Review

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Drug substitution and adjuvant therapy in patients with genetics related infertility: A review

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SUMMARY With the in-depth study of the human genome and the increasing popularity of gene sequencing, it has been gradually confirmed that genetics can play a crucial role in infertility. To provide references for clinical treatment, we have focused on genes and drug therapy for genetic infertility. This review recommends adjuvant therapy and drug substitution. Examples of these therapies include antioxidants (such as folic acid, vitamin D, vitamin E, inositol, coenzyme Q10 *etc.*), metformin, anticoagulants, levothyroxine, dehydroepiandrosterone, glucocorticoids, and gonadotropins *etc.* Based on the pathogenesis, we provide an overview of the current knowledge, including randomized controlled trials and systematic reviews, and predict potential target genes and signaling pathways, proposing possible future strategies for the use of targeted drugs to treat infertility. Non-coding RNAs are anticipated to become a novel target for the treatment of reproductive illnesses since they have a significant role in controlling the occurrence and development of reproductive diseases.

Keywords Genetics, infertility, drug substitution, adjuvant therapy, targeted drugs

1. Infertility overview

Infertility, a state of subfertility, refers to the inability to establish a clinical pregnancy after 12 months of regular unprotected sexual intercourse with a healthy partner. Unfortunately, 15% of newlywed couples struggle with infertility. The cause of infertility is specifically associated with females 25%-35% of the time, males 30% of the time, co-contributing factors 25%-40% of the time, and unexplained or idiopathic causes 10%-20% of the time (*1*). Infertility is a complex disease caused by genetics, anatomical abnormalities, immunological abnormalities, endocrine problems, and the environment, with genetics being one of the main areas of study regarding this topic.

2. Literature on infertility and genetics

With the in-depth study of the human genome and the increasing popularity of gene sequencing, it has been gradually confirmed that genetics can play a crucial role in infertility. Clinical phenotypes are complicated and dynamic, and infertility diagnosis and treatment frequently include interdisciplinary approaches. To provide references for clinical treatment, we focus on genes connected to infertility.

2.1. Female

Infertility can arise from abnormalities in any of the processes that control ovarian growth, oocyte maturation, fertilization ability, and early embryonic developmental in females. An increasing number of mutations in genes (such as *FSHR*, *mT-TRNA*, *PATL2*, *TUBB8*, *TRIP13*, *CDC20*, *ZP*, *TLE6*, *WEE2*, *BTG4*, *PANX1*, *PADI6*, *NLRP2*, *NLRP5*, *KHDC3L*, *REC114* and *MTHFR*) have been proven to be pathogenic causes of endometrial receptivity deficiency, oocyte maturation arrest, fertilization disorder and early embryo arrest (*2*).

Uterine dysfunction can prevent embryo implantation. Aside from abnormal uterine structure, the endometrium seems to be an important factor in implantation failure and later stages of pregnancy, including placentation, foetal development, pregnancy outcome and postnatal health after implantation. Importantly, it has been reported that many genetic factors are implicated in endometrial receptivity. During the implantation window in Chinese patients with polycystic ovary syndrome

(PCOS), TM4SF4 and MMP26 are particularly downregulated, indicating that differential endometrial gene expression contributes to endometrial receptivity dysfunction (*3*). In addition, the *ICAM-1* gene and *MUC1* gene play crucial roles in cellular adhesion in the endometrium, however, it has been proven that genetic polymorphism in the *ICAM-1* and *MUC1* domain may be proven that genetic polymorphisms in the *ICAM-1* and *MUC1* domains may be associated with susceptibility to endometriosis, leading to implantation failure or endometriosis, recurrence (*4*). In addition, the expression of other altered genes, including those encoding MME and WWP1, were greatly enhanced, whereas the expression of the gene encoding TNC, LIF and HOXA-10 were decreased in recurrent implantation failure samples during the implantation window (*5,6*). Collectively, these altered genes may serve as targets for improving endometrial receptivity.

2.1.1. Oocyte maturation arrest

Human oocytes can be fertilized through three stages: the germinal vesicle (GV), metaphase I (MI) stage and metaphase II (MII) stage. The genetic origin of oocyte maturation arrest, a major kind of infertility characterized by the production of immature oocytes, is yet unknown. In certain close families with a GV-blocking phenotype, *PATL2* mutations have been identified that lead to improper oocyte maturation. PATL2 deficiency disrupted oocyte maturation, according to a study by Marie *et al.* (*7*) utilizing *PALT2* knockout mice. Additionally, *TUBB8* mutations disrupted oocyte meiotic spindle assembly, oocyte maturation and microtubule behaviour, causing MI arrest in oocytes and female infertility (*8*). Another theory links a *TRIP13* mutation to female infertility characterized by oocyte meiotic arrest (*9*). The primary downstream target of spindle assembly checkpoint inhibition is the mitotic activator CDC20. As a result, suppression of CDC20 could impede sister chromatin separation, resulting in female infertility due to oocyte maturation arrest, fertilization problems, and early embryo arrest (*9*).

2.1.2. Fertilization disorder

To our knowledge, the zona pellucida (ZP) is crucial to the development of the preimplantation embryo and to other reproductive processes. There are four glycoproteins in Z*P* (ZP1, ZP2, ZP3 and ZP4). Infertility caused by abnormal ZP-free oocytes, such as oocyte degeneration and empty follicle syndrome, is caused by mutations in human genes *ZP1*, *ZP2* and *ZP3* (*2*). WEE2 is a crucial meiotic regulator. Numerous *WEE2* mutations have recently been found to be responsible for failed human fertilization. Reduced WEE2 protein levels or cellular compartment relocation caused by mutated *WEE2* in HeLa cells resulted in lower levels of phosphorylated cell division control protein 2 homologue and failed fertilization (*10*).

