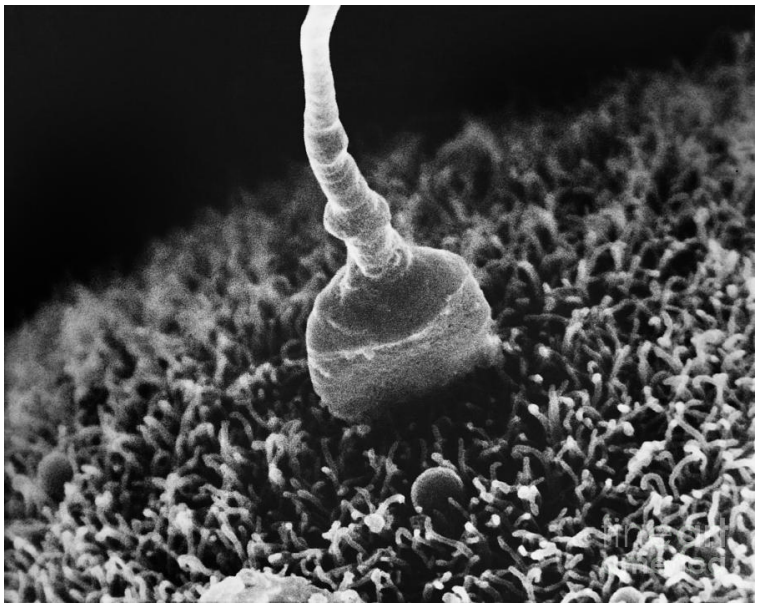
HELLENIC REPUBLIC
National and Kapodistrian University of Athens

National and Kapodistrian University of Athens, School of Health Sciences, School of Medicine

MSc Reproductive - Regenerative Medicine



Thesis:

"*Pharmacogenomics and In Vitro Fertilisation*"

"*Φαρμακογενωμική και εξωσωματική γονιμοποίηση*"

Karatasos Charalampos

Supervisor: Mavrogianni Despoina

Athens 2024

CONTENTS

Abstract

In vitro fertilisation (IVF) has revolutionised assisted reproductive technology, offering hope to couples struggling with infertility. Despite all advancements, IVF outcomes remain variable and are, influenced by numerous factors, including genetic variations such as polymorphisms. This thesis explores the role of various polymorphisms in determining the success rates of IVF procedures, by mainly focusing on studies published since 2017. Through an extensive review of the literature, genetic databases, and clinical data, this thesis aims to study the relationship between specific polymorphisms and key IVF outcomes, including fertilisation rates, embryo quality, implantation success, and live birth rates. Additionally, this thesis explores the underlying mechanisms by which these genetic variations impact reproductive processes, shedding light on potential biomarkers for predicting IVF success and guiding personalised treatment strategies. By providing insights into the genetic determinants of IVF outcomes, this thesis contributes to the optimisation of assisted reproductive techniques, ultimately improving the chances of success for couples undergoing IVF procedures.

Περίληψη

Η εξωσωματική γονιμοποίηση (IVF) έχει επαναστατήσει στην τεχνολογία υποβοηθούμενης αναπαραγωγής, προσφέροντας ελπίδα σε ζευγάρια που αντιμετωπίζουν προβλήματα υπογονιμότητας. Παρά τις εξελίξεις, οι εκβάσεις της εξωσωματικής γονιμοποίησης (IVF) παραμένουν μεταβλητές, επηρεαζόμενες από πολλούς παράγοντες, συμπεριλαμβανομένων γενετικών παραλλαγών όπως οι πολυμορφισμοί. Αυτή η διπλωματική εργασία αναλύει το ρόλο διαφόρων πολυμορφισμών στον καθορισμό της επιτυχίας ή της αποτυχίας των διαδικασιών IVF, εστιάζοντας κυρίως σε μελέτες που δημοσιεύτηκαν από το 2017. Μέσω μιας ολοκληρωμένης ανασκόπησης της βιβλιογραφίας, γενετικών βάσεων δεδομένων και κλινικών δεδομένων, αυτή η μελέτη στοχεύει να διαλευκάνει τη σχέση μεταξύ συγκεκριμένων πολυμορφισμών και κύριων εκβάσεων της IVF, συμπεριλαμβανομένων των ποσοστών γονιμοποίησης, της ποιότητας των εμβρύων, της επιτυχίας της εμφύτευσης και των ζώντων γεννήσεων. Επιπλέον, η διατριβή εξερευνά τους υποκείμενους μηχανισμούς με τους οποίους αυτές οι γενετικές παραλλαγές επηρεάζουν τις αναπαραγωγικές διαδικασίες, αναδεικνύοντας πιθανούς βιοδείκτες για την πρόβλεψη της επιτυχίας της IVF και καθοδηγώντας σε εξατομικευμένες στρατηγικές θεραπείας. Παρέχοντας πληροφορίες για τους γενετικούς καθοριστικούς παράγοντες των αποτελεσμάτων της εξωσηματικής γονιμοποίησης, αυτή η έρευνα συντελεί στη βελτίωση των τεχνικών υποβοηθούμενης αναπαραγωγή, βελτιώνοντας τις πιθανότητες επιτυχίας για τα ζευγάρια που υποβάλλονται σε διαδικασίες IVF.

Introduction

Embryonic development

Mammalian embryo development starts when a female's haploid cell, the oocyte, and a male's haploid cell, the spermatozoon, fuse to make the diploid zygote. The oocyte and the sperm are the gametes of the female and male respectively. The union of the two gametes, is called fertilisation and is achieved through the fusion of their membranes. For the fertilisation to be successful, both the female and male DNA are necessary.

The haploid gametes derive from diploid cells through a process called gametogenesis. Gametogenesis in females is called oogenesis and in males spermatogenesis.

I humans, oogenesis starts before birth. Approximately 8-20 weeks after fertilisation, the cells that will potentially become mature oocytes are proliferating. By the time the female is born, all the oocytes that will be released during the active reproductive years of the female are already in the ovaries. Those primordial ovum are about 400.000 and they will remain dormant until ovulation when only 1 oocyte is released from the ovaries. The egg will remain a primordial oocyte until its release from the ovary. Then it undergoes cell division. The nucleus is divided and so half of its chromosomes goes to one cell and half to another. One of these new cells usually is bigger than the other and is also known as secondary ovum. The smaller cell is known as the polar body. The secondary ovum will remain in the ovary until it reaches maturation. It then is released in the fallopian tubes. Once in the tubes the oocyte is ready for fertilisation [1, 2].

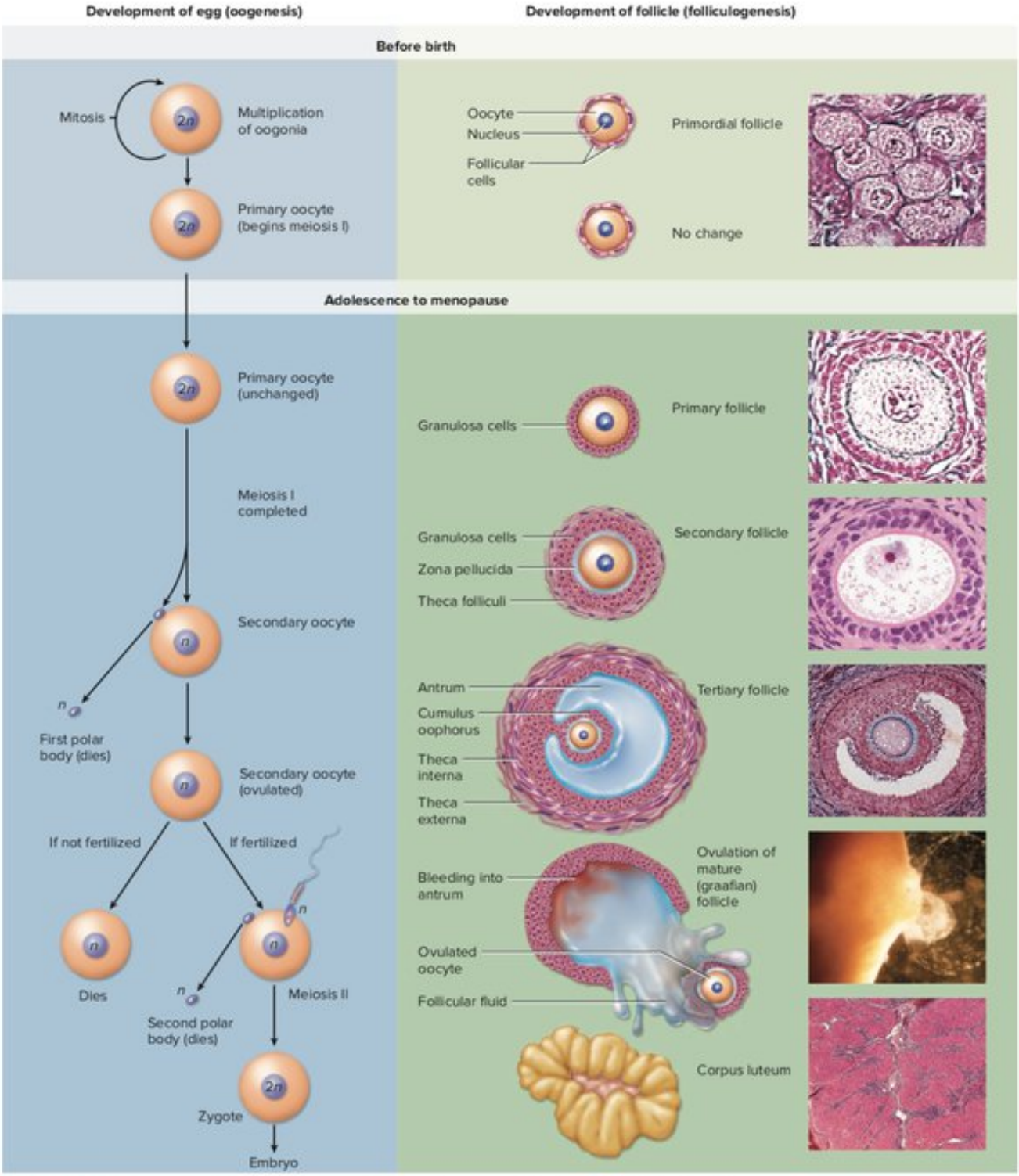


Fig 1: Maturation of human oocyte (https://www.quora.com/What-is-the-difference-between-oogenesis-and-folliculogenesis)

Spermatozoa, in the male reproductive system, begin and finish their development in the testes. The testes are composed of many thin tightly coiled tubules, known as seminiferous tubules. Sperm cells are produced on the walls of those tubules. In the seminiferous tubules, are numerous scattered Sertoli cells, whose function is mainly to support and nourish immature sperm cells. As the young immature gametes grow, Sertoli cells help them transport from the outer space of the seminiferous tubule to the central channel. One immature germ cell needs about 74 days to reach its final maturation stage. The process initiates with spermatogonia, immature cells originating from stem cells situated in the outer seminiferous tubule wall. Through mitosis, the stem cells duplicate, with half of the new cells evolving into future sperm cells and the remainder persisting as stem cells to maintain a continuous supply of germ cells. The spermatogonia designated for maturation, termed primary sperm cells, migrate from the outer to the central seminiferous tubule regions, attaching themselves to Sertoli cells. These primary cells undergo development by increasing cytoplasmic content and the presence of organelles. After a resting phase, the primary cells undergo division, resulting in the formation of secondary sperm cells. During cell division, the nuclear material undergoes splitting, reducing the chromosome count from 46 in the primary sperm cells to 23 in each secondary sperm cell [3].

The eventual union of egg and sperm during fertilisation combines their chromosomes, leading to a blending of characteristics from both individuals and the commencement of growth in the new organism.

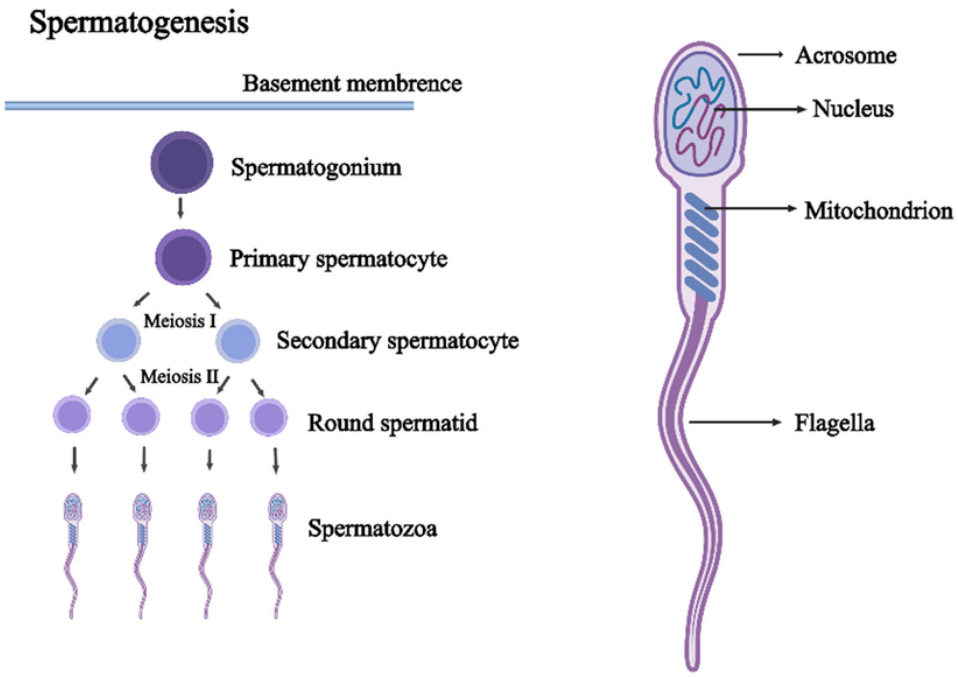


Fig 2: Spermatogenesis (left) and Spermatozoa structure (right) (https://www.researchgate.net/figure/The-process-of-spermatogenesis-and-the-structure-of-spermatozoa-During-spermatogenesis_fig1_366009879

Infertility and *In-Vitro* Fertilisation

Human infertility refers to the inability of a couple to conceive a child despite regular, unprotected sexual intercourse for at least 12 months. Infertility can affect both men and women, and it may result from a variety of factors. Common causes in women include ovulatory disorders, issues with the fallopian tubes or uterus, endometriosis and age - related decline in fertility. Male infertility can be due to low sperm count, poor sperm motility, abnormal sperm shape, or issues with sperm production and ejaculation. In many cases, infertility results from a combination of male and female factors or unexplained causes.

Infertility is categorised into primary and secondary. Primary infertility pertains to a couple unable to achieve pregnancy after a year of regular sexual intercourse during their childbearing age. On the other hand, secondary infertility is recognised when attempts to conceive are unsuccessful for 6–12 months. Secondary infertility specifically refers to the challenge of conceiving or sustaining a pregnancy after previously giving birth. Notably, the prior pregnancy should have occurred naturally, without the use of fertility drugs or procedures such as *in vitro* fertilisation, for it to be classified as secondary infertility. A longitudinal study spanning from 1993 to 2017 revealed a global trend wherein the prevalence of primary and secondary infertility was lower among men compared to women. Additionally, there was a decline in infertility rates, particularly in high-income countries.

Treatment options for infertility depend on the underlying cause. Common interventions include fertility drugs, assisted reproductive technologies (ART) such as *in vitro* fertilisation (IVF), intrauterine insemination (IUI) and surgery to address structural issues.

In vitro fertilisation (IVF) is a complex assisted reproductive technology that helps individuals and couples overcome fertility issues by facilitating the conception of a child outside the body. IVF involves several key steps. First of all the woman undergoes hormonal treatments to stimulate the ovaries to produce multiple eggs. Fertility medications, such as follicle - stimulating hormone (FSH) and luteinising hormone (LH) are administered. Once the eggs reach maturity, a minor surgical procedure known as egg retrieval or follicular aspiration is performed. A thin needle is inserted through the vaginal wall into the ovaries to extract the eggs. On the same day as egg retrieval, the male partner or a sperm donor provides a semen sample. The sperm is then processed to isolate healthy,

motile spermatozoa. In the laboratory, the eggs and sperm are combined in a culture dish for fertilisation. This can be done through traditional insemination, where sperm is added to the eggs, or through intracytoplasmic sperm injection (ICSI), where a single sperm is directly injected into each egg. Fertilised eggs, now embryos, are cultured for a few days. The embryologist monitors their development and selects the healthiest embryos for transfer. One or more selected embryos are transferred into the woman's uterus through the cervix. This is typically done 3 to 5 days after egg retrieval. The number of embryos transferred depends on factors such as the woman's age and/or the quality of the embryos. Hormonal medications are often prescribed to support the luteal phase of the menstrual cycle and enhance the chances of successful implantation. Finally, about 10 to 14 days after embryo transfer, a pregnancy test is conducted to determine if the procedure was successful.

