The Role of Nanotechnology and Biotechnology to Reduce Male Infertility: Novel Solutions for Complex Challenges

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Abstract

Infertility is the failure of a couple to conceive after a year of unprotected sexual activity. Male infertility is the failure of a male to conceive a female after at least a year of consistent, unprotected sexual activity. There are various causes of male infertility. Males may be the only cause of infertility in 20% to 30% of cases, with a 50% overall contribution in couples. Some infertile couples are still able to conceive if they do not receive treatment. Various treatments for male infertility include lifestyle changes, oral medications, hormonal therapies, gonadal treatments, and other options. The VF and ICSI treatments for male infertility primarily target sperm malfunction but often overlook underlying genetic, epigenetic, or physiological issues. However, advances in biotechnology and nanotechnology have opened new avenues for more effective and targeted therapies. Biotechnology is essential to developing gene therapies, regenerative medicine, and diagnostic tools to provide individualized treatments for male infertility. With its exceptional capacity for molecular-level interaction, nanotechnology has the potential to improve medicine delivery, minimize adverse effects, and improve sperm function. This chapter examines how these state-of-the-art technologies can be used to diagnose, treat, and possibly cure male infertility, emphasizing these technologies' difficulties and therapeutic uses.

Keywords: Male infertility, Biotechnology, Nanotechnology, Sperm function, Gene therapy.

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Introduction

Infertility is the inability of a couple to conceive after a year of consistent, unprotected sexual activity (Practice Committee of the American Society for Reproductive Medicine, 2020). In the United States, infertility upsets almost 15% of couples (Thonneau et al., 1991; Louis et al., 2013). Male infertility refers to the failure of a male to achieve pregnancy with a female partner after at least a year of consistent defenseless intercourse. Male factors are the cause of about 20% of infertility cases and contribute to an additional 30–40% of cases (Hull et al., 1985; Agarwal et al., 2015). Overall, male factors account for around 50% of all infertility case's (Schlegel et al., 2021). The WHO defines normal semen parameters, as outlined in Table 1 (Cooper et al., 2010).

Table 1: Normal semen parameters in accordance with WHO

Parameters	Values	95% CI
Volume	1.5 mL	mL 1.4–1.7
Sperm Morphology	4% Normal	3–4
Sperm vitality	58% alive	55–63
Concentration of Sperm	15 M spermatozoa/mL	12–16
Number of sperm/ejaculation	39 M spermatozoa	33–46
Progressive motility	32%	31–34
Progressive and nonprogressive sperm motility	40%	38–42

1.1 Etiology

Male infertility can arise from various causes. Primary testicular defects, including irregular sperm parameters, account for 65% to 80% of cases. Endocrine disorders, typically resulting from hypogonadism, contribute to 2% to 5%, while sperm transport disorders represent about 5%. Idiopathic infertility, where the male has normal semen parameters but remains infertile, makes up 10% to 20% of cases (Winters & Walsh, 2014).

1.2 Epidemiology

Globally, around 13% to 15% of couples experience infertility, with 1 in 5 couples unable to conceive within their first year of trying (Esteves et al., 2011). However, even without specific treatment, nearly half of young couples in the United States who do not conceive through their initial year of sexual activity will be able to conceive within the following year (Evers, 2002). Male factors are the sole cause of infertility in 20% to 30% of cases and contribute to about 50% of infertility cases overall (Agarwal et al., 2015).

1.3 Pathophysiology

Male infertility can be classified into the following categories:

- Pre-testicular causes: These include chromosomal abnormalities, genetic factors, retrograde ejaculation, anejaculation, hypogonadotrophic hypogonadosm and erectile dysfunction.
- Testicular syndromes: Examples comprise orchiectomy, primary testicular dysfunction, cryptorchidism, and atrophic testes.

Post-testicular causes: These involve seminal tract obstructions, post-vasectomy complications, congenital lack of vas deferens, premature ejaculation, erectile dysfunction, plus the usage of condoms or diaphragms (Leslie et al., 2024).