2.1.3. Early embryo arrest

Several mutations in the subcortical maternal complex genes, including *FLOPED*, *PADI6*, *NLRP2*, *TLE6* and *NLRP5*, have been linked to early embryo arrest-related female infertility (*11*). A specific early embryonic phenotype known as zygotic cleavage failure is characterized by normal oocytes that could be successfully fertilized but were unable to complete the first cleavage. The connection of CNOT7 and eIF4E, two essential translation initiation factors, and BTG4, a crucial adaptor of carbon catabolite repression 4-negative on tata-less, promotes the degradation of maternal mRNAs (*12*). The eggs of *BTG4*-null females can be fertilized successfully but fail to be cleaved. Four homozygous mutations of *BTG4* (c.73C>T (p.Gln25Ter), c.1A>G (p.?), and c.475_478del (p.Ile159LeufsTer15), c.166G>A (p.Ala56Thr)) are related to the phenotype of zygotic cleavage failure (*12*). Mutations in CDC20 have also been discovered to cause early embryonic arrest (*12*). It has previously been demonstrated that early embryonic arrest results from mutations in *REC114* and *KHDC3L* (*13*).

2.2. Male

Spermatogenesis is a multistep process that includes three primary stages: spermatogonia growth, spermatocyte meiosis, and mature sperm cell differentiation following meiosis. Azoospermia, oligospermia, asthenospermia, and teratospermia are a few examples of poor semen quality that are associated with male infertility. Idiopathic male infertility may be caused by a variety of factors, including abnormal genes and sperm mitochondria, the impact of environmental contaminants, and abnormal hormone metabolism.

2.2.1. Azoospermia and oligospermia

The total lack of spermatozoa in ejaculated semen is known as azoospermia. Sperm density < 15 million/mL is known as oligospermia. As a rule, chromosomal abnormalities, Y chromosome microdeletions in the azoospermia factor region, and mutations in the cystic fibrosis gene are the most prevalent genetic abnormalities. Azoospermia can be separated into obstructive azoospermia and nonobstructive azoospermia. Congenital bilateral absence of the vas deferens (CBAVD) is the most common cause of obstructive azoospermia; 68%-80% of cases of CBAVD are caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (*14*). In addition, follicle-stimulating hormone (FSH) signaling may be regulated by polymorphisms of *FSHB* genes (rs1394205, c.-29G

>A; rs6165, c.919A>G; rs6166, c.2039 A>G) (*15*) and *FSHR* genes (rs10835638, c.-211G>T) (*16*), which are always associated with azoospermia.

The most prevalent hereditary cause of male infertility, Creutzfeldt-Jakob syndrome, is characterized by severe oligozoospermia or azoospermia (> 90%) (*17*). Creutzfeldt-Jakob syndrome patients may experience infertility as a result of spermatogenesis issues, including sex hormone imbalance, serological cell failure, and spermatogonial stem cell loss. Additionally, the congenital absence of the vas deferens, idiopathic hypogonadotropic hypogonadism, isolated hypogonadotropic hypogonadism, androgen insensitivity syndrome, immotile cilia syndrome, and round head spermatozoa are genetic mutations linked to male infertility caused by Creutzfeldt-Jakob syndrome (*17,18*).

A significant contributing factor to azoospermia or severe oligozoospermia is Y chromosome microdeletion. Its prevalence is second only to that of Creutzfeldt-Jakob disease (*18*). The term "Y chromosome microdeletion" refers to the loss of azoospermia components on the long arm of the Y chromophobe; this deletion accounts for 60%-70% of all cases. Clinical symptoms caused by Y chromosomal microdeletions are diverse, resulting in phenotypes that range from azoospermia to normal sperm count but abnormal morphology (*18*).

2.2.2. Asthenozoospermia

Males with asthenozoospermia have sperm motility problems, such as reduced or absent motility that worsens over time. Numerous genetic variables, including as chromosomal mutations, sperm motility-related gene mutations, mtDNA abnormalities, epigenetic aberrations, and micro RNAs (miRNAs), contribute to the aetiology of asthenozoospermia (*19*).

2.2.3. Teratozoospermia

The abnormal morphology of more than 85% of sperm cells following ejaculation is referred to as teratozoospermia. Sperm cells with abnormal morphology are influenced by genetic factors such as aneuploidy, sperm DNA breaks, and mutations. It is crucial for gamete fusion, sperm flagellum movement, and the acrosome reaction to be regulated by the protein CRISP2, which is found in sperm. Asthenoteratozoospermic males exhibit low levels of CRISP2 protein expression in their ejaculated sperm, which is associated with reduced sperm motility, abnormal morphology, and infertility (*20*).

2.3. Genetic disorders associated with infertility

2.3.1. *MTHFR* genetic polymorphism-induced infertility

A number of enzymes are needed to catalyse the metabolic activity of folic acid in the body. Folic acid is an essential B vitamin that is involved in the production of purine and pyrimidine and is crucial for protein synthesis and cell division. In the metabolism of folic acid, methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme. Homocysteine (Hcy) accumulation (*21*) and DNA hypomethylation (*22*) are caused by incorrect catalytic steps of gene-related enzymes and low folic acid levels caused by the *MTHFR* gene mutation.

Hcy results in poor villui vascularization and hypercoagulability by destroying vascular endothelial cells. More research revealed that the thrombogenic effect of Hcy was predominantly closely related to Hcy harming vascular endothelial cells, inducing the formation of thromboxane and prostaglandin through thiolactone, thereby encouraging platelet aggregation, increasing the activity of coagulation Factor V, and inhibiting the fibrinolytic system. Therefore, Hcy affects embryo implantation by decreasing endometrial receptivity (*23*).

