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Effect of Follicle Stimulating Hormone Receptor Gene Polymorphisms on Response of FSH Therapy in Iraqi Infertile Women

A Thesis

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Dedication

To the soul of my father

To my beloved mother

who taught me how to hold the pen

To my husband the source of my power

To my children... the light of my way

Abeer 2022

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List of abbreviations

Abbreviation	Full name
%	Percentage
A	Adenine
AFC	Antral follicles count
Ala	Alanine
AMH	Anti Müllerian hormone
AMHR	Anti Müllerian hormone receptor
ANOVA	Analysis of variance
Asn	Asparagine
BMI	Body mass index
bp	Base pair
C	Cytosine
CI	Confidence interval
E_2	Estradiol
ECD	Extracellular domain
ELs	Extracellular loops
ES	Estrogen
ESR	Estrogen receptor
FSH	Follicular stimulating hormone
FSHR	Follicular stimulating hormone receptor

G	Guanine
GnRH	Gonadotropin releasing hormone
GPHRs	Glycoprotein hormone receptors
hCG	Human chorionic gonadotropin
ILs	Intracellular loops
IM	Intramuscular
IU	International unit
IV	Intravenous
Kb	Kilobase
kg	Kilogram
L	Liter
LH	Luteinizing hormone
LHR	Luteinizing hormone receptor
m^2	Square meter
mg/L	Milligram/liter
ml	Milliliters
mmol/L	Millimole/liter
N	Number
OHSS	Ovarian hyperstimulation syndrome
OR	Odds ratio
PCR	Polymerase chain reactions

rFSH	Recombinant follicle stimulating hormone
rhFSH	Recombinant Human FSH
rpm	Revolutions per minute
SC	Subcutaneous
SD	Standard deviation
Ser	Serine
SNPs	Single nucleotide polymorphisms
T	Thymine
Taq	Thermus aquaticus
TBE	Tris borate EDTA
Thr	Threonine
TMD	Transmembrane domain
TSH	Thyroid stimulating hormone
µL	Microliters

Abstract

Background: Female infertility is a multifactorial condition constituting a worldwide public health problem. Follicle-stimulating hormone (FSH) is produced in the pituitary gland and is essential for reproduction. It specifically binds to a membrane receptor (FSHR) expressed in somatic cells of the gonads. The FSH/FSHR system presents many features compared to classical G protein-coupled receptors (GPCRs). Polymorphisms at codons 307 and 680 are the most commonly encountered allelic variants of the follicle-stimulating hormone receptor (FSHR) gene.

Aims of study: The aims of this study are to detect the genetic polymorphisms of FSHR rs6166 (C> T) and rs6165 (C> T) gene particularly that associated with the response to FSH treatment and their effects on the pathogenesis of infertility in Iraqi women.

Subjects and methods: In this prospective observational study, two hundred sixty women were selected to participate in this study all of them have no differences in age, BMI, age of menarche, fifty of enrolled women apparently healthy that considered as control group and the rest 210 were recently diagnosed infertile women in which they divided into three groups according to ovarian response to treatment into poor responder (70 infertile women), moderate responder (61 infertile women), and high responder (79 infertile women). All the participants had signed informed consent before enrolled in this study, blood samples were obtained from all groups on second day of the menstrual cycle for genetic, hormonal (follicle stimulating hormone , luteinizing hormone, anti-mullerian hormone, thyroid stimulating hormone, prolactin, and estradiol) analysis, all infertile women included in the study were subjected to routine ultrasonography for measurement of

antral follicle count, blood sample drawn again for measurement of E_2 after six days of treatment by FSH and ultrasonography also done for measurement of size and number of graafian follicles. This study used Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS PCR) for detection of FSHR SNPs, rs6166(C>T) and rs6165 (C>T).

Results: The findings of this study in infertile women, clearly indicates that multiple genotypes of FSHR gene particularly (rs6166) (C>T) and (rs6165) (C>T), that include the homozygous wild genotype (CC), homozygous mutant (TT) and heterozygous (CT) genotype. The T allele was significantly increased ($P<0.05$) in poor responder infertile women for both rs6166 and rs6165 in FSHR which associated significantly with poor response to FSH in Iraqi infertile women.

Conclusion: Polymorphisms in FSHR gene may be associated with decrease in response to FSH treatment and it was associated with pathogenesis of infertility in Iraqi women/ Kerbala province.

CHAPTER ONE

Introduction

1.Introduction

1.1.Infertility

It is defined as a disorder of the reproductive system described by the failure to obtain a clinical pregnancy after twelve months or more of regular unprotected sexual intercourse, according to the World Health Organization(1). Infertility is a public health problem that affects approximately 8 to 12% of couples globally (2). Infertility may exert a negative effect on financial ,physical, emotional and social welfare of affected couples also it affects marriage stability ,job performance and family relationships (3).

Infertility in females can be classified into primary infertility which happens when a woman is unable to bear a child , either because she is incapable to conceive or because she is unable to carry a pregnancy to term and the other one is secondary infertility; concerning women who have previously been pregnant. Primary infertility affected 1.9% of couples, whereas secondary infertility affected 10.5% (2,4) . Despite the belief that infertility affects women more than men, the causes of infertility are equally divided between the genders. In 40% of infertile couples, the female partner is either the sole cause of infertility or a contributing factor, in another 40% it is the male partner and in the remaining 20% there is no recognized causes, which is referred to as unexplained infertility (5) .

1.1.1. Risk factors of infertility

Many factors can increase a woman's risk of becoming infertile such as:

A. Age: It is a more prevalent factor of infertility in women. Fertility problems are more likely in women over the age of 35. The reasons of this include: lower number and quality of eggs, more eggs have an unusual number of chromosomes and an increased risk of other health problems (6).

B. **High caffeine consumption**: Caffeine is a pharmacological active chemical that is frequently used , which is found in tea, coffee, cocoa, soft drinks and prescription drugs. Excessive caffeine uptake, increases the risk of infertility (7) .

C. **Taking illicit drugs:** Substance addiction and recreational drug not used for medical purposes (heroin, cannabis, cocaine, marijuana, opiates, and other similar substances) are frequently linked to physical, behavioral and social problems .The route of administration ,duration of exposure, age, dose, mental and physical status of an individual are all elements that influence their effects(8). It disturbs the reproductive processes leading to primary infertility by affecting folliculogenesis, implantation failure, embryo development, and spontaneous abortion(9).

D. inappropriate timing of sexual intercourse for reproductive purpose: The time of intercourse in relation, to ovulation has strongly impact on chance of conception, intercourse during five days before ovulation or on the day of ovulation is responsible for nearly all pregnancies in healthy women seeking to conceive(10).

E. Infectious agents: Sexually transmitted infections that are left untreated are one of the factors, that cause damage, to the reproductive system. Chlamydia and gonorrhea are most common infections leading to infertility and, if untreated the women will be at risk of pelvic inflammatory disease which causes chronic pelvic pain and ectopic ,pregnancy (11).

F. **Smoking tobacco:** Smoking may reduce the probability of pregnancy, also make fertility treatments less effective. Ovulatory problems are more likely in young females who smoke excessively, nicotine in smoke has been proven to reduce estrogen bioavailability or inhibit aromatase action in granulosa cells important for estrogen production ,besides miscarriages are also more common in women who smoke (12,13).

G. **Stress:** Hormonal changes caused by stress may cause mood swings, menstrual dysregulation,functional hypothalamic amenorrhea, anovulation and reductions in gonadotropin-releasing hormone, follicular stimulating hormone (FSH) and luteinizing hormone (LH) can all result in infertility as a result of prolonged stress (14).

H. **Alcohol:** Excessive alcohol consumption causes estrogen levels to increase, which lower the FSH levels and affect follicular development and ovulation process (15).

I. **Overweight**: A sedentary lifestyle, lack of exercise and being overweight lead to obesity, which increases the probability of infertility (16)**.**

J. Underweight: Women with eating disorders such as bulimia or anorexia, as well as those who adopt an extremely low-calorie or restrictive diet, are at risk for fertility problems .Exercise is very important, .However ,excessive exercise is harmful ,because it changes hormonal level which affects the fertility (17).

1.1.2 Etiology of infertility

There are many causes of infertility; however, it can be difficult to identify the exact cause, and some couples, have unexplained or multifactorial infertility (several causes, frequently including both male and female factors). Female factor infertility can be caused by a variety of factors include:(18)

A. **Hormonal Disorders;** there are many hormonal disorders that cause infertility. Hypothyroidism and hyperprolactinemia are a few examples of these disorders which consider a major cause of infertility in women. The inability of women at ovulation and regulation of hormone levels leads to too high or too low production of hormones. These hormonal disorders are characterized with symptoms such as irregular menstrual cycles, excessive bleeding, or very little bleeding, pelvic and abdominal cramps, absence of menstruation or long menstruation and excessive weight loss or weight gain (19,20).

B. **Abnormalities of the uterus or the cervical region;** includes uterine polyps, abnormalities with the cervix or the shape of the uterus**.** Uterine fibroids are benign tumors in the wall of uterus that can cause infertility by obstructing the fallopian tubes or preventing a fertilized egg from implanting in the uterus (21).

C. **Damage or obstruction of the fallopian tube;** this is often caused by inflammation of the fallopian tube .This can result from pelvic inflammatory diseases, which is usually caused by asexually transmitted infections, adhesions or endometriosis (22).

D. **Endometriosis;** which happens when endometrial tissue starts to grows outside of the uterus ,which may interfere with the function of uterus ,ovaries and fallopian tubes (23,24).

E. Primary ovarian insufficiency (early menopause); which occurs when the ovaries stop working and menstruation ends before age 40. Although the cause is generally unknown, specific factors are linked to early menopause, involve some genetic conditions such as Turner syndrome ,immune system diseases, , and treatment with radiation or chemotherapy(25).

F. Pelvic adhesions; the pelvic anatomy is malformed and fertility decreased through mechanical adhesion like pelvic adhesion, these adhesions cause damage to ovule release or selection and disruption in myometrium contraction like fertilization disorders (26) .

G. Cancer and its treatment, Certain cancers especially reproductive cancers frequently impair female fertility, both chemotherapy and radiation may affect fertility(27) .

H. Genetic factors, the genomic base of infertility is very complicated and determined by a variety of factors ,these factors affect the development of embryo, gametes and reproductive organs (28).

The genetic disorders can affect females ,males or both, causing infertility. Many of the 25–30 % of couples with idiopathic infertility are likely have a genetic etiology for their condition (29).

Genetic causes of infertility can be divided into gene defect, cytogenetic anomalies and epigenetic aberrances, several genes have been associated with infertility including Bone Morphogenetic Protein 15 and Fragile X Mental Retardation Genes .The genetic cause of infertility may be due to inactivation mutations in the gonadotropin and their receptor genes such as Follicle stimulating hormone and its receptor (FSHR), estrogen and its receptor (ESR) , Anti Müllerian hormone and its receptor (AMHR), luteinizing hormone and its receptor (LHR) (30–32).

1.1.3. Diagnosis of infertility

1.1.3.1. Case history

Initial infertility assessment include a detailed history, that consist of the infertility duration, family history of infertility, gynecologic history (chronic pelvic pain, pelvic inflammatory disease, fibroid uterus) medical history (thyroid disease, hyperprolactinemia), medication history (cancer treatment ,prolonged use of steroid, hormonal therapy, certain antihypertensive,anti-obesity,antidepressant/antipsychotic) and surgical history (appendectomy, pelvic or abdominal surgery)(33).

1.1.3.2. Physical examination

The physical examination give important hint to the causes of infertility. Physical conditions such as BMI ,vital signs , examination of breast for galactorrhea ,signs of androgen raise (dermatological and examination of external genitalia), the appearance of abnormal cervical or vaginal anatomy and pelvic tenderness or masses(34).

1.1.3.3. Gynecologic ultrasonography

It is used extensively to assess pelvic organs to diagnose and manage gynecologic problems such as ovarian cysts, endometriosis, lesions, gynecological cancer and in infertility management to monitor the response of ovarian follicles to fertility drugs (35).

Ultrasonographic measures the ovarian volume and antral follicles count (AFC), it is the total number of antral follicles in both ovaries as determined by transvaginal ultrasonography through the early-follicular phase.

AFC has been suggested as prognostic indicator of clinical outcome and ovarian reserve in variety types of infertility states , also play an important role in detection of ovarian response (36,37).

1.1.3.4. Laboratory tests

Infertility has been diagnosed using a variety of laboratory investigations, such as:

A. Prolactin

Prolactin is a polypeptide hormone that is generated and secreted from specific cells of the anterior pituitary gland, it promotes the breasts to grow and produce milk during pregnancy and after delivery ,prolactin levels are usually high in pregnant women and new mothers, it is usually low for non-pregnant women(38).

Elevated prolactin has an effect on reproduction through its negative effect on hypothalamic, gonadotropin releasing hormone (GnRH) neurons and on the pituitary gland which lead to decrease secretion of the gonadotropins [follicle-stimulating hormone (FSH) and luteinizing hormone (LH)]. Prolactin hypersecretion causes galactorrhea ,amenorrhea and infertility (39).

B. Thyroid stimulating hormone (TSH)

It is synthesized and secreted by the pituitary gland , its importance in controlling the secretion of thyroid hormones via the thyroid stimulating hormone receptors, TSH levels are the most sensitive, accurate and diagnostic indicator of thyroid function(40).

TSH has a synergetic impact with follicle stimulating hormone in the promotion of granulosa cell proliferation , regulates the FSH stimulation in follicles and inhibits their apoptosis . In follicular fluid ,TSH has an important role in the follicle development, while its dysregulation may effect on the follicular development, miscarriage risk and fertility by interfering with the follicular growth(41).

C. Estradiol (E2)

Estradiol is the main form of estrogen, another name is 17 beta-estradiol, it's produced by placenta, ovaries, adrenal glands, breasts and during pregnancy. Estradiol promotes the development and growth of female sex organs, including breasts, vagina, uterus and fallopian tubes. Normal levels of E2 in females are critical to health and function of sexual organs and affects on other marker of health such as bone strength(42,43).

D. Anti-Mullerian hormone (AMH).

AMH is a dimeric glycoprotein belonging to transforming growth factor-beta superfamily, which acts on tissue growth and differentiation, it is produced by the granulosa cells from pre antral and small antral follicles. AMH is a, clinically beneficial marker of ovarian reserve and hence of clinical value in the treatment of infertility ,when it is crucial to measure the follicles reserve. Serum AMH is used as a diagnostic test in infertile women undergoing controlled ovarian stimulation , assessment risk of ovarian hyperstimulation syndrome, predicting of menopause and monitoring the effect of radiotherapy and chemotherapy on ovarian function (44,45). AMH levels decrease with age from adulthood toward menopause demonstrating the size of the ovarian follicle pool (46).

AMH levels remain constant throughout the menstrual cycle and thus can be accurately measured at any time unlike other hormone markers that must be tested in early follicular phase (47).

E. Luteinizing hormone (LH)

LH is a glycoprotein hormone produced from the anterior pituitary gland, it helps in the regulation of the menstrual cycle and triggers the release of an egg from the ovary. LH works closely with follicle stimulating hormone to regulate sexual functions and its level rapidly rise before ovulation(48).

F. Follicle-stimulating hormone

FSH is a dimeric glycoprotein with a molecular weight of 35.5 kilodalton (KDa), that is composed of two polypeptide subunits alpha and beta, its structure is similar with other hormones including thyroid stimulating hormone, luteinizing hormone and human chorionic gonadotropin (hCG). The alpha subunits of the glycoproteins TSH, FSH, LH and hCG are similar and consist of 96 amino acids , whereas the beta subunits differ which are hormone specific, both alpha and beta subunits are desired for biological activity. The 111-amino-acid beta subunit of FSH is responsible for interaction with the follicle-stimulating hormone receptor and confers its unique biologic action (49) .

FSH is a gonadotropin hormone produced by gonadotropic cells in the anterior pituitary, it plays essential role in the regulation of male and female reproduction and its synthesis is controlled by gonadotropin-releasing hormone (GnRH) pulse that is produced in hypothalamus(50).

FSH regulates the growth, pubertal maturation, development and reproductive processes of the body. During the follicular phase of the menstrual cycle, it stimulates the growth and maturation of eggs (follicles) in the ovaries(51).

Disorders affecting the ovaries ,pituitary gland and hypothalamus can cause the production excessive or insufficient FSH, resulting in different conditions such as abnormal menstrual cycles, infertility , delayed puberty or precocious (52).

In females, FSH deficiency causes impaired folliculogenesis prior to the antral stage and infertility through the adult life (53)

FSH levels increase in late luteal phase through midfollicular phase of menstrual cycle, in addition to preovulatory rise corresponding to recruitment of a cohort of follicles to the growing pool. FSH plays a key role during ovarian folliculogenesis, antral follicle development and it stimulates preovulatory follicular growth when combined with luteinizing hormone (54).

FSH has an effect on the proliferation of granulosa cells, which produce estrogen essential for follicle growth ,development and maturation of antral follicles, as well as preparing the dominant follicle for ovulation, in response to LH surge. Usually in humans, only one follicle becomes predominant and remain to grow to 18–28 mm in size and ovulate(55).

As serum estradiol levels increase , FSH production falls and LH production rises, the smaller follicles undergo atresia as a result of the fall in serum FSH levels, as they lack the sensitivity to FSH to survive(56).

1.2. Recombinant follicle stimulating hormone (rFSH)

Recombinant DNA technology is a technique for combining DNA molecules from two different species and inserting them into a host organism to produce new genetic combinations, useful in medicine, science, agriculture and industry, also it plays a vital role in improving health conditions by producing new vaccinations, medications, and treatment strategies by developing monitoring devices, diagnostic kits, and new therapy approaches(57).

The development of new varieties of experimental mutant mice for research purposes and the synthesis of synthetic human insulin, erythropoietin, and FSH by genetically modified bacteria are the most common examples of genetic engineering in health(58).

Exogenous FSH plays a crucial role in the treatment of infertility, it has been dispensed to induce follicular growth both when monofollicular development was required and when multifollicular growth was desired to generate multiple oocytes (59,60).

Several techniques can be used to obtain follicle stimulating hormone, one of them is by extraction from women's urine in menopausal age and comprising FSH:LH activity at a ratio of 1:1 in addition to a variety of urinary proteins, but ovulation induction may be negatively affected by LH. As a result, only FSH must be extracted from urine (61,62). Usage recombinant DNA technique has better outcomes because it is not contaminated with other hormones and differences among batches are minimized (63).

Recombinant FSH is comparable to pituitary or urinary FSH in receptor binding capacity, glycosylation site ,amino acid sequence and in vitro biologic activity, while the recombinant and natural carbohydrate structure are the same(64).

Early formulations were derived from animal sources such pregnant mare serum or post-mortem extracts of the human pituitary gland, in the beginning the use of recombinant human FSH (r-hFSH) was widely recognized as a further progress in hFSH pharmacology, having higher purity, more intense specific activity, and superior efficacy in terms of pregnancy rate when compared with urinary human follicle stimulating hormone u-hFSH (65,66) .Currently, there are different r-hFSH products on the market: follitropin α, β and δ (67).

Follitropin α, β and δ have the same amino acid sequence; nevertheless, they differ in glycosylation, composition of sialic acid residues and isoelectric coefficients: follitropin α is more acidic than follitropin β, resulting in slightly different biological activity, half-life and metabolic clearance (68,69).

1.3. Follitropin-alfa

It is a human FSH preparation obtained by recombinant DNA technology, which consists of non-identical glycoprotein designed as α and β subunits which linked non-covalently, the two subunits consist of 92 and 111 amino acids for α and β subunits respectively. The primary and tertiary structure of these subunits are indistinguishable from those native human follicle stimulating hormone, Recombinant FSH production occurs in genetically modified Chinese hamsters ovary cells are cultured in bioreactors ,the product Purified by immune chromatography by using an antibody specifically binding to FSH, the resulted preparation is highly purified with a consistent FSH isoform profile and a high specific activity (70,71).

1.3.1. Therapeutic indication

Follitropin α is indicated for treatment of an ovulatory infertility in women who have not been responded to clomiphene citrate or when clomiphene citrate is contraindicated , and used for controlled ovarian hyperstimulation in women undergoing assisted reproductive technology(72).

1.3.2. Pharmacokinetic properties

After administration of intravenous follitropin α, it distributed to the extracellular fluid space with an initial half-life of about two hours and eliminated from the body with terminal half-life around one day .The fraction of follitropin α excreted in urine is one-eighth of the dose (73).

Maximal concentration of follitropin α reached earlier by subcutaneous (SC) administration which about sixteen hours ,as compared to intramuscular (IM) route which approximately twenty five hours, this situation was associated with the longer half-life observed with the IM administration (eight hours) than with the SC route (five hours). Thirty seven hours are the terminal half-life of IM and SC routes, and the greatest interindividual variability(70%) occurs in these routes(74).

After multiple SC administration, the terminal half-life about 24 hours with little interindividual variability of (30 %) and comparable with the terminal half-life that recorded beyond single intravenous(IV) dose. The renal clearance accounts for 10% of total clearance(73)

The primary volume of distribution is 4L, which is corresponded with serum volume. At steady state, the volume of distribution is 11L, and 20 hours are the mean residence time , there is a strong correlation between increasing maximum serum estradiol level with maximal total follicular volume beyond SC administering of recombinant FSH along seven days (75,76), administered daily dose of rFSH cannot reach to maximal effect until three or four days after multiple doses, before increasing the dose it is advisable to wait for at least four days for efficacy effects (77).

1.3.3. Side effects

The main side effects of rFSH are listed below:

Ovarian hyperstimulation syndrome (OHSS): is a prevalent and dangerous side effect, that occurs as the ovaries produce very high number of eggs (overstimulated), and can cause suddenly accumulation of fluid in the heart, chest, and, stomach also development of blood clots may occur (78–80).

Twisting of ovary: It occurs when the woman has specific conditions like past abdominal surgery, pregnancy and OHSS. Fewer than 2% of the gonadotropin cycles the provoked ovary may spin on itself, so the ovary is heavier because the presence of more follicles, this twisting can cause cut off blood supply to the ovaries, surgery is required in order to untwist the ovary and in severe situations, may need to remove it (81,82).

Multiple babies pregnancy: Exogenous gonadotropin treatment elevates the occurrence of multifollicular formation, that leads to multiple pregnancy for 5-20% of periods. A 50% increase in rate of twin birth has been detected during the last three decades accompanied with high-order rates of multiple birth increasing even significantly (83,84).

