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REVIEW ARTICLE

Engineered nanoparticles potentials in male reproduction

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Abstract

Background: The escalating prevalence of fertility problems in the aging population necessitates a comprehensive exploration of contributing factors, extending beyond environmental concerns, work-related stress, and unhealthy lifestyles. Among these, the rising incidence of testicular disorders emerges as a pivotal determinant of fertility issues. Current treatment challenges are underscored by the limitations of high-dose and frequent drug administration, coupled with substantial side effects and irreversible trauma inflicted by surgical interventions on testicular tissue.

Material and methods: The formidable barrier posed by the blood–testis barrier compounds the complexities of treating testicular diseases, presenting a significant therapeutic obstacle. The advent of nanocarriers, with their distinctive attributes, holds promise in overcoming this impediment. These nanocarriers exhibit exceptional biocompatibility, and membrane penetration capabilities, and can strategically target the blood–testis barrier through surface ligand modification, thereby augmenting drug bioavailability and enhancing therapeutic efficacy.

Results and discussion: This review concentrates on the transformative potential of nanocarriers in the delivery of therapeutic agents to testicular tissue. By summarizing key applications, we illuminate the strides made in utilizing nanocarriers as a novel avenue to effectively treat testicular diseases.

Conclusions: Nanocarriers are critical in delivering therapeutic agents to testicular tissue.

KEYWORDS
blood–testis barrier, drug delivery, infertility diseases, nanoparticles, testicular diseases

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1 | INTRODUCTION: CURRENT CHALLENGES OF INFERTILITY AND THEIR CAUSES

Infertility, as a pervasive global health challenge,[1] is impacting millions of couples and individuals worldwide. Current statistics reveal that 48 million couples and 186 million individuals are challenged with infertility (www.who.int/publications/i/item/9789200683 15), with male-related factors contributing to nearly half of these cases.[2–4] The etiology of male infertility is intricate, with a myriad of factors influencing both its onset and progression.

Testicular disorders encompass a spectrum of conditions affecting the physiological function of the testicles and their ancillary structures, including the epididymis, spermatic cord, and scrotum. Categorically, testicular dysfunction manifests through congenital genetic conditions including point mutations,[5] like microdeletions and translocations of chromosomes,[6] and acquired disorders, such as genitourinary infections (epididymitis or orchitis, etc.), chemotherapy for malignant tumors,[7] varicocoele,[8] and various kinds of medication treatment (statins[9] and psychoactive drugs[10]) Poor lifestyle including smoking, excessive alcohol consumption,[11] obesity,[12] and substance abuse and chronic exposure to chemicals, radiation, and heavy metals can also exert significant influences on fertility.[13] For instance, anabolic steroids can cause testicular atrophy,[14] while drugs like cocaine and marijuana can temporarily reduce sperm count or quality,[15] and significantly affect fertility.[16,17] Moreover, a variety of medical conditions and surgical interventions can impede male fertility,[18] ranging from infections like epididymitis or orchitis to scarring that hinders sperm passage through the excurrent duct system.[19]

The multifaceted nature of male infertility extends to various medical treatments, including radiation therapy or chemotherapy forcancer,[20] antibody attacks on spermatozoa, and abnormal blood flow resulting from conditions like varicocoele. Specific medication regimens may interfere with testicular endocrine function, altering reproductive or supportive cellular mechanisms and impacting sperm production.[21]

In essence, male infertility emerges from a complex interplay of factors, encompassing testicular ailments, chronic health conditions, lifestyle preferences, therapeutic interventions, environmental conditions, de novo genetic mutations, and so forth. A comprehensive classification identifies three main categories of male infertility: secondary hypogonadism due to hypothalamic–pituitary disorders,[22] obstruction of semen outflow, and testicular dysfunction. Among these, testicular dysfunction stands out as the most prevalent cause, thereby shaping the primary treatment strategy based on its specific underlying causes. In the meantime, controlled male contraception from a medication-based approach has long been lacking and requires considerable attention. Any approaches that could unlock the blood–testis barrier (BTB) would be of immense help in all the above issues in male reproduction.