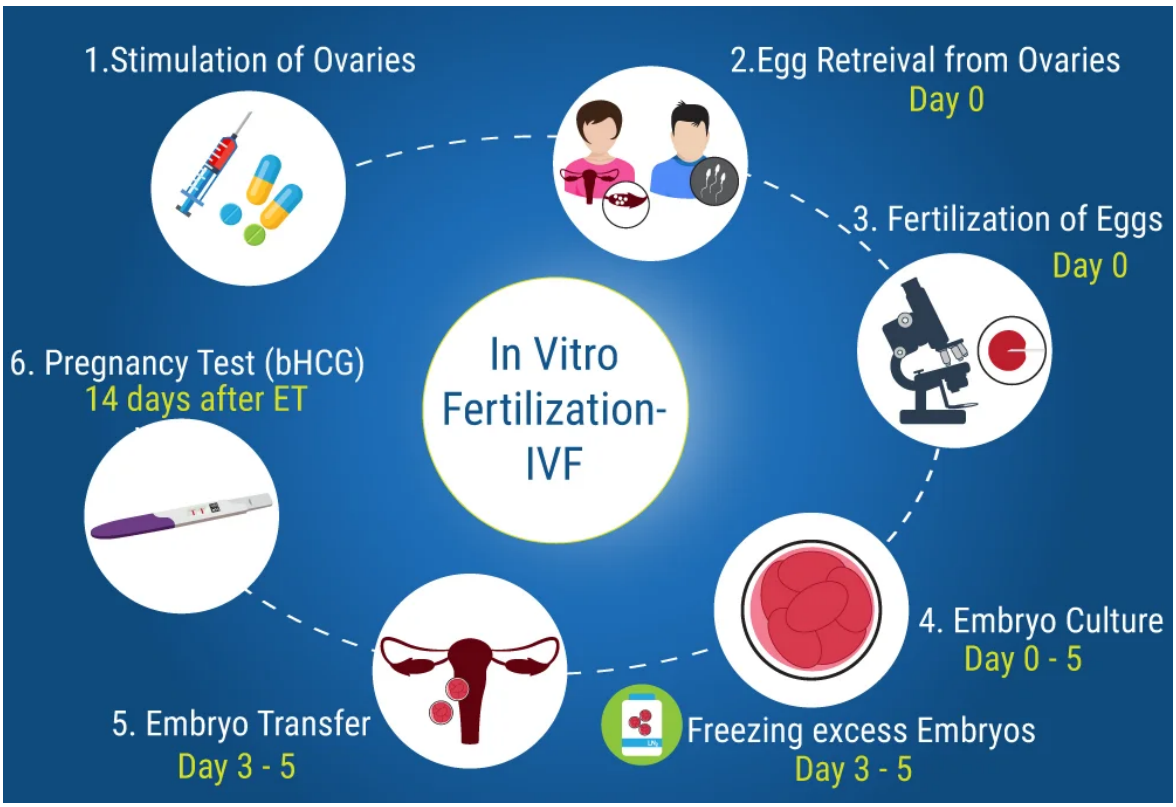


Fig. 3: A complete IVF cycle (https://selectivf.com/ivf-process-georgia/)

IVF is used to address various fertility issues, such as blocked fallopian tubes, male factor infertility, endometriosis, and unexplained infertility. It offers a viable option for individuals and couples who have not achieved pregnancy through other fertility treatments, such as IUI. It is important to note that the success of IVF can vary based on factors such as the age of the patients, the cause of infertility and the overall health of the individuals involved. Additionally, IVF carries ethical, emotional and financial considerations and individuals often work closely with reproductive specialists to navigate the process.

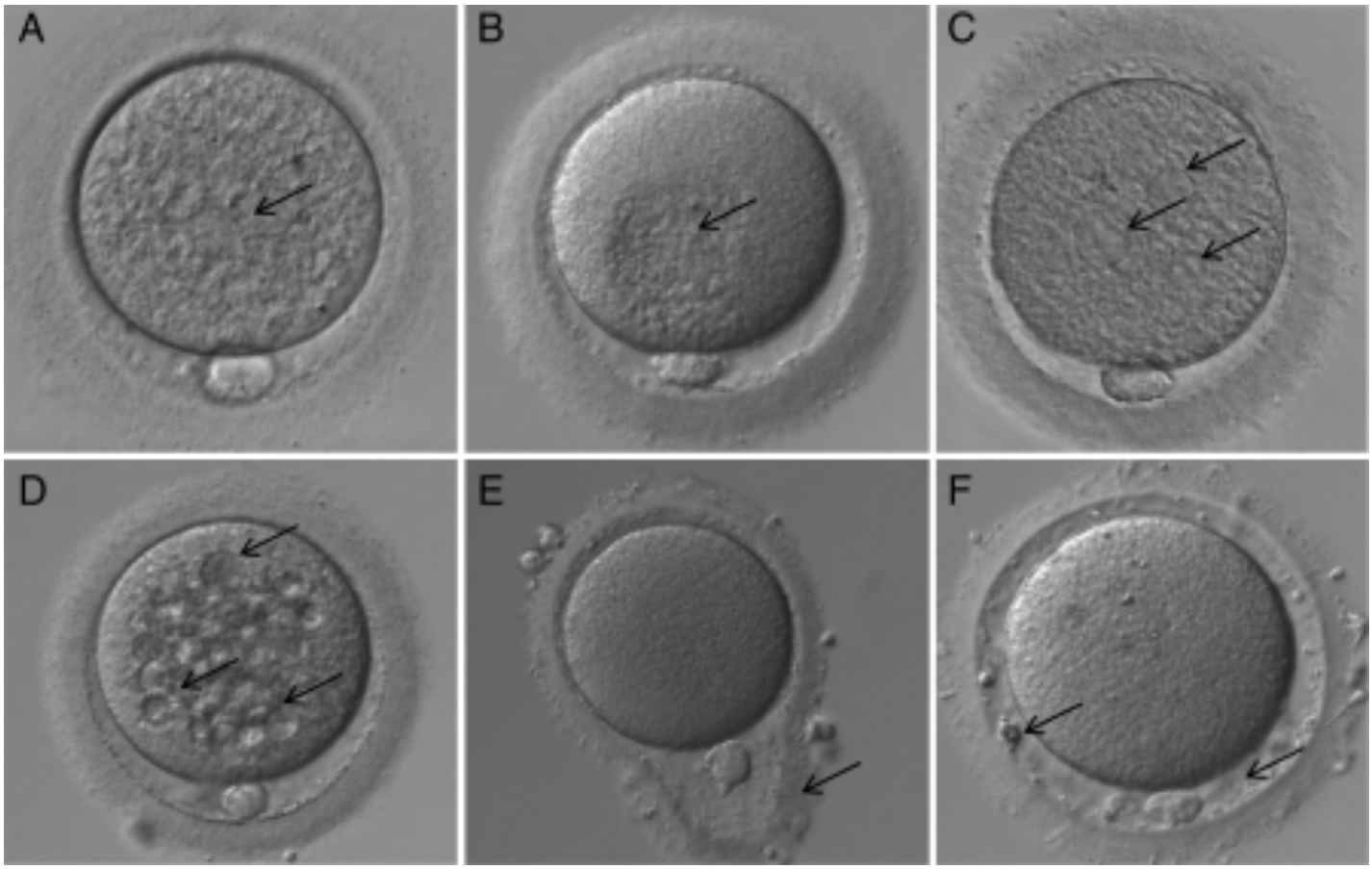
One common stimulation protocol used in IVF is the **long GnRH Agonist protocol**: A GnRH agonist is administered to suppress the natural hormonal cycle. After suppression, gonadotropins (FSH and/or LH) are given to stimulate the ovaries. Ovarian response is monitored through ultrasounds and hormone levels. When the follicles of proper size, human chorionic gonadotropin (hCG) is administered to trigger their final maturation and ovulation. Another protocol is the so-called **GnRH antagonist protocol**. Gonadotropin stimulation begins on day 2 or 3 of the menstrual cycle and a GnRH antagonist is added later to prevent premature ovulation. Gonadotropins are continued until the eggs are mature. Ovarian response is monitored, and hCG is administered when proper for the final maturation trigger. Another commonly used protocol is the **natural cycle or minimal stimulation protocol**. Low doses of gonadotropins are used to stimulate the developments of a few eggs. Ovarian response is also monitored and hCG will be used to trigger maturation. [4]

The choice of the stimulation protocol, is individualised based on the patient's specific characteristics and the fertility clinic's protocols. Close monitoring throughout the process is essential to adjust medication doses and timing as needed. The goal is to retrieve a suitable number of mature eggs for fertilisation during IVF

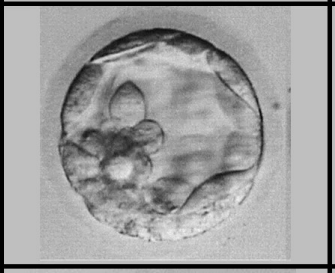
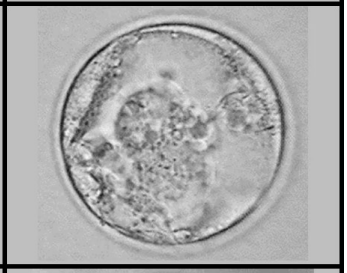
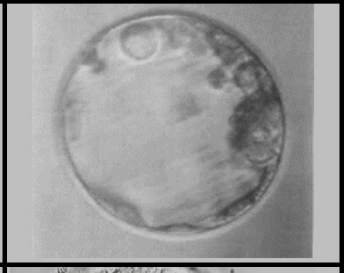
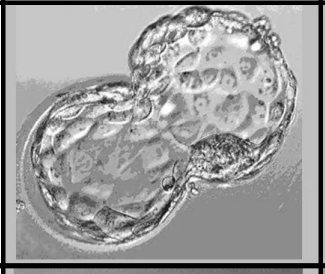
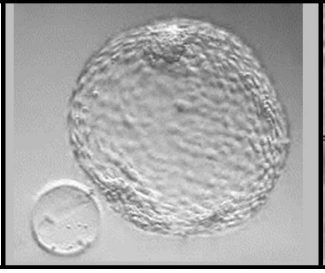
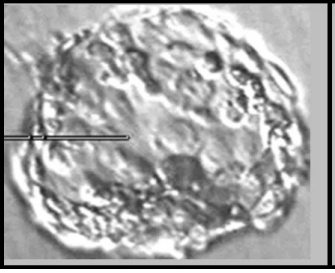
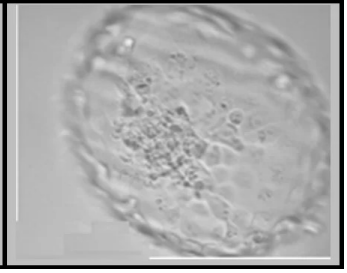
Challenges of IVF

In vitro fertilisation (IVF), a groundbreaking assisted reproductive technology, has offered hope to countless couples facing infertility. However, the journey towards successful conception through IVF is not without its formidable challenges. Understanding and addressing these challenges are crucial for enhancing the efficacy of IVF treatments and ensuring the well-being of both patients and offspring. One of the foremost challenges in IVF is the variability in success rates. Numerous factors, including maternal age, underlying causes of infertility, and the quality of embryos, contribute to the outcomes of IVF cycles. Couples often face the emotional rollercoaster of repeated attempts, underscoring the need for research to identify factors influencing success and refine treatment protocols. The financial cost, in addition associated with IVF remains a substantial hurdle for many prospective parents. Multiple treatment cycles, medications, and associated medical expenses contribute to the overall financial burden. Limited insurance coverage exacerbates the strain, making accessibility a significant challenge for a broader demographic. The emotional toll on couples undergoing IVF is profound. The combination of uncertainty, repeated disappointments, and the demanding nature of the treatment process can lead to heightened stress and anxiety. Acknowledging and addressing the emotional well-being of patients is paramount to comprehensive fertility care. While IVF increases the likelihood of successful pregnancies, it also elevates the risk of multiple pregnancies. This presents medical challenges for both mothers and infants, including a higher incidence of complications during pregnancy and childbirth. Balancing the desire for a successful pregnancy with the associated risks requires careful consideration. Also, Ovarian Hyper - Stimulation Syndrome (OHSS) is a potential complication arising from the use of fertility medications to stimulate egg production. This

syndrome can manifest with symptoms ranging from discomfort to severe complications, underscoring the need for precise hormonal management.



The success of IVF hinges on the quality and quantity of retrieved eggs. Women with diminished ovarian reserve or poor egg quality (see Fig 4) may face challenges in producing viable embryos. Understanding the factors influencing egg quality and developing strategies to optimise it are critical aspects of IVF research. Even with the availability of high-quality embryos (see Fig 5), successful implantation remains uncertain. Factors such as uterine abnormalities, endometrial receptivity, and the overall health of the uterus contribute to the complexity of the implantation process. Advancements in understanding and improving the implantation phase are essential for enhancing IVF success rates. Despite pre-implantation genetic testing (PGT), IVF does not eliminate the risk of genetic abnormalities in embryos. The ethical implications and the potential impact on the health of the offspring necessitate ongoing

Inner Cell Mass (ICM)	***A*** *Numerous and tightly packed*	***B*** *Several and loosely packed cells*	***C*** *Few cells*
Trophectoderm (TE)	***A*** *Many tightly packed cells organised into epithelium*	***B*** *Several cells organised into loose epithelium*	***C*** *Few cells*
Morula			
Early Blastocyst			
Blastocyst			
Expanded Blastocyst			
Hatching Blastocyst			
Fully Hatched Blastocyst			

research into refining genetic screening methods and addressing associated concerns. In the pursuit of advancing IVF as a reliable solution

for infertility, navigating these challenges requires a multidisciplinary approach. This thesis aims to contribute to this ongoing dialogue by exploring the intersection of IVF and pharmacogenomics, shedding light on potential avenues for improvement in treatment outcomes and patient experiences.

Fig 4: Different human oocyte morphological abnormalities (arrows) observed by light microscopy: (A) scarce cytoplasmic granularity, (B) centrally located cytoplasmic granular area, (C) smooth endoplasmic reticulum clusters, (D) vacuoles, (E) abnormal zona pellucida shape, (F) large perivitelline space with fragments. (https://www.researchgate.net/figure/Different-human-oocyte-morphological-abnormalities-arrows-observed-by-light-microscopy_fig2_45271886)

Fig 5: Gardner's blastocyst grading system assigns 3 quality scores to each blastocyst: 1)Blastocyst developmental stage, expansion and hatching status, 2)Inner Cell Mass (ICM) quality, 3) Trophectoderm (TE) quality (https://fertilitysolutions.com.au/choosing-embryos-for-transfer-or-freezing/)

Possible causes of infertility

Polycystic Ovary Syndrome, PCOS

Polycystic Ovary Syndrome (PCOS) is defined as an endocrine disorder that impacts around 1 in 10 women of reproductive age. Hyperandrogenism, insulin resistance, enlarged and often dysfunctional ovaries are some of the problems that are caused by PCOS. The precise aetiology and pathology of the syndrome has not been fully described, although it's main causes seem to be the abnormally high ratio of luteinising hormone to follicle-stimulating hormone (LH/FSH) and the increased frequency of gonadotropin-releasing hormone (GnRH). Type 2 diabetes mellitus, metabolic syndrome, cardiovascular diseases, obesity and many others have been mentioned as further complications of PCOS. Therefore, regular exercise and weight loss seem as an important precaution that is recommended to every woman with PCOS. Apart from that physicians use antiandrogen agents, insulin sensitisers, oral contraceptives and ovulation inducers as an off-label medication to fight the negative effects of PCOS. There is no specific medication for PCOS. Many women suffering from PCOS tend to use assisted reproductive technology in order to have the chance of becoming parents.[5]

Premature Ovarian Insufficiency, POI

Premature Ovarian Insufficiency (POI) is defined as the loss of ovarian function before the age of 40 and is mostly characterised by amenorrhea or secondary oligomenorrhoea and serum FSH levels more than 25 IU/L. The chance of spontaneous conception for women diagnosed with POI is about 5-10%. The clinical symptoms may include amenorrhea,

oligomenorrhoea, vasomotor instability (hot flushes, night sweats),low libido, sleep disturbances, vulvovaginal atrophy, altered urinary frequency, dyspareunia, and lack of energy. All these are due to estrogen deficiency. 1:10,000 women before the age of 25, 1:1000 before 30 and 1:100 before 40, suffer from POI. The cause of POI remains mainly undefined but potential aetiologies can be divided into different groups: idiopathic, genetic, autoimmune, environmental or iatrogenic [6].