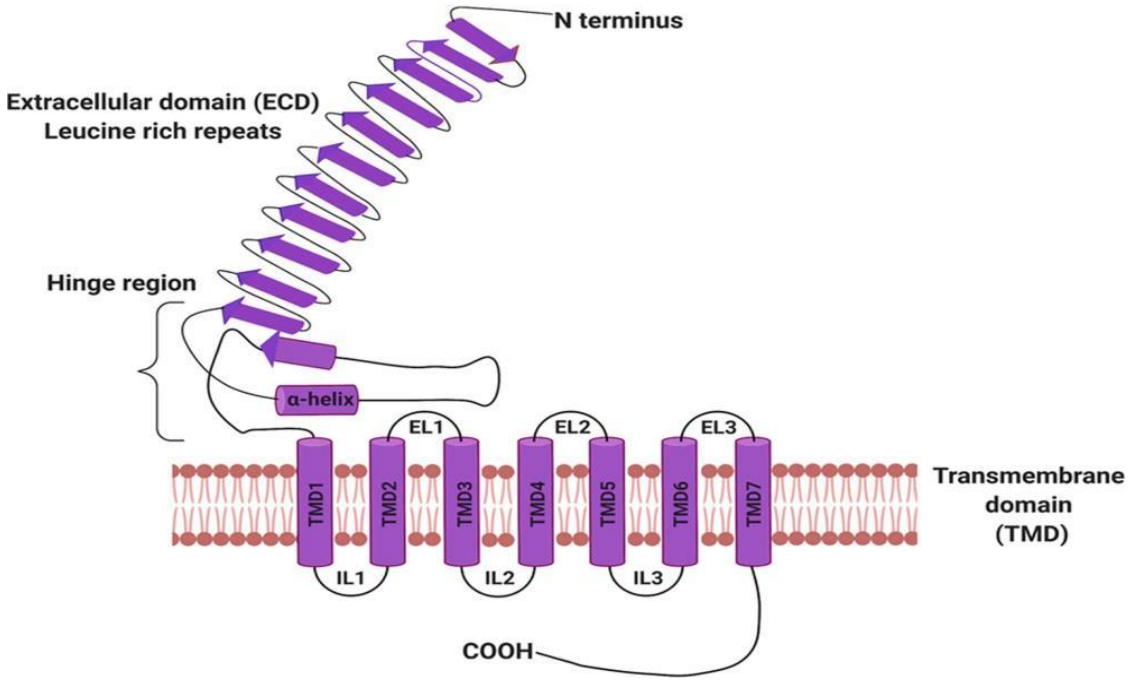
Ectopic pregnancy: It means pregnancy outside womb, the occurrence of conceiving outside of uterus is elevated if there is fallopian tube problem. Ectopic pregnancies develop in normally occurring pregnancies for about 1-2 % ,the rate is slightly elevated in gonadotropin cycles (85,86).

Another side effects also may occur but in low rates such as miscarriage, , bleeding between periods ,tumors of the ovary**,** headache, ovarian cyst , pelvic pain ,stomach pain , nausea. The most commonly local side effects include pain, itching, erythema, and bruising which appear around the site injection(87).

1.4. Follicle stimulating hormone receptor (FSHR)

FSHR is a type of G protein-coupled receptor (GPCR) belonging to glycoprotein hormone receptors (GPHRs) family, with a long extracellular domain (ECD), that consist mainly of 7 transmembrane domain (TMD), 3 of which are short intracellular loops, 3 extra loops and an intracellular tail (88) .The molecular weight of FSHR is approximately 75 KDa ,that comprises 695 amino acids , which include a 17 amino acids signal peptide with three to four potential glycosylation sites (89,90).

The ECD is connected to the TMD which is membrane spanning, by means of a hinge region. The hinge region carries a hairpin loop and an a-helix surrounded on each sides by two motifs of cysteine box that form cysteine bonds. The TMD which is responsible for transduction of signal is composed of seven α -helices joined to each other through three extracellular loops ELs (EL1, EL2 and EL3) and three intracellular loops ILs (IL1, IL2 and IL3) (56) , in the cytoplasm a short C-terminal tail is present as shown in Figure(1-1).



Figure(1-1): Tertiary structure and functional domains of FSHR (91)**.**

The human FSHR gene is located on chromosome 2p21 and it is single copy gene composed from 54 Kilobase (kb) in length, the human FSHR gene involves 10 exons and 9 introns with a promoter region. The extracellular domain encoded by nine exons while the C-terminus and intracellular domain of extracellular domain, and transmembrane domain all of them are encoded by exon 10 (92).

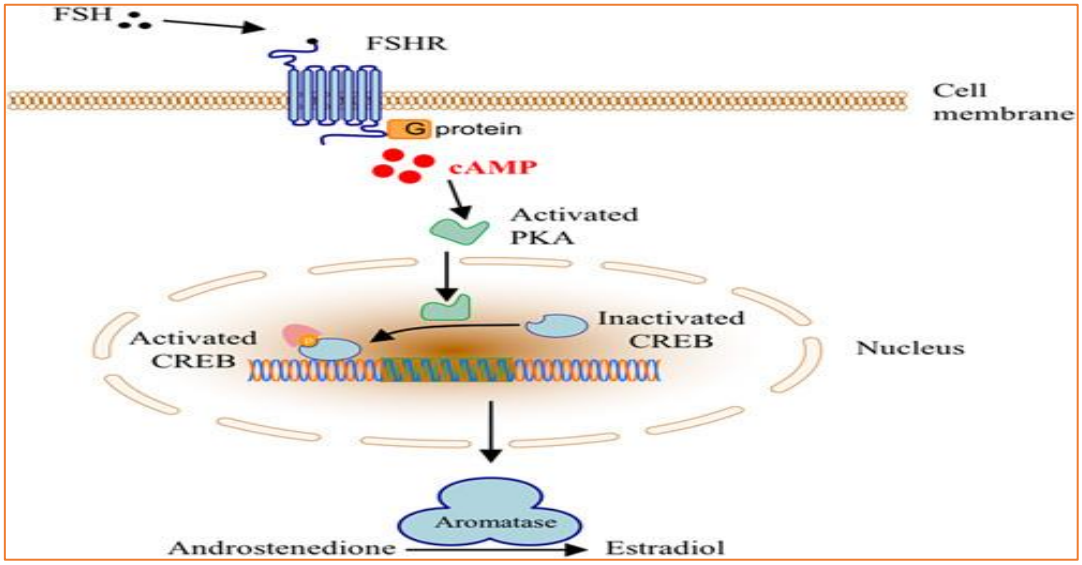
Multiple isoforms of FSHR have been recorded and FSHR expressed on extra gonadal tissues that involve placenta, prostate, uterus, ovarian epithelium and bone tissue also ovarian cancer. FSHR has also been found to be selectively expressed on the surfaces of many tumor blood vessels, and related to tumor metastasis .FSH has high liability for binding to leucine rich repeats of beta strand in the ECD of FSHR which form a horseshoe structure (93).

Comparison of the motifs sequences across GPHRs revealed little sequence identity for the ECD region that consistent with the stability of ligand binding specificity, while higher identity observed in the transmembrane region indicates a common stimulation mechanism(94,95).

The FSHR life cycle includes its synthesis, then appropriates folding, followed by post-translational modifications and formation of highly-ordered di/oligomers in the Golgi apparatus and endoplasmic reticulum, subsequently followed by anchoring to the cell surface in order to become functional for FSH signaling events and binding(96).

Following FSH binding the Gas protein is divorced from the receptor, and triggers pathways subsequently to Gas activation, namely protein kinase A mediated by phosphorylation of extracellular signal-regulated kinase and cyclic adenosine mono phosphate (cAMP) (97,98), that regulate transcription of cAMP response element-binding proteins (CREB) as phosphorylation and aromatase of downstream effectors of the mitogen-activated protein kinase pathway essential for luteinization and ovulation events (Figure1-2). The desensitized receptor followed by phosphorylation in its C-terminal tail and ILs by means of G protein-related kinases , the phosphorylated receptor subsequently acted upon by proteins is called beta arrestin to mediate its conjucation with clathrin-coated pits for endocytosis(99,100).

Most of the FSH–FSHR complexes upon internalization are reserved to a plasma membrane for recycling pathway, and only a little fraction is routed for degradation in lysosomal pathway (101).

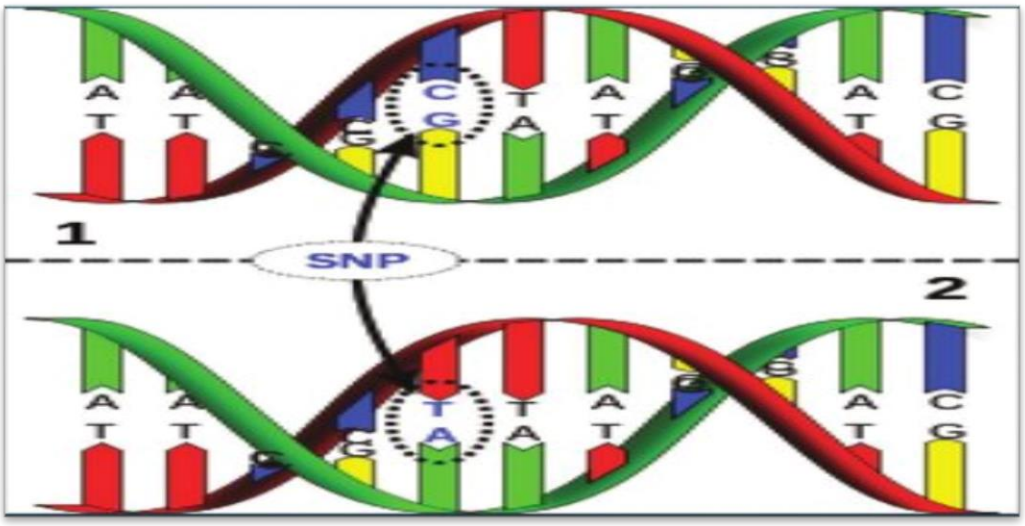


Figure(1-2): Signaling pathway (cellular action of FSH and FSHR) by the usual cyclic AMP/ protein kinase A (102)**.**

1.5. Genetic variation (polymorphism)

Genetic polymorphism is difference in DNA sequence occurring among individuals, populations, groups, it can affect single phenotypes such as susceptible to diseases, color of skin or eyes, and respond to drug, chemical, pathogen, and vaccine. Its frequency ≥ 1% and occurs more widely than mutations amongst different individuals. Several forms of genetic variants are present in the human genome as deletions, sequence repeats, insertions, and the most common type of genetic variation is single nucleotide polymorphisms (SNPs) (103,104).

SNPs mean change in a genetic sequence which affects only one of basic building blocks cytosine (C), guanine (G), adenine (A), or thymine (T) in a segment of a DNA molecule and that occurs more than 1 percent of population, two of every three SNPs involve the replacement of C with T as shown in Figure (1-3) (105,106).



Figure(1-3): Single nucleotide polymorphism(107)**.**

Approximately 90% of all human genetic variation is SNP, occurs at rate of one in each 100-300 nucleotides of human genome. The SNPs in the human genome can occur in both coding(exon) and non coding(intron) regions. SNPs falling in the coding regions of genome can be further divided into: nonsynonymous and synonymous SNPs. The synonymous SNPs do not change in amino acid sequence of protein, or not affect the function of protein. The nonsynonymous can divided into two types: nonsense and missense (108,109) .

The missense SNP lead to amino acid changes that usually has pathogenic impacts on the structure, and/or function of protein by development of diseases and affect physiological responses to drugs. The nonsense point mutation in DNA sequence that changes to a stop codon which results in nonfunctional protein product (110,111).

1.6. FSH receptor polymorphism and therapeutic response to FSH.

Pharmacogenetics explain the relationship between genetic variability and drug responses, also can be used to individualize pharmacological therapies to the genetic characteristics of an individual patient as well as enhance the desired actions and reduce the side effects of such treatments. Drug-genome interactions can occur in several ways including:

a. genetic variation in the direct molecular target of a drug class.

b. class of genes are those involved in drug absorption ,distribution, metabolism and excretion. Considering the large variability of the ovarian stimulation outcome, pharmacogenetics has a broad applicability in treatment of infertility also to develop diagnostic tests that can predict drug action and modify therapy appropriately (112,113).

Genetic factors can explain the differences among individuals in the terms of drug response; although many non-genetic factors affect the action of medications, these differences evidently occur because of sequence variants in the genes that encoding drug targets (114).

Within the human genome more than 19 million of SNPs have been identified some of these SNPs already have been associated with changes in the effects of drugs (115).

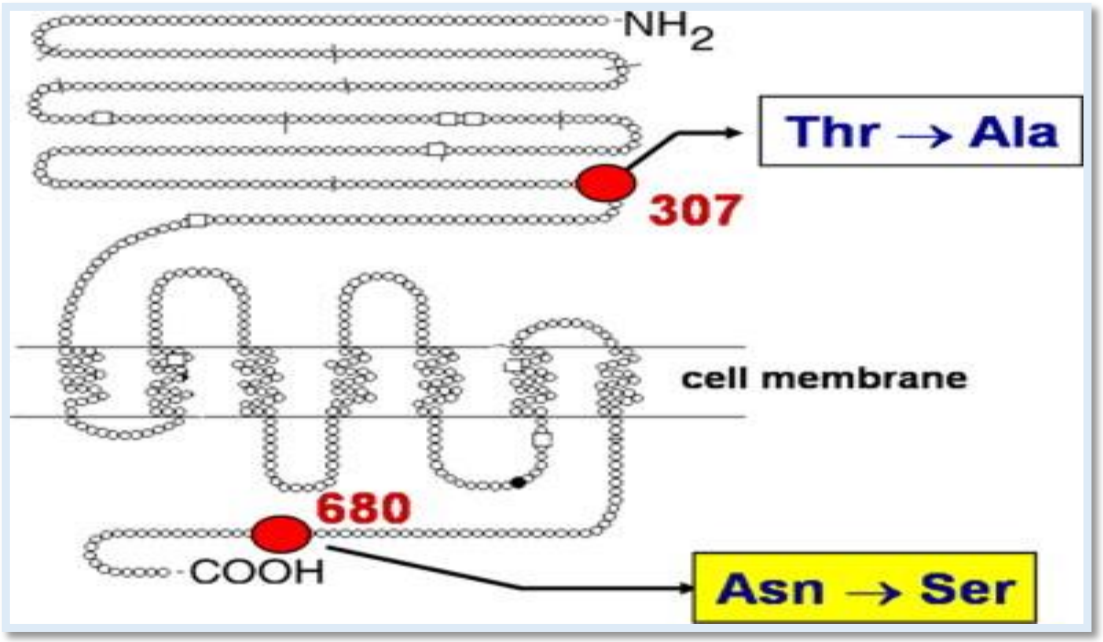
Defects in structure of FSHR as a result of polymorphisms, mutations and other anomalies might modify hormone action, endocrine feedback systems, and influence the ovarian action for the exogenous and endogenous FSH. This could result in variation between individuals in reproductive performance that could influence the success of assisted reproductive treatment (116,117).

Several variants that involve inactivating and activating of FSH-R have been identified. The most of the inactivating variants are located on exons 7 and 10. The major typical clinical manifestations of inactivating variants are infertility, elevated FSH levels, primary amenorrhea and premature ovarian failure; whereas activating mutations can predispose to ovarian hyper stimulation syndrome, as a consequence of exogenous FSH administration, or to a spontaneous onset(112,118,119).

The activating variants in FSH-R gene can appear in heterozygotes, while the inactivating variants change the phenotype only when found in the homozygous, or compound heterozygous form (42).

Recent genetic studies have revealed that the pathogenesis of infertility can be due to mutations in the follicle-stimulating hormone receptor (FSHR) gene. While mutations affecting FSHR are sporadic; polymorphism of the FSHR gene seems to be a common phenomenon. The FSH-R gene is highly polymorphic in nature and carries single nucleotide variants more than 2000, the most common of these SNPs are located at codon 307 and 680 of exon 10, which are related to ovarian response (120,121).

The first SNP is found in the extracellular domain codon 307 (rs6165), and the second lies in the intracellular domain codon 680(rs6166).Both SNPs affect function of gene by changing the properties of gene product, and consequently modifying the response to FSH. Threonine (T) can be substituted by alanine (A) at position 307 and asparagine (N) can be substituted by serine (S) at position 680. These polymorphisms are in linkage disequilibrium, resulting in the most frequent allelic combination of T307-N680 and A307-S680. For the purpose of simplification, most studies focus almost exclusively on polymorphisms at codon680 as shown in figure (1-4) (122,123) .



Figure(1-4): FSH receptor protein and usual sites for amino acid changes due to (SNP) (124).

1.7. Aims of study

The aims of this study was to investigate the effect of FSHR polymorphisms (rs6166 and rs6165) on the therapeutic response to FSH in Iraqi infertile women and the role of FSHR polymorphisms in the pathogenesis of infertility.

CHAPTER TWO

Materials and Methods

2. Materials and Methods

2.1. Patients and Control

This prospective observational study was conducted on 210 Iraqi women aged 20-34 years with newly diagnosed infertility and 50 healthy women clinically without any diseases served as control group. All participated women were recruited by consultation of gynecologist according to the inclusion and exclusion criteria of the study. This study was conducted from November 2021 till June 2022. Samples were collected from private clinic in Kerbala city. The practical part was conducted at research laboratories in the University of Kerbala, College of Pharmacy, department of Pharmacology and Toxicology.

This research was approved by the Scientific and Ethical Committee, and all subjects were given an idea about the study and their written informed consent was taken. Demographic parameters (questionnaire) were taken from all patients before taking the treatment which include (Age, body mass index(BMI), acne, age of menarche, family history of infertility, family history of other disease, Hirsutism, education, menstrual irregularity, history of previous conception, medication taken for another diseases)

All participants subjected to physical examination, vaginal ultrasonography for measurement of AFC by consultant gynecologist and basal levels of laboratory investigations (FSH, LH, Prolactin, TSH, E_2, AMH) were measured in the follicular phase (2nd day of cycle).

At the third day of menstrual cycle all infertile women were given 75 international unit of follitropin- α (subcutaneously)(125,126) and after six days of stimulation, infertile women subjected to vaginal ultrasonography and E_2 measurement. Follicular development was monitored by transvaginal sonography every other day until at least one follicle reach 17 mm then hCG 10,000 IU was given as a single I.M injection to trigger ovulation.

2.1.1.Study groups

The study subjects divided into two groups:

1.Control group: which include 50 healthy women.
2.Patient group: which include 210 infertile women that distributed into three groups according to treatment response(127).

-Poor responder: Which includes 70 infertile women suffering from failure to respond adequately to ovarian stimulation treatment ,low ovarian reserve based on elevated basal FSH , AFC < 5 and/or AMH < 0.5.

-Moderate responder: Which includes 61 infertile women with AFC 5-12 and/or AMH >2.

-High responder: Which includes 79 infertile women were predicted to yield high ovarian response based on AFC >12 and/or AMH > 5.

2.1.2. Patients criteria: All patients selected or excluded according to the following criteria:

2.1.2.A. Inclusion criteria: women included in this study must have the following criteria**:**

- Women with infertility have newly been diagnosed based on uterine ultrasonography and hormonal levels.
- age ≤34 years.
- BMI ≤25.
- cycle length 27–32 days.

2.1.2.B. Exclusion criteria: women are excluded if they have one or more of the following criteria:

- previous ovarian surgery.
- Ovarian endometriosis.
- Endocrine, and systemic disorders (diabetes mellitus, renal or cardiovascular diseases) .
- Male factor infertility.
- Poly cystic ovarian syndrome.

2.2. Materials

2.2.1. Instruments, Equipments and their Suppliers

All instruments and equipments used in this study were listed in table (2-1) with their manufacturing company.

Table (2-1): Instruments and equipments with their manufacturing companies and countries

Equipments	Company	Country
Centrifuge	Hettich	Germany
Cobas e411 analyzer	Roche	Germany
Nanodrop	Bio drop	England
Digital camera	Canon	England
Distillatory	GEL	Germany
Hood	Lab Tech	Korea
Incubator	Binder	Germany
Micropipettes	Slamed	Japan
Nano pac 500 power supplierfor electrophoresis	Cleaver	UK
PCR machine(Thermocycler)	Verity	United state
Sensitive balance	AND	Taiwan
UV-trans illuminator	Syngene	England
Vortex mixer	Human twist	Germany
Disposable syringe	Slamed	Germany
Microcentrifuge tube	Slamed	Germany
Disposable test tube	Afma.despo	Jordan
Deep freezer	GFL	Germany

2.2.2. Chemicals, Kits and their Suppliers

All chemicals and kits used in this study with their manufacturing company were listed in the table (2-2)

Table (2-2) Chemicals and kits and their producing companies

	Chemicals and Kits	Company	Country
Chemicals	follitropin α	Merck	Switzerland
	Agarose powder	Bio Basic	Canada
	Ethanol 99.9 %	Hayman Kimia	UK
	Ethedium Bromide	Sigma	USA
	Nuclease free water	Bioneer	Korea
	TBE buffer	Bioneer	Korea
Hormonal Kit	FSH kit	Roche	Germany
	LH kit	Roche	Germany
	Prolactin kit	Roche	Germany
	TSH kit	Roche	Germany
	Estradiol Kit	Roche	Germany
	AMH Kit	Roche	Germany
Kits For Genetic Study	DNA extraction kit	Favor	China
	DNA ladder(100bp)	Bioneer	Korea
	PCR Pre Mix Kit	Bioneer	Korea
	Primers	Macrogen	Korea

2.3. Methods

2.3.1. Samples Collections

Seven milliliters (7ml) of venous blood was withdrawn from all patients and healthy controls at the second day of menstrual cycle, (2 ml) was placed in EDTA tube for DNA extraction and (5ml) was placed in plain tube for serum analysis (FSH, LH, Prolactin, TSH, E_2, AMH) ,serum was aspirated after centrifugation of blood at 3000 rpm for 10 minutes were it used for measurement. After six days of ovarian stimulation (on day nine of menstrual cycle) another two milliliters (2 ml) of venous blood were drawn from all infertile women included in this study for serum analysis of E_2.

2.3.2. Biochemical Assay Methods

2.3.2.1. Measurement of Serum Follicle Stimulating Hormone

The quantitative determination of FSH in human serum is by an (Elecsys FSH) kit and Cobas e 411 analyzer and dependent upon electro chemiluminescence immunoassay method (128).

Principle

Sandwich principle, total duration of assay: 18 minutes.

- **1st incubation:** 40 microliter (μL) of sample, a biotinylated monoclonal FSH-specific antibody, and a monoclonal FSH-specific antibody labeled with aruthenium complex form a sandwich complex.
- **2nd incubation:** After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

- The reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured, onto the surface of the electrode.

Unbound substances were then removed with ProCell/ProCell, M. voltage Applicated to the electrode then induces chemiluminescent emission which was measured by a photomultiplier.

- Results were determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode**.**

Reagents

The reagent rack pack was labeled as FSH.

M Streptavidin-coated microparticles: one bottle(6.5mL) of Streptavidin-coated microparticles 0.72 milligram / milliliter(mg/mL).

R1 Anti-FSH-Ab~biotin: one bottle(10mL) of Biotinylated monoclonal anti-FSH antibody, 0.5 mg/L, MES buffer 50 *millimole/Liter* (mmol/L).

R2 Anti-FSH-Ab~Ru(bpy): one bottle(10mL) of Monoclonal anti-FSH antibody (mouse) labeled with ruthenium complex 0.8milligram/liter(mg/L), MES buffer 50 mmol/L.

2.3.2.2. Measurement of Luteinizing Hormone

The quantitative determination of luteinizing (LH) in human serum was done by an (Elecsys LH) kit and Cobas e 411 analyzer and dependent upon electro chemiluminescence immunoassay method (128).

Principle

Sandwich principle. Total duration of assay: 18 minutes.

- **1st incubation:** 20 µL of sample, a biotinylated monoclonal LH-specific antibody, and a monoclonal LH-specific antibody labeled with aruthenium complex form a sandwich complex.
- **2nd incubation:** After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. voltage were applicated to the electrode then induces chemiluminescent emission which is measured by a photomultiplier
- Results are determined via a calibration curve which was instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode

Reagents

The reagent rack pack labeled as LH.

M Streptavidin-coated microparticles: one bottle (6.5mL) of Streptavidin-coated microparticles 0.72 mg/mL.

R1 Anti-LH-Ab~biotin: one bottle (10mL) of Biotinylated monoclonal anti-LH antibody (mouse) 2.0 mg/L; TRIS buffer 50 mmol/L.