Reactive oxygen species (ROS) synthesis and removal in semen are dynamically balanced under physiological conditions, and the right amount of ROS is necessary for various physiological sperm functions, including energy acquisition and the acrosomal response. When the dynamic equilibrium is disturbed, an excess of ROS causes oxidative damage to proteins, DNA, and sperm membranes. Increased ROS can also influence the expression of associated genes, which in turn modify the quantity of specific chemicals in semen. Increased ROS can also disrupt various signaling pathways in cells. Recent research has indicated that variations in the *MTHFR* gene, particularly at the polymorphic loci of *MTHFR* C677T, and A1298C, are linked to male infertility (*24*). Folic acid deficiency lowered MTHFR activity and led to hyperhomocysteinaemia, which disrupted ROS homeostasis in semen and caused DNA damage to sperm (*25*). Additionally, hyperhomocysteinemia is significantly associated with disease severity and is closely related to cardiovascular and cerebrovascular diseases (*26*). Atherosclerosis in the arteries of the testis will disrupt spermatogenesis if there is insufficient blood flow (Figure 1).

Non-coding RNAs (NcRNAs) play pivotal roles in the aetiology of diseases, including those associated with hyperhomocysteinaemia. A previous study confirmed that miRNAs played essential roles in the regulation of gene expression and certain transitions between the main phases of spermatogenesis in mammals (*27*). The *MTHFR* 3'-UTR rs55763075 polymorphism was associated with folate and Hcy in men with idiopathic azoospermia, which may modify the susceptibility to male infertility (*28*).

2.3.2. Premature ovarian insufficiency

Premature ovarian insufficiency (POI) refers to

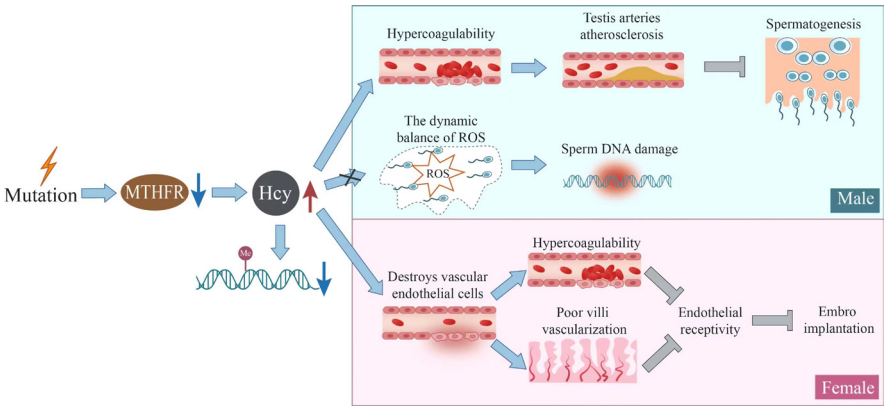

Figure 1. Mutation in *MTHFR* gene decreases MTHFR, resulting in Hcy and DNA hypomethylation. For female, Hcy destroys vascular endothelial cells, leading to poor villi vascularization and hypercoagulability. Therefore, Hcy results in reducing endometrial receptivity and influencing embryo implantation. For male, Hcy destroys the dynamic balance of ROS in semen, leading to sperm DNA damage. In addition, hypercoagulability caused by Hcy may lead to atherosclerosis in the arteries of testis, affecting spermatogenesis.

the occurrence of ovarian dysfunction in women before the age of 40 years. Patients with POI have significantly reduced or lost fertility due to severe ovulatory dysfunction. This condition has a solid genetic foundation. Genetic factors include mitochondrial dysfunction, X chromosome abnormalities, gene mutation, and other elements. Up to 94% of POI cases are caused by pathogenic chromosomal abnormalities. One of the significant genetic causes of POI is gene mutation. Mitochondrial DNA (MtDNA) is closely related to the quality of oocytes, and a decrease in mtDNA copy number in oocytes leads to a decrease in mitochondrial number, oocyte apoptosis and arrest. The mtDNA of oocytes is more susceptible to assault by ROS than that of other cells because of the low level of oxidative phosphorylation and absence of histone protection. Particularly when there is oxidative stress, the likelihood of mtDNA deletion or mutation will be considerably increased (*3*). Current studies have found *mT-TRNA* mutations in POI patients, such as MFN2, DNM1L, C3303T, A4435G, T4363C, G5821A and A15951G (*29,30*).

Increasing evidence has demonstrated that circular RNAs (circRNAs) are dysregulated in many diseases, such as cancer, cardiovascular diseases and neurological diseases. Moreover, a recent study identified 133 upregulated and 424 downregulated circRNAs in patients with POI, suggesting that differentially expressed circRNAs in the pathogenesis of POI (*31*). Additionally, miRNAs also participate in the development of POI. Numerous miRNAs including miR-23a, miR-27a, miR-22-3p, miR-146a, miR-196a, miR-290-295, miR-423, and miR-608 are significantly associated with POI (*32*). MiRNA-379-5p is implicated in the pathophysiology of biochemical POI by modulating PARP1 and XRCC6 (*33*). Therefore, dysregulated circRNAs and miRNAs are imperative in the development and progression of POI in women.

2.3.3. Hereditary prethrombotic state

A class of hereditary disorders known as hereditary prethrombotic states is predisposed to thrombosis and a hypercoagulable state of blood as a result of numerous genetic abnormalities. The development of follicle blood vessels, known as angiogenesis, is known to play a significant role in both the follicular and luteal stages. Ovarian function may be affected by genes related to thrombosis and vascular homeostasis, and enhanced Factor v activation brought on by *Factor v leiden* mutations may result in ovarian microthrombosis (*34*), which in turn alters the vascularization of the follicular system. Once insufficiently vascularized, the follicular microenvironment experiences a decrease in oxygen levels that eventually causes the oocyte/follicular pool to shrink more quickly, which results in the depletion of the follicular/follicular reserve (*35*). Additionally, the prethrombotic state, which impairs microcirculation and lowers patients' endometrial receptivity, is one of the risk factors for repeated implantation failure (*23*).