Male infertility

Around 72.4 million people worldwide have some sort of fertility disorder. Male factor accounts for about 50% of cases. Although genetic mutations, modern lifestyle and medications can be contributing factors that can lead to male infertility, idiopathic sperm abnormalities are blamed for about 30% of male sub-fertility cases. Obesity can play a crucial role on sperm parameters and as shown overweight or obese men have higher chances of oligo-azoo-teratospermia (OAT) than men with normal body mass index (BMI). In addition, higher DNA fragmentation index has been associated with obesity. As a result lower pregnancy rates and many pregnancy losses are observed in couples which undergo IVF and the man is overweight or obese. Until now studies were focused on maternal age and how it can negatively affect pregnancy rates and outcome. However, paternal age has recently been associated with infertility since sperm parameters seem to be worse in older men. Moreover, offspring of older men have been shown to have increased risk for various conditions [7].
Idiopathic male infertility is diagnosed when there are unexplained sperm abnormalities, without any female factor infertility, as opposed to unexplained male infertility, where sperm parameters are normal. Oxidative stress has been identified as a potential mechanism for idiopathic male infertility. Previous research indicates that spermatozoa

with morphological defects are more likely to produce excessive reactive oxygen species (ROS) and have a diminished antioxidant capacity. Additionally, oxidative stress is frequently observed in males with idiopathic infertility, showing an imbalance in ROS levels and antioxidant capacity compared to fertile males. Despite recognising the association between oxidative stress and idiopathic infertility, there remains a lack of definitive treatment. For example, there is uncertainty about which patients should undergo screening for oxidative stress and what tests should be employed to measure ROS levels in semen samples. Furthermore, there is controversy surrounding the type, dosage, and duration of antioxidant treatment for individuals with elevated ROS levels. [8]

Male infertility is a complex condition that impacts approximately 7% of the general male population. Recent classifications of impaired male reproductive function emphasise the undeniable significance of genetic factors, constituting around 15% of male infertility cases across various causes [9]. Notably, men with non-obstructive azoospermia face a heightened risk (20%) of carrying numerical and structural chromosomal anomalies, such as Klinefelter syndrome and Y chromosome-linked Azoospermia Factor deletions (AZF) [10]. Standard genetic diagnostic procedures, including karyotype and AZF deletion screening, also incorporate mutational analysis of the CFTR gene for individuals with congenital agenesis of vas deferens (CAVD) [11].

Despite these advancements, about 40% of cases involving oligo/ azoospermia are categorioed as idiopathic infertility, where the cause is unknown. Genetic factors are strongly implicated in these instances, given that approximately 2000 genes are projected to be involved in spermato-genesis pathways, though only a minority have been identified thus far. Previous attempts using Sanger sequencing to uncover recurrent monogenic causes were largely unsuccessful. However, the recent widespread adoption of Next-Generation Sequencing (NGS) has facilitated

the validation of previously identified candidate genes and the discovery of novel genetic causes across all four aetiological categories

The Hap - Map project

The deciphering of the complete human genome has enabled our ongoing endeavor to create a haplotype map of the human genome, known as the "HapMap." This tool facilitates the identification of genes and genetic variations associated with health and disease.

While the DNA sequence of any two individuals is 99.5 percent identical, variations in single DNA bases, known as single nucleotide polymorphisms (SNPs), can significantly influence an individual's susceptibility to disease. SNPs located close together on the same chromosome are inherited in blocks, forming distinct haplotypes. Although these blocks may contain numerous SNPs, only a few are necessary to uniquely identify the haplotypes within them. The HapMap catalogs these haplotype blocks, with specific SNPs—referred to as tag SNPs—serving as markers for identifying the haplotypes.

The HapMap's value lies in its ability to reduce the number of SNPs needed to explore the entire genome's association with various phenotypes, from approximately 10 million SNPs to roughly 500,000 tag SNPs. This streamlines genome-wide scans for regions harboring disease-related genes, making the process more efficient and inclusive of all genomic regions.

Beyond its role in studying genetic disease associations, the HapMap serves as a robust resource for investigating genetic factors affecting responses to environmental stimuli, susceptibility to infections, and the efficacy and adverse reactions to drugs and vaccines. These studies rely on the premise that individuals with a particular disease or response exhibit higher frequencies of contributing genetic components compared to those without. By utilising tag SNPs, researchers can identify

chromosome regions with divergent haplotype distributions between affected and unaffected groups, leading to detailed investigations into specific gene variants contributing to diseases or responses. This knowledge aids in the development of more targeted interventions and predictive tests for drug efficacy based on individuals' genotypes for genes involved in drug metabolism.

Pharmacogenomics

Pharmacogenomics, a groundbreaking discipline within the broader realm of pharmacology, represents a paradigm shift in the way we approach and administer medications. This field delves deep into the intricate relationship between an individual's genetic makeup and their response to pharmaceutical interventions. By examining genetic variations that influence drug metabolism, efficacy, and potential side effects, pharmacogenomics seeks to unravel the complexities of inter-individual differences in drug response. The realisation that genetic factors play a pivotal role in determining how individuals react to medications has paved the way for a more personalised and precise approach to healthcare. The key premise of pharmacogenomics is to tailor medical treatments based on a patient's unique genetic profile, thereby optimising therapeutic outcomes while minimising the risk of adverse reactions. This emerging field is propelled by advancements in genomics, bioinformatics, and high-throughput technologies, enabling researchers and healthcare professionals to identify genetic markers associated with drug response variability. The ultimate goal is to move away from the traditional one-size-fits-all model of drug prescribing, allowing for a more nuanced and individualised treatment strategy.

One of the primary applications of pharmacogenomics lies in guiding drug development and prescription practices. Understanding how specific genetic variations influence drug metabolism pathways allows for the identification of individuals who may be more or less responsive to certain medications. This knowledge can be particularly crucial in the development of drugs with a narrow therapeutic window, where small variations in drug concentrations can lead to either inadequate therapeutic effects or increased toxicity. Additionally, pharmacogenomics has the potential to enhance the safety profile of medications by identifying patients who may be predisposed to adverse reactions based on their genetic predisposition.

In clinical settings, pharmacogenomic testing is increasingly being integrated into patient care to inform treatment decisions. By analysing a patient's genetic profile, healthcare providers can predict how an individual is likely to respond to a specific drug and adjust dosage or choose alternative medications accordingly. This personalised approach not only improves the overall efficacy of treatments but also mitigates the risk of adverse drug reactions, ultimately leading to better patient outcomes.

Despite its transformative potential, challenges remain in the widespread adoption of pharmacogenomics in clinical practice. Issues such as cost, accessibility, and the need for standardised guidelines for interpreting genetic data pose hurdles to its integration into routine healthcare. However, ongoing research and technological advancements are steadily addressing these challenges, paving the way for a future where pharmacogenomics plays a central role in revolutionising drug therapy, making it more tailored, effective, and safer for each individual.

Luteinising Hormone (LH)

Luteinising Hormone, is a glycoprotein. It is composed of the same α-subunit as FSH, TSH and hCG and a unique, specific for the hormone β subunit. The *LHβ* gene is located on chromosome 19 and it consists of 3 exons and 2 introns. LH has a fundamental role in follicular maturation and ovulation and its participation in ovarian and testicular regulation is also very important. Furthermore, it has a distinct impact on the modification of synthesis of growth factors, steroid hormones and cytokines.[11]

Some polymorphisms in the *LHβ* gene have been studied and were shown to have an impact on the fertility of men and women. For instance, in an meta-analysis performed by Alviggi *et al.* they found in 2 studies that the minor allele carriers of the LHB c.82 A>G Trp8Arg (rs1800447) had used significantly higher FSH dosage during controlled ovarian stimulation (COS) [13]. Nevertheless, the meta-analysis could not find a significant difference between the genotypes, concerning the rs1800447, probably due to the small amount of studies associating this polymorphism with infertility.

Luteinising Hormone Choriogonadotropin Receptor (LHCGR)

The β subunit of LH gives the hormone the ability to interact specifically with its receptor, luteinising hormone choriogonadotropin receptor (LHCGR) LHCGR is a G-protein coupled receptor and is expressed in several tissues such as the fallopian tubes, the uterus, the gonads, the

placenta and even the fetus. Its expression is increasing just before ovulation, when FSH and E2 increase the pituitary LH and LHCGR expression in the ovaries. The receptor seems to have an impact on follicle maturation, luteinisation and ovulation so its role in female fertility is crucial. [13]. Inactivation of the *LHCGR* gene in mice embryonic stem cells can affect the fertility of both males and females [15]. 172 patients of several ethnicities that underwent IVF procedures were recruited and genotyped in a study trying to associate several polymorphisms in LH and LHCGR genes with ovarian hyperstimulation syndrome. The two polymorphisms that were studied were the following; the insertion of 6 base pairs CTGCAG at position 54 of the LHCGR (insLQ, rs4539842) and +28G>C (rs4073366). Although the first polymorphism could not be linked with OHSS, the minor allele "C" carriers of the rs4073366 were shown to have 3 times higher chance of developing OHSS compared. It is concluded that this is happening probably due to the alterations caused by the rs4073366 to apolipoprotein E, which is an important factor of cholesterol uptake and steroidogenesis and has been associated with reproductive efficiency.[14]

Recently, in 2019, a study on Iranian women was published. This study included 100 women undergoing IVF procedures and compared the results of the IVF with their genotypes. Three polymorphisms were tested. The insLQ rs4539842, the LHCGR c.827A>G Asn291Ser (rs12470652) and the LHCGR c.935A>G Asn312Ser (rs2293275). An association has been observed between the G allele of rs2293275 and PCOS [16,17] and higher testosterone levels. The results showed a significant association between women carrying the rs2293275 and/or the rs4539842 and their success rate in IVF. Concerning the rs4539842, failure in IVF was observed in the group of women having no insertion of the 6bp CTGCAG while women heterozygous or homozygous for the insertion had higher chances of success. As for the c935A>G p.Asn312Ser, the A allele seemed to have an positive impact on the IVF success rate since 18% of women carrying

the AA genotype had successful results and 0% of them had failed results in IVF . On the contrary 46% of women having the GG genotype were unsuccessful compared to 18% in the successful group [15]. Although, the GG genotype was associated with higher clinical pregnancy rate when it comes to fresh embryo transfer and compared to the heterozygous AG. [18]. In 2016 it was shown that women with the AA genotype needed lower doses of rFSH in order to achieve adequate response while undergoing IVF procedures [19]. These results were supported from data in a later study on OHSS. Genotyping women with OHSS and normal ovarian response resulted in an association of the GG genotype of rs2293275 with higher chances of OHSS, thus supporting that the A allele may increase the sensitivity of the receptor to the hormone [20]. In a later study by the same authors, a significant association between the GG genotype and poor ovarian response was observed. Another study by Lindrigen *et al.* in 2019, combined two polymorphisms, the FSHR Asn680Ser (rs6166) and the *LHCGR* Asn312Ser (rs2293275) and the results supported that women carrying the Serine residue for both SNPs had a higher chance of a live birth. So, if a woman was homozygous S for both polymorphisms, she had the highest chance of a live birth[21]. On the contrary, no significant difference occurred in a later study of 2022 concerning the *LHCGR* rs2293275 and the live birth rate of the outcome of ART among people of various ethnicities [22]. Different studies seem to have contradictory results associating these polymorphisms and ART procedures. However, these statements should be studied more extensively. In order to have a better understanding of what could affect the fertility and the IVF procedures and more data associating different variants and ART would be very helpful.

Estrogen Receptors (ERs)

Estrogen is a steroid hormone, one of the most important of the sex hormones. It is produced by the gonads and the adrenal cortex. Estradiol (E2) is the most common of estrogens and plays a crucial role in the fertility of both females and males. Development of the secondary female sex characteristics, regulation of the menstrual cycle and growth of the endometrial lining starting from the menarche to menopause and also regulating spermatogenesis in males are some of the basic functions of E2 [23]. Estradiol effects are mediated by transcription factors and estrogen receptors (ER)α and (ER)β. Those receptors are encoded by distinct genes *ESR1* and *ESR2* respectively. ESR1 gene is located on chromosome 6, includes 8 exons and encodes for a 66 kDa protein of 595 amino-acids. ESR2 gene is located on chromosome 14 also consists of 8 exons and is translated into a 60 kDa protein of 530 amino acids [24]. Several polymorphisms in both genes have been associated with conditions that have a huge impact on female fertility. These conditions include endometriosis, PCOS, recurrent pregnancy loss, the result of the ovarian stimulation and even the result of the ART procedure. Although many studies have been conducted and observed the possible association of polymorphisms in the ESR genes, the results are contradictory. Women homozygous for the G allele of *ESR1* -351A>G (rs9340799), for example, seem to have 4 times higher chance of endometriosis [25]. This association was also observed in a case-control study from 2021, in which women with the GA genotype for rs9340799 seem to have e prevalence for endometriosis. In the same study, another polymorphism was associated with endometriosis. The *ESR2* +1730G>A (rs4986938) was observed to have a significant effect on the occurrence of endometriosis. [26]. These results are confirmed by another study of 2020 that recruited 100 women with endometrioses and 100 controls and the results showed

a decreased risk of endometrioses in women with the AA genotype for the rs4986938 [28] Furthermore, in a recent narrative review published in 2022 the rs9340799 was associated with endometriosis as well as migraines only in the Asian population whereas the association was not observed in Caucasians. On the contrary, endometriosis and migraine in the Caucasian population were associated with the rs4986938 while Asians showed no significant association. Another polymorphism, the *ESR1* -397T>C rs2234693, was shown to have a significant association with endometriosis in both Asian and Caucasian populations [27]. A trend towards the lack of association of rs2234693 and endometriosis was stated in a systematic review by Mear *et al.* published in 2020. Although it is also stated that further studies are needed in order to find a possible association between these polymorphisms and endometriosis[29].

Polymorphisms on the genes of estrogen receptors have been strongly associated with PCOS as well, with controversial results. The GA genotype of rs9340799, for instance, have been associated with a decreased risk of PCOS and in the same study an intron substitution of guanine with adenine on chromosome 6 (rs1999805) was associated with an increased risk of developing PCOS in Chinese population[30]. Recently, in 2020, in a study concerning Tunisian women, several ESR SNPs were included in the list of polymorphisms associated with PCOS. The *ESR2* rs1256049, *ESR1* rs3798577 and rs2234693 were correlated with higher chance of PCOS The strongest association, however, was that of the latter, where the C allele seemed pathogenic while the T protective. In addition, haplotype combinations of SNPs in the ESR1 gene (rs2234693 C/T, rs9340799 G/A, rs3798577 T/C, rs3020314 T/C) were observed to have an important impact on some of the effects of PCOS. TATC were strongly associated with obesity, CGTT and CATC with high blood pressure and some seemed to have a trend towards association with insulin resistance (TACT), dyslipidemia (TATT) and cholesterol levels (CACC)[31]. Higher levels of testosterone were observed in women homozygous for the minor

allele of 1730 G>A rs4986938 than heterozygous or homozygous for the major allele [32].

Pregnancy loss is another issue that increases the problem of infertility in couples. Many studies have found correlations between polymorphisms on the ER genes and recurrent pregnancy loss (RPL). 444 Tunisian women that had at least 3 or more consecutive pregnancy losses and 446 controls were enrolled in a study of 2020 and genotyped for three polymorphisms on ER genes, *ESR1* rs2234693, *ESR1* rs3020314 and *ESR2* rs928554. No association was found for the last two. However, the C allele of rs2234693 had a significant impact on the pregnancy result. Women homozygous for this allele were more likely to have a pregnancy loss than the other genotypes[33]. Another interesting finding was that the same polymorphism can have extremely different results when population groups are compared. Seven case-control studies were combined in a cohort by Yin Quian-Xun *et al.* the rs9340799 was shown to have an association with an increased risk of RPL in non Asian group in the homozygous genetic model. The same SNP, in heterozygous and dominant genetic models, was associated with a decreased risk of spontaneous abortion[34]. In two meta-analyses, published in 2021 and 2022 an association between the rs4986938 (in Caucasian population) and rs2234693 respectively with recurrent pregnancy loss was observed. [35]. A finding that was supported by a cohort in Egyptian women, published in 2021 that found an association between the rs2234693 and rs9340799 with RSA [36]. On the contrary, in a case-control study recruiting 258 women who had experienced 3 or more miscarriages and 264 healthy controls with 2 or more successful pregnancies, no association was found concerning three polymorphisms of the ESR1 genes (*ESR1* rs9340799, rs2234693 and rs3798759) and recurrent spontaneous abortion. However, for these SNPs, a haplotype analysis showed that the haplotype ACA had an increased risk of RSA, whereas the haplotype GCA had lower chance RSA in the Chinese population [37]. Controversial

results from different studies increase the need for more studies recruiting bigger populations for significant results.

