرمونية. لدى مرضى متلازمة تكيس المبايض مستويات أقل من هرمون البروجسترون مقارنة بالنساء الأصحاء. اظهر بحثنا أن السيسترين 2، وهو علامة الالتهام الذاتي، لا يتم تنظيمه في متلازمة تكيس المبايض

وقد يكون بمثابة تشخيص جديد للمتلازمة. قد يكون انخفاض مستويات السيسترين 2 في الدم بمثابة علامة بيولوجية تشخيصية جديدة لمتلازمة تكيس المبايض. تساعد الالتهام الذاتي في الحفاظ على صحة المبيض وتعمل على عملية انحدار الجسم الأصفر. كما أنه يؤثر على قدرة الخلايا الحبيبية على العيش. وينظم بشكل غير مباشر بواسطة السيسترين . وفقا للنتائج الحالية، قد تكون متلازمة تكيس المبايض قد بدأت نتيجة لاعتلال سيسترين 1 في مصل مرضى متلازمة تكيس المبايض.

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جامعة كربلاء

كلية العلوم الطبية التطبيقية

تقييم مستويات مصل السيسترين 1 و السيسترين 2 و البكلين 1 بتنظيم الالتهام الذاتي عند النساء المصابات بمتلازمة تكيس المبايض (PCOS)

رسالة مقدمة

الى مجلس كلية العلوم الطبية التطبيقية _جامعة كربلاء

وهي جزء من متطلبات نيل درجة الماجستير في علوم التحليلات المرضية

كتبت بواسطة

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بكالوريوس كلية التقنيات الصحية والطبية/تحليلات مرضية/٢٠١٧

بأشراف

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1445هـ   2023 م