Republic of Iraq

Ministry of Higher Education

and Scientific Research

University of Babylon

College of Medicine

Department of Chemistry and Biochemistry

The Effect of Obesity on Androgenic Hormones and Androgen Receptor Level and gene in Sub. Infertile Women.

A Thesis

Submitted to the Council of the College of Medicine/ University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master of Science / Clinical Biochemistry

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

بسم الله الرحمن الرحيم

﴿قَالَ رَبِّ اشْرَحْ لِي صَدْرِي ﴿٢٥﴾
وَيَسِّرْ لِي أَمْرِي ﴿٢٦﴾ وَاحْلُلْ عُقْدَةً
مِّن لِّسَانِي ﴿٢٧﴾ يَفْقَهُوا قَوْلِي ﴿٢٨﴾﴾

صدق الله العلي العظيم

سورة طه (٢٥-٢٨)

Dedication

To the deceased of my soul, my husband, Dr. Shabeer Hassan, may God bless him with his vast mercy..

To my mother and father for their love, support, kindness, and motivation...

To my roses Ali, Muhammad, and Jumana..

To my brothers and friends, and to everyone who supported me, even with a smile... I dedicate this work.

Zainab

Acknowledgements

Praise and thanks to almighty Allah (swt) for all grace and mercy he showers me with….

And prayer and peace upon prophet Mohammed peace being upon him and his pedigree….

It is to my pleasure to express my thanks to my supervisors

Prof. Dr.Maha Smaism & Prof. Dr. Nadia Al-Hilli for their supervision and not sparing any efforts to overcome all the difficulties during the theoretical and practical parts ... without their critical discussions, comments, and great help, this work would not have been completed…

My great appreciation to the staff working in different clinics, hospital and laboratories who participated in the study as well as the candidates of this work…

Last, but not least, I would like to acknowledge the staff at the Biochemistry Department at the College of medicine, University of Babylon for their cooperation…

Supervisor Certification

We certify that this thesis entitled (The Effect of Obesity on Androgen Hormone and Androgen Receptor Level and Androgen Receptor gene in Infertile Women.) has been prepared under our supervision at the Department of Biochemistry, College of Medicine, University of Babylon, in partial requirements for the degree of master in Clinical Biochemistry.

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In the view of the available recommendation, I forward this thesis for debate by the Examination Committee.

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Summary

Infertility was a problem of the male or female reproductive system. Obesity is associated with multiple alterations of the endocrine systems, including abnormal circulating blood hormone concentrations, due to changes in their pattern of secretion and/or metabolism As well as obesity is also associated with an increased testosterone production rate in women. The Androgen receptor plays a role in regulating female sexual, somatic, and behavioral functions. The polymorphic appear to influence the function of the receptor as a transcription factor.

The aim of study is effect of obesity on free testosterone(fT) , testosterone(T), dehydroepiandrosterone sulfate (DHEAS) and androgen receptor (AR) levels in infertile obese women and to evaluate the risk of single nucleotide polymorphism of androgen receptor gene (rs6152 G > A) on infertile obese women in Babylon Province.

To achieve this aim, the present study included 90 women the first group included 45 sub infertile obese women BMI(30-39)$Kg_{/m}^{2}$,and age (20-30)years the second group was 45 apparently healthy individual's BMI (20-24.5) $Kg_{/m}^{2}$and thier age(20-30)years as control groups. The inclusion and exclusion criteria for this study were as follows:

Inclusion criteria*:* All females in this study included: obese sub infertile women, reproductive age (20-30)y,Normal weight and non-infertile women for control.

Exclusion criteria: patients have the following conditions were excluded: Hypertension, Thyroid disease, PCOs, patients who were taken any hormonal medication.

Free testosterone, testosterone, dehydroepiandrosterone sulfate and Androgen receptor concentration were determined by enzyme linked immunosorbent assay method .DNA was extracted from whole blood

form all womens .genotyping of androgen receptor gene SNP was carried out by Restriction Fragment Length Polymorphism. Genotyping was achieved with specific primers to amplify fragments.

In this study, results found there was a significant increase in free Testosterone, Dehydroepiandrosterone sulfate concentration compared to the control group ($P< 0.05$).

Also found significant decrease in concentration in total testosterone, and androgen receptor in patients with obese infertile group compared to the control group ($P< 0.05$).

The homoozygous genotype (GG) of AR gene (rs6152 G > A) SNP was found to be No-significantly difference ($p>0.05$) with The homoozygous genotype (AA) and the heterozygous genotype (GA) in two groups.

The minor allele frequencies (A) of AR *gene* **(**rs6152 G > A) SNP in patient and control group were found to be 22.2 % , and 14.5 % respectively. It was No-significantly difference($p>0.05$) in obese infertile group when compared with that of the control group.

The age no-significantly difference ($p>0.05$) between obese infertile group compared to the control group.

We concluded from this study that obesity affects on Free testosterone, testosterone, dehydroepiandrosteronesulfate and androgen receptor ($P< 0.05$). and the SNP(6152) androgen receptor gene, not-associated with obesity ($p>0.05$), The age no-significantly difference ($p>0.05$) between obese infertile group compared to the control group.

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List of Abbreviations

Abbreviation	Meaning
Abs	Absorbance
BMI	body mass index
CDC	Centers for Disease Control
CVD	cardiovascular diseases
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immune sorbent assay
GnRH	gonadotropin-releasing hormone
HRP	Horseradish peroxidase
IGF-1	insulin-like growth factor-1
M	Mean
NR3C4	nuclear receptor subfamily 3
OD	optical density
PCOS	polycystic ovarian syndrome
PGH	hypothalamic-pituitary-gonadal
PID	Pelvic Inflammatory Disease
r	Correlation-coefficient
SHBG	Sex hormone-binding globulin
SNP	Single nucleotide polymorphism
T2DM	Type2 diabetes mellitus

Chapter One

Introduction

1. Introduction

1.1. Infertility

The World Health Organization defines infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (and there is no other reason, such as breast feeding or postpartum amenorrhea) **(1).**

There are many causes of infertility, including some that medical intervention can treat. Various hypothalamus, pituitary, thyroid, adrenal, and ovarian problems, drug use, advanced age, and obesity can all have a deleterious impact on fertility. Female-related disorders are categorized as tubular peritoneal and ovulatory among the main causes of infertility in women **(2).**

Extremes in body weight have an impactite because they are associated with multiple alterations of the endocrine systems, including abnormal circulating blood hormone concentrations **(3).**

Male infertility is responsible for 20–30% of infertility cases, while 20–35% are due to female infertility. The most common cause of female infertility is ovulatory problems, which generally manifest themselves by sparse or absent menstrual periods. Male infertility is most commonly due to deficiencies in the semen, and semen quality is used as a surrogate measure of male fecundity **(4).**

In the USA, up to 20% of infertile couples have unexplained infertility. In these cases, abnormalities are likely to be present but not detected by current methods. Possible problems could be that the egg is not released at the optimum time for fertilization, that it may not reach the fallopian tube, sperm may not be able to reach the egg, fertilization may fail to occur, transport of the zygote may be disturbed, or implantation

fails **(5).**It is increasingly recognized that egg quality is of critical importance and women of advanced maternal age have eggs of reduced capacity for normal and successful fertilization **(6).**

Obesity is a heterogeneous disorder with wide variations in risks for complicating diseases. The recognition of the marked differences between excess fat localized in different parts of the body has markedly increased the knowledge of mechanisms by which metabolic and cardiovascular risk factors and diseases can be associated with obesity. At the same time, emerging scientific interest has increased our understanding of the main metabolic and hormonal factors involved in the pathophysiology of obesity (**7).**

Obesity is associated with multiple alterations of the endocrine systems, including abnormal circulating blood hormone concentrations, due to changes in their pattern of secretion and/or metabolism, altered hormone transport, and/or action at the level of target tissues. Although it was thought for a long time that these alterations were secondary to obesity, usually being improved after weight loss, it has recently become evident that they may conversely play a cardinal role in the development of obesity and associated metabolic abnormalities **(8)**(Fig1- 1).

The investigation of the relationship between androgens and obesity is of interest for several reasons:

Obesity has a profound impact on sex hormone secretion and metabolism.

androgens play a pivotal role in the regulation of different body fat distribution pattern according to sex .

Changes in androgen levels in males and females may favor the development of specific associated endocrine disorders.

Sex hormone imbalance may favor infertility in both females and males **(9).**

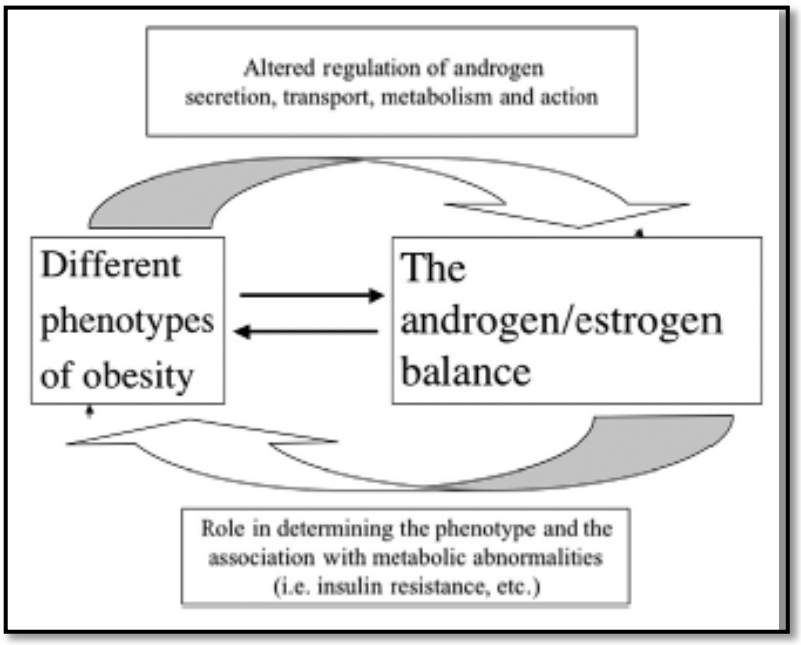


Fig1- 1 :Obesity and androgens(9) .

1.2.Type and Effect of Infertility

Primary infertility is infertility in a couple who have never had a child. Secondary infertility is failure to conceive following a previous pregnancy. Secondary Infertility may be caused by infection in the man or woman, but often there is no obvious underlying cause **(11)(12).**

1.3.The Main Causes of Infertility

The Centers for Disease Control (CDC) states that there are three major areas into which the reasons of female infertility can be broken down: faulty ovulation, transport, and implantation. Those categories are covered in more detail below **(13).**

1.3.1.Defective Ovulation

The following factors can lead to defective ovulation:

Endocrine conditions: An excess of prolactin caused by hypothalamic and pituitary gland dysfunction may impede ovulation.

The thyroid and other endocrine glands including the adrenals might postpone ovulation.

Infertility can result from the corpus luteus's inability to produce enough progesterone to thicken the uterine lining, which prevents the fertilized egg from implanting

decreases glucose uptake by muscle, might induce infertility **(14).** As a result, the pancreas produces a lot of insulin, lead to Increases LH levels and testosterone and Low FSH levels can also prevent ovarian follicles from producing eggs, which can result in fluid-filled ovarian cysts that eventually cover the entire ovary and impede conception **(15).**

World Health Organization subdivided ovulatory disorders into four classes.

- Hypogonadotropic hypogonadal anovulation: i.e., hypothalamic amenorrhea
- Norm gonadotropic norm estrogenic anovulation: i.e., polycystic ovarian syndrome (PCOS)
- Hypergonadotropic hypoestrogenic anovulation: i.e., premature ovarian failure
- Hyperprolactinemic anovulation: i.e., pituitary adenoma**(16).**

1.3.2.Defective Transport

The following can result in faulty sperm and egg transport:

Ovum: Fimbria adhesions, gonorrhea, peritonitis, Pelvic Inflammatory Disease (PID), and prior tubal surgery can all result in tubal obstruction, which prevents the release or trapping of the egg and delays conception(16).

Scar tissue following abdominal surgery may affect the mobility of the ovaries, fallopian tubes, and uterus, leading to infertility(16).

1.3.3.Defective Implantation

Following factors can lead to defective implantation:

Bicornuate uterus and uterine fibroids close to the fallopian tubes or cervix are examples of congenital uterine anomalies that might affect embryo implantation and result infertility**(18)**.

Obesity, anorexia nervosa, and extreme exercise are an example of physical disorders that can cause overweight or malnutrition, later disrupt the menstrual cycle, and ultimately render a couple infertile **(19).**

Other factors that can affect a woman's chances of conceiving include being overweight or underweight, or her age as female fertility declines after the age of 30. Sometimes it can be a combination of factors, and sometimes a clear cause is never established **(20).**

It is well known that an increase in body weight and fat tissue is associated with several abnormalities of sex steroid balance in both premenopausal and postmenopausal women. Such alterations involve both androgens and estrogens and their carrier protein, sex hormone–binding globulin (SHBG), which binds testosterone and dihydrotestosterone with high affinity and estrogens with lower affinity.

Changes in SHBG concentrations lead to an alteration of androgen and estrogen delivery to target tissues **(21).**

Body fat distribution has been demonstrated to substantially affect SHBG concentrations in obese women. In fact, female subjects with obesity usually have lower SHBG concentrations in comparison with their age- and weight matched **(22).**

This seems to be partly dependent on higher circulating insulin and on the inhibiting capacity of insulin on SHBG liver synthesis.

The pattern of body fat distribution can regulate androgen production and metabolism to a significant extent. Obesity has higher testosterone production rates **(23).**

The maintenance of normal circulating levels of these hormones in obesity may lead to predicting the presence of a sophisticated regulation, which can adjust both the production rate and the metabolic clearance rate of these hormones to body size. Due to the greater reduction of SHBG concentrations, the percentage free testosterone fraction tends to be higher in women with obesity, testosterone or SHBG concentrations, regardless of body mass index (BMI) values. Therefore, a condition of "relative functional hyperandrogenism" appears to be associated with the abdominal obesity phenotype in women **(24)**.

1.4. Epidemiology

Infertility is a global reproductive health problem and the prevalence rate increased and varies widely by geographic location around the world. With environment and lifestyle changes, the incidence of infertility might be associated with the delay of marriage and giving birth to the first child , environmental pollution , and unhealthy lifestyles . For patients diagnosed with infertility, psychological pressure rises and the relationships between family members deteriorate, which may greatly affect the quality of life. In addition, the declining birth rate could potentially worsen the aging problem **(25).**

According to recent studies of the World Health Organization (WHO), about (8-10%) of couples in the world suffer from one of the types of infertility **(26).**

Infertile female with overweight were maximum (42.65%), followed by female with normal weight (41.67%), underweight (8.33%), and obese (7.35%). (67.2%) had primary infertility and (32.8%) had secondary infertility. Of female with primary infertility, (8.8%) were underweight, (40.1%) were normal weight (45.3%) were overweight, and (5.8%) were obese. Of female with secondary infertility, (7.5%) were underweight,

(44.8%) were normal weight (37.3%) were overweight, and (10.4%) were obese **(27).**

Prevalence of primary infertility and secondary infertility in Iran were respectively 5.2% and 3.2% **(28),(29).**

1.5. Risk factor

Female fertility is known to decline with

- Age: About 1 in 5 (22%) couples in which the woman is 30-39 have problems conceiving their first child, compared to about 1 in 8 (13%) couples in which the woman is younger than 30. Fertility declines with age primarily because egg quality declines over time. In addition, older women have fewer eggs left and they are more likely to have health conditions that can cause fertility problems **(30).** Smoking, excessive alcohol use, people with overweight or obesity or underweight, extreme weight gain or loss, excessive physical or emotional stress that results in amenorrhea (absent periods) **(31).**

1.6.Obesity

Obesity is a medical condition, sometimes considered a disease, in which abnormal or excess body fat has accumulated to such an extent that it may have a negative effect on health **(32).**

Obesity is a major cause of disability and is correlated with various diseases and conditions, particularly cardiovascular diseases, type 2 diabetes, obstructive sleep apnea, certain types of cancer, and osteoarthritis. High body mass index BMI is a marker of risk for, but not a direct cause of, diseases caused by diet and physical activity. A reciprocal link has been found between obesity and depression, with

obesity increasing the risk of clinical depression, and also depression leading to a higher chance of developing obesity **(33).**

Obesity has individual, socioeconomic, and environmental causes. Some of the known causes are diet, physical activity, urbanization, genetic susceptibility, medications, mental disorders, economic policies, endocrine disorders, and exposure to endocrine-disrupting chemicals**(34**).

Diet quality can be improved by reducing the consumption of energy-dense foods, such as those high in fat or sugars, and by increasing the intake of dietary fiber **(35) (36).**

In pathologic states, changes in hormone concentrations in the body can occur depending on altered secretion, transport, metabolism or action. In the preceding sections it was noted that androgen balance is completely different in obese subjects according to their sex. in women, in whom a condition of functional hyperandrogenism may take place, particularly in those presenting with the abdominal obesity (**37)**.

A reduction of SHBG, which occurs in obese individuals of both sexes, cannot, however, explain this disparate behavior of androgens, although in both men and, particularly, women, the abdominal obesity phenotype is associated with significantly lower SHBG levels than in their peripheral fat distribution counterparts **(38).**

It is possible therefore that a different estrogen/androgen ratio in obese women to that in obese men may be partly responsible for a sex difference in the SHBG concentrations, as reported in some studies. Undoubtedly other factors need to be involved to explain the disparate androgen status in obesity according to sex. Gonadotropins are the most important regulatory factors determining androgen synthesis in both men and women.

There are many other factors potentially responsible for the reduced hypothalamic-pituitary-gonadal (HPG) axis in male obesity and the development of the relative hyperandrogenism associated with obesity in females **(39).**

Obesity is a state of the excess buildup of fats where it leads to chronic low-grade inflammation, lipotoxicity, deranged endocrine system and the female sex hormones. It is speculated that each of these aspects plays a contributory role to infertility in obese. changes in the endocrine system and the hypothalamic-pituitary-ovarian (HPO) axis in obese women affect the female reproductive system **(40).**

Obese women are associated with higher levels of androgens that effects poor follicular growth, premature luteinization, atresia of ovarian cells and follicles, as well as poor endometrial development **(41).** The high circulating free fatty acids found in obesity is able to infiltrate.

Non -adipose tissues, leading to an effect known as "lipotoxicity", which causes oxidative stresses to the endoplasmic reticulum and mitochondria of those cells, leading to apoptosis. The stated effects could potentially explain how fat buildup can be linked to the many chronic conditions associated with obesity like atherosclerosis, stroke, diabetes, malignancies and even infertility **(42).**

With the excess of adipose tissue in obesity, the peripheral proud-action of androgens is raised leading to increased production of estrogens as well through peripheral aromatization. Resultant increase in production of estrogen may cause disruptions to the endometrial receptor- ivity as well as produce inhibitory feedback to the HPO axis, which disrupts gona otropin secretion and pituitary LH pulse amplitude. This leads to poor oocyte recruitment and poor follicular growth **(43).**

So adiposity is the main culprit for infertility in obese women, losing weight should be effective in the resumption of fertility. Weight

reduction is possible through negative energy balance, where one's energy expenditure is greater than energy intake. Weight loss is associated with better ovulation and pregnancy rates, live birth rates and menstrual regula rity(**44).**

Excess adipose tissues can stimulate insulin secretion from pancreatic beta cells . Furthermore, the excess circulating fatty acids may amplify hepatic lipid production and disrupt hepatic function, which leads to compensatory mechanism of hyperinsulinemia **(45).**

The ovaries are still sensitive to the effects of insulin, where insulin acts directly on the theca and granulosa cells by upregulating CYP17A1 enzymes to enhance steroidogenesis. Insulin is also able to enhance the effects of LH on steroidogenesis by increasing LH receptors and improveing ovarian LH-binding capacity. Moreover, insulin enhances the effects of FSH which results in greater production of androgen substrates and thus elevates es trogen levels in the developing follicles (**46).**

The pituitary gland also remains its sensitivity to insulin, where insulin can amplify the sensitivity of gonadotroph cells to gonadotropin-releasing hormones (GnRH), which then increases LH production and further stimulating ovarian steroidogenesis. As consequences of elevated steroidogenesis and hyperandrogenemia, follicular growth is arrested, differentiation and mitosis of granulosa cells are restricted, premature luteinization occurs,which is followed by premature follicular atresia **(47**).