One of the most important issues concerning ART procedures is the ovarian response to gonadotropins. A significant association of the CT genotype of rs2234693 and poor responders was revealed in the Egyptian population [38]. In 2020, a correlation of ESR2 +1730 G>A rs4986938 and the ovarian response in IVF cycles was studied. No association was revealed, however, since the number of follicles, the number of oocytes, the number of embryos, the size of follicles and the pregnancy rates seemed to have no difference among the genotypes [87]. Interestingly, in 2018, a genotype that seems protective in the IVF procedure was revealed. The allele combination of T for rs2234693, A for rs9340799, S for EST1 (TA), A for rs6166, G for rs6165 and del of PROGINS seem to be more frequent in women who had successful results in IVF [39].

Follicle Stimulating Hormone (FSH)

Follicle Stimulating Hormone (FSH), like LH, is a heterodimeric glycoprotein hormone which shares the same alpha subunit with LH, TSH, and hCG and a unique specific beta subunit. FSH binds specifically to the FSHR and their interaction is important for ovarian stimulation and function [40] by promoting proliferation and differentiation of granulosa cells as well as E2 synthesis[41]. The beta (β) subunit of the FSH molecule is crucial for the hormone-specific biological properties and is encoded by the FSHβ gene which lies on chromosome 11 [42]. Polymorphisms on the FSHβ gene and/or its promoter have been shown to affect fertility of both males and females in various ways. One of the known SNPs that have an impact in fertility is the -211 G>T (rs10835638), which refers to the substitution of guanine to thymine in the promoter of the FSHβ gene. Mainly this SNP has been shown to affect

male fertility but in a study of 2016 from the United Kingdom it was shown to also affect women. Longer menstrual cycle, later menopause and altered FSH serum levels were associated with the rs10835638. Specifically, women carrying the minor allele (T) had a longer menstrual cycle (approximately by 1 day), later age at menopause (0.13 per minor allele), and lower levels of serum FSH. In addition, increased nulliparity was associated with the minor allele. This means that these women had a reduced chance of pregnancy. On the other hand, the T allele was shown to have a protective impact against endometriosis[43]. Another study, also in 2016 on healthy girls of peripubertal age, did not find any association of the rs10835638 with the FSH serum levels, although they showed a positive association with LH levels. Carriers and homozygotes of the T allele had significantly higher LH levels compared with GG homozygotes. LH/FSH ratio was, also, impacted by this polymorphism, which means that the *FSHB* c.-211G>T is involved in PCOS since LH/FSH ratio is considered its biochemical hallmark[41].

A meta-analysis was performed in 2018 and gathered data from 33 studies. In these studies, many polymorphisms in the FSHR gene were associated with various markers of fertility such as the FSH consumption during COS, the stimulation duration, the number of retrieved oocytes, the number of mature MII oocytes and the ongoing pregnancy rate (OPR) which is defined as "a pregnancy diagnosed by ultrasonographic visualization of at least one gestational sac". Most of the studies resulted in the FSHR 919 A>G Thr307Ala (rs6165) having a distinct impact on the above markers. Five studies showed that the AA homozygotes had more oocytes retrieved than the GG and AG carriers. Three studies supported that the stimulation protocol lasted longer in the AG heterozygotes compared with the AA. A majority of studies found that the number of oocytes retrieved (21 studies) as well as the number of metaphase II oocytes (5 studies) were higher in women having the non variant AA genotype than the variant GG. Another SNP that was observed to affect

the stimulation protocol in some studies was the FSHR -29 G>A (rs1394205). The FSH consumption during the stimulation protocol was significantly higher in the minor allele homozygotes as opposed to the GG and AG genotypes. Although, the same results were observed for the other two SNPs of FSHR, the rs6165 and . Higher amounts of FSH were required during controlled ovarian stimulation in T carriers for the rs6165 and G carriers for the rs6166 [44].

Genetic factors play a crucial role on the fertility of men, hence various studies are focusing on associating polymorphisms on different genes with conditions that cause male infertility. One of these studies, published in 2021 used 2742 men with idiopathic infertility and studied its association with FSHB c.-211G>T (rs10835638). Men were divided into two cohorts based on total sperm count (TSC). In cohort A eligible were men with TSC ≥1mil/ejac and in cohort B men with TSC<1mil/ejac. The results showed that the FSHB T allele carriers as well as higher median FSH level and lower bitesticular volume were observed in men in cohort B than in cohort A . They concluded that the polymorphism has a positive effect on serum FSH levels and a negative effect on testicular size and sperm count. [45]

In 2020, a cross-sectional study gathered data from 2020 Danish men and divided them into two groups based on their sperm parameters, their reproductive hormones and testicular size. Genotyping was performed for 3 polymorphisms:

1. FSHB c.-211 G>T (rs10835638),
2. FSHR c.-29 G>A (rs1394205) and
3. FSHR c.2039 A>G (rs6166)

For FSHB polymorphism the results showed that heterozygous and homozygous T allele carriers had lower levels of serum FSH compared to homozygous G carriers. Also the percentage of T allele carriers was higher amongst men with FSH levels lower than median FSH. The same

results were obtained for inhibin B, since the percentage of T allele carriers were also higher in the group of men with levels of inhibin B below median. Carriers of the T allele had significantly lower number of progressively motile spermatozoa than GG homozygous men. Although it would be obvious that the TT homozygous would have the worst phenotype concerning the motility of the spermatozoa, no significant result could be obtained probably due to insufficient number of men with this genotype.

As for the FSHR polymorphisms (rs6166 & rs1394205) they were both associated with higher FSH levels and smaller testis size but no significant difference was observed concerning semen parameters.

In addition, minor allele carriers, for FSHB -211G>T and FSHR 2039A>G SNPs were shown to have lower free testosterone/LH ratio[46].

A Genome Wide Association Study that was performed in men with idiopathic or/and unexplained infertility. Their approach was about identifying SNPs that may affect FSH serum levels and therefore may be contributing factors of infertility among men. The results showed a strong association of polymorphisms on FSHB gene and FSH serum levels. 9 SNPs were identified and associated with FSH serum levels in the 11p14.1 region, all of them located upstream and downstream of FSHB gene and seemingly in strong LD. Two of them, rs11031005 and rs10835638, were shown to be in strong association with FSH levels and FSH/LH ratio. The first of these variations can explain about the 6.95% of the serum FSH level variance in a subgroup of oligozoospermic men. It is also stated in this study that the rs11031005 may be a better predictor of serum FSH levels than the rs10835638 because the second has a lower frequency among oligozoospermic men and the two variations are in a strong but not complete LD of 74% and also because the rs11031005. The study concludes that variations found on the genomic region on chromosome 11p14.1, which embeds the FSHB gene, are eligible of use for diagnostic

purposes in order to find factors that contribute to infertility of unknown origin amongst men [47].

Follicle Stimulating Hormone Receptor (FSHR)

Follicle Stimulating Hormone Receptor (FSHR) is an transmembrane G-protein coupled receptor that consists of 695 amino acids and weighs 76 kDa. It is a member of the subfamily of rhodopsin - like receptors. The FSHR gene has more than 190Kb and is located in chromosome 2 p.21 - p.16. It consists of 10 exons and 9 introns. Exons 1 - 9 encode for the long extracellular ligand binding domain, whereas exon 10 encodes for the 7 transmembrane spanning domains and for the intracellular C-terminal tail. It is expressed mainly in granulosa and sertoli cells and it is a crucial factor of steroid synthesis and gametogenesis (see Fig. 6). The genomic region that embeds the FSHR gene is considered a hot spot for ovarian response to gonadotropins. Many SNPs falling into this region have been studied and shown to modulate the receptor's expression and signaling.

Many polymorphisms that modulate this receptor have been shown to have an impact on the fertility of either males or females. One of the most extensively studied polymorphism is the c.2039 A>G (rs6166) on exon 10 and features the substitution of Asparagine to Serine on the position 680 (Asn680Ser), which is located on the receptor's intracellular domain[48]. Recent study in 161 ovulatory women showed that in those with the Ser/Ser variant higher levels of FSH serum were detected and also they required higher doses of exogenous rFSH in order to accomplish the same results with the other genotypes [48]. In another study of 2014 on primary granulosa lutein cells, different kinetics of the receptor's response to exogenous FSH were observed in homozygous Ser/Ser

variants. The early increase of intracellular cAMP is vital for the progesterone production and the homozygous variants were shown to reach the plateau with about 1h delay after FSH treatment compared to the other variants. Different kinetics of the receptor's response to FSH and the late cAMP increase have an impact on the phosphorylation of ERK1/2 and CREB, the progesterone production and gene expression [49]. Another study of 2021 by Polyzos *et al.* included 368 patients from Europe and Asia. They underwent ovarian stimulation with a GnRH antagonist protocol and followed by oocyte retrieval. A fixed daily dose of 150 IU rFSH was administered and no dose adjustments were made during the stimulation. They showed that patients homozygous for the G/G variant had statistically significant fewer oocytes retrieved than A/A variants (about 1 oocyte less). They also observed that carriers of the G variant were more often in the group of hypo - responders than A/A homozygotes. The G allele was also associated with low Follicle to Oocyte Index (FOI), as the G carriers (G/A & G/G) had a lower FOI than women with A/A genotype [50]. Another observation of this study was the higher frequency of G/G homozygotes in the Caucasian population (23.2%) compared to the Asian population (9.5%). The same results were associated with another polymorphism studied by Polyzos *et al.,* 919A>G which results in a substitution of Threonine by Alanine at position 307 (Thr307Ala, rs6165). The frequency of homozygous G/G patients was 34.8% and the polymorphism was significantly higher in Caucasian compared to Asian patients (44.5% vs 23.2%). The FOI was also lower in carriers A/G than A/A women 79.75±3.35 vs 92.08±6.23. There was no significant association of the rs6165 with ovarian response, number of oocytes retrieved and Follicular output rate (FORT) which is defined as the "ratio between the number of follicles that reach preovulatory maturation in response to FSH and the available pool of FSH-sensitive follicles'' [50] [51]. Another study of 2021 in 210 infertile women, observed that as for the c.2039 A>G Asn680Ser(rs6166),the frequency of the AA, AG and GG

genotypes was 49%, 45.7% and 5.2% respectively. There was no statistical significant difference in the frequency of the FSHR c.2039 Asn680Ser polymorphism amongst the three ovarian response groups:

1. Low prognosis (number of retrieved oocytes ≤9)
2. Normal response (number of oocytes retrieved 10-15)
3. High response (number of oocytes retrieved ≥15)

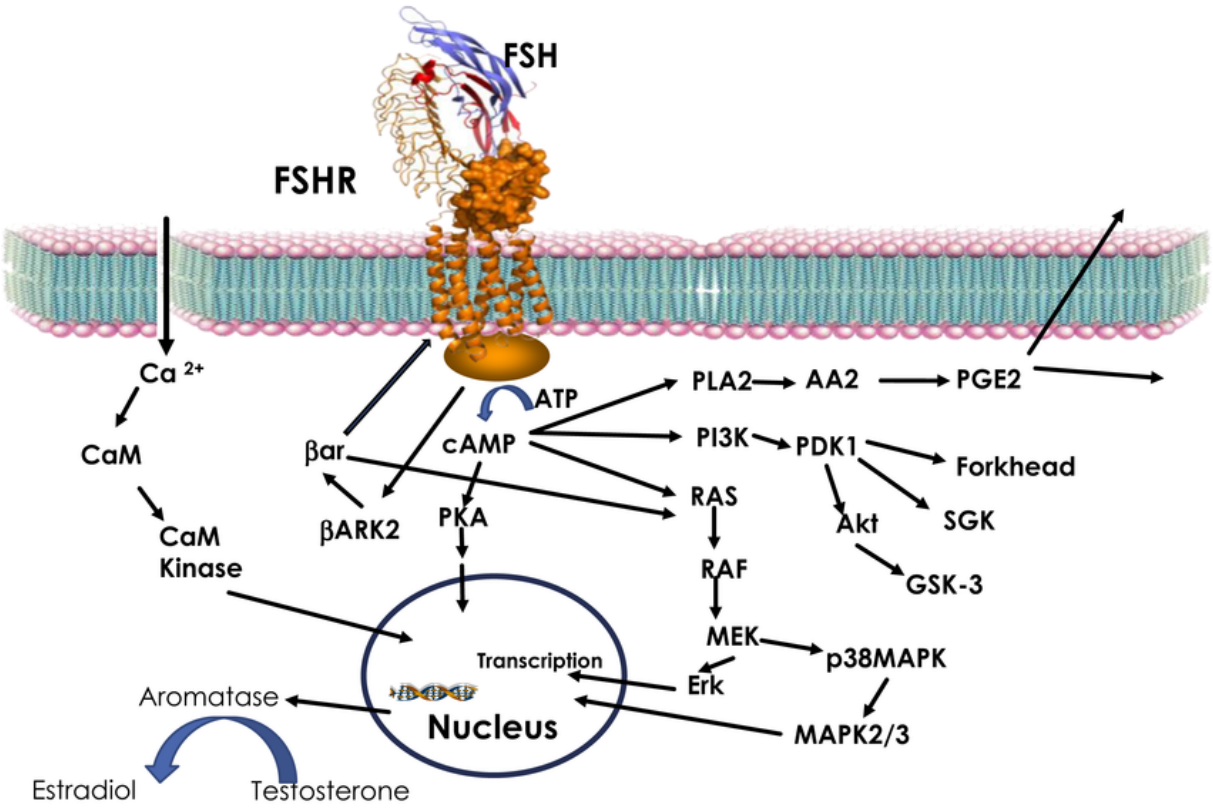
However, when they combined the heterozygous and homozygous women (AG & GG) they observed that these women were significantly more in the low prognosis group than the AA homozygotes. The AA women also had significantly higher AMH levels than AG (4.24±3.79 vs 2.72±2.86)[52]. Significant differences were also observed concerning the AFC, the number of oocytes retrieved, the number of mature MII oocytes, the number of fertilized 2PN and the total number of embryos given amongst the AA genotype the AG genotype and the combination of heterozygous and homozygous variants AG & GG. Patients with the AA genotype had higher AFC, gave more oocytes per retrieval, more Metaphase II, more 2PN and more Embryos than than the other genotypes.

	AA	AG	AG/GG	GG	p value
AMH	4.24±3.79	2.72±2.86	2.82±3.55	3.66±7.30	0.006 (AA vs AG) 0.007 (AA vs AG/GG)
AFC	11.94 ±6.95	8.32±5.54	8.82±6.55	9.90±4.86	<0.001 (AA vs AG) 0.001 (AA vs AG/GG)
Oocytes Retrieved	11.80±7.89	9.14±7.31	9.33±7.37	11.00±8.07	0.015 (AA vs AG) 0.002 (AA vs AG/GG)
MII	10.34±7.30	7.56±6.16	7.80±6.34	9.91±7.75	0.005 (AA vs AG) 0.008 (AA vs AG/GG)
2PN	7.53±5.53	5.67±5.03	5.72±5.00	6.18±4.85	0.013 (AA vs AG) 0.013 (AA vs AG/GG
Embryos	7.84±5.87	5.82±5.15	5.87±5.10	6.27±4.84	0.011 (AA vs AG) 0.01 (AA vs AG/GG)

Table 1. Differences between the 3 genotypes and their combination concerning the FSHR c.2039 A>G Asn680Ser (rs6166) as for AMH levels, Antral Follicular Count (AFC), number of oocytes retrieved, number of mature MII, number of fertilized oocytes (2PN), and embryos given.[18]

Another SNP that has a distinct impact on the receptor and is in strong linkage disequilibrium with the rs6166, is the substitution of Adenine to Guanine at nucleotide 919 which leads to Threonine 307 being replaced by Alanine (919 A>G Thr307Ala, rs6165). In the same study by Polyzos *et al.* which included 368 patients from Asia and Europe the homozygous GG patients were 34.8%. Also in the group of AG carriers the FOI were significantly smaller than the AA homozygous (79.75±3.35 vs 92.08±6.23

respectively). The frequency difference was also obvious between the Caucasian and Asian women, since the frequency of the polymorphism in Caucasian was 44.5% whereas in Asian was 23.2%. No statistically significant difference was observed regarding ovarian reserve, number of oocytes retrieved or the FORT among the FSHR re6165 genotypes.



