***Review Paper:***

# Genetic Causes of Male Infertility and Emerging Potential of Stem Cell Therapeutics

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## *Abstract*

*Infertility has haunted mankind from ancient times. In 40 to 50% of the cases resulting in failure to conceive, the cause can be attributed to male infertility. The rates of infertility have risen in the past few decades among both among men and women. The reasons can be attributed to life style changes and environmental pollution apart from genetic causes. Scientists have found out few possible causes of male infertility and there are few treatment measures currently being carried out. The success of these depends on the primary cause of the infertility problem. In the past few decades, a number of genes playing roles in different stages of sperm development and differentiation were functionally analysed.*

*This knowledge coupled with the advancements in stem cell technology and in vitro gametogenesis has led to new frontiers in possible therapeutic measures of male infertility, especially those with genetic causes. The present study focuses on the causes and treatment of male infertility focussing mainly on important genetic regulations and emerging stem cell-based therapeutics.*

**Keywords:** Male infertility, spermatogenesis, genetic causes, stem cells.

## Introduction

Male infertility refers to a male's inability to cause pregnancy in a fertile female. In humans it accounts for 40–50% of infertility. The causes of male infertility can be genetic or environmental. For successful treatment, there should be accurate diagnosis of the cause. The various treatment measures available these days vary according to the underlying disease and the degree of impairment of the person's fertility. In most of the cases, the exact cause could not be pinpointed. Such cases of male infertility are called idiopathic infertility[15]. The present study focuses on the causes and treatment of male infertility specifically using stem cell technology. Female infertility is also highly prevalent in the current situation, but many treatment measures are available and oogonia has successfully been produced *in vitro* too.

**Causes of Male Infertility:** Male infertility can be caused by a number of genetic and non-genetic attributes. These include endocrine issues, sperm transport and spermatogenesis errors, sperm antibodies, intercourse disorders etc. Endocrine disorders include deficiency of gonadotropin, some pituitary diseases and hypothalamic disorders. Sperm transport problem consists of congenital and acquired problems - congenital is when the vas deferens is absent and acquired is when some surgery or genital infection interferes with normal transport of sperm cells.

Acquired problems include vasectomy, prostatectomy, inguinal hernia, hydrocele repair etc. Spermatogenesis factors also could be genetic or acquired. The genetic ones include Klinefelter syndrome, Noonan syndrome etc. Acquired factors include systemic illness, drugs or medications, gonadotoxins (like cyclophosphamide), infections, vascular abnormalities, heat exposure etc.

Sperm antibodies cause the agglutination of sperms in the epididymis and vas deferens leading to disturbances in sperm motility, acrosome reaction etc. The sperm antibody count is found to be more in infertile males than in normal males. Few medications and faulty diet can lead to infertility. For example, those suffering from Celiac disease have chances of having low fertility rates. Such problems can be rectified by adopting a gluten-free diet. The main cause of immunological infertility is the formation of antisperm antibodies (ASA), which affects the capability of fertilization of spermatozoa[21]. People who have Celiac disease have greater risk of developing one or more of the associated autoimmune diseases[7]. A significant association of antisperm antibody or ASA as etiological factor for infertility with silent Celiac disease was proven in a research study conducted in Iraq[2].

One of the most common reversible cause of male infertility is varicocele (swelling of the veins that drain the testicle). This is related to abnormal testicular temperature regulation. Treating the varicocele can improve sperm numbers and function and may potentially improve outcomes when using assisted reproductive techniques such as *in vitro* fertilization. Infection which can cause sperm blockage is yet another reason which can be due to the inflammation of the epididymis (epididymitis) or testicles (orchitis) and due to gonorrhoea or HIV. Ejaculation issue can be another cause of male infertility.

Various health conditions can cause retrograde ejaculation including diabetes, spinal injuries, medications and surgery of the bladder, prostate or urethra. Spinal cord injuries can result in faulty ejaculation though sperm production will not be compromised. In such cases, sperm can still be retrieved for use in assisted reproductive techniques. Intercourse

disorders including erectile and ejaculator problems and psychological problems are also possible causes of male infertility. Deviations from the normal anatomy of the male reproductive organs like abnormal penile curvature or angulation also can lead to male infertility. Almost twenty percentage of male fertility issues are related to reduced sexual drive, erectile malfunction, immature ejaculation or the failure of intromission.