2 | TESTICULAR DISORDERS AND CURRENT TREATMENTS

Prominent testicular disorders are orchitis, epididymis—orchitis,[23] testicular cancer, testicular torsion, varicocoele, and testicular effusion. Orchitis,[24] an inflammation often triggered by microorganisms like bacteria, viruses,[25] fungi, and parasites, demands tailored interventions. Antibiotics play a pivotal role in bacterial orchitis, while supportive care, including analgesics and hot or cold compresses, is essential for other infections. Severe cases may necessitate surgical drainage or excision.

Testicular cancer,[26] the most prevalent solid malignant tumor in young males, primarily comprises germ cell tumors.[27] Its uncertain etiology intertwines innate factors such as cryptorchidism, heredity, and chromosomal abnormalities with acquired factors like infections, trauma, environmental pollution, and nutritional deficiencies. Orchiectomy, serving both diagnostic and therapeutic purposes, guides subsequent treatments such as active surveillance, chemotherapy, retroperitoneal lymph node dissection, and radiation therapy. Yet, these interventions come with significant side effects, including infertility and various toxicities.

Cyclophosphamide (CP), a key component in cancer treatment, induces testosterone deficiency (TD) and fertility issues. Testosterone replacement therapy (TRT) addresses TD,[28] but its adverse effects underscore the need for more effective treatments post-CPTD.[29] Balancing the efficacy of common cancer treatments against their potential long-term toxicities, including cardiovascular disease, secondary cancers, and reduced fertility, remains a critical consideration.

Azoospermia, encompassing testicular spermatogenic dysfunction and obstructive azoospermia (OA),[30] presents distinct challenges. Non-obstructive azoospermia (NOA), linked to endocrine conditions, varicocoele-induced damage, undescended testes, and various factors, requires tailored clinical approaches. OA can involve surgical reconstruction or sperm retrieval for those desiring fertility, although the effectiveness of surgical correction varies.[31,32]

Asthenozoospermia, characterized by poor sperm motility,[33] arises from diverse etiologies, including inflammation, hormonal imbalances, lifestyle factors, and exposure to environmental stressors. Potential therapeutic strategies involve improving energy metabolism[34,35] and reducing oxidative stress,[36] with antioxidants and supplements such as *N*-acetylcysteine, coenzyme Q10, vitamin E,[37] and L-carnitine showing promise.

Oligospermia, marked by a reduced sperm count, stems from various causes, including endocrine dysfunction, infections, heredity, varicocoele,[38] sperm agglutination, and environmental factors.[39] Treatment options range from surgical interventions for varicocoele and cryptorchidism to immunosuppressants for anti-sperm antibodies.[40,41]

In summary, treatments for male infertility, ranging from medication to surgery, target specific underlying causes. Hormones,