The levels of Progesterone and Estradiol in the late follicular phase have been associated with the ovarian reserve[50]. In a multicentre study by Neves *et al.* 366 predicted normoresponders underwent ovarian stimulation protocol with GnRH antagonist protocol, they were genotyped for three polymorphisms of the FSH receptor (rs6165, rs6166 and rs1394205) and their serum progesterone and estradiol was measured at the day of the trigger. The study showed no significant difference concerning the serum progesterone levels in the mean late follicular phase or the estradiol levels on the day of trigger in dominant, co-dominant and recessive models for the three polymorphisms of FSHR. However, haplotype analysis for the rs6165/rs6166 haplotypes AA, GA and GG when

compared to AG revealed lower E2 levels on the day of trigger. The two polymorphisms (rs6165 and rs6166) have been combined before [54]. In 2014 a study which included 69 women aged 15-38 (mean age 28y/o) from which follicular fluid and granulosa cells were collected from various stages of the menstrual cycle under physiological FSH conditions and then and correlated the different genotypes with:follicle diameter, AMH levels, PRG levels, E2 levels, testosterone and androstenedione and FSHR, LHR, androgen receptor, aromatase cytochrome p450 (CYP19A1), AMH & AMHR2 gene expression. The three genotypes and their frequencies were:

Genotype	Frequency
A/A (p.307 Thr/Thr, p.680 Asn/Asn)	35%
A/G (p.307 Thr/Ala, p.680 Asn/Ser)	42%
G/G (p.307 Ala/Ala, p.680 Ser/Ser)	23%

The G/G genotype was associated with high expression of LHR and low expression of AMHR2 in all follicle sizes. In addition, in follicles 3-6mm diameter high LHR expression and low AMH expression was correlated with G/G genotype as well as in follicles of >6mm diameter showed significantly higher E2 and CYP19A1 gene expression. [53]

Another study from Iran published in 2017, correlated the rs6165 with the ovarian response. 198 women who underwent Assisted Reproduction Technology were categorised into two groups according to the number of oocytes that were retrieved, the poor responders (>5 mature oocytes)and the good responders(<5 mature oocytes). The results showed lower levels of FSH and LH in good responders. On the contrary, good responders showed higher numbers of oocytes retrieved, MI and MII mature oocytes, total embryos and AMH. After genotyping these patients, the frequency of FSHR rs6165 AA homozygous patients was higher in the group of poor responders (32%) vs their frequency in good responders

(17%) p=0.029. The frequency, also, of the A allele was significantly higher in the group of poor responders (52%) vs that of good responders (40%) p=0.026. These findings led to the proposition that genotyping the FSHR rs6165 can be a possible predictor of ovarian response in ART [55].

Polycystic Ovary Syndrome (PCOS) is a common endocrine disorder in women and can be a serious reason for infertility in women of reproductive age. A study from 2017 recruited 377 women with PCOS and 388 controls and investigated whether the FSHR rs6165 and rs6166 were associated with susceptibility to PCOS. They also found that these 2 polymorphisms are in almost complete linkage disequilibrium (r^2=99%). The genotype distributions of rs6165 and rs6166 revealed a significant correlation of the homozygous variants of Asn680Ser, Thr307Ala and PCOS since the frequency of Ser/Ser in women with PCOS vs controls was 13.3% vs 8.2% (p=0.035) and the frequency of Ala/Ala was 14.9% vs 8.0% (p=0.005) respectively. This study could not find an association between the FSHR polymorphisms and serum FSH, AFC and ovarian size. Although, a larger group of subjects may be needed to reveal any small differences in serum FSH [56].

Fig. 6: FSH signaling. Activation of FSHR by FSH leads to increase in intracellular cAMP through Gs-adenylate cyclase. Increased cAMP leads to PKA activation, which regulate expression of several genes through phosphorylation of transcription factors like CREBP. FSH also causes increase in Ca^{2+} by depolarization of Ca channels. Increased Ca^{2+} can upregulate calmodulin kinase leading to modulation of downstream effectors. In addition to cAMP, FSH has also been shown to modulate PLA, Erk, p38 MAPK, and PI3Kinase pathways. Activated FSHR is phosphorylated by BARK, which in turn recruits β-arrestin to the receptor and lead to down regulation of FSHR, in addition, β-arrestin independently can activate Erk pathway.
(Nataraja, Selvaraj & Yu, Henry & Palmer, Stephen. (2015). Discovery and Development of Small Molecule Allosteric Modulators of Glycoprotein Hormone Receptors. Frontiers in Endocrinology. 6. 142. 10.3389/fendo.2015.00142.)

Anti-Müllerian Hormone (AMH)

One of the most important molecules concerning fertility and therefore ART is Anti-Mullerian Hormone (AMH). AMH plays a crucial role in sexual differentiation of both sexes. In males, it is critical for the involution of the Mullerian ducts and in females functions as a regulator of follicular development and as a predictor of ovarian reserve. AMH is a dimeric glycoprotein and a member of the transforming growth factor β superfamily. It is produced by granulosa cells of the early developing follicles in the ovary. When the follicles reach the size of 4-6mm and a state of differentiation at which AMH becomes receptive for FSH they may be selected for dominance. AMH may have a pivotal role in the inhibition of primordial follicle development since studies have shown that in the absence of AMH, follicles are recruited faster and are more sensitive to FSH [57].

The AMH c.146 G>T lies on the promoter of the gene and leads to an amino acid substitution of isoleucine 49 to serine (Ile49Ser) (rs10407022). This region is responsible for protein stability and folding. There is not much data concerning this SNP, however it seems that it might affect the action of the protein. The bioactivity is compromised but its processing is not. The results that concern the rs10407022 are contradictory. A study in Dutch women showed that those with the Ser/ Ser genotype had significantly higher estradiol levels on day 3 of the menstrual cycle [58]. On the contrary, in a later study in Chinese women, no significant correlation between this polymorphism and estradiol levels was found [59]. In 2015, in a paper by Peluso *et al* it was stated that the rs10407022 is not significantly associated with the oocyte retrieval rate or the antral follicular count, but the embryos produced were statistically different among the genotypes. Also the T/Ser allele's frequency was higher amongst infertile patients compared with controls [57]. In 2020, a study concerning Premature Ovarian Insufficiency (POI) in Iraqi women,

concluded that the GT and TT genotypes of rs10407022 are positively associated with POI [60]. A meta - analysis was conducted by Di Chen *et al.* and included 7 studies with 2078 total participants. They found that in the Asian population women with the TT genotype had fewer oocytes retrieved than those with the GT/GG genotype. However, more metaphase II (MII) oocytes were retrieved in the II genotype [61].

Anti-Müllerian Hormone Receptors (AMHR)

Like most TGFβ family members, AMH signals through a heterodimeric receptor complex consisting of two kinase receptors. These related Ser/ Thr receptors are type I and type II receptors. Ligand binding to the type II receptor leads to the recruitment of the type I and its phosphorylation by the first. Phosphorylation of downstream Smad proteins follows and they interact with the common Smad4. This complex translocates into the nucleus in which it regulates gene expression [62].

There have been few studies comparing the AMH and AMHR polymorphisms with the outcome of ART. Two SNPs are more extensively studied in them. The AMH c146G>T Ile49Ser (rs10407022) and the AMHRII c-482 A>G (rs2002555).

As for the AMH receptors there are several polymorphisms affecting fertility. One of the most extensively studied is an A>G change in the position c-482 of the gene promoter (rs2002555), which was shown to affect the length of the stimulation as well as the amount of gonadotropins needed. Women with the AA genotype were stimulated, on average, 9.1 +1.4 days vs women with the AG/GG genotype were stimulated for 9.7 +1.3 days. In addition the AG/GG genotype required higher units of gonadotropins than AA genotype [63]. The minor allele (G) has, also, been associated with poor ovarian response [64]. However, there was no

significant difference in the number of oocytes retrieved after FSH administration, or with the ovarian reserve. The rs3741664 is another SNP that has been correlated with fertility dysfunctions. It refers to an amino acid change from adenine to guanine in the non-coding position 4952 of the AMHRII gene. In a recent study of 2019, women with the GG genotype needed higher doses of rFSH during COS in comparison with patients with the AG genotype [65]. This polymorphism is another sample of contradictory results in different studies. Although it was stated that the GG genotype needs higher doses of FSH during COS, another study from 2020 from Rafaa *et al* showed that the AG and GG genotypes have a decreased risk of POI [60].

Sex Hormone - Binding Globulin (SHBG)

Sex Hormone-Binding Globulin (SHBG) is a homodimeric 90-kDa glycoprotein, largely synthesised in the liver. It consists of two 373 amino acid subunits and its main function is steroid transportation. Each dimer of SHBG has 1 steroid binding site, therefore can transport 2 steroid molecules. At any time zero, one or both binding sites may be occupied and it is possible that different steroids may be bound to the same SHBG protein at the same time. It is specific for testosterone, 5-alpha-dehydroandrosterone and 17-beta-estradiol however its affinity of testosterone is approximately double that of estradiol. It regulates the plasma metabolic clearance rate of these hormones by controlling their plasma concentration. The gene encoding for SHBG is located on chromosome 17p13.

The protein levels are regulated at transcriptional and translational level. Missense mutations may alter these levels. Since SHBG transports testosterone, it is associated with conditions like metabolic syndrome (MetS) and PCOS both of which are hyperandrogenic. In a study of 2018,

from China, 478 women that underwent IVF procedures where selected for a case-control study that tried to associate 2 polymorphisms of SHBG with the outcome of in-vitro fertilisation-embryo transfer for PCOS patients. Those polymorphisms where the rs6259, a missense mutation at nucleotide 5790 in exon 8 where a G is substituted by either A or C, leading to the substitution of Aspartic acid by Asparagine at 327 position (D327N) and rs727428. The second did not show significant association with the outcome of IVF in PCOS patients. However rs6259 adenine carriers (GA or AA) where found to be elevated in the PCOS group suggesting that the A allele might be a risk factor for PCOS. In addition, in contrast to individuals with the TT genotype at the SHBG rs6259 locus, those with the GA/AA genotype exhibited a decrease in the number of retrieved oocytes and embryos given, as well as a lower fertility rate. Conversely, there was an increase in the abortion rate, incidence of ovarian hyperstimulation syndrome, transplant rejection rate, serum estradiol, serum testosterone and testosterone in the follicular fluid among carriers of the A allele. The study concludes that the rs6259 might have an impact of the outcome of the IVF treatment for PCOS patients [66]. The studies that associate the rs6259 and rs727428 with PCOS have contradictory results. Although in the previous study by Liu Y *et al* in 2019 showed a significant association of rs6259 with PCOS, a year later a meta-analysis found no significant connection of these two[67]. This statement is also supported by another meta-analysis by Li Y, conducted in 2021 [68]. Another broadly studied polymorphism of SHBG is a (TAAAA)n polymorphism on the promoter of the gene which has been found to affect transcriptional activity. The number of the pentanuclotide repeats is highly variable with studies reporting 6-11 repeats in the human SHBG gene[68]. In addition >8 repeats seem to be significantly associated with high risk of PCOS since women in the PCOS group seemed to carry 8 or more TAAAA repeats with significant higher frequency than the control group[68, 69].

P450 Aromatase, CYP19

Aromatase cytochrome P450 is a key enzyme in the conversion and metabolism of androgens to estrogens. The CYP19A1 gene, located on the short arm of chromosome 15 (15q21.2), encodes the crucial enzyme aromatase, responsible for synthesising estrogen. This gene is expressed in vital organs such as ovaries, testicular, adipose, bone, placental, and cerebrum tissues, with the highest activity observed in female ovaries.
Aromatase, organised by a 93 kb gene regulative factor and controlled by nine consecutive exons, plays a pivotal role in estrogen production. The promoter region, positioned 1 kb upstream of exon two, governs aromatase enzyme production in the ovaries. Mutations in the CYP19A1 gene have been linked to aromatase deficiency, characterised by low estrogen and elevated androgen levels.
The irregular activation of the promoter may contribute to estrogen-stimulating disorders, including conditions such as breast cancer and endometriosis. Therefore, understanding the genetic and regulatory aspects of CYP19A1 is crucial for comprehending hormone synthesis and its implications in various health conditions [70].

The gene of CYP19A1 has been studied as of its correlation with several conditions that might affect the outcome of IVF, such as PCOS, endometriosis, oocyte yield after COS or estradiol levels. It has been found that the TT genotype for c.*19C>T CYP19A1 (rs10046) was associated with fewer oocytes after ovarian stimulation and therefore lower ovarian response compared to the controls [71]. Although different studies may have different results as for the same polymorphisms. No statistical significance between the rs10046 and the oocytes retrieved was found in a study conducted by Amaro *et al.* Elevated estradiol level was

found in women carrying the AA genotype in the same study[88]. The same polymorphism has been studied for its possible connection with endometriosis. However several studies could not correlate the rs10046 with endometriosis [27,28]. More studies are needed in order to spot a possible association of this SNP with endometriosis. [72].

Superoxide dismutase 2 (SOD2)

The SOD2 gene, also known as MnSOD, encodes for manganese superoxide dismutase, a mitochondrial protein that functions as a homotetramer. Each monomer of this protein requires a manganese ion as a cofactor. SOD2 plays a crucial role in mitigating mitochondrial reactive oxygen species (mtROS) by catalyzing the dismutation of superoxide ($O_2^{\cdot -}$)generated by the electron transport chain (ETC) into H_2O_2, along with the production of O2. This process occurs in both the mitochondrial matrix and intermembrane space, contributing significantly to mitoochondrial reactive oxygen species (mtROS) detoxification. This enzyme is an endogenous antioxidant that is synthesised within the cytosol and post transcriptionally is transported in the mitochondria. Altered expression or activity of SOD2 can have profound effects on mitochondrial function and may be implicated in the development of various diseases.

Although the knockout of SOD2 does not impact embryonic development, it leads to post-birth mortality and a substantial reduction in ETC activity in mice. Therefore, SOD2 activity is crucial for maintaining the functionality of mitochondrial complexes, especially in the presence of oxidative stress, highlighting its pivotal role in preserving mitochondrial function.[73] Superoxide dismutase 2 gene is located on chromosome 6q25 and the protein consists of 222 amino acids. SOD2 plays a major

role in the defence of the organism against oxidative stress and as shown by several studies it is up-regulated in case of oxidative stress. For instance, in a study that compared gene expression between smoking and non smoking women undergoing IVF procedures, it was found that in the case of smokers SOD2 along with interleukin 6 (IL6) are up-regulated [74]. These 2 enzymes play a pivotal role in the battle against oxidative stress. Also in another study the effects of citrate clomiphene (CC) and letrozole were investigated with and without the presence of estradiol. It was found that in cumulus cells cultured with CC and letrozole without estradiol, SOD2 and Bax genes were up-regulated. A phenomenon that seem to be reversed in the presence of estradiol [75].

As such, the results indicate that these drugs increase the oxidative stress in cumulus cells, therefore SOD2 is up-regulated in order to decrease it.

Polymorphisms in the SOD2 gene have been associated with conditions that affect the fertility of both females and males. Specifically the polymorphism that is referring to a C substitution from a T on the second exon of SOD2 gene and leads to an amino acid substitution of Alanine 16 to Valine (Ala16Val) can impair the activity of the enzyme. The Ala16 variant of the SOD2 gene is noted for boosting the antioxidant enzymatic activity of the SOD2 protein in comparison to the Val16 variant. Individuals with the Ala16 variant may experience more effective neutralization of reactive oxygen species. Furthermore, it appears that the protein carrying the Ala16 variant exhibits enhanced capability to enter the mitochondrial matrix, the innermost compartment of mitochondria where crucial biochemical processes occur. This improved entry into the matrix could contribute to the protein's heightened efficiency in dealing with superoxide radicals. Additionally, the interaction of the SOD2 protein with the import channel in the inner mitochondrial membrane seems to be influenced by the Ala16 variant, potentially affecting the efficiency of protein import into the mitochondrial matrix. In summary, the Ala16 variant is associated with improved antioxidant activity, enhanced entry

into the mitochondrial matrix, and altered interaction with the import channel, collectively suggesting a more efficient role in counteracting oxidative stress within the mitochondria compared to the Val16 variant [76]. The *SOD2* pAla16Val (rs4880) have been correlated in recent studies in Slovenian [77], Turkish [76] and Saudi women [78] with an increased risk of developing PCOS. In fact, in the study conducted by Polat *et al*. the risk of PCOS in women with the TT genotype for rs4880 was 2 times greater than women with the AA genotype. In the same study, another polymorphism of SOD2 was associated with an increased risk of PCOS. The 3' UTR A>G (rs5746136) refers to a substitution of adenine by guanine and is located in the 65th base of the 3' untranslated region of the SOD2 gene downstream of the poly-A site. In this case, the G allele, also doubles the risk of developing PCOS in Turkish women. Combining these two polymorphisms,, having the AG/GG genotype for rs5746136 and the TT genotype for rs4880, results to a 3-fold increased risk of PCOS.

