Follicle stimulating hormone receptor: impact of genetic variations and gene expression levels in fertility

Mariagrazia Orlando[2*], Giulia D'Alessandro[1], Sofia De Girolamo[1], Emanuela Giannusa[2*], Flavia Piccini[2], Maurizio Ferrari[2*], Lucia De Santis[1]

[1] Centro Scienze Natalità, Ginecologia e Ostetricia, IRCCS Ospedale San Raffaele, Milano, Italy; [2] UOC Genomica Clinica, Servizio di Medicina di Laboratorio – Citogenetica, IRCCS Ospedale San Raffaele, Milano, Italy; [*] Affiliation at the time of the first draft of the present manuscript

ABSTRACT

To date, Assisted Reproductive Technologies (ARTs) increase the probability of conception starting from the collection of more than a single oocyte produced in a regular ovulatory cycle. To achieve this aim many ovarian stimulation protocols have been proposed, some with very good performances but also with some important side effects and, more important, with not many possibilities to personalize the hormonal treatment according to patients characteristics.

Although the hormonal and clinical parameters remain the only proven factors to aid in the selection of the best possible hormone stimulation for each patient, none of the commonly used markers has an optimal predictive value if considered individually. Therefore, a complementary strategy that is emerging in recent years is pharmacogenetics. The candidate genes to date are follicle hormone (FSH) and its receptor (FSHR), in which single nucleotide polymorphisms (SNPs) are able to modulate the expression and functions of the genes.

The FSH-FSHR complex initiates a cascade of molecular events in the gonads, from the increase of cyclic AMP (cAMP) to the transduction of enzyme-encoding mRNA products, which modulate the synthesis of steroid hormones. In this way, FSH stimulates folliculogenesis and steroidogenesis in the ovary and testicular development and spermatogenesis in the testis. The administration of FSH in the treatment of infertility, in both sexes, aims to induce these activities in order to allow infertile couples to carry out the pregnancy.

Many studies on the genetic polymorphisms of FSH and its receptor identified which of these variants could be considered as a marker able to predict the individual responses of patients undergoing ovarian stimulation.

KEYWORDS

Clinical embryology, FSH, Polymorphism, Ovarian functions.

Introduction

Physiological and molecular aspects of FSH

The stimulating FSH is a glycoprotein with a fundamental role in the development of the gonads and sexual maturation during puberty in male and female.

In particular, FSH in women induces the maturation of ovarian follicles, which are egg cells surrounded by follicular cells. During the fertile period of the woman, about every 28 days, an ovarian follicle is brought to maturity. While the follicle grows releases the Inhibin that, trough a negative feedback, stops the release of FSH into the adenohypophysis.

In males, FSH activate the production of androgen binding protein (ABP) in Sertoli cells, in seminiferous tubules, and it is essential for spermatogenesis.

FSH is a heterodimer composed of two glycoproteins. Its structure is similar to LH, TSH and hCG, all hormones produced by the pituitary gland. The two polypeptide units that make up the FSH dimer are called alpha and beta subunits. The alpha subunit is the same for all LH, TSH and hCG hormones, and contains 96 amino acids. The beta subunit, on the other hand, is specific for each hormone and in the case of FSH it contains 118 amino acids and is called FSHB. The activity of FSH is mediated by the binding of FSHB with its receptor (FSHR) located in the plasmalemma of Sertoli cells in the testes and in the cells of Granulosa in the ovaries. The FSHR protein is produced following the transcription of the FSHR gene mapped on the chromosome 2p16.3

The National Centre of Biotechnology Information database reports 731 point mutations at the FSHR gene level [(1)]. Since the research for these mutations occurs by studying patients with alterations in the reproductive function, mutations

Article history

Received 4 Jul 2023 – Accepted 6 Feb 2024

Contact

D'Alessandro Giulia; dalessandro.giulia@hsr.it

Master's Degree, Centro Scienze Natalità, Ginecologia e Ostericia, IRCCS Ospedale San Raffaele, Tel.: +39 02 2643 4308

are classified as activating, inactivating or neutral, depending on the function of FSHR [2]. Point mutations in the encoding DNA of FSHR gene induce amino acid changes that modulate the receptor signalling expression and pathway with important clinical consequences.

In addition, allelic variants or SNPs have been discovered giving rise to different haplotypes that modify the effect of FSH in tissues.

Polymorphisms

Polymorphism means the presence of two or more different gene forms in the same locus with a frequency within the population of at least 1%. The polymorphisms may be due to mutations, insertion or deletion of more or less long stretches of the DNA sequence, which may induce modifications of the amino acid produced by a single codon. The frequencies of genetic polymorphisms within a population are determined by evolution and by the process of natural selection. However, these are allelic variants that can be transmitted to offspring, therefore inheritable. In fact, some analyses of these variants take advantage of the chromosome property called Linkage disequilibrium (LD) which is based on the observation that two neighbouring variants tend to be segregated together more frequently than two distant variants whose probability of recombination is greater. Allelic variation involving a single nucleotide is called SNPs (Single Nucleotide Polymorphisms).

SNPs can occur within a coding sequence of a gene, within an intronic region or an intergenic region. In any case, SNPs within a gene do not necessarily modify the encoded amino acid sequence, since the genetic code is degenerated. The presence of these variants is fundamental to define haplotypes of a population, particular combinations of alleles determined by the specific combination of SNPs in a given genomic sequence.

The studies carried out on the gene sequence coding for the FSHR have detected eight polymorphic sites at the level of the coding regions but six of these are asymptomatic while the remaining two are relevant in the variation of the receptor responses to the stimulation with FSH. One of these polymorphisms is found at the level of the gene coding for the amino acid residue at position 307 occupied by an Ala or Thr, important for interaction with the ligand. The second one is located in the coding portion for the amino acid residue 680 occupied by an Asn or a Ser, important in signal transduction [3].

Analysis strategies for the identification of polymorphisms

The study of polymorphisms allows to make predictive investigations on the development of a disease or to provide valuable information on how the disease could develop. Indeed, the polymorphisms that are located near a disease gene can be used to find the disease gene, so they are therefore very frequently used in association study maps. The most common association studies are the ones cases-control, in which the comparison between the allele frequencies of genetic polymorphisms within the genome of subjects with pathology and healthy control subjects. Many polymorphisms are specific to each population; therefore, in 2002 the "International HapMap project" was created to guarantee to genetic research a database of useful information to lead large-scale genome-wide association studies. In fact, the decrease in costs and the increase in the density of polymorphisms in the genotyping panels makes it possible to analyse the whole genome for the study of complex pathologies, substituting this approach to the used analysis of a candidate gene. Another useful tool for association studies is the National Centre for Biotechnology Information (NCBI) SNPs database (http://www.ncbi.nlm.nih.gov) where SNPs are registered with a specific nomenclature [4].