**Genetic Causes of Male Infertility:** More than one thousand genes are found to be involved in human spermatogenesis as the number of proteins and enzymes including regulatory molecules required for this process is manifold. Among these, only few genes are well studied. For example, those implicated in the processes of testis determination, testis descent and spermatogenesis are clinically well recognized. These genes include the *CFTR* (cystic fibrosis transmembrane conductance regulator) gene, whose mutations cause cystic fibrosis and absence of vas deferens[17] and the *AR* gene (androgen receptor). Mutations in the latter can cause androgen insensitivity syndrome and spermatogenic damage[5]. Many research teams are coming up with newly identified gene functions implicated in the whole process of sperm formation starting from the sperm stem cells.

**Genes in Azoospermia:** Azoospermia is a condition wherein the semen does not contain any sperm. This affects 1% of the human male population and can be classified as Obstructive or Non-Obstructive Azoospermia. The most commonly known genetic causes of male infertility are the chromosomal anomalies and microdeletions of the azoospermia factor (AZF) of the Y chromosome. The frequency of these abnormalities can go up to 30%, corresponding with the severity of the spermatogenic defect in azoospermic men. Y chromosome microdeletions can be the cause of about 10% of the male infertility cases[14].

**AZF region of Y chromosome:** The AZF locus of the long arm of the human Y chromosome which plays a significant role in sperm production was mapped in 1996[48]. Deletions in this region are associated with inability to produce sperm[24]. Scientists consider the AZF locus as one of the most genetically dynamic regions in the human genome. This is because it can serve as a counter against the genetic degeneracy due to the absence of a partner chromosome during meiosis. It also presents a possible threat as some rearrangements represent a risk factor of spermatogenic disruption[37]. A 2010 study has shown that a specific partial deletion of AZFc called *gr/gr deletion* was found to be associated with male infertility among Caucasians in Europe and the West Pacific[44]. Sometimes duplications in the AZF region can also lead to male infertility as in the case reported in a study conducted in Taiwan[32].

Cytogenetic studies are usually done to screen for chromosomal abnormalities to detect deletions, duplications etc. PCR analyses of AZF regions can detect several STSs in the three AZF regions. Simultaneous detection of the most common genetic causes of male infertility i.e. sex chromosomal aneuploidies and AZFb and AZFc deletions, partial AZFc deletions/duplications and *AR* CAG repeats is made possible by quantitative fluorescent (QF)-PCR method[39]. One of the significant factors of spermatogenic failure is the microdeletion of azoospermia factor, a (AZFa) region of the Y chromosome. Recently the malfunctioning of the genes Ddx3x and Ddx3y was found to affect the process of spermatogenesis in mice[34].

One of the genes directly responsible for azoospermia is the *Sycp3* gene located on the Y chromosome outside of the AZF region[35]. Mutations in this gene cause mitotic arrest in men and in turn pregnancy loss for the female partner. Some women with recurrent pregnancy loss were tested and it was found that some of them had the nucleotide alterations in *Sycp3* gene for independent heterozygous chromosome.[3.]

Another gene implicated in idiopathic azoospermia is *HSF2*. One heterozygous mutation in - R502H - has been found to be the cause of the loss in functionality of *HSF*2 gene and only the mutant allele was found[36]. *Tex11* is one of the genes responsible for chiasma and synaptonemal complex formation during chromosomal crossing over. This gene is located on the X chromosome[1]. Mutations in this gene have been found to cause mitotic arrest in infertile mice[38]. In a study involving 15 human males suffering from azoospermia, two were found to have recurrent 99kb *T Tex11* intragenic deletion, Xq13.2[51].

**Genes in Oligospermia:** Oligozoospermia refers to the significant decrease in the total number of spermatozoa in the ejaculate. One of the genes affecting sperm count is *KLHL10*. This gene is expressed in the cytoplasm of elongating spermatids. The process by which the round spermatids differentiate into elongated spermatids is called spermiogenesis, during which the haploid male germ cells undergo several unique morphological changes. The changes include acrosome formation, nuclear condensation and packaging, tail formation, reorganization of cytoplasm and organelles and spermiation[28]. Spermiation is the process by which mature spermatids are released from Sertoli cells into the seminiferous tubule lumen, prior to their passage to the epididymis.

An important gene responsible for oligospermia is the *KLHL10* gene (kelch homolog 10) which codes for a substrate-specific adapter of a ubiquitin-protein ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of target proteins during spermatogenesis. *KLHL10* displays high evolutionary conservation in mammals as evidenced by 98.7% amino acid identity between mouse and human KLHL10 proteins[50]. Mutation of this gene at 17g21 was found to cause homodimerization resulting in oligospermia. Reverse transcription polymerase chain reaction (RTPCR) was used as a technique for testing this defect. Yet another reason for

oligospermia was attributed to the abnormal number of CAG repeat sequences in exon 1 of the androgen receptor gene.