Glutathione S-transferase (GST)

Detoxification of electrophiles by glutathione conjugation is considered to be the main function of GST genes. The GST enzymes have traditionally been considered as part of the cellular defence mechanism against various harmful chemicals produced both within the body and in the environment. The general function of GST enzymes involves the addition of GSH to electrophiles with a diverse range of chemical structures. Key substrates include polycyclic aromatic hydrocarbon epoxides, which are produced through the catalytic actions of phase 1 cytochrome P-450s, as well as numerous by-products of oxidative stress. Oxidative stress, characterised by increased production of ROS and reduced activity of antioxidant

defence enzymes, is identified as a significant mechanism through which environmental substances affect male reproductive function, potentially leading to infertility. This suggests that variations in polymorphic genes encoding antioxidant and biotransformation enzymes, which influence individual differences in the ability to metabolise and eliminate environmental chemicals, could serve as modifiers of susceptibility to infertility in men [79]. While the precise physiological role of any specific GST cannot be accurately defined, *in vitro* data suggests that these proteins typically operate as dimeric enzymes. However, this inhibitory function is compromised during oxidative stress due to the covalent dimerisation of GSTP monomers. This alteration has consequential effects on c-jun, influencing cell proliferation and the expression of cell cycle regulators such as CCND1. There are two distinct supergene families responsible for encoding proteins with glutathione-S-transferase (GST) activity. Firstly, there are at least 16 genes that encode proteins expressed in tissue cytosols, and secondly, there are at least six genes expressed in membranes. In humans, eight unique gene families are involved in encoding soluble GST. These include alpha on chromosome 6, mu on chromosome 1, theta on chromosome 22, pi on chromosome 11, zeta on chromosome 14, sigma on chromosome 4, kappa (with an unknown chromosomal location), and chi (also known as omega) on chromosome 1. The kappa enzymes, although likely soluble, are expressed in mitochondria. Polymorphism has been identified in many genes within these families. However, the focus has primarily been on allelism in the mu, theta, and pi families so far. While extensive research has been conducted on the molecular basis for variation in the mu and theta class clusters, uncertainties remain regarding the importance of linkage dysequilibrium in the mu gene cluster and the potential basis for polymorphism in GSTT2 [80].

Polymorphisms on two very important, for the detoxification of oxidative stress, members of the GST genes, GST Mu-1 (*GSTM1*) and GST Theta-1 (*GSTT1*), located o chromosome 1p13.3 and 22q11, have been reported to lead in enzymatic loss of function. Therefore, there might be a correlation between idiopathic infertility and these polymorphisms. [81]. In a meta - analysis from 2010 by Economopoulos *et al*. GSTM1 was positively associated with idiopathic male infertility, although the results were controversial. This controversy existed probably due to the low number of studies used in the analysis [82]. However, 2 years later, a meta - analysis by Tang *et al*., used 15 case - control studies with 1897 cases and 1785 controls, also resulted in a positive correlation of *GSTM1 null* polymorphism and male idiopathic infertility [81]. GSTM1 and GSTT1 genes seem to have the highest rate of heterogeity among the GST genes. In a study of 2017, deletion variants of GSTT1 and GSTM1 were shown to have a significant effect on male infertility. The frequency of GSTT1 deletion variant was 2 times higher in the group of infertile men and the GSTM1 deletion variant was 1.4 times in the same group compared with the group of men with no fertility issue. In 19% of infertile men, complete deletion of both genes was observed compared to only 6% of fertile men having this genotype [83]. Another member of the GST genes is the GSTP1, located on chromosome 11q13. The GSTP1 polymorphism is a SNP located at c.313 A>G and lead to an Ile substitution from Val at position 105. This substitution may affect the binding affinity or the cellular activity of the enzyme and therefore disturb the balance of the detoxification system. As a result the individual is more susceptible to damage from oxidative stress and this could possibly lead to male infertility. A recent case - control study enrolled 246 males with idiopathic infertility and 117 controls and compared the semen quality according to their genotype. Three of the polymorphisms of GST genes were studied, GSTM1(-/+), GSTT1(-/+) and GSTP1 (c.313 A>G, Ile105Val, rs 1695). The frequency of the different genotypes in each group is shown on Table 2:

	INFERTILE MEN	CONTROLS	P value
GSTM1(-)	60.57%	41.88%	0.001
GSTT1(-)	62.60%	47.86%	0.008
GSTM1(-) & GSTT1(-)	38.62%	14.53%	<0.0001
GSTP1 (AG/GG)	32.11%	27.35%	0.847

Table 2: Frequency of different genotypes in the groups of infertile men and controls

The frequency of GSTM1(-) and GSTT1(-) genotypes are significantly higher in the group of men with idiopathic infertility. In addition, sperm concentration, motility and viability were significantly lower in the GSTM1(-) and GSTT1(-) groups compared to the GSTM1(+) and GSTT1(+). Sperm normality (the percentage of spermatozoa with normal morphology) was also lower in the group of GSTT1(-) than in the GSTT1(+). Comparing men with both GSTM1(-) and GSTT1(-) resulted obviously in lower sperm concentration, motility, viability and percentage of spermatozoa with normal morphology than men with GSTM1(+) and GSTT1(+). Sperm motility, also, was lower in the group of men with the GSTP1 (AG or GG) genotype than in the GSTP1 (AA) group. Other sperm parameters such as linearity (LN), curvilinear velocity (VCL), path velocity (VAP), straight - line velocity (VSL), beat cross frequency (BCF), straightness (STR) and wobble (WOB) were lower and the amplitude of lateral head displacement (ALH) was significantly higher in the GSTM1(-), GSTT1(-) and GSTM1(-)/GSTT1(-) groups compared with the GSTM1(+),GSTT1(+) and GSTM1(+)/GSTT1(+). Furthermore, straightness (STR) was significantly lower and amplitude of lateral head displacement (ALH) significantly higher in men with GSTP1 (AG or GG) genotype than wild type GSTP1 (AA). In addition, several parameters concerning semen Oxidative Stress (OS) were studied. Nitric Oxide (NO), a free radical synthesised by nitric oxide synthases and at high concentrations have been found to contribute to OS [84], malodialdehyde

(MDA), a lipid aldehyde the production of which is induced by the lipid peroxidation cascade, 8-hydroxy-2’-deoxyguanosine (8-OHdG) which facilitates DNA damage by limiting the repairing capacity of spermatozoa [85] and the total antioxidant capacity (TAC) were measured and compared according to the different genotypes. Levels of NO and 8-OHdG were elevated in the GSTM1(-), GSTT1(-) and GSTM1(-)/GSTT1(-) genotype groups compared to those in the GSTM1(+), GSTT1(+) and GSTM1(+)/GSTT1(+). MDA activity showed higher levels in the GSTT1(-) and GSTM1(-)/GSTT1(-) genotype groups compared toy the GSTT1(+) and GSTM1(+)/GSTT1(+). TAC was significantly lower in the GSTM1(-)/ GSTT1(-) group compared to the GSTM1(+)/GSTT1(+). Furthermore, the GSTP1 (A/G, G/G) genotype group exhibited higher levels of NO and 8-OHdG and lower TAC compared to the GSTP1 (A/A) group. The DNA fragmentation index (DFI) was also significantly elevated in the GSTT1(-) and GSTM1(-)/GSTT1(-) genotype groups compared to the GSTT1(+) and GSTM1(+)/GSTT1(+). Another recent study of 2023 comparing 345 men with idiopathic infertility and 215 healthy individuals had the same results concerning the GSTM1, GSTT1 and GSTP1 genotypes. In this study, OS, sperm parameters and fluoride - induced toxicity were measured. The results were similar with the ones before, since in the mutant genotypes OS, mitochondrial membrane potential (MMP) and DFI were significantly higher and simultaneously sperm parameters and testosterone were also significantly different than the wild type genotypes [GSTM1(+),GSTT1(+) and GSTP1 (A/A)] [86].

Table 3: Polymorphisms on several genes and their effect on fertility

Gene	Polymorphism	dbSNP code	Reference	Complication in IVF
LHβ	c.82 A>G Trp8Arg	rs1800447	Alviggi *et al.* 2018[13]	↑ FSH consumption during COS
LHCGR	insLQ	rs4539842	Javadi-Arjmand *et al,* 2019[15]	(+insertion) ↑ success rate inIVF
	+28G>C	rs4073366	O'Brien *et al.* (2013)[14]	↑ OHSS
	c.935A>G Asn312Ser	rs2293275	Thathapudi *et al.* (2015)[16], Atoum *et al.* (2021)[17]	↑ risk of PCOS
			Javadi-Arjmand *et al, (*2019)[15]	↑ success rate in IVF AA ⇒ ↓ higher FSH consumption GG ⇒ Premature Ovarian Failure
			Lindgren *et al.* (2019[19]	Ser⇒ ↑ live birth
			Ghaderian *et al.* (2019)[20]	↑ risk for OHSS
ESR1	-351A>G	rs9340799	Vassilopoulou *et al.* (2019)[25], Eldafira *et al.* (2021)[26], Szaflik *et al.* (2020)[28], van der Vaart *et al.* (2022)[27]	Endometriosis
			Jiao et al. (2018) [30]	↓ risk for PCOS
			Yin *et al.* (2018) [34], Refeat *et al.* (2021)[36]	↑ risk for Recurrent Pregnancy Loss
	-397T>C	rs2234693	van der Vaart *et al.* (2022)[27]	Endometriosis
			Douma *et al.* (2020)[31]	↑ risk for PCOS
			Bahia *et al.* (2020), Mu *et al.* (2021) [35], Refeat *et al.* (2021)[36]	↑ risk for RPL
			Ahmed *et al.* (2021)[38]	↑ frequency of poor responders
	Intron sub G>A	rs1999805	Jiao et al. (2018) [30]	↑ risk for PCOS
	Intron substitution	rs3798577	Douma et al. (2020)[31]	↑ risk for PCOS
	Intron substitution C>T / C>G	rs3020314	Douma et al. (2020)[31]	↑ risk for PCOS
ESR2	+1730G>A	rs4986938	Mu *et al.* (2021)[35]	↑ risk for RPL
			Lidaka *et al* (2021) [32]	↑ testosterone levels
		rs1256049	Douma et al. (2020)[31]	↑ risk for PCOS

Table 3: Polymorphisms on several genes and their effect on fertility

Gene	Polymorphism	dbSNP code	Reference	Complication in IVF
FSHβ	-211 G > T	rs10835638	Ruth *et al.* (2016) [43]	Longer menstrual cycle, Later age of menopause, Increased nulliparity BUT Protective against endometrioses
			Krenz *et al.* (2021) [45], Bang *et al.* (2021)[46]	Idiopathic male infertility ↓ FSH levels in men ↓ sperm motility
			Busch *et al.* (2016) [41]	↑ risk for PCOS
FSHR	919 A>G Thr307Ala	rs6165	Alviggi et al. (2018) [44]	↓ number of oocytes retrieved AG →longer stimulation ↑ FSH consumption during
			Polyzos *et al.* (2021)[50], Grynberg *et al.* (2023)[51]	↓ FOI (follicle - oocyte index)
			Kim *et al.* (2017) [56]	↑ risk for PCOS
	-29 G>A	rs1394205	Alviggi et al. (2018) [44]	↑ FSH consumption during COS
	c.2039 A>G	rs6166	Alviggi et al. (2018) [44]	↑ FSH consumption during COS
			Santi *et al.* (2018) [48]	↑ serum FSH ↑ FSH consumption during
			Kim *et al.* (2017) [56]	↑ risk for PCOS
			Guo *et al.* (2021) [18]	↓ number of oocytes retrieved ↑ number of hypo responders ↓ FOI (follicle - oocyte index) ↓ AMH
AMH	c.146 G>T Ile49Ser	rs10407022	Pelyso *et al.* (2015) [57]	Bad quality embryos More frequent in infertile patients
			Rafaa *et al.* (2020) [60]	↑ risk for Premature Ovarian Failure (POF)
			Chen *et al.* (2020) [61]	↓ number of oocytes retrieved
AMHR	c-482 A>G	rs2002555	Lledó *et al.* (2019) [63]	↑ time of stimulation ↑ gonadotrophin consumption during COS
			Ghaderian et al. (2019)[64]	↑ risk for POF
	c.4952 A>G	rs3741644	Čuš *et al.* (2019) [65], Rafaa *et al.* (2020)[60]	↑ FSH consumption during COS but

Table 3: Polymorphisms on several genes and their effect on fertility

Gene	Polymorphism	dbSNP code	Reference	Complication in IVF
SHBG	c.5790 G>A,C D327N	rs6259	Liu *et al.* (2019) [66]	↑ risk for PCOS ↓ number of oocytes retrieved and embryos given ↑ RPL, OHSS
	(TAAAA)n	rs35785886	Li *et al.* (2021)[68], Kalinderi *et al.* (2019)[69]	>8 repeats→PCOS
CYP19A1	c.*19C>T	rs10046	Song *et al.* (2019) [71], Amaro *et al.* (2019)[88]	↓ number of oocytes retrieved (contradictory)
SOD2	C>T Ala16Val	rs4880	Polat *et al.* (2020) [76], Herman *et al.* (2020)[77], Alkhuriji *et al.* (2021)[78]	↑ risk for PCOS
	3'UTR A>G	rs5746136	Polat *et al.* (2020) [76]	↑ risk for PCOS
GSTM1	GSTM1(-)		Tang *et al.* (2012) [81], Economopoulos *et al.* (2010)[82], Kolesnikova *et al.* 2017)[83], Zini *et al* (1995)[84], He *et al.* (2023)[86]	Idiopathic male infertility ↑ Oxidative stress
GSTT1	GSTT1(-)		Tang *et al.* (2012) [81], Economopoulos *et al.* (2010)[82], Kolesnikova *et al.* 2017)[83], Zini *et al* (1995)[84], He *et al.* (2023)[86]	Idiopathic male infertility ↑ Oxidative stress
GSTP1	c.313 A>G, Ile105Val	rs1695	Zini *et al* (1995) [84], He *et al.* (2023)[86]	Idiopathic male infertility ↑ Oxidative stress

Discussion

Investigation of genetic variations in genes linked to drug metabolism and fertility has been a major focus of research conducted in the past few years. All of the results suggest a strong correlation between certain genetic variants and several aspects of the outcomes of in vitro fertilisation (IVF). In order to further our knowledge of pharmacogenomics in the context of assisted reproduction, there is a definite need to collect and interpret these findings. The impact of polymorphisms on the ovarian response to IVF stimulation techniques has been the subject of several investigations. Interestingly, differences in genes related to hormone and gonadotropin receptors have been identified as potential indicators of the sensitivity of patients to ovarian stimulation. Comprehending these genetic variances can direct customised strategies for personalising stimulation protocols to maximise results for every patient. Research into the genetic variables that influence endometrial receptivity and embryo implantation has revealed considerable correlations between certain genetic polymorphisms and successful implantation. Differences in genes associated with uterine receptivity, cytokine production, and immune response have been found, indicating prospective areas for targeted therapy to increase implantation rates. Research into the genetic factors that affect fertility and IVF procedures has given us very promising results that could improve our strategies. Variations in genes involved in drug metabolism (like CYP450 enzymes), follicle maturation (such as FSH, FSHR, LH, and LHCGR), sex differentiation and ovarian reserve (like ERs and AMH) have been linked to differences in how people respond to fertility drugs. This knowledge could lead to personalised medication plans that reduce side effects and improve treatment results. Despite the promising findings, it's important to recognise the limitations of current research in this field. Variations in sample sizes, patient populations and methodologies, can lead to inconsistencies in results. Larger and more

precise investigations should be the goal of future research, in order to improve the accuracy of the already found, and maybe new correlations. Additionally, the inclusion of a variety of ethnicities will enhance the applicability of pharmacogenomic findings in the process of *in vitro* fertilisation. The idea that taking into consideration the genetic profile of each person into IVF protocols has great potential to improve outcomes. However, large genome - wide association studies (GWAS) are crucial in order to identify genetic variants associated with different drug responses in IVF. These studies should include diverse populations to identify population - specific genetic markers and also that the results are generalisable. Apart from identifying genetic variants, it is crucial to understand how these affect drug responses. Using genomic approaches—like transcriptomics, proteomics, and metabolomics—can reveal the pathways and networks affected by specific genetic differences. This research could lead to discovering more biomarkers for ovarian response and endometrial receptivity. The clinical utility and cost - effectiveness of personalised treatment strategies are aspects that need to be examined. Randomised controlled trials (RCTs) that incorporate pharmacogenomic testing into IVF protocols are essential in order to demonstrate these aspects. These trials should compare the standard IVF protocols with pharmacogenomic-regulated protocols as for the pregnancy rates, live birth rates and the frequency of side effects. Patients would be better equipped to make decisions about their course of therapy if they had access to thorough patient-clinician communication and extensive genetic counselling. As we move forward, a coordinated effort for standardisation, replication and further exploration of genetic correlations will be crucial for the utilisation of the full potential of pharmacogenomics in optimising IVF outcomes.