2.3.4. Obesity

The *leptin* gene produces the adipokine leptin. Patients with obesity may develop insulin resistance (IR) and hyperinsulinaemia as a result of excessive fat buildup. The body is in a chronic inflammatory state as a result of the increased fat release of pro-inflammatory and decreased anti-inflammatory substances in obese patients, which ultimately affects the quality and function of the oocyte. Additionally, a study discovered that obese women had significantly higher levels of oxidized low density lipoprotein, which could raise the body's level of ROS by interacting with the appropriate receptors (*36*). As a result, an oxidative stress response occurred, causing an increase in granulosa cell apoptosis in follicular cells and lowering reproductive potential.

A large body of studies report that numerous miRNAs, circRNAs and long non-coding RNAs (lncRNAs) contribute to the regulation of obesity-related metabolic pathways. For example, obese individuals

exhibit decreased levels of lncRNAs such as Mist, lincIRS2, lncRNA-p5549, H19, GAS5 and SNHG9 in adipose tissues or other biological samples (*37*). Abnormal levels of miRNA-192, miRNA-122, and miRNA-221 were observed in obesity, diabetes, and cardiovascular diseases (CVD), which increase the risk of development of diabetes and MetS and its progression to CVD in patients with obesity (*38*).

2.3.5. Autoimmune thyroid diseases

According to modern medicine, other elements that contribute to the aetiology of autoimmune thyroid disorders (AITDs) primarily include genetic, immunological, dietary, and apoptotic factors. Since there is clear family aggregation in the aetiology of AITDs, genetic factors likely play a significant role in the disease's pathogenesis and may be linked to a few particularly susceptible genes. Several susceptibility genes for AITDs, including *CTLA-4, CD40*, and *HLA-DR*, have been discovered (*39*). Meanwhile, various miRNAs (such as miR-154, miR-376b, miR-22, miR-183, miR-101, miR-197, and miR-660), lncRNAs (IFNG-AS1 and SAS-ZFAT), and circ 0089172 have been identified to be dysregulated in T-cell metabolic reprogramming during AITDs (*40*).

Endometrium, ovulation, and fertility are all negatively impacted by thyroid dysfunction from implantation to birthing. However, uncertainty surrounds the proposed pathogenic mechanism.

2.3.6. Congenital adrenal hyperplasia

CYP21A2 mutation, which results in steroid 21-hydroxylase production problems, is the main cause of congenital adrenal hyperplasia (CAH), a set of autosomal recessive genetic illnesses. Reduced corticosteroid 21-hydroxylase triggers compensatory increases in pituitary and hypothalamic corticotropin-releasing hormone release, which ultimately results in increased androgen and progesterone levels (*41*). The primary cause of the infertility problems experienced by 10%-30% of CAH women of reproductive age is testosterone excess-associated anovulatory cycles (*42*). Through androgen receptors, androgens may directly influence follicular development, while aromatase expression and oestrogen aromatization indirectly influence follicular development (*42*). The viability of fallopian tubes, endometrial receptivity, and cervical thickness are also impacted by higher progesterone levels, which have an impact on embryo implantation (*43*).

3. Drug substitution and adjuvant therapy

A significant societal issue and ongoing medical concern is infertility. Currently, fertility preservation and assisted reproductive technology (ART) are the main treatments for infertility caused by genetics. These methods have been successfully employed to treat infertility in recent years. Despite impressive advancements in infertility, ART failure rates remain high. Additionally, the consequences of ovarian stimulation and accompanying adverse effects are relatively unclear to clinicians. Numerous natural substances have been shown to reduce infertility in a variety of ways. In this review, adjuvant therapy and drug substitution are advised. Examples of these therapies include antioxidants (such as folic acid, vitamin D, vitamin E, inositol, coenzyme Q10 *etc.*), metformin, anticoagulants, levothyroxine (LT4), dehydroepiandrosterone (DHEA), glucocorticoids, gonadotropins *etc.* (Figure 2). The use of these medications in the field of assisted reproduction is still in the exploratory phase. On the basis of the literature, this article reviews the use of medications in infertility.

3.1. Antioxidants

Utilizing oxidant/activating chemicals is one of the treatments for infertility as the importance of oxidative stress energy release in the pathophysiology of infertility is coming to treatment. One way is that the right nutrients can enhance ovarian function. However, optimal supplements may also contain elements that affect the levels of plasma testosterone, activating agents, and antioxidants that increase sperm motility.

3.1.1. Folic acid

Folic acid is an antioxidant whose metabolic route is crucial for nucleotide anabolism, DNA methylation and repair, and the preservation of genomic stability. The treatment of infertility is highly contested. Preovulation folic acid supplementation decreased the rate of embryonic retardation, and it boosted glutathione production in oocytes in animal models where preconception folic acid deficiency impeded ovulation and increased the number of vestigial follicles. This suggests that folic acid is crucial for the growth of follicles. Folic acid is used to support reproductive health through affordable and efficient techniques, and how it is used is influenced by its genetic polymorphisms. Studies have demonstrated that folic acid produced metabolic disorders in women with *MTHFR* gene mutations; nevertheless, even when given in the same amount, folic acid levels in serum and red blood cells were lower than those of healthy individuals (*44*). Recently, Ye *et al.* (*37*) found that *MTHFR* polymorphism rs1801133 was not related to the pregnancy rate or pregnancy outcomes of women undergoing *in vitro* fertilization/intracytoplasmic sperm injection-embryo transfer with adequate synthetic folic acid supplementation by analysing 692 women undergoing *in vitro* fertilization/intracytoplasmic sperm injection-embryo transfer and taking adequate folic