Although the adrenal gland and gonads serve as the primary source of circulating steroid hormones, adipose tissue expresses a full arsenal of enzymes for activation, inter conversion, and inactivation of steroid hormones. Traditionally the primary determinants of steroid hormone action were thought to be circulating free steroid hormone concentrations and tissue-specific expression of steroid hormone receptors **(48).**

An additional determinant of steroid hormone action is tissue-specific prereceptor steroid hormone metabolism. Several steroidogenic enzymes are expressed in adipose tissue including cytochrome P450-dependent aromatase, 3-hydroxysteroid dehydrogenase (HSD), 3HSD, 11HSD1, 17HSD, 7-hydroxylase, 17-hydroxylase, 5- reductase, and UDP- glucuronosyltransferase 2B15**(49) .**

Cytochrome P450- dependent aromatase and 17HSD are two enzymes that are highly expressed in adipose tissue stromal cells and preadipocytes **(50).**

Aromatase mediates the conversion of androgens to estrogens: androstenedione to estrone and testosterone to estradiol. 17HSD mediates the conversion of weak androgens or estrogens to their more potent counterparts: androstenedione to testosterone and estrone to estradiol **(51).** Expression of 17HSD is decreased relative to aromatase in adipose tissue but increased relative to aromatase in visceraladipose tissue. The ratio of 17HSD to aromatase is positively correlated with central adiposity, implicating increased local androgen production in visceral adipose tissue. Thus, adipose tissue is an important site for both metabolism and secretion of sex steroids **(52).**

1.6.1. Classification of Obesity

Obesity is typically defined as a substantial accumulation of body fat that could impact health. Medical organizations tend to classify people as obese based on body mass index (BMI) – a ratio of a person's weight in kilograms to the square of their height in meters **(53).**

The classification of obesity is:

Underweight < 18.5, Normal weight 18.5 – 24.9**,** Overweight 25.0 – 29.9**,** Obese (class I) 30.0 – 34.9**,** Obese (class II) 35.0 – 39.9**,** Obese (class III) ≥ 40.0**(53).**

1.6.1.1 Effect of obesity on Endocrinology and system:

- Diabetes mellitus
- Polycystic ovarian syndrome
- Menstrual disorders
- Infertility
- Complications during pregnancy
- Birth defects
- Intrauterine fetal death **(54).**

1.6.2. Causes

The "a calorie is a calorie" model of obesity posits a combination of excessive food energy intake and a lack of physical activity as the cause of most cases of obesity. A limited number of cases are due primarily to genetics, medical reasons, or psychiatric illness. In contrast, increasing rates of obesity at a social level are felt to be due to an easily accessible and palatable diet, increased reliance on cars, and mechanized manufacturing**(55).**

A 2006 review identified ten other possible contributors to the recent increase of obesity: insufficient sleep, endocrine disruptors (environmental pollutants that interfere with lipid metabolism), decreased variability in ambient temperature, decreased rates of smoking, because smoking suppresses appetite, increased use of medications that can cause weight gain (e.g., atypical antipsychotics**)(56),** proportional increases in ethnic and age groups that tend to be heavier, pregnancy at a later age (which may cause susceptibility to obesity in children), epigenetic risk factors passed on generationally, natural selection for higher BMI.

According to the Endocrine Society, there is "growing evidence suggesting that obesity is a disorder of the energy homeostasis system, rather than simply arising from the passive accumulation of excess weight" **(57).**

1.6.3. Pathophysiology of Obesity

At a biological level, there are many possible pathophysiological mechanisms involved in the development and maintenance of obesity. This field of research had been almost unapproached until the leptin gene was discovered in 1994 by J. M**(58).** Friedman's laboratory. While leptin and ghrelin are produced peripherally, they control appetite through their actions on the central nervous system **(59).** They and other appetite-related hormones act on the hypothalamus, a region of the brain central to the regulation of food intake and energy expenditure. There are several circuits within the hypothalamus that contribute to its role in integrating appetite, the melanocortin pathway being the most well understood. The circuit begins with an area of the hypothalamus **(60).**

1.7. Androgens

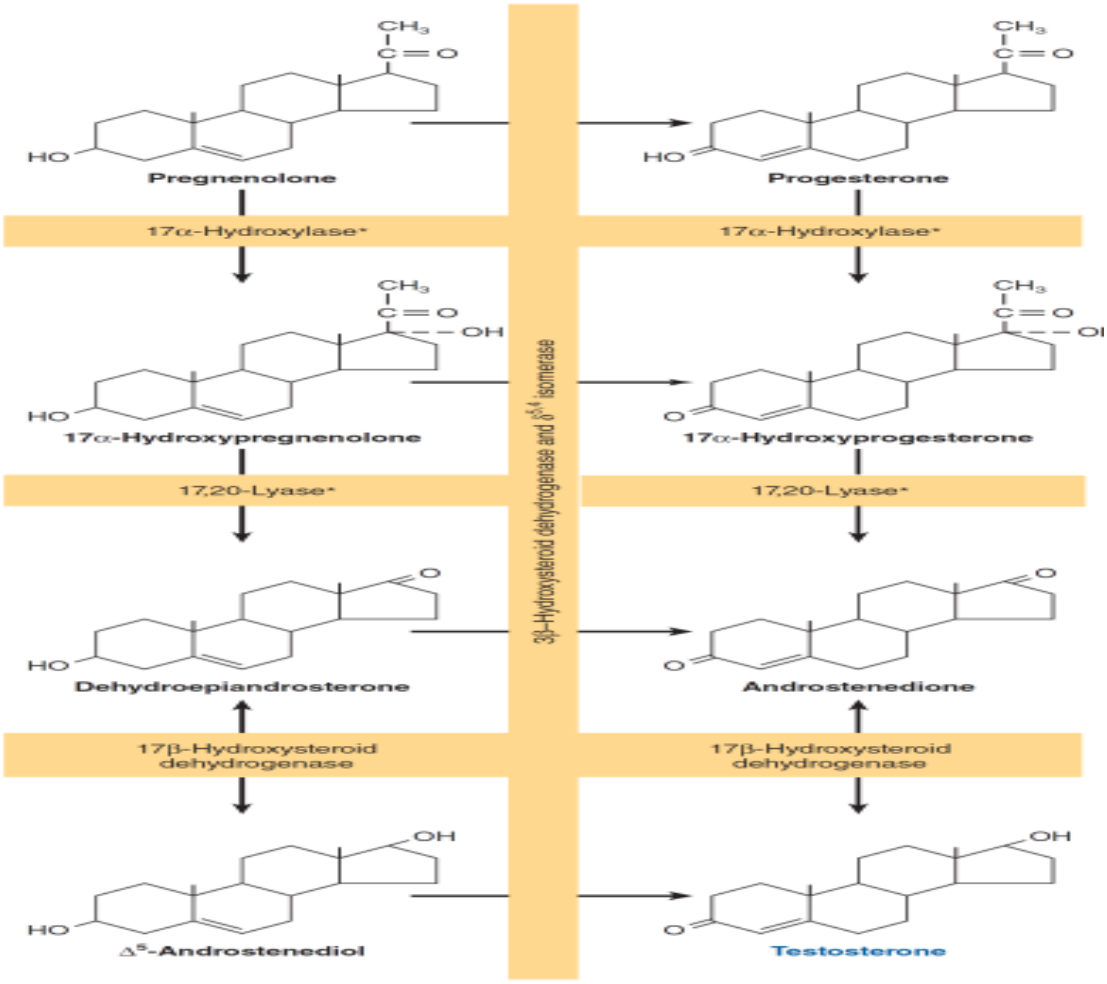
The adrenal cortex synthesizes the major androgens DHEA-S, DHEA, androstenedione, and testosterone, dihydrotestosterone (DHT) in the ovaries. The DHEAS, DHEA, and androstenedione are really hormone precursors, that are converted to total testosterone and DHT which are the active form **(61)**.

Testosterone and other androgens that are important for maintaining ovarian function, bone metabolism, sexual function, and female reproduction. As well as the androgens lead to increase levels of receptors

for the FSH hormone in the follicles, which is responsible for inducing follicular growth and development. So high androgen levels can lead to excessive growth with dysfunctional formation of antral follicles. On the other hand, too low levels of androgen are associated with less ovarian response and ovarian insufficiency **(62).**

Approximately (90-99%) of DHEA is produced from the adrenal cortex while androstenedione is produced in both ovaries and adrenal gland, 25% of total testosterone is synthesized in ovaries while 25% in the adrenal gland and the remaining part is produced through peripheral conversion from androstenedione in the skin, adipose tissue, and liver **(63).**

As well as obesity is also associated with an increased testosterone production rate. Synthesis of androgen from cholesterol which converted to pregnenolone then is converted to 17α-hydroxy pregnenolone and follows the removal of the two-carbon side chain through the action of 17,20-lyase **(64)**. The lyase activity catalyzes 17α-hydroxylation that important in both the adrenals and the gonads and acts exclusively on 17α-hydroxy-containing molecules, to produce DHEA-S by the actions of 17α-hydroxysteroid dehydrogenase, convert the DHEA into the androstenedione, reduction of androstenedione at the C17 position results in the formation of testosterone, that summarized in the figure (1-2) **(65).**



1-2:Biosynthasis of testosterone and dehydroepiandrosterone in ovary(66).

Testosterone is androstane class containing a ketone and a hydroxyl group at positions three and seventeen respectively. It is biosynthesis in several steps and is converted in the liver to inactive metabolites. It exerts its action through binding to and activation of the androgen receptor **(67).** In adult males, levels of testosterone are about seven to eight times as great as in adult females. As the metabolism of testosterone in males is more pronounced, the daily production is about 20 times greater in men. Females are also more sensitive to the hormone **(68).**

The largest amounts of testosterone (>95%) are produced by the testes in men, while the adrenal glands account for most of the remainder. Testosterone is also synthesized in far smaller total quantities

in women by the adrenal glands, thecal cells of the ovaries, and, during pregnancy, by the placenta. Like most hormones, testosterone is supplied to target tissues in the blood where much of it is transported bound to a specific plasma protein, sex hormone-binding globulin (SHBG) **(69).**

In general, androgens such as testosterone promote protein synthesis and thus growth of tissues with androgen receptors. Testosterone can be described as having virilising and anabolic effects.

Anabolic effects :include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation **(70).**

Androgenic effect :Include maturation of the sex organs, particularly the penis, and the formation of the scrotum in the fetus, and after birth (usually at puberty) a deepening of the voice, growth of facial hair (such as the beard) and axillary hair. Many of these fall into the category of male secondary sex characteristics**(71).**

Testosterone effects can also be classified by the age of usual occurrence. For postnatal effects in both males and females, these are mostly dependent on the levels and duration of circulating free testosterone **(72).**

Free testosterone lipophilic hormones (soluble in lipids but not in water), such as steroid hormones, including testosterone, are transported in water-based blood plasma through specific and non-specific proteins. Specific proteins include sex hormone-binding globulin (SHBG), which bind testosterone, dihydrotestosterone, estradiol, and other sex steroids **(73).**

1.7.1 Factors affecting testosterone levels may include:

Age: Testosterone levels gradually reduce as men age this effect is sometimes referred to as andropause or late-onset hypogonadism **(74).**

Exercise: Resistance training increases testosterone levels acutely **(75).**

Nutrients: Vitamin A deficiency may lead to sub-optimal plasma testosterone levels. The secosteroid vitamin D in levels of 400–1000 IU/d (10–25 µg/d) raises testosterone levels **(76).** Zinc deficiency lowers testosterone levels but over-supplementation has no effect on serum testosterone. There is limited evidence that low-fat diets may reduce total and free testosterone levels in men, and moderate evidence that short-term, very high protein diets (≥35% protein) decrease total testosterone levels in men(**77).**

Weight: Obesity has a profound impact on sex hormone secretion and metabolism.

Androgens play a pivotal role in the regulation of different body fat distribution pattern according to sex **(78).**

Miscellaneous: Sleep increases nocturnal testosterone levels.

Medical Herbs:

Natural or made antiandrogens including spearmint tea reduce testosterone levels. Licorice can decrease the production of testosterone and this effect is greater in females **(79).**

The plasma protein binding of testosterone is 98.0 to 98.5%, with 1.5 to 2.0% free or unbound. It is bound 65% to sex hormone-binding globulin (SHBG) and 33% bound weakly to albumin**(80).**

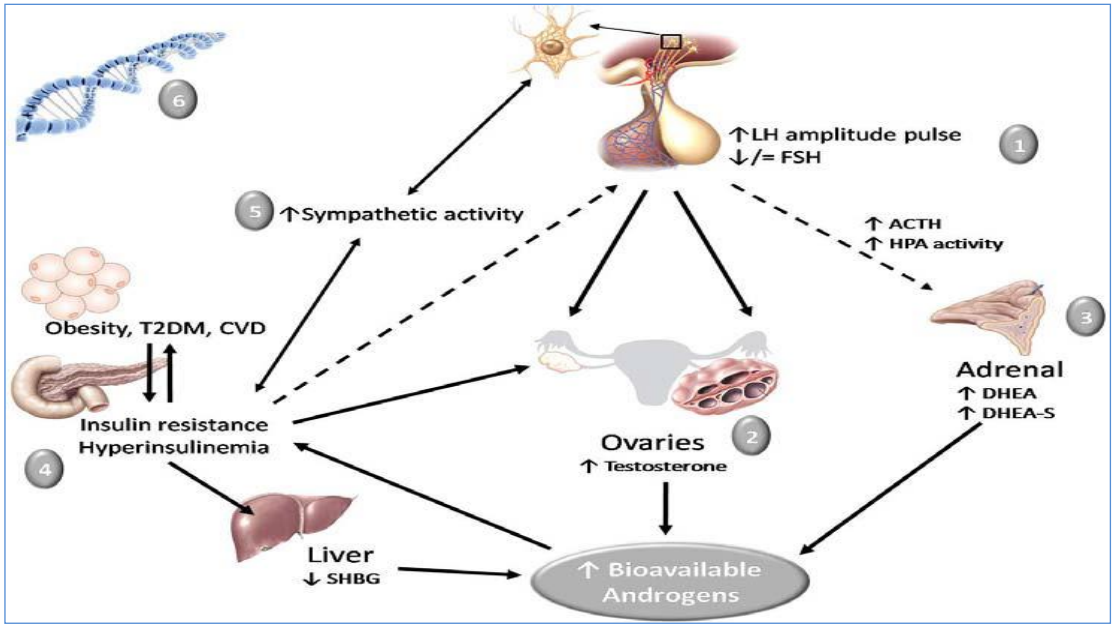


Figure 1-3 : Hyperandrogenism in patients with Obesity (81) Sympol: ACTH adreno corticotrophic hormone, CVD – cardiovascular disease, DHEA – dehydroepiandrosterone, DHEAS – dehydroepiandros- terone sulfate, FSH – follicle stimulating hormone, HPA – hypothalamic-pituitary-adrenal, LH – luteinizing hormone, SHBG – sex hormone binding globulin, T2DM – type 2 diabetes mellitus.

Figure 1-3 show as:

(1) Neuroendocrine defects: impaired the normal negative feed -back from sex-steroids on the hypothalamus and resulting high GnRH pulse frequencies high LH and limited production of FSH which promote androgen production **(81).**

(2) the ovary's theca cell response to high level of LH, a key factor in the hyperandrogenemia. The quantity of testosterone secreted varies

approximately in proportion to the amount of LH available. Progesterone is the primary regulator of gonadotropin-releasing hormone (GnRH) pulse frequency by the negative feedback effects of progesterone**,** produced a large androgens level causing disrupt the menstrual cycle (**82)**. Follicular atresia and leading to anovulatory process by inhibit follicle growth. 17β-hydroxysteroid dehydrogenases (17β-HSD) enzymes found in both theca and granulosa cell responsible for converting androstenedione to testosterone and converted testosterone in theca cell and converting estrone to estradiol in granulosa cell and the aromatase enzyme found only in granulosa cell response to convert the androgen to estrogen therefor the defect of FSH in granulosa cell lead to inhibition the aromatase enzyme and accumulation of androgen as shown in Figure 1-4**(83).**

(2) Impaired adrenal androgen production.

(3) Insulin resistance with hyperinsulinemia which effect directly or synergistically with LH stimulates androgen production from the ovarian theca cells and inhibits the hepatic synthesis of sex hormone-binding globulin (SHBG) may cause increases free testosterone concentration in the ovary **(84).**

(4) Increase sympathetic nerve activity.

(5) Genetic defects**(85).**

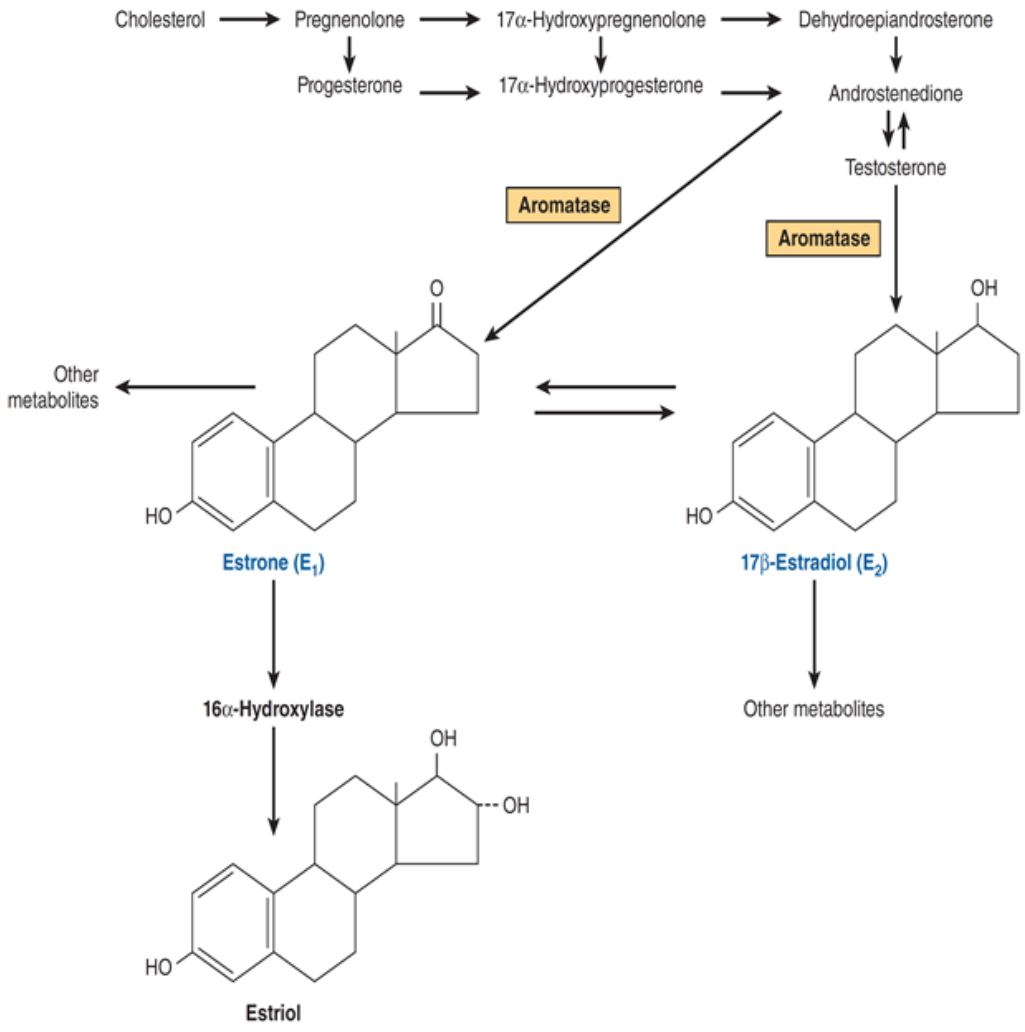


Figure (1-4): Biosynthasis of estrogen from testosterone (86)

1.7.2. The androgen receptor (AR)

The androgen receptor (AR), also known as NR3C4 (nuclear receptor subfamily 3, group C, member 4), is a type of nuclear recep- tor. that is activated by binding any of the androgenic hormones, including testosterone and dihydrotestosterone. in the cytoplasm and then translocating into the nucleus. The androgen receptor is most closely related to the progesterone receptor, and progestins in higher dosages can block the androgen receptor**(87).**

The main function of the androgen receptor is as a DNA binding transcription factor that regulates gene expression.