References

1. Britannica, The Editors of Encyclopaedia. "gametogenesis". Encyclopedia Britannica, 8 Jan. 2019, https://www.britannica.com/science/gametogenesis. Accessed 1 February 2023.
2. Britannica, The Editors of Encyclopaedia. "oogenesis". Encyclopedia Britannica, 17 Feb. 2019, https://www.britannica.com/science/oogenesis. Accessed 1 February 2023.
3. Britannica, The Editors of Encyclopaedia. "spermatogenesis". Encyclopedia Britannica, 21 Sep. 2022, https://www.britannica.com/science/spermatogenesis. Accessed 1 February 2023.
4. OVARIAN STIMULATION FOR IVF/ICSI Guideline of the European Society of Human Reproduction and Embryology. (n.d.). October 2019
5. Sadeghi, H. M., Adeli, I., Calina, D., Docea, A. O., Mousavi, T., Daniali, M., Nikfar, S., Tsatsakis, A., & Abdollahi, M. (2022). Polycystic Ovary Syndrome: A Comprehensive Review of Pathogenesis, Management, and Drug Repurposing. In International Journal of Molecular Sciences (Vol. 23, Issue 2). MDPI. https://doi.org/10.3390/ijms23020583
6. Szeliga, A., Calik-Ksepka, A., Maciejewska-Jeske, M., Grymowicz, M., Smolarczyk, K., Kostrzak, A., Smolarczyk, R., Rudnicka, E., & Meczekalski, B. (2021). Autoimmune diseases in patients with premature ovarian insufficiency—our current state of knowledge. In International Journal of Molecular Sciences (Vol. 22, Issue 5, pp. 1–11). MDPI AG. https://doi.org/10.3390/ijms22052594
7. Fainberg, J., & Kashanian, J. A. (2019). Recent advances in understanding and managing male infertility. F1000Research, 8. https://doi.org/10.12688/f1000research.17076.1
8. Takalani, N. B., Monaneng, E. M., Mohlala, K., Monsees, T. K., Henkel, R., & Opuwari, C. S. (2023). Role of oxidative stress in male infertility. In Reproduction and Fertility (Vol. 4, Issue 3). BioScientifica Ltd. https://doi.org/10.1530/RAF-23-0024
9. Tournaye, H., Krausz, C., & Oates, R. D. (2017). Novel concepts in the aetiology of male reproductive impairment. In The Lancet Diabetes and Endocrinology (Vol. 5, Issue 7, pp. 544–553). Lancet Publishing Group. https://doi.org/10.1016/S2213-8587(16)30040-7
10. Krausz, C., & Riera-Escamilla, A. (2018). Genetics of male infertility. In Nature Reviews Urology (Vol. 15, Issue 6, pp. 369–384). Nature Publishing Group. https://doi.org/10.1038/s41585-018-0003-3
11. Bieth, E., Hamdi, S. M., & Mieusset, R. (2021). Genetics of the congenital absence of the vas deferens. In Human Genetics (Vol. 140, Issue 1, pp. 59–76). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s00439-020-02122-w
12. Perera-Marín, G., Murcia, C., & González-Padilla, E. (2007). Luteinizing hormone (LH) isoforms in ruminants: Characterization and physiological relevance. In Animal Reproduction Science (Vol. 101, Issues 3–4, pp. 187–207). https://doi.org/10.1016/j.anireprosci.2007.03.011

13. Alviggi, C., Conforti, A., Santi, D., Esteves, S. C., Andersen, C. Y., Humaidan, P., Chiodini, P., de Placido, G., & Simoni, M. (2018). Clinical relevance of genetic variants of gonadotrophins and their receptors in controlled ovarian stimulation: A systematic review and meta-analysis. Human Reproduction Update, 24(5), 599–614. https://doi.org/10.1093/humupd/dmy019
14. O'Brien, T. J., Kalmin, M. M., Harralson, A. F., Clark, A. M., Gindoff, I., Simmens, S. J., Frankfurter, D., & Gindoff, P. (2013). Association between the luteinizing hormone/chorionic gonadotropin receptor (LHCGR) rs4073366 polymorphism and ovarian hyperstimulation syndrome during controlled ovarian hyperstimulation. Reproductive Biology and Endocrinology, 11(1). https://doi.org/10.1186/1477-7827-11-71
15. Javadi-Arjmand, M., Damavandi, E., Choobineh, H., Sarafrazi-Esfandabadi, F., Kabuli, M., Mahdavi, A., & Ghadami, M. (2019). Evaluation of the Prevalence of Exons 1 And 10 Polymorphisms of LHCGR Gene and Its Relationship with IVF Success. In J Reprod Infertil (Vol. 20, Issue 4).
16. Thathapudi, S., Kodati, V., Erukkambattu, J., Addepally, U., & Qurratulain, H. (2015). Association of luteinizing hormone chorionic gonadotropin receptor gene polymorphism (rs2293275) with polycystic ovarian syndrome. Genetic Testing and Molecular Biomarkers, 19(3), 128–132. https://doi.org/10.1089/gtmb.2014.0249
17. Atoum, M. F., Alajlouni, M. M., & Alzoughool, F. (2022). A Case-Control Study of the Luteinizing Hormone Level in Luteinizing Hormone Receptor Gene (rs2293275) Polymorphism in Polycystic Ovarian Syndrome Females. Public Health Genomics, 25(3), 89–97. https://doi.org/10.1159/000521971
18. Guo, C., Yu, H., Feng, G., Lv, Q., Liu, X., & Liu, X. (2021). Associations of FSHR and LHCGR gene variants with ovarian reserve and clinical pregnancy rates. Reproductive BioMedicine Online, 43(3), 561–569. https://doi.org/10.1016/j.rbmo.2021.06.016
19. Lindgren, I., Baath, M., Uvebrant, K., Dejmek, A., Kjaer, L., Henic, E., Bungum, M., Bungum, L., Cilio, C., Leijonhufvud, I., Skouby, S., Andersen, C. Y., & Giwercman, Y. L. (2016). Combined assessment of polymorphisms in the LHCGR and FSHR genes predict chance of pregnancy after *in vitro* fertilization. Human Reproduction, 31(3), 672–683. https://doi.org/10.1093/humrep/dev342
20. Ghaderian, S. M. H., Akbarzadeh, R., Mohajerani, F., Khodaii, Z., & Salehpour, S. (2019). The implication of single-nucleotide polymorphisms in ovarian hyperstimulation syndrome. Molecular Reproduction and Development, 86(8), 964–971. https://doi.org/10.1002/mrd.23171
21. Lindgren, I., Nenonen, H., Henic, E., Bungum, L., Prahl, A., Bungum, M., Leijonhufvud, I., Huhtaniemi, I., Yding Andersen, C., & Lundberg Giwercman, Y. (2019). Gonadotropin receptor variants are linked to cumulative live birth rate after *in vitro* fertilization. Journal of Assisted Reproduction and Genetics, 36(1), 29–38. https://doi.org/10.1007/s10815-018-1318-y
22. Pirtea, P., de Ziegler, D., Marin, D., Sun, L., Tao, X., Ayoubi, J. M., Franasiak, J., & Scott, R. T. (2022). Gonadotropin receptor polymorphisms (FSHR N680S and LHCGR N312S) are not predictive of clinical outcome and

live birth in assisted reproductive technology. Fertility and Sterility, 118(3), 494–503. https://doi.org/10.1016/j.fertnstert.2022.06.011
23. Chen, P., Li, B., & Ou-Yang, L. (2022). Role of estrogen receptors in health and disease. In Frontiers in Endocrinology (Vol. 13). Frontiers Media S.A. https://doi.org/10.3389/fendo.2022.839005
24. Yaşar, P., Ayaz, G., User, S. D., Güpür, G., & Muyan, M. (2017). Molecular mechanism of estrogen–estrogen receptor signaling. In Reproductive Medicine and Biology (Vol. 16, Issue 1, pp. 4–20). John Wiley and Sons Ltd. https://doi.org/10.1002/rmb2.12006
25. Vassilopoulou, L., Matalliotakis, M., Zervou, M., Matalliotaki, C., Krithinakis, K., Matalliotakis, I., Spandidos, D., & Goulielmos, G. (2019). Defining the genetic profile of endometriosis (Review). Experimental and Therapeutic Medicine. https://doi.org/10.3892/etm.2019.7346
26. Eldafira, E., Prasasty, V. D., Abinawanto, A., Syahfirdi, L., & Pujianto, D. A. (2021). Polymorphisms of estrogen receptor-α and estrogen receptor-β genes and its expression in endometriosis. Turkish Journal of Pharmaceutical Sciences, 18(1), 91–95. https://doi.org/10.4274/tjps.galenos.2019.94914
27. van der Vaart, J. F., & Merki-Feld, G. S. (2022). Sex hormone-related polymorphisms in endometriosis and migraine: A narrative review. In Women's Health (Vol. 18). SAGE Publications Ltd. https://doi.org/10.1177/17455057221111315
28. Szaflik, T., Smolarz, B., Romanowicz, H., Bryś, M., Forma, E., & Szyłło, K. (2020). Polymorphisms in the 3'UTR Region of ESR2 and CYP19A1 Genes in Women With Endometriosis. European Journal of Obstetrics and Gynecology and Reproductive Biology, 250, 241–245. https://doi.org/10.1016/j.ejogrb.2020.05.023
29. Méar, L., Herr, M., Fauconnier, A., Pineau, C., & Vialard, F. (2020). Polymorphisms and endometriosis: A systematic review and meta-analyses. Human Reproduction Update, 26(1), 73–103. https://doi.org/10.1093/humupd/dmz034
30. Jiao, X., Chen, W., Zhang, J., Wang, W., Song, J., Chen, D., Zhu, W., Shi, Y., & Yu, X. (2018). Variant alleles of the ESR1, PPARG, HMGA2, and MTHFR genes are associated with polycystic ovary syndrome risk in a Chinese population: A case-control study. Frontiers in Endocrinology, 9(AUG). https://doi.org/10.3389/fendo.2018.00504
31. Douma, Z., Dallel, M., Bahia, W., ben Salem, A., Hachani Ben Ali, F., Almawi, W. Y., Lautier, C., Haydar, S., Grigorescu, F., & Mahjoub, T. (2020). Association of estrogen receptor gene variants (ESR1 and ESR2) with polycystic ovary syndrome in Tunisia. Gene, 741. https://doi.org/10.1016/j.gene.2020.144560
32. Lidaka, L., Bekere, L., Rota, A., Isakova, J., Lazdane, G., Kivite-Urtane, A., Dzivite-Krisane, I., Kempa, I., Dobele, Z., & Gailite, L. (2021). Role of single nucleotide variants in fshr, gnrhr, esr2 and lhcgr genes in adolescents with polycystic ovary syndrome. Diagnostics, 11(12). https://doi.org/10.3390/diagnostics11122327
33. Bahia, W., Soltani, I., Haddad, A., Soua, A., Radhouani, A., Mahdhi, A., & Ferchichi, S. (2020). Association of genetic variants in Estrogen receptor

(ESR)1 and ESR2 with susceptibility to recurrent pregnancy loss in Tunisian women: A case control study. Gene, 736. https://doi.org/10.1016/j.gene.2020.144406
34. Yin, X. Q., Ju, H. M., Guo, Q., Zhao, L., Zhu, X. X., Wei, R., Zhang, Z., Zhang, Y. H., Wang, B., & Li, X. (2018). Association of estrogen receptor 1 genetic polymorphisms with recurrent spontaneous abortion risk. Chinese Medical Journal, 131(15), 1857–1865. https://doi.org/10.4103/0366-6999.237412
35. Mu, F., Shi, M., Huang, L., Wang, D., & Shen, A. (2021). The role of estrogen receptor-beta gene +1730G/ A polymorphisms in recurrent pregnancy loss A protocol for systematic review and meta-analysis. https://doi.org/10.17605/OSF.IO/EW9FB
36. Refeat, M. M., Shalabi, T., El-Bassyouni, H. T., & Shaker, M. (2021). The correlation of estrogen receptor 1 and progesterone receptor genes polymorphisms with recurrent pregnancy loss in a cohort of Egyptian women. Molecular Biology Reports, 48(5), 4413–4420. https://doi.org/10.1007/s11033-021-06459-x
37. Tang, L., Xiang, Q., Xiang, J., & Li, J. (2022). The haplotypes GCA and ACA in ESR1 gene are associated with the susceptibility of recurrent spontaneous abortion (RSA) in Chinese Han: A case-control study and meta-analysis. Medicine (United States), 101(21), E29168. https://doi.org/10.1097/MD.0000000000029168
38. Ahmed, I., Abdelateef, S., Abdel-Lah, M. A. K., Amor, H., & Hammadeh, M. E. (2021). Association between FSHR and ESR1 gene variants and ovarian response to gonadotropin in Egyptian women undergoing ICSI treatment. Reproductive Biology, 21(2). https://doi.org/10.1016/j.repbio.2021.100499
39. Ganesh, V., Venkatesan, V., Koshy, T., Reddy, S. N., Muthumuthiah, S., & Paul, S. F. D. (2018). Association of estrogen, progesterone and follicle stimulating hormone receptor polymorphisms with *in vitro* fertilization outcomes. Systems Biology in Reproductive Medicine, 64(4), 260–265. https://doi.org/10.1080/19396368.2018.1482030
40. Tanase, A., Nemescu, D., Popescu, R., Toma, B. F., Matasariu, R. D., & Onofriescu, M. (2020). Fsh receptor and fsh beta chain polymorphism involvement in infertility and endometriosis disease. Acta Endocrinologica, 16(2), 142–147. https://doi.org/10.4183/aeb.2020.142
41. Busch, A. S., Hagen, C. P., Almstrup, K., Main, K. M., & Juul, A. (2016). Genetic variations altering FSH action affect circulating hormone levels as well as follicle growth in healthy peripubertal girls. Human Reproduction, 31(4), 897–904. https://doi.org/10.1093/humrep/dew022
42. Polyzos, N. P., Neves, A. R., Drakopoulos, P., Spits, C., Alvaro Mercadal, B., Garcia, S., Ma, P. Q. M., Le, L. H., Ho, M. T., Mertens, J., Stoop, D., Tournaye, H., & Vuong, N. L. (2021). The effect of polymorphisms in FSHR and FSHB genes on ovarian response: a prospective multicenter multinational study in Europe and Asia. Human Reproduction, 36(6), 1711–1721. https://doi.org/10.1093/humrep/deab068
43. Ruth, K. S., Beaumont, R. N., Tyrrell, J., Jones, S. E., Tuke, M. A., Yaghootkar, H., Wood, A. R., Freathy, R. M., Weedon, M. N., Frayling, T.