Treatmen

Some infertile couples can still achieve pregnancy without treatment (Figure 1). Research shows that 23% of infertile pairs conceive within two years without intervention, and this percentage increases to 33% after four years (Matorras et al., 1996).

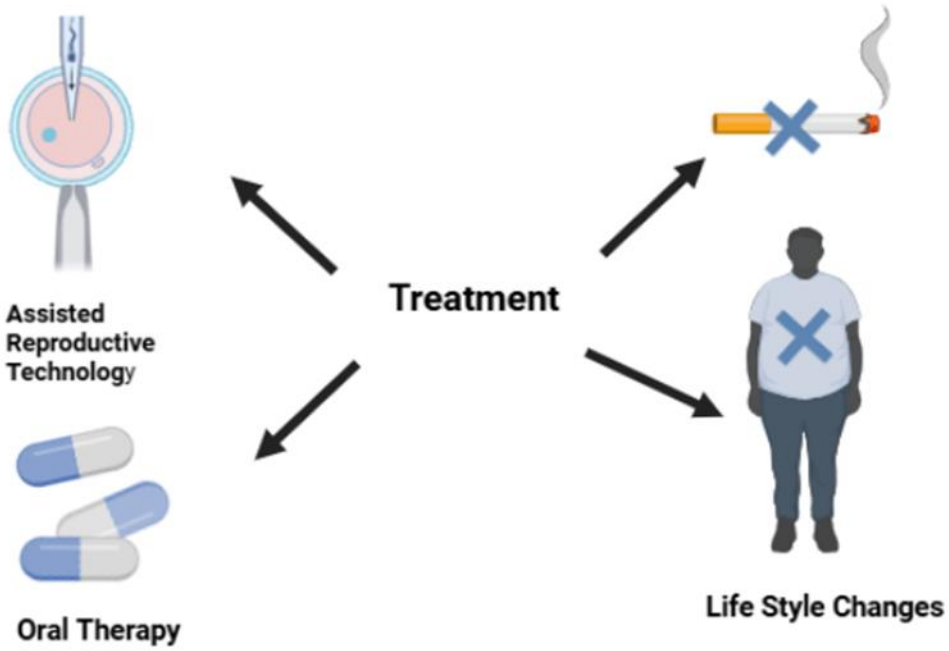

Fig. 1: Ways of Treating Male Infertility (Biorender)

2.1 Lifestyle Changes

Lifestyle changes that may improve male fertility include leaving smoking, decreasing or eliminating alcohol drinking, adopting a healthier diet, losing weight if overweight, increasing physical activity, avoiding harmful artificial lubricants throughout intercourse, decreasing stress, stopping recreational and illegal drug usage (such as marijuana), limiting prescription medications, and minimizing contact to pesticides, heavy metals (like boron, mercury, lead, and cadmium), and excessive chemicals (Penzias et al., 2018). While the exact impact of these factors on male infertility remains uncertain, it is reasonable to assume that maintaining a healthy lifestyle can enhance overall male fertility (Sharma et al., 2013).

2.2 Oral Therapies

In addition to being cost-effective, these therapies may provide psychological benefits. There is sufficient evidence suggesting potential advantages to justify clinical exploration for infertile men who cannot afford alternative treatments or are not suitable applicants for them. Such cures also allow physicians to offer a form of "treatment" while giving the pair more time to achieve a natural pregnancy (Whitten et al., 2006; Chua et al., 2013). Oral treatments are deliberated optional and are categorized into nutrition-based, antioxidant-based, and hormonal therapies.

While some studies present conflicting results, reducing oxidative stress on sperm and semen through antioxidant therapy appears to be a promising approach for treating male infertility (Qamar et al., 2023). However, in a three-year, multi-institutional trial involving 174 infertile men across nine reproductive centers, antioxidant therapy without L-carnitine did not lead to important improvements in semen parameters or pregnancy rates over a six-month observation period (Steiner et al., 2020). Despite this, additional key findings support using antioxidants in male infertility treatment.