The AR plays a role in regulating female sexual, somatic, and behavioral functions. the promotion of cardiac growth, kidney hypertrophy, cortical bone growth and regulation of trabecular bone structure is a result of DNA-binding-dependent actions of the AR in females **(88).**

The mutations cause the inactivation of AR due to mutations conferring resistance to circulating testosterone, with more than 400 different AR mutations reported (**89).**

In some cell types, testosterone interacts directly with androgen receptors, whereas, in others, testosterone is converted by 5-alpha-reductase to dihydrotestosterone, an even more potent agonist for androgen receptor activation. Testosterone appears to be the primary androgen receptor-activating hormone, whereas dihydrotestosterone is the main androgenic hormone **(90) .**

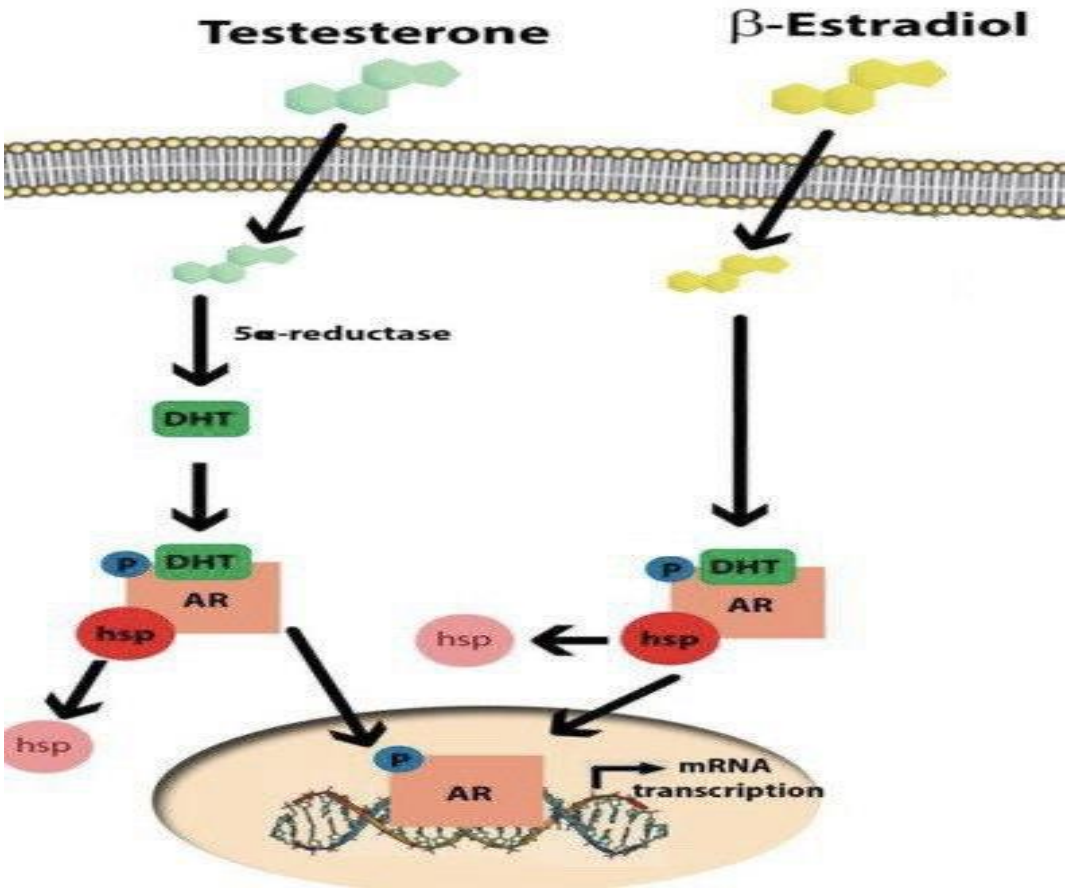
Androgens cause slow maturation of the bones, but more of the potent maturation effect comes from the estrogen produced by aromatization of androgens**(91).**

The binding of an androgen to the androgen receptor results in a conformational change in the receptor that, in turn, causes dissociation of heat shock proteins, transport from the cytosol into the cell nucleus, and dimerization. The androgen receptor dimer binds to a specific sequence of DNA known as a hormone response element**(92).** Androgen receptors interact with other proteins in the nucleus, resulting in up- or down-regulation of specific gene transcription. Up-regulation or activation of transcription results in increased synthesis of messenger RNA, which, in turn, is translated by ribosomes to produce specific

proteins**(93).** One of the known target genes of androgen receptor activation is the insulin-like growth factor 1 receptor (IGF-1R). Thus, changes in levels of specific proteins in cells is one way that androgen receptors control cell behavior. One function of androgen receptor that is independent of direct binding to its target DNA sequence is facilitated by recruitment via other DNA-binding proteins. One example is serum response factor, a protein that activates several genes that cause muscle growth **(94).**

More recently, androgen receptors have been shown to have a second mode of action. As has been also found for other steroid hormone receptors such as estrogen receptors, androgen receptors can have actions that are independent of their interactions with DNA. Androgen receptors interact with certain signal transduction proteins in the cytoplasm **(95).**

Androgen binding to cytoplasmic androgen receptors can cause rapid changes in cell function independent of changes in gene transcription on, such as changes in ion transport. Regulation of signal transduction pathways by cytoplasmic androgen receptors can indirectly lead to changes in gene transcription, for example, by leading to phosphorylation of other transcription factors **(96).**



Figure(1-5): Normal function of the androgen receptor. Testosterone (T) enters the cell and, if 5-alpha-reductase is present, is converted into dihydrotestosterone (DHT). Upon steroid binding, the androgen receptor (AR) undergoes a conformational change and releases heat-shock proteins (hsps). Phosphorylation (P) occurs before or after steroid binding. The AR translocates to the nucleus where dimerization, DNA binding, and the recruitment of coactivators occur. Target genes are transcribed (mRNA) and translated into proteins (97)

The androgen receptor (AR) plays an important role in regulating androgen action in men and women. The AR gene codes for a protein that has three major functional domains: an N-terminal domain, a DNA-binding domain, and an androgen-binding domain. The AR functions as a steroid hormone–activated transcription factor **(98).**

The polymorphic appear to influence the function of the receptor as a transcription factor, so that relatively long fragments are associated with low levels of receptor function and have been related to alterations in blood levels of testosterone **(99)**.

Androgen receptor dysfunction causes a diverse range of clinical conditions, including testicular feminization mutation, prostate cancer, and motor neuron disease (Kennedy's disease) **(100).**

In women, associations between AR polymorphism and hirsutism, acne, androgenic alopecia, bone mass density, and breast cancer have been suggested **(101).**

Available studies therefore suggest some relationship between AR polymorphism, excess body fat, and fat distribution pattern in both sexes. Whether altered androgen action mediated by AR dysfunctions may play a role in the pathophysiology of different obesity phenotypes is, however, still controversial **(102).**

The effects of testosterone in humans and other vertebrates occur by way of multiple mechanisms: by activation of the androgen receptor (directly or as dihydrotestosterone), and by conversion to estradiol and activation of certain estrogen receptors. Androgens such as testosterone have also been found to bind to and activate membrane androgen receptors **(103).**

Free testosterone(ft) is transported into the cytoplasm of target tissue cells, where it can bind to the androgen receptor as in figure 1-5,or can be reduced to 5α-dihydrotestosterone (DHT) by the cytoplasmic enzyme 5α-reductase. DHT binds to the same androgen receptor even more strongly than testosterone, so that its androgenic potency is about 5 times that of T**(104).** The T-receptor or DHT-receptor

complex undergoes a structural change that allows it to move into the cell nucleus and bind directly to specific nucleotide sequences of the chromosomal DNA. The areas of binding are called hormone response elements (HREs), and influence transcriptional activity of certain genes, producing the androgen effects**(105).**

Androgen receptors occur in many different vertebrate body system tissues, and both males and females respond similarly to similar levels. Greatly differing amounts of testosterone prenatally, at puberty, and throughout life account for a share of biological differences between males and females**(106).**

The bones and the brain are two important tissues in humans where the primary effect of testosterone is by way of aromatization to estradiol. In the bones, estradiol accelerates ossification of cartilage into bone, leading to closure of the epiphyses and conclusion of growth. In the central nervous system, testosterone is aromatized to estradiol. Estradiol rather than testosterone serves as the most important feedback signal to the hypothalamus (especially affecting LH secretion**)(107).**

In many mammals, prenatal or perinatal "masculinization" of the sexually dimorphic areas of the brain by estradiol derived from testosterone programs later male sexual behavior **(108).**

1.8. Single Nucleotide Polymorphism:

Single nucleotide polymorphisms or SNPs is a substitution of a single nucleotide at a specific position in the genome, that is present in a sufficiently large fraction of the population **(109)**. DNA sequence variations that occur when a single nucleotide (A,T,C, or G) in the genome sequence is altered. For example a SNP might change the DNA sequence AAGGCTAA to ATGGCTAA. For a variation to be considered

a SNP, it must occur in at least 1% of the population. SNPs, which make up about 90% of all human genetic variation, occur every 100 to 300 bases along the 3-billion-base human genome. Two of every three. About 12 million of SNPs have been known **(110).** Mutations defined as a sequence variations that occur in less than 1% of the population while the more widespread variation are known as polymorphisms. The main common genetic variations are single nucleotide polymorphisms (SNPs) **(111)(112).**

The common form of SNPs are transition (pyramidine-pyramidine C↔T or purine-purine A↔G) or transversion (pyramidine-purine or purine-pyramidine) replacements. About 2/3 of SNPs are transition replacements, while 1/3 of SNPs are transversion replacements **(113).**

The SNPs classification is based on their genomic site. The coding SNPs (cSNP) are situated in translated sequences (exons) of DNA and non-coding SNPs are located in untranslated sequences (introns) of DNA. SNPs may be either synonymous or non-synonymous**(114) .**

The synonymous cSNPs are classically silent and change the sequence of DNA, but do not change the sequence of amino acid in protein while the non-synonymous cSNPs change the sequence of DNA in a coding region and it change the sequence of amino acid in protein. Because of a changes in the amino acid function and structure, these cSNPs are considered as genetic markers for several diseases **(115).**

The bulk of SNPs are situated in the non-coding region of genome. On the other hand, some of these intronic SNPs have no recognized role but may participate in a regulation of the gene expression of coding regions, these SNPs are known as regulatory SNPs (rSNPs). Regulatory

SNPs situated in the promoter area may influence on the sites of transcription factor(116).

In general the non-synonymous SNPs in a coding sequence are more likely to affect the function or availability of a protein than other SNP classes **(116).**

1.9. Androgen receptor Gene:

The gene coding for AR is located in the X-chromosome at Xq11.2-q12 and is over 90 kb long Fig 1-6 as shown **(117).**

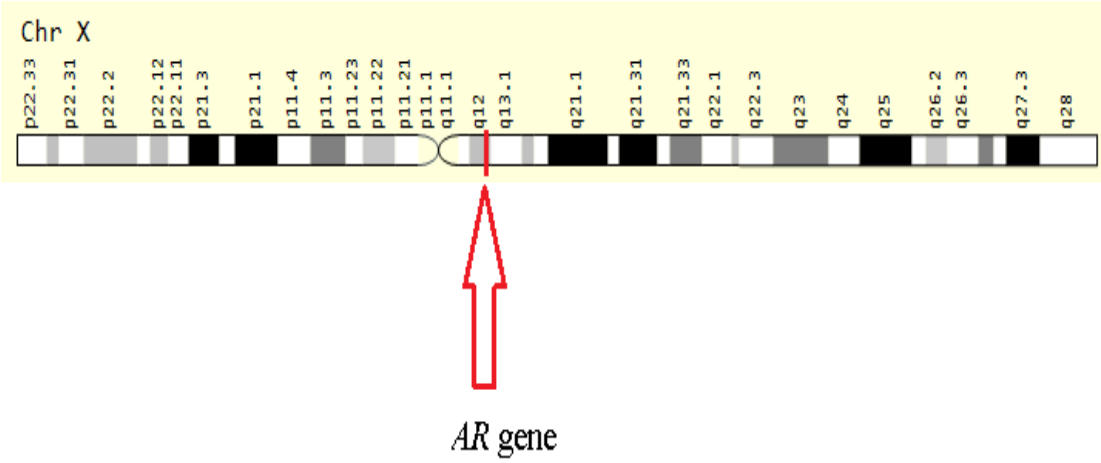


Figure 1-6 : Location of Androgen recptor gene in chromosome Xq12

Aim of the study

1. The effect of obesity on free testosterone(ft) , total testosterone(t), dehydroepiandrosterone sulfate (DHEAS) and androgen receptor (AR) levels in infertile obese women.
2. Evaluate the risk of single nucleotide polymorphism of Androgen receptor gene (rs6152 G > A) on infertile obese women and comparison with control group in Babylon Province.

Chapter Two

Materials and Methods

2.1 Materials and Methods

2.1.1 Chemicals and Kits

Chemicals and kits that used in this study were listed in Table 2-1:

Table 2-1: Chemical and kits used in the study

No.	Chemicals	Origin
1.	Agarose	BioBasic (Canada)
2.	Absolute ethanol	Germany
3.	Androgen receptor ELISA kit	Elabscience
4.	DNA extraction kit (blood)	Favorgen (Taiwan)
5.	Ethidium bromide	Intron biotechnology korea
6.	Free Testosterone ELISA kit	DRG (USA)
7.	Human Dehydroepiandrosterone sulfate ELISA kit	Maglumi / China
8.	Nuclease free water	Cyntol russian
9.	Primer Forward, Revars	(korea)
10.	Proteinase K (10 mg/mL)	Promega
11.	PCR Master Premix kit	Cyntol russian
12.	Restriction enzyme	sibEnzyme russian
13.	TBE buffer(tris base-boric acid-EDTA)	thomas baker india
13.	TE buffer	Intron biotechnology (Korea)
15.	Testosterone ELISA kit	DRG (USA)
16.	100 bp DNA ladder	Cyntol(Russian)

2.1.2 Instruments and Material

The instruments and equipment used in this study are shown in Table 2-2:

Table 2-2: Instruments and equipment used in the study.

Autoclave	**Haramaya / Japan**
Centrifuge EBA 20	**Hettich (Germany)**
Chemiluminescence Immunoassay (CLIA) analyzers	**Maglumi 800/ China**
Disposable syringes (5 mL)	**Medical jet (Syria)**
Distillator	**Bibby science (England)**
Deep Freeze	**GFL / Germany**
Digital scale	**China**
EDTA tube	**AFCO , Jordan**
ELISA system	**Bio-tech instruments (USA)**
Eppindorf tube (0.5 ml)	**China**
Horizontal gel electrophoresis (agarose)	**ATTO/ Japan**
Hood	**labtech / Korea**
Incubator	**Fisher scientific (germany)**
Multiple micropipettes	**Watson Nexty (Japan)**
Micropipettes	**Dragon lab china**
Magnetic Stirrer with Hot plate	**Grant / England**
Nanodrop	**Nanodrop 2000 (USA)**
PCR Thermo cycler	**Biometra Germany**
Photo documentation	**E-Graph/ Japan**
PH meter	**Hanna /China**
Vortex (Electronic)	**Kunkel /Germany**
Water bath	**GFL / Germany**
(0.01 ml ,0.1 ml, 1ml) pipette tips	**China**

2.1.3 Place of Study

This study was carried out for patients attended to Babylon Teaching Hospital for Maternity and Children in Hilla city and private clinics. The practical side of the study was performed at the laboratory of Biochemistry Department/ College of Medicine.

2.1.4 Study Design

This is a case control study.

2.1.5 Study individuals

The present study included 90 women. The first group included 45 patients with obese infertility, BMI (30-39) Kg/m^2 and age (20-30)y . The second group included 45 apparently healthy individuals, BMI(20-24.9) Kg/m^2 and age (20-30)y which was the control group.

2.1.6 Ethical Issues

Depends on the following:

a- Approval of scientific committee in Chemistry and Biochemistry Department College of Medicine, University of Babylon.

b- The objectives and methodology of this study were explained to all participants in the current study to gain their verbal acceptance.

c-Written approval from the Babylon Health Department.

d- Written approval from the Research Ethics Committee of the College of Medicine.

2.1.7 Data Collection

The inclusion and exclusion criteria for this study were as follows:

- ***Inclusion criteria:*** All females in this study included:
  - ❖ Obese infertile women.

- Reproductive age (20-30)y.
- Normal weight and infertile women for control.

Exclusion criteria: patients have the following conditions were excluded:

- Hypertension
- Thyroid disease
- PCOs
- Patients who were taken any hormonal medicine.

Questionnaire

The socio- demographic characteristics that composed of age, family history , residence .

2.1.8 Sample Collection

The blood samples were collected during the period extended July 1/7/2022 to January 30/1/2023 .

Five milliliters of venous blood was obtained by 5ml disposable syringe, and for these two milliliters of the blood was drained into EDTA tube and mixed gently, the blood in EDTA (ethylene diamine tetra acetic acid) tube stored at -20 ˚C and used for DNA extraction. While the rest 3ml of blood was drained into gel plain tube for serum preparation, which would be used in biochemical test.

2.2 Methods (Biochemical part)

2.2.1 Determination of Free Testosterone Concentration

Free testosterone concentration is measured by enzyme linked immunosorbent assay kit [**DRG**].

2.2.1.1.A. Assay Principles:

The DRG Free Testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with an antibody directed towards an antigenic site on the testosterone molecule.

Endogenous Free testosterone of a sample competes with a testosterone-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of free testosterone in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of free testosterone in the sample. **[118]**.

2.2.1.2.A. Reagents Preparation

Wash buffer: A volume of 1 ml of concentrated wash buffer was diluted 20 ml with distilled water.

Standard: The standard vial was centrifuged at 14000 rpm for 1 minute, and reconstituted the standard with 1.0 ml of reference standard and sample diluent. The lid was tightened and the standard was let to stand for 10 minutes and turned it upside down for several times. Reconstitution was produced a stock solution of 100 pg/ml. Then serial dilutions were made as needed. The recommended concentrations were as follows: 0 - 0.2 - 1.0 - 4.0 - 20.0 - 100.0 pg/mL. The standard was prepared within 15 minute before use.

Biotinylated detection Ab.: The biotin-antibody vial was centrifuged before opening, and then diluted to the working concentration using biotin-antibody diluents (1:100)ml.

Concentrated HRP conjugate: The concentrated HRP conjugate was diluted to the working concentration using HRP conjugate diluent (1:100)ml.

A. Assay procedure :

1. All reagents were brought to room temperature before use .
2. Twenty μL of each Standard, control and samples were added to the standard well.
3. A volume of 100 μL Enzyme Conjugate into each well, except for the blank well.
4. Incubated for 60 minutes at 37°C.
5. The wash process was repeated for three times by filling each well with was buffer (approximately 300 μL)
6. A 100 μL volume of *Substrate Solution* was add to each well.
7. The microplate wells coverd with a new sealer then incubated for 10Minutes at 37 C.
8. The reaction was Stopped by adding 100 μL of Stop Solution to each well.
9. The absorbance was read at 450nm using microtiter plate reader within 15 minutes. The standard curve is depicted in figure 2-1.

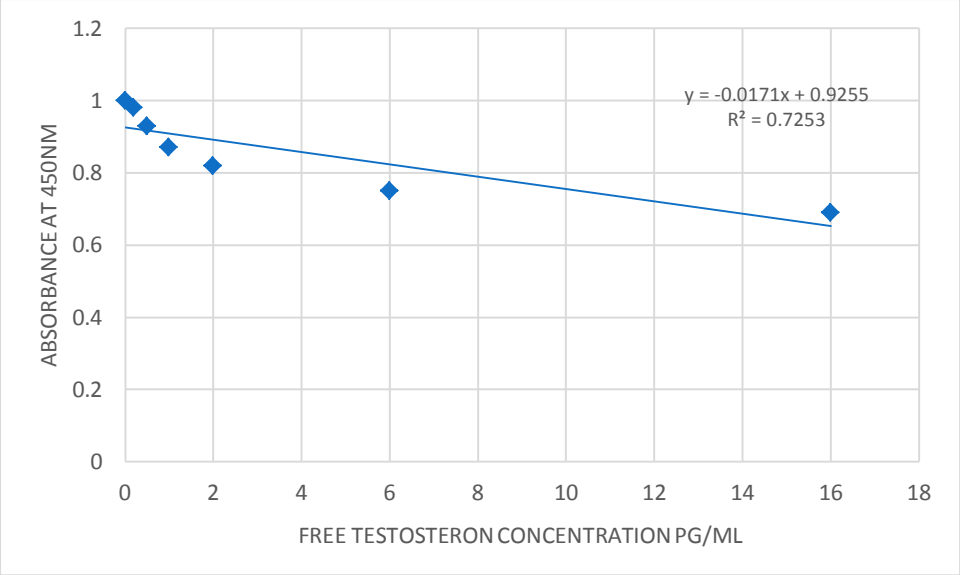


Figure 2-1 : **Standard Curve for Free Testosterone Concentration by ELISA**

2.2.2 Determination of Testosterone concentration

The DRG testosterone ELISA is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of Testosterone in serum and plasma.

2.2.2.1. A. Assay Principles:

The DRG testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a monoclonal [mouse] antibody directed towards an unique antigenic site on the testosterone molecule. Endogenous testosterone of a patient sample competes with a testosterone horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is reverse proportional to the concentration of testosterone in the sample. After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of testosterone in the patient sample.