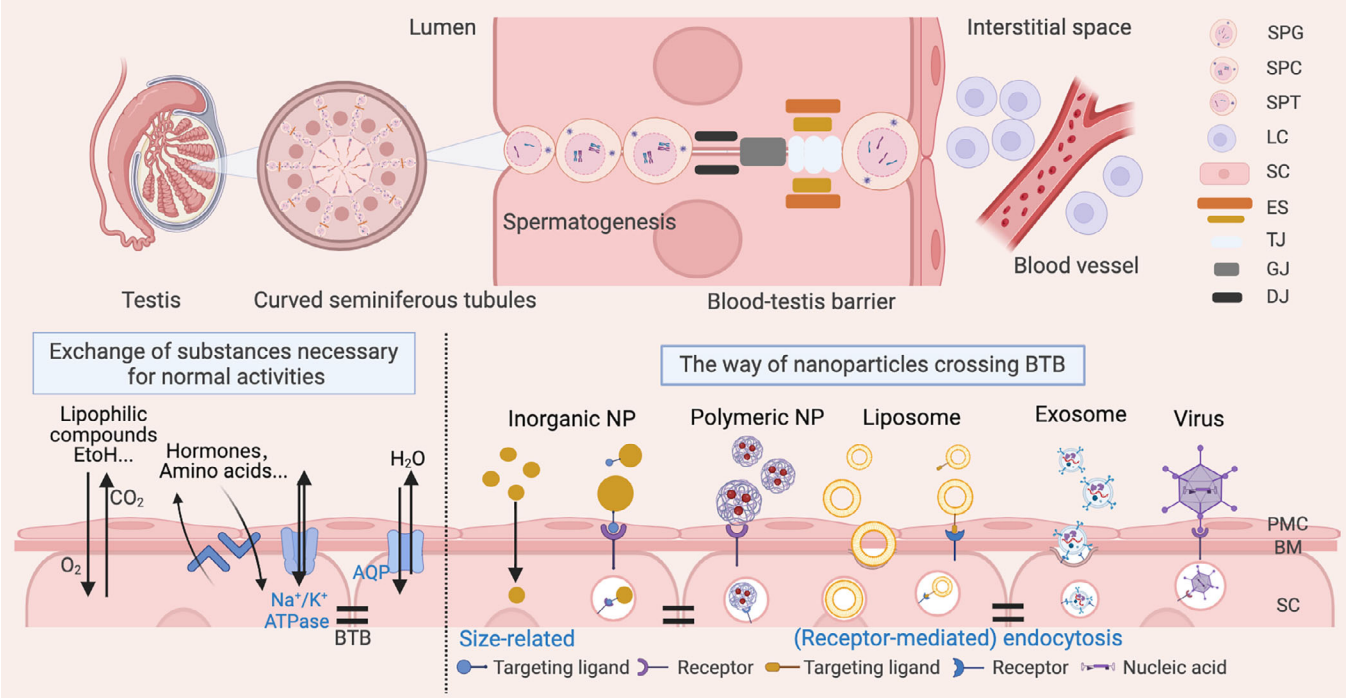

FIGURE 1 Schematic diagram of the blood–testis barrier (BTB) and mode of transportation of substances across the BTB. The BTB is located near the basement membrane of the germinal epithelium of the seminiferous tubules. It is composed of tight junctions, gap junctions (GJs), ectoplasmic specialization (ES), and desmosome-like junctions (DJs). BTB can regulate the transport of various substances, in which nutrients essential for spermatogenesis are transported through simple diffusion, solute carrier-mediated transport, and so forth. Nanoparticles of varying sizes can also pass through the BTB, due to their small size or surface-modified ligands that bind to the receptor. LC, leydig cell; SC, Sertoli cell; SPC, spermatocyte; SPG, spermatogonia; SPT, spermatid; TJ, tight junctions.

antibiotics, and supplements address endocrine dysfunction, reproductive tract inflammation, and sperm function, while surgeries like descending testicular fixation and varicocoelectomy promote spermatogenic function.[42,43] However, the formidable BTB remains a critical challenge, limiting therapeutic efficacy and exacerbating drug toxicity and side effects.

3 | OBSTRUCTION OF DRUG DELIVERY BY THE BLOOD–TESTIS BARRIER

The BTB, is a barrier between the seminiferous tubules and the circulation, located near the base of the Sertoli cells, and consists of tight junctions (TJs), ectoplasmic specializations (ESs), desmosomes, and gap junctions (GJs; Figure 1),[44] it plays a pivotal role in orchestrating the testicular microenvironment. Spermatogonia and preleptotene spermatocytes are located in the basal zone compartment, while other primary and secondary spermatocytes, round spermatids, and elongated spermatids are located in the intraluminal compartment.[45] TJs are the most important components of the BTB, acting simultaneously as a gate and a fence. TJs prevent the passage of water, solutes, and other macromolecules through the interstitial space of the cell and restrict the movement of proteins and lipids between the apical and basolateral domains. ES consists of F-actin microfilaments arranged in a hexagonal pattern between the plasma membrane and the endoplasmic reticulum (Figure 1). ESs that exist between SCs are called the basal ESs while those that exist between SCs and elongated spermatids are called the apical ESsx. GJs are intercellular channels that allow the diffusion of metabolites, second messengers, ions, and molecules smaller than 1 kDa.[46] Regulating the influx of nutrients (O_2, H_2O, and ion) essential for spermatogenesis, the BTB acts as a guardian, preventing the entry of deleterious substances[47]—particularly chemicals that might impede sperm production.[48] Furthermore, it governs the transportation of biological macromolecules and modulates the concentration of bioactive substances within the seminiferous tubules,[45] thus fostering an optimal milieu for spermatogenesis.[49] The protective role of the BTB extends to shielding spermatogenic cells from immune system assaults and thwarting the invasion of toxic substances and pathogens into the seminiferous ducts, essential for preserving male fertility.[50]