The most extensively considered vitamins, antioxidants, and minerals, include coenzyme Q10, lycopene, N-acetyl cysteine, folic acid, L-carnitine, vitamin C, vitamin E, selenium, and zinc. Hormonal therapies, which are considered optional, include clomiphene, tamoxifen, and aromatase inhibitors.

2. 3 Sperm Abnormalities and Suggested Treatment

2.3.1 Standard semen analysis or Normospermia (15 million sperm/mL)

Men with standard semen analysis may also have an infertile spouse or idiopathic male infertility. In these cases, assisted reproductive technologies like IVF combined with ICSI should be used (Sunder & Leslie, 2022).

2.3.2 Low Motility or Asthenozoospermia

N-acetyl cysteine and L-carnitine can considerably increase sperm morphology and motility. Other cures include condom use to decrease exposure, immunity suppress steroid treatment for both spouses, and specific sperm processing for direct use in IUI (Sunder & Leslie, 2022).

2.3.3 Low Morphology or Teratozoospermia

N-acetyl cysteine and L-carnitine improve the morphology of isolated sperm. IVF with ICSI, may also be viable cure options (Sunder & Leslie, 2022).

2.3.4 Low sperm count or Oligozoospermia, (<15 million sperm/mL)

Hormone levels, including FSH, testosterone, LH, and prolactin, should be assessed. Low testosterone go along with by high LH and FSH may indicate Klinefelter syndrome, particularly if both testes are small. In these cases, karyotyping is suggested. If karyotyping results are normal, ART, including IVF with ICSI, should be used (Sunder & Leslie, 2022).

2.4 Gonadotropic Therapy

Gonadotropin therapy for men with idiopathic infertility remains a topic of debate. A meta-analysis of six randomized trials showed higher pregnancy rates with this therapy related to placebo (Finkelstein et al., 2013). While FSH promotes Sertoli cell action and sperm manufacture, its effectiveness is limited when used alone (Santi et al., 2015).

Effective gonadotropin therapy typically involves a combination of HCG, LH, FSH, GnRH, and HMG. For men with idiopathic hypogonadotropic hypogonadism (IHH), exogenous testosterone supplementation is discontinued. Treatment for male infertility in IHH has been successfully achieved using groupings of HCG, FSH, GnRH, and HMG.

Medications to accelerate the recovery of spermatogenesis are recommended in cases of delayed or prolonged recovery, older patients, individuals with azoospermia, and those with extended testosterone use. HCG, FSH, and SERMs are commonly used to stimulate the testes to produce endogenous testosterone naturally. Amongst these, as clomiphene in aiding sperm recovery, tamoxifen may not be as active.

2.5 Assisted Reproductive Technologies

Assisted reproductive technologies (ART), like as in vitro fertilization (IVF), intrauterine insemination (IUI), and intracytoplasmic sperm injection (ICSI), deliver traditional solutions for male infertility. These practices efficiently manage conditions like oligospermia and azoospermia, and poor sperm motility. For azoospermia (obstructive and non-obstructive), surgical sperm retrieval methods enable the usage of viable sperm.

Cryopreservation, artificial gametes, nanotechnology-based sperm organization, and gene-editing technologies improve ART's achievement. Together with hormonal treatments, antioxidant supplementation, and lifestyle adaptations, these improvements offer targeted treatments for male infertility, improving effective conception while decreasing the emotional burden on affected individuals (Jain & Singh, 2025).

3. Limitations of Male Infertility Treatments

IVF and ICSI treatments for male infertility primarily address sperm dysfunction but often fail to address underlying genetic, epigenetic, or physiological factors. Invasive sperm retrieval methods, such as TESE, do not resolve genetic abnormalities in sperm, which may compromise embryo quality and pose risks to the health of offspring. Hormonal therapies can be costly, have side effects, and require multiple cycles, making them emotionally and physically demanding for patients (Mehta et al., 2016).

4. Application of Nanotechnology in Treatment of Male Infertility

The fascinating multidisciplinary topic of nanotechnology is concerned with altering specific, size-dependent qualities at the nanometer scale (1–100 nm). Their ability at nono scale to manipulate and alter matter's behavior is unparalleled. Proper material control can be used to manage and improve solubility, targeted release, and other important features. The development of nanoscience has had a significant impact on several scientific fields, most notably medicine.