[119].

2.2.2.2 .B. Reagents Preparation

Wash buffer: A volume of 1 ml of concentrated wash buffer was diluted with 20ml distilled water.

Standard: The standard vial was centrifuged at 14000 rpm for 1 minute, and reconstituted the standard with 1.0 ml of reference standard & sample diluent. The lid was tightened and the standard was let to stand for 10 minutes and turned it upside down for several times. Reconstitution was produced a stock solution of 100 ng/ml. Then serial dilutions were made as needed. The recommended concentrations were as follows: 0 , 0.2 , 0.5 ,1 , 2 , 6 ,16 ng/mL. The standard was prepared within 15 minutes before use.

Biotinylated detection Ab.: The biotin-antibody vial was centrifuged before opening, and then diluted to the working concentration using biotin-antibody diluents (1:100).

Concentrated HRP conjugate: The concentrated HRP conjugate was diluted to the working concentration using HRP conjugate diluent (1:100).

A. Assay procedure :

1. All reagents were brought to room temperature before use.
2. Twenty five μL of each Standard, control and samples were added to the standard well.
3. A volume of 200 μL Enzyme Conjugate into each well, except for the blank well.
4. Incubated well for 60 minutes at 37°C.
5. The wash process was repeated for three times by filling each well with was buffer (approximately 400 μL)
6. A 100 μL volume of Substrate Solution was add to each well.

7. The microplate wells coverd with a new sealer then incubated for 10Minutes at 37 C^0.
8. The reaction was stopped by adding 100 µL of Stop Solution to each well.
9. The absorbance was read at 450nm using microtiter plate reader within 15 minutes. The standard curve is depicted in figure 2-2.

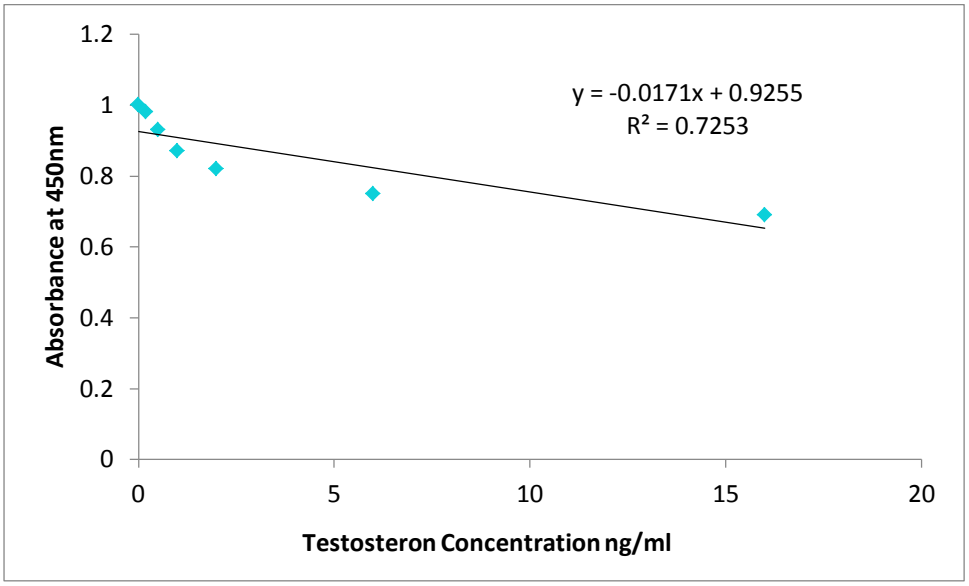


Figure 2-2: The Standard Curve for Testosterone Concentration.

2.2.3 Determination of Serum Dehydroepiandrosterone-Sulfate Concentration

2.2.3.1.A. Principle

Competitive immunoluminometric assay; label fluorescein isothiocyanate (FITC), combines with amine groups to produce protein conjugates (an anti-DHEA-S antigen labeled FITC), and ABEI coated with a purified DHEA-S monoclonal antibody while the magnetic microbeads that coated with anti-FITC polyclonal antibody.

Herein, sample, calibrators or control, FITC label, ABEI label, and nanomagnetic microbeads are completely combined and incubated at 37°C to form complexes Ab-Ag, then sediment in a magnetic field, decant the supernatant, and cycle wash.

The beginning reagents are then added, and the flash chemiluminescent process is started, within three seconds, a photomultiplier measures the light signal as RLU which is proportional to the amount of DHEA-S in the samples, the half-life of DHEA-S is 7-10 hours, whereas the half-life of DHEA is only 15-30 minutes**[120]**.

2.2.3.2.A. Preparation of the Reagent Integral

Microbeads were mixed prior to loading the reagent assembly onto the system for the first time to resuspend the microbeads that had settled during shipment.

The micro wheel in the microbeads chamber was rotated back and forth until the color of the suspension changed to brown, waited for 30 minutes for the integrator to sit in the reagent compartment, the magnetic microbeads were automatically agitated and completely resuspend during this period.

To preparation the wash buffer was added one bottle of concentrated washing to 10 liters of distal water.

2.2.3.3 . B. Calibration and Traceability

The instrument was calibrated by pressing the calibration button, as well as standard solution was added instead of the sample by replacing the sample with a standard solution.

The control for the maglumi system has been added to ensure that it was within the expected range, and if the room temperature changes more than 5 °C, the control must be added to ensure an accurate result via Maglumi's fully automatic chemiluminescence immunoassay (CLIA) analyzer.

2.2.3.4.C. Procedure

1- The reagents had been loaded into the device after scanning the code with the automatic area scanning of the device until, the device took the test information, the group containing the bead was suspended in the solution by automatic mixing that occurs after loading for 30 minutes.

2- A volume of 10μL of sample and control had been placed into the collection tube, entered into the device and then the starting was determined.

3- The samples had been withdrawn by needle pipettes and 50 μL ABEI Label, 50 μL FITC Label, 20 μL nanomagnetic into the cuvette; antigen of serum is competitive with FITC-labeled DHEA-S antigen for fusion with ferromagnetic nanomicrobeads 20 μL of the maglumi system array and then combined with 50 μL of purified ABEI-labeled DHEA-S monoclonal antibody.

4- The mixture had been incubated at 37°C for 15min.

5- The cuvettes were transferred to the washing station and the cycle was washed three times with 400 μL wash buffer to remove unconjugated contents.

6- The cuvettes had been moved to a three-second measuring room, which was a dark room, starters 1 and 2 had been added, they reacted with the ABEI chemiluminescence label by oxidative reaction of NaOH and H_2O_2 to indicate the chemical product.

7- The cuvettes were pushed into the waste bag after being measured and tested.

2.2.3.5 Calculation Result

Concentration was calculated automatically by the device, the concentration of DHEA-S in each sample was calculated using a

calibration curve generated by a two-point titration curve procedure. which results had been measured in a photomultiplier as RLUs proportional to the concentration of DHEA-S present in the sample.

2.2.4 Determination of Androgen Receptor concentration

A. Assay Principles:

This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to human androgen Receptor AR. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for human AR and avidin-horseradish peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain human AR, biotinylated detection antibody and avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The absorbance is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. the absorbance value is proportional to the concentration of human AR(**121)**.

2.2.4.2 B. Reagents preparation

Wash buffer: A volume of 1 ml of concentrated wash buffer was diluted into 20 ml of distilled water .

Standard: The standard vial was centrifuged at 14000 rpm for 1 minute, and reconstituted the standard with reference standard and sample diluent. The lid was tightened and the standard was let to stand for 15 minutes and turned it upside down for several times.

Reconstitution was produced a stock solution of 20 ng/ml. Then serial dilutions were made as needed. The recommended concentrations were as follows: 20, 10, 5, 2.5, 1.25, 0.63, 0.31 and 0 ng/ml. The standard was prepared within 15 minute before use.

2.2.4.3 C. Assay procedure

1. All reagents were brought to room temperature before use.
2. A volume of 100 microliter of the each standard and sample was added to the standard well.
3. A volume of 100 µl of antibody were add to testing sample wells.
4. A volume of 100 µl of streptavidin HRP reagent was dispensed into each well. Covered with a sealer and incubated for 60 minutes at 37 C^0.
5. The wash process was repeated for five times by filling each well with wash buffer (approximately 300 µl).
6. The liquid was removed at each step was essential to good performance then remaining wash solution was removed by aspirating and the plate was invert and blot it against clean paper towels .
7. A 90 µl volume of substrate was add to each well.
8. The microplate wells covered with a new sealer then incubated for 10 minutes at 37 $_oC$.
9. The reaction was stopped by adding 50 µl of stop solution to each well. The color was changed from blue to yellow color.
10. The absorbance was read the optical density at 450 nm using microtiter plate reader within 10 minutes. The standard curve is depicted in figure 2-3.

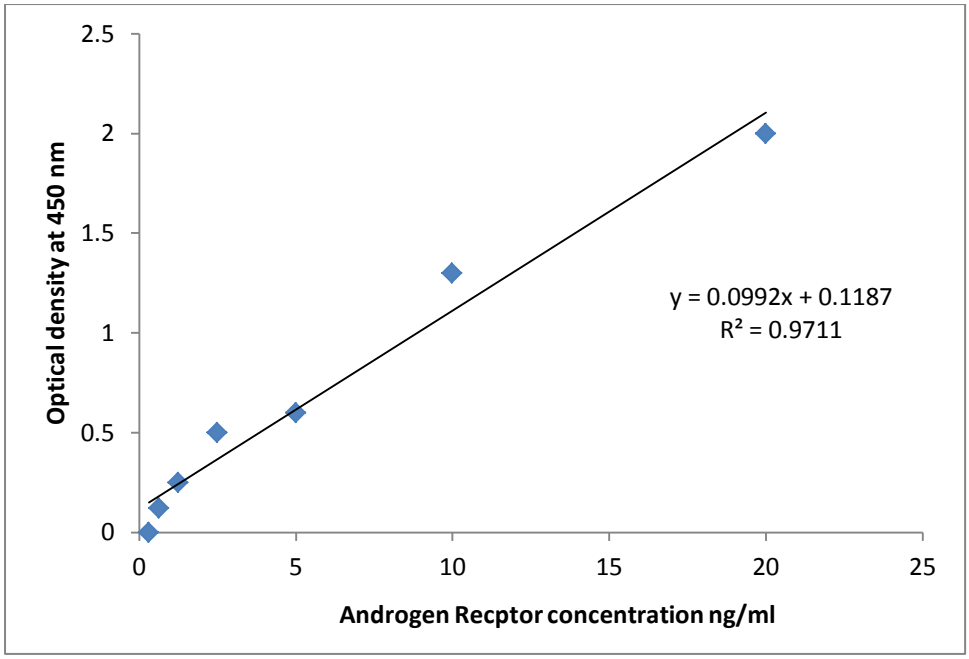


Figure 2-3: The Standard Curve for Androgen Receptor Concentration.

2.3 Methods (Genetic part)

2.3.1 DNA Extraction

The DNA was extracted from the venous blood by genomic DNA mini kit (Favorgen, Taiwan).

A. Principle (Silica Based Technology):

Silica matrices have special properties for DNA binding. They have positive charge and consequently they have large affinity for the negative charge of the DNA. High salt conditions and pH are performed to lyse cells and degrade protein, allowing DNA to bind to the glass fiber matrix of the spin column, by using sodium cations that bind strongly to the negatively charged oxygen of the DNA phosphate. Contaminants were removed with multiple washing steps by using a wash buffer (containing ethanol) and the purified genomic DNA was eluted by low ionic strength (pH ≥7) through TE buffer. These methods was faster and simpler procedure than other methods like organic extraction method **[122].**

B. Preparation of proteinase K and wash buffer:

Proteinase K was dissolved in 1.1 ml of DNase Deionized-Free Water ddH2O and 100ml of ethanol (96-100%) was added to the concentrated wash buffer .

C. DNA Extraction Procedure (For Frozen Blood):

The protocol of DNA extraction from blood involved several steps :

1- Two hundred µl of blood was carry over to a 1.5 ml micro centrifuge tube.

2- Thirty µl of proteinase K (10mg/ml) was added and briefly mixed. Then it was incubated for 15 minutes at 60℃.

3- Two hundred µl of FABG buffer was added to the samples then mixed by shaking vigorously.

4- The sample was incubated in a 70℃ water bath for 15minutes, during incubation the sample was inverted every 3 minutes.

5- Two hundred µl of ethanol(96-100%) was added to the test and vortex for 10 second.

6- Favorgene blood genomic (FABG) column was set to a 2ml collection tube.

7- The entire mixture (containing any precipitate) was transferred to the FABG column.

8- The sample were centrifuged for 5 minutes at full speed (10,000 x g).

9- The 2ml collection tubes containing the flow-through were discared, after that the FABG column was placed in a new 2ml collection tube.

10- Favorgene blood genomic column was washed with 400 µl W1 buffer and centrifuged for 30 seconds at full speed (10,000 x g) then the flow-through was discarded.

11- Favorgene blood genomic column was putted back in the 2ml collection tube and washed with 600 µl wash buffer (ethanol added)

then centrifuged for 30 seconds at full speed and discarded the flow-through.

12- The FABG column was placed back in the 2ml collection tube and centrifuged for additional 3 min to dry the column.

13- The FABG column was placed to a new 1.5 ml microcentrifuge tube.

14- One hundred µl of pre-heated elution buffer was added to the membrane center of FABG column and for 3-5 minutes the FABG column stayed standing to ensure the elution buffer was absorbed by the membrane.

15- FABG column Centrifuged for 30 seconds at full speed in order to elute the purified DNA.

16- The DNA fragment was stored at 4C ^{0}or -20C^{0}.

2.3.2 Determination of Concentration and Purity of DNA

Agarose gel electrophoresis and spectrophotometric methods were used to measure the concentration and purity of extracted DNA.

- **Agarose Gel Electrophoresis Method**

The quality of the isolated DNA was assessed by running 8 µL of each DNA sample on 1% agarose gel stained with Red Safe nucleic acid staining solution, then DNA sample was visualized by U.V. transilluminator **[123]**.

- **Spectrophotometric Method**

The purity and concentration of DNA were measured by nanodrop instrument (absorbance method). Absorbance readings were accomplished at 260 nm and at 280 nm. At 260 nm, the DNA strongly absorbs light while at 280 nm, the protein absorbs light most strongly. DNA purity can be estimated by A260/A280 ratio. The A260/A280 ratio

between 1.7 and 2.0 is generally accepted and it represent a high-quality DNA sample **[124,125]**.

Procedure

- One microliter of distilled water was applied on the highly sensitive micro detector of nanodrop as blank.
- The micro detector was cleaned up from blank.
- Then 1 µL of sample was applied on the micro detector of nanodrop.
- The concentration and A260/A280 ratio of DNA were recorded from the instrument **[126]**.

2.3.3 Detection of Single Nucleotide Polymorphism

Polymerase Chain Reaction-Restriction Fragment Length Polymorp-hism (PCR-RFLP) was used to investigate the rs6152G>A

2.3.4 Polymerase chain reaction (PCR).

PCR is a biochemical technique used to amplify a DNA piece for generating thousands to millions copies of a particular DNA sequence **[127]**. Several reagents and components are required for PCR, these components consist of DNA target (DNA template) that includes the region of DNA to be amplified, primers (forward and reverse primer) which are complementary to the DNA template, Thermus aquaticus polymerase (*Taq* polymerase), deoxynucleoside triphosphates (dNTPs) and buffer solution which provide an appropriate chemical environment for most favorable stability and activity of the DNA polymerase **[128]**.

The basic PCR steps are:

- **Initialization Step**

In this step, the reaction was heated to about 94-96 °C. It is required for DNA polymerases activation **[129]**.

- **Denaturation step**

This step includes heating the reaction to about 94-98°C for melting the DNA template by distraction of hydrogen bonds. The single stranded DNA molecules are yielded in this step.

- **Annealing step**

The temperature of reaction is lowered to 50-65 °C allowing for the primers to annealing with single stranded of DNA template. The *Taq* polymerase attaches to the primer-template hybrid and begins the formation of DNA.

- **Extension/Elongation step**

The optimum activity of *Taq* polymerase occurs at 72-80 °C and usually 72 °C is used with this enzyme. DNA polymerase synthesizes a new DNA strand that complementary to the DNA template strand by adding dNTPs in 5' to 3' direction. The extension time depends on both the length of the DNA target and the type of DNA polymerase.

- **Final elongation**

After the last cycle of PCR, this step is done to ensure that any remaining single stranded DNA is fully extended **[130,131]**.

2.3.5 Primers of Amplification

The AR gene was amplified by conventional PCR with specific primer for each SNP. The SNP (rs6152G>A) was amplified by specific primer by primer 3 program, as shown in table 2- 3.

Table 2-3 :The forward and reverse primers for *AR* gene SNP with their product size.

Markers	Primers	Primer sequence 5'-3'	Product size	GC%
rs6152G>A	F	5'-CCGCTGACCTTAAAGACATCCT-3'	206 bp	50
	R	5'-GGACACCGACACTGCCTTAC-3'		60

CCGCTGACCTTAAAGACATCCTGAGCGAGGC
CAGCACCATGCAACTCCTTCAGCAACAGCAG
CAGGAAGCAGTATCCGAAGGCAGCAGCAGCG
GGAGAGCGAGGGAgGCCTCGGGGGCTCCCAC
TTCCTCCAAGGACAATTACTTAGGGGGCACT
TCGACCATTTCTGACAACGCCAAGGAGTTGT
GTAAGGCAGTGTCGGTGTCC

G= 108 + 98
A= 206

2.3.6 Primer Pairs preparation and Storage

DNase Deionized-Free Water dd H2O was added to each primer to obtain primer stock tube that would be used again to obtain a working stock.

The following steps were followed for reconstituting and diluting the primers :

1- The tubes were spin down before opening the caps.
2- The desired amount of DNase Deionized-Free Water dd H2O were added according to the manufacturer to obtain a 100 pico moles/µl (Master Stock).
3- The tubes was vortex properly for re-suspend the primers evenly.
4- A volume of 10µl of the primers stock was transferred to a 1.5 ml Eppendorf tube that contains 90µl of sterile, nuclease-free water (Working Stock).
5- The primer stock was stored at -20 C^0.
6- The working stock was stored at 4 C^0.
7- The working stock was thawed on ice and vortexed before using in PCR and then stored at 4 C^0.

2.3.7 PCR Components of Amplification

Maxim PCR Pre-Mix Kit was used for amplification of AR gene **rs6152G>A**. Maxim PCR Pre-Mix Kit is a convenient premix type PCR amplification kit which containing i-Taq DNA Polymerase, dNTP mixture and reaction buffer in one tube for one reaction PCR. The reaction components for PCR amplification of AR genes that give the best results were described in Table 2-4.

Protocol

. template DNA and primers were added into Maxime PCR PreMixtubes (*i*-Taq).

- Nuclease free water was added into the tubes to a total volume of 20 µL.

Table 2-4 : Contents of the PCR Reaction Mixture

SNPs **rs6152G>A**	**Green master mix**	**Upstream primer**	**Downstream primer**	**DNA template**	**Nuclease free water**	**Total volume (µl)**
concentration	2.5x	10 Pmol/ml	10 Pmol/ml	10-20 ng/ µl		
Volume	8µl	1 µl	1 µl	2 µl	8	20

2.3.8 Optimization of PCR condition

Conventional PCR were used to amplify the target DNA using specific primer pairs. It includes three consecutive steps that repeated for specific number of cycles to get PCR product (amplicon) which can be finally visualized after agarose gel electrophoresis. The PCR thermocycler program that gave the best results of amplification of AR Gene **rs6152G>A** are shown in Table 2-5.

Table 2-5: Thermocycler Program for Optimization PCR Amplification of AR Gene (rs6152G>A) SNP

Step		**Temperature**	**Time**	**No. of Cycles**
1	Initial Denaturation	95°C	4 minutes	1 cycle
2	Denaturation	95°C	30 sec.	35 cycles
3	Annealing	55-67°C	30 sec	
4	Extension	72°C	30 sec.	