The techniques used to study the receptor for FSH and its gene are different depending on whether the gene sequence and its variants are investigated, or the structure and function of the receptor protein encoded by these variants. Several research groups have developed techniques based on "Polymerase Chain Reaction" (PCR), which allows amplifying the whole FSHR gene or parts of it. Numerous studies refer to the methods originally used by Gromoll, author of the primers and protocols for 16 PCR procedures that amplify the whole FSHR gene [5].

Most of the primers used in these studies have exon 10 as a target sequence because it encodes as many as 410 of the 695 amino acids that make-up the FSHR. The identification of some SNPs within the exons of the gene has allowed the development of different Restriction Fragment Length Polymorphisms (RFLP) methods that through a simple enzymatic digestion followed by gel electrophoretic run make possible to distinguish individuals homozygous from the heterozygous for each point mutation of the FSHR gene that is intended to be analyse. Another technique is Real-Time PCR (RT-PCR) that allows amplifying and quantifying DNA simultaneously [6].

Although PCR is the first approach to study the gene for the FSHR, others techniques are able to achieve this goal. After amplification, DNA can be sequenced or used for some analyses in order to identify the nature of the point mutations. The analysis of possible genetic mutations involves the use of different techniques; one of these is the Denaturing High Performance Liquid Chromatography (DHPLC), a very sensitive and accurate technique based on separation of DNA homoduplex from DNA heteroduplex for the detection of mutations and SNPs. Another DNA screening system is the analysis using Single Strad Conformational Polymorphism (SSCP) which consists in the heat denaturation of the DNA fragments followed by a rapid process of cold renaturation to maintain the structural conformation given by the coupling between the bases. This conformation could be altered by an exchange of bases that changes the electrophoretic run of the fragments.

When the mutations have been identified, the region of mutated DNA can be transferred through the process of DNA transfection into host cells, in order to study the differences in expression and binding with FSH between the "wild-type" receptor and the receptor encoded by the mutated gene sequence. Over the years, many cell types have been used as acceptors of these transfection procedures, such as COS cells, 293-T and MSC-1. Once a transfected cell line that expresses the mutated receptor in a stable manner has been obtained, it is possible to proceed with studies of kinetics of the binding with FSH or it can be studied how the mutations result in a variation of the cellular response in terms of synthesis of cAMP or other second messengers [7].

Follicle stimulating hormone receptor

Molecular structure of FSHR

The FSHR belongs to the family of G Protein Coupled Receptors (GPCR) and consists in a mature protein of 678 amino acids with a molecular weight of 75 kDa. FSHR consists of three domains: an intracellular domain, a 7 α-helical transmembrane domain, and an extracellular domain.

The FSHR portion involved in the interactions with FSH is located towards the extreme N-terminal of the extracellular region. The extracellular domain is composed by 349 amino acids and consists of 10 repetitions of about 24 amino acids with the characteristic LLR motif (leucine-rich-repeats) that allows the interaction both with the transmembrane domain and the hormone. There are several cysteine residues; eight are perfectly preserved even in the luteinizing hormone and thyroid hormone receptors, suggesting their fundamental role in preserving the integrity of the receptor conformation.

In addition, the extracellular domain has three potential glycosylation sites at the level of the 191, 199 and 293 residues important for receptor expression in the plasmalemma and for the binding of FSH. The specificity of binding with FSH is given by the residues of Phe165 and His274 with FS22's Val221.

The transmembrane domain has the typical characteristic structure of the GPCR, made up of 246 amino acids grouped in 7 hydrophobic α-helices alternated by extracellular and intracellular loops. The two residues of Cys, 442 and 517, at the level of the first and second loops, form a sulfide bridge important for the structural stability of the receptor. Following the binding of the receptor with FSH, the signalling pattern is activated by intracellular domain at the C-terminal region of the protein. The intracellular domain consists of 65 amino acids and is rich in Thr and Ser residues that are phosphorylated by intracellular kinases to activate signal transduction.

FSHR isoforms: potential roles in healthy tissues

There are different transcripts of the FSHR gene that appear to be generated by the presence of several polyadenylation sites and by alternative splicing phenomena. The FSHR isoforms that have been identified are:

1. FSHR1 is the most common receptor isoform and has a typical GPCR structure. It is a receptor expressed only in ovary granulosa cells and it is involved in follicle development and granulosa cell differentiation.
2. FSHR2 is a variant of the receptor that lacks the intracellular domain, for this reason it is able to bind the substrate (FSH) but not to activate the G protein. This isoform is also called "dominant negative" because it is thought that two pairs of FSHR2 go to activate the inhibitory G protein instead of the stimulatory G protein.
3. FSHR3 is a receptor isoform that has only one transmembrane domain; it works as type I growth factor receptor because it activates the signalling pathway of MAP kinases and the calcium dependent channels in epithelial cells of the ovarian tissue.
4. FSHR4 is a soluble isoform of the receptor that lacks the transmembrane domain, but the function of this receptor is still unknown.

Signal transduction pathways

The stimulating follicle hormone receptor is synthesized in granulosa cells, glycosylated and then transported and evenly distributed on the plasma membrane of cells. FSHR is activated after a conformational change of its molecular structure; although receptor dimerization is not always necessary, it can stabilize the binding with FSH and ease signal transduction.

Following the binding of the hormone, the receptor interacts with the protein G that binds the cofactor guanosine triphosphate (GTP) and stimulates the enzyme adenylate cyclase to synthesize the second messenger: the cAMP. The cAMP, in turn, activates the protein kinase A (PKA) that recruits the regulatory-extracellular kinase 1/2 (ERK1/2) and phosphorylates structural proteins, enzymes and transcriptional modulators belonging to the family of the cAMP responsive elements (CRE). CRE binding proteins (CREB) are transcription factors that mediate the expression of genes that encode for enzymes that modulate steroidogenesis and signal activation/deactivation, such as steroid acute regulatory protein (StAR) and aromatases that induce the synthesis of steroids and P53 and the cyclins that block their synthesis. Depending on the endocrine status and the stage of maturation of the gametes, different isoforms of the modulators and CRE proteins are preferentially expressed.

Instead, ERK1/2 is involved in the regulation of steroid signals, in receptor desensitization and proliferative events.

In addition to the signalling pathways activated by cAMP/PKA and ERK, there are other intracellular events, such as the increase of intracellular calcium, the activation of protein kinase B (AKT) or Epidermal Growth Factor Receptor (EGFR) and the activation of the mTOR pathway who allows a balance of pro- and anti-apoptotic signals.

The signal is turned off by a negative feedback c-AMP dependent that causes a down regulation of the receptor expression. In particular, after about 1-4 hours from FSH stimulation, the receptor-hormone complex is sequestrated in the lysosomes.

Molecular biology of the FSHR gene

The human FSHR gene was mapped on chromosome 2p16.3 [MIM *136435].The gene occupies a very large region of about 200kb and includes 10 exons and 9 introns. The extracellular domain of the protein is encoded by the first 9 exons of the gene, while the C-terminal part of the extracellular domain, of the intracellular and transmembrane domain are coded by the exon 10.