Nanoparticles (NPs) offer a treatment for infertility in couples due to their long and load-carrying ability. They have shown promise in reproductive medicine, preserving fertility, diagnosing and treating infertility, puberty, menopause, STIs, and reproductive tract malignancies (Baig et al., 2021).

4.1 Nanoparticle-mediated L-carnitine Transfer

Male infertility impacts 40% of couples, and L-carnitine (LC) is considered a potential treatment. However, traditional LC supplementation faces limitations due to absorption issues. This study examines the usage of nanoparticles to enhance the transfer and efficiency of LC in male

fertility treatments. While there are challenges related to safety and scalability, nanoparticle-based LC delivery shows promising potential for future clinical applications (Moghadam et al., 2024).

4.2 Antioxidant Nanoparticles

Antioxidant nanoparticles can improve sperm function and male fertility. Cryopreservation increases oxidative stress in sperm cells, which decreases the capacity for fertilization. Cerium oxide (CeO_2) NPs can act like reactive oxygen species (ROS) and accumulate oxygen keep sperm viability in cooling. This improves motility, enhance sperm velocity, and keeps both plasma membranes and DNA integrity protected, boosting efficiency in small sheep farms that use liquid-cooled semen (Khalil et al., 2018).

Nano-selenium (SeNPs) has also been utilized as a ROS scavenger to defend sperm cells from oxidative stress. It increases semen quality subsequently thawing and decrease oxidative damage in semen of rooster. Oral supplementation of SeNPs defends spermatogenesis from oxidative stress due to an anticancer drug, cisplatin (Safa et al., 2016).

4.3 Transfer of Functionalized Materials through Sperm-transported Nanoparticles

Nanotechnology is progressively being used to develop Nano platforms for transferring biological materials to cells or particular tissues. These treatments hold important potential for improving assisted reproductive technologies in farm animals. The distinguishing features of sperm cells, including their motility, limited intracellular uptake, and capacity to interlink with various compounds, have enhancing research into using nano carriers to effectively load sperm with proteins and nucleic acids. Studies have confirmed that polyvinyl alcohol (PVA)-Fe3O4 and PVA-Eu2O3 nanoparticles can be burdened into bovine sperm cells devoid of affecting their motility or acrosome integrity. Moreover, mesoporous silica nanoparticles (MSNPs) carrying fluorescent nucleic acids or proteins can interrelate with sperm membranes while preserving normal function. These results could pave the way for generating diagnostic and therapeutic tools targeted at enhancing sperm quality and treating male fertility issues (Ben-David Makhluf et al., 2006; Makhluf et al., 2008; Barkalina et al., 2014).

4.4 Controversies Surrounding the Usage of Nanotechnology in Reproductive Medicine: Environmental Effect of Nanoparticle Dispersal and Reprotoxicity"

Metal oxide nanoparticles (NPs), are gradually used in everyday life, including electronics, cosmetics, and food packing. There are ecological worries about their dispersion and distinctive biological effects on living organisms. Zinc oxide NPs have been revealed to apply cytotoxic action on murine testicular germ cells, affecting the maturing procedure of sperm in the epididymis (Talebi et al., 2013). Silver NPs are a possible cytotoxic cause for sperm cells, causing oxidative stress and reducing the achievement rate of in vitro fertilization (Yoisungnern et al., 2015). Exposure to TiO_2NPs can cause decrease in sperm production, testicular toxicity, and sperm lesions in mouse testes (Hong et al., 2015). AgNPs and Gold are widely used metal NPs, with the administration of AuNPs in mice causing an important decline in motility and normal morphology of sperm, chromatin remodeling, increased DNA damage, and lower chromatin stability. TiO_2NPs can cause DNA damage in male mice and affect the potency of male offspring after maternal exposure. Other metal NPs, CeO_2NPs, and dimercaptosuccinic acid-coated maghemite do not affect ram or bull sperm function or structure when added *in-vitro*.