5	Final Extension	72°C	5 minutes	1 cycle

2.3.9. Agarose gel Electrophoresis

1. 1.6 grams of agarose were weighed and placed into a glass beaker and then 80 mL of 0.5 X TBE buffer (tris borate EDTA) were added. 0.5 X TBE buffer was prepared by diluting 5 X TBE buffer with distal water (one volume of 5 X TBE buffer with nine volume of distal water).
2. The solution was stirred on a microwave until dissolving the agarose and a clear solution was appeared.
3. ethedium bromide Staining Solution was added (2.5 µL).
4. The comb was placed in the gel tray about 1 inch from one end of the tray.
5. The gel solution was poured into the tray to a depth of about 5 mm.
6. The gel was allowed to solidify at room temperature for about 20 minutes.
7. The comb was gently removed and then the DNA Ladder (10 µL) and PCR product (10 µL) was loaded on each well with extreme cautions to avoid damages of the wells and cross contamination of neighboring wells.
8. The tray was placed in the chamber of electrophoresis and covered with a buffer (the same buffer used to prepare the agarose).
9. The cathode was connected to the well side of the unit and the anode to the other side.
10. Electrophoresis was done at 70 volts for 50 minutes **[132].**

2.3.10. Restriction Fragment Length Polymorphism

The basic technique of RFLPs involves fragmenting a DNA sample by a restriction enzyme, which can be distinguished. The fragments of

digested DNA are separated by agarose gel electrophoresis depending on the length of this fragments **[133]**.

The restriction enzymes that used in this study were:

Pce-I (high fidelity): this restriction enzyme cleave the sequence**:**
AGG↓ CCT

TCC↓ GGA

2.3.11 RFLP Program and Digestion Conditions

The (**rs6152G>A**) SNP of AR gene was assessed by using Pce-I restriction enzyme, the homozygotes genotype (AA) remains uncut (206 bp) whereas the wild genotype (GG) is digested at 108 and 98 bp fragments. the heterozygotes genotype (GA) produced three bands size 206, 108, and 98 bp [**134].**

RFLP program and reagents used in digestion reaction of AR gene **rs6152G>A** SNP that gave the best results include: 0.5 µL restriction enzyme was added to 15 µL of PCR product then 1.5 µL of dilution buffer and of nuclease free water 8 µL were added the incubated for 2 hr at 50℃. The restriction digestion products were analyzed on 2% agarose gel electrophoresis.

2.3.12. Analysis of the Digested Products by Agarose Gel Electrophoresis

After digestion with the restriction enzyme, the digested product was estimated by agarose gels electrophoresis. The rate of migration or mobility of DNA in gel through the electric field depends on its molecular weight, concentration of the agarose, voltage applied and strength of the electrophoresis buffer. The location of DNA within the gel

can be determined directly by staining with ethidium bromide nucleic acid staining solution, then gel was exposed to UV light and the photos was captured **[135]**.

Procedure

The same protocol was used for preparing 1% and 2 % agarose.

2.7 Statistical Analysis

The results of phenotypes data were expressed as mean ± SD. Student's t-test and the linear regression analysis were used for the evaluation of data.

The sample size was determined according to the online software OSSE (online sample size estimator) (osse.bii.a-star.edu.sg).

The output data expressed as odd ratio (OR), confidence interval (CI) 95% and P value. Statistical analyses were performed with SPSS (version 22). A P value less than 0.05 was considered to be statistically significant.

Chapter Three

Results and Discussion

3. Results and Discussion

3.1 General Characteristic of the Study Group

3.1.1 Body Mass Index (BMI)

In this study, the significant difference in BMI (P< 0.05) among patients and control groups, mean ± SD were (32.22±1.44), (22.0±1.47) respectively as in Table 3-1.

Table 3-1: BMI level

Subjects	Number	BMI Range	BMI Km/m^2 Mean ± SD	Age Years	P-value
Patients group	**45**	**30-39**	**32.22±1.44**	**20-30**	**P < 0.05**
Control group	**45**	**20-24.5**	**22.0±1.47**	**20-30**	

BMI: Body mass index; significant at p <0.05

Ferlitsch, et al. 2020, suggested that with each reduction of unit BMI, there is an increment in pregnancy rates. Studies implied reduction in weight is sufficient to improve ovulation rate, menstrual regularity and pregnancy rates **(134).**

which finding agreement with previous study by *Carlson, etal,2019* **(137)** who found, obese women, hardly become pregnant and have low chance of infertility treatment. In women with a body mass index BMI >30, compared with BMI <25.

3.1.2 Age

The present study included 45 patients with obese infertility with a mean age (28.488 ±7.41) years and an age range of (20-30) years. Besides, the study included 45 apparently healthy women with a mean

age of (26.66 ± 5.90) years and an age range of (20-30) years, the results show no significant differences ($p > 0.05$) in age between control group and patients group shown in Table 3-2.

This age matching helps to eliminate difference in parameters, results that may originate due to the significant variation in age.

Table 3-2: Means Age ± SD of obese infertility and control groups.

Subjects	Number	Age (Years) Means ±SD	Range (Years)	P-value
patients group	45	28.488 ±7.41	(20-30)	**P > 0.05**
Control group	45	26.66 ± 5.90	(20-30)	

SD: standard deviation; **non-significant at p > 0.05**

Age is a very important factor with regard to fertility, and even with all the advancements in assisted reproduction, it still remains an insurmountable barrier. Public awareness of this fact is important as the age-related decline **(138).** Age distribution in this study showed that most people were in the age range (20-30) years and this is relabel with another reviews like Lauren J, *et al*. **(139),** who found in their early teens, girls often have irregular ovulation resulting in irregular menstrual cycles, but by age 16 they should have established regular ovulation resulting in regular periods. A woman's cycles will remain regular, 26 to 35 days, until her late 30s to early 40s when she may notice that her cycles become shorter. Today, age-related infertility is becoming more common because, for a variety of reasons, many women wait until their 30s to begin their families **(140).**

Even though women today are healthier and taking better care of themselves than ever before, improved health in later life does not offset

the natural age-related decline in fertility. It is important to understand that fertility declines as a woman age due to the normal age-related decrease in the number of eggs that remain in her ovaries. This decline may take place much sooner than most women expect **(141).**

Both males and females become fertile in their teens following puberty. For girls, the beginning of their reproductive years is marked by the onset of ovulation and menstruation. It is commonly understood that after menopause women are no longer able to become pregnant (**141).**

Generally, reproductive potential decreases as women get older, and fertility can be expected to end 5 to 10 years before menopause **(142).** Naturally, there is an age-related decline in fecundity, the decrease usually starting at the age of 32 with a dramatic fall after the age of 37. Spelt differently, the natural monthly fecundity rate which is about 25% between 20 and 30 years of age decreases to below 10% above the age of 35 **(143).**

As time passes, she will begin to skip ovulation resulting in missed periods. Ultimately, periods become increasingly infrequent until they cease completely. When a woman has not had a menstrual period for 1 full year, she is said to be in menopause **(144).**

Age is perhaps the single most important factor in assessing an ovarian reserve and reflects both the quantity and quality of oocytes. Not surprisingly, in IVF cycles, older women tend to produce lesser number of oocytes and embryos derived from them have lower implantation potential. Further reflecting on the oocyte quality, women who conceive, experience higher miscarriage rates and increased incidence of congenital anomalies **(145).**

A diminished ovarian reserve can be either physiological (age related) or due to a premature decline in the reserve. The clinician needs

to be attuned to the effect of female age on fertility outcomes and render appropriate advice with early referral to an infertility specialist when required. Although infertility is defined as an inability to conceive following a year of unprotected intercourse, this definition needs to be modified in the older woman. Investigation and treatment should be initiated earlier in women 35 and above **(146).**

The assessment of the ovarian reserve helps in counselling as well as determining appropriate therapy. There is also a clear distinction between physiological and premature diminished ovarian reserves with the latter having a better prognosis in terms of clinical pregnancies. Establishing a woman's ovarian reserve is important as the patient would want to know the probability of success with treatment **(147).**

In contrast, active sperm production in men continues throughout the adult life, with age causing mainly a decline in function **(148)**.

3.2 Biochemical Results

3.2.1 Free Testosterone (FT) Concentration in Infertile Women and Control Group

Testosterone and other related hormones in the body (also known as androgens) clearly have important roles in healthy women. It is generally known that testosterone is important for muscle and bone strength and for growth of normal body hair. But testosterone may have favorable effects on mood, well-being, energy and 'vitality' in women **(149)**.

Testosterone mainly circulates in the blood bound to a protein called sex hormone binding globulin (SHBG). In women the

concentration of SHBG usually greatly exceeds the total testosterone level and very little free testosterone is present.

Androgens, both in excessive and depleted states, have been implicated in female reproductive health disorders. As such, serum testosterone measurements are frequently ordered by physicians in cases of sexual dysfunction and in women presenting with hirsutism **(150)**.

The present study revealed significant difference (P<0.05) in free testosterone level among patients and control groups as in Table 3-3.

Table 3-3: Free testosterone level of the studied Groups

Variables	Group	No	Mean± SD	p- value
FT(pg/ml)	patients	45	3.12±0.88	p < 0.05
	control	45	1.03 ±0.86	
Correlation of free testosterone with BMI in patients group				
	Correlation (r)		**p- value**	
BMI vs FT	0.378		p < 0.05	

FT: Free testosterone; BMI: body mass index; P< 0.05 significant

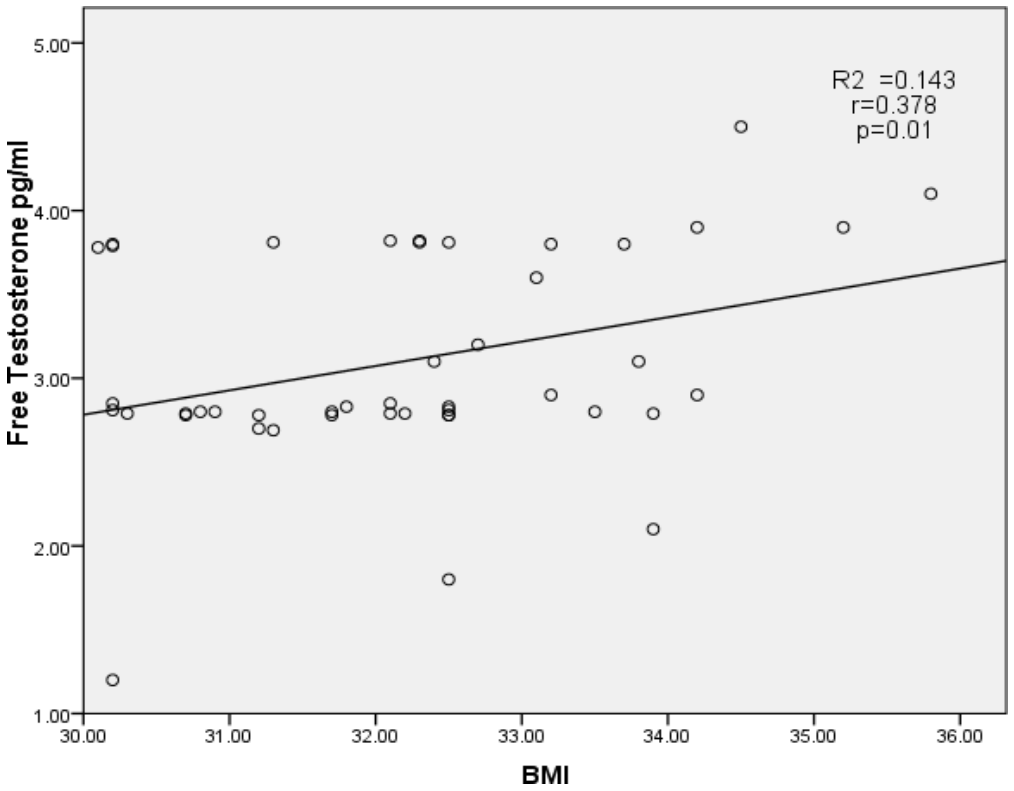


Figure 3- 1: Correlation between Free testosterone and BMI in patients group

These results might be attributed to impaired the normal negative feed-back from sex-steroids on the hypothalamus and resulting high GnRH pulse frequencies high LH and limited production of FSH which promote androgen production **(151).**

The present study observed significant positive correlation ($P<0.05$) of free testosterone level with body mass index in patients group Table 3-3, figure 3-1.

That means obesity causes this increment in free testosterone, this finding in agreement with previous studies by Eilerman, *et al.* 2009 **(150),** shows that obese females had higher free testosterone compared to normal weight females.

This may be because in obese female the serum levels of sex hormone-binding globulin (SHBG) tend to be lower, especially those with central obesity. It can be elevated by estrogens, iodothyronines and

growth hormones whereas insulin and androgens reduce it **(153).**

The hyperinsulinemic state associated with obesity may be the leading cause in reduced levels of SHBG, through the reduction in hepatic synthesis. This leads to more free circulating sex steroids, which have a higher tendency to get metabolic- ally cleared. With increased clearance, the body produces more androgens as compensation, resulting in a state of relative functional hyperandrogenism. Similarly, hyperandrogenism would then lead to poor ovarian function through premature luteinization and apoptosis of granulosa cells and follicles **(154).**

The adipose tissue is the biggest endocrine organ in the body where it is associated with glucose homeostasis, steroid production and metabolism, regulation of immune system and reproduction. It is able to produce androgens, store sex hormones and act as a conversion site for androgens, estradiol and dihydroepiandrosterone to be converted to estrogens, estrone, and androstenediol, respectively **(155) (156).**

3.2.2 Total Testosterone (T) Concentration in Infertile Women and Control Group

Androgens play important roles in the regulation of ovarian function. AR, expressed in oocytes, granulosa cells(GCs)and theca cells, is pivotal for normal follicular development. AR is most highly expressed in the GCs of preantral and early antral follicles, and its expression decreases as the follicles grow. by decreasing follicle atresia and GC apoptosis and stimulating the proliferation and differentiation of GC. Although AR is not expressed in primordial follicles, androgens promote primordial follicle initiation **(157) (158).**

The results of the present study showed significant differences between Infertile patients and controls ($p < 0.05$) regarding the value of total testosterone and BMI correlated significantly as in Table 3-4, figure 3-2.

Table 3-4: Total Testosterone Level in the stadied Groupe

Variables	Group	No	Mean± SD	p-value
T (ng/ml)	patients	45	0.14±0.185	p < 0.05
	control	45	0.41±0.12	
Correlation of total testosterone with BMI in patients group				
	Correlation (r)		**p- value**	
BMI vs T	-0.55		p < 0.05	

T: Total testosterone; BMI: body mass index; P< 0.05 significant

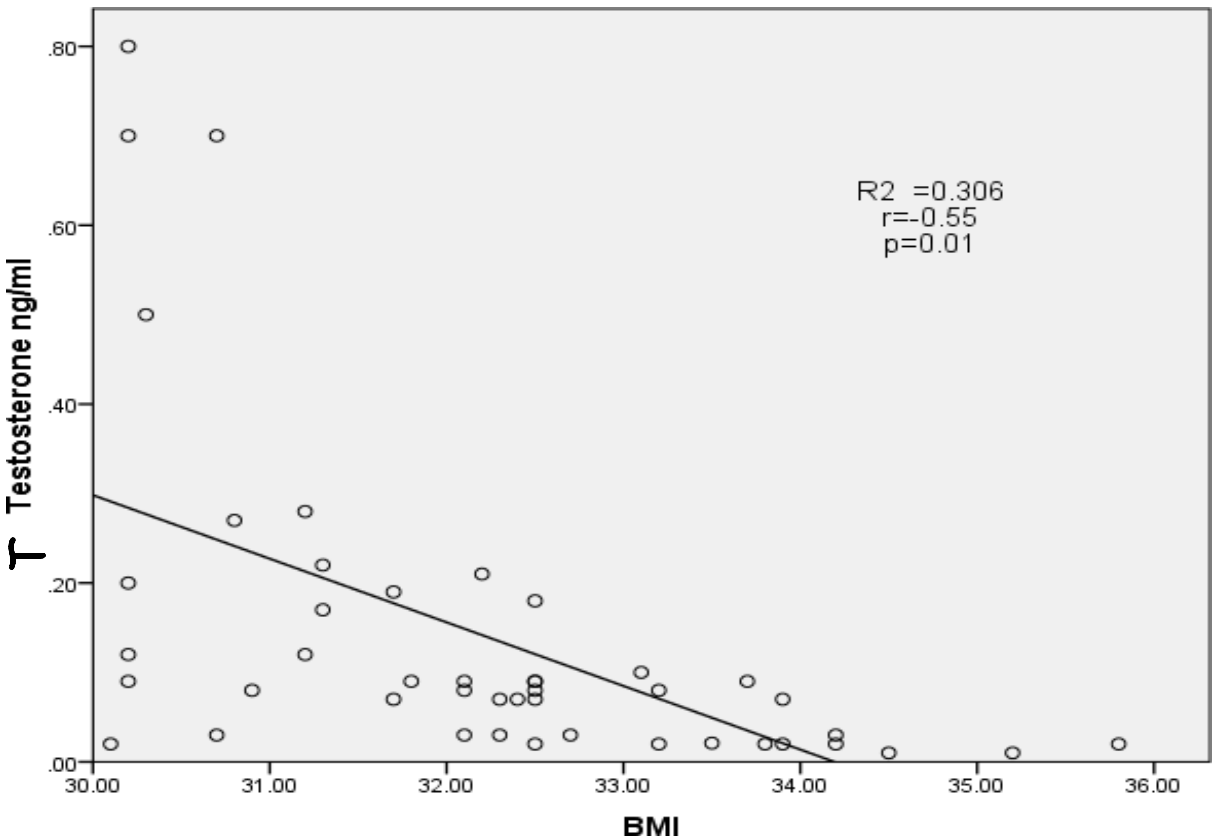


Figure 3- 2: Correlation between Totel. Testosterone and BMI in patients' group

This result might be caused by insulin resistance with hyperinsulinemia which effect directly or synergistically with LH stimulates androgen production from the ovarian theca cells and inhibits the hepatic synthesis of sex hormone-binding globulin (SHBG) that cause increases free testosterone concentration in the ovary **(159).**

On the other hand, androgen excess may lead to impaired ovarian function and dysregulated follicle development, displaying irregular cycles, oligo-ovulation and polycystic ovaries. These findings agree with our results that infertile women with higher testosterone T levels. Thus, an optimal balance in androgenic actions is necessary for maintaining normal ovarian function and androgens support follicle health **(160).**

The significant correlation ($P<0.05$) of BMI with total testosterone in infertile women means that obesity causes this decrease in testosterone, this finding agreement with previous studies by Sadeghi, *et al.*2022 **(159)** who show that obese females had higher testosterone compared to normal weight females.

Obesity causes decrease the level of total testosterone, this finding agreement with previous studies by Brewer C.J, *et al***.** 2010 **(160)** who found that the relative contribution of adipose tissue to whole body steroid metabolism is quite significant, with adipose tissue contributing up to 100% of circulating estrogen in postmen pausal women and 50% of circulating testosterone in premenopausal women**.** Lastly, free testosterone (FT) levels are usually higher in obese women without hirsutism, raising the possibility that increased concentrations of FT may have a negative effect on fertility.

3.2.3 (DHEAS) Concentration in Infertile Women and

Dehydroepiandrosterone Sulfate Control Group

dehydroepiandrosterone Sulfate DHEA helps fertility by supporting egg quality and improving. pregnancy rates in women with diminished ovarian reserve (DOR) DOR, also commonly called age-related infertility by fertility specialists, refers to small-sized follicles and a reduced ovarian follicular pool size at a given age **(149)**.

The results of the current study presented significant differences between infertile patients and controls ($p < 0.05$) regarding the value of DHEAS, BMI correlated significantly, as in Table 3-5, figure 3-3.