Low doses of FSH activate signalling pathways that induce FSHR mRNA synthesis, whereas high doses of FSH down-regulate mRNA levels and consequently decrease the number of binding sites for FSH on the receptor.

Studies performed on adult rats during the phases of the estrous cycle have shown that the FSHR concentration increases linearly respect to the follicle maturation, which reaches the maximum peak in the ovulatory phase, and decreases drastically in the luteinizing phase. In humans, studies on perimenopausal patients with irregular cycle have identified a correlation between an increase of serum FSH and a reduction of FSHR mRNA levels associated with follicular atrophy [8].

In the regulation of FSHR expression are also involved some paracrine factors: activin and Transforming growth factor b (TGF-b) which are potent inducers of gene transcription, and Epidermal growth factor (EGF), insulin-like growth factor (IGF) and Fibroblast growth factor (FGF) which are factors that regulate negatively the transcription to lower the response induced by FSH.

Allelic variants of the follicle stimulating hormone receptor

SNPs in the FSHR promoter

The promoter of FSHR belongs to that class of promoters that lack the conventional TATA box but it has numerous sites of transcription initiation. The promoter core is located respect to the start codon between position 225 and position 1 and presents two responsive elements: the consensus sequence E-box (CACATG) that binds a group of transcription factors called "basic helix-loop-helix" like upstream stimulatory factor (USF) and the Initiator element (Inr), typical region of housekeeping genes.

Comparative studies on different species have shown the presence of a G/A variant in position -29 of the FSHR gene promoter where is present GG / AAA consensus sequence, the binding site for the cE factor- twenty-six specific (c-ETS). The presence of a homozygous AA genotype at position -29 is associated with an increase of serum FSH and consequently a decrease in the receptor response. Another highly polymorphic region at the promoter level results to be the A-stretch oligo (A11-17), but in this case there are no significant changes in receptor functions.

Frequency and ethnic distribution of FSHR polymorphisms

Studies on the frequency distribution of FSHR polymorphisms show contrasting results. This suggests that the ethnic background of the subjects examined can strongly influence these results.

The FSHR gene presents more than 900 SNPs, organized in different blocks in linkage disequilibrium according to ethnic groups.

Considering the distribution of the SNP rs6166 polymorphism (p.N680S), according to the data obtained from the Hap Map Database (http://hapmap.ncbi.nlm.nih.gov), the ancestral allele A is predominant in the South-East Asia, while the G allele is prevalent in other populations, particularly in Kalash (North-West Pakistan), Yakuts (Siberia), Suruì Paiter (Brazil) and Melanesia (Oceania) [9].

It is interesting to note that these ethnic groups are geographically isolated and genetically distinct, and this certainly makes us think about events of genetic drift positive selection. While for other populations, such as Europe, Central Asia and Oceania, they show a higher frequency of the G allele, in North America instead the frequency is slightly lower. In particular in the Asian population (including both Japanese and Chinese) the Ser680Ser receptor variant is much less common, with a frequency of 10.5% compared to the Caucasian (21.5%) and Mediterranean (22.3%) population [10].

Data related to rs6165 polymorphism (p.A307T) [6,7] show that the ancestral allele G is predominant in sub-Saharan Africa with a MAF of 0.274, while in other populations MAF is similar to rs6166 polymorphism. This suggests that the two SNPs with the exception of the African population are in Linkage Disequilibrium.

The third SNP rs1394205 (c.-29G> A) present on the FSHR promoter does not appear to be in linkage disequilibrium with the two SNPs present on the exon 10. Comparative alignment analyses show that the allele G is the ancestral allele. The homozygous AA genotype, associated with an increase in serum FSH, is more frequent in the United States of America and East Asia than in the European and African populations.

The most studied polymorphism is the Ser680Asn variant because it has been associated with evident effects in the response to ovarian stimulation provided by the IVF protocols [11].

The results obtained from different metanalytical studies [12] suggest that when studying and evaluating the effects of these polymorphisms, it is essential to consider also the ethnic origin and environmental factors that can change from one geographical area to another.

Clinical consequences in reproductive functions

SNP effects in exon 10 of the FSH receptor in normal ovarian functions

The secretion of gonadotropins from the pituitary gland determines the progression of the ovarian cycle and the activity of the gland is controlled by a feedback mechanism from the ovarian hormones. The highest peak in serum levels of stimulating follicle hormone occurs when a group of follicles are recruited at the antral stage, and then allowed to mature. Generally, only follicles with low FSH levels can grow and become Graafian follicle following stimulation with LH.

The biological activity of the hormone depends on the expression of FSHR in the granulosa cells, for this reason numerous studies have focused on the influence of these receptors both in the dynamics of the normal menstrual cycle and in the pathologies linked to the ovarian functions.

Given its functions, FSH is used extensively in ovarian stimulation protocols, both as urinary FSH and as recombinant FSH. The main biomarkers used in clinical practice to predict a normal, low or high ovarian response to the stimulation treatment are: the woman's age, the Anti-Müllerian hormone (AMH), the Antral Follicle Counts (AFC) and the levels of Basal FSH (assessed on the third day of the cycle). AFC and AMH are considered the main predictors of ovarian response with gonadotropins. However, these biomarkers alone do not allow an optimization of IVF therapy for all patients, given that some patients may be over-responsive to a low dose of FSH and hypo-responsive in cases of stimulation with a high dose of gonadotropins [13]. It has been demonstrated that the sensitivity of follicles to exogenous FSH, measured through Follicular Output RaTe (FORT), may be very different between patients with different clinical features [14].

This indicates that genetic factors play a fundamental role

in the ovarian response to gonadotropins. Different studies have analysed the correlation between FSHR polymorphisms, ovarian reserve markers and the outcome of the ovarian response to gonadotropins during in Vitro Fertilization (IVF) or Intracytoplasmic Sperm Injection (ICSI) cycles. Most of these studies focus on the c.2039G>A (p.Ser680Asn) variant [NM_000145.3], as the presence of a G/G (Ser/Ser) genotype appears to be associated with higher basal FSH levels and a reduction in exogenous FSH sensitivity. This was demonstrated by an increase in the total dose of gonadotropins used during IVF ovarian stimulation treatment, by a decrease in estradiol peak following hCG administration and by a decrease in oocyte numbers and a high incidence of G/G genotype in hypo-responsive patients.