4.5 Additional Challenges in the Practical Application of Nanoparticles

Nanoparticles (NPs) have limitations in in vivo drug delivery, targeting, and immune clearance. However, strategies like protein corona and PEGylation have been developed to improve targeting. This approach has not been broadly used for male infertility cure but has shown potential in testicular functions. Understanding the mechanisms of NPs' uptake and intracellular fate is vital for optimizing their planned functions. Further study is needed to translate this technology into effective in vivo products (Baig et al., 2021).

5. Application of Biotechnology in the Treatment of Male Infertility

In 1919, Hungarian engineer Karl Ereky introduced the term Biotechnology. Biotechnology uses live creatures or the parts of them to produce novel products. It is an integration of bio science with engineering through which live cells are used to produce novel products (Gupta et al., 2017)

5.1 Applications in Medication

Biotechnology has become vital tool in medicine, driving remarkable progress in health. Some medical applications are mentioned below:

5.1.1 Gene Therapy

Gene therapy is the delivery of specific genes to the cells to cure diseases. This can be done by various methods, such as viral or non-viral techniques. Applications of gene therapy have targeted conditions such as cystic fibrosis, muscular dystrophy, and certain cancers (Sayed et al., 2022).

5.1.2 Recombinant Protein Production

Biotechnology has facilitie the large-scale symthesis of recombinant proteins used as therapeutic agents. By genetically modifyied bacteria, or mammalian cells, these systems produce specific proteins for medical use. Examples include insulin for diabetes management, growth hormone for treating growth deficiencies, and clotting factors for hemophilia patients (Huang et al., 2012).

5.1.3 Genome Editing

The capacity to modify genetic code has revolutionized medicine. Advanced techniques like CRISPR-Cas9 enable precise gene editing in cells or organisms, paving the way for potential treatments for genetic syndromes, cancer, and contagious (Kolanu, 2024).

5.1.4 Personalized Medicine

Biotechnology advancements have led to the emergence of modified medicine, which tailors cures to an individual's genetic profile. This approach employs techniques like genetic testing and pharmacogenomics to evaluate how a person's genes disturb their reaction to specific medications (Imran, 2024).

5.1.5 Stem Cell Therapy

Stem cells are unique in their ability to alter into various cell types, making them a capable option for addressing numerous diseases and injuries. Advances in biotechnology have facilitated the large-scale production and modification of stem cells, which enables their specific application in therapeutic treatments (Zweigerdt, 2009).

5.2 Role of Biotechnology in Male Infertility

Louis Ignarro in 1992 showed that nitric oxide (NO) has an important function in human male penile tissue and that the inhibitor of cyclic GMP degradation potentiates the effect of release neuronal NO (2). This work aided in the creation of very effective remedies for men who suffer from the condition (ED) (Goldstein et al., 1998).

5.3 Gene Editing Techniques

5.3.1 Microarray

Microarray a laboratory techniques that make us in analyses of thousands of gene expression simultaneously. The use of this technique is increased in identification of genes related with human male infertility. After creation of cDNA microarray for human testis, Chen and his colleagues discover *tsMCAK* a novel testis-specific gene that is homologous to *HsMCAK*, this gene encodes human testis-specific mitotic centromere-associated kinesin (Cheng et al., 2002).

In testes tsMCAK gene was found to be expressed with spermatogenic arrest at spermatocyte stage. However, this gene is absents in patients that are suffering from sertoli-cell-only syndrome and also in patient with spermatogenic arrest at spermatid stage. This shows that *tsMCAK* have a critical role in the later phases of spermatogenesis, and absence of this gene might cause male infertility.

These results show that microarray technology is an important tool in gene identifications associated with male infertility. This technique provides molecular markers for future diagnosis and therapeutic application.it help in the discovery of novel genes their pathological mechanism of action, and their role in human male infertility (García-Giménez et al., 2017).

Microarray technology allows a quicker, more accurate diagnosis while also permitting the advancement of better treatment methods for various diseases. This progress is of great benefit to researchers and clinicians aiming to enhance diagnostic procedures and develop new therapies for various reasons for male infertility. So far, many of the known and new genes were analyzed using microarray technologies as possible causes of male infertility, they were made both in mice and humans and it gives hope for diagnosis and treatment.