Large number of CAG repeats were found to cause oligozoospermia as they lead to impaired androgen receptor activity[53]. *BRCA*2, the human breast cancer susceptibility gene 2 is involved in homologous recombinational repair of DNA damages during meiosis. One of the common SNPs (single-nucleotide polymorphism) of *BRCA*2 is associated with severe oligospermia[55].

**Genes in Oligoasthenozoospermia:** This is a combination of asthenozoospermia (reduced sperm motility) and oligozoospermia (low spermatozoan count). *SEPT12* and NANOS1 are two important genes involved in this condition. Septins are members of the GTPase superfamily whose functions include cytokinesis and morphogenesis. One of the members of this family, Septin 12 (*SEPT12*) is a testis-specific gene critical for the terminal differentiation of male germ cells. A missense mutation in *SEPT12* (16p13.3), was reported in men with infertility due to oligoasthenozoospermia[29]. Nanos are RNA-binding proteins playing crucial roles in germ cell development and maintenance. Mutations in *NANOS1* gene have been implicated in patients with oligoasthenoteratozoospermia. It was interesting to note that different patients showed mutations in different regions of this gene[30].

**Genes in Globozoospermia:** Globozoospermia is a rare and severe form of monomorphic teratozoospermia wherein over 85% of spermatozoa have an abnormality. This condition is responsible for less than 0.1% of male infertility. It is characterised by round-headed spermatozoa without acrosome and an abnormal nuclear membrane. Affected males therefore suffer from either reduced fertility or infertility. Mutations or deletions in many genes can give rise to this condition[4]. The significant ones include *PICK1, SPATA16* and *DPY19L2*[3,9,11]. *DPY*19L2 is a transmembrane protein localised on the acrosome of spermatids. It contributes to normal acrosome formation by anchoring the acrosome to the spermatozoa nucleus. Mutations in this gene can lead to failure of sperm head elongation and acrosome formation causing a round-headed sperm to form. *Pick*1 is a cytosolic protein found in the proacrosomal vesicles of round spermatids. It functions during protein trafficking.

Mutations in *Pick1* can lead to the proacrosomal vesicles failing to merge causing a round-headed sperm. *SPATA*16 codes for a protein-protein interaction domain are located in proacrosomal vesicles. The gene was first identified in a family with three out of six brothers being homozygous for the mutation; their sperms were without acrosomes and showed round headedness. Mutations in any of these three genes will result in sperms being unable to bind to the zona pellucida and fail to fertilise the oocyte[8].

**Genes in Macrozoospermia:** The condition wherein spermatozoa are large-headed, multiflagellar and polyploid is called macrozoospermia. Aurora kinase C encoded by the *AURKC* gene ensures that the mechanisms for cell division are in place and help chromosomes properly align with each other so that every new sperm cell contains one copy of each chromosome. Homozygous mutation in single nucleotide deletion caused in *AURKC* gene encoding region was found to be associated with this condition. Termination of translation was also reported in some mutations with loss of function of the protein, causing macrozoospermia or large headed polyploid spermatozoa[10]. A list of important genes involved in different genetic causes of male infertility is depicted in figure 1.