Dynamic alterations in BTB occur throughout spermatogenesis, influenced by peptides and signaling molecules that impact its barrier function and facilitate the transport of reproductive cells.[51] Despite its crucial protective functions, the BTB poses a formidable challenge in the context of drug delivery for testicular disorders. Certain viruses and tumors have been able to find refuge within the testes due to the BTB's immune privilege, rendering it difficult for water-soluble or large-molecule drugs, including antibiotics, antivirals, and antitumor agents, to penetrate the barrier and achieve therapeutically effective concentrations.[52]

Efflux proteins stationed at vital barriers in vivo further complicate drug delivery, exporting waste products of normal metabolism and preventing the entry of various harmful compounds into the protected tissues.[53] The germ cells of the BTB and testis express multiple drug transporter proteins, including the multidrug resistance protein P-glycoprotein,[48,54] actively expelling drugs from the testis and diminishing their therapeutic efficacy. Despite the surge in the variety and incidence of testicular disorders, the development of testicular drug delivery systems has seen limited progress.

Historically, treatment modalities for testicular diseases heavily relied on oral and intravenous administration.[55] However, these approaches presented notable drawbacks, such as systemic side effects, frequent dosing, challenges in achieving sufficient intratesticular concentration for therapeutic impact, and the incapacity of macromolecules to traverse the formidable BTB. Drugs targeting oxidative stress and energy metabolism enhancement, although frequently employed, often lack organ specificity and exhibit limited efficacy at elevated dosages. The BTB, acting as a formidable guardian, hampers the treatment of reproductive system diseases, including testicular cancer, asthenozoospermia, and viral infections, by impeding the entry of small-molecule compounds and nucleic acid drugs into reproductive cells.[56]

4 | ADVANTAGES OF NANOPARTICLES IN DRUG DELIVERY

The formidable presence of the BTB poses a significant challenge for conventional drugs to traverse and reach the targeted lesion site effectively. Nanoparticles, characterized by their ability to surmount biological barriers, offer a promising avenue for overcoming this obstacle.[57,58] Nanocarriers, specifically designed to ferry functional therapeutics, exhibit the capability to breach biological barriers through endocytosis[59] and physical methods that aid in the transient opening of biological barriers and deliver therapeutic payloads to tissues safeguarded by such barriers. These nanocarriers are adept at carrying small molecules of chemical drugs, nucleic acids, peptides, proteins, antibodies, and CRISPR/Cas9.

Nanocarriers can be broadly classified into four main categories based on their composition: organic materials, inorganic materials, extracellular vesicles (EVs), and viral vectors.[60] Organic nanoparticles, including lipid-based nanodelivery vectors, micelles, and dendritic macromolecules formed from lipids, polymers, and small molecules, can be surface-modified to enhance critical properties like surface charge,[61] lipophilicity, biocompatibility, tissue and organ targeting, and cell permeability.[62] These carriers can also achieve controlled release of drugs under specific conditions,[63,64] protecting them from rapid clearance by the body and prolonging their circulation time[65] (Figure 2).