This effect was first observed in a non-randomized study and subsequently in a randomized trial involving women undergoing assisted human reproduction. In the non-randomized study [15] it was observed that the amount of FSH required to induce controlled ovarian hyper-stimulation (COH) and obtain a similar peak of estradiol is low for women homozygous for the Asn680 variant compared to women homozygous for the Ser680 variant. This data were corroborated with a randomized trial in women undergoing COH who received the same dose of FSH (16), showing that FSH is less efficient in women with the p.N680S variant at the receptor level. However, an increase in FSH dose from 150 to 225 U / day exceeds the low estradiol response in women with Ser / Ser (group II, 7804 ± 983 pmol / l). Even considering the different levels of estradiol, there were not significant differences in the number of follicles or oocytes, variations in fertilization rate and pregnancy. This indicates that some women may receive an excessive amount of FSH, which increases the risk of developing ovarian hyperstimulation syndrome (OHSS), which indirectly depends on excessive stimulation with FSH.

The production of estradiol, an important hormone for the maintenance of normal ovarian functions, depends on the availability of androgen substrates that are LH-dependent. FSH stimulates the expression of LHR. The presence of p.N680S polymorphism in the FSHR induces a low expression of the FSHR and consequently a low expression of LH receptors and/or the expression of aromatases. The modulation of this pathway, given the presence of allelic changes in the FSHR would explain the difference in estradiol levels depending on whether it is a homozygous or heterozygous Asn680 or Ser680 genotype.

This concept has been confirmed by studies performed on women with mono-ovulatory cycles going to monitor a normal menstrual cycle.

SNP effects in exon 10 of the FSHR in ovarian functions under pathological conditions

A change in the reference values of FSH may indicate some pathologies. In non-pathological conditions FSH in males has a concentration of 1.0-17 mIU/mL, while in women it can vary according to the phase of the cell cycle and obviously to the aging: in the follicular phase the concentration of FSH is equal to 1.4-18 mUI/mL, in the ovulatory phase is equal to 9.4-42 mIU/mL, in the lutein phase 1.7-10 mIU/mL and in the menopause 37.0-167 mIU/mL [13].

Generally, in women with pathological conditions, high FSH levels indicate primary ovarian failure and low FSH levels indicate secondary ovarian failure due to a hypophyseal or hypothalamic problem. Furthermore, low blood levels of FSH have been associated with an increased risk of ovarian cancer. In man under pathological conditions an increase in FSH levels may be due to primary testicular failure. Low levels instead are consistent with hypophyseal and hypothalamic disorders. A change in FSH levels may in turn be linked to a change in the receptor conformation. For this reason, several studies have been performed on the effects of the main polymorphic variants of the FSHR, such as Asn680Ser and Ala307Thr, in the various pathologies linked to ovarian functions. Among the pathologies considered there are: the OHSS, which may occur following a stimulation of follicular growth and dehiscence and the Polycystic Ovary Syndrome (PCOS) and the Premature Ovarian Failure (POF), which are characterized by hormonal imbalances in reproductive age. Studies have also been carried out on the possible association of ovarian cancer with the presence of FSHR polymorphisms, demonstrated by the fact that in ovarian carcinoma tissues was observed an increase in FSHR expression, compared to healthy tissues.

Ovarian Hyperstimulation Syndrome (OHSS)

OHSS is a disorder linked to ovarian stimulation during the treatment of medically assisted reproduction (MAR). The severe form of OHSS occurs in 0.1-5% of the stimulation cycles and its clinical consequences are: a volumetric increase in the ovaries with the formation of multiple cysts and an increase in the permeability of the capillaries at the ovarian level which in turn increases the transfer of fluids, from the intravascular to the extravascular compartment. This results in a state of concentration of the solid components of the blood which on the one hand exposes the patient to a high risk of thrombosis and emboli and on the other causes a reduction in blood flow in organs with risk of ischemia. Several vascular and inflammatory molecules contribute to the increase in vascular permeability, including interleukin (IL)-6, interleukin (IL)-8, tumour necrosis factor (TNF) and angiotensin II [7].

The development of OHSS is mainly associated with the administration of hCG for ovulation induction, because it stimulates the production of VEGF which, in turn, stimulates the formation of new blood vessels and increases vascular permeability. Based on the development of the symptomatology, we can distinguish two types of OHSS: "early" OHSS, with onset in the middle-luteal phase, deriving from the administration of exogenous hCG to stimulate ovulation and "late" OHSS, for the production of endogenous hCG in the initial phase of any pregnancy.

Although the causes of OHSS are not yet fully understood, to date are considered as risk factors: young age at conception, low body weight and lower body mass index (BMI), polycystic ovary syndrome, an increase of estradiol levels and all the stimulations that can compete to vascular damage.

However, there are no absolute predictors to prevent the syndrome which is currently the most serious adverse event in ART.

Recently, three different allelic variants on FSHR associ-

ated with patients presenting recurrent forms of OHSS have been identified. In particular, it has been observed that mutated forms of the FSHR are hyperstimulated at high doses of hCG, whereas instead during pregnancy FSHR levels are either absent or very low. This has prompted several researchers to investigate the possible correlation between FSHR polymorphisms and iatrogenic OHSS.

A recent study has shown that the Ser680 variant occurs very often in women who develop OHSS, but it is the Asn680 variant that is associated with a more severe form of OHSS [17]. In another study [16], also the Ala307 polymorphism was associated with a higher risk in iatrogenic OHSS due to a higher sensitization of the FSHR [18]. These results indicate that subjects homozygous for the Alaine at position 307 (NM_000145.3 (FSHR):c.919G>A (p.Ala307Thr) could make subjects susceptible to the development of OHSS.

Polycystic Ovary Syndrome (PCOS) and Premature Ovarian Failure (POF)

The duration of the fertile age is another parameter that can be considered as a plausible effect of polymorphisms. Among the diseases that may be related to the presence of the most common allelic variants of the FSHR are PCOS and POF.

PCOS is a disorder that affects about 10% of women during reproductive age. It is a syndrome associated with metabolic and endocrinological abnormalities such as hypergonadism, chronic inovulation, abnormal menstrual cycle and polycystic ovaries. Despite numerous studies on the probable genetic causes related to this syndrome, the key factors involved in PCOS are not yet fully understood. The study on the allelic frequency of the Thr307Ala and Asn680Ser variants reveals that the haplotype Ala307/Asn680 is significantly bound with PCOS and could be a risk factor for the disease. A study of 193 PCOS patients shows that women homozygous for the Ser680 allelic variant have a double chance of developing resistance to clomiphene citrate (CC) compared to women carriers of the Asn680 variant [19].

CC is the first choice treatment for patients with PCOS and is a competitive antagonist of 17 ß-estradiol at the receptor complex. By blocking the estrogen receptor, FSH and LH can be released allowing follicle growth and ovulation. In about 80% of patients treated with CC ovulation is recovered, while 20% of patients are resistant to the maximum dose of 150 mg of CC. The exact causes of clomiphene resistance cited are still unknown. Among the possible causes, a probable role of polymorphic variants at FSHR level has been hypothesised [20]. In fact, the presence of SNPs could explain the inability of FSHR to respond to the hormone during ovulation. However, larger-scale studies are needed to further deepen and clarify the correlation between FSHR allelic variants and women with PCOS.