R2 Anti-LH-Ab~Ru(bpy): one bottle(10mL) of Monoclonal anti-LH antibody (mouse) labeled with ruthenium complex 0.3 mg/L; TRIS buffer 50 mmol/L.

2.3.2.3. Measurement of Estradiol

The quantitative determination of estradiol in human serum was done by an (Elecsys Esradiol III) kit and Cobas e 411 analyzer and dependent upon electro chemiluminescence immunoassay method (129).

Principle

Competition principle. Total duration of assay: 18 minutes.

- **1st incubation:** 25 µL of sample incubated with two estradiol specific biotinylated antibodies, immunocomplexes were formed, the amount of which is dependent upon the analyte concentration in the sample.

- **2nd incubation:** After addition of streptavidin-coated microparticles and an estradiol derivative labeled with a ruthenium complex, the still-vacant sites of the biotinylated, antibodies become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances were then removed with ProCell/ProCell M. Voltage were applicated to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results were determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

Reagents

The reagent rack pack labeled as E2 III.

M Streptavidin-coated microparticles: one bottle (6.5mL) of Streptavidin-coated microparticles 0.72 mg/mL.

R1 Anti-estradiol-Ab~biotin: one bottle(9 mL) of two biotinylated monoclonal anti-estradiol antibodies (rabbit) 2.5 ng/mL and 4.5 ng/mL; mesterolone 130 ng/mL; MESb) buffer 50 mmol/L.

R2 Estradiol-peptide~$Ru(bpy)^{2+}$3: one bottle(9 mL) of Estradiol derivative, labeled with ruthenium complex 4.5 ng/mL; MES buffer 50 mmol/L.

2.3.2.4. Measurement of AMH

The quantitative determination of anti-Müllerian hormone (AMH) in human serum was done by an (Elecsys AMH Plus) kit and Cobas e411 analyzer and dependent upon electro chemiluminescence immunoassay method(128).

Principle

Sandwich principle. Total duration of assay : 18 minutes.

▪ **1st incubation**: 50 µL of sample , a biotinylated monoclonal AMH-specific antibody, and a monoclonal AMH-specific antibody labeled with a ruthenium complex form a sandwich complex.

▪ **2nd incubation**: After addition of streptavidin-coated microparticles, the complex, becomes bound to the solid phase via interaction of biotin and streptavidin.

▪ The reaction mixture was, aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Avoltage were applicated to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

▪ Results were determined via a calibration curve which was instrument specifically generated, by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

Reagents

The reagent rack pack labeled as AMHP.

-**M** Streptavidin-coated microparticles: one bottle(6.5 mL) of Streptavidin-coated microparticles 0.72 mg/mL; preservative.

-**R1** Anti-AMH-Ab~biotin: one bottle (8 mL) of Biotinylated monoclonal anti-AMH antibody (mouse) 1.0 mg/L, phosphate buffer 50 mmol/L, pH 7.5; preservative.

-**R2** Anti-AMH-Ab~Ru(bpy): one bottle (8 mL) of Monoclonal anti-AMH antibody, (mouse) labeled with ruthenium complex 1.0 mg/L, phosphate buffer 50 mmol/L, pH 7.5; preservative.

2.3.2.5. Measurement of Prolactin

The quantitative determination of prolactin in human serum was done by an (Elecsys Prolactin II) kit and Cobas e 411 analyzer and dependent upon electro chemilumine-scence immunoassay method (128).

Principle

Sandwich principle. Total duration of assay: 18 minutes.

▪ **1st incubation:** 10 µL of sample and a biotinylated monoclonal prolactin specific antibody form a first complex.

▪ **2nd incubation:** After addition of a monoclonal prolactin-specific antibody, labeled with a ruthenium complexa and streptavidin-coated microparticles, a sandwich complex was formed and becomes bound to the solid phase via interaction of biotin and streptavidin.

▪ The reaction mixture was aspirated, into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances then removed with ProCell/ProCell M. Avoltage were applicated to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

▪ Results were determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

Reagents

The reagent rack pack labeled as PRL II.

M. Streptavidin-coated microparticles: one bottle (6.5 mL) of Streptavidin-coated microparticles 0.72 mg/mL.

R1. Anti-prolactin-Ab~biotin: one bottle(10mL) of Biotinylated monoclonal anti-prolactin antibody (mouse) 0.7 mg/L; phosphate buffer 50 mmol/L.

R2. Anti-prolactin-Ab~Ru(bpy) one bottle(10mL) of Monoclonal anti-prolactin antibody (mouse) labeled with ruthenium complex 0.35 mg/L; phosphate buffer 50 mmol/L.

2.3.2.6. Measurement of Thyroid Stimulating Hormone

The quantitative determination of thyroid stimulating hormone (TSH) in human serum was by an (Elecsys TSH) kit and Cobas e 411 analyzer and dependent upon electro chemiluminescence immunoassay method (128).

Principle

Sandwich principle. Total duration of assay: 18 minutes

- **1st incubation:** 50 µL of sample, a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with aruthenium complex react to form a sandwich complex.

2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes, bound to the solid phase via interaction of biotin and streptavidin.

- The reaction mixture was aspirated, into the measuring cell where the micro particles were magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell.
- A voltage were applicated to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results were determined via a calibration curve which is instrument specifically generated by 2-point calibration, and a master curve provided via the reagent barcode or e-barcode.

Reagents

The reagent rack pack labeled as TSH.

M. Streptavidin-coated microparticles: one bottle (12 mL) of Streptavidin-coated microparticles 0.72 mg/mL.

R1. Anti-TSH-Ab~biotin: one bottle (14 mL) of Biotinylated monoclonal anti-TSH antibody (mouse) 2.0 mg/L; phosphate buffer 100 mmol/L.

R2. Anti-TSH-Ab~Ru(bpy): one bottle (12 mL) of Monoclonal anti-TSH antibody (mouse/human) labeled with ruthenium complex 1.2 mg/L; phosphate buffer 100 mmol/L.

2.3.3. Determination of Body Mass Index (BMI)

It is a value derived from the mass (weight) and height of an individual. The BMI was calculated by dividing the body weight by the square of the body height, according to the following equation:

BMI in kg/m ^{2} = Weight (kg) / Square Height (m ^{2})

Normal weight falls between BMI values of 18.5-24.9, overweight between 25-30 and obese above 30 (130).

2.3.4. Genetic Analysis

2.3.4.1.The selection of FSHR gene SNPs

The SNPs that involved in the current study were selected depending on NCBI (national center for Biotechnology Information), the clinical var reported a higher susceptibility for modulating ovarian response and FSHR function ,these two SNPs rs6166 and rs6165 were the most common in the FSHR gene. Table (2-3).

Table (2-3): SNPs ,Gene ,Nucleotide change ,Amino acid substitution that involved in current study(108)**.**

SNP	Gene	Nucleotide change	Amino acid substitution	Consequence
rs6166	FSHR	C>T	Asn680Ser	missense variant
rs6165	FSHR	C>T	Thr307Ala	missense variant

The position of rs6166 (C\T) SNP on FSHR gene was illustrated in figure (2-1) and for rs6165 (C\T) on FSHR in figure (2-2)

[AGGCAAGACTGAATTATCATTCAATACTCAGATACATTTTCAC
ATTGTGTTTTAGTTTTGGGCTAAATGACTTAGAGGGACAAGTAT
GTAAGTGGAACCA[C>T]TGGTGACTCTGGGAGCTGAAGAGCAG
TGGCCATTCCTTGGATGGGTGTTGTGGACAGTGGATGAAGTTTC
TGTCCTATAAATTTGGGCTTGCATTTCATA]

Figure (2-1): Position of SNP rs6166 (C\T) SNP on FSHR gene.

[TCATTGCATAAGTCATAGTCAAACTCAGTGTACGTCATGTCAA
ATCCTCTGCTGTAGCTGGACTCATTGTCTTCTGCCAGAGAGGAT
CTCTGACCCCTAG[C>T]CTGAGTCATATAATCAACTTCTTGCCTT
AAAATAGATTTGTTGCAAATTGGATGAAGCTCAGAGCTAGAAA
AATACAAAAAGAAATAGAATCAACATCTC]

Figure (2-2): Position of SNP rs6165 (C\T) SNP on FSHR gene.

2.3.4.2. Extraction of Genomic DNA from Blood Sample

In most genetic analytical research and recombinant DNA techniques, the initial step is extraction of nucleic acids, A numerous number of procedures, based on different methods and principles, exists for the extraction and purification of nucleic acid, all of them share the common requirements that the biological material must be lysed, cellular nucleases must be inactivated, and the desired nucleic acid must be purified from the cellular debris(131).

The DNA extraction was accomplished at College of Pharmacy / University of Kerbela. Genomic DNA was extracted from a blood sample according to the protocol of (FavorPrep™) genomic DNA extraction kit for blood.

Extraction procedure was carried out in four steps including : lyses, binding, washing and elution as the following steps:

- 200 µl of whole blood was added into 1.5 ml microcentrifuge tube.
- 30µl of proteinase K enzyme was added to the sample tube then they were mixed briefly and incubating for 15 minutes at 60 ºc.
- 200µl of FABG buffer was added into the sample tube then mixed thoroughly by vortexing to produce a lysis solution.
- The mixture was incubated at 70 ºc temperature for 15 minutes.
- 200µl of absolute ethanol was added to the sample then mixed by vortex.

- The mixture was carefully added to the spin column, and centrifuged at 14,000 rpm for 1 min, filtrate was then discarded and a new collection tube was placed

- 400µl of W1 buffer was added to the spin column and centrifuged for 30 sec. at 14,000 rpm and discard the filtrate.

- 600µl of wash buffer was added to the spin column and centrifuged for 30 sec. at 14,000 rpm and discard the filtrate then the collection tube was backed to spin column for additional centrifuged for 3 min to dry the column.

- The spin-column was placed to a new 1.5 ml microcentrifuge tube then 100 µl of preheated elution buffer was added directly to membrane center of spin column and incubated for 10 min at 37 ºc then centrifuged for 1min at 14,000 rpm to elute the DNA, then stored at -20ºc(deep freezing).

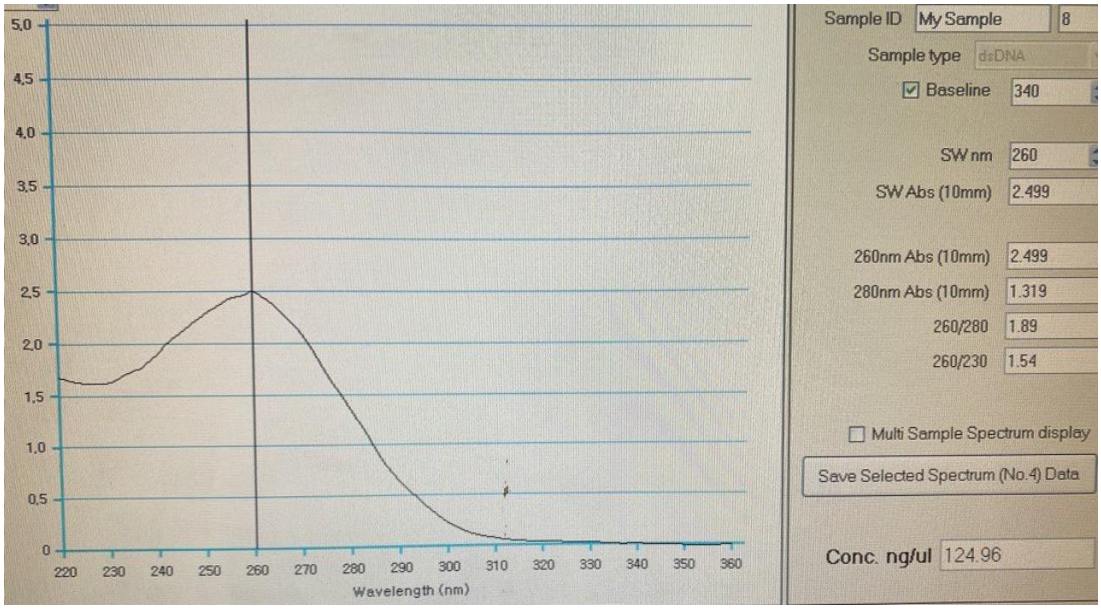
2.3.4.3. Determination of purity and concentration of DNA

The concentration and purity of DNA were determined by using spectrophotometric method (NanoDrop).

Principle:

The Nano drop instrument was used to measure the purity and concentration of extracted DNA samples by absorbance method. The absorbance readings were done at 260 nm and 280 nm. The DNA absorbs light significantly at 260 nm, while the protein absorbs light most strongly at 280 nm. The purity of DNA was measured by the A260/A280 ratio(132).

The A260/A280 ratio 1.8-2.0 was commonly accepted as a good indicator of a high-quality DNA sample. 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 documented from the instrument(133).(Fig 2-3) illustrate the results of nano drop which expressed the (purity and concentration) of DNA.



Figure(2-3):Nano drop results(purity and concentration) of DNA.

2.3.4.4. Polymerase Chain Reactions

Polymerase chain reactions (PCR) were accomplished using thermocycler, which amplified a desired region of the genome. The concentration of required target sequence rises from one molecule to many million copies. Any PCR cycled 25-45 times has three steps, which include:

A. Denaturation: This step involves unwinding double strands DNA into two single strands at 94-95oC.

B. Annealing: This step takes place at 55-65^{o}C. A pair of short nucleotide sequences (primers) anneal to the ends of strands of DNA and start the reaction.

C. Extension: This step takes place at 72-74^{o}C and the primers were extended to generate a new strand that is complementary to the template strand. This happens when Taq DNA polymerase was present(134).

2.3.4.5. Allele Specific Polymerase Chain reaction

Allele specific PCR is a polymerase chain reaction (PCR) application that allows for the detection of any a mutation or a polymorphism in human DNA, also called as an ARMS-PCR (amplification refractory mutation system) or PASA (PCR amplification of specific allele). Some reagents and components are crucial for ARMS-PCR, these components consist of DNA target (DNA template), primers (forward and reverse primer), *Thermus aquaticus* DNA polymerase (*Taq polymerase*) enzyme, deoxy nucleotide tri- phosphates (dNTPs) and buffer solution ,This technique is mostly used for detecting known SNPs. Allele-specific PCR was used for detection of FSHR (rs6166 C>T) and (rs6165 C> T) genetic polymorphisms (135).

2.3.4.5.A. Primer Design.

Polymerase Chain Reaction (PCR) was accomplished by using a specific primers to amplify FSHR gene rs6166 and rs6165. The primers of this study were designed by Asst. Prof. Dr. Ahmad Abdul Jabar using primer-blast software, and purchased from Macrogen , Korea as a lyophilized product of different picomols concentrations(136).

The primers were dissolved in 250 µl of nuclease-free water to get a concentration of 100 pmol/µl (stock solution) then, 10 µl of each primer (stock solution) was diluted with 90µl of nuclease-free water to get a (work solution) . Both stock and work solution were stored at -20°C until further use. Table (2-4) illustrates the primers were used to amplify the gene alleles.

Table (2-4) Primers sequences of FSHR rs6166 and rs6165.

Primers		Sequence (5' ⟶ 3')	Product Size (bp)
Primers sequences of FSHR rs6166	Reverse	CTGCTATGAAATGCAAGCCCAAATTTAT	-
	Allele C F1	TTAGAGGGACAAGTATGTAAGTGGAACCAC	134
	Allele T F2	TTAGAGGGACAAGTATGTAAGTGGAACCAT	134
Primers sequences of FSHR rs6165	Reverse	CCCTTGAGGTTAGCCTCAAGGGCAGG	-
	Allele C F1	CTG CCA GAG AGG ATC TCT GAC CCC	140
	Allele T F2	CTG CCA GAG AGG ATC TCT GAC CCT	140

2.3.4.5.B. Optimization of PCR Conditions.

Optimization of PCR was accomplished after several trials, to determine the best annealing temperature, number of amplification cycles that was appropriate for the allele-specific PCR reaction and the best concentration for both DNA and primer .The total reaction volume was(25µl) which centrifuged for 10 seconds at 2000 rpm in a microcentrifuge for mixing the sample tubes and then placed in thermocycler. The components of PCR for genotyping of FSHR rs6166 and rs6165 were shown in Tables (2-5).

Table (2-5): The components of PCR , for genotyping of FSHR rs6166 C>T and rs6165 C>T.

Component	Volume (µl)
Forward primer	1.5
Reverse primer	1.5
DNA template	4
Nuclease free water	13
Premix	5
Total	25

2.3.4.5.C. Polymerase Chain Reaction Run

The reaction was carried out by mixing PCR ingredients with the optimized concentrations and used the optimized PCR programs as displayed in the Tables (2-6),(2-7) PCR Run for genotyping of FSHR gene rs6166 and rs6165.

Table (2-6): PCR run conditions for genotyping of FSHR gene rs6166 C>T.

Steps	Temperature(c)	Time	Cycle
Initial denaturation	94	3minutes	1
Denaturation	94	30Sec	30
Annealing	63	40Sec	
Extension	72	55Sec	
Final extension	72	5minutes	1

Table (2-7): PCR run conditions for genotyping of FSHR gene rs6165. C>T.

Steps	Temperatures(c)	Time	Cycle
Initial denaturation	94	3 minutes	1
Denaturation	94	30 sec	30
Annealing	58	45 sec	
Extension	72	55 sec	
Final extension	72	5 minutes	1

2.3.4.6. Agarose Gel Electrophoresis

Electrophoresis is a typical method for identifying, separating, and purifying DNA fragments of varying sizes ranging from 100 bp to 1000 bp throughout the agarose gels. Agarose gel electrophoresis was selected to validate the presence of PCR amplification (137).

1X TBE buffer (tris borate EDTA) was obtained by diluting 10X TBE buffer with deionized water (one volume of 10X TBE buffer, with 9 volume of deionized water: 1:10 dilution).

• In order to prepare agarose gel, 1.5 g of agarose powder was dissolved in 100 ml of 1x TBE buffer (pH 8) (138).

•The solution heated by using heater till all of the gel particles were dissolved and the gel solution was looked clear and pure.

• The solution was cooled to 50˚C.

• 3.5 microliters of ethidium bromide were added to solution

• The comb was placed to the end of the tray to create wells for the loading of PCR products.

• The agarose was poured into the tray and allowed to solidify at room temperature for 30 min.

• The comb was removed carefully from the tray.

• The tray was fixed in an electrophoresis chamber and it was filled with a TBE buffer.

• PCR products were directly loaded into the wells

• The voltage of the electrophoresis device was fixed to make sure an electrical field regulated with 5 v.cm-1 for distance between cathode and anode.

• At the end of the run, bands were detected using an ultraviolet trans-illuminator at 320–336 nm.

• A digital camera was used to take pictures of the gel.

2.4 Statistical Analysis

By using Statistical Package for Social Science (SPSS 26 IBM, Armonk, USA), one-sample Kolmogorov-Smirnov test is used to know how the values are distributed. If the values are distributed normally, then t- test is used and if the values are not distribution normally, so Mann-Whitney test should be applied. In this study the t-test was used for study data.

The results were expressed as mean ± standard deviation (SD). The differences in means of the variables between control and patient groups (poor, moderate, and high responder groups) were analyzed by analysis of variance (one-way ANOVA) test. Differences in biochemical parameter for patient groups (poor, moderate, and high responder groups) before and after treatment with FSH were compared using paired t-test.

The genotyping results were expressed as frequency and percentage and Hardy-Weinberg equilibrium online calculator used for all genotypes in the study to obtain allele frequency. Odds ratio (OR) and confidence interval 95% (CI-95) were used to examine the association of these genotypes on the study clinical and biochemical markers also on the development of infertility. P value of less than 0.05 was regarded to be statistically significant ($P<0.05$).

CHAPTER THREE

Results

3. Results

3.1. Socio-demographic Data

Socio-demographic data for 210 infertile women (patients group) and 50 healthy women (control group) demonstrated in the table (3.1). The mean ± SD of age was 25.78 ±2.66 years for control group and for patients group were 26.27 ±2.95, 25.39±3.09, 26.42 ±3.16 years in (Poor, Moderate, and high responder infertile women) respectively, there was non-significant statistical difference in age between the two groups ($p>0.05$).The mean ± SD result of BMI for control group was 24.62 ±0.74 Kg/m^2 and for patient groups were 24.13±3.05, 23.96±2.38, 24.26. ±2.68 years for (Poor, Moderate, and high responder infertile women) respectively, there were non-significant statistical difference in BMI between the two groups ($p>0.05$).

The age of menarche was expressed as mean ± SD for control group 12.76±1.3 years and were for infertile group 13.19 ±1.49, 12.95 ±1.37, 13.13 ±1.1 years for (Poor, Moderate, and high responder infertile women) respectively, there were non-significant statistical difference in age of menarche between the two groups ($p>0.05$).

Forty-eight of women in control group had no family history of infertility and two had family history of infertility while in patient group the number and percent (%) of women had no family history of infertility for (Poor, Moderate, and high responder infertile women) were 66 (94.3%),59 (96.7%), and 77 (97.5%) respectively, and those had family history for the same group were 4 (5.7%),2 (3.3%), and 2 (2.5%) respectively, these results were obtained statistically considered non-significant.

All women in control group have previous conception while all patients group participants have no previous conception. There were no significant statistical differences regarding socio-demographic characteristics like smoking and family history ($p>0.05$) of the infertile and control groups. All the participants in this study (control and patient) groups have regular menses, have no Hirsutism, and no Acne.

Table (3-1): Socio-demographic data of control and patient groups (Poor, Moderate, and high responder infertile women)

Mean ± SD		Control	Poor responder	Moderate responder	High responder
Number		50	70	61	79
Age(year)		25.78 ±2.66	26.27 ±2.95	25.39±3.09	26.42 ±3.16
Body mass index (Kg/m2)		24.62 ±0.74	24.13±3.05	23.96±2.38	24.26. ±2.68
Age of menarche (Year)		12.76±1.3	13.19 ±1.49	12.95 ±1.37	13.13 ±1.1
Family History N (%)	**No**	48(96%)	66(94.3%)	59(96.7%)	77(97.5%)
	Yes	2(4%)	4(5.7%)	2(3.3%)	2(2.5%)

N=Number, %= percent

3.2. Results of study markers

3.2.1.Hormonal and clinical parameters in control and patient groups Before treatment.

The results of biochemical markers for present study were shown in table (3.2), all results were expressed as mean ± standard deviation (SD) for serum levels of FSH, LH, TSH, prolactin, AMH, E_2, and antral follicle count for both control group and patient groups (poor, moderate, and high responder infertile women) before treatment.

The mean ±SD of serum FSH levels for control and patient groups (poor, moderate, and high responder infertile women) were 6.27 ±(1.43) mIU/mL, 9.64 ± (0.45) mIU/mL, 6.64±(1.48) mIU/mL, 5.67 ± (0.26) mIU/mL respectively.

There were insignificant statistical differences ($P > 0.05$) in means of serum levels of FSH for control and moderate groups ,while there were very highly significant increase ($P < 0.001$) in mean serum levels of FSH for poor responder group as compared with control group, and highly significant drop ($P < 0.01$) occur in high responder infertile women group when compared with control group, very highly significant decrease ($P < 0.001$) occurs between (moderate, and high responder infertile women) when compared to poor responder group, also there were very highly significant statistical decrease ($P < 0.001$) in mean serum levels of FSH for high responder group as compared with moderate responder group.

The mean ±SD of serum LH levels for control and patient groups (Poor, Moderate, and high responder infertile women) were 5.85 ± (0.72) mIU/mL, 8.09± (1.14) mIU/mL, 5.48± (1.52) mIU/mL, 7.42. ±(0.66) mIU/mL respectively.