In previous studies, each type of nanoparticle is capable of loading drugs of different nature, such as some biomolecules with therapeutic effects (siRNA, mRNA, plasmids and proteins, etc.) and small molecule inhibitors. The characteristics of different types of nanoparticles vary, liposomes have the advantage of flexible drug loading and high biosafety, but they also face poor biodistribution and low drug loading rates. Inorganic metal nanoparticles have the advantage of flexible size and shape, but due to their poor solubility, they often need to be modified to improve their biocompatibility. Various nanoparticles have been prepared in a variety of ways, as summarized in Table 1.

Inorganic nanoparticles, encompassing metals, metal oxides, and silica,[60] leverage their unique physical, chemical, optical, electronic, and magnetic properties for applications such as drug delivery, bioimaging,[81] therapeutics, and cancer immunotherapy,[82] The size, shape, and surface properties of inorganic nanoparticles can be controlled for optimal interaction with target cells. These nanomaterials offer biostability and non-degradability, allowing internal or external control of transport and drug release through factors like temperature, pH, magnetic fields, and light.[83]

EVs, originating from eukaryotic cells and bacteria, present a natural and endogenous option for drug delivery.[84] Comprising lipid bilayer membranes containing organelle-free cytoplasmic lysosomes, these vesicles can transport drugs across cell membranes via various pathways, ensuring good biocompatibility and low immunogenicity.[85]

Viral vectors, including adenovirus,[86] lentivirus,[87] and retrovirus,[88] exhibit unique structures and have been explored for their potential in vaccine development.[89] Virus-like particles (VLPs), lacking a genome and being non-infectious, offer a safer and less immunogenic alternative. Viral nanostructures serve as scaffolds for diverse materials, enhancing their modifiability and functionalization.[89]

Nanoparticles are significant in overcoming biological barriers, such as the blood–brain barrier (BBB), BTB, and placental barrier, to deliver drugs. Nanoparticles typically cross biological barriers to the lesion site through the transcellular pathway encompassing transcytosis and receptor-mediated transportation.[90] Alternatively, they may rely on physical means to assist them through the paracellular pathway. The transcytosis pathway can be identified as specific receptor-mediated and non-specific receptor-mediated. Specific endocytosis involves modifying the surface with targeting molecules such as glucose,[91] amino acids,[92,93] apolipoprotein E,[94,95] and ferritin,[96] These molecules bind specifically to their corresponding receptors expressed by targeting cells to achieve efficient transport. Non-specific transcytosis includes adsorption-mediated, clathrin-dependent, or clathrin-independent transcytosis pathways. For instance, cationic liposomes[97] and erythrocyte-derived EVs cross the biological barrier through adsorption-mediated cytosis.[98] Delivery of nanoparticles through the paracellular pathway can be affected by various factors, including size,[99] surface charge,[100] surface modification,[101] and magnetic[102] and photothermal properties.[103] Furthermore, physical methods, such as ultrasonic waves and electromagnetic pulses (EMPs), can be utilized to momentarily open the barrier and facilitate the delivery of nanoparticles.[104] Although there are limited studies on how nanoparticles penetrate the BTB, it is important to reference the crossing of other biological barriers (e.g., the extensively studied BBB) for the development of drug delivery vectors relevant to testicular diseases. Modification strategy for targeting specific cells in the testis (Figure 3) mostly involves the initial step to identify the target cells, followed by