Premature Ovarian Insufficiency (POI)

Another very frequent pathology is premature ovarian failure (POF), recently named as POI, a syndrome that is expressed with ovarian failure and oocyte depletion before the age of physiological menopause. This syndrome is therefore characterized by primary or secondary amenorrhea with anovulation lasting at least 6 months, elevated serum gonadotropins (FSH> 40 IU/L in at least two doses) and hypoestrogenism in women aged less than 40 years. About 1% of women under 40 and 0.1% of women under 30 are affected by this disease. The causes of POF can be very variable and may be also sought in familial genetics, autoimmune diseases or X-chromosome abnormalities. A recent study shows that there is a clear correlation between POF and the FSHR. In particular, knockout mice for the FSHR receptor have been developed which show the classic POF phenotype with an increase in serum FSH levels and a decrease in estrogen levels. However, after the intra-ovarian injection of an adenovirus expressing a copy of human FSHR, mice are able to induce folliculogenesis, recorded with an increase in the number of follicles and serum estrogen levels and a decrease in Serum FSH [21]. However, a meta-analysis study of 157 cases and 633 controls reveals that the two most important FSHR polymorphisms are not associated with POF in humans, but probably only at a premature appearance of the clinical phenotype of the disease. Ultimately, it appears that the presence of Ala307Thr polymorphism is associated with an early onset of the disease, but longitudinal studies are needed to confirm these findings [22].

Ovarian endometriosis

Endometriosis is a chronic estrogen-dependent inflammatory disease that affects 10-20% of women of childbearing age. It rising up in women between 25 and 35 years and is practically absent in the prepuberal and post-menopausal age. It is so called because it is characterized by the presence of endometrial tissue (the tissue normally found only inside the uterine cavity) in ectopic site that is different from the physiological one. The mechanism that leads to the formation of endometrial tissue is not yet completely clear, one of the hypotheses credited is the passage, caused by uterine contractions that occur during menstruation, of endometrial fragments from the uterus in the tubes and from these in the abdomen, with implant on the peritoneum and on the surface of the pelvic organs. More than 50% of women with endometriosis show changes in fertility. Infertility can be associated to several aetiologies, such as: mechanical factors given by the subversion of the pelvic organs and the formation of adhesions with consequent alteration of the relations between the tubes and the ovaries; or from ovulatory factors in which endometriomas could counteract the normal ovulatory mechanisms that for example could negatively impact with the possibility of implantation of a pregnancy. In recent years, several studies in different ethnic groups have evaluated a possible correlation between the presence of SNPs and the risk of developing endometriosis. These hypotheses emerge from recent discoveries involving allelic variants of FSHR in serum FSH levels and in response to ovarian stimulation. In particular, the 680Asn/Asn variant induces an increase in the aromatase activity compared to the 680Ser/Ser variant, leading to a higher production of estrogens and to the stimulation of the proliferation of the endometric tissue [23]. Through the pharmacological suppression of estradiol it is possible to reduce the symptoms of endometriosis. This suggests that suppression of the functions of FSH in combination with the inhibition of estrogen production could lead to a new therapeutic

strategy for endometriosis. For the first time, a study published in 2018 (24), demonstrates a possible correlation between the presence of the haplotype GG/307Ala580Ser and an increased risk of developing endometriosis. The study was performed on 352 women with endometriosis and 510 fertile women used as controls, all of Brazilian origin. From the analysis it results a non-significant difference in the polymorphisms Ala307Thr and Asn680Ser between the two groups. However, when the group of women suffering from endometriosis was divided according to fertility status and disease stage, a positive association between 680Ser/Ser or GG genotype and fertile women with endometriosis was observed (P = 0.004).

Ovarian cancer

Ovarian cancer is one of the most common cancers in the female population. The risk of developing ovarian cancer increases after 35 years and decreases after 59 years. The causes of ovarian cancer are mainly sporadic, but they can also be genetic as in the case of hereditary high penetrance mutations of the BRCA1 and BRCA2 genes. In recent years the hypothesis has been evaluated that the stimulating effects of gonadotropins and female steroid hormones involved in cell proliferation may play a fundamental role in the development of ovarian cancer. This hypothesis was supported by *in vitro* experiments demonstrating that stimulation with FSH leads to growth of the epithelial surface of the ovarian surface and an over-expression of FSHR in Chinese hamster ovary (CHO) [25]. In fact, numerous immunohistochemistry studies [26] confirm that there is an increase in FSHR expression not only in ovarian cancer tissue but also in vascular endothelium cells of different cancer tissues. The lack of FSHR expression in many non-cancerous cells indicates that the receptor could play a key role in both diagnostic and therapeutic terms. It appears that FSHR promotes the link between the Vascular Endothelial Growth Factor (VEGF) and its VEGFR-2 receptor, inducing the process of tumour neoangiogenesis [27]. Furthermore, activation of the FSH/FSHR pathway induces Gq/11 protein activation in endothelial cells, which in turn induces VEGFR-2 activation even in the absence of VEGF [28]. Considering all these mechanisms, one might suspect to block the FSHR pathway and acting against cancer. In fact, some researchers have designed a molecule able to selectively block the FSHR [29]. *In vitro* results suggest that it is possible to reduce the growth of FSH-responsive cancers through a small inhibitory RNA (siRNA), which could increase the effect of chemotherapeutics in the treatment of cancer, but many studies are still needed to validate these findings. The SNPs present on the FSHR gene were also investigated, in particular a study carried out on subjects of the Chinese population of Hong Kong examined the possible association between the variant Ala307Thr and Asn680Ser and the different subtypes of ovarian cancer [30]. This association study is based on genotyping techniques using DNA restriction enzymes extracted from 202 patients with ovarian carcinoma of different types and 266 controls. The study shows that there is an association between the Ala307/Ser680 haplotype and the susceptibility to ovarian cancer, especially in two cancer subtypes: serous and mucinous (P <0.0005, OR = 2.60, 95% CI = 1, 56 - 4,34; and P <0,0005, OR = 2.89, 95% CI = 1.73 - 4.84). However, the results obtained in Asian patients do not coincide perfectly with the association studies in Caucasian patients, suggesting that the cancer risk may vary depending on the ethnicity of the patient's origin.