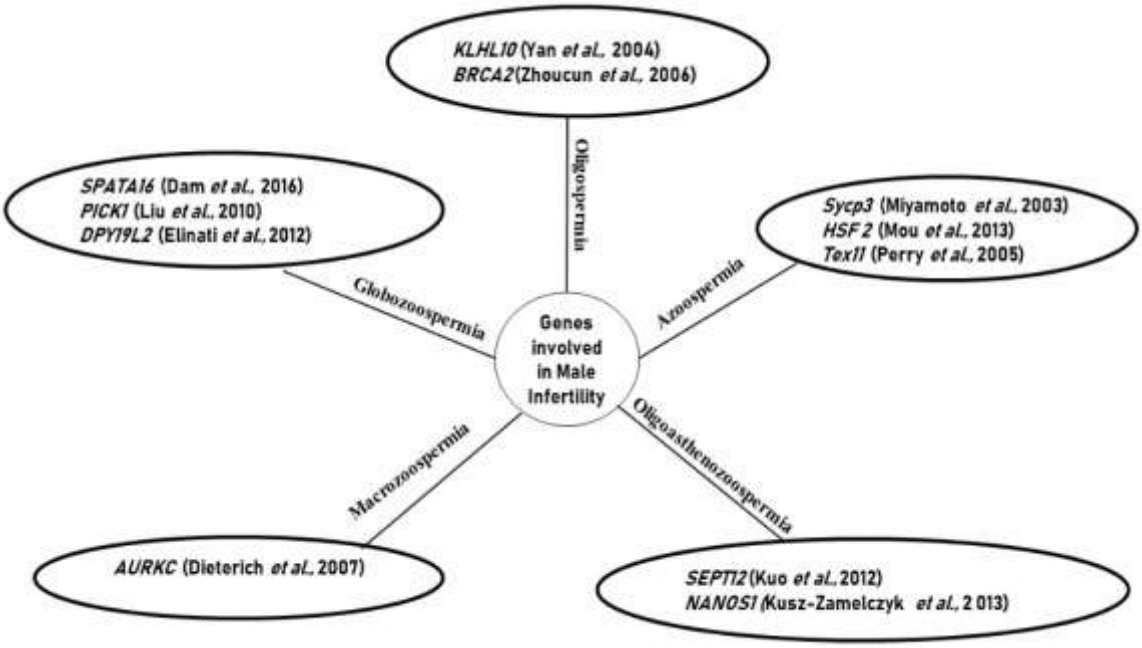


**Fig. 1: List of important genes involved in male infertility**

**Stem Cells in Male Infertility:** The complex body of human beings is comprised of over 200 different cell types that are organized into tissues and organs. All the differentiated cells in the animal body arise from stem cells. In many tissues in the animal body, there is cellular heterogeneity. Those tissues are maintained by stem cells which have extensive renewal capacity and the ability to generate daughter cells that undergo further differentiation[31]. Such cells generate only the differentiated lineages appropriate for the tissue in which they reside and are thus referred to as multipotent or unipotent. Stem cells originate from two main sources - adult body tissues and embryos.

In 1998, it was first reported that embryonic stem cells could be derived from human blastocysts[43]. These had the pluripotent ability and hence could be tuned to generate many other types of cells. Later the concept of iPSCs or Induced Pluripotent Stem Cells was proved by coaxing the mouse fibroblast cells to become pluripotent by addition of 4 transcription factors[45]. This remarkable discovery has revolutionized the field of stem cell technology offering the possibility of replacing damaged tissues to treat diseases like Alzheimer's[27], diabetes[42] etc.

Scientists all over the world are still in pursuit of trying out possibilities of differentiating iPSCs to tune them into yielding different cell types in the animal body to treat different diseases. The key questions which are still not fully answered include the thorough understanding of genetic and epigenetic regulators of cellular differentiation, role of extrinsic factors in the renewal and development of these cells and also how to tap this potential for tissue repair without creating side effects like tumour growth. In the past two decades, a thrust area of research in this domain is to understand the factors controlling the differentiation of germline cells and ways to manipulate it. Most of the experimental data from mice models can be extrapolated to humans too, since the mechanisms align in most cases.

Since humans are more evolutionarily ahead, some of the differentiations can be more complex when compared to murine models. The differentiation dynamics of human germ cells in the embryonic period can be compared to those of the mouse germ cells, but human germ cells have been found to be more heterogenous in their developmental timing. Also, human germ cells undergo some developments which occur in mice only after birth due to the long gestational time period of humans[13].

Though some researchers were able to show the induction of PGCs (primordial germ cells) or round spermatids within embryoid bodies[16,47], these attempts could select only few cells (less than 1%) expressing germ cell markers. In humans, the number of germ cells are around 1000 at week four and more than 1.5 lakhs at week nine of embryonic development. Hence, from the practical point of view, such initiatives may not yield fruitful cues to patterns of germ cell development. Studies on mouse iPSCs have shown that they can be induced into primordial germ cell like cells (mPGCLCs) which could in turn contribute to spermatogenesis and oogenesis and to healthy offspring[19,20].

Human embryonic stem cells cultured in the presence of inhibitors for four kinases (MAPK, GSK3, p38 and JNK) could be efficiently induced into hPGCLCs (human primordial germ cell-like cells) using a procedure identical to that for mPGCLC induction whereas hPSCs cultured under conventional conditions were unable to form hPGCLCs[25].