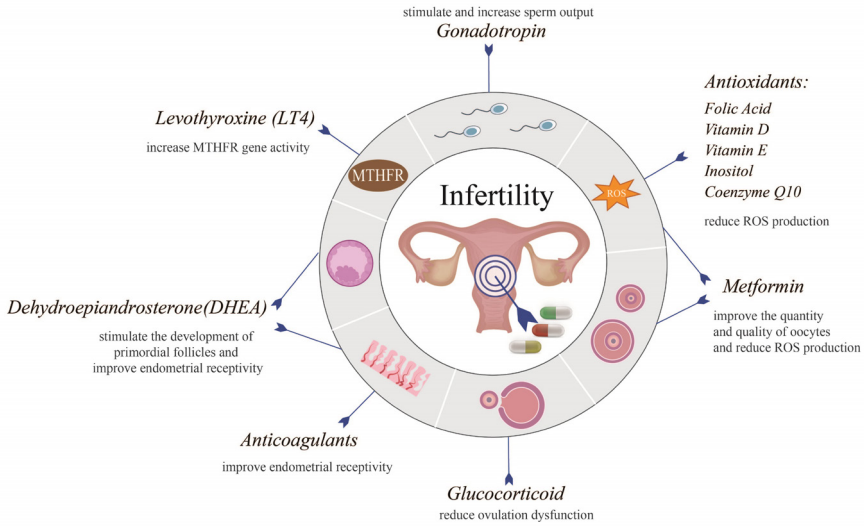

Figure 2. Adjuvant therapy and drug substitution are advised. Examples of these therapies include antioxidants (such as folic acid, vitamin D, vitamin E, inositol, coenzyme Q10 *etc.*), metformin, anticoagulants, levothyroxine, dehydroepiandrosterone, glucocorticoids, and gonadotropins *etc.*

acid. The United States Preventive Services Task Force advises that all women take a multivitamin containing folic acid at least one month before to becoming pregnant to boost a woman's chances of getting pregnant due to the safety of folic acid supplementation (*45*).

Folic acid may enhance sperm characteristics and increase fertility. In oligozoospermia with the *MTHFR* 677TT genotype, folic acid treatment improved semen characteristics, semen malondialdehyde, sperm DNA fragmentation, and pregnancy outcome (*46*). However, several research have claimed that folic acid is unable to enhance the DNA integrity or semen characteristics of male infertility (*47*).

3.1.2. Vitamin D

The biological purpose of vitamin D in the body is to maintain calcium homeostasis, which has an impact on cell differentiation and death. All of these tasks are predominantly carried out by vitamin D receptors, which have been found in some reproductive and endocrine organs including the ovaries, uterus, testes, hypothalamus, and pituitary gland in addition to calcium-regulated tissues (*48*). There are vitamin D receptors in the endometrium. According to a study by Karine *et al.* (*49*), the endometrium becomes thinner as vitamin D levels rise. Supplementing with vitamin D may increase the success rates of *in vitro* fertilization in infertile women (*50*). Only a small percentage of follicles mature into sinus follicles and ovulate at this time, whereas the majority spontaneously degenerate into atresia. Studies have showed a connection between vitamin D administration and the quantity of sinus follicles (*48*).

It is the term used to describe the diminished sensitivity of peripheral tissues to insulin, which reduces the biological impact of insulin. Insufficient use of glucose causes high blood glucose levels, which in turn trigger the pancreatic B cells to secrete more insulin as a balancing mechanism, causing hyperinsulinaemia and infertility. Numerous randomized controlled trials (*51*) have demonstrated that vitamin D administration can lower IR and blood glucose levels, although the precise mechanism relating vitamin D and IR is still unknown. According to some studies (*52*) the mechanism of vitamin D's impact on insulin metabolism may involve directly or indirectly stimulating IR by increasing the expression of the vitamin D receptor, improving insulin's sensitivity to transporting glucose, and reducing the release of pro-inflammatory cytokines. To fully realize the protective action of islet B cells, vitamin D can block their death (*53*) .

In patients with asthenospermia infertility, vitamin D administration can increase the mean sperm concentration and sperm motility as well as the overall clinical pregnancy rate. In fact, some authors claimed that vitamin D treatment had positive benefits on patients' progressing sperm motility and morphology, while others did not (*54*). Other research revealed a connection between the quality, motility, and functionality of mature sperm and the enzymes in charge of vitamin D metabolism in the human sperm flagellum (*55*).

Supplementation with vitamin D could improve the function of endothelial cells in in type 2 diabetes by affecting the expression profiles of 1,791 mRNAs, 2,726 lncRNAs, 205 circRNAs, and 45 miRNAs, and these differentially expressed RNAs were related to matrix metalloproteinase and guanosine triphosphatase activities, specific signaling pathways, and components of actin, extracellular matrix, or adherens junctions (*56*). However, the role of these natural products, nutrients, and supplements on ncRNAs requires further study in infertility.

3.1.3. Vitamin E

An essential antioxidant molecule known as vitamin E (-tocopherol) is thought to stop lipid peroxidation and increase the action of several free radical-scavenging antioxidants. According to Bahadori *et al*. (*57*), vitamin E levels were associated with both the frequency of high-quality embryos and the maturation rate of oocytes. The maturation rate of oocytes with 0.35-1 mg/dL and 1.5-2 mg/dL vitamin E in follicular fluid was greater, while the rate of high-quality embryos with 10-15 mg/dL vitamin E in serum was higher. The outcomes agree with those of Ashraf *et al.* (*58*). Experiments have showed that vitamin E protects sperm from oxidative damage, and improves sperm performance, and is effective in idiopathic infertility (*59*). However, some studies have suggested that vitamin E does not improve semen parameters (*47*).

3.1.4. Inositol

Mammalian oocyte synthesis of intracellular signals, the completion of poly spermatozoa meiosis, and embryo development all depend on inositol. Myo-inositol (MI), D-chiro-inositol, L-chiro-inositol, and shar-inositol are the four isomers that have currently been identified. In clinical settings, inositol drugs mostly refer to MI, which has properties that improve insulin sensitivity as well as anti-oxidation and anti-inflammatory properties. Researchers inquired whether inositol may enhance the quality of eggs and discovered that the levels of MI in follicular fluid were positively associated with those of oestradiol and the quality of the embryos, indicating that high concentrations of MI may be involved in follicular maturation.