The test of ANOVA showed that there were no statistical differences ($P > 0.05$) in mean serum levels of LH between moderate responder group and control group, in contrast there were high significant increases ($P < 0.001$) in mean of LH in high and poor responder groups when compared to moderate responder and control groups, there were very high significant decrease ($P < 0.001$) in mean of LH in high responder group in comparison with poor responder groups.

The mean ±SD of serum TSH levels for control and patient groups (Poor, Moderate, and high responder infertile women) were 1.9 ± (0.39) mIU/mL, 2.06 ± (0.57) mIU/mL, 2.04 ± (0.41) mIU/mL, and 2.06 ± (0.44) mIU/mL respectively and for prolactin were19.55±2.26 ng/mL, 19.91 ±2.38 ng/mL, 20.08 ±2.51 ng/mL, and 19.23 ±3.22 ng/mL respectively.

There were insignificant statistical differences ($P > 0.05$) in these means of serum levels of TSH and prolactin for control group and patient groups (Poor, Moderate, and high responder infertile women), also there were insignificant statistical differences ($P > 0.05$) in mean serum levels of TSH and prolactin between patient groups.

Table 3.2 demonstrated the levels of serum AMH as mean ±SD were 3.24±0.96 ng/mL, 0.63±0.12 ng/mL, 2.89±0.66 ng/mL, and 7.98±1.46 ng/mL for control and patient groups (Poor, Moderate, and high responder infertile women) respectively.

Very high significant decrease ($P < 0.001$) in mean of AMH found in poor responder group compared with the control group, while no significant difference between moderate responder group compared with control group ($P > 0.05$), but very high significant increase ($P < 0.001$) in mean serum AMH level found in high responder group when compared with control group.

Very high significant increase was found ($P < 0.001$) in mean of AMH for high and moderate responder groups compared with the poor responder group, also for high responder group compared with moderate responder group.

The mean ±SD for serum levels of basal E_2 for control and patient groups (Poor, Moderate, and high responder infertile women) were 31.17±6.35 pg/mL, 38.96±6.52 pg/mL, 33.22±5.62 pg/mL, and 30.23±5.24 pg/mL respectively.

Comparison of the results of the basal E_2 using ANOVA showed very high significant increase ($P < 0.001$) in the mean of basal E_2 in the poor responder group as compared with control group, yet there is insignificant statistical difference ($P>0.05$) between high and moderate responder groups and control group, there was very high significant decrease ($P < 0.001$) in mean of basal E_2 in moderate responder group compared with poor responder group.

There was very high significant decrease ($P < 0.001$) in mean of basal E_2 reported in the serum of high responder group as compared to moderate and poor responder groups.

The mean ±SD of Antral follicle count for control and patient groups (Poor, Moderate, and high responder infertile women) were 11.7±1.23, 4.14±0.72, 11.16±1.01, and 16.59±2.16 respectively.

The ANOVA showed that there were no statistical differences ($P > 0.05$) in mean of antral follicle count between moderate responder group and control group, in contrast there was very high significant decrease ($P < 0.001$) in mean of antral follicle count in poor responder groups when compared to control group, while there was very high significant increase ($P < 0.001$) in mean of antral follicle count in high responder group in comparison with control group.

Very high significant increase reported ($P < 0.001$) in mean of antral follicle count for high and moderate responder groups compared with the poor responder group, also for high responder group compared with moderate responder group.

Table (3-2): Comparison between hormonal and clinical parameters in control and patient groups (Poor, Moderate, and high responder infertile women) using ANOVA test, all data expressed as mean ±SD.

Hormonal and clinical parameters	Control N=50	Poor responder N=70	Moderate responder N=61	High responder N=79
FSH (mIU/mL)	6.27 ±1.43	9.64 ±0.45 **a▲*****	6.64±1.48 a NS, b**▼*****	5.67 ±0.26 a**▼**,b▼***,c▼*****
LH (mIU/mL)	5.85 ±0.72	8.09±1.14 **a▲*****	5.48±1.52 a NS, b**▼*****	7.42. ±0.66 **a▲***,b▼***,c▲*****
TSH (mIU/mL)	1.9 ±0.39	2.06 ±0.57 a NS	2.04 ±0.41 a NS, b NS	2.06 ±0.44 a NS, b NS, c NS
Prolactin (ng/mL)	19.55±2.26	19.91 ±2.38 a NS	20.08 ±2.51 a NS, b NS	19.23 ±3.22 a NS, b NS, c NS
AMH (ng/mL)	3.24±0.96	0.63±0.12 **a▼*****	2.89±0.66 **a** NS, b**▲*****	7.98±1.46 **a▲***,b▲***,c▲*****
Basal E2 (pg/mL)	31.17±6.35	38.96±6.52 **a▲*****	33.22±5.62 **a NS, b▼*****	30.23±5.24 **aNS,b ▼***,c▼*****
Antral follicle count	11.7±1.23	4.14±0.72 a**▼*****	11.16±1.01 **a** NS, b**▲*****	16.59±2.16 **a▲***** b**▲*****,c**▲*****

a= ANOVA test between poor, moderate, high responder groups versus control group.

b= ANOVA test between moderate, high responder groups versus poor group.

c= ANOVA test between high and moderate responder groups.

▼***= very high significant decrease (P<0.001); **▼****= high significant decrease (P<0.01); **▲*****=very high significant increase (P<0.001); NS= non-significant difference.

3.2.2. Biochemical and clinical parameters in patient groups after treatment.

The concentrations of E2 for the studied groups (Poor, Moderate, and high responder infertile women) were re-measured. The results were compared with their respective mean values prior to treatment.

Table (3-3) expressed the results of E2 after six days of stimulation with FSH as mean ± SD as the following 80.26±4.67 pg/mL, 311.02±34.61 pg/mL, 704.78±138.85 pg/mL for (Poor, Moderate, and high responder infertile women) groups respectively.

The ANOVA test revealed very high significant increase ($P < 0.001$) in the mean of E2 after six days of stimulation with FSH for moderate and high responder groups when compared with poor responder group, also there were very high statistical significant increase ($P < 0.001$) in the mean of E2 after six days of stimulation with FSH for high responder group in comparison with moderate responder group.

Comparison of the results of the basal E2 and after 6 days of stimulation treatment with FSH using paired t-test showed very high significant increase ($P < 0.001$) in the mean of E2 in the poor, high, and moderate responder groups after six days of stimulation compared with the E2 mean value of the same groups before treatment with FSH.

The mean ± SD of size of graafian follicle for patient groups (Poor, Moderate, and high responder infertile women) after treatment with FSH were (9.76±2.23) mm, (18.31±1.16) mm, (21.91 ±0.9) mm, respectively. Using ANOVA test there were very high significant increase ($P < 0.001$) in the mean size of graafian follicle for moderate and high responder groups when compared with poor responder group.

The mean ± SD of number of graafian follicle for patient groups (Poor, Moderate, and high responder infertile women) after treatment with FSH were (1.04±0.2), (2.07±0.25), and (3.05±0.22), respectively. ANOVA test revealed very high significant increase ($P < 0.001$) in mean number of graafian follicle in moderate and high responder groups as compared with poor responder group.

Table (3-3): The mean ±SD of basal E2 and after 6 days of stimulation, Size of graafian follicle, Number of graafian follicle in patient groups (Poor, Moderate, and high responder infertile women).

Hormonal and clinical parameters		Poor responder	Moderate responder	High responder
		N=70	N=61	N=79
E2 (pg/mL)	Basal	38.96±6.52	33.22±5.62	30.23±5.24
	After 6 days of stimulation	80.26±4.67 c▲***	311.02±34.61 a▲***,c▲***	704.78±138.85 a▲***,b▲***,c▲***
Size of graafian follicle (mm)		9.76±2.23	18.31±1.16 a▲***	21.91 ±0.9 a▲***,b▲***
Number of graafian follicle		1.04±0.2	2.07±0.25 a▲***	3.05±0.22 a▲***,b▲***

a= ANOVA test between moderate, high responder infertile women groups versus poor responder group.

b= ANOVA test between moderate and high infertile women groups.

c= Paired t-test between basal E2 and after 6 days of stimulation for poor, moderate and high responder infertile women groups.

▲*** =very high significant increase.

3.3.Result of Genotypes Analysis

3.3.1 Results of amplification reactions among the genotypes of rs6166 and rs6165.

The amplification of SNPs of FSH receptor gene: FSHR (C>T) (rs6166) was shown in 134 bp as presented in figure (3-1) and FSHR (C>T) (rs6165) genetic polymorphism was shown in 140 bp as presented in figure (3-2). The size of amplicons was determined by comparing with molecular weight marker (ladder) 100 - 1000 bp.

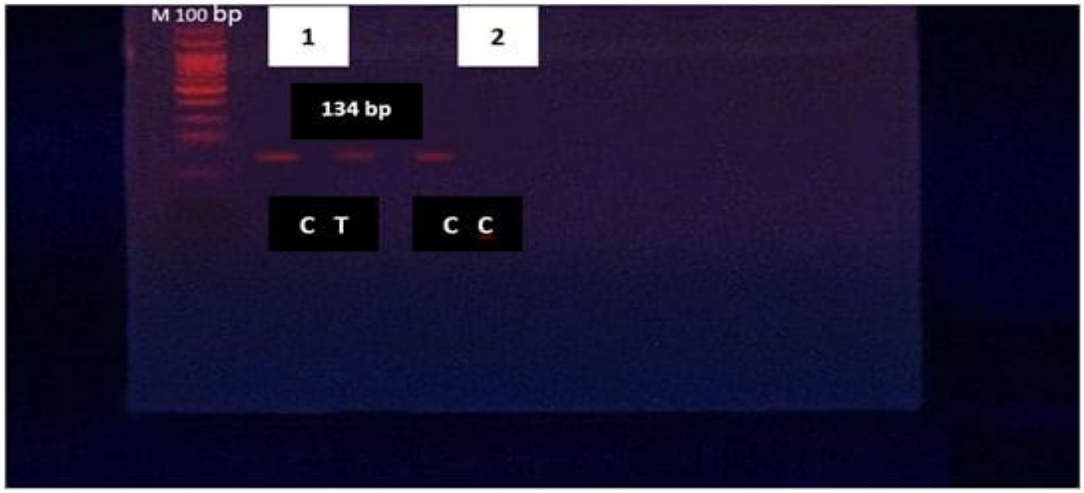


Figure (3-1): Agarose gel electrophoresis for allele specific PCR product of FSH receptor gene polymorphisms for (rs6166):
(M): represented molecular weight marker 100 - 1000 bp, Lane (1): represented (one sample) which was heterozygous CT (Cyt\Thy) genotype was shown in (134bp),Lane(2): represented (one sample) homozygous (Wild type) CC (Cyt\Cyt) genotype was shown in (134bp).

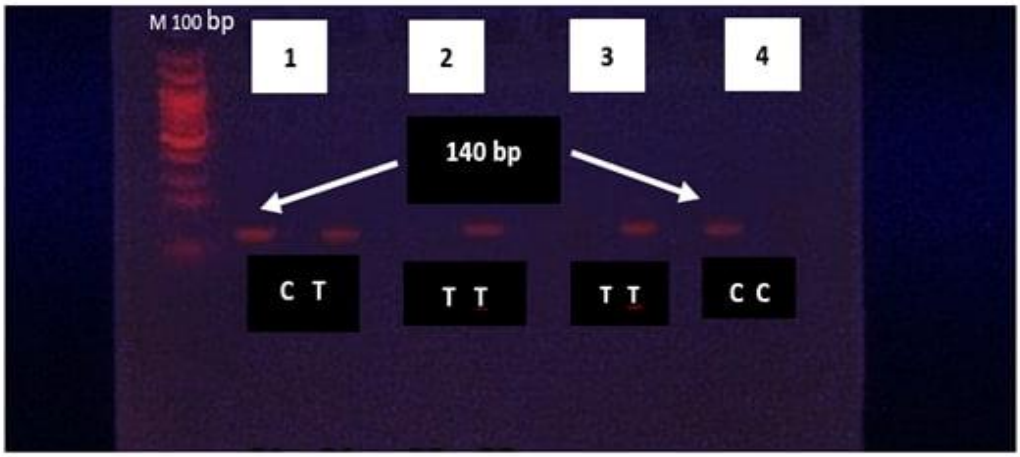


Figure (3-2): Agarose gel electrophoresis for allele specific PCR product of FSH receptor gene polymorphisms for(rs6165):

(M): represented molecular weight marker 100 - 1000 bp, Lane (1): represented (one sample) heterozygous CT (Cyt\Thy) genotype ,Lane(2,3): each two lane represented (one sample) which was homozygous (Mutant type) TT (Thy\Thy) genotype were shown in (140bp), Lane (4): represented (one sample) which was homozygous genotype (Wild type) CC (Cyt\Cyt) genotype was shown in (140bp).

3.3.2. Distribution of genotyping and allele frequencies of FSHR gene polymorphism (C>T) (rs6166) in control and patient groups (Poor, Moderate, and high responder infertile women):

All subjects were categorized as being (CC) for homozygous wild genotype of (rs6166) FSHR gene, (CT) for heterozygous polymorphism, and (TT) for homozygous mutated genotype.

Table (3.4) summarize the genotyping and allele frequencies genotyping of different study subjects. The heterozygous genotype (CT) of FSHR gene was more abundant than (CC) and (TT) genotypes in control group with a frequency of (46,40,14%) respectively with major allele frequency of 63(63%) and minor allele frequency of 37(37%).

In poor responder group there were 21 homozygous patients (30%), 32 heterozygous (45.7%), and 17(24.3%) homozygote mutant type with an allele frequency of 74(53%) for (C) allele and 66(47%) for (T) allele, while in moderate responder group (39.3%) had homozygote wild type, (44.3%) heterozygous and (16.4%) with homozygous mutated genotype, the allele frequency was 75 (61%) for major allele (C) and 47 (39%) for minor allele (T).

Among 79 high responder patients, there were 36 heterozygous (CT) genotypes (45.6%),30 (CC) genotypes (38%) and 13 (TT) genotypes (16.4%) for the SNP rs6166 gene, so minor allele frequency was 62(39%)for allele T, and major allele frequency was 96 (61%).

Table (3-4): Genotypes and allele frequencies expressed as number and percentage detected of FSHR Gene (rs6166) in control and patient groups (Poor, Moderate, and high responder infertile women).

Genotypes		Group			
		Control	Poor responder	Moderate responder	High responder
		(n=50)	(n=70)	(n=61)	(n=79)
CC Homozygote wild type		20 (40%)	21 (30%)	24(39.3%)	30 (38%)
CT Heterozygous		23 (46%)	32(45.7%)	27(44.3%)	36(45.6%)
TT (homozygote mutant type)		7 (14%)	17(24.3%)	10(16.4%)	13(16.4%)
Allele frequency N (%)	Allele C	63(63%)	74(53%)	75(61%)	96 (61%)
	T	37(37%)	66(47%)	47(39%)	62(39%)
Total		100(100%)	140(100%)	122(100%)	158(100%)

N=number, %= percentage

3.3.3. Distribution of genotyping and allele frequencies of FSHR gene polymorphism (C>T) (rs6165) in control and patient groups (Poor, Moderate, and high responder infertile women):

All groups subjects were categorized as being (CC) for homozygous wild genotype of (rs6165) FSHR gene, (CT) for heterozygous polymorphism, and (TT) for homozygous mutated genotype.

The allele frequencies and genotyping of different study subjects were listed in table (3.5) .In control group the heterozygous genotype (CT) of FSHR gene was more abundant than (CC) and (TT) genotypes with a frequency of (58,34,8%) respectively with major allele frequency of (63%) and minor allele frequency of (37%).

There were 16 homozygous patients (22.9%), 41 heterozygous (58.6%), and 13(18.5%) homozygote mutant type with an allele frequency of 73 (52%)for (C) allele and 67 (48%) for (T) allele in poor responder group, while in moderate responder group 21(34.4%) had homozygote wild type, 35(57.4%) heterozygous and 5 (8.2%)with homozygous mutated genotype, the allele frequency was 77 (63%) for major allele (C) and 45 (37%) for minor allele (T).

Among 79 high responder patients, there were 45 heterozygous (CT) genotypes (57%),27 (CC) genotypes (34.2%) and 7 (TT) genotypes (8.8%) for the SNP rs6165 gene, so minor allele frequency was 59(38%) for allele T, and major allele frequency was 99 (62%).

Table (3-5): Genotypes and Allele frequencies expressed as number and percentage detected of FSHR Gene (rs6165) in control and patient groups (Poor, Moderate, and high responder infertile women).

Variables		Group			
		Control	Poor responder	Moderate responder	High responder
genotype		(n=50)	(n=70)	(n=61)	(n=79)
CC Homozygote, wild type		17 (34%)	16(22.9%)	21(34.4%)	27(34.2%)
CT heterozygous		29 (58%)	41 (58.6%)	35(57.4%)	45(57%)
TT (homozygote, mutant type)		4 (8%)	13 (18.5%)	5 (8.2%)	7(8.8%)
Allele frequency N (%)	Allele C	63(63%)	73 (52%)	77 (63%)	99 (62%)
	T	37 (37%)	67 (48%)	45 (37%)	59(38%)
Total		100(100%)	140(100%)	122(100%)	158(100%)

N=number, %= percentage

3.3.4. Association Between Allele Frequencies in (poor, moderate, and high responder) and control groups with FSHR(rs6166) (C > T) Genetic Polymorphism

In the present study, the women enrolled in final data analysis were subdivided into control and patient (poor, moderate and high responder) groups, then comparison between those groups related to their genotyping were made, the results is best presented in table (3-6).

In poor responder group when compared with control group the odd ratio (OR) of CT genotype was 1.32, CI 95% was 0.58-2.98 and (P =0.49) as compared to wild (reference) which represented by CC genotype, while the TT genotype of this SNP has the odd ratio of 2.31, CI 95% was 1.79-6.75and (P =0.04) as compared to wild genotype(reference).

As shown in table (3-6), the (OR), CI 95% and P value of CT genotype for moderate responder group when compared with control group were 0.97, 0.43-2.2 and (0.95) respectively as compared to wild (reference) genotype that represented by CC genotype, while the TT genotype of this SNP has the odd ratio of 1.63, CI 95% was 0.52-5.05and (P =0.39) as compared to (CC) wild genotype that considered as (reference).

The high responder group as compared with control group show odd ratio (OR) of CT genotype equal to 1.04, CI 95% was 0.48-2.25 and (P =0.91) as compared to wild (reference) which represented by CC genotype, as well as the TT genotype of this C>T polymorphism has the odd ratio of 1.48, CI 95% was 0.51-4.3 and (P =0.46) as compared to wild genotype(reference).

Table (3-6): Odds ratio (OR), P-value and Confidence interval (CI) of the FSHR (rs6166) C/T genotypes in different (poor, moderate, and high responder) groups versus control group.

Groups	Genotype	OR	CI 95%	P value
Poor responder versus control group	CC(Reference)	---	---	---
	CT	1.32	0.58-2.98	0.49
	TT	2.31	1.79-6.75	0.04
moderate responder versus control group	CC(Reference)	---	---	---
	CT	0.97	0.43-2.2	0.95
	TT	1.63	0.52-5.05	0.39
High responder versus control group	CC(Reference)	---	---	---
	CT	1.04	0.48-2.25	0.91
	TT	1.48	0.51-4.3	0.46

OR (odd ratio), CI 95% (confidence interval)

3.3.5. Association Between Allele Frequencies in (poor, moderate, and high responder) and control groups with FSHR(rs6165) (C > T) Genetic Polymorphism

Table (3-7) represents the results of comparison related to genotype differences between all women enrolled in the study that subdivided into control and patient (poor, moderate, and high responder) groups were made, the results is best presented as odd ratio, confidence interval, and p value.

The first comparison was made for poor responder group for (CT) genotype when compared with control group the odd ratio (OR) of was 1.5, CI 95% was 0.65-3.45 and (P =0.33) as compared to wild (reference) which represented by CC genotype, while the TT genotype of this single nucleotide polymorphism has the odd ratio of 3.45, CI 95% was 1.92-12.82 and (P =0.03) as compared to wild (CC) genotype(reference).

Odd ratio (OR) of CT genotype was 0.97, CI 95% was 0.43-2.18 and (P =0.95) for moderate responder group when compared with control group, the (CC) genotype taken as wild (reference) for the comparison, while the odd ratio of 1.01, CI 95% equal to 0.23-4.36 and (P =0.98) for TT genotype of this SNP when compared to wild (CC) (reference genotype).

In high responder group when compared with control group the odd ratio (OR) of CT genotype was 0.97, CI 95% was 0.45-2.1 and (P =0.95) as compared to wild (reference) which represented by CC genotype, while the TT genotype of this SNP has the odd ratio of 1.1, CI 95% was 0.27-4.33 and (P =0.88) as compared to wild genotype(reference).

Table (3-7): Odds ratio (OR), P-value and Confidence interval (CI) of the FSHR (rs6165) C/T genotypes in different (poor, moderate, and high responder) groups versus control group.

Groups	Genotype	OR	CI 95%	P value
Poor responder versus control group	CC(Reference)	---	---	---
	CT	1.5	0.65-3.45	0.33
	TT	3.45	1.92-12.82	0.03
moderate responder versus control group	CC(Reference)	---	---	---
	CT	0.97	0.43-2.18	0.95
	TT	1.01	0.23-4.36	0.98
High responder versus control group	CC(Reference)	---	---	---
	CT	0.97	0.45-2.1	0.95
	TT	1.1	0.27-4.33	0.88

OR (odd ratio), CI 95% (confidence interval)

3.3.6. Effect of genetic polymorphism of FSHR gene (C>T) (rs6166) on biochemical parameters among patient groups (poor, moderate, high responder).

The results of the present study were shown in tables (3-8 and 3-9) using one-way ANOVA, the table showed biochemical parameters in each genotype of FSHR (C> T) (rs6166). To examine the individual differences between patients, the association between genotypes and the levels of biochemical markers including (FSH, LH, TSH, prolactin, AMH, basal E2 and antral follicle count) should be detected.

The mean ± standard deviation (SD) of FSH serum level for (CC, CT, and TT) genotype for poor responder group were 8.56 (±0.46) mIU/mL, 9.37 (±0.28) mIU/mL, 10.99 (±0.34) mIU/mL, respectively.

The test of ANOVA showed in poor responder group a high significant increases ($P < 0.01$) in mean serum FSH in (TT) genotype compared with the (CC) genotype, while there were significant increases ($P < 0.05$) as compared with CT genotype, yet there were insignificant differences ($P > 0.05$) between CC and CT genotype, also there were no significant differences ($P > 0.05$) for FSH serum levels in moderate and high responder groups between all genotypes (CC, CT, and TT).

The mean ± (SD) of serum LH levels in (mIU/mL) for (CC, CT, and TT) genotype in poor responder group were 6.76±0.63 mIU/mL, 7.51 ±0.96 mIU/mL, 9.68 ±0.74 mIU/mL, respectively, by applying ANOVA test, there was non-significant changes in serum level of LH between (CC and CT).

The pattern of changes and the level of statistical significance in the concentration of LH for (TT) genotype revealed very high significant increase ($P < 0.001$) as compared with (CC and CT) genotypes belong to poor responder group.

The ANOVA test when applied to compare the statistical differences in TSH levels (mIU/mL) and serum prolactin (ng/mL) reveal insignificant statistical difference ($P>0.05$), between (CC, CT, and TT) genotypes when compared in each group alone for (poor, high ,and moderate responder) groups.

Table (3-8): Comparison between mean ± SD of the studied biochemical parameters (FSH, LH, TSH, and prolactin) with different genotypes of (rs6166) SNP of FSHR gene in patient groups (poor, moderate, high responder).