Scientists were able to successfully derive GSCs from mouse ESCs[26]. A more robust system was tested successfully by Zhou et al[54] in which haploid round spermatid could be derived from mouse ESCs and the resultant spermatids when injected into eggs were able to produce offsprings. Gene mutations in GSCs could be corrected (by technologies like CRISPR editing etc.) and the resultant clones could be selected, thereby getting rid of any kind of gene mutations[6,49]. There should be sufficient studies on the ethical and biosafety aspects especially with regard to off target gene expression before such ventures.

Spermatogenesis is a tightly regulated multistep process under the control of many transcriptional regulators. Elaborate studies using single-cell RNA sequencing data from around 35,000 cells from the adult mouse testis could identify almost all known germline and somatic cells involved in spermatogenesis with their candidate transcriptional regulators during differentiation. Such studies could be useful in understanding the transformation of stem cells to functional sperms[18]. A recent research on monkeys has shown that the sperms isolated from grafted macaque testicular tissue fragments have the full potential to produce a healthy baby by using ICSI or Intra Cytoplasmic Sperm Injection[12].

This gives hope for the possibility of autografting of immature testicular tissue as an option for human male fertility preservation. Identification of the role of individual protein's contribution to the proper functioning of sperms is very critical. A group of scientists have identified the role of a protein, CENTROBIN, which is well conserved between humans and flies. This protein plays a critical role in the assembly of a subset of microtubules within the basal bodies. Mutations in the gene coding for CENTROBIN can prevent microtubule function and results in non-motile tails of sperms.

Consequently, CENTROBIN mutant males are sterile. Such a defect is called "easily decapitated spermatozoa defect" wherein the sperm heads are separated from their tails and thus they cannot swim[41]. Testicular torsion is a urological emergency that may lead to infertility as a result of decreased amount of sperm, low motility, poor F-actin expression and reduced content of ATP in sperms. Local injection of mesenchymal stem cells (MSCs) have been shown to

improve sperm function, particularly the motility of sperms[23]. An understanding of the root cause of such defects can help in stem cell based therapeutic measures.

## *In vitro* gametogenesis (IVG)

IVG is the process of collecting adult human cells like skin cells or cheek cells and converting those cells into artificial gametes by genetic manipulation. The key benefits of IVG research include the ability to generate gametes *in vitro* to be used for reproductive purposes and to understand the biochemical pathways and genes involved in gamete formation. Ever since Yamanaka's remarkable discovery of induced pluripotent stem cells, scientists world over are trying to replicate each stage of cellular development and trying to lure these stem cells to differentiate into different cell types in the body by modifying the transcription regulatory cocktails.

Experiments conducted in mice have shown that functional sperm could be obtained from frozen-thawed testicular tissues by an organ culture system[52]. Stanford research team used skin from infertile men's forearms, reprogrammed the skin cells to become iPSCs and transplanted them into the testicles of mice to create human germ cells, the primitive precursors to eggs and sperm[40]. They did not make embryos using these. But after two more years, two Japanese scientists, Mitinori Saitou and Katsuhiko Hayashi, described how they had got iPSCs from mouse tail which in turn were converted into eggs[22]. Using these eggs, eight healthy, fertile pups were produced.

Though such breakthroughs appear highly promising in treating infertility problems, several generations of resultant offsprings of experimental animals like mice must be monitored to rule out any phenotypic abnormalities due to genetic and epigenetic malfunctions. Nonetheless, discoveries like IVG give hope to couples facing genetic problems of sperm production, cancer patients, old people etc. to have their biological offspring.

## Conclusion

Stem cell therapy to treat male infertility is required in cases of genetic defects and also in cases where the individuals have to undergo cancer therapies. In the case of cancer chemotherapy, the sperms can be collected and stored to use later in the case of adult men, but prepubertal patients do not have this option. Currently, the only option is to cryopreserve the testicular tissue containing spermatogonia with a view to preserve the individual's germline cells. But, till now, no standardised protocols have been developed to derive functional sperms from such preserved germline cells, though many studies are going on testing possible differentiation methods. One hope in such cases relies on the fact that intracytoplasmic sperm injection (ICSI) procedure requires much less quantity of spermatozoa when compared to that needed for natural fertilization and hence less number of properly functional sperms can also result in fertilization. Also, techniques like *in vitro* gametogenesis offer chances for cancer patients undergoing chemotherapy and those with genetic male infertility problems to use their own sperms to have a son or daughter. Long term monitoring and detailed studies of these in model organisms are required before translating this kind of research to benefit humankind.

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