SNP effects in FSHR in testicular functions

The stimulating follicle hormone induces the proliferation of Sertoli cells, the mitotic activity of spermatogonia and supports cell differentiation and mitosis up to the spermatid stage. Male infertility affects 7% (https://www.iss.it/) of the population and compared to the past today in 1 case out of 2 the difficulty in obtaining a pregnancy depends on male reproductive problems such as insufficient sperm or the qualitatively altered nature of sperm (for reduced motility, altered morphology, damaged DNA) that hinder conception. In about 80% of cases of male infertility the cause of infertility cannot be identified and therefore a specific therapy cannot be used. In case of hormonal alterations, such as hypogonadotropic hypogonadism (a rare inherited condition characterized by deficiency of some hormones) it is possible to intervene with a medical therapy based on hormones. In the most severe situations are used MAR. To date, an increasing number of scientific evidences supports the efficacy of FSH therapy [31, 32]. The treatment with FSH allows to increase the count and sperm motility in infertile subjects with idiopathic oligozoospermia [33]. FSH levels above the normal range are universally considered to be predictive of a lack of therapeutic response to treatment with FSH, which should therefore not be administered in these cases. The numerous studies in favour of FSH therapy have led scientists to investigate the possible effects that FSHR polymorphisms could have in the hormonal treatment of patients with idiopathic infertility. Contrary to what is observed in women with normal ovarian functioning, the SNPs of FSHR in exon 10 in men have no effect on the serum FSH levels and on other clinical parameters, generating a discrepancy between the two sex genders that remains inexplicable. Various polymorphisms have been investigated in man including the variant at the G-29A promoter level, but the results among them have been very contradictory, suggesting that the correlation between testicular dysfunctions and polymorphisms are evident only in association with particular genetic backgrounds or environmental factors.

The mechanism by which the FSHR haplotype explicates its effect remains to be determined. One of the possible explanations could be that the transcriptional activity of the FSHR promoter is influenced by the SNP present in the promoter's core in the -29 position and in the same way the FSH becomes less efficient in the presence of the Ala307/Ser680 variants and more efficient in presence of the Thr307/Asn680 receptor isoform. Although it has been reported that the polymorphisms of the FSH and FSHR gene may influence the response to therapy, there are no definitive data on the use of a pharmacogenetic approach to identify therapy responders a priori.

Potential therapeutic implications of FSHR

FSHR as a predictive marker of infertility

IVF is a complex process that involves the collection of oocytes after controlled ovarian hyperstimulation, oocyte fertilization,

embryo development and the transfer of embryos produced in utero. All these steps are fundamental to the success of the IVF. However, one of the most critical phases of this complex procedure is precisely the COH, whose objective is to obtain, without risks, a large number of mature oocytes and to allow the selection of embryos suitable for transfer or cryopreservation [34]. The efficacy and safety of IVF treatments depend substantially on the ovarian response to exogenous FSH. A response to the controlled ovarian stimulation of a normal-responder patient should allow not only adequate ovarian recovery based on the previously assessed ovarian reserve, but also an adequate number of embryos. However, the ovarian response to stimulation with gonadotropins is very variable, a low or high response may occur depending on the patients treated. This heterogeneity may depend on several factors such as patient age, ovarian reserve or endocrine status. Furthermore, the standard dose of gonadotropins may not be suitable for all patients, so using the right dose of gonadotropin could play a very important role in determining the success of COH and then IVF. Based on these considerations, women are generally classified into three groups: high-responders, poor-responders, normal-responders.

Women who are not responsive to stimulation could lead to maturing only a few follicles such that many times they do not respond adequately to treatment. In contrast, women with an ovarian response too high may incur the risk of developing ovarian hyperstimulation syndrome. Optimizing protocols based on individual patient needs would minimize the risk of OHSS and maximize the likelihood of completing IVF programs.

For this reason, the research is turning an increasing interest to the identification of new methods for assessing the state of ovarian reserve of the woman before the beginning of stimulation, and to identify the pharmacological therapy that can guarantee the best outcome of the treatment. Several serum and ultrasound markers were postulated as predictors of ovarian response [35]. However, these markers seem useful for identifying the appropriate follicle-stimulating hormone dose to be administered but fail to accurately predict the type of response to COH, pregnancy rates or reproductive outcome assisted.

By definition (http://www.fda.gov/cder/genomics/genomic_biomarkers_table.htm), a biomarker is considered valid when it is measured by an analytical test that has well-known yield characteristics and for which there is scientific evidence to demonstrate the physiological, toxicological, pharmacological or clinical significance of the test results. Genetic biomarkers considered "valid" play an important role in the identification of subjects capable or unable to respond to a specific treatment and in the identification of the optimal dose to be administered in order to optimize the efficacy and tolerability of the chosen therapy.

Currently, the AMH level is the most widely used marker, although it has been widely demonstrated that the individual genetic background greatly influences the different responses of women to gonadotrophin stimulation. In fact, it has recently been observed that some genetic variants such as SNPs present in genes encoding LH, estrogen receptors, FSH and the related receptor may affect both the reserve ovarian that the response to COH and, consequently, the IVF outcome.

Although basal serum FSH and/or estradiol concentrations on the third day of the cycle are universally associated with a reduced ovarian reserve and the prospect of low yield in MAR cycles, a significant proportion of hypo-responsive women is not identified by these tests, making the search for new and more sensitive markers crucial. In this regard, FSH remains the most promising factor as responsible for the growth of antral follicles. FSH exists in various isoforms, dissimilar for isoelectric points, for example acidic isoforms appear more secreted during follicular recruitment while the most basic ones appear after the follicle has been selected.

An important role has also FSHR gene that is considered the first candidate to explain changes in ovarian response [36].

Numerous studies have evaluated the role of the genetic polymorphisms of FSHR showing that in homozygous ovulatory patients for the Ser680 variant, treated with a fixed dose of FSH to obtain an ovarian response, they show lower estradiol levels than the homozygous patient Asn680 treated with the same dose of FSH. This indicates that a higher dose of FSH is required to obtain an ovarian response in patients homozygous for the Ser680 variant.

For this reason, FSHR polymorphisms could be good predictors for a hypo-response or hyper-response to FSH treatment during ovarian stimulation and FSHR genotype analysis would allow individual modulation of the administration of FSH and therefore to increase the efficacy and safety of the therapy. The most relevant and significant results on the action of polymorphisms in the conditions of female infertility has been obtained by Manuela Simoni studying the ovarian response of patients undergoing COH [37]. Other scientists have tried to study a possible correlation between allelic variants of the FSHR to other infertility-related disorders, first of all ovarian hyperstimulation, in which it seems that the presence of Ala307Thr variant in homozygous form Ala/Ala induces an increased sensitivity of the receptor which makes the carriers most at risk in the development of OHSS.

A genetic association study on Ala680Ser variant show that this polymorphism, rather than be held as a predictor of risk of incurring OHSS, should be regarded as a marker of the severity of the syndrome. In fact, some studies show that there is a clear and significant correlation between the presence of the variant in homozygous Ser/Ser and the more severe forms of OHSS. Regarding the Polycystic Ovary Syndrome, Endometriosis or ovarian cancer, further studies will be needed to deepen and clarify the role of the genetic polymorphisms of FSHR in these gynaecological syndromes.