Table 3-5: Dehydroepiandrosterone Sulfate level DHEAS in the studied Groupe

	Group	No	Mean± SD	p- value
DHEA-S µg/dl	patients	45	249.6±87.33	p < 0.05
	control	45	169± 61.63	
Correlation of DHEA-S with BMI in patients' group				
	Correlation (r)		**p- value**	
BMI vs DHEA-S	0.295		p < 0.05	

DHEA-S: Dehydroepiandrosterone; BMI: body mass index; P< 0.05 significant

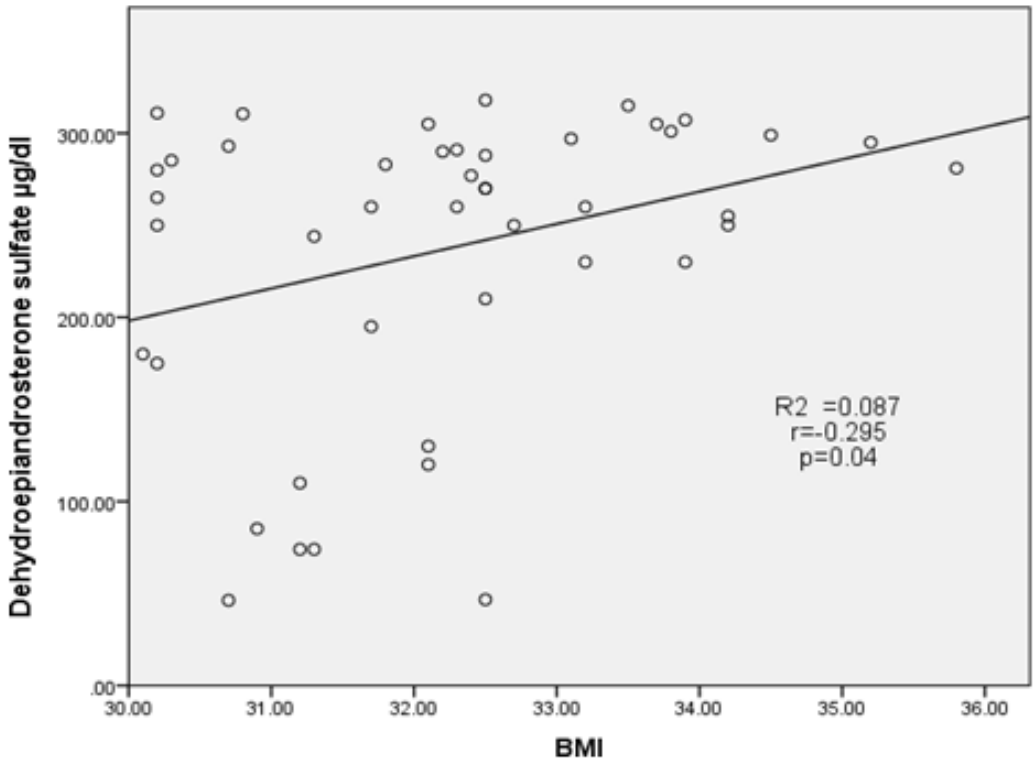


Figure 3- 3: Correlation between DHEAS and BMI in patients group

The main causes of elevated DHEA-S in infertile women may be due to impaired the normal negative feed-back from sex-steroids on the hypothalamus and resulting high GnRH pulse frequencies high ACTH and limited production of FSH which promote androgen production **(151).**

Our finding in harmony with Lizneva D, *et al* 2016 **(161)** who conducted that DHEA-S is bound to albumin and require conversion to testosterone to exert androgenic effects. Androstenedione is the most important precursor of testosterone, while DHEA accounts for only 5% and 13% of circulating testosterone in reproductive age women, therefor, the level of DHEA-S remaining higher in serum.

Body mass index BMI correlated significantly with DHEAS and these result consistence with Mäntyselkä A, *et al.* 2014 **(162),** who

revealed that the conversion to active androgens in peripheral adipose tissue may be enhanced by obesity (165,166).

3.2.4 Androgen Receptor (AR) Concentration in Infertile Women and Control g Group

The androgen receptor (AR) is expressed in multiple reproductive tissues, it is not surprising that androgens have important effects on multiple organ systems. These play critical roles in the regulation of many male, and female sexual, somatic and behavioral functions critical to lifelong health, functions as a steroid hormone–activated transcription factor **(167).**

Androgen receptor AR, is a type of nuclear receptor that is activated by binding any of the androgenic hormones. Activation of the AR has diverse biological effects in health and disease. As such, understanding the role of androgen action mediated via both the DNA binding-dependent and non-DNA binding-dependent actions of the AR**(168).**

Androgen deficiency in males and androgen excess in females produce metabolic dysfunction via deficient or excessive AR action, respectively, in multiple tissues including the central nervous system, liver, skeletal muscle, adipose and B-cells **(169)**.

In this study the level of AR decrease significantly in infertile women group compared to control, BMI correlated significantly with AR while age non significantly correlated, as in Table 3-6, figure 3-4.

Table 3-6: Androgen receptor level in the studied Groupe

	Group	No	Mean± SD	p- value
AR ng/ml	patients	45	1.09±0.82	p < 0.05
	control	45	2.11±0.73	
Correlation of Androgen receptor with BMI in patients group				
	Correlation (r)		**p- value**	
BMI vs AR	-0.340		p < 0.05	

AR: Androgen receotor; BMI: body mass index; P< 0.05 significant

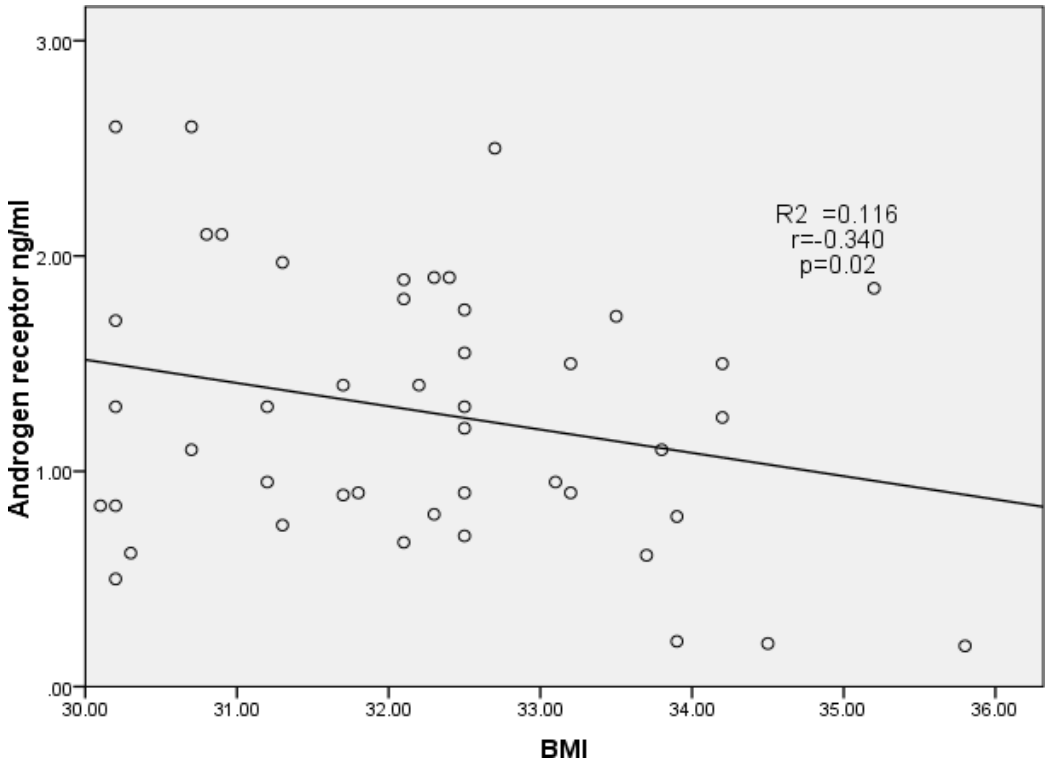


Figure 3-4: Correlation between AR and BMI in patients group

Interestingly, most studies have shown that obesity tends to be correlated with negative AR, even in premenopausal women, possibly due to the apoptosis to the ovarian cell due to permanent stimulation of ovarian cells to produce testosterone **(170)**.

Our finding agreement with previous studies **(160,171),** they found AR deficiency leads to dramatic metabolic dysfunction in aging males but in females it does not probably reflecting the lower concentration of testosterone and DHT**(172)**.

3.3 Genetic Results

3.3.1 Measurement of Concentration and Purity of Deoxyribonucleic Acid

A ratio of absorbance at 260 nm and 280 nm was measured for the estimation of DNA concentration and purity. Data was demonstrated in Table 3-7.

Table 3-7: DNA concentration and purity.

DNA concentration and purity	Mean ± SD
DNA concentration ng/µL	30.06 ± 7.27
DNA purity (260/280)	1.85 ± 0.08

3.3.2 *AR* receptor gene polymorphism in studied group

In current study the gene polymorphism of AR was studied in Iraqi patients with infertility and healthy control groups. The genotyping were Restriction Fragment Length Polymorphism (PCR-RFLP) technique used to confirm the results.

rs6152 G>A SNP within the AR gene included in present study considered as a new SNPs studied in infertile patients.

3.3.3 AR Receptor Gene (rs6152 G > A) Polymorphism

The amplification of *AR* receptor gene exhibited an amplicon of 206 bp. It contained the target SNP (rs6152G > A), figure 3-5 and figure 3-6.

The digestion of the product by a restriction enzyme explored three patterns:

- One band, the uncut fragment (206 bp) is the homozygous genotype (AA). This indicated the presence of polymorphism.
- Two bands (108 and 98 bp) are the wild genotype (GG). This indicated the absence of polymorphism.
- Three bands (206, 108 and 98 bp) are heterozygous genotype (GA). This indicated the presence of polymorphism.

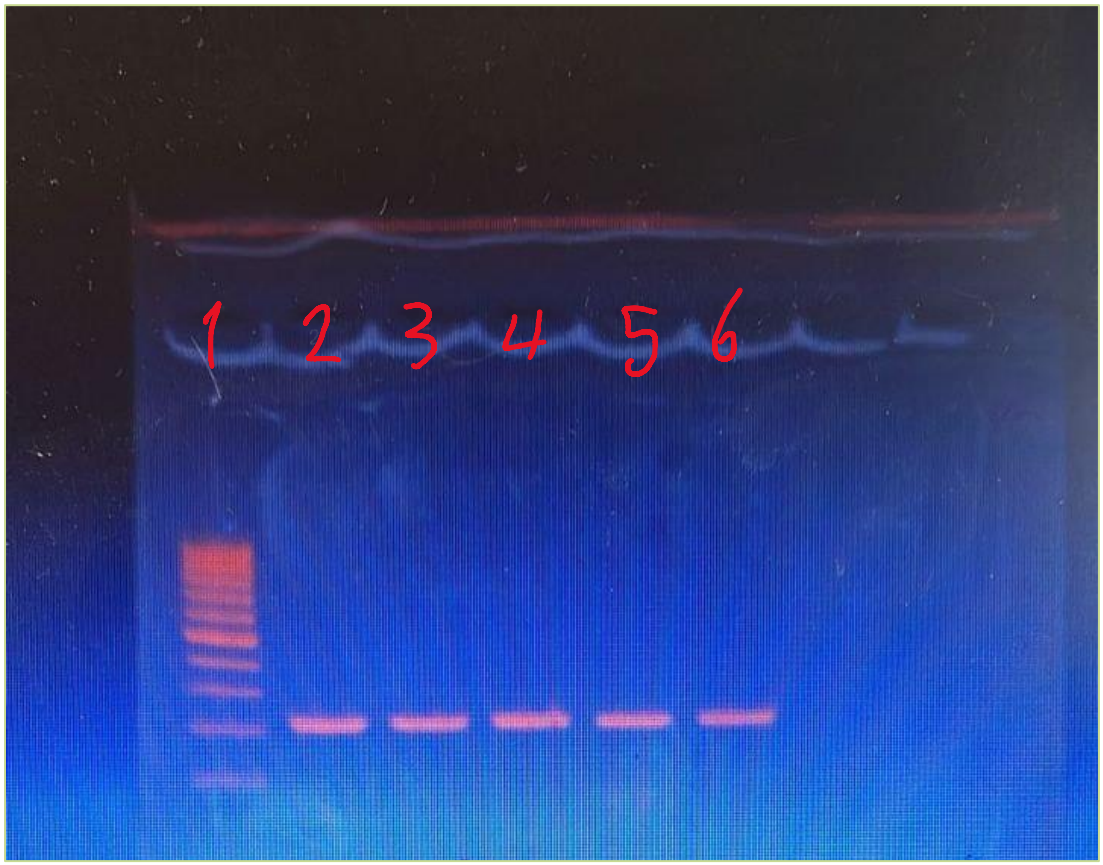


Figure 3-5: The PCR Product of AR **Gene (rs6152 G>A) SNP in 2%Agarose Gel Electrophoresis. Lane 1: Ladder. Lane 2-6: PCR product (one band 206 bp)**

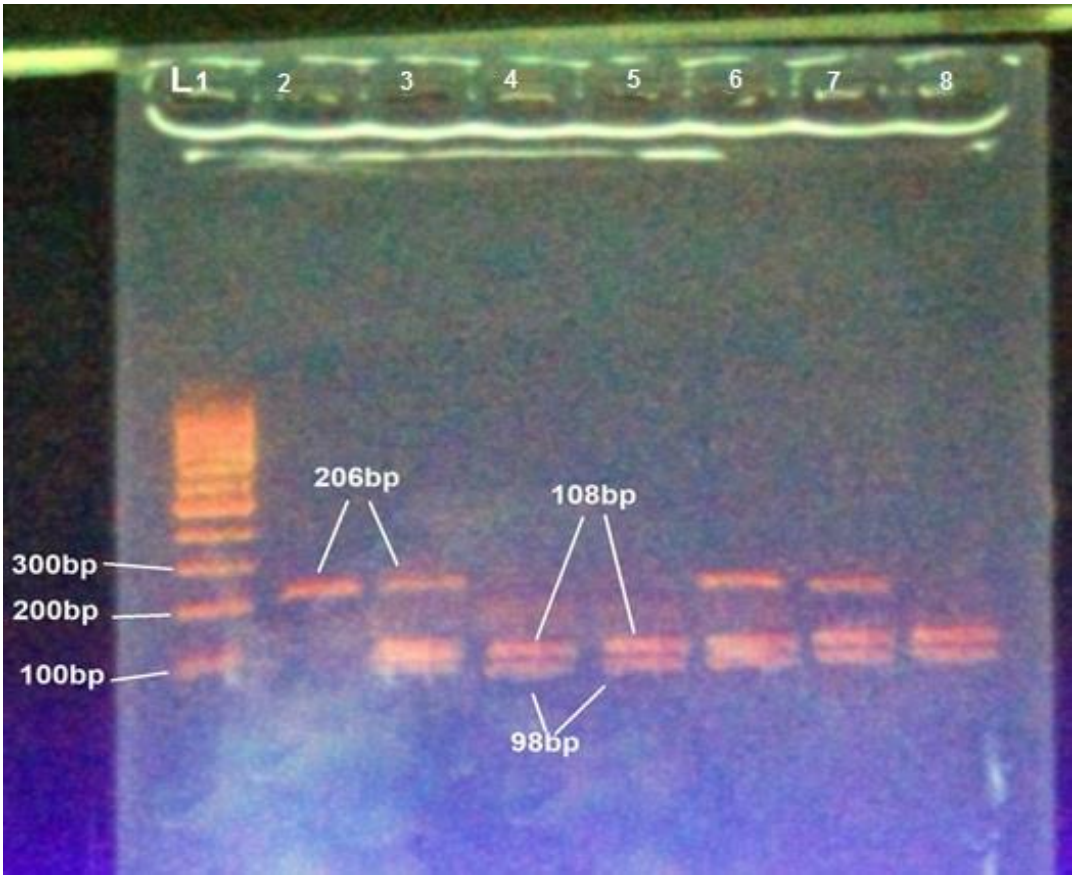


Figure 3-6: Restriction Digestion of PCR Product of *AR* (rs6152G>A) SNP in 1% Agarose Gel Electrophoresis. Lane 1: Ladder. Lane 2: One band, the uncut fragment (206 bp) are homozygous genotype (AA). Lane 3,6 and 7: Three bands (206, 108 and 98 bp) are heterozygous genotype (GA). Lane 4,5 and 8: Two bands (108 and 98 bp) are wild genotype (GG).

3.3.4 The Hardy–Weinberg Equilibrium (WHE) model for (rs6152G>A).

The result from Hardy-Weinberg equilibrium (WHE) exact test revealed the control group the genotype frequency follow the Hardy-Weinberg equilibrium, but not follow with the Hardy-Weinberg equilibrium in patient group indicating that the investigated allele frequencies are constant between generations, as in Table 3-8.

Table 3-8: Results of Hardy Weinberg Equilibrium for *AR* Gene (rs6152) SNP genotypes in Patients and the Controls Groups

Group	X^2	P-value
Control	5.73	0.057
Patient	6.181	0.04

3.3.5 Relevance of *AR* Gene (rs6152 G>A) Polymorphism with obese Infertile

The genotypes distribution and frequency of *AR* gene (rs6152 G>A) SNP as shown in Table 3-9. The analysis of results indicated that the AR gene (rs6152 G>A) SNP genotype frequencies of wild genotype (GG), heterozygous (GA) and homozygous genotype (AA) were, (66.6%,22.2 % and 11.2%) and control group, (77.7.%,15.5 % and 6.6 %) patient group respectively.

The heterozygous genotype (GA) of AR gene (rs6152 G>A) was found to be non-significantly difference (OR = 0.60 , CI 95% , (0.20-1.77) ,P =0.35) the risk of infertility with respect to those of the wild genotype (GG) of *AR* gene.

The homozygous genotype (AA) of AR gene (rs6152) SNP was found to be non-significantly difference (OR = 0.51 , CI 95% , (0.113-2.3) ,P =0.38). the risk of infertility with respect to those of the wild genotype (GG) of *AR* gene.

Table 3-9: Genotypes Distribution and Frequency of AR Gene (rs6152G>A) SNP in Groups.

Genotype	Control N=45	Patient N=45	OR	P-value
GG	30 66.6%	35 77.7 %	Refe	Refe
GA	10 22.2 %	7 15.5 %	0.60	0.35
AA	5 11.2%	3 6.6 %	0.51	0.38

O.R.:Odds Ratio; non-significant at p > 0.05

GG: Wild genotype, GA: Heterozygous and AA : Homozygous genotype

The allele distribution and frequency of AR gene (rs6152 G > A) SNP shown in Table 3-10. The allele frequencies of G and A of AR gene (rs6152 G > A) SNP were found to be 85.5 % and 14.4 % in patients respectively and 77.77% and 22.2% in the control group respectively. It was non-significantly change (P > 0.05) in patients when compared with that of the controls group.

Table 3-10: Alleles Distribution and Frequency of *AR* Gene (rs6152 G>A) SNP in Patients and the Controls Groups

Allele		control	patient	OR	P-value
G	No.	70	77	Refe	Refe
	%	77.77	85.5		
A	No.	20	13	0.59	0.180
	%	22.2	14.5		
Total	NO	90	90		

O.R.: Odds Ratio; non-significant at p > 0.05

The AR gene is located on the long arm of the X chromosome specifically at its region (q 11.2-q12) as shown in figure 1-6 this gene consists of 8 exons separated by relatively long introns, AR is isolated by heat shock proteins in the cytoplasm that help to stabilize and protect gene from degradation, any defect in this gene leads to an increase in the level of androgen **(173)**.

Previous studies have demonstrated that androgens serve crucial roles in the regulatory process of follicle development. They mediate their regulatory actions mainly via their specific AR receptors, affecting the transcription and translation of multiple female follicles. Development testosterone t associated downstream target genes **(174).** Recent studies in human emphasize the importance of AR in follicle growth and survival. Important in regulating female fertility and ovarian function **(175).**

The AR is considered to be highly vulnerable for genetic alteration. This alteration is featured by the presence of effective SNPs at specific location of the exon and change in CAG repeats number **(176).**

Previous study found association between (rs6152) polymorphism in androgen receptor gene and recurrent spontaneous abortions in Mexican population **(175)** other study found Investigating pathogenic SNPs in androgen receptor with direct influence on polycystic ovary syndrome (PCOS) in women **(178).**

Available studies therefore suggest some relationship between AR polymorphism, excess body fat, and fat distribution pattern in both sexes. altered androgen action mediated by AR dysfunctions play a role in the pathophysiology of different obesity phenotypes **(179)**

Conclusion and Recommendation

Conclusion

1. Obesity in patients in Babylon province may be increase serum level of free testosterone, dehydroepiandrosterone sulfate comparing to normal subjects and may be decrees serum level of testosterone(T) and androgen receptor comparing to normal subjects.
2. The Androgen receptor gene SNPs (rs6152 G > A) do not associate with infertile obese women in Babylon province.

Recommendations:

1. Future studies are needed for all categories of obese infertile to see its relations with another genetic mutation.
2. Increase sample size and collect samples from other different hospitals and Provinces
3. Study Androgen receptor gene on other population from different regions
4. Use more advance molecular techniques for detection of other omentin-1 polymorphisms (Real time PCR and sequencing).
5. More research on pharmacological agents that target weight reduction with the effects of fertility resumption in obese women without PCOS.