Due to its ability to regulate hormones and its antioxidant properties, inositol has been shown to have positive impacts on mitochondrial function and sperm motility. Antioxidant therapy is currently a common treatment option for oligospermia and asthenospermia. As a typical antioxidant, MI has a considerable protective effect against DNA oxidative damage and can lessen the effects of oxidative stress on sperm quality and fertility in infertile men. According to Canepa *et al.* (*60*), subfertile male semen quality (sperm concentration, number of spermatozoa, progressive motility, total motile sperm count, and normal sperm morphology) can be improved by a nutraceutical supplement containing MI, folic acid, alpha-lipoic acid, betaine, and vitamins. In a different study, oligoasthenoteratozoospermic individuals who consumed supplements primarily made of MI and added MI to *in vitro* sperm culture demonstrated improved sperm motility both *in vitro* and *in vivo* (*61*). In light of this, MI may enhance sperm function in oligoasthenoteratozoospermic individuals both *in vitro* and *in vivo*. Additionally, Montanino *et al.* (*62*) discovered that MI delivered vaginally could increase pregnancies while also being safe for both the mother and the foetus by boosting total sperm motility and cervical mucus quality.

3.1.5. Coenzyme Q10

Coenzyme Q10 is a crucial coenzyme of the body's oxidative respiratory chain as well as a vital fat-soluble antioxidant. Therefore, by enhancing mitochondrial function, coenzyme Q10 supplementation may enhance pregnancy outcomes in infertile patients. With age, plasma coenzyme Q10 levels decline. In older women, coenzyme Q10 supplementation accelerated oocyte maturation and decreased post-meiotic aneuploidy. Additionally, coenzyme Q10 supplementation can increase the amount of coenzyme Q10 in the follicular fluid of people with infertility. Coenzyme Q10 supplementation boosted clinical pregnancy rates, but there was no discernible difference in miscarriage or live birth rates, according to a systematic review and meta-analysis (*63*).

Coenzyme Q10 has well-known antioxidant properties, and while semen can shield sperm from oxidative stress, treatment with this coenzyme can increase sperm motility (*64*). Coenzyme Q10 is present in sperm cells and spermatoplasm in a predetermined amount, and its degree of concentration is associated with specific sperm characteristics. Therefore, a reduction in coenzyme Q10 levels in sperm may result in a reduction in sperm motility. Improved pregnancy outcomes can be achieved by using coenzyme Q10 to boost sperm mitochondrial energy production and neutralize the ROS generated (*65*). Despite being affordable and secure, coenzyme Q10 is currently not utilized extensively in clinical practice since more patients and improved clinical trial designs are required to completely quantify and confirm its impact on human fertility.

3.1.6. Other antioxidants

When taken in supplement form, vitamin C has been shown to reduce endogenous oxidative damage and enhance sperm quality in infertile men. An important antioxidant is carnitine. To speed up the oxidation process, carnitine functions as a crucial cofactor in the transport of long-chain fatty acids in the mitochondrial matrix. Therefore, carnitine can improve postgonadal maturation, sperm production, and cellular energy generation. According to previous studies, patients with idiopathic asthenospermia who took L-carnitine and acetyl-L-carnitine together experienced significant improvements in their overall motor function (*66*). Resveratrol enhances mitochondrial homeostasis in oocytes and granulosa cells, stimulates mitochondrial biosynthesis, and improves ovarian reserve in older women by regulating the balance between mitochondrial biogenesis and autophagy by activating sirtuin-1 (*67*). Additionally, resveratrol enhances clinical infertility

caused by follicular growth by stimulating nuclear factor-κB and phosphoinositide 3-kinase/protein kinase B. By blocking nuclear factor-κB signaling, the nuclear factor-κB signaling pathway reduces ovarian dysfunction in POI patients and prevents granulosa cell apoptosis (*68*). Its anti-inflammatory qualities, however, might prevent the inflammatory response of decidualization, which would lower endometrial receptivity and have negative consequences on implantation and endometrial decidualization.

3.1.7. Metformin

Caloric restriction (CR), an antioxidant strategy, limits a person's daily calorie intake to between 25% and 50% of their average calorie intake. CR can promote fertility, postpone ovarian senescence, and extend reproductive life. According to Weeg *et al.* (*69*), obese female oocytes have aberrant telomeres, which impact both the amount and quality of oocytes. Telomere shortening and the activation trend of the compensatory mechanism for telomere shortening were more noticeable in the high body mass index group in 20 women who had ART for pregnancy, and telomere length was adversely linked with the number of follicles and fertilization rate. It is difficult to put CR into practice, but there are substitutes that have the same impact. For instance, metformin inhibits mitochondrial ETC complex I and lowers the generation of hepatic glucose, simulating the impact of CR and lowering the production of ROS (*70*).

Meanwhile, the application of metformin attenuates PCOS through the downregulation of miR-122, miR-223 and miR-29a in women (*71*). For patients with recurrent endometriosis, lncRNA H19 is highly expressed in the ectopic endometrium and is an independent prognostic factor of endometriosis (*72*), and the combination therapy of metformin and sitagliptin upregulates lncRNA-H19 by suppressing the PI3K/AKT-DNMT1 pathway in patients with PCOS accompanied by IR (*73*). Thus these ncRNAs can be considered as potential markers in molecular PCOS research and treatment approach development.

3.2. Anticoagulants

Anticoagulants can increase endometrial receptivity during the "window of implantation" and be used to boost implantation rates empirically. Examples include aspirin and low-molecular-weight heparin. Aspirin is a non-selective cyclooxygenase inhibitor that can alter the ratio of prostacyclin to thromboxane, increase blood flow, and enhance endometrial tolerance. The use of low-dose aspirin during *in-vitro* fertilization/intracytoplasmic sperm injection could enhance pregnancy rates, according to the findings of a meta-analysis of 13 randomized controlled studies performed by Li *et al.* (*74*); the clinical dose that was advised was 100 mg/d. In patients with repeated implantation failure, endometrial and uterine artery blood flow resistance was dramatically reduced following low-dose aspirin treatment compared to before treatment (*75*).