While several studies have shown in women the presence of polymorphisms in the FSH-receptor gene that can influence circulating FSH levels and the sensitivity of the hormone receptor, in the male, the meaning of these polymorphisms is still in doubt. The genetic investigation of the variants on the FSHR and FSH genes allows to evaluate a possible treatment of male infertility with the FSH hormone in particular, azoo/oligospermic patients with normal levels of FSH and having at least one serine at position 680 on the gene FSHR (p.Ser680Asn) benefit from the administration of FSH in terms of: number and concentration of spermatozoa, progressive motility and percentage of spermatozoa with normal morphology.

Pharmacogenetics and personalization of MAR therapy

Different subjects respond differently to the same drug; this diversity is linked to the large number of proteins involved in the response to drug therapy.

The variability in the efficacy and tolerability of a drug seems to be, at least in part, the result of the genetic differences of individuals.

Pharmacogenetics aims to study DNA sequence variations that can influence an individual's response to a given drug.

The ultimate goal is to provide the clinician with routine tests to be included in normal clinical practice with the aim of predicting how a patient will respond to a certain therapy both in terms of efficacy and side effects.

The identification, on the basis of a genetic test, of which drug to administer and in what dose would be the solution to a problem both health and socio-economic:

- A safer use of drugs by clinicians, who will be able to choose specific therapies according to the genetic patrimony of the individual patient;
- A research aimed at developing new drugs to be administered to individuals who do not respond or cannot take a specific treatment;
- A more rational use of resources due to the non-use of ineffective drugs and the reduction of hospitalization time due to the side effects linked to the different therapies.

The treatment of infertility is a clear and modern example of personalized medicine, in which the use of the stimulating follicle hormone is widely used for both sexes, although a standardisable approach is still far from being established. The main objective is to obtain the maximum therapeutic result minimizing, at the same time, the risk of potential side effects, including the hyperstimulation syndrome. Given the proven correlation between the amount of FSH necessary to obtain an adequate follicular response, it could be hypothesized to screen the treated patients, so as to provide specific stimulation protocols. In other words, it would be possible to pre-select patients to be treated with higher doses of FSH, with a method that would be more sensitive than the FSH dose in the early follicular phase [38]. By amplification in PCR of the DNA extracted from the peripheral leucocytes, it is in fact relatively simple to identify polymorphisms of single nucleotides.

Finally, we can also think of the possibility of reducing the incidence and severity of ovarian hyperstimulation syndromes in those patients who, on the contrary, would be negative for screening. The studies of future genetic research will bring about the polymorphisms of FSH and its receptor will be able to broaden the already fascinating panorama of the possible clinical implications that currently exist in the endocrinology of reproduction.

To date, have already been made of genetic testing, can be used in the clinic, which allow the detection of numerous allelic variants, including those of the FSHR, to allow patients who undergo MAR starting a program of stimulation with FSH using a dose that guarantees the success of the therapy.

Despite numerous evidences of the usefulness of pharmacogenetic tests to define an increasingly personalized therapy, the introduction of this discipline in the laboratory routine is still in its infancy.

There are many reasons to explain why pharmacogenetics has a limited impact on clinical practice:

- The studies aimed at evaluating the validity of the identified genetic markers are population-specific and often of small size and not enough statistical power.
- The identified biomarkers, although apparently "valid" (showing that relationship cause-effect between the presence of a specific genetic variation and differences in drug response) are not always significant when compared to individuals from different ethnic groups or in larger series wide.

Pharmacogenetics alone is not sufficient to explain all the inter-individual differences in the efficacy and tolerability of the treatments; environmental factors such as sex, age, organ function, etc., contribute significantly to the success of the therapy. From this it follows that the presence of the same genetic markers in individuals affected by the same pathology and treated with the same drug but with different physiological characteristics, differently influences the response that each of these individuals manifests towards the therapy. The use of genetic tests in the field of infertility in couples has primarily diagnostic purposes but may also allow to formulate predictions on reproductive risk. Therefore, the analysis of the genotype of the FSHR gene allows to individually dose the hormone and thus increase the efficacy and safety of the therapy.

To conclude, the pharmacogenetics in reproductive medicine opens a new and promising era in the concept of personalized medicine of treatment. The known knowledge of the genetic heterogeneity of infertile women can contribute significantly to the design of an appropriate ovarian stimulation protocol for individual patients. At present, the protocols are demitted in accordance with a series of parameters, such as age, body mass index, ovarian reserve and the hormonal profile. The aim of pharmacogenetics is to derive further information from a genetic point of view to allow the right amount of drug to be used in women who respond inappropriately to standard ovarian stimulation protocols. For this purpose, in recent years genetic tests have been developed to which women are subjected before an ovarian stimulation in order to guarantee a greater percentage of success of the therapy.

Ideally, pharmacogenetic studies will project us into a new era in which, on the basis of its own DNA sequence, it will be possible to determine the correct therapy.

The aim is to combine these new genetic tests to the assessment of the parameters traditionally considered (age, AFC, AMH, baseline FSH and patient's history) in order to modulate a therapeutic scheme and obtain the ideal ovarian response that should consist of an optimal balance between high number of oocytes recovered and therefore better clinical outcome and low risk of OHSS.

References

1. Heubner M, Riemann K, Otterbach F, et al. The haplotype of two FSHR polymorphisms in ovarian cancer--a potential role of ethnology in risk modification. Gynecol Oncol 2009;112(3):486-9.
2. Bhartiya D, Patel H. An overview of FSH-FSHR biology and explaining the existing conundrums. J Ovarian Res. 2021;14(1):144.