References

References

1. Verma P, Verma R, Nair RR et al: Altered crosstalk of estradiol and progesterone with Myeloid-derived suppressor cells and Th1/Th2 cytokines in early miscarriage is associated with early breakdown of maternal-fetal tolerance. Am J Reprod Immunol, 2019; 8(12).
2. Caleb D. Ayers , Karen S. Carlson, Spontaneous Pregnancy in the Setting of Primary Ovarian Insufficiency and Breastfeeding: Does Immunosuppression Play a Role?, Spontaneous pregnancy in POI Am J Case Rep, 2020; 21.
3. Segal TR, Giudice LC. Before the beginning: environmental exposures and reproductive and obstetrical outcomes. Fertility and Sterility 2019;112(4):613-21.
4. World Health Organization (WHO). International Classification of Diseases, 11th Revision (ICD-11) Geneva: WHO 2018.
5. Matthew H. Walker; Kyle J. Tobler., Female Infertility,23,4- May 26, 2022.(557) 342 -6.
6. Kuohung W, MD, Wendy Kuohung, MD, Patient education: Evaluation of infertility in couples (Beyond the Basics), Aug 12, 2021.
7. Zorena K, Adipokines and Obesity. Potential Link to Metabolic Disorders and Chronic Complications, Int. J. Mol. Sci. 2020, 21(10), 3570.
8. Goldsammler M, Merhi Z, and Erkan Buyuk, Role of hormonal and inflammatory alterations in obesity-related reproductive dysfunction at the level of the hypothalamic-pituitary-ovarian axis, Goldsammler et al. Reproductive Biology and Endocrinology :2018, 16:45.
9. Renato Pasquali, M.D., Obesity and androgens: facts and perspectives. Vol. 85, No. 5, May 2006.

10. Li Joon T, Naganathan Pillai, Christina Gertrude Yap, Nowrozy Kamar Jahan, Obesity and Female Infertility—A Review on Mechanisms (Endocrinology),.1108817 Jun. 24, 2022.
11. Institute for Public Health National Health and Morbidity Survey (NHMS): Non-Communicable Diseases, Healthcare Demand, and Health Literacy—Key Findings. 40 p,2020.
12. Zorena, K., Jachimowicz-Duda, O., Slezak, D., Robakowska, M. and Mrugacz, M. Adipokines and Obesity. Potential Link to Metabolic Disorders and Chronic Complications. International Journal of Molecular Sciences , ,2020,21, Article No. 3570.
13. Sandra Ann Carson, MD, Amanda N. Kallen, MD, Diagnosis and Management of Infertility: JAMA. 2021 July 06; 326(1): 65–76. 2021.4788.
14. Penzias A, Bendikson K, Falcone T, et al. ; Practice Committee of the American Society for Reproductive Medicine. Evidence-based treatments for couples with unexplained infertility: a guideline. Fertil Steril. 2020;113(2):305–322.
15. Khanna, D. and Rehman, A. Pathophysiology of Obesity. StatPearls Publishing, Treasure Island, FL,2021.
16. Weir, C.B. and Jan, A. BMI Classification Percentile and Cut off Points. StatPearls Publishing, Treasure Island, FL,30,2021.
17. Shahnaz Anwar and Ayesha Anwar, Infertility: A Review on Causes, Treatment and Management, 2016 Volume 2 • Issue 6 • 040.
18. Zorena, K., Jachimowicz-Duda, O., Slezak, D., Robakowska, M. and Mrugacz, M. Adipokines and Obesity. Potential Link to Metabolic Disorders and Chronic Complications. International Journal of Molecular 2020 :Sciences , 21, Article No. 3570.

19. Gambineri, A., Laudisio, D., Marocco, C., Radellini, S., Colao, A. and Savastano, S. Female Infertility: Which Role for Obesity? 2019:International Journal of ObesitySupplements , 9, 65-72.
20. Glenn, T., Harris, A.L. and Lindheim, S.R. Impact of Obesity on Male and Female Reproductive Outcomes. Current Opinion in Obstetrics and Gynecology2019, 31, 201-206.
21. Jun Song M and Jong Young Choi, Androgen dysfunction in non-alcoholic fatty liver disease: Role of sex hormone binding globulin ,Endocrinology .2022.1053709,
22. Vuppalanchi R, Noureddin M, Alkhouri N, Sanyal AJ. Therapeutic pipeline in nonalcoholic steatohepatitis. Nat Rev Gastroenterol Hepatol (2021) 18:373–92.
23. Seidu T, McWhorter P, Myer J, Alamgir R, Eregha N, Bogle D, et al. DHT causes liver steatosis via transcriptional regulation of SCAP in normal weight female mice. J Endocrinol (2021) 250:49–65.
24. Cui P, Hu W, Ma T, Hu M, Tong X, Zhang F, et al. Long-term androgen,excess induces insulin resistance and non-alcoholic fatty liver disease in PCOS-like rats. J Steroid Biochem Mol Biol (2021) 208:105829.
25. C.M. Cox 1, M.E. Thoma , N. Tchangalova, Infertility prevalence and the methods of estimation from 1990 to 2021: a systematic review and meta-analysis , Human Reproduction Open, pp. 1–24, 2022.
26. 26-Hanan Abdul Jabar AlTaee , Ban Jabir Edan and Daniah Wisam Al-Sallami, Prevalence and risk factors of infertility in women from Al-Mahaweel district, Babylon \ Iraq, International Journal of Science and Research Archive, 2022, 05(02), 096–102 .
27. Keerthana Dhandapani, Bhagyalakshmi Kodavanji, NA. Vinodini, Association of body mass index with primary and secondary infertility among infertile women in Mangalore: A cross-sectional study, ational

Journal of Physiology, Pharmacy and Pharmacology 2016 | Vol 6 | Issue 1.

28. Ali Delpisheh, Kourosh Sayehmiri, The prevalence of infertility in Iran. Asystematic Review, International Journal of Epidemiologic Research, 2014; 1 (1): 35-43.
29. Ilse Delbaerea , Sarah Verbiestb and Tanja Tyd_enc, Knowledge about the impact of age on fertility: a brief review, upsala journal of medical sciences 2020, vol. 125, no. 2, 167–174.
30. UNICEF. Sweden, Norway, Iceland, Estonia and Portugal rank highest for family-friendly policies in OECD and EU countries [Internet].UNICEF press release; 12 June 2019. Available from.
31. Angela K. Fitch a , Harold E. Bays b, Obesity definition, diagnosis, bias, standard operating procedures (SOPs), and telehealth: An Obesity Medicine Association (OMA) Clinical Practice Statement (CPS) 2022, A.K. Fitch, H.E. Bays Obesity Pillars 1 (2022) 100004.
32. Bays HE, McCarthy W, Burridge K, Tondt J, Karjoo S, Christensen S, Ng J, Golden A, Davisson L, Richardson L. Obesity Algorithm eBook. ,2021,presented by the Obesity Medicine Association,
33. Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of obesity and severe obesity among adults: United States, 2017-2018. NCHS Data Brief; 2020. p. 1–8.
34. Ross R, Neeland IJ, Yamashita S, Shai I, Seidell J, Magni P, et al. Waist circumference as a vital sign in clinical practice: a consensus statement from the IAS and ICCR working group on visceral obesity. Nat Rev Endocrinol 2020;16: 177–89.
35. Muscogiuri G, Pugliese G, Barrea L, Savastano S, Colao A. Obesity: the "achilles heel" for COVID-19? Metabol Clin Exp 2020;108:154251.

36. Butler MJ, Barrientos RM. The impact of nutrition on COVID-19 susceptibility and long-term consequences. Brain Behav Immun 2020 Jul;87:53–4 32311498.
37. Sommer I, Teufer B, Szelag M, Nussbaumer-Streit B, Titscher V, Klerings I, et al. The performance of anthropometric tools to determine obesity: a systematic review and meta-analysis. Sci Rep 2020;10:12699.
38. Ofenheimer A, Breyer-Kohansal R,et al. Reference values of body composition parameters and visceral adipose tissue (VAT) by DXA in adults aged 18-81 yearsresultsfrom the LEAD cohort. Eur J Clin Nutr. 2020 Aug;74(8):1181–91.
39. Tong Li Joon, Naganathan Pillai, Christina Gertrude Yap, Obesity and Female Infertility—A Review on Mechanisms Open Access Library Journal,, Jun. 24, 2022.
40. Institute for Public Health ,National Health and Morbidity Survey (NHMS) 2019: Non-Communicable Diseases, 2020 Healthcare Demand, and Health Literacy—Key Findings. 40 p.
41. Practice Committee of the American Society for Reproductive Medicine : Definitions of Infertility and Recurrent Pregnancy Loss: A Committee Opinion. Fertility and Sterility 2013, 99, 63.
42. Romero Ramos, R., Romero Gutierrez, G., Abortes Monroy, I. and Medina Sanchez,H.G. [Risk Factors Associated to Female Infertility]. Ginecologia y Obstetricia de Mexico2008 , 76, 717-721.
43. Direkvand-Moghadam, A., Delpisheh, A. and Khosravi, A. (2013) Epidemiology of Female Infertility: A Review of Literature. Biosciences Biotechnology Research Asia, 10, 559-567.
44. Jain, A., Polotsky, A.J., Rochester, D., Berga, S.L., Loucks, T., Zeitlian, G., et al . (2007) Pulsatile Luteinizing Hormone Amplitude and Progesterone Metabolite Excretion Are Reduced in Obese

Women. The Journal of Clinical Endocrinology & Metabolism, 92, 2468-2473.

45. Freeman, E.W., Sammel, M.D., Lin, H. and Gracia, C.R. (2010) Obesity and Reproductive Hormone Levels in the Transition to Menopause. Menopause , 17, 718-726.
46. van der Steeg, J.W., Steures, P., Eijkemans, M.J., Habbema, J.D., Hompes, P.G., Burggraaff, J.M., et al . (2008) Obesity Affects Spontaneous Pregnancy Chances in Subfertile, Ovulatory Women. Human Reproduction, 23, 324-328.
47. Pantasri, T. and Norman, R.J. (2014) The Effects of Being Over-weight and Obese on Female Reproduction: A Review. Gynecological Endocrinology, 30, 90-94.
48. Sasaki, R.S.A., Approbato, M.S., Maia, M.C.S., Ferreira, E. and Zanluchi, N. (2019) Ovulatory Status of Overweight Women without Polycystic Ovary Syndrome. JBRA Assisted Reproduction, 23, 2-6
49. Broughton, D.E. and Moley, K.H. (2017) Obesity and Female Infertility: Potential Mediators of Obesity's Impact. Fertility and Sterility , 107, 840-847.
50. Petersen, G.L., Schmidt, L., Pinborg, A. and Kamper-Jorgensen, M. (2013) The Influence of Female and Male Body Mass Index on Live Births after Assisted Reproductive Technology Treatment: A Nationwide Register-Based Cohort Study. Fertility and Sterility , 99, 1654-1662.
51. Dag, Z.O. and Dilbaz, B. (2015) Impact of Obesity on Infertility in Women. Journal of the Turkish German Gynecological Association, 16, 111-117.
52. Candido FG, Coelho OGL, Balbino KP, Hermsdorff HHM. Improving applicability of the new obesity classification based on weight history in severe obesity. Arch Endocrinol Metab. 2022;66(6):934-5.

53. Halpern B, Mancini MC, Melo ME, Lamounier RN, Moreiro RO, Carra MK, et al. Proposal of an obesity classification based on weight history: an official document by the Brazilian Society of Endocrinology and Metabolism (SBEM) and the Brazilian Society for the Study of Obesity and Metabolic Syndrome (ABESO). Arc. Endocrionol Metab. 2022;66(2):139-51
54. Look AHEAD Research Group; Wing RR, Bray GA, Cassidy-Begay M, Clark JM, Coday M, Egan C, et al. Effects of Intensive Lifestyle Intervention on All-Cause Mortality in Older Adults With Type 2 Diabetes and Overweight/Obesity: Results From the Look AHEAD Study. Diabetes Care. 2022;45(5):1252-9.
55. Müller TD, Blüher M, Tschöp MH, DiMarchi RD. Anti-obesity drug discovery: advances and challenges. Nat Rev Drug Discov. 2022;21(3):201-23.
56. Weir, C.B.; Jan, A. BMI Classification Percentile and Cut Off Points. In StatPearls; Statpearls Publishing: Tampa, FL, USA, 2020.
57. Itani, L.; Kreidieh, D.; El Masri, D.; Tannir, H.; Chehade, L.; El Ghoch, M. Revising BMI Cut-Off Points for Obesity in aWeight Management Setting in Lebanon. Int. J. Environ. Res. Public Health 2020, 17, 3832.
58. Pellegrini, M.; Ponzo, V.; Rosato, R.; Scumaci, E.; Goitre, I.; Benso, A.; Belcastro, S.; Crespi, C.; De Michieli, F.; Ghigo, E.; et al. Changes in Weight and Nutritional Habits in Adults with Obesity during the "Lockdown" Period Caused by the COVID-19 Virus Emergency. Nutrients 2020, 12, 2016.
59. Abdelaal, M.; le Roux, C.W.; Docherty, N.G. Morbidity and mortality associated with obesity. Ann. Transl. Med. 2017, 5, 161.
60. Laura Di Renzo , Leila Itani , Paola Gualtieri 1, Massimo Pellegrini , Marwan El Ghoch and Antonino De Lorenzo, New BMI Cut-Off

Points for Obesity in Middle-Aged and Older Adults in Clinical Nutrition Settings in Italy: A Cross-Sectional Study, Nutrients 2022, 14, 4848.

61. E. S. Han and A. goleman, daniel; boyatzis, Richard; Mckee, Illustrated Biochemistry, vol. 53, no. 9. 2019.
62. E. Nieschlag and S. Nieschlag, "The history of discovery, synthesis and development of testosterone for clinical use," European Journal of Endocrinology, vol. 180, no. 6. pp. R201–R212, 2019.
63. H. G. Burger, "Androgen production in women," Fertil. Steril., vol. 77, pp. 3–5, 2002.
64. Schiffer a L , Arlt a, W , -Heinz Storbeck K, Intracrine androgen biosynthesis, metabolism and action revisited, Molecular and Cellular Endocrinology 465 ,2018, 4e26.
65. Zhang H , Yi Zhou , Xing Z , Kumar Sah R, Androgen Metabolism and Response in Prostate Cancer Anti-Androgen Therapy Resistance, Int. J. Mol. Sci. 2022, 23, 13521.
66. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Prostate Cancer Version 1.2022; NCCN: Plymouth Meeting, PA, USA, 2022.
67. Clark, R.; Vesprini, D.; Narod, S.A. The Effect of Age on Prostate Cancer Survival. Cancers 2022, 14, 4149.
68. Fenner, A. Intestinal microbiota drive CRPC. Nat. Rev. Urol. 2021, 18, 705.
69. He, Y.D.; Wei, T.; Ye, Z.Q.; Orme, J.J.; Lin, D.; Sheng, H.Y.; Fazli, L.; Karnes, R.J.; Jimenez, R.; Wang, L.G.; et al. A noncanonical AR addiction drives enzalutamide resistance in prostate cancer. Nat. Commun. 2021, 12, 1521

70. Geoffrey L Hammond, Plasma steroid-binding proteins: primary gatekeepers of steroid hormone action 2016 Society for Endocrinology Printed in Great Britain.
71. Stephan J. Schoech, L. Michael Romero, Ignacio T. Constraints, concerns and considerations aboutthe necessity of estimating free glucocorticoidconcentrations forfield endocrine studies, Functional Ecology2013,27, 1100–1106.
72. E. Nieschlag, Late-onset hypogonadism: a concept comes of age, Andrology, 2021, 8, 1506–1511.
73. Nieschlag E & Nieschlag S. Endocrine History: The history ofdiscovery, synthesis and development of testosterone for clinical use.2019 Eur J Endocrinol 180, R201–R212.
74. Dohle GR, Arver S, Bettocchi C, Kliesch S,Punab M, & de Ronde W. 2017. EAU Guidelines on male hypogonadism. (last visited July 15, 2019.
75. Bhasin S, Brito JP, CunninghamGR, Hayes FJ, Hodis HN, Matsumoto AM, Snyder PJ, Swerdloff RS, Wu FC & Yialamas MA. Testosterone therapy in men with hypogonadism: An Endocrine Society Clinical Practice Guideline2018. J Clin EndocrinolMetab 103, 1715–1744.
76. Maria C. Ysrraelit and Jorge Correale, Impact of Andropause on Multiple Sclerosis, Frontiers in Neurology.org 1 November 2021 | Volume 12 | Article 766308.
77. Yang L, Zhou R, Tong Y, Chen P, Shen Y, Miao S, et al. Neuroprot ection by dihydrotestosterone in LPS-induced neuroinflammation. Neurobiol Dis. (2020) 140:104814. doi: 10.1016/j.nbd.2020.104814
78. G. L. Hortin and B. A. Goldberger, Tietz Textbook of Clinical Chemistry and Molecular Diagnosis. 2015.
79. V. L. Nelson et al., “The biochemical basis for increased testosterone production in theca cells propagated from patients with polycystic

ovary syndrome," J. Clin. Endocrinol. Metab., vol. 86, no. 12, pp. 5925–5933, 2001.

80. C. R. McCartney, M.D., J. C. Marshall, M.B., Ch.B, and M.D., "Polycystic Ovary Syndrome," vol. 44, p.54-64, 2016.
81. R. Azziz, E. Carmina, D. Dewailly, E. Diamanti-Kandarakis, H. F. Escobar-Morreale, and W. Futterweit, "Androgen Excess Society. Position statement: Criteria for defining pcos as a predominantly hyperandrogenic syndrome: An Androgen Excess Society guideline," J Clin Endocrinol Metab, vol. 91, pp. 4237–4245, 2006.
82. L. M. Holm, "Polycystic ovary syndrome Studies of metabolic and ovarian disturbances and effects of physical exercise and electro-acupuncture." 2010.
83. A. Kozlova T and David B. Levin, Effect of 17β-Estradiol on Growth and Biosynthesis of Microalgae Scenedesmus quadricauda (CPCC-158) and Duckweed Lemna minor (CPCC-490) Grown in Three Different Media: Plants 2022, 11, 1669.
84. Sujun Wu , Kun Yu , Zhengxing Lian, Molecular Regulation of Androgen Receptors in Major Female Reproductive System Cancers, Int. J. Mol. Sci. 2022.
85. Chung, W.M.; Chen, L.; Chang, W.C.; Su, S.Y.; Hung, Y.C.; Ma, W.L. Androgen/Androgen Receptor Signaling in Ovarian Cancer :Molecular Regulation and Therapeutic Potentials. Int. J. Mol. Sci. 2021, 22, 7748.
86. Ehsani, M.; David, F.O.; Baniahmad, A. Androgen Receptor-Dependent Mechanisms Mediating Drug Resistance in Prostate.Cancer. Cancers 2021, 13, 1534.
87. Nita, I.; Nitipir, C.; Toma, S.A.; Limbau, A.M.; Pirvu, E.; Badarau, I.A. The importance of androgen receptors in breast cancer.Med. Pharm. Rep. 2021, 94, 273–281.

88. Hu, H.; Zhou, H.; Xu, D. A review of the effects and molecular mechanisms of dimethylcurcumin (ASC-J9) on androgen receptor-related diseases. Chem. Biol. Drug Des. 2021, 97, 821–835.
89. Wu, M.J.; Chen, C.J.; Lin, T.Y.; Liu, Y.Y.; Tseng, L.L.; Cheng, M.L.; Chuu, C.P.; Tsai, H.K.; Kuo,W.L.; Kung, H.J.; et al. Targeting KDM4B that coactivates c-Myc-regulated metabolism to suppress tumor growth in castration-resistant prostate cancer. Theranostics 2021, 11, 7779–7796
90. John McCoy, Carlos G. Wambier, et al, Racial variations in COVID-19 deaths may be due to androgen receptor genetic variants associated with prostate cancer and androgenetic alopecia. Are anti-androgens a potential treatment for COVID-19?, J Cosmet Dermatol. 2020;19:1542–1543.
91. Guan W, Ni Z, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382:1708-1720.
92. Emily A. Kolyvas, Carlos Caldas, Androgen receptor function and targeted therapeutics across breast cancer subtypes, Kolyvas et al. Breast Cancer Research (2022) 24:79.
93. Handelsman DJ, Hirschberg AL, Bermon S. Circulating testosterone as the hormonal basis of sex differences in athletic performance. EndocRev. 2018;39(5):803–29
94. Ricciardelli C, Bianco-Miotto T, Jindal S, Butler LM, Leung S, McNeil CM, et al. The magnitude of androgen receptor positivity in breast cancer is critical for reliable prediction of disease outcome. ClinCancer Res. 2018;24(10):2328–41.
95. Meehan KL, Sadar MD . "Androgens and androgen receptor in prostate and ovarian malignancies".May 2003 Frontiers in Bioscience. 8 (1–3): d780–800.