By interacting with antithrombin, the sulfated polysaccharide heparin can prevent the activity of clotting proteins such as factor Xa and factor IIa. Insulin-like growth Factor 1 (IGF-1) may control the differentiation and implantation of endometrial tissue. In addition, IGF-1 is upregulated during the early stages of decidualization, which helps the endometrial stromal cells differentiate and is important for endometrial receptivity. Low-molecular-weight heparin can increase gonadal hormone expression while decreasing IGF-1 expression, which can increase the pregnancy rate (*76*). Furthermore, heparin-binding malignant growth factors can encourage angiogenesis and the development of a capillary network at the implantation site of embryos (*77*).

3.3. Levothyroxine

There is currently insufficient evidence for LT4 to increase fertility in females with normal thyroid function and positive thyroid autoimmune antibodies. Hypothyroidism was consistently linked to low folate and high Hcy levels (above 100%). In addition, Lien *et al.* (*78*) showed a correlation between thyroid function, which is elevated in hyperthyroidism and depressed in hypothyroidism, and *MTHFR* gene activity. Therefore, we speculate that adding thyroxine to your diet may boost the activity of the *MTHFR* gene. All women seeking treatment for infertility should be examined for thyroid disease, if found, and treated with low dose LT4, whose initial dose is 25-50 μg/d, due to the safety of this medication (*79*).

3.4. Dehydroepiandrosterone

DHEA is an endogenous steroid that is produced by ovarian follicular membrane cells and the adrenal reticular zone. It serves as a precursor hormone for the production of testosterone and oestradiol. A recent meta-analysis revealed that DHEA improved pregnancy outcomes in patients with decreased ovarian reserve by raising anti-müllerian hormone levels and the number of antral follicles (*80*). Studies have shown that DHEA supplementation increases the number of primordial and primary follicles in aged mice by inhibiting follicular apoptosis (*80*). By upregulating the expression of HOXA-10 in the uterus, Celik *et al.* (*81*) discovered for the first time that oral DHEA supplementation might improve endometrial receptivity in women who were not good responders. DHEA's safety (regular oral dose and usage time) is unclear.

3.5. Glucocorticoid

Due to their ability to control excessive androgen

expression by blocking adrenocorticotropic hormone activation, glucocorticoids have been the primary treatment for CAH. In people with non-classic adrenal hyperplasia, studies have shown that glucocorticoids can increase fertility by lowering excessive levels of testosterone and progesterone (*43*). Ovulation dysfunction in CAH women and supplemention with glucocorticoids may help to induce ovulation. Retrospective studies revealed that glucocorticoids improved fertility by lowering ovulation dysfunction and preventing overexposure of the endometrium to progesterone and androgens from the adrenal glands. Patients with CAH who received glucocorticoids had more regular periods, became pregnant, and had healthy babies (*41*). Additionally, hormone therapy can be used throughout pregnancy with the main goals of restoring cortisol insufficiency, suppressing androgen overproduction, preventing the masculinization of female foetuses and preventing long-term consequences on child fertility (*82*).

Hsa-circ 001533, spliced from FKBP51, is crucial in cell proliferation, migration and apoptosis. It has been reported that FKBP51 interacts with the glucocorticoid receptor (*83*). A recent study has identified that hsa-circ 001533 contributes to the cumulus cell apoptosis and nuclear maturation of oocytes by inhibiting the expression of FKBP51 in infertile women with endometriosis (*84*). Thus, the regulatory effect of glucocorticoids on circRNAs needs further validation.

3.6. Gonadotropin

Due to the oligosaccharide transferase complex of pituitary gonadotropin cell glycosylation of α and β subunits during protein synthesis, FSH is a complex, highly diverse glycoprotein hormone. Hormones control spermatogenesis, therefore, when exogenous gonadotropin or pulsing gonadotropin-releasing hormone is used, testosterone levels in the testis and serum rise and sperm are present during ejaculation. Theoretically, hormones can stimulate and enhance sperm output, which may be the rationale behind FSH treatment of male infertility caused by hypogonadotropic hypogonadism (*85*). Male idiopathic infertility can benefit from gonadotropin treatment by increasing sperm count and function, but not in all cases. More randomized controlled trials are required to prove this, but it may rely on the aetiology of oligoasthenoteratozoospermia and/or the genetic background that affects FSH action. Overproduction of FSH can cause ovarian hyperstimulation syndrome, which can trigger *FSHR* mutations and cause tumours that secrete FSH (*86*). Fortunately, no available research has documented negative effects on men following FSH stimulation.

Granulosa cells in follicles respond to gonadotropin signaling and participate in follicular formation and oocyte maturation. In mice with knockout of miRNA-200b and miRNA-429, the female mice were sterile and regained fertility only after application of exogenous gonadotropins for superovulation (*87*). In anovulatory women, these miRNAs were overexpressed, while exogenous gonadotropins decreased the levels of these miRNAs to a normal level in normal ovulating women (*87*). Therefore, exogenous gonadotropin treatment provides a promising strategy for fertility by inhibiting miRNA-200b and miRNA-429 expression.

4. Future directions

On the basis of the pathogenesis, we outline the current knowledge, including randomized controlled trials and systematic reviews, and suggest potential future approaches for the use of targeted medications on infertility. LncRNA, miRNA, and circRNA are examples of RNA that does not translate proteins and are referred to as ncRNA. They are anticipated to become a novel target for the treatment of reproductive illnesses since they have a significant role in controlling the occurrence and development of reproductive diseases (Figure 3).