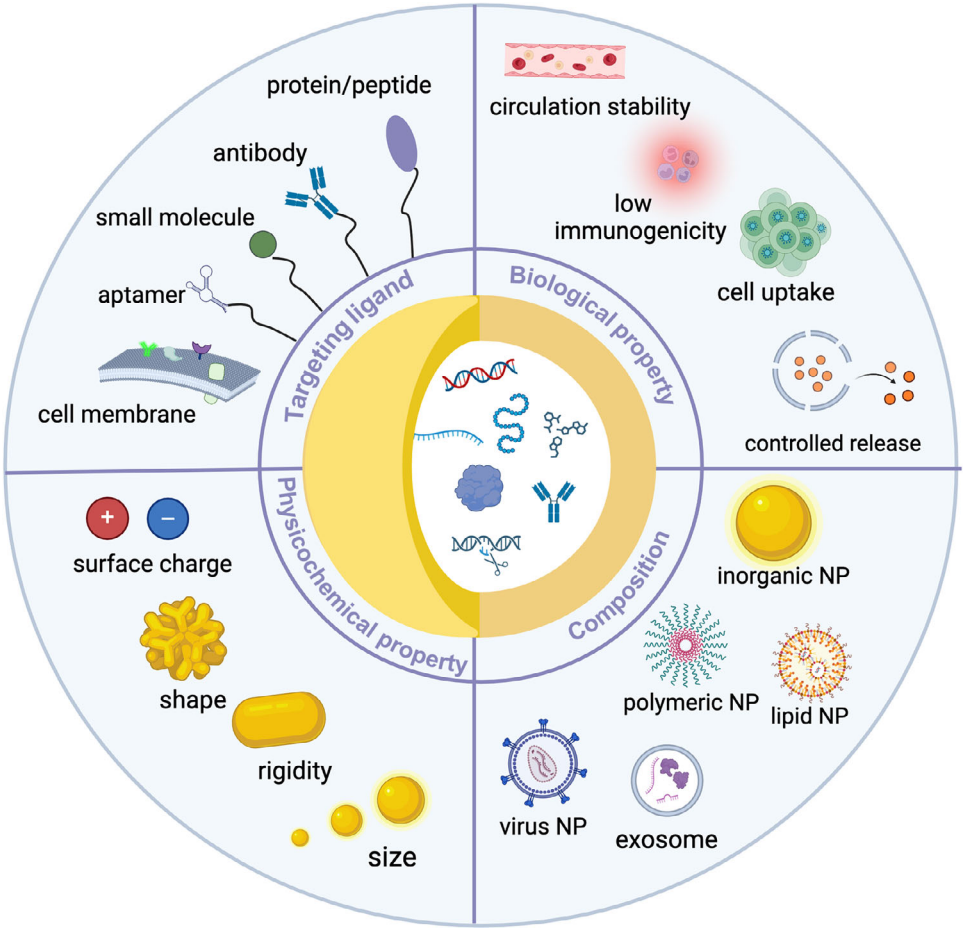

FIGURE 2 Common types of nanoparticles (NPs) and properties. NPs can be loaded with different properties and types of drugs, such as proteins, small molecules, nucleic acids, and antibodies; Common types of NPs include liposomes, polymeric NPs, and exosomes. The surface of NPs can be positively or negatively charged, and their particle size, shape, and hardness can vary. In addition, the surface of NPs can be modified with small molecules, proteins, antibodies, and aptamers to enhance targeting. These properties enable in vivo applications such as extended biological half-life, efficient cellular uptake, tissue penetration, and controlled drug release.