Biochemical parameters	Genotype	Poor responder N=70	Moderate responder N=61	High responder N=79
FSH (mIU/mL)	CC	8.56 ±0.46	6.59±0.72	5.39 ±0.17
	CT	9.37 ±0.28 c NS	7 ±1.01 c NS	5.83±0.09 c NS
	TT	10.99 ±0.34 c▲**, d▲*	7.29 ±0.12 c NS, d NS	5.93±0.35 c NS, d NS
LH (mIU/mL)	CC	6.76±0.63	5.46±1.24	7.05 ±0.92
	CT	7.51 ±0.96 c NS	5.37 ±0.92 c NS	7.64±0.2 c NS
	TT	9.68 ±0.74 c ▲***, d▲***	6.01 ±0.69 c NS, d NS	7.66±0.24 c NS, d NS
TSH (mIU/mL)	CC	1.82±0.31	1.83 ±0.16	1.96 ±0.37
	CT	1.95 ±0.66 c NS	2.25 ±0.25 c NS	2.06±0.52 c NS
	TT	2.26 ±0.59 c NS, d NS	2.24 ±0.32 c NS, d NS	2.14±0.42 c NS, d NS
Prolactin (ng/mL)	CC	19.33 ±1.4	20.26 ±2.28	18.74 ±3.37
	CT	19.87 ±1.56 c NS	20.08 ±2.17 c NS	19.14±3.52 c NS
	TT	20.01 ±1.41 c NS, d NS	21.05 ±3.03 c NS, d NS	20.61±1.12 c NS, d NS

c= ANOVA test between (CT, TT) and CC genotype.

d= ANOVA test between TT and CT genotype.

▲*= significant increase (P<0.05); ▲**= high significant increase (P<0.01); ▲***= very high significant increase (P<0.001); NS= non-significant difference (P > 0.05).

The results in table (3-9) expressed the mean ±SD of serum levels for AMH (ng/mL), basal E2 (pg/mL), and antral follicle count for patient groups (poor, moderate, high responder).

The mean ± standard deviation (SD) of serum AMH level for (CC, CT, and TT) genotypes were 0.78 (±0.08) mIU/mL, 0.65 (±0.07) mIU/mL, 0.46 (±0.18) mIU/mL, respectively for poor responder group.

The ANOVA showed in poor responder group a significant decrease ($P < 0.05$) in mean serum AMH in (TT) genotype compared with the (CC and CT) genotypes, while there was insignificant difference ($P > 0.05$) between CC and CT genotype, also there were no significant differences ($P > 0.05$) for AMH serum levels in moderate and high responder groups between all genotypes (CC, CT, and TT).

The mean ± standard deviation (SD) of serum basal E2 (pg/mL) concentration for (CC) genotype in poor responder group was 36.36±10.17 (pg/mL), while 37.29±3.19 (pg/mL), and 41.77±4.2 (pg/mL), for (CT and TT) genotypes respectively.

The ANOVA showed in poor responder group there was insignificant difference ($P > 0.05$) in mean serum of basal E2 in (CT) genotype compared with the (CC) genotype, while there were very high significant increase ($P < 0.001$) in mean serum levels of basal E2 in (TT) genotype as compared with CT, and CC genotypes. ANOVA revealed insignificant differences ($P > 0.05$) in serum levels of basal E2 in moderate and high responder groups between all genotypes (CC, CT, and TT).

In poor responder group, the mean ± (SD) of antral follicle count for (CC, CT, and TT) genotypes were 5.41 (±0.61), 4.19(±0.79), 2.9 (±0.75) respectively.

By using ANOVA test to compare the differences in antral follicle count between genotypes in poor responder group, the test show a non-significant statistical difference ($P > 0.05$) in mean of antral follicle count in (CT) genotype compared with the (CC) genotype, while there were very high significant decrease ($P < 0.001$) in mean of antral follicle count in (TT) genotype as compared with (CC and CT) genotypes, also there were no significant differences ($P > 0.05$) for antral follicle count in moderate and high responder groups between all genotypes (CC, CT, and TT).

Table (3-9): Comparison between mean ± SD of the studied parameters (AMH, basal E2 , and antral follicle count) with different genotypes of (rs6166) SNP of FSHR gene in patient groups (poor, moderate, high responder).

Biochemical parameters	Genotype	Poor responder N=70	Moderate responder N=61	High responder N=79
AMH (ng/mL)	CC	0.78±0.08	3.19±0.65	8.51±0.32
	CT	0.65±0.07 c NS	3.1±0.57 c NS	8.56±1.16 c NS
	TT	0.46±0.18 c ▼*, d ▼*	2.47±0.49 c NS, d NS	7.61±0.91 c NS, d NS
Basal E2 (pg/mL)	CC	36.36±10.17	33.53±5.96	30.76±4.1
	CT	37.29±3.19 c NS	33.25±2.19 c NS	30.25±5.38 c NS
	TT	41.77±4.2 c ▲***, d ▲***	34.26±0.03 c NS, d NS	31.56±4.31 c NS, d NS
Antral follicle count	CC	5.41±0.61	11.7±1.13	17.84±1.28
	CT	4.19±0.79 c NS	10.75±0.73 c NS	16.44±1.52 c NS
	TT	2.9±0.75 c ▼***, d ▼***	10.7±0.48 c NS, d NS	16.44±1.52 c NS, d NS

c= ANOVA test between (CT, TT) and CC genotype.

d= ANOVA test between TT and CT genotype.

▼*= significant decrease (P<0.05); ▼***= very high significant decrease (P<0.001); ▲***=very high significant increase (P<0.001); NS= non-significant difference.

3.3.7. Effect of genetic polymorphism of FSHR gene (C>T) (rs6165) on biochemical parameters among patient groups (poor, moderate, high responder).

The results of the present study were shown in tables (3-10, 3-11), using one-way ANOVA, the table showed biochemical parameters in each genotype of FSHR (C> T) (rs6165), to examine the individual differences between patients, the association between genotypes and the levels of clinical and biochemical markers including (FSH, LH, TSH, prolactin, AMH, basal E2 and antral follicle count) should be detected.

The levels of serum FSH were expressed in table (3-10), the mean ± standard deviation (SD) of FSH serum level for (CC, CT, and TT) genotype in poor responder group were 8.38 (±0.35) mIU/mL, 8.59 (±0.44) mIU/mL, 11.96 (±0.38) mIU/mL, respectively.

ANOVA showed in poor responder group very high significant increases ($P < 0.001$) in mean serum FSH in (TT) genotype compared with the (CC and CT) genotypes, while there were insignificant differences ($P > 0.05$) between CC and CT genotype, also there were no significant differences ($P > 0.05$) for FSH serum levels in moderate and high responder groups between all genotypes (CC, CT, and TT) within same group.

The mean ± (SD) of serum LH levels in (mIU/mL) for (CC, CT, and TT) genotype in poor responder group were 7.58±0.59 mIU/mL, 7.25 ±1.14 mIU/mL, 9.63 ±0.72 mIU/mL, respectively.

By applying ANOVA test, there was non-significant change ($p>0.05$) in serum level of LH between (CC and CT), yet, the pattern of changes and the level of statistical significance in the concentration of LH for (TT) genotype revealed high significant increase (P value< 0.01) as compared with (CC and CT) genotypes belong to poor responder group. There were no significant differences ($P > 0.05$) in LH concentrations for moderate and high responder groups among all genotypes (CC, CT, and TT) within same group.

The levels of TSH (mIU/mL) were expressed in table (3-10) and ANOVA test applied to compare the statistical differences between serum levels among (CC, CT, and TT) genotypes, when compared in each group alone for (poor, high, and moderate responder) groups, revealed non-significant differences ($P>0.05$).

ANOVA test applied to make comparison in serum prolactin (ng/mL) levels for finding the statistical differences among (CC, CT, and TT) genotypes and showed non- significant differences ($P>0.05$) when compared these genotypes for each group alone (poor, high, and moderate responder) groups.

Table (3-10): Comparison between mean ± SD of the studied biochemical parameters with different genotypes of (rs6165) SNP of FSHR gene in the study groups.

Biochemical parameters	Genotype	Poor responder N=70	Moderate responder N=61	High responder N=79
FSH (mIU/mL)	CC	8.38 ±0.35	6.3±0.64	5.36 ±0.15
	CT	8.59 ±0.44 c NS	7.34 ±1.01 c NS	5.83±0.1 c NS
	TT	11.96 ±0.38 c▲***, d▲***	7.2 ±0.53 c NS, d NS	5.91±0.24 c NS, d NS
LH (mIU/mL)	CC	7.58±0.59	4.9±1.27	6.99 ±0.23
	CT	7.25 ±1.14 c NS	5.56 ±0.55 c NS	7.59±0.2 c NS
	TT	9.63 ±0.72 c ▲**, d▲**	5.41 ±0.81 c NS, d NS	7.66±0.23 c NS, d NS
TSH (mIU/mL)	CC	1.73 ±0.26 NS	1.59 ±0.14	1.9 ±0.34
	CT	1.9 ±0.67 c NS	2.24 ±0.27 c NS	1.9±0.51 c NS
	TT	2.25 ±0.56 c NS, d NS	2.52 ±0.34 c NS, d NS	2.18±0.46 c NS, d NS
Prolactin (ng/mL)	CC	18.37 ±3.79	19.23 ±2.42	18.33 ±3.20
	CT	19.87 ±1.2 c NS	20.2 ±2.94 c NS	19.61±3.39 c NS
	TT	20.52 ±1.61 c NS, d NS	20.58 ±2.43 c NS, d NS	20.28±0.48 c NS, d NS

c= ANOVA test between (CT, TT) and CC genotype.

d= ANOVA test between TT and CT genotype.

▲** = high significant increase (P<0.01); ▲*** = very high significant increase (P<0.001); NS= non-significant difference (P > 0.05).

The results in table (3-11) expressed the mean ±SD of serum levels for AMH (ng/mL), basal E2 (pg/mL), and antral follicle count for patient groups (poor, moderate, high responder).

The mean ± standard deviation (SD) of serum AMH level for (CC, CT, and TT) genotypes for poor responder group were 0.7 (±0.09) mIU/mL, 0.73 (±0.04) mIU/mL, 0.48 (±0.1) mIU/mL, respectively.

ANOVA showed a significant decrease ($P < 0.05$) in mean serum AMH level for (TT) genotype compared with the (CC and CT) genotype in poor responder group, while there were insignificant difference ($P > 0.05$) when comparing CC and CT genotypes, also there were no significant differences ($P > 0.05$) for AMH serum levels in moderate and high responder groups between all genotypes (CC, CT, and TT).

Table (3-11) involve the mean ± standard deviation (SD) of serum basal E2 in (pg/mL) concentration for (CC) genotype in poor responder group was 36.83±11.22 (pg/mL), while 37.27±3.84 (pg/mL), and 42.84±3.94 **(**pg/mL), for (CT and TT) genotypes respectively.

ANOVA showed in poor responder group there was insignificant difference ($P > 0.05$) in mean serum of basal E2 in (CT) genotype compared with the (CC) genotype, while there were very high significant increase ($P < 0.001$) in mean serum levels of basal E2 in (TT) genotype as compared with CT, and CC genotypes, also in ANOVA test there were in-significant differences ($P > 0.05$) in serum levels of basal E2 in moderate and high responder groups between all genotypes (CC, CT, and TT).

All results of antral follicle count were listed in table (3-11) and mean ± (SD) for (CC, CT, and TT) genotypes in poor responder group were 5.3 (±0.25), 4.14(±0.72), 3.02 (±0.42) respectively.

ANOVA test used to compare the differences in antral follicle count between genotypes in poor responder group, the test showed a non-significant statistical difference ($P > 0.05$) in mean of antral follicle count in (CT) genotype compared with the (CC) genotype.

The levels of antral follicle count mean in (TT) genotype for poor responder group show high significant decrease ($P < 0.01$) as compared with (CC) genotype, while there was significant decrease ($P < 0.05$) in (TT) genotype in comparison to (CT) genotype.

ANOVA test results reveal insignificant statistical differences ($P > 0.05$) for antral follicle count in moderate and high responder groups between all genotypes (CC, CT, and TT).

Table (3-11): Comparison between mean ± SD of the studied parameters (AMH, basal E2, and antral follicle count) with different genotypes of (rs6166) SNP of FSHR gene in patient groups (poor, moderate, and high responder).

Biochemical parameters	Genotype	Poor responder N=70	Moderate responder N=61	High responder N=79
AMH (ng/mL)	CC	0.7±0.09	3.9±0.71	8.37±0.18
	CT	0.73±0.04 c NS	2.96±0.32 c NS	8.63±1.02 c NS
	TT	0.48±0.1 c ▼*, d ▼*	2.54±0.49 c NS, d NS	7.44±0.78 c NS, d NS
Basal E2 (pg/mL)	CC	36.83±11.22	32.91±6.13	29.13±4.9
	CT	37.27±3.84 c NS	33.15±2.56 c NS	30.8±3.35 c NS
	TT	42.84±3.94 c ▲***, d ▲***	33.99±2.07 c NS, d NS	30.8±5.01 c NS, d NS
Antral follicle count	CC	5.3±0.25	11.54±1.12	16.99±1.51
	CT	4.14±0.72 c NS	11.01±0.92 c NS	16.57±1.13 c NS
	TT	3.02±0.42 c ▼**, d ▼*	10.57±0.59 c NS, d NS	15.96±2.16 c NS, d NS

c= ANOVA test between (CT, TT) and CC genotype.

d= ANOVA test between TT and CT genotype.

▼*= significant decrease (P<0.05); ▲***= very high significant increase (P<0.001); NS= non-significant difference (P > 0.05); ▼**= high significant decrease (P<0.01).

3.3.8. Effect of genetic polymorphism of FSHR gene (C>T) (rs6166) on response to FSH in studied parameters (E2 after six days of stimulation , Size of graafian follicle and Number of graafian follicle) among patient groups (poor, moderate, high responder).

Table 3-12 expressed the results of E2 after six days of stimulation in (pg/mL), size of graafian follicle in (mm) and number of graafian follicle for patient groups (poor, moderate, high responder) after six days of stimulation with FSH that allowing study the effect of SNP in FSHR gene (6166) on response to FSH.

The mean ± standard deviation (SD) of serum E2 levels after six days of stimulation with FSH in (CC, CT, and TT) genotypes for poor responder group were 83.74±5.31 (pg/mL), 82.83±4.64 (pg/mL), 74.54±4.11 (pg/mL), respectively.

ANOVA showed a non-significant difference ($P > 0.05$) in mean serum E2 levels after six days of stimulation for (CT) genotype compared with the (CC) genotype in poor responder group, while there was high significant decrease ($P < 0.01$) when comparing TT genotype with (CC and CT) genotypes in the same group.

The results of comparison in means of E2 levels after six days of stimulation between genotypes (CC, CT, and TT) for each group in moderate and high responder groups reveal no significant differences ($P > 0.05$).

Table (3-12) involves size of graafian follicle levels in (mm) after treatment with FSH for all groups, mean ± (SD) for poor responder group in (CC, CT, and TT) genotypes were 11.37±1.86, 10.94±2.4, 6.64±2.16, consequently.

By applying ANOVA test, the results for size of graafian follicle in (mm) for poor responder group reveal there was non-significant changes ($p>0.05$) in between (CC and CT) levels, yet, the pattern of changes and the level of statistical significance in the mean size of graafian follicle in (TT) genotype revealed very high significant decrease ($P < 0.001$) in comparison to (CC and CT) genotypes belong to poor responder group. In moderate and high responder groups, the results of comparison in mean size of graafian follicle levels after treatment between genotypes (CC, CT, and TT) for individual group reveal no significant differences ($P > 0.05$).

The results in table (3-12) listed the mean ±SD of number of graafian follicle for patient groups (poor, moderate, high responder), for poor responder group mean ± standard deviation (SD) levels for number of graafian follicle to (CC, CT, and TT) genotypes were 1.21 (±0.25), 1.17 (±0.72), 0.75 (±0.53), subsequently.

ANOVA test when applied to compare CT with CC genotypes for number of graafian follicle in poor responder group showed insignificant difference ($P > 0.05$), while there were significant decrease ($P < 0.05$) for (TT) genotype when compared with the (CC and CT) genotypes. The results of comparison in means number of graafian follicle after treatment for moderate and high responder groups between genotypes (CC, CT, and TT) in individual group reveal insignificant differences ($P>0.05$).

Table (3-12): Comparison between mean ± SD of the studied parameters (E2 after six days of stimulation , size of graafian follicle and number of graafian follicle) after treatment with FSH on different genotypes of (rs6166) SNP of FSHR gene in patient groups (poor, moderate, high responder).

Biochemical and clinical parameters	Genotype	Poor responder N=70	Moderate responder N=61	High responder N=79
E2 after 6 days of stimulation (pg/mL)	CC	83.74±5.31	302.51±13.49	762.1±97.45
	CT	82.83±4.64 c NS	331.36±15.88 c NS	721±109.46 c NS
	TT	74.54±4.11 c ▼**, d ▼**	318.5±6.23 c NS, d NS	691.73±43.53 c NS, d NS
Size of Graafian follicle (mm)	CC	11.37±1.86	19.15±1.29	22.75±0.94
	CT	10.94±2.4 c NS	18.56±0.94 cNS	21.9±0.9 c NS
	TT	6.64±2.16 c ▼***, d ▼***	17.91±0.78 c NS, d NS	21.3±0.74 c NS, d NS
Number of Graafian follicle	CC	1.21±0.25	2.2±0.12	3.1±1.51
	CT	1.17±0.72 c NS	2.01 ±0.9 c NS	3.09±1.13 c NS
	TT	0.75±0.53 c ▼*, d ▼*	2.09±0.51 c NS, d NS	3.01±0.48 c NS, d NS

c= ANOVA test between (CT, TT) and CC genotype.

d= ANOVA test between TT and CT genotype.

▼*= significant decrease (P<0.05); ▼**= high significant decrease (P<0.01); ▼***=very high significant decrease (P<0.001); NS= non-significant difference.

3.3.9. Effect of genetic polymorphism of FSHR gene (C>T) (rs6165) on response to FSH in studied parameters (E2 after six days of stimulation , Size of graafian follicle and Number of graafian follicle) among patient groups (poor, moderate, high responder).

The results of E2 after six days of stimulation in (pg/mL), size of graafian follicle in (mm) and number of graafian follicle were expressed in table (3-13) for patient groups (poor, moderate, high responder) after six days of stimulation with FSH that allowing study the effect of SNP in FSHR gene (6165) on response to FSH.

The mean ± standard deviation (SD) of serum E2 levels after six days of stimulation with FSH in (CC, CT, and TT) genotypes for poor responder group were 85.74±5.55 (pg/mL), 84.59±4.33 (pg/mL), 71.79±4.5 (pg/mL), respectively.

By using ANOVA test the results exhibit insignificant difference ($P > 0.05$) in mean serum E2 levels after six days of stimulation for (CT) genotype compared with the (CC) genotype in poor responder group, although there was very high significant decrease ($P < 0.001$) when comparing TT genotype with (CC and CT) genotypes in the same group.

The results of comparison in means of E2 levels after six days of stimulation between genotypes (CC, CT, and TT) for each group in moderate and high responder groups reveal no significant differences ($P > 0.05$).

Size of graafian follicle in (mm) results after treatment with FSH were involved in table (3-13) which expressed as mean ± (SD) for (CC, CT, and TT) genotypes that belong to poor responder group were 11.96±1.61, 11.04±2.33, 5.84±2.44, consequently.

By applying ANOVA test, the results for size of graafian follicle in (mm) for poor responder group reveal there were non-significant changes ($p>0.05$) between (CC and CT) levels, yet, the pattern of changes and the level of statistical significance in the mean size of graafian follicle in (TT) genotype revealed very high significant decrease (P value< 0.001) in comparison along (CC and CT) genotypes belong to poor responder group. In moderate and high responder groups, the results of comparison in means size of graafian follicle levels after treatment between genotypes (CC, CT, and TT) for individual group reveal no significant differences ($P > 0.05$).

The results in table (3-13) listed the mean ±SD of number of graafian follicle for poor responder group and the mean ± standard deviation (SD) for (CC, CT, and TT) genotypes were 1.23±0.43, 1.27±0.72, 0.65±0.49, subsequently.

ANOVA test when applied to compare CT with CC genotypes for number of graafian follicle in poor responder group showed insignificant difference ($P > 0.05$), while there was high significant decrease ($P < 0.01$) for (TT) genotype when compared with the (CC and CT) genotypes.

The results of comparison in mean number of graafian follicle after treatment for moderate and high responder groups between genotypes (CC, CT, and TT) in individual group reveal insignificant differences ($P>0.05$).

Table (3-13): Comparison between mean ± SD of the studied parameters (E2 after six days of stimulation , Size of graafian follicle and Number of graafian follicle) after treatment with FSH on different genotypes of (rs6165) SNP of FSHR gene in patient groups (poor, moderate, high responder).

Biochemical and clinical parameters	Genotype	Poor responder N=70	Moderate responder N=61	High responder N=79
E2 after 6 days of stimulation (pg/mL)	CC	85.74±5.55	319.62± 1.78	763.89± 86.65
	CT	84.59±4.33 c NS	329.76± 18.57 c NS	734.26±98.75 c NS
	TT	71.79±4.5 c▼***, d ▼***	303.66± 1.78 c NS , d NS	695.84± 21.43 c NS, d NS
Size of graafian follicle (mm)	CC	11.96±1.61	18.11±1.29	21.75±0.94
	CT	11.04±2.33 c NS	19.01±0.93 c NS	21.97±0.86 c NS
	TT	5.84±2.44 c ▼***, d ▼***	18.6±0.41 c NS, d NS	22.29±0.79 c NS, d NS
Number of graafian follicle	CC	1.23±0.43	2.09±1.12	3.17±0.53
	CT	1.27±0.72 c NS	2.06±0.92 c NS	3.11±1.13 c NS
	TT	0.65±0.49 c ▼**, d ▼**	2.01±0.44 c NS, d NS	3.01±0.48 c NS, d NS

c= ANOVA test between (CT, TT) and CC genotype.

d= ANOVA test between TT and CT genotype.

▼*= significant decrease (P<0.05); ▼**= high significant decrease (P<0.01); ▼***=very high significant decrease (P<0.001); NS= non-significant difference.

CHAPTER FOUR

Discussion

4. Discussion

One of the main hormones influencing the growth and enhancement of eggs in the ovaries is the FSH hormone and its action is mediated by FSH receptor (FSHR).Genetic defects are caused by mutations in the FSHR which result in gain or loss of function(139).

4.1.Socio Demographic characteristics of the study groups.

Women below the age of 34 were selected for the present study because the prevalence of poor ovarian response increase with age and women above the age of 40 have more than 50% possibility of poor ovarian response. Follicle depletion increases six times once the individual passes the age of 39, and a great number of clinical experiments have found important fluctuations regarding the ovarian response after the age of 38 (140), so any woman above 34 and below 20 years old were excluded from this study.

The results of this prospective observational study showed that there were insignificant statistically differences in patients groups (poor, moderate, high responder) when compared with control group regarding age, BMI and age of menarche ,also there were non-significant differences between poor, moderate and high responder groups (Table 3-1).

Siddiqui *et al*. reported that age had weak related to number of follicles restored and not be used as index for ovarian response to ovarian stimulation treatment(141), other study showed that the average age in poor responder group was higher than in good and hyper responder groups and reported that this marker of ovarian reserve was one of the important predictive parameters in ovarian response(142).