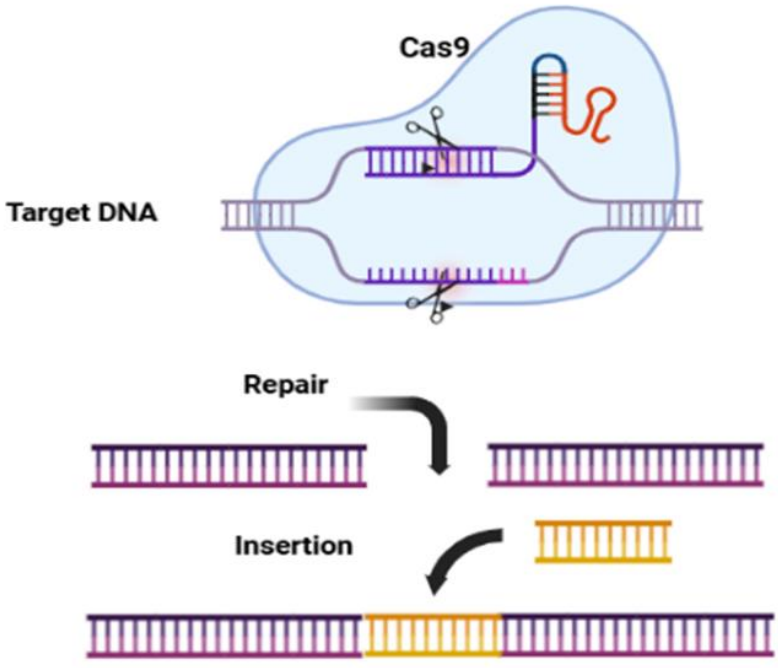

Fig. 2: Mechanism of CRISPR in Gene Editing (biorender)

5.3.2 CRISPR/Cas9

The procedure of cutting external viral nucleic acids in clustered repeatedly interspaced short palindromic repeats (CRISPR) has revealed its potential for gene editing. An RNA-guided tool, the CRISPR/Cas system is designed to modify specific DNA sequences. It functions through Cas proteins and includes CRISPR clusters, leader sequences, repeat regions (tracers), and a conserved set of CRISPR linked genes (Cas genes) (Liu et al., 2022).

The CRISPR/Cas system, especially CRISPR/Cas9, became highly popular among researchers soon after its debut in 2013. This technology has transformed genetic manipulation, making it easier to delete, insert, and substitute genes in various organisms, as illustrated in Figure 2.

In 2013, Yuxuan Wu and his team laid the groundwork for using CRISPR/Cas9 to repair genetic defects (Wu et al., 2013). By 2015, they had successfully used CRISPR/Cas9 to edit the Crygc gene in mouse spermatogonial stem cells (SSCs), effectively repairing genetic issues in mice (Wu et al., 2015). This innovative use of CRISPR/Cas9 for homology-directed repair (HDR) in SSCs offered a new beneficial approach to tackle male infertility linked to genetic problems in germ cells. In 2019, Xiaoyu Li and his colleagues applied CRISPR/Cas9 in vitro to correct a point mutation in the SSCs of Kitw/Kitwv mice (Li et al., 2019). Later being displaced back into the testes, these corrected SSCs effectively restored the normal fertility of the mice.

Conclusion

The combination of nanotechnology and biotechnology in addressing male infertility offers a promising strategy for treatment. Nanotechnology enables targeted drug delivery systems, accurate imaging, and biomimetic environments that enhance spermatogenesis, thereby improving both the effectiveness and safety of treatments. On the other hand, biotechnology drives innovations in gene editing, stem cell therapy, and assisted reproductive technologies. Recent developments, such as CRISPR-Cas9 for gene editing and nanoparticles for localized drug delivery, highlight the merging of these disciplines. Nonetheless, challenges such as ethical issues and regulatory obstacles persist. To fully leverage these technologies, collaborative efforts in research, clinical trials, and policy-making are essential.

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