3. Jiang X, Liu H, Chen X, et al. Structure of follicle-stimulating hormone in complex with the entire ectodomain of its receptor. Proc Natl Acad Sci U S A. 2012;109(31):12491-6.
4. Neves R, Vuong NL, Blockeel C, et al. The effect of polymorphisms in FSHR gene on late follicular phase progesterone and estradiol serum levels in predicted normoresponders. Hum Reprod. 2022;37(11):2646-54.
5. Gromoll J, Simoni M. Genetic complexity of FSH receptor function. Trends Endocrinol Metab. 2005;16(8):368-73.
6. Sharifiyazdi H, Mirzaei A, Ghanaatian Z. Characterization of polymorphism in the FSH receptor gene and its impact on some reproductive indices in dairy cows. Anim Reprod Sci. 2018;188:45-50.
7. Lussiana C, Guani B, Mari C, Restagno G, Massobrio M, Revelli A. Mutations and polymorphisms of the FSH receptor (FSHR) gene: clinical implications in female fecundity and molecular biology of FSHR protein and gene. Obstet Gynecol Surv. 2008;63(12):785-95.
8. Meyer JM, Eaves LJ, Heath AC, Martin NG. Estimating genetic influences on the age-at-menarche: a survival analysis approach. Am J Med Genet. 1991;39(2):148-54.
9. Simoni M, Casarini L. Mechanisms in endocrinology: genetics of FSH action: a 2014-and-beyond view. Eur J Endocrinol. 2014;170(3):R91-107.
10. Kuijper EA, Blankenstein MA, Luttikhof LJ, et al. Frequency distribution of polymorphisms in the FSHR gene in infertility patients of different ethnicity. Reprod Biomed Online. 2011;22 Suppl 1:S60-5.
11. Wunsch A, Sonntag B, Simoni M. Polymorphism of the FSH receptor and ovarian response to FSH. Ann Endocrinol (Paris). 2007;68(2-3):160-6.
12. Chaudhary S, Walia R, Bhansali A, et al. FSH-stimulated Inhibin B (FSH-iB): a novel marker for the accurate prediction of pubertal outcome in delayed puberty. J Clin Endocrinol Metab. 2021;106(9): e3495-e3505.
13. Tang H, Yan Y, Wang T, et al. Effect of follicle-stimulating hormone receptor Asn680Ser polymorphism on the outcomes of controlled ovarian hyperstimulation: an updated meta-analysis of 16 cohort studies. J Assist Reprod Genet. 2015;32(12):1801-10.
14. Maheshwari A, Gibreel A, Bhattacharya S, Johnson NP. Dynamic tests of ovarian reserve: a systematic review of diagnostic accuracy. Reprod Biomed Online. 2009;18(5):717-34.
15. Perez Mayorga M, Gromoll J, Behre HM, Gassner C, Nieschlag E, Simoni M. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. J Clin Endocrinol Metab. 2000;85(9):3365-9.
16. Greb RR, Grieshaber K, Gromoll J, 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. J Clin Endocrinol Metab. 2005;90(8):4866-72.
17. Daelemans C, Smits G, de Maertelaer V, et al. Prediction of severity of symptoms in iatrogenic ovarian hyperstimulation syndrome by follicle-stimulating hormone receptor Ser680Asn polymorphism. J Clin Endocrinol Metab. 2004;89(12):6310-5.
18. Achrekar SK, Modi DN, Desai SK, Mangoli VS, Mangoli RV, Mahale SD. Follicle-stimulating hormone receptor polymorphism (Thr307Ala) is associated with variable ovarian response and ovarian hyperstimulation syndrome in Indian women. Fertil Steril. 2009;91(2):432-9.
19. Overbeek A, Kuijper EA, Hendriks ML, et al. Clomiphene citrate resistance in relation to follicle-stimulating hormone receptor Ser680Ser- polymorphism in polycystic ovary syndrome. Hum Reprod 2009;24(8):2007-13.
20. Overbeek A, Kuijper EA, Hendriks ML, et al. Clomiphene citrate resistance in relation to follicle-stimulating hormone receptor Ser680Ser- polymorphism in polycystic ovary syndrome. Hum Reprod 2009;24(8):2007-13. UGUALE 19
21. Ghadami M, El-Demerdash E, Salama SA, et al. Toward gene therapy of premature ovarian failure: intraovarian injection of adenovirus expressing human FSH receptor restores folliculogenesis in FSHR(-/-) FORKO mice. Mol Hum Reprod. 2010;16(4):241-50.
22. Vilodre LC, Kohek MB, Spritzer PM. Screening of follicle-stimulating hormone receptor gene in women with premature ovarian failure in southern Brazil and associations with phenotype. J Endocrinol Invest. 2008;31(6):552-7.
23. Wang HS, Cheng BH, Wu HM, et al. A mutant single nucleotide polymorphism of follicle-stimulating hormone receptor is associated with a lower risk of endometriosis. Fertil Steril. 2011;95(1):455-7.
24. André GM, Martins Trevisan C, Pedruzzi IN, et al. The impact of FSHR gene polymorphisms Ala307Thr and Asn680Ser in the endometriosis development. DNA Cell Biol. 2018;37(6):584-91.
25. Horn D, Fitzpatrick WC, Gompper PT, et al. Regulation of cell growth by recombinant oncostatin M. Growth Factors. 1990;2(2-3):157-65.
26. Papadimitriou K, Kountourakis P, Kottorou AE, et al. Follicle-Stimulating Hormone Receptor (FSHR): a promising tool in oncology? Mol Diagn Ther. 2016;20(6):523-30.
27. Shibuya M. Vascular Endothelial Growth Factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti- and pro-angiogenic therapies. Genes Cancer. 2011;2(12):1097-105.
28. Zeng H, Zhao D, Yang S, Datta K, Mukhopadhyay D. Heterotrimeric G alpha q/G alpha 11 proteins function upstream of vascular endothelial growth factor (VEGF) receptor-2 (KDR) phosphorylation in vascular permeability factor/VEGF signaling. J Biol Chem. 2003;278(23):20738-45.
29. Hong SS, Zhang MX, Zhang M, et al. Follicle-stimulating hormone peptide-conjugated nanoparticles for targeted shRNA delivery lead to effective gro-α silencing and antitumor activity against ovarian cancer. Drug Deliv. 2018;25(1):576-84.
30. Yang CQ, Chan KY, Ngan HY, et al. Single nucleotide polymorphisms of follicle-stimulating hormone receptor are associated with ovarian cancer susceptibility. Carcinogenesis. 2006;27(7):1502-6.
31. Ding YM, Zhang XJ, Li JP, et al. Treatment of idiopathic oligozoospermia with recombinant human follicle-stimulating hormone: a prospective, randomized, double-blind, placebo-controlled clinical study in Chinese population. Clin Endocrinol (Oxf). 2015;83(6):866-71.
32. Nieschlag E, Simoni M, Gromoll J, Weinbauer GF. Role of FSH in the regulation of spermatogenesis: clinical aspects. Clin Endocrinol (Oxf). 1999;51(2):139-46.
33. Aljuhayman A, Almardawi A, Fallatah M, Alhathal N. Efficacy of follicle-stimulating hormone as a treatment of severe idiopathic oligospermia: a retrospective study. Urol Ann. 2020;12(1):69-72.
34. Huang X, Li L, Hong L, et al. The Ser680Asn polymorphism in the follicle-stimulating hormone receptor gene is associated with the ovarian response in controlled ovarian hyperstimulation. Clin Endocrinol (Oxf). 2015;82(4):577-83.
35. Altmäe S, Hovatta O, Stavreus-Evers A, Salumets A. Genetic predictors of controlled ovarian hyperstimulation: where do we stand today? Hum Reprod Update. 2011;17(6):813-28.
36. Morón FJ, Ruiz A. Pharmacogenetics of controlled ovarian hyperstimulation: time to corroborate the clinical utility of FSHR genetic markers. Pharmacogenomics. 2010;11(11):1613-8.
37. Simoni M, Casarini L. Mechanisms in endocrinology: Genetics of FSH action: a 2014-and-beyond view. Eur J Endocrinol. 2014 Feb 4;170(3):R91-107. Uguale a 9.
38. Santi D, Potì F, Simoni M, Casarini L. Pharmacogenetics of G-protein-coupled receptors variants: FSH receptor and infertility treatment. Best Pract Res Clin Endocrinol Metab. 2018;32(2):189-200.

Conflict of interest statement: *None Declared*