BMI is a further indicator of ovarian response, a greater BMI decreases the number of mature oocytes that can be produced, also obesity is a significant risk factor for female infertility because obesity is associated with menstrual disorders, miscarriage, an ovulation, and hormonal disturbances and all of which could contribute to infertility (143), therefore women with BMI more than 25 were excluded from the present study.

4.2. Biochemical analyses of the study groups.

In current study, there were very high significant increase in mean of serum FSH in poor responder group as compared with control group, while there was very high significant decrease occurred between (moderate and high responder infertile women) when compared to poor responder group as shown in table (3-2),as egg quality and quantity declined the pituitary gland increases the level of FSH in order to maintain normal follicular development, so the basal FSH level give an indicate of response to ovarian stimulation treatment (144),these results were in line with **Jaiswar *et al.*** who conducted that basal serum FSH concentration were important in predicting ovarian reserve/response and infertile women with high serum FSH level were at high risk of development poor response to ovarian stimulation treatment (145).

The results of the current study were also in agreement with studies (144,146) which stated that patients having high serum FSH levels had decreased fecundity ,while the observation of current study was different from study(147) which demonstrated that no significant differences in the level of FSH among different groups of responders to ovarian stimulation treatment.

In this study there was significant increase in mean of LH serum level in poor responder group and in high responder group as compared to control group . FSH and LH are crucial for follicles development in female. LH enhances the growth of large follicles as well as increases granulosa cell FSH activity by increasing androgen synthesis then promotes the recruitment of follicles, so when LH level was abnormal that lead to abnormal follicular development(148).These results were in agreement with findings of study (149) which showed that level of FSH and LH were higher in poor responder group than in high responder group, while these findings were different from study(150) that found no significant differences in the level of LH between these group.

The result of present study showed that there was no significant difference in the mean of serum TSH and prolactin in patients with poor, moderate and high responder compared with the control group, also there was insignificant difference between (poor, moderate and high responder)groups, among the factors affecting the functional of the ovary is thyroid disorders .TSH regulates the stimulation role of FSH in follicular growth and development (41).

Thyroid diseases are one of most common endocrine problems which may cause menstrual ,ovulation disorders and infertility in reproductive age(151).**Kuroda *et al***. reported that increase in TSH serum level was associated with diminished ovarian reserve and function(152),according to these findings all the subjects included in this study are within the normal TSH level .

High prolactin levels can alter or interrupt ovulation as well as cause irregular or missing the period by interfering with the normal production of other hormones like estrogen ,the hyperprolactinemic state could be contributed to the cause of infertility and this is to be considered in the management of infertility (153), so all women included in this study are within normal prolactin level.

The observed data in table (3-2) indicate very high significant decrease in AMH serum level in the poor responder group as compared to the control group, while there was very high significant increase occur in (moderate and high responder infertile women) when compared to poor responder group, AMH is an indicator of ovarian reserve and it is a single best predictor of poor response to ovarian stimulation treatment(154).AMH is produced by granulosa cells of pre antral and small antral follicles and poor responder group have lower number of antral follicles, these results suggested that poor responder group be more likely to have decreased ovarian reserve.

There was very highly significant increase in AMH level in high responder group when compared to poor responder group, that means high responder group have high level of ovarian reserve ,this finding is consistent with studies (155,156) that listed the AMH level which was different between poor responder and normal responder and it was an accurate marker for poor response to ovarian stimulation .

Vase-parab *et al.* showed that in poor responders, with decreasing AMH levels the antral follicle and mature follicles would also decrease so the poor ovarian response and low serum AMH are connected tightly(157).

The basal level of E2 in current study revealed that there were very high significant increase in poor responder group as compared with control group ,yet there were very high significant decrease occurred in (moderate and high responder infertile women) when compared to poor responder group as shown in table (3-2) while there was insignificant difference in moderate and high responder groups as compared to control group. Basal E2 serum levels in women was measured as part of ovarian reserve testing, it may be used to guide the clinician as to whether the stimulation with gonadotropins can be started or not(158).

Carvalho *et al.* has demonstrated the measurement of both basal FSH and E2 may help to diminish the incidence of false-negative tests based on measurement of FSH alone, when both markers are precociously elevated, poor ovarian response is likely to occur (159), **Prasad et al.** shows that the number of graafian follicles and pregnancy rate decrease with increasing basal level of E2 . In addition, alternative stimulation protocols for patients with elevated basal E2 levels should be considered (160).

This study predicted that AFC which measured on second day of menstrual cycle showed very highly significant increase in high responder and no significant differences in moderate responders compared to control group.

The number of antral follicles that measured by vaginal ultrasound is depend on the primordial follicle pool from which they are recruited, the more primordial follicles which are available mean more follicles will grow. Therefore, the diminishing primordial follicle pool is reflected in the declining antral follicle count, this may help to explain why the AFC is a marker for ovarian response prediction(161).

Decreased number of AFC indicates lower ovarian reserve and lower response to ovarian stimulating drugs (162) these finding suggested that high and moderate responder groups have adequate ovarian reserve while there was very highly significant decrease in poor responder group as compared to control, moderate and high responder groups , these results mean that poor responder group have low ovarian reserve. These findings were in line with study (163) which showed that groups with low AFC may have higher opportunity of diminished ovarian reserve ,ultrasound determination of AFC is an effective technique for the prediction of ovarian response.

Barbakadze *et al*. reported that the use of AMH combined with AFC may improve ovarian reserve evaluation and there is significant correlation between serum AMH and AFC (164).

In the current study, the serum level of estradiol following the administration of exogenous FSH for six days in patient groups (poor ,moderate and high responder) were expressed in table (3-3) which revealed very high significant increase in moderate and high responder groups as compared to poor responder group and very high significant increase in high responder when compared to moderate responder group.

Measurement of E2 level after ovarian stimulation treatment would be helpful to estimate follicle maturation and to predict the ovarian response to treatment. E2 is a steroid hormone secreted by granulosa cells of developing ovarian follicles. The low level of estradiol indicates a reduction in the ability of ovarian follicles to grow and produce estradiol in response to FSH (165), because the main functions of FSH is follicular development and stimulation of estradiol production which might be uncoupled and/or involve different downstream pathways of the FSH receptor

The findings of present study were similar to study(166) which found that decreased estradiol level in poor responder group was associated with decrease maturation of the follicles and significantly decrease pregnancy rate. **Malathi *et al.*** reported that estradiol levels were important clinical tool in the prediction of mature follicles after ovarian stimulation treatment and it is strongly correlated with size and numbers of graafian follicles(42).

The results of current study after treatment of exogenous FSH regarding size and number of graafian follicles showed that very highly significant increase in moderate and high responder group as compared to poor responder group and very high significant decrease in moderate responder group when compared to high responder group.

FSH promotes the follicular growth through the process of folliculogenesis(maturation of ovarian follicles from primordial follicles to their final stage of maturation) (167)and this action is mediated by binding to its receptor (FSHR) so any factor affects this process leads to decreased ovarian growth and maturation.

These findings suggested that decreased sensitivity of ovarian follicles to FSH in poor responder group and decreased stimulation of granulosa cell ,this contributes to poor follicle development these results were in agreement with **Abbara *et al.*** who reported that elevated FSH level in poor responder group was associated with decreased size and number of mature follicles (168).

4.3. The Association of Follicle Stimulating Hormone Receptor Gene Polymorphisms with the Incidence of Infertility.

The results of present study that show the allele frequency were listed in table(3-4) and (3-5)for rs6166 and rs6165 revealed that heterozygous genotype of both SNPs were more abundant than homozygous mutant and wild genotypes in control and patient groups. These results were consistent with those of **Andre *et al.*** which shows that the heterozygous genotype frequency of rs6166and rs6165 was higher than wild and homozygous mutant genotypes in both control and infertile women groups(169).

In poor responder group TT genotype is higher than TT genotype in control ,moderate and high responder group in rs6166 ;yet these increment in TT genotype was much higher in rs6165 for poor responder group as compared with TT genotype in control, moderate and high responder groups, these observations were consistent with the results obtained by **Rod *et al.*** who noticed that position 307 of FSHR SNP(rs6165) was more representative than position 680 of SNP(rs6166)(170),**Motawi *et al.*** indicated that the mutant genotype was 2.5 fold higher in poor responders group than in good responders group(171).

The results of this study disagree with study(172)finding that no statistically significant differences in genotypes distributions between infertile women with poor ovarian response and control group regarding rs6166 and rs6165.

The expression of odd ratio and association between allele frequencies for rs6166 in (poor ,moderate and high responder groups) and control group displayed in table (3-6).Odd ratio for CT genotype of rs6166 for moderate, high and poor responder groups reveal insignificant differences that mean no association between CT genotype in all groups with infertility occurrence.

The odd ratio for the TT genotype in the moderate and high responder groups have the same level of insignificancy. The odd ratio for TT genotype in the group of poor responder was greater than two indicating a link between this genotype with occurrence of infertility ,this significant difference means that polymorphism occurrence (C>T) in rs6166 play a role in pathogenesis of infertility.

Table (3-7)enrolled the results of odd ratio and allele frequency for rs6165 showed insignificant differences in the odd ratio of rs6165 for CT genotype for moderate, high and poor responder groups that mean no link between CT genotype in all group with infertility prevalence ,as well as the odd ratio for the TT genotype in the moderate and high responder groups which revealed insignificant differences .The odd ratio for TT genotype in poor responder group was more than three which indicate an association between this genotype with prevalence of infertility.

The odd ratio in poor responder group for mutant genotype (TT) that belong to rs6165 have more risk than rs6166 on the occurrence of infertility in Iraqi infertile women, these findings were in agreement with **Kaviani *et al.*** which reported that rs6165 was associated with increased risk of infertility(149).

This study revealed that the minor allele frequency for rs6166 and rs6165 were very high for T allele in poor responder group when compared with T allele in control, moderate and high responder groups as shown in tables(3-4) and(3-5),these findings suggested that the (T allele) of rs6165 and rs6166 was associated with possible risk of female infertility in Iraqi infertile women and these SNPs seem to exert pathogenic contribution to female infertility.

These results were in contrast to a study performed in the Indian population 2019 which states that rs6166 had no association with infertility pathogenesis (173), while results of current study were agree with several studies that show the T allele of rs6166,rs6165 were associated with increased risk to infertility, in several population as stated by **Kaviani *et al.*** and **Motawi *et al.*** (149,171).

4.4.Influence of FSHR Polymorphisms on FSH Response.

Understanding of genetic factors related to infertility will affect on the clinical management of infertility ,also it will help to make sure that families with or at risk of genetic disorders related to infertility(174).

Exogenous FSH is utilized for controlled ovarian stimulation for various infertility treatment in female. Despite similar dose of exogenous FSH are used, the ovarian response varies greatly ranging from poor to hyper responder and it's critical to identify the factors that contribute to this variability, especially in the field of personalized medicine(175).

Several parameters such as age ,basal serum FSH level , serum levels of AMH and AFC ,have been used as an indicator to estimate the ovarian response, but haven't been proven(176). FSH receptor has a major role in regulating the physiological response of FSH in the ovary .The gene encoding FSH receptor was found on second chromosome, so any changes in the structure of this region lead to amino acid configuration change for FSH receptor gene resulting in changes in the function of gene, many of these changes may enhance the function of receptor ,yet other changes reduce gene function(89). Polymorphisms in FSHR gene are the most extensively studied in relation to ovarian response in different population(177).

The present study which has been done to illustrate and evaluate the effect of FSHR genetic polymorphisms on FSH response in Iraqi infertile women.

In the current study we investigated the relationship between the FSHR polymorphisms (rs6166,rs6165) with the clinical and endocrinologic parameters of the patient groups ,there were great individual variations among infertile women which were carrying different FSHR SNPs (rs6166 and rs6165) genotypes.

As the two SNPs of FSHR(rs6166 and rs6165) are located on the same exon, they have been suggested and even shown to be linked in several studies(122,178).

It has been reported that FSH levels differ significantly among the rs6166 and rs6165 genotype variants as shown in tables (3-8)(3-10), there were significant increment of FSH level in poor responder group with TT genotype rather than in the CC genotype and in the CT genotype for both (rs6166) and (rs6165), basal FSH serum level was used to evaluate the ovarian reserve ,in which high serum FSH level usually predicts a high ovarian threshold to exogenous FSH responses, these findings suggested that poor responder group with TT genotype were less responsive to FSH, also the analysis of allele frequency revealed that poor responders had a greater frequency of the allele T so the T allele is associated, with poor ovarian response to FSH treatment .The presence of polymorphisms for both (rs6166,rs6165)in poor responder group were related to elevated level of FSH and decreased sensitivity to it, while there was no significant difference in FSH level between the three genotypes(CC, CT and TT) in moderate and high responder groups, this means that no association between FSHR genotypes with ovarian response in moderate and high responder groups.

The findings of present study were similar to studies reported by many researches (179–181),which show that the infertile women carriers mutant variant in poor responder group have high basal FSH level and poor response to exogenous FSH. **Jun *et al.*** and **Achrekar *et al.*** which conclude that ovarian response to exogenous FSH may be depended on FSHR genotypes and these genotypes used as marker to predict individual responses to FSH (182,183).

The results of the current study disagreed with **Trevisan et al.**which concludes that polymorphisms of Thr307Ala(rs6165) and Asn680Ser(rs6166) did not affect the FSH and estradiol serum levels and not to be associated with ovarian response(184).

In this study, the AMH levels were different among genotypes variants tables(3-9)(3-11), infertile women with TT genotype in poor responder group had lower AMH level compared to women with CC and CT genotypes in the same group for both FSHR SNPs(rs6166and rs6165) .AMH serum level may describe both the number and quality of ovarian follicles pool ,so decreased level of it represents decreasing the ovarian reserve and decreases response to FSH, it was a good predictor of poor ovarian response (155).It can be concluded that TT genotype was strongly associated with poor response to FSH treatment. However, the presence of CC and CT among poor responders indicated that TT may not be the only cause of poor response to FSH ,other genetic and environmental factors may contribute to the low ovarian response to FSH ,furthermore, there were no significant differences in CT and TT genotypes in moderate and high responder groups, so in these groups there was no association between FSHR genotypes and ovarian response for both SNPs(rs6166,rs6165).

The data of current study were similar to **Tanase *et al.*** who showed that FSH receptor gene polymorphisms (rs6166,rs6165) influenced AMH serum level in response to FSH treatment in patients with homozygous mutant (TT)(185).

The present study disagreed with **Mohiyiddeen *et al.*** which listed no association between variants in the FSH receptor polymorphisms and AMH levels as marker of ovarian reserve (186).

In this study, there was highly significant reduction in AFC value in poor responder group with TT genotype compared to CC,CT genotype in the same group for both rs6166 and rs6165 tables(3-9)(3-11). AFC is a good indicator of the number of mature follicles that will be able to stimulate in women's ovary(163). Ovarian response to gonadotropin stimulation is influenced by a number of factors such as diminished ovarian reserve which thought to be the principal factor of poor ovarian response . The most frequently utilized predictive tests for evaluating ovarian reserve are basal serum FSH and antral follicle count(187).

These finding suggested that FSHR polymorphisms affect the number of the antral follicle and it was strongly linked to poor response to exogenous FSH treatment, while there was no significant difference between three genotype in moderate and high responder groups for both rs6166and rs6165.These results mean no association between these SNPs and ovarian response to FSH in moderate and high responder groups. These findings were in agreement with **Mohiyiddeen *et al.*** research findings (188),but the results of this study were different from study(122) which reported that no association between FSHR polymorphisms and value of AFC.

The present study was revealed a statistically insignificant variation in TSH and prolactin genotypes in poor ,moderate and high responder groups for both FSHR gene SNPs ,these finding mean there was no effect of these SNPs on the level of TSH and prolactin hormones.

In the present study, the same FSH dose for ovarian stimulation resulted insignificantly lower serum levels of estradiol in infertile women with the TT genotype in poor responder group compared to women with the CC and CT genotypes.

The important role of FSH in the regulation of female reproduction by binding to its specific FSHR, which is located in granulosa cells of the ovary. Interaction between FSH and FSHR induces intracellular signaling pathway to determine differentiation and proliferation of granulosa cells. The FSH stimulated granulosa cells produce E2.Therefore, impaired FSHR activity by rs6166 and rs6165 leads to poor proliferation and differentiation of granulosa cells and reduced production of E2 (168,189).These results suggested that the polymorphisms in FSHR (rs6166and rs6165) was associated with poor response to FSH and the T allele may be responsible for decrease sensitivity of FSH receptor to FSH.

Regarding the CC,CT and TT genotypes in moderate and high responder groups ,there was no significant difference between them suggested that no association between these genotypes and estradiol serum level in moderate and high responder groups for both rs6166 and rs6165.

The current study was in line with many studies such as (182,190,191) which realized that infertile women with homozygous mutant genotypes for both study SNPs (rs6166 and rs6165) showed lower E2 level and fewer mature oocyte than other genotypes ,but the data of present study disagreed with **Trevisan *et al.*** findings which showed FSH and estradiol serum levels were not associated with polymorphisms of FSHR (rs6166 and rs6165)(184).

In the present study the size and number of graafian follicles was significantly lower in FSHR (rs6166 and rs6165) TT genotype carriers in poor responder group than in CC and CT carriers .

The dominance of estradiol and FSH in follicular fluid is essential for sustained accumulation in granulosa cells ,continued follicular growth and estradiol production (192) so the genetic change involved in FSHR SNPs (rs6166 and rs6165) was suggested to potentially decreased sensitivity of FSHR to FSH, hence decrease the action of FSH reflected by decreased size and number of graafian follicles while there was no significant difference among CC,CT and TT carriers in moderate and high responder groups ,that mean no association of FSHR polymorphisms with genotypes of these groups.

These results were in agreement with **Sheikhha *et al.*** that showed the lower ovarian response in poor responder group with TT genotype including high basal FSH and lower number of mature oocytes(193). **Ahmed *et al.*** and **Boudjenah *et al.*** showed that SNPs of FSH receptor impact on number of graafian follicles and this affects the response to ovarian stimulation in infertile women(150,179).

The findings of current study were different from **Allegra *et al.*** showed that no association between FSHR polymorphisms and mature follicles (194).

Conclusions

The findings of this study revealed:

1. The FSHR polymorphisms(rs6166 and rs6165) can be considered as one of the genetic factors responsible for variability in response to FSH in Iraqi infertile women.
2. The heterozygous genotype CT was more predominant than other genotypes CC and TT for both SNPs.
3. The odd ratio indicated an association between presence of both SNPs in FSHR gene and incidence of infertility in Iraqi women.
4. The TT genotype of rs6166 and rs6165 are strongly linked to poor ovarian response to FSH treatment in clinical and hormonal parameters as observed by significant reduction in mean of AFC, AMH ,E2 and significant increase in basal FSH levels.

Recommendations

1.Study another SNPs of FSHR gene with their effects on response to FSH.

2.Use genetic test to help the clinician to individualize person's response according to genotype and developing a personalized drug that is more effective and safe.

3. Larger scale studies including more infertile women and healthy control participants from different Iraqi cities are preferred.

REFERENCES

References

1. Wadadekar G, Inamdar D, Nimbargi V. Assessment of impact of infertility & its treatment on quality of life of infertile couples using fertility quality of life questionnaire. Journal of Human Reproductive Sciences. 2021;14(1):3–10.
2. Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. Clinical Biochemistry. 2018;62:2–10.
3. Garolla A, Pizzol D, Carosso AR, Borini A, Ubaldi FM, Calogero AE, et al. Practical Clinical and Diagnostic Pathway for the Investigation of the Infertile Couple. Frontiers in Endocrinology. 2021;11:1–15.
4. Szkodziak F, Krzyżanowski J, Szkodziak P. Psychological aspects of infertility. A systematic review. Journal of International Medical Research. 2020;48(6):1–13.
5. Abdelazim IA, Purohit P, Farag RH, Zhurabekova G. Unexplained infertility: prevalence, possible causes and treatment options. A review of the literature. Journal of Obstetric Gynecological Investigation. 2018;1(1):17–22.
6. Leridon H, Kremlin-bice L, Cedex Ã. Can assisted reproduction technology compensate for the natural decline in fertility with age ? A model assessment. Human Reproduction. 2004;19(7):1548–1553.
7. Lassi ZS, Imam AM, Dean S V, Bhutta ZA. Preconception care : caffeine , smoking , alcohol , drugs and other environmental chemical / radiation exposure. Reproductive Health. 2014;11(3):1–12.
8. Downer EJ, Campbell VA. Phytocannabinoids , CNS cells and development : A dead issue ? Drug and Alcohol. 2010;29(1):91–98.
9. Brents LK. Marijuana , the Endocannabinoid System and the Female Reproductive System.Yale Journal of biology and medicine.2016;89(2):175–191.
10. Stanford JB, Dunson DB. Effects of sexual intercourse patterns in time to pregnancy studies. American Journal of Epidemiology. 2007;165(9):1088–1095.
11. Bala R, Singh V, Rajender S. Environment, Lifestyle, and Female Infertility. Reproductive Sciences. 2020;28(3):617–638.
12. Freour T, Masson D, Mirallie S, Jean M, Bach K, Dejoie T, et al.

References

Active smoking compromises IVF outcome and affects ovarian reserve. Reproductive BioMedicine. 2008;16(1):96–102.

13. Waylen AL, Metwally M, Jones GL, Wilkinson AJ, Ledger WL. Effects of cigarette smoking upon clinical outcomes of assisted reproduction : a meta-analysis. Human Reproduction Update. 2009;15(1):31–44.

14. Sheiner EK, Sheiner E. The Effect of Women ' s Occupational Psychologic Stress on Outcome of Fertility Treatments. Occupational and Enviromental Medicine. 2006;48(1):56–62.

15. Harrison, Patricia A. PAH. Alcohol and Drug Use Before and During Pregnancy : An Examination of Use Patterns and Predictors of Cessation. Maternal and Child Health. 2009;13:386–394.

16. Zauner G, Girardi G. Potential causes of male and female infertility in Qatar. Journal of Reproductive and Immunology. 2020;141:1–13.

17. Boutari C, Pappas PD, Mintziori G, Nigdelis MP, Athanasiadis L, Goulis DG, et al. The effect of underweight on female and male reproduction. Metabolism. 2020;107:1–13.

18. Madziyire MG, Magwali TL, Chikwasha V, Mhlanga T. The causes of infertility in women presenting to gynaecology clinics in Harare, Zimbabwe; a cross sectional study. Fertility Research and Practice. 2021;7(1):1–8.

19. Puttabyatappa M, Padmanabhan V. Developmental Programming of Ovarian Functions and Dysfunctions. Vitamins and Hormones. 2018;107: 377–422.

20. Zhu J, Liu J, Cao X, Wang X. An Efficacy and Feasibility Analysis of Chinese Patent Medicine Combined With Letrozole in the Treatment of Women With Ovulation Disorders : A Network Meta-Analysis. Frontiers in Pharmacology. 2021;12:1–12.

21. Devesa-peiro A, Sebastian-leon P, Garcia-garcia F.Uterine disorders affecting female fertility : what are the molecular functions altered in endometrium ? Fertility and Sterility.2020;113(6):1261–1274.

22. Shen H, Cai M, Chen T, Zheng D, Huang S, Zhou M, et al. Factors affecting the success of fallopian tube recanalization in treatment of tubal obstructive infertility. International Medical Research. 2020;48(12):1–10.

References

23. Maddern J, Grundy L, Castro J, Brierley SM. Pain in Endometriosis. Frontiers in Cellular Neuroscience. 2020;14:1–16.

24. Malvezzi H, Marengo EB, Podgaec S, Piccinato CDA. Endometriosis : current challenges in modeling a multifactorial disease of unknown etiology. Journal of Translation Medicine. 2020;18(311):1–21.