96. A Davey R and Grossmann M, Androgen Receptor Structure, Function and Biology: From Bench to Bedside, Clin Biochem Rev 37 (1) 2016.
97. Brock O, De Mees C, Bakker J. Hypothalamic expression of oestrogen receptor alpha and androgen receptor is sex-, age- and region-dependent in mice. J Neuroendocrinol. 2015;27:264–76.
98. Daniel M, Dehm SM. Lessons from tissue compartment-specific analysis of androgen receptor alterations in prostate cancer. J Steroid Biochem Mol Biol. 2016.
99. Guillaumier, S.; Peters, M.; Arya, M.; Afzal, N.; Charman, S.; Dudderidge, T.; Hosking-Jervis, F.; Hindley, R.G.; Lewi, H.; McCartan, N.; et al. A Multicentre Study of 5-year Outcomes Following Focal Therapy in Treating Clinically Significant Nonmetastatic Prostate Cancer. Eur. Urol. 2018, 74, 422–429.
100. Fuentes-Prior, P.; Rojas, A.; Hagler, A.T.; Estebanez-Perpina, E. Diversity of Quaternary Structures Regulates Nuclear Receptor Activities. Trends Biochem. Sci. 2019, 44, 2–6.
101. Kregel, S.; Bagamasbad, P.D.; He, S.; la Pensee et al. Differential modulation of the androgen receptor for prostate cancer therapy depends on the DNA response element. Nucleic Acids Res. 2020, 48, 4741–4755
102. Jimenez-Panizo, A.; Perez, P.; Rojas, A.M.; Fuentes-Prior, P.; Estebanez-Perpina, E. Non-Canonical Dimerization of the An-drogen Receptor and Other Nuclear Receptors: Implications for Human Disease. Endocr. Relat. Cancer 2019, 26, R479–R497
103. De Mol, E.; Szulc, E.; di Sanza, C.; Marttnez-Cristtbal, P; et al. Regulation of Androgen Receptor Activity by Transient Interact ions of Its Transactivation Domain with General Transcription Regulat ors. SSRN Electron. J. 2018, 26, 145

104. Yu, X.; Yi, P.; Hamilton, R.A.; Shen, H.; Chen,et al. Structural Insights of Transcriptionally Active, Full-Length Androgen Receptor Coactiv- ator Complexes. Mol. Cell 2020, 79, 812–823.e4.
105. O'Shaughnessy, P.J.; Antignac, J.P.; Le Bizec, B.; et al. Alternative (backdoor) androgen production and masculinization in the human fetus. PLoS Biol. 2019,
106. Minozzi, S.; Lunardini, A.; Caldarini, C. et al. Metastatic Prostate Carcinoma from Imperial Rome (1st to 2nd Centuries AD). Pathobiology 2018, 85, 289–299.
107. Chen Z, Wang G, Li M, Peng Z, Ali H, et al. Development of Single Nucleotide Polymorphism (SNP) Markers for Analysis of Population Structure and Invasion Pathway in the Coconut Leaf Beetle Brontispa longissima (Gestro) Using Restriction Site-Associated DNA (RAD) Genotyping in Southern China, Insects 2020, 11, 230.
108. Chistyakov D, and Sergeev G, The Polymorphism of Drugs: New Approaches to the Synthesis of Nanostructured Polymorphs. : 7 Novem- ber 2019; Published: 1 January 2020.
109. Josephat K. Bungei a,b , Victor A. Mobegi b, , Steven G. Nyanjom, Single-nucleotide polymorphism characterization of gametocyte develop ment 1 gene in Plasmodium falciparum isolates from Baringo, Uasin- Gishu, and Nandi Counties, (2020)Kenya, Heliyon 6 .
110. B. Gopi, Vir Singh R, Kumar S, Sushil Kumar, Anuj ChauhanSingle nucleotid polymorphismsin CLEC7A, CD209 and TLR4 gene and their association with susceptibility to paratuberculosis in Indian cattle Journal of Genetics volume 99, Article number:2020, 14 .
111. Buijs S B , Jansen A F M , Oosterheert J J , T. Schoffelen , et al, Single nucleotide polymorphism (SNP) rs3751143 in P2RX7 is associa- ted with therapy failure in chronic Q fever while rs7125062 in

MMP1 is associated with fewer complications, Clinical Microbiology and Infection volume 9, Article number: 17, 2019.

112. Bing Tang. Preview of Single Nucleotide Polymorphism (Snp) and Potential Correlation between Human Genomes and Evolutionary History As Well As Religious Behaviors, Symbiosis November 12, 2018; Accep ted: March 4, 2019; Published: April .
113. Beata Mrozikiewicz‐Rakowska, Agnieszka Sobczyk‐Kopcioł. Role of the rs2274907 allelic variant of the ITLN1 gene in patients with diabetic foot, polish archives of internal medicine 2017; 127 (5)
114. Stenson P, Mort M ,Ball E et al ,The human mutation database ,2008 update ,Genome medicine ,2009.1(13):1-6.
115. Cynthia a. heinlein and chawnshang chang, The Roles of Androgen Receptors and AndrogenBinding Proteins in Nongenomic Androgen Actions, Heinlein and Chang Minireview Mol Endocrinol, October 2002, 16(10):2181–2187.
116. DRG International, Testosterone , (EIA-1559), Revised 22 July 2009.
117. DRG International ,Free testosterone , (EIA-2924) 2010 rm (Vers. 15.1)
118. L. Shenzhen New Industries Biomedical Engineering Co., "MAGLUMITM DHEA-S (CLIA)." 2019.
119. Elabscience Biotechnology Co. Ltd. Androgen recptor ELISA kit user manual. Wuhan: Elabscience Biotechnology Co, Ltd.
120. Chacon-Cortes D, Griffiths LR. Methods for extracting genomic DNA from whole blood samples: current perspectives. Journal of Biorepository Science for Applied Medicine. 2014;2:1-9.
121. Harisha S. Biotechnology procedures and experiments handbook. Infinity Science Press LLC, India. 2007.

122. Adams D. In Lab. Math.: A handbook of measurements, calculations and other quantitative skills. Cold Spring Harbor Laboratory Press, NY. 2003; 127-145.
123. Tataurov A., You Y. and Owczarzy R. Predicting ultraviolet spectrum of single stranded and double stranded deoxyribonucleic acids. Biophysical Chemistry. 2008; 133 (1-3): 66-70.
124. User's Manual. Thermo Fisher Scientific NanoDrop Spectrophotometer. 2008.
125. Bartlett J. and Stirling D. A short history of the polymerase chain reaction. PCR Protocols. 2003; 226: 3-6.
126. Joseph S. and David W. Molecular cloning: a laboratory manual. cold spring harbor, N.Y. In vitro amplification of DNA by the polymerase chain reaction. 2001; 3:8.
127. Sharkey D, Scalice E, Christy K, et al. Antibodies as thermolabile switches: high temperature triggering for the polymerase chain reaction. Bio/Technology. 1994; 12: 506-509.
128. Jon B, David S, Wu-bo L, et al. PCR from problematic templates. Focus. 2000; 22 (1): 10.
129. Holly A. Helpful tips for PCR. Focus. 2000; 22 (1): 12.
130. Harisha S. Biotechnology procedures and experiments handbook. Infinity Science Press LLC, India. 2007.
131. Pingoud A., Jeltsch A. Structure and function of type II restriction endonucleases. Nucleic Acids Res. 2001; 29: 3705-3727.
132. Mei Zhao X, Lin Xiang Z, Xing Chen Y, Yang P, Chong Zeng Y & Z. A. sequence polymorphism on 8q24 is associated with survival in hepatocellular carcinoma patients who received radiation therapy. Scientific Reports .2018; 2264.

133. Sambrook J, Russell D. Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory press. 2001; 3nd: 1.
134. Ferlitsch A, Bond R T, Nachef A. Obesity and Infertility: A Metabolic Assessment Strategy to Improve Pregnancy Rate, J Reprod Infertil. 2020;21(1):34-41.
135. 137-Rabenberg M, Scheidt-Nave C, Busch MA, Rieckmann N, Hintzpeter B, Mensink GB. Vitamin D status among adults in Germany-results from the German health interview and examination survey for adults (DEGS1). BMC Public Health. 2010; 15:641.
136. WHO. Infertility is a global public health issue. WHO. World Health Organization; 2020.
137. Carlson L J, Natalie D. Shaw MD. Development of Ovulatory Menstrual Cycles in Adolescent Girls, J Pediatr Adolesc Gynecol. 2019; 32(3): 249–253.
138. Abdelazim IA, Purohit P, Farag RH, Zhurabekova G. Unexplained infertility: prevalence, possible causes and treatment options. A review of the literature. J Obstet Gynecol Investig. 2018;1(1):17–22
139. Harare,Zimbabwe Population(2020) -Population Stat. cited 2020.
140. Infertility Workup for the Women ’s Health Specialist 2020.
141. Elhussein OG, Ahmed MA, Suliman SO, Yahya leena I, Adam I. Epidemiology of infertility and characteristics of infertile couples requesting assisted reproduction in a low-resource setting in Africa, Sudan. Fertil Res Pract. 2018 .
142. Sedighiyan M, Abdolahi M, Taheri E, Qorbani M, Omidian P and Hosseini S. The French Maritime Pine Bark Extract Reduce Metabolic Syndrome Risk and Improve Body Composition in Obesity: A New Clinical Approach 2018. Acta Med. Iranica 56 (3), 196–203.

143. American College of Obstetricians and Gynecologists, Committee Opinion No 589: Female age-related fertility decline, 2020.
144. Du Fosse NA, Hoorn M, et al. Advanced paternal age is associated with an increased risk of spontaneous miscarriage: a systematic review and meta-analysis. Hum Reprod Update. 2020;26:650-669.
145. Newman J, Paul R, Chambers G. Assisted reproductive technology in Australia and New Zealand 2018. National Perinatal Epidemiology and Statistics Unit. the University of New South Wales. 2020; 17.
146. Morris G, Mavrelos D, Theodorou E, et al. Effect of paternal age on outcomes in assisted reproductive technology cycles: systematic review and meta-analysis. F&S Reviews, 2020;1:16-34.
147. Zhang H , Yi Zhou , Xing Z , Kumar Sah R, Androgen Metabolism and Response in Prostate Cancer Anti-Androgen Therapy Resistance. Int. J. Mol. Sci. 2022; 23:13521.
148. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Prostate Cancer Version 1.2022.
149. Nelson V. L. et al.The biochemical basis for increased testosterone production in theca cells propagated from patients with polycystic ovary syndrome. J. Clin. Endocrinol. Metab.2001; 86(12):5925–5933.
150. Eilerman B, Salehi M and Tomer Y.The genetics of polycystic ovary syndrome. Diagnosis and Management of Polycystic Ovary Syndrome.2009; 83–91.
151. Cooper L A, Page S T Amory J K. The association of obesity with sex hormone-binding globulin is stronger than the association with ageing – implications for the interpretation of total testosterone measurements. Clin Endocrinol (Oxf). 2015; 83(6): 828–833.
152. Bourebaba N , AgnieszkaN, Lynda S. Sex hormone binding globulin as a potential drug candidate for liver-related metabolic disorders treatment. Biomedicine & Pharmacotherapy. 2022;113261.

153. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol 2006; 7: 85-96 .
154. Sowers M F, Beebe J L, McConnell D, et al. Testosterone Concentrations in Women Aged 25–50 Years: Associations with Lifestyle, Body Composition, and Ovarian Status . *American Journal of Epidemiology. 2001;*153(3): 256–264.
155. Goldsammler, M., Merhi, Z. and Buyuk, E. Role of Hormonal and Inflammatory Alterations in Obesity-Related Reproductive Dysfunction at the Level of the Hypothalamic-Pituitary-Ovarian Axis. Reproductive Biology and Endocrinology.2018
156. Wu S, Divall S, Nwaopara A, Radovick S, Wondisford F, Ko C. and Wolfe A. Obesity-Induced Infertility anHyper androgen- ism Are Corrected by Deletion of the Insulin Receptor in the Ovarian Theca Cell. Diabetes. 2014; 63: 1270-1282.
157. Holm L M. Polycystic ovary syndrome Studies of metabolic and ovarian disturbances and effects of physical exercise and electro-acupuncture. 2010.
158. Poretsky L, Cataldo N.A, Rosenwaks Z. and Giudice L.C. The Insulin-Related Ovarian Regulatory System in Health and Disease. Endocrine Reviews. 1999;20: 535-582.
159. Sadeghi H M et al. Polycystic Ovary Syndrome: A Comprehensive Review of Pathogenesis, Management, and Drug Repurposing. International Journal of Molecular Sciences Review. 2022.
160. Brewer C.J. and Balen A.H. The Adverse Effects of Obesity on Conception and Implantation. Reproduction The Official Journal of the Society for the Study of Fertility.2010;140, 347-364.

161. Lizneva D, Gavrilova-Jordan L, Walker W, and Azziz R. Androgen excess: Investigations and management. Best Practice and Research: Clinical Obstetrics and Gynaecology.2016;37:pp. 98–118.
162. Mäntyselkä A, Jääskeläinen J, Lindi V, Viitasalo A, Tompuri T, Voutilainen R, et al. The presentation of adrenarche is sexually dimorphic and modified by body adiposity. J Clin Endocrinol Metab. 2014;99:3889–94.
163. Orentreich N, Brind JL, Rizer RL, Vogelman JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab. 1984;59:551–5.
164. Orentreich N, Brind JL, Vogelman JH, Andres R, Baldwin H. Long-term longitudinal measurements of plasma dehydroepiandrosterone sulfate in normal men. J Clin Endocrinol Metab. 1992;75:1002–4.
165. Navarro G, Allard C, Xu W, and Mauvais-Jarvis F. The Role of Androgens in Metabolism, Obesity,and Diabetes in Males and Females. Obesity.2015; 23(4).
166. Ademuyiwa F.O., Groman A., O'Connor T., Ambrosone C., Watroba N., Edge S.B. Impact of body mass index on clinical outcomes in triple-negative breast cancer. Cancer. 2011;117:4132–4140.
167. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2006;295: 1288-1299.
168. Chen L., Cook L.S., Tang M.T., Porter P.L., Hill D.A., Wiggins C.L., Li C.I. Body mass index and risk of luminal, HER2-overexpressing, and triple negative breast cancer. Breast Cancer Res. Treat. 2016;157:545–554.

169. Rachel A Davey and Mathis Grossmann, Androgen Receptor Structure, Function and Biology: From Bench to Bedside, Clin Biochem Rev.2016; 37 (1).
170. Davison SL, Bell R,Montalto JG, et al. Measurement of total testosterone in women: comparison of a direct radioimmunoassay versus radioimmunoassay after organic solvent extraction and celite column partition chromatography. Fertil Steril 2005;84(6):1698–1704
171. Ding D, Xu L, Menon M, Reddy GP, Barrack ER. Effect of GGC (glycine) repeat length polymorphism in the human androgen receptor on androgen action. Prostate. 2005;62:133–9.
172. Cloke B, Huhtinen K, Fusi L, Kajihara T, Yliheikkilä M, Ho KK, et al. The androgen and progesterone receptors regulate distinct gene networks and cellular functions in decidualizing endometrium. Endocrinology. 2008;149:4462–74.
173. Esencan E , Beroukhim G, Seifer D B, Age-related changes in Folliculogenesis and potential modifers to improve fertility outcomes - A narrative review, Esencan et al. Reproductive Biology and Endocrinology.2022; 20:156.
174. Livadas S, Pappas C, Karachalios A, Marinakis E,et al. Prevale nce and impact of hyperandrogenemia in 1218 women . Endocrine. 2014; 47:631–638.
175. Porras-Dorantes A, Brambila-Tapia A, et al. Association between G1733A (rs6152) polymorphism in androgen receptor gene and recurrent spontaneous abortions in Mexican population, J Assist Reprod Genet.2017; 34:1303–1306.
176. RuiWang ,MarkO.Goodarzi, TingXiong, Negative association between androgen receptor gene CAG repeat polymorphism and polycystic ovary syndrome? A systematic review and meta-analysis, Molecular Human Reproduction. 2012;18(10): 498–509.

177. Frank A, Santos R, Palmer B, Clegg D. Determinants of body fat distribution in humans may provide insight about obesity-related health risks. Journal of Lipid Research. 2019; 60.

الخلاصة

قلة الخصوبة هي مشكلة يعاني منها الازواج. ترتبط السمنة بتغيرات متعددة في أنظمة الغدد الصماء ، بما في ذلك تركيزات هرمون الدم غير الطبيعية ، بسبب التغيرات في نمط إفرازها و / أو التمثيل الغذائي ، وكذلك ترتبط السمنة أيضا بزيادة معدل إنتاج هرمون التستوستيرون. يلعب مستقبل الأندروجين دورا في تنظيم الوظائف الجنسية والجسدية والسلوكية للإناث. يبدو أن متعدد الأشكال يؤثر على وظيفة المستقبل كعامل نسخ.

الهدف من الدراسة هو معرفة تاثير السمنة على مستويات التستوستيرون الحر (FT) والتستوستيرون (T) وكبريتات ديهيدروبياندروستيرون (DHEAS) ومستقبلات الأندروجين (AR) في النساء المصابات بالسمنة ولتقييم خطر تعدد أشكال نيوكليوتيد أحادي لجين الأندروجين (G > A ٦١٥٢rs) على النساء المصابات بالسمنة المفرطة في محافظة بابل.

ولتحقيق هذا الهدف، شملت الدراسة الحالية ٩٠ نقطة فرعية. المجموعة الأولى تشمل ٤٥ المرضى مع النساء البدينات العقم , BMI (30-39)Kg/m^2والعمر(٢٠-٣٠)، والمجموعة الثانية و ٤٥ فردا على ما يبدو الأصحاء كمجموعات ضابطة BMI(20-24.5) Kg/m^2 والعمرy(٢٠-٣٠). وتضمنت المعاييرفي هذه الدراسة النساء السمينات العقيمات والعمرy (٢٠-٣٠) بالمقارنة مع نساء ذات وزن طبيعي ولا تعاني من العقم وكذلك تم استبعاد النساء المرضى بمتلازمة المبيض المتعدد الكيسات والضغط المرتفع وامراض الغدة الدرقية والعلاج الهرموني ،في الدراسة تم تحديد التستوستيرون الحر والتستوستيرون وكبريتات ديهيدروبياندروستيرون وتركيز مستقبلات الأندروجين بطريقة مقايسة الممتز المناعي المرتبط بالإنزيم. تم استخراج الحمض النووي من الدم الكامل من جميع الموضوعات . تم تنفيذ التنميط الجيني لجين مستقبلات الأندروجين SNP عن طريق تعدد الأشكال بطول جزء التقييد. تم تحقيق التنميط الجيني باستخدام بادئات محددة لتضخيم الأجزاء. تم تحليل المنتج عن طريق هلام agarose الكهربائي. تم تطبيق تحليل إحصائي مختلف لتحليل البيانات.

في هذه الدراسة، وجدت النتائج أن هناك زيادة في تركيز التستوستيرون الحر ، ديهيدر وبياندر وستيرون كبريتات P<٠.٠٥.

وجد أيضا انخفاضا في التركيز في هرمون التستوستيرون ، ومستقبلات الأندروجين في المرضى الذين يعانون من مجموعة العقم البدينة مقارنة بمجموعة التحكم (P<٠.٠٥).

تم العثور على النمط الجيني المتماثل (GG) لجين (rs ٦١٥٢AR SNP (A > G ليكون فرقا غير معنوي (<p٠.٠٥) مع النمط الجيني المتماثل (AA) والنمط الجيني غير المتجانس (GA) في مجموعتين.

تم العثور على ترددات الأليل الصغرى (A) لجين AR (rs٦١٥٢ SNP (A > G في المريض ومجموعة التحكم ٢٢,٢٪ و١٤.٥٪ على التوالي. لم يكن هناك فرق معنوي (p>0.05) في مجموعة العقم البدينة بالمقارنة مع المجموعة الضابطة.

العمر غير معنوي.(p>0.05)بين المرضى والمجموعة الضابطة.

استنتجنا في هذه الدراسة ان السمنة توثر على

التستوستيرون الحر، والتستوستيرون الكلي، داي هايدرواندروستيرون سولفات ،و الاندروجين ريسيبتور ،(P< 0.05).

SNP (6152) اندروجين ريسبتر جين ليس له علاقة في السمنة.(p>0.05)

وان العمر غير معنوي(p>0.05) بين المرضى والمجموعة الضابطة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل/ كلية الطب
فرع الكيمياء والكيمياء الحياتية السريرية

تأثير السمنة على الهرمونات الذكرية وتركيز مستقبل وجين الاندروجين في النساء القليلات الخصوبة

رسالة مقدمة إلى عمادة كلية الطب في جامعة بابل
وهي جزء من متطلبات نيل درجة الماجستير
في العلوم / الكيمياء الحياتية السريرية

من قبل

زينب محمد مرعي الخزاعي

بكالوريوس علوم الكيمياء/ جامعة بابل سنة (٢٠٠٤_٢٠٠٥)

إشراف

الأستاذ الدكتور	الأستاذ الدكتور
مها فاضل سميسم	**نادية مضر الحلي**

٢٠٢٣ م ١٤٤٥ هـ