M., & Murray, A. (2016). Genetic evidence that lower circulating FSH levels lengthen menstrual cycle, increase age at menopause and impact female reproductive health. Human Reproduction, 31(2), 473–481. https://doi.org/10.1093/humrep/dev318

44. Alviggi, C., Conforti, A., Santi, D., Esteves, S. C., Andersen, C. Y., Humaidan, P., Chiodini, P., de Placido, G., & Simoni, M. (2018). Clinical relevance of genetic variants of gonadotrophins and their receptors in controlled ovarian stimulation: A systematic review and meta-analysis. Human Reproduction Update, 24(5), 599–614. https://doi.org/10.1093/humupd/dmy019
45. Krenz, H., Sansone, A., Kliesch, S., Gromoll, J., & Schubert, M. (2021). FSHB Genotype Identified as a Relevant Diagnostic Parameter Revealed by Cluster Analysis of Men With Idiopathic Infertility. Frontiers in Endocrinology, 12. https://doi.org/10.3389/fendo.2021.780403
46. Bang, A. K., Almstrup, K., Nordkap, L., Priskorn, L., Petersen, J. H., Blomberg Jensen, M., Krause, M., Holmboe, S. A., Egeberg Palme, D. L., Winge, S. B., Joensen, U. N., Olesen, I. A., Hvidman, H. W., Juul, A., Rajpert-De Meyts, E., & Jørgensen, N. (2021). FSHB and FSHR gene variants exert mild modulatory effect on reproductive hormone levels and testis size but not on semen quality: A study of 2020 men from the general Danish population. Andrology, 9(2), 618–631. https://doi.org/10.1111/andr.12949
47. Schubert, M., Lanuza, L. P., Woste, M., Dugas, M., David Carmona, F., Palomino-Morales, R. J., Rassam, Y., Heilmann-Heimbach, S., Tuttelmann, F., Kliesch, S., & Gromoll, J. (2022). A GWAS in Idiopathic/Unexplained Infertile Men Detects a Genomic Region Determining Follicle-Stimulating Hormone Levels. Journal of Clinical Endocrinology and Metabolism, 107(8), 2350–2361. https://doi.org/10.1210/clinem/dgac165
48. Santi, D., Potì, F., Simoni, M., & Casarini, L. (2018). Pharmacogenetics of G-protein-coupled receptors variants: FSH receptor and infertility treatment. In Best Practice and Research: Clinical Endocrinology and Metabolism (Vol. 32, Issue 2, pp. 189–200). Bailliere Tindall Ltd. https://doi.org/10.1016/j.beem.2018.01.001
49. Casarini, L., Moriondo, V., Marino, M., Adversi, F., Capodanno, F., Grisolia, C., la Marca, A., la Sala, G. B., & Simoni, M. (2014). FSHR polymorphism p.N680S mediates different responses to FSH *in vitro*. Molecular and Cellular Endocrinology, 393(1–2), 83–91. https://doi.org/10.1016/j.mce.2014.06.013
50. Polyzos, N. P., Neves, A. R., Drakopoulos, P., Spits, C., Alvaro Mercadal, B., Garcia, S., Ma, P. Q. M., Le, L. H., Ho, M. T., Mertens, J., Stoop, D., Tournaye, H., & Vuong, N. L. (2021). The effect of polymorphisms in FSHR and FSHB genes on ovarian response: a prospective multicenter multinational study in Europe and Asia. Human Reproduction, 36(6), 1711–1721. https://doi.org/10.1093/humrep/deab068
51. Grynberg, M., Pytel, S., Peigne, M., & Sonigo, C. (2023). The follicular output rate in normo-ovulating women undergoing ovarian stimulation is increased after unilateral oophorectomy. Human Reproduction, 38(6), 1162–1167. https://doi.org/10.1093/humrep/dead056

52. Guo, C., Yu, H., Feng, G., Lv, Q., Liu, X., & Liu, X. (2021). Associations of FSHR and LHCGR gene variants with ovarian reserve and clinical pregnancy rates. Reproductive BioMedicine Online, 43(3), 561–569. https://doi.org/10.1016/j.rbmo.2021.06.016
53. Borgbo, T., Jeppesen, J. v., Lindgren, I., Lundberg Giwercman, Y., Hansen, L. L., & Yding Andersen, C. (2014). 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, 21(3), 255–261. https://doi.org/10.1093/molehr/gau106
54. Neves, A. R., Vuong, N. L., Blockeel, C., Garcia, S., Alviggi, C., Spits, C., Ma, P. Q. M., Ho, M. T., Tournaye, H., & Polyzos, N. P. (2022). The effect of polymorphisms in FSHR gene on late follicular phase progesterone and estradiol serum levels in predicted normoresponders. Human Reproduction, 37(11), 2646–2654. https://doi.org/10.1093/humrep/deac193
55. Kaviani, M., Ghaderian, S. M. H., Arefi, S., Hashemi, M., & Afjeh, S. S. A. (2017). Role of FSHR rs6165 and ESR2 rs4986938 polymorphisms in ovarian stimulation of Iranian women who underwent assisted reproduction treatment. Human Antibodies, 26(3), 121–126. https://doi.org/10.3233/HAB-170329
56. Kim, J. J., Choi, Y. M., Hong, M. A., Chae, S. J., Hwang, K., Yoon, S. H., Ku, S. Y., Suh, C. S., & Kim, S. H. (2017). FSH receptor gene p. Thr307Ala and p. Asn680Ser polymorphisms are associated with the risk of polycystic ovary syndrome. Journal of Assisted Reproduction and Genetics, 34(8), 1087–1093. https://doi.org/10.1007/s10815-017-0953-z
57. Peluso, C., Fonseca, F. L. A., Gastaldo, G. G., Christofolini, D. M., Cordts, E. B., Barbosa, C. P., & Bianco, B. (2015). AMH and AMHR2 polymorphisms and AMH serum level can predict assisted reproduction outcomes: A cross-sectional study. Cellular Physiology and Biochemistry, 35(4), 1401–1412. https://doi.org/10.1159/000373961
58. Kevenaar, M. E., Themmen, A. P. N., Laven, J. S. E., Sonntag, B., Fong, S. L., Uitterlinden, A. G., de Jong, F. H., Pols, H. A. P., Simoni, M., & Visser, J. A. (2007). Anti-Müllerian hormone and anti-Müllerian hormone type II receptor polymorphisms are associated with follicular phase estradiol levels in normo-ovulatory women. Human Reproduction, 22(6), 1547–1554. https://doi.org/10.1093/humrep/dem036
59. Xu, P., Shen, S. M., Zhang, X. L., Liang, F., Xie, G. bin, Yi, L., Gao, Q., & Wang, Y. (2013). Haplotype analysis of single nucleotide polymorphisms in anti-Müllerian hormone gene in Chinese PCOS women. Archives of Gynecology and Obstetrics, 288(1), 125–130. https://doi.org/10.1007/s00404-013-2734-y
60. Rafaa, T. A., Suleiman, A. A. J., Dawood, M. F., & Al-Rawi, A. M. (2020). Association of two single nucleotide polymorphisms rs10407022 and rs3741664 with the risk of primary ovarian insufficiency in a sample of Iraqi women. Molecular Biology Research Communications, 9(4), 141–144. https://doi.org/10.22099/mbrc.2020.36371.1477
61. Chen, D., Zhu, X., & Wu, J. (2020). Can polymorphisms of AMH/AMHR2 affect ovarian stimulation outcomes? A systematic review and meta-

analysis. Journal of Ovarian Research, 13(1). https://doi.org/10.1186/s13048-020-00699-4
62. Visser, J. A. (2003). AMH signaling: From receptor to target gene. Molecular and Cellular Endocrinology, 211(1–2), 65–73. https://doi.org/10.1016/j.mce.2003.09.012
63. Lledó, B., Llácer, J., Blanco, L., Ortiz, J. A., Morales, R., Fabregat, A., Guerrero, J., & Bernabeu, R. (2019). The relevance of the individual screening for genetic variants in predicting ovarian response. Pharmacogenetics and Genomics, 29(9), 216–223. https://doi.org/10.1097/FPC.0000000000000386
64. Ghaderian, S. M. H., Akbarzadeh, R., Mohajerani, F., Khodaii, Z., & Salehpour, S. (2019). The implication of single-nucleotide polymorphisms in ovarian hyperstimulation syndrome. Molecular Reproduction and Development, 86(8), 964–971. https://doi.org/10.1002/mrd.23171
65. Čuš, M., Vlaisavljević, V., Repnik, K., Potočnik, U., & Kovačič, B. (2019). Could polymorphisms of some hormonal receptor genes, involved in folliculogenesis help in predicting patient response to controlled ovarian stimulation? Journal of Assisted Reproduction and Genetics, 36(1), 47–55. https://doi.org/10.1007/s10815-018-1357-4
66. Liu, Y., Zhao, X. X., Hu, X. J., Yang, F., Lin, P., Cui, S. C., Zhao, W., Cao, X. Y., & Wang, Y. S. (2019). Effect of sex hormone–binding globulin polymorphisms on the outcome of *in vitro* fertilization-embryo transfer for polycystic ovary syndrome patients: A case-control study. Journal of Cellular Biochemistry, 120(3), 4675–4686. https://doi.org/10.1002/jcb.27756
67. Liao, X., & Cao, S. (2020). Association of the genetic polymorphisms rs6259 and rs727428 of the SHBG Gene with polycystic ovary syndrome risk: A meta-analysis. Genetic Testing and Molecular Biomarkers, 24(8), 492–501. https://doi.org/10.1089/gtmb.2019.0229
68. Li, Y., Fang, L., Yan, Y., Wang, Z., Wu, Z., Jia, Q., Cheng, J. C., & Sun, Y. P. (2021). Association between human SHBG gene polymorphisms and risk of PCOS: a meta-analysis. In Reproductive BioMedicine Online (Vol. 42, Issue 1, pp. 227–236). Elsevier Ltd. https://doi.org/10.1016/j.rbmo.2020.10.003
69. Kalinderi, K., Asimakopoulos, B., Nikolettos, N., & Manolopoulos, V. G. (2019). Pharmacogenomics in IVF: A New Era in the Concept of Personalized Medicine. Reproductive Sciences, 26(10), 1313–1325. https://doi.org/10.1177/1933719118765970
70. Heidarzadehpilehrood, R., Pirhoushiaran, M., Abdollahzadeh, R., Osman, M. B., Sakinah, M., Nordin, N., & Hamid, H. A. (2022). A Review on CYP11A1, CYP17A1, and CYP19A1 Polymorphism Studies: Candidate Susceptibility Genes for Polycystic Ovary Syndrome (PCOS) and Infertility. In Genes (Vol. 13, Issue 2). MDPI. https://doi.org/10.3390/genes13020302
71. Song, D., Huang, X. liang, Hong, L., Yu, J. min, Zhang, Z. feng, Zhang, H. qin, Sun, Z. gui, & Du, J. (2019). Sequence variants in FSHR and CYP19A1 genes and the ovarian response to controlled ovarian stimulation. Fertility

and Sterility, 112(4), 749-757.e2. https://doi.org/10.1016/j.fertnstert.2019.05.017
72. Méar, L., Herr, M., Fauconnier, A., Pineau, C., & Vialard, F. (2020). Polymorphisms and endometriosis: A systematic review and meta-analyses. Human Reproduction Update, 26(1), 73–103. https://doi.org/10.1093/humupd/dmz034
73. Yi Chen, Jiawen Lu, Chunchao Nie, 6-Mitochondrial dysfunction and underlying molecular mechanisms in acrylamide-induced toxicity, Editor(s): Marcos Roberto de Oliveira, Mitochondrial Intoxication, Academic Press, 2023, Pages 133-146, ISBN 9780323884624, https://doi.org/10.1016/B978-0-323-88462-4.00028-6. (https://www.sciencedirect.com/science/article/pii/B9780323884624000286) , Keywords: Acrylamide; Mitochondria; Molecular mechanisms; Oxidative stress
74. Konstantinidou, F., Budani, M. C., Sarra, A., Stuppia, L., Tiboni, G. M., & Gatta, V. (2021). Impact of cigarette smoking on the expression of oxidative stress-related genes in cumulus cells retrieved from healthy women undergoing ivf. International Journal of Molecular Sciences, 22(23). https://doi.org/10.3390/ijms222313147
75. Silveira CO, Oliveira RM, Moraes LM, Oliveira GG, Aguiar LPT, Souza FHS, Coelho SS, Prazeres PHDM, Del Puerto HL, Ferreira MCF. The Effect of Clomiphene Citrate and Letrozole in Apoptotic Pathways and Cell Cycle in Human Primary Cumulus Cells and the Protective Effect of Estradiol. Reprod Sci. 2022 Aug;29(8):2272-2281. doi: 10.1007/s43032-022-00961-8. Epub 2022 May 5. PMID: 35513593.
76. Polat, S., & Şimşek, Y. (2020). Five variants of the superoxide dismutase genes in Turkish women with polycystic ovary syndrome. Free Radical Research, 54(6), 467–476. https://doi.org/10.1080/10715762.2020.1802022
77. Herman, R., Jensterle, M., Janež, A., Goričar, K., & Dolžan, V. (2020). Genetic variability in antioxidative and inflammatory pathways modifies the risk for PCOs and influences metabolic profile of the syndrome. Metabolites, 10(11), 1–18. https://doi.org/10.3390/metabo10110439
78. Alkhuriji, A. F., Alomar, S. Y., Babay, Z. A., El-Khadragy, M. F., Alsharidah, A. R., Hanan, A., Alnafjan, A. A., & Mansour, L. (2021). Association SOD2 and PON1 Gene Polymorphisms with Polycystic Ovary Syndrome in Saudi Women. Molecular Syndromology. https://doi.org/10.1159/000519527
79. Yarosh, S. L., Kokhtenko, E. v., Churnosov, M. I., Solodilova, M. A., & Polonikov, A. v. (2015). Joint effect of glutathione S-transferase genotypes and cigarette smoking on idiopathic male infertility. Andrologia, 47(9), 980–986. https://doi.org/10.1111/and.12367
80. Strange, R. C., Spiteri, M. A., Ramachandran, S., & Fryer, A. A. (2001). Glutathione-S-transferase family of enzymes. In Mutation Research (Vol. 482).
81. Tang, M., Wang, S., Wang, W., Cao, Q., Qin, C., Liu, B., Li, P., & Zhang, W. (2012). The glutathione-S-transferase gene polymorphisms (GSTM1 and GSTT1) and idiopathic male infertility risk: A meta-analysis. Gene, 511(2), 218–223. https://doi.org/10.1016/j.gene.2012.09.054

82. Economopoulos, K. P., Sergentanis, T. N., & Choussein, S. (2010). Glutathione-S-transferase gene polymorphisms (GSTM1, GSTT1, GSTP1) and idiopathic male infertility: Novel perspectives versus facts. In Journal of Human Genetics (Vol. 55, Issue 9, pp. 557–558). https://doi.org/10.1038/jhg.2010.89
83. Kolesnikova, L. I., Kurashova, N. A., Bairova, T. A., Dolgikh, M. I., Ershova, O. A., Dashiev, B. G., Korytov, L. I., & Koroleva, N. v. (2017). Role of Glutathione-S-Transferase Family Genes in Male Infertility. Bulletin of Experimental Biology and Medicine, 163(5), 643–645. https://doi.org/10.1007/s10517-017-3869-9
84. Zini, A., de Lamirande, E., & Gagnon, C. (1995). Low Levels of Nitric Oxide Promote Human Sperm Capacitation *In Vitro*. Journal of Andrology, 16(5), 424–431. https://doi.org/10.1002/j.1939-4640.1995.tb00558.x
85. Alahmar, A. (2019). Role of oxidative stress in male infertility: An updated review. In Journal of Human Reproductive Sciences (Vol. 12, Issue 1, pp. 4–18). Wolters Kluwer Medknow Publications. https://doi.org/10.4103/jhrs.JHRS_150_18
86. He, J., Mu, Y., Liu, M., Che, B. W., Zhang, W. J., Chen, K. H., & Tang, K. F. (2023). Glutathione S-transferase genetic polymorphisms and fluoride-induced reproductive toxicity in men with idiopathic infertility. Asian Journal of Andrology, 25(3), 404–409. https://doi.org/10.4103/aja202271
87. Parsa, E., Hoseini, S. M., Namayandeh, S. M., Akhavansales, Z., & Sheikhha, M. H. (2020). Investigating the rate of different ovarian response in *in vitro* fertilization cycles based on estrogen receptor beta +1730 polymorphism: A cross-sectional study. International Journal of Reproductive BioMedicine, 18(7), 509–516. https://doi.org/10.18502/ijrm.v13i7.7368
88. Amaro, A., Polerá, D., Figueiredo, F. W. D. S., Bianco, B., Christofolini, D. M., & Barbosa, C. P. (2019). The Impact of Variants in Genes Associated with Estradiol Synthesis on Hormone Levels and Oocyte Retrieval in Patients Who Underwent Controlled Ovarian Hyperstimulation. *Genetic Testing and Molecular Biomarkers*, *23*(2), 145–149. https://doi.org/10.1089/gtmb.2018.0205