25. Chon SJ, Umair Z, Yoon M. Premature Ovarian Insufficiency : Past , Present , and Future. Frontiers in Cell and Developmental Biology. 2021;9:1–13.

26. Al-Kuran O, Al- Mehaisen L, Al-duraidi H,Al- Husban N, Attarakih B, et al. How prevalent are symptoms and risk factors of pelvic inflammatory disease in a sexually conservative population. Reproductive Health. 2021;18(109):1–7.

27. Waimey KE, Smith BM, Confino R, Jeruss JS, Pavone ME. Understanding Fertility in Young Female Cancer Patients.Journal of Women's Healh . 2015;24(10):812–818.

28. Mallepaly R, Butler PR, Herati AS, Lamb DJ. Genetic Basis of Male and Female Infertility. Monographs in Human Genetics. 2017;21:1–16.

29. Badar A. An Overview on the Genetic Determinants of Infertility. Biomedical Journal of Scientific and Technical Research. 2018;10(4):7960–7964.

30. Manuscript A. The Genetics of Infertility : Current Status of the Field. Current Genetic Medicine Report. 2013;1(4):1–22.

31. Szymańska K, Kałafut J, Rivero-Müller A. The gonadotropin system, lessons from animal models and clinical cases. Minerva Obstetrics and Gynecology. 2018;70(5):561–587.

32. He WB, Du J, Yang XW, Li W, Tang WL, Dai C, et al. Novel inactivating mutations in the FSH receptor cause premature ovarian insufficiency with resistant ovary syndrome. Reproductive Biomedicine Online. 2019;38(3):397–406.

33. Carson SA, Kallen AN. Diagnosis and Management of Infertility. Journal American Medical Association. 2022;326(1):65–76.

34. Marshburn PB. Counseling and Diagnostic Evaluation for the Infertile Couple. Obstet Gynecol Clin NA. 2015;42(1):1–14.

References

35. Mohammad SH, Sommerich CM, Butwin AN, Evans KD. Assessment of Obstetrics and Gynecology Residents' Knowledge of Basic Obstetric Sonography and Psychomotor Evaluation With Motion Analysis Using a Computer-Based Simulator. Journal of Diagnostic Medical Sonography. 2020;36(4):312–321.

36. Marca A La, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers : from theory to practice. Human Reproductive Update . 2014;20(1):124–140.

37. Penzias A, Azziz R, Bendikson K, Falcone T, Hansen K, Hill M, et al. Testing and interpreting measures of ovarian reserve. Fertility and Sterility. 2020;114(6):1151–1157.

38. Auriemma RS, Del Vecchio G, Scairati R, Pirchio R, Liccardi A, Verde N, et al. The Interplay Between Prolactin and Reproductive System: Focus on Uterine Pathophysiology. Frontiers in Endocrinology. 2020;11:1–8.

39. Martinez-Alarcon O, García-Lopez G, Guerra Mora JR, Molina-Hernandez A, Diaz-Martinez NE, Portillo W, et al. Prolactin from Pluripotency to Central Nervous System Development. Neuroendocrinology. 2021;112(3):201–214.

40. Vissenberg R, Manders VD, Mastenbroek S, Fliers E, Afink GB, Ris-Stalpers C, et al. Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction. Human Reproductive Update. 2015;21(3):378–387.

41. Kabodmehri R, Sharami SH, Sorouri ZZ, Gashti NG, Milani F, Chaypaz Z, et al. The relationship between thyroid function and ovarian reserve: a prospective cross-sectional study. Thyroid Research. 2021;14(1):4–9.

42. Malathi A, Balakrishnan S, B. S L. Correlation between estradiol levels on day of HCG trigger and the number of mature follicles, number of oocytes retrieved, and the number of mature oocytes (M2) after oocyte aspiration in ICSI cycles. Middle East Fertil Society. 2021;26(34):1–10.

43. Ying Y, Wu Y, Liu S, Huang Q, Liu H. The timing for initiating estrogen stimulation in artificial cycle for frozen-thawed embryo transfer can be flexible. Reproductive Health. 2021;18(1):1–7.

44. Moolhuijsen LM, Visser JA. Anti-Müllerian Hormone and Ovarian

References

Reserve: Update on Assessing Ovarian Function. Journal of Clinical Endocrinology and Metabolism. 2020;105(11):3361–3373.

45. Umarsingh S, Adam JK, Krishna SBN. The relationship between anti-Müllerian hormone (AMH) levels and pregnancy outcomes in patients undergoing assisted reproductive techniques (ART). Life and enviromental Journal. 2020;8:1–14.

46. Li HWR, Robertson DM, Burns C, Ledger WL. Challenges in Measuring AMH in the Clinical Setting. Frontiers in Endocrinology. 2021;12:1–8.

47. Xu H, Zhang M, Zhang H, Alpadi K, Wang L, Li R, et al. Clinical Applications of Serum Anti-Müllerian Hormone Measurements in Both Males and Females: An Update. Innovation. 2021;2(1):1–10.

48. Liu M, Liu S, Li L, Wang P, Li H, Li Y. LH levels may be used as an indicator for the time of antagonist administration in GnRH antagonist protocols—a proof-of-concept study. Frontiers in Endocrinology. 2019;10(67):1–8.

49. Bhartiya D, Patel H. An overview of FSH-FSHR biology and explaining the existing conundrums. Journal of Ovarian Research. 2021;14(1):1–14.

50. Das N, Kumar TR. Molecular regulation of follicle-stimulating hormone synthesis, secretion and action. Journal of Molecular Endocrinology. 2018;60(3):131–155.

51. Oduwole OO, Huhtaniemi IT, Misrahi M. The roles of luteinizing hormone, follicle-stimulating hormone and testosterone in spermatogenesis and folliculogenesis revisited. International Journal of Molecular Sciences. 2021;22(23):1–30.

52. Bosch E, Alviggi C, Lispi M, Conforti A, Hanyaloglu AC, Chuderland D, et al. Reduced FSH and LH action: implications for medically assisted reproduction. Human Reproduction. 2021;36(6):1469–1480.

53. Banerjee AA, Mahale SD. Role of the extracellular and intracellular loops of follicle-stimulating hormone receptor in its function. Frontiers in Endocrinology. 2015;6(110):1–10.

54. Singhasena W, Pantasri T, Piromlertamorn W, Samchimchom S, Vutyavanich T. Follicle-stimulating hormone receptor gene polymorphism in chronic anovulatory women, with or without

polycystic ovary syndrome: A cross-sectional study. Reproductive Biology and Endocrinology. 2014;12(1):1–7.

55. Lizneva D, Rahimova A, Kim SM, Atabiekov I, Javaid S, Alamoush B, et al. FSH beyond fertility. Frontiers in Endocrinology. 2019;10(136):1–10.

56. Ulloa-Aguirre A, Zariñán T. The follitropin receptor: Matching structure and function. Molecular Pharmacology. 2016;90(5):596–608.

57. Winstel R, Wieland J, Gertz B, Mueller A. Manufacturing of Recombinant Human Follicle-Stimulating Hormone Ovaleap (XM17), Comparability with Gonal-f , and Performance / Consistency. Drugs R D. 2017;17(2):305–312.

58. Shinde.S.A, Chavhan.S.A, Sapkal.S.B. Recombinant DNA Technology and its Applications. Medi Pharm Research. 2018;4(2):79–88.

59. Barros.F.R, Leao.F, Sandro.C.L. Gonadotropin therapy in assisted reproduction : an evolutionary perspective from biologics to biotech. Clinics. 2014;69(4):279–293.

60. Bergandi L, Canosa S, Carosso AR, Paschero C, Gennarelli G, Silvagno F, et al. Human recombinant FSH and its biosimilars: Clinical efficacy, safety, and cost-effectiveness in controlled ovarian stimulation for in vitro fertilization. Pharmaceuticals. 2020;13(7):1–23.

61. Butnev VY, Butnev VY, May J V., Shuai B, Tran P, White WK, et al. Production, purification, and characterization of recombinant hFSH glycoforms for functional studies. Molecular and Cellular Endocrinology. 2015;405:42–51.

62. Revelli A, Pettinau G, Basso G, Carosso A, Ferrero A, Dallan C, et al. Controlled Ovarian Stimulation with recombinant-FSH plus recombinant-LH vs . human Menopausal Gonadotropin based on the number of retrieved oocytes : results from a routine clinical practice in a real-life population. Reproductive Biology and Endocrinology. 2015;13(77):1–8.

63. Orlova NA, Kovnir S V., Khodak YA, Polzikov MA, Nikitina VA, Skryabin KG, et al. High-level expression of biologically active human follicle stimulating hormone in the Chinese hamster ovary

cell line by a pair of tricistronic and monocistronic vectors. PLoS One. 2019;14(7):1–20.

64. Taketani Y, Kelly E, Yoshimura Y, Hoshiai H, Irahara M, Mizunuma H, et al. Recombinant follicle-stimulating hormone (follitropin alfa) versus purified urinary follicle-stimulating hormone in a low-dose step-up regimen to induce ovulation in Japanese women with anti-estrogen-ineffective oligo- or anovulatory infertility: results . Reproductive Medicine Biology. 2010;9(2):99–106.

65. Pan W, Tu H, Jin L, Hu C, Xiong J, Pan W, et al. Comparison of recombinant and urinary follicle-stimulating hormones over 2000 gonadotropin-releasing hormone antagonist cycles: a retrospective study. Scientifics Report. 2019;9(1):1–9.

66. Rashidi M, Aaleyasin A, Aghahosseini M, Loloi S, Kokab A, Najmi Z. Advantages of recombinant follicle-stimulating hormone over human menopausal gonadotropin for ovarian stimulation in intrauterine insemination: A randomized clinical trial in unexplained infertility. European Journal of Obstetric and Gynecology and Reproductive Biology. 2013;169(2):244–247.

67. Santi D, Simoni M. Biosimilar recombinant follicle stimulating hormones in infertility treatment. Expert Opinin on Biological Therapy. 2014;14(10):1399–409.

68. Dias JA, Ulloa-Aguirre A. New Human Follitropin Preparations: How Glycan Structural Differences May Affect Biochemical and Biological Function and Clinical Effect. Frontiers in Endocrinology. 2021;12:1–16.

69. Chua SJ, Mol BW, Longobardi S, Orvieto R, Venetis CA, Lispi M, et al. Biosimilar recombinant follitropin alfa preparations versus the reference product in couples undergoing assisted reproductive technology treatment: a systematic review and meta-analysis. Reproductive Biology and Endocrinology. 2021;19(51):1–13.

70. Rettenbacher M, Andersen AN, Garcia-Velasco JA, Sator M, Barri P, Lindenberg S, et al. A multi-centre phase 3 study comparing efficacy and safety of Bemfola versus Gonal-f in women undergoing ovarian stimulation for IVF. Reproductive Biomedicine Online. 2015;30(5):504–513.

References

71. Kurji HA, Al Khames Aga QA, Al-Taie MJM, Bataineh YA, Hasan MK, Abdulkareem AH, et al. Efficacy of follitropin-a (Gonal-f) versus follitropin-b (puregon) for controlled ovarian stimulation in women undergoing in vitro fertilization (ivf). International Journal of Pharmaceutical Quality Assurance. 2019;10(4):681–685.

72. Haute.L, Drakopoulos P, Verheyen G, Vos M, Tournaye H, Blockeel C. Follitropin alpha versus beta in a first GnRH antagonist ICSI cycle: a retrospective cohort study. Reproductive Biomedicine. 2021;43(4):655–662.

73. Olsson H, Sandström R, Grundemar L. Different pharmacokinetic and pharmacodynamic properties of recombinant follicle-stimulating hormone (rFSH) derived from a human cell line compared with rFSH from a non-human cell line. Journal of Clinical Pharmacology. 2014;54(11):1299–1307.

74. Koechling W, Plaksin D, Croston GE, Jeppesen J V., Macklon KT, Andersen CY. Comparative pharmacology of a new recombinant FSH expressed by a human cell line. Endocrine Connection. 2017;6(5):297–305.

75. Nahuis M, Van Der Veen F, Oosterhuis J, Mol BW, Hompes P, Van Wely M. Review of the safety, efficacy, costs and patient acceptability of recombinant follicle-stimulating hormone for injection in assisting ovulation induction in infertile women. International Journalof Womens Health. 2009;1(1):205–211.

76. Lunenfeld B, Bilger W, Longobardi S, Alam V, D'Hooghe T, Sunkara SK. The development of gonadotropins for clinical use in the treatment of infertility. Frontiers in Endocrinology. 2019;10(429):1–15.

77. Wolzt M, Gouya G, Sator M, Hemetsberger T, Irps C, Rettenbacher M, et al. Comparison of pharmacokinetic and safety profiles between Bemfola®and Gonal-f®after subcutaneous application. European Journal of Drug Metabolism and Pharmacokinetics. 2016;41(3):259–265.

78. Griesinger G, Verweij PJM, Gates D, Devroey P, Gordon K, Stegmann BJ, et al. Prediction of ovarian hyperstimulation syndrome in patients treated with corifollitropin alfa or rFSH in a GnRH antagonist protocol. PLoS One. 2016;11(3):1–14.

79. Fiedler K, Ezcurra D. Predicting and preventing ovarian

hyperstimulation syndrome (OHSS): The need for individualized not standardized treatment. Reproductive Biology and Endocrinology. 2012;10(32):1–10.

80. Pfeifer S, Butts S, Dumesic D, Fossum G, Gracia C, La Barbera A, et al. Prevention and treatment of moderate and severe ovarian hyperstimulation syndrome. Fertility and Sterility. 2016;106(7):1634–1647.

81. Krishnan S, Kaur H, Bali J, Rao K. Ovarian torsion in infertility management - Missing the diagnosis means losing the ovary: A high price to pay. Journal of Human Reproductive Sciences. 2011;4(1):39–42.

82. Pirtea P, de Ziegler D, Poulain M, Ayoubi JM. New Twists in Ovarian Stimulation and Their Practical Implications. Frontires in Medicine. 2019;6(197):1–6.

83. Fauser BC, Devroey P, Macklon NS. Multiple birth resulting from ovarian stimulation for subfertility treatment. Lancet. 2005 ;365(9473):1807–1816.

84. Dickey RP. Strategies to reduce multiple pregnancies due to ovulation stimulation. Fertility and Sterility. 2009;91(1):1–17.

85. Bu Z, Xiong Y, Wang K, Sun Y. Risk factors for ectopic pregnancy in assisted reproductive technology: a 6-year, single-center study. Fertility and Sterility. 2016;106(1):90–94.

86. Jin XY, Li C, Xu W, Liu L, Wei ML, Fei HY, et al. Factors associated with the incidence of ectopic pregnancy in women undergoing assisted reproductive treatment. Chinese Medical Journal. 2020;133(17):2054–2060.

87. Buhler KF, Fischer R, Verpillat P, Allignol A, Guedes S, Boutmy E, et al. Comparative effectiveness of recombinant human follicle-stimulating hormone alfa (r-hFSH-alfa) versus highly purified urinary human menopausal gonadotropin (hMG HP) in assisted reproductive technology (ART) treatments: a non-interventional study in German. Reproductive Biology and Endocrinology. 2021;19(1):1–14.

88. Tafazoli A, Miltyk W, Wołczyński S, Wawrusiewicz-Kurylonek N, Esmaeili SA. Pharmacogenomic biomarkers of follicle-stimulating hormone receptor malfunction in females with impaired ovarian

response-A genetic survey. Journal of Clinical Medicine. 2021;10(2):1–14.

89. Sever B, Simsek M, Akar ME, Alper O, Leblebici IM. Comparison of FSH receptor polymorphisms between infertile and fertile women. Biomedical Research. 2014;25(1):121–126.

90. Dias JA, Cohen BD, Lindau-Shepard B, Nechamen CA, Peterson AJ, Schmidt A. Molecular, structural, and cellular biology of follitropin and follitropin receptor. Vitamins and Hormones. 2002;64:249–322.

91. Banerjee AA, Joseph S, Mahale SD. From cell surface to signalling and back: the life of the mammalian FSH receptor. FEBS Journal. 2021;288(8):2673–2696.

92. Althumairy D, Zhang X, Baez N, Barisas G, Roess DA, Bousfield GR, et al. Glycoprotein G-protein Coupled Receptors in Disease: Luteinizing Hormone Receptors and Follicle Stimulating Hormone Receptors. Diseases. 2020;8(3):1–24.

93. Jiang X, Dias JA, He X. Structural biology of glycoprotein hormones and their receptors: insights to signaling. Molecular and Cellular Endocrinology. 2014;382(1):424–451.

94. Vassart G, Pardo L, Costagliola S. A molecular dissection of the glycoprotein hormone receptors. Trends in Biochemical Sciences. 2004;29(3):119–126.

95. Feng X, Zhang M, Guan R, Segaloff DL. Heterodimerization Between the Lutropin and Follitropin Receptors is Associated With an Attenuation of Hormone-Dependent Signaling. Endocrinology. 2013;154(10):3925–3930.

96. Haldar S, Agrawal H, Saha S, Straughn AR, Roy P, Kakar SS. Overview of follicle stimulating hormone and its receptors in reproduction and in stem cells and cancer stem cells. International Journal Biological Sciences. 2022;18(2):675–692.

97. Zarinan T, Butnev VY, Gutiérrez-Sagal R, Maravillas-Montero JL, Martínez-Luis I, Mejía-Domínguez NR, et al. In Vitro Impact of FSH Glycosylation Variants on FSH Receptor-stimulated Signal Transduction and Functional Selectivity. Journal of Endocrine Society. 2020 ;4(5):1–23.

98. Puri P, Little-ihrig L, Chandran U, Law NC, Hunzicker-dunn M,

References

Zeleznik AJ. Protein Kinase A : A Master Kinase of Granulosa Cell Differentiation. Journal of Scientific Reports. 2016;6:1–12.

99. Kara E, Cre P, Gauthier C, Martinat N, Piketty V, Guillou F, et al. A Phosphorylation Cluster of Five Serine and Threonine Residues in the C-Terminus of the Follicle-Stimulating Hormone Receptor Is Important for Desensitization But Not for Beta-Arrestin-Mediated ERK Activation. Molecular Endocrinology. 2006;20(11):3014–3026.

100. Landomiel F, Pascali F De, Raynaud P, Jean-alphonse F, Poupon A, Reiter E. Biased Signaling and Allosteric Modulation at the FSHR. Frontiers in Endocrinology. 2019;10(148):1–15.

101. George JW, Dille EA, Heckert LL. Current concepts of follicle-stimulating hormone receptor gene regulation. Biology of Reproduction. 2011;84(1):7–17.

102. Nenonen HA, Lindgren IA, Prahl AS, Trzybulska D, Kharraziha I, Hultén M, et al. The N680S variant in the follicle-stimulating hormone receptor gene identifies hyperresponders to controlled ovarian stimulation. Pharmacogenetics and Genomics. 2019;29(5):114–120.

103. Jin Y, Wang J, Bachtiar M, Chong SS, Lee CGL. Architecture of polymorphisms in the human genome reveals functionally important and positively selected variants in immune response and drug transporter genes. Human Genomics. 2018;12(1):1–13.

104. Albert PR. What is a functional genetic polymorphism? Defining classes of functionality. Journal of Psychiatry and Neuroscience. 2011;36(6):363–365.

105. Robert F, Pelletier J, Alain T. Exploring the Impact of Single-Nucleotide Polymorphisms on Translation. Frontiers in Genetics. 2018;9(507):1–11.

106. Chen AY, Huang CW, Liu SH, Liu AC, Chaung HC. Single nucleotide polymorphisms of immunity-related genes and their effects on immunophenotypes in different pig breeds. Genes (Basel). 2021;12(9):1–17.

107. Doo M, Kim Y. Obesity: Interactions of genome and nutrients intake. Preventive Nutrition and Food Science. 2015;20(1):1–7.

108. Conforti A, Tüttelmann F, Alviggi C, Behre HM, Fischer R, Hu L, et

References

al. Effect of Genetic Variants of Gonadotropins and Their Receptors on Ovarian Stimulation Outcomes: A Delphi Consensus. Frontiers in Endocrinology. 2022;12:1–18.

109. Chu D, Wei L. Nonsynonymous, synonymous and nonsense mutations in human cancer-related genes undergo stronger purifying selections than expectation. BMC Cancer. 2019;19(1):1–12.

110. Ismail S, Essawi M. Genetic polymorphism studies in humans. Middle East Journal of Medical Genetics. 2012;1:57–63.

111. Bello JR, Jimenez M. Functional implications of single nucleotide polymorphisms (SNPs) in protein-coding and non-coding RNA genes in multifactorial diseases. Gac Med Mex. 2017;153:218–229.

112. Conforti A, Vaiarelli A, Cimadomo D, Bagnulo F, Peluso S, Carbone L, et al. Pharmacogenetics of FSH action in the female. Frontiers in Endocrinology. 2019;10:1–7.

113. Lledo B, Guerrero J, Turienzo A, Ortiz JA, Morales R, Ten J, et al. Effect of follicle-stimulating hormone receptor N680S polymorphism on the efficacy of follicle-stimulating hormone stimulation on donor ovarian response. Pharmacogenetic Genomics. 2013;23(5):262–268.

114. Mutsatsa S, Currid TJ. Pharmacogenetics: a reality or misplaced optimism? Journal of Psychiatric and Mental Health Nursing. 2013;20(4):314–320.

115. Wang J, Pang GSY, Chong SS, Lee CGL. SNP web resources and their potential applications in personalized medicine. Current Drug Metabolism. 2012;13(7):978–990.

116. Ilgaz NS, Sena O, Aydos E, Karadag A, Taspinar M, Eryilmaz OG, et al. Impact of follicle-stimulating hormone receptor variants in female infertility. Assisted Reproduction and Genetics. 2015;32(11):1659–1668.

117. Casarini L, Santi D, Marino M. Impact of gene polymorphisms of gonadotropins and their receptors on human reproductive success. Reproduction. 2015;150(6):175–184.

118. Desai SS, Roy BS, Mahale SD. Mutations and polymorphisms in FSH receptor: Functional implications in human reproduction. Reproduction. 2013;146(6):235–248.

References

119. Leener A De, Montanelli L, Durme J Van, Chae H, Smits G, Vassart G, et al. Presence and absence of follicle-stimulating hormone receptor mutations provide some insights into spontaneous ovarian hyperstimulation syndrome physiopathology. Clinical Endocrinology and Metabolism. 2006;91(2):555–562.

120. Jimenez G, Zarinan T, valentin R, Dominguez NR, Sagal R, Montes G, et al. Frequency of the T307A , N680S , and -29G > A single-nucleotide polymorphisms in the follicle-stimulating hormone receptor in Mexican subjects of Hispanic ancestry. Reproductive Biology and Endocrinology. 2018;16(100):1–12.

121. Zilaitiene B, Dirzauskas M, Verkauskiene R, Ostrauskas R, Gromoll J, Nieschlag E. The impact of FSH receptor polymorphism on time-to-pregnancy: A cross-sectional single-centre study. BMC Pregnancy Childbirth. 2018;18(1):1–8.

122. Yan Y, Gong Z, Zhang L, Li Y, Li X, Zhu L, et al. Association of Follicle-Stimulating Hormone Receptor Polymorphisms with Ovarian Response in Chinese Women: A Prospective Clinical Study. PLoS One. 2013;8(10):1–8.