4.1. LncRNA

A target gene's mRNA expression is influenced by lncRNA through the competitive binding of miRNA, posttranscriptional regulation, and epigenetic alteration. Yao *et al.* (*88*) discovered 52 differentially expressed messenger RNA transcripts and 20 differentially expressed messenger RNAs that were associated with granulosa cell activity and follicular development. Furthermore, they compared the RNA transcripts of the ovarian cortex and serum between POI patients and normal controls and then found that the expressions of lncRNA-ADAMTS1-1:1/ADAMTS1 and lncRNA-PHLDA3-3:2/CSRP1 in POI patients' ovarian cortex were upregulated significantly and the expressions of lncRNA-COL1A1-5:1/COL1A1, lncRNA-SAMD14-5:3/COL1A1, and lncRNA-GULP1-2:1/COL3A1 were downregulated, according with the results of serological examination. Subsequently, lncRNA-GULP1-2:1 and other potential target genes showed a positive correlation with ovarian tissues, both of which were significantly downregulated. In a rat experiment, Xiong *et al.* (*89*) discovered that lncRNA-MEG3 prematurely caused ovarian failure by inhibiting the proliferation of ovarian granulosa cells and activating the p53-p66Shc pathway and the apoptosis-related protein caspase-3.The synthesis of oestradiol was enhanced when the lncRNA HCG26 was downregulated in granule cells, which also hindered cell growth and cell cycle progression (*90*). In mouse ovarian granule cells, Li *et al.* (*91*) discovered that overexpression of the lncRNA SRA could increase the levels of oestradiol and progesterone as well as the production of vital enzymes (YP19A1 and CYP11A1). The novel lncRNA CASC7 is a potential

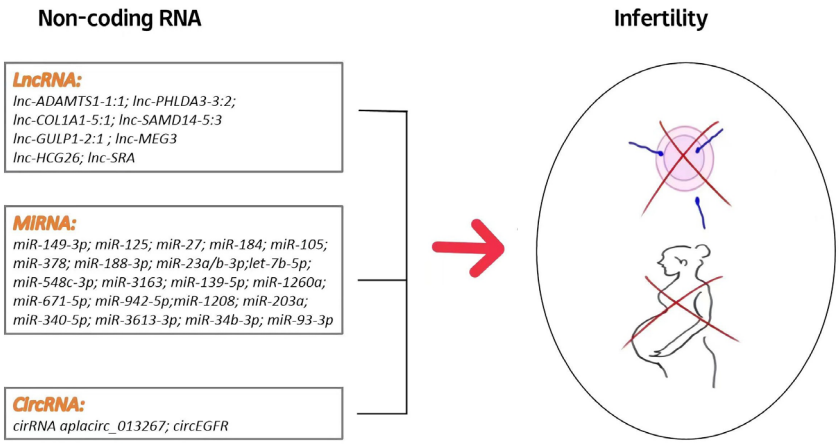

Figure 3. NcRNAs includes lncRNA, miRNA and circRNA, which have a significant role in controlling the occurrence and development of reproductive diseases.

therapeutic target and promising biomarker for male infertility (*92*).

4.2. miRNA

Endogenous miRNAs are short, ncRNAs that affect gene expression at the posttranscriptional or translational level. They are involved in steroidogenesis and germ cell development and contribute to infertility. The miRNA-125 family also plays a crucial regulatory role in oocyte activation. Mature miRNA-125b has two precursors, miRNA-125b-1 and miRNA-125b-2. Li *et al.* (*93*) also found that miRNA-27, miRNA-125b, miRNA-184 and miRNA-105 inhibited the release of progesterone and oestrogen in ovarian granulosa cells and that miRNA-378 could directly regulate oestradiol production.

During various spermatogenic phases, miRNAs are highly expressed. By attaching to the 3' untranslated region of CRISP2 and inhibiting CRISP2 production following transcription, miRNA-27a selectively targets CRISP2, a protein important for sperm motility. A study of oligosthenospermia patients showed that miRNA-23a/b-3p expression was negatively correlated with sperm motility, morphology, and sperm count, and some of the direct targets identified by miRNA-23a/b-3p, including PFKFB4, HMMR, SPATA6, and TEX15, play a fundamental role in sperm function (*94*). Let-7b-5p expression was observed to be lower in asthenozoospermia patients than in healthy males and additional research revealed that low let-7b-5p expression prevented glycolysis by specifically targeting AURKB in people with asthenozoospermia (*95*). In addition, miRNAs have been predicted in infertile males including azoospermia (miRNA-548c-3p), oligozoospermia (miRNA-3163, miRNA-548c-3p, miRNA-139-5p, miRNA-1260a), asthenozoospermia (miRNA-671-5p, miRNA-942-5p, miRNA-1208), and teratozoospermia (miRNA-203a, miRNA-340-5p, miRNA-3613-3p, miRNA-34b-3p, miRNA-93-3p) (*96,97*). Therefore, these miRNAs can be used as novel potential therapeutic targets for patients with infertility.

4.3. CircRNA

CircRNAs are ncRNAs that control posttranscriptional regulation of gene expression. CircRNAs have a crucial role in ovarian growth and function, according to research on circRNAs in the ovary over the past ten years. Comparing granule cells from women with biochemical premature ovarian insufficiency (bPOI) and control women's, Zhou *et al.* (*31*) discovered that in bPOI patients, a total of 133 circRNAs were elevated and 424 circRNAs were downregulated. Further study revealed that the Foxo signaling pathway is one of the signaling pathways that is highly enriched in cellular senescence. Therefore, circRNAs may be involved in the pathogenesis of bPOI. Wu *et al.* (*98*) showed that the circRNA aplacirc_013267 inhibited apla-mi-1-13 and increased the expression of THBS1, upregulating granulosa cell apoptosis. Jia *et al.* (*99*) discovered that *circEGFR* gene knockdown increased progesterone production while inhibiting granulosa cells' ability to secrete oestrogen. Conversely, circEGFR overexpression improved the proliferation and oestrogen production of granulosa cells in mouse ovaries.

5. Conclusion

Adjuvant treatment and drug substitution have been used to treat infertility successfully. To better assist infertile couples in improving the success of pregnancy, it will be necessary to conduct additional research on the effectiveness and safety of these drugs before they can be used as adjuncts for pregnancy. Additionally, the dosage and methods of use of these drugs should also be standardized. Future tailored medications to treat infertility may be guided by the projected potential target genes and signaling pathways discussed above, but more extensive studies are still needed.

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