123. Zalewski G, Wołczyński S, Chyczewski L. Association of rs6166 polymorphism with FSH receptor transcript variants and steroid production in human granulosa cell cultures. System Biology in Reproductive Medicine. 2013;59(4):191–198.

124. Wunsch A, Sonntag B, Simoni M. Polymorphism of the FSH receptor and ovarian response to FSH. Annales Endocrinology. 2007;68:160–166.

125. Bruna-Catalán I, Menabrito M. Ovulation induction with minimal dose of follitropin alfa: A case series study. Reproductive Biology and Endocrinology. 2011;9(142):1–7.

126. Velthuis E, Hubbard J, Longobardi S, D'Hooghe T. The Frequency of Ovarian Hyperstimulation Syndrome and Thromboembolism with Originator Recombinant Human Follitropin Alfa (GONAL-f) for Medically Assisted Reproduction: A Systematic Review. Advances in Therapy. 2020;37(12):4831–4847.

127. Datta AK, Maheshwari A, Campbell S, Nargund G, Felix N, Campbell S, et al. Mild versus conventional ovarian stimulation for IVF in poor, normal and hyper-responders: a systematic review and meta-analysis. Human Reproduction Update. 2021;27(2):229–253.

References

128. Burtis. C, Bruns D. Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics. 2014; 1–2412.

129. Xin T, Chen H, Lin Z, Liang S, Lin J. A secondary antibody format chemiluminescence immunoassay for the determination of estradiol in human serum. Talanta. 2010;82(4):1472–1477.

130. Karnes J, Arora A, Feng J, Steiner HE, Sulieman L, Boerwinkle E, et al. Racial , ethnic , and gender differences in obesity and body fat distribution : An All of Us Research Program demonstration project. PLoS One. 2021;16(8):1–13.

131. Nair A, Mary A,Yadav S. A meta-review on principles of recombinant DNA technology. World Journal Pharmaceutical Research. 2021;10(4):902–926.

132. Tataurov A V, You Y, Owczarzy R. Predicting ultraviolet spectrum of single stranded and double stranded deoxyribonucleic acids. Biophysical Chemistry. 2008;133(3):66–70.

133. Sharma A, Tapadia K, Sahin R, Shrivas K. Surfactant-Assisted Nanodrop Spectrophotometer Determination of Iron(III) in a Single Drop of Food, Biological, and Environmental Samples. Journal of Applied Spectroscopy. 2016;82(6):1064–1071.

134. Atawodi S, Atawodi J, Dzikwi A. Polymerase chain reaction: Theory, practice and application: A review. Sahel Medical Journal. 2011;13(2):54–63.

135. Darawi MN, Ai-Vyrn C, Ramasamy K, Hua PPJ, Pin TM, Kamaruzzaman SB, et al. Allele-specific polymerase chain reaction for the detection of Alzheimer's disease-related single nucleotide polymorphisms. BMC Medical Genetics. 2013;14(1):1–8.

136. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics. 2012;13(134):1–11.

137. Ylmaz M, Ozic C, Gok L. Principles of Nucleic Acid Separation by Agarose Gel Electrophoresis. Gel Electrophoresis - Principles and Basics. 2012;1-378

138. Lee PY, Costumbrado J, Hsu CY, Kim YH. Agarose gel electrophoresis for the separation of DNA fragments. Journal of Visualized Experiments. 2012;(62):1–5.

References

139. Alviggi C, Conforti A, Esteves SC, Vallone R, Venturella R, Staiano S, et al. Understanding ovarian hypo-response to exogenous gonadotropin in ovarian stimulation and its new proposed marker-the follicle-to-oocyte (FOI) index. Frontiers in Endocrinology. 2018;9:1–7.

140. Massarotti C, La Pica V, Sozzi F, Scaruffi P, Remorgida V, Anserini P. Influence of age on response to controlled ovarian stimulation in women with low levels of serum anti-Müllerian hormone. Gynecological Endocrinology. 2020;36(12):1074–1078.

141. Siddiqui Q, Anjum S, Zahra F, Yousuf SM. Ovarian reserve parameters and response to controlled ovarian stimulation in infertile patients. Pakistan J Med Sci. 2019;35(4):958–962.

142. Kligman I, Rosenwaks Z. Differentiating clinical profiles: Predicting good responders, poor responders, and hyperresponders. Fertility and Sterility. 2001;76(6):1185–1190.

143. Musa S, Osman S. Risk profile of Qatari women treated for infertility in a tertiary hospital: a case-control study. Fertility Research and Practice. 2020;6(12):1–17.

144. Catteau A, Bach-Ngohou K, Blin J, Barrière P, Fréour T, Masson D. Abnormally Elevated Follicle-Stimulating Hormone (FSH) level in an infertile woman. Cases Reports in Endocrinology. 2019;102:1–5.

145. Jaiswar SP, Natu SM, Sujata, Sankhwar PL, Manjari G. Prediction of Poor Ovarian response by Biochemical and Biophysical Markers: A Logistic Regression Model. Journal of Obstetrics and Gynecology. 2015;65(6):411–416.

146. Labarta E, Marin D, Remohí J, Bosch E. Conventional versus minimal ovarian stimulation: an intra-patient comparison of ovarian response in poor-responder women according to Bologna Criteria. Reproductive Biomedicine Online. 2018;37(4):434–441.

147. Cai J, Lou H, Dong M, Lu X, Zhu Y, Gao H, et al. Poor ovarian response to gonadotropin stimulation is associated with low expression of follicle-stimulating hormone receptor in granulosa cells. Fertility and Sterility. 2007;87(6):1350–1356.

148. Zhang W, Liu Z, Liu M, Li J, Guan Y. Is it necessary to monitor the serum luteinizing hormone (LH) concentration on the human chorionic gonadotropin (HCG) day among young women during

the follicular- phase long protocol ? A retrospective cohort study. Reproductive Biology and Endocrinology. 2022;20(24):1–7.

149. Kaviani M, Ghaderian SMH, Arefi S, Hashemi M, Afjeh SSA. Role of FSHR rs6165 and ESR2 rs4986938 polymorphisms in ovarian stimulation of Iranian women who underwent assisted reproduction treatment. Human Antibodies. 2017;26(3):121–126.

150. Ahmed I, Abdelateef S, Abdel-Lah MAK, Amor H, Hammadeh ME. Association between FSHR and ESR1 gene variants and ovarian response to gonadotropin in Egyptian women undergoing ICSI treatment. Reproductive Biology. 2021;21(2):1–6.

151. Korevaar T, Minguez-Alarcon L, Messerlian C, Poortere RA, Williams PL, Broeren MA, et al. Association of Thyroid Function and Autoimmunity with Ovarian Reserve in Women Seeking Infertility Care. Thyroid. 2018;28(10):1349–1358.

152. Kuroda K, Uchida T, Nagai S, Ozaki R, Yamaguchi T, Sato Y, et al. Elevated serum thyroid-stimulating hormone is associated with decreased anti-Müllerian hormone in infertile women of reproductive age. Journal of Assisted Reproduction and Genetics. 2015;32(2):243–247.

153. Parijatham S, Saikumar P. Serum levels of follicular stimulating hormone, luteinising hormone and prolactin in primary female infertility in rural population. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2014;5(2):1155–1158.

154. Punchoo R, Bhoora S, Bias I. Variation in the Measurement of Anti-Müllerian Hormone – What Are the Laboratory Issues ? Frontiers in Endocrinology. 2021;12:1–9.

155. Knez J, Kovačič B, Medved M, Vlaisavljević V. What is the value of anti-Müllerian hormone in predicting the response to ovarian stimulation with GnRH agonist and antagonist protocols? Reproductive Biology and Endocrinology. 2015;13(58):1–7.

156. Ho LM, Aw TC, Hum SC, Lee SN, Chung SC, Yu SL. Basal serum anti-müllerian hormone and antral follicle count are predictors of ovarian response for Asian women in Singapore. Asian Pacific Journal of Reproduction. 2014;3(3):169–175.

157. Vaze-Parab A, Baldawa P, Bhatt P, Parihar M. Anti-mullerian hormone, follicular phase follicle stimulating hormone and antral

follicular count as predictors of ovarian response in assisted reproductive technique cycles. Minimal Stimulation IVF. 2014;1(1):32–35.

158. Frattarelli JL, Bergh PA, Drews MR. Evaluation of basal estradiol levels in assisted reproductive technology cycles. Fertility and Sterility. 2000;74(3):518–524.

159. Carvalho BR, Sobrinho DB, Vieira ADD, Resende MPS, Barbosa ACP, Silva AA, et al. Ovarian Reserve Assessment for Infertility Investigation. ISRN Obstetrics and Gynecology. 2012;10(54):1–10.

160. Prasad S, Kumar Y, Singhal M, Sharma S. Estradiol level on day 2 and day of trigger: A potential predictor of the IVF-ET success. Journal of Obstetrics and Gynecology. 2014;64(3):202–207.

161. Ersahin AA, Arpaci H, Ersahin SS, Celik N, Acet M. AFC vs. AMH: prediction of ovarian response in women with endometrioma undergoing controlled ovarian stimulation. European Review for Medical and Pharmacological Sciences. 2017;21:2499–2503.

162. Lai Q, Chen C, Zhang Z, Zhang S, Yu Q, Yang P, et al. The significance of antral follicle size prior to stimulation in predicting ovarian response in a multiple dose GnRH antagonist protocol. International Journal of Clinical and Experimental Pathology. 2013;6(2):258–266.

163. Hsu A, Arny M, Knee AB, Bell C, Cook E, Novak AL, et al. Antral follicle count in clinical practice: Analyzing clinical relevance. Fertility and Sterility. 2011;95(2):474–479.

164. Barbakadze L, Kristesashvili J, Khonelidze N, Tsagareishvili G. The correlations of anti-mullerian hormone, follicle-stimulating hormone and antral follicle count in different age groups of infertile women. International Journal of Fertility and Sterility. 2015;8(4):393–398.

165. Huang Q, Niu Y, Xu L, Chen B, Zhang Y, Song LJ, et al. Relationship between a low ratio of serum estradiol to follicle number and fertility treatment outcomes. Medicine. 2018;97(34):1–8.

166. Kondapalli LA, Molinaro TA, Sammel MD, Dokras A. A decrease in serum estradiol levels after human chorionic gonadotrophin administration predicts significantly lower clinical pregnancy and live birth rates in in vitro fertilization cycles. Human Reproduction. 2012;27(9):2690–2697.

References

167. Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle : a review. Human Reproductive Update. 2012;18(1):73–91.

168. Abbara A, Patel A, Hunjan T, Clarke SA, Chia G, Eng PC, et al. FSH Requirements for Follicle Growth During Controlled Ovarian Stimulation. Frontiers in Endocrinology. 2019;10(579):1–11.

169. Andre G, Trevisan C, Pedruzzi I, Fernades R,et al.The impact of Follicle-stimulating hormone receptor gene polymorphisms Ala307Thr and Asn680Ser in endometriosis developments. DNA and Cell Biology. 2018;37(6):584–591.

170. Rod A, Jarzabek K, Wolczynski S, Benhaim A, Reznik Y, Herlicoviez M, et al. ESR1 and FSHR Gene Polymorphisms Influence Ovarian Response to FSH in Poor Responder Women with Normal FSH Levels. Endocrinology and Metabolic Syndrome. 2014;3(3):1–5.

171. Motawi TMK, Rizk SM, Maurice NW, Maged AM, Raslan AN, Sawaf AH. The role of gene polymorphisms and AMH level in prediction of poor ovarian response in Egyptian women undergoing IVF procedure. Journal of Assisted Reproduction Genetics. 2017;34(12):1659–1566.

172. Sindiani AM, Batiha O, Al-zoubi E, Khadrawi S, Alsoukhni G, Alkofahi A, et al. Association of single-nucleotide polymorphisms in the ESR2 and FSHR genes with poor ovarian response in infertile Jordanian women. Clinical and Experimental Reproductive Medicine. 2021;48(1):69–79.

173. Sangeeta Rai A, , Preeti Kumari AS, Singh R. Correlation of follicle-stimulating hormone receptor gene Asn 680 Ser (rs6166) polymorphism with female infertility. Journal of Family Medicine and Primary Care. 2019;8(10):3356–3361.

174. Goetz LH, Schork NJ. Personalized Medicine: Motivation, Challenges and Progress. Fertility and Sterility. 2019;109(6):952–963.

175. Song D, Huang X liang, Hong L, Yu J min, Zhang Z feng, Zhang H qin, et al. Sequence variants in FSHR and CYP19A1 genes and the ovarian response to controlled ovarian stimulation. Fertility and Sterility. 2019;112(4):749-757.

References

176. Badawy A, Wageah A,EL Gharib M, Osman EE. Prediction and diagnosis of poor ovarian response: The dilemma. Journal of Reproduction and Infertility. 2011;12(4):241–248.

177. Papanikolaou IG, Giannelou P, Anagnostou E, Mavrogianni D. Combined study on the single nucleotide polymorphisms in the follicle-stimulating hormone receptor (Asn680Ser) and anti-Müllerian hormone receptor type II (− 482A > G) as genetic markers in assisted reproduction. Hormone Molecular Biology and Clinical Investigation. 2019;38(1):1–9.

178. Livshyts G, Podlesnaja S. A distribution of two SNPs in exon 10 of the FSHR gene among the women with a diminished ovarian reserve in Ukraine. Journal of Assisted Reproduction and Genetics. 2009;26(1):29–34.

179. Boudjenah R, Molina-gomes D, Torre A, Bergere M, Bailly M, Wainer R, et al. Genetic Polymorphisms Influence the Ovarian Response to rFSH Stimulation in Patients Undergoing In Vitro Fertilization Programs with ICSI. PLoS One. 2012;7(6):1–16.

180. Loutradis D, Patsoula E, Minas V, Koussidis GA, Antsaklis A, Michalas S, et al. FSH receptor gene polymorphisms have a role for different ovarian response to stimulation in patients entering IVF / ICSI-ET programs. Assisted Reproduction and Genetics. 2006;23(4):177–184.

181. Yao Y, Ma C, Tang H, Hu Y. Influence of follicle-stimulating hormone receptor (FSHR) Asn680Ser polymorphism on ovarian function and in-vitro fertilization outcome : A meta-analysis. Molecular Genetics and Metabolism. 2011;103(4):388–393.

182. Jun JK, Yoon JS, Ku SY, Choi YM, Hwang KR, Park SY, et al. Follicle-stimulating hormone receptor gene polymorphism and ovarian responses to controlled ovarian hyperstimulation for IVF-ET. Journal of Human Genetics. 2006;51(8):665–670.

183. Achrekar SK, Modi DN, Desai SK, Mangoli VS, Mangoli R V., Mahale SD. Follicle-stimulating hormone receptor polymorphism (Thr307Ala) is associated with variable ovarian response and ovarian hyperstimulation syndrome in Indian women. Fertility and Sterility. 2009;91(2):432–439.

184. Trevisan CM, Peluso C, Cordts EB, De Oliveira R, Christofolini DM, Barbosa CP, et al. Ala307Thr and Asn680Ser polymorphisms

of FSHR gene in human reproduction outcomes. Cellular Physiology and Biochemistry. 2014;34(5):1527–1535.

185. Tanase A, Nemescu D, Popescu R, Carauleanu A, Mogos R, Luca A, et al. Follicle-stimulating hormone receptor gene polymorphisms of ovarian reserve markers in Romanian population. Experimental and Therapeutic Medicine. 2020;20(6):1–6.

186. Mohiyiddeen L, Newman WG, Mcburney H, Mulugeta B, et al. Follicle-stimulating hormone receptor gene polymorphisms are not associated with ovarian reserve markers. Fertility and Sterility. 2012;97(3):677–681.

187. Agarwal A, Verma A, Agarwal S, Shukla RC, Jain M, Srivastava A. Antral follicle count in normal (fertility - proven) and infertile Indian women. Journal of Radiology and Imaging. 2014;24(3):297–302.

188. Mohiyiddeen L, Nardo LG. Single-nucleotide polymorphisms in the FSH receptor gene and ovarian performance : Future role in IVF. Human Fertility. 2010;13(2):72–78.

189. Polyzos NP, Neves AR, Drakopoulos P, Spits C, Mercadal BA, Garcia S, et al. The effect of polymorphisms in FSHR and FSHB genes on ovarian response : a prospective multicenter multinational study in Europe and Asia. Human Reproduction. 2021;36(6):1711–1721.

190. Behre HM, Greb RR, Mempel A, Sonntag B, Kiesel L, Kaltwaßer P, et al. Significance of a common single nucleotide polymorphism in exon 10 of the follicle-stimulating hormone (FSH) receptor gene for the ovarian response to FSH: A pharmacogenetic approach to controlled ovarian hyperstimulation. Pharmacogenetics and Genomics. 2005;15(7):451–456.

191. Greb RR, Grieshaber K, Gromoll J, Sonntag B, Nieschlag E, Kiesel L, et al. A common single nucleotide polymorphism in exon 10 of the human follicle stimulating hormone receptor is a major determinant of length and hormonal dynamics of the menstrual cycle. Journal of Clinical Endocrinology and Metabolism. 2005;90(8):4866–4872.

192. Borgbo T, Jeppesen J V., Lindgren I, Lundberg Giwercman Y, Hansen LL, Yding Andersen C. Effect of the FSH receptor single nucleotide polymorphisms (FSHR 307/680) on the follicular fluid

hormone profile and the granulosa cell gene expression in human small antral follicles. Molecular Human Reproduction. 2014;21(3):255–261.

193. Sheikhha MH, Eftekhar M, Kalantar SM. Investigating the association between polymorphism of follicle-stimulating hormone receptor gene and ovarian response in controlled ovarian hyperstimulation. Journal of Human Reproductive Sciences. 2011;4(2):86–90.

194. Allegra A, Marino A, Raimondo S, Maiorana A, Gullo S, Scaglione P, et al. The carriers of the A/G-G/G allelic combination of the c.2039 A>G and c.-29 G>A FSH receptor polymorphisms retrieve the highest number of oocytes in IVF/ICSI cycles. Journal of Assisted Reproduction and Genetics. 2017;34(2):263–273.

APPENDIX

Research questionnaire for all participants

Name: mobile:

Age:

Weight: Height:

Age of menarche:

Acne yes/no	
Family history for infertility yes/no	
Family history for other disease yes/no	
Hirsutism yes/no	
Menstrual irregularity yes/no	
Smoking yes/no	
Education primary/secondary/college	
History of previous Conception	

Clinical and hormonal results

FSH		TSH	
LH		Basal estradiol	
AMH		Estradiol (after treatment)	
Prolactin		Size of grafiaan follicles	
AFC		number of grafiaan follicles	

الخلاصة

خلفية البحث: يعد العقم عند النساء حالة متعددة العوامل والتي تشكل مشكلة صحية عامة عالمية . ينتج الهرمون المنبه للجريب (FSH) في الغدة النخامية وهو اساسي للتكاثر و يرتبط هذا الهرمون على وجه التحديد بمستقبلات الغشاء(FSHR) معبرا عنها في الخلايا الجسدية للغدد التناسلية و يتميز نظام FSH / FSHR بالعديد من الخصائص المميزة مقارنة بمستقبلات G الكلاسيكية المقترنة بالبروتين (GPCRs) حيث تعد الأشكال المتعددة في الكودونين 307 و 680 هي المتغيرات الأليلية الأكثر شيوعًا لجين مستقبل الهرمون المنبه للجريب(FSHR).

اهداف الدراسة:

تهدف هذه الدراسة الى الكشف عن تعدد الأشكال الجينية لمستقبل الهرمون المنبه للجريب FSHR rs6166 (C> T) و rs6165 (C> T) خاصة تلك المرتبطة بالاستجابة لعلاج FSH وتأثيراتها في مسببات العقم عند النساء العراقيات.

الاشخاص وطرق العمل:

اختير في هذه الدراسة القائمة على المراقبة مئتان وستون امرأة للمشاركة في هذه الدراسة جميعهم ليس لديهن فارق في العمرو كذلك في مؤشر كتلة الجسم و سن الحيض،حيث أن خمسين من النساء المشاركات يتمتعن بصحة جيدة اما البقية مئتان وعشرة من النساء فقد شخصت مؤخرًا بالعقم حيث تم تقسيمهم إلى ثلاث مجاميع حسب استجابة المبيض للعلاج إلى ضعيف الاستجابة (70 امرأة مصابة بالعقم) معتدل الاستجابة (61 امرأة مصابة بالعقم) وعالي الاستجابة (79 امرأة مصابة بالعقم). وقد اخذت الموافقات المسبقة لجميع المشاركات في الدراسة و سحبت عينات الدم من جميع المجاميع في اليوم الثاني من الدورة الشهرية لأجراء التحاليل الوراثية والهرمونية (الهرمون المنبه للجريب ، الهرمون اللوتيني، الهرمون المضاد للمولريان ، هرمون الغدة الدرقية ، البرولاكتين والاستراديول)،تم فحص جميع النساء المصابات بالعقم اللائي شملتهن الدراسة بواسطة السونار الروتيني لقياس عدد الحويصلات وسحبت عينة الدم مرة أخرى لقياس مستوى الاستراديول بعد ستة أيام من العلاج ، اجري التصوير بالموجات فوق الصوتية أيضًا لقياس حجم وعدد الحويصلات الناضجة. استخدم في هذه الدراسة تفاعل البوليميراز المتسلسل لنظام الطفرة التضخمية(ARMS PCR) للكشف عن طفرات مستقبل الهرمون المنبه للجريب FSHR rs6166 (C> T) و rs6165 (C> T).

النتائج:

اشارت نتائج هذه الدراسة بوضوح عند النساء المصابات بالعقم إلى أن الأنماط الجينية المتعددة لجين الهرمون المنبه للجريب (FSHR) بشكل خاص (rs6166) (C> T) و (rs6165) (C> T)، التي تشمل النمط الجيني البري متماثل (CC)، متماثل الزيجوت متحولة (TT) والنمط الجيني متغاير الزيجوت (CT). حيث تم زيادة أليل T بشكل ملحوظ (P <0.05) في النساء المصابات بالعقم ذوات الاستجابة الضعيفة لكل من (rs6166) و (rs6165) في (FSHR) والتي ترتبط بشكل كبير بضعف الاستجابة لـ FSH في النساء العراقيات المصابات بالعقم.

الاستنتاج:

استنتج من هذه الدراسة بأن التغاير الجيني في مستقبلات الهرمون المنبه للجريب (FSHR) يرتبط بقلة الاستجابة للهرمون المحفز للجريب (FSH) وان هذا التغاير الجيني كان له علاقة بإمراضية العقم عند النساء العراقيات في محافظة كربلاء.

بسمِ اللهِ الرحمنِ الرحيمِ

قَالُواْ سبحَانكَ لَا علمَ لَناَ إِلَّا مَا
عَلَّمتَنَا إِنَكَ أَنتَ العَلِيمُ الحَكِيمُ

صَدقَ اللهُ العلـيُ العظيمُ

سُورةُ البقرة

الآيـة (٣٢)

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الصيدلة

فرع الادوية والسموم

تأثير تعدد الأشكال الجيني لمستقبلات الهرمون المحفز للجريبات في الاستجابة للعلاج بعقار FSH عند النساء العراقيات المصابات بالعقم

رسالة

مقدمة الى مجلس كلية الصيدلة / جامعة كربلاء
كجزء من متطلبات نيل درجة الماجستير في علم الادوية والسموم

من قبل

عبير حسين هليجي عبود

بكالوريوس صيدلة (الجامعة المستنصرية 2007)

بإشراف

الأستاذ المساعد

مازن حامد عودة

الطبيبة الاستشارية

د. حميدة هادي عبد الواحد

2022ميلادي **1444 هجري**