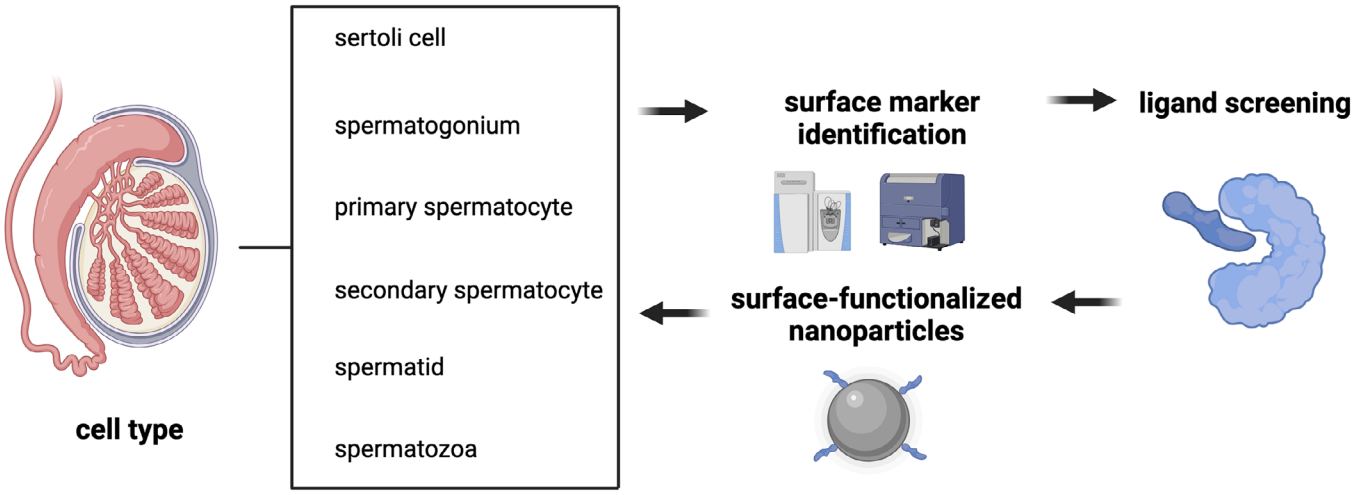

FIGURE 3 Rational design for cell type-specific targeting nanoparticle.

TABLE 1 Summarization of drug loading, characterization, and preparation of different nanoparticle types.

NP type	Therapeutic cargo delivered	Advantages	Disadvantages	Production	References
LNP	siRNA, mRNA, ASO, protein, small molecules, vaccines	Simple formulation, self-assembly, good biocompatibility, high bioavailability, ability to carry drugs with different physical properties	Low drug loading and biodistribution that results in high uptake to the liver and spleen	Microfluidic methods, extrusion of lipid vesicles, nanoprecipitation	66–68
Polymer NPs	Small molecules, siRNA, mRNA, ASO	Biocompatible and have simple formulation parameters	The increased risk of particle aggregation and toxicity	Solvent evaporation method, dialysis	69–71
Inorganic NPs	Small molecules, siRNA, DNA protein	The wide variety of sizes, structures and geometries, unique physical, electrical, magnetic and optical properties	Low solubility and toxicity concerns	Method of precipitation, ultrasonic method, self-assembly	70, 72, 73
EVs	siRNA, antibody, protein, plasmid, small molecules	Low immunological response; potential intrinsic therapeutic activity	Low separation efficiency, Limited mechanism studies	Density gradient centrifugation, differential centrifugation, kit method	74–77
Virus vectors and VLPs	mRNA, protein, small molecules	Favorable biocompatibility and biodegradability	Be recognized and attacked by the immune system	Modification using genetic engineering techniques	78–80

Abbreviations: ASO, antisense oligonucleotides; EVs, extracellular vesicles; LNP, lipid nanoparticle; NP, nanoparticle; siRNA, small interfering RNA; VLPs, virus-like particles.

identifying their corresponding unique surface markers, and a proper ligand that can bind to the surface marker of choice using techniques such as computer simulation, antigen–antibody conjugation, and so forth. The selected ligand can then be used to decorate and "functionalize" the surfaces of nanoparticles, thus enabling them to transport across the BTB.

In summary, nanoparticle-based carrier systems provide a multitude of advantages, including protection of drugs from degradation, prolongation of drug circulation time, improvement of bioavailability, controlled drug release, and targeted delivery to specific cell types. Recognizing these advantages, nanodelivery carriers emerge as a promising approach to overcoming biological barriers. This review focuses on several nanocarriers capable of crossing the BTB and delivering small-molecule inhibitors, nucleic acid drugs, and male contraceptives to testicular tissue (Table 2).

4.1 | Extracellular vesicles

EVs are membranous structures released by cells, facilitating the transport of bioactive cargoes—proteins, lipids, and nucleic acids—to regulate diverse biological functions. Categorized into microvesicles, apoptotic bodies, and exosomes based on their diameter and origin,[85] EVs serve as signal carriers for intercellular communication, playing a crucial role in maintaining cellular homeostasis. Their ability to fuse with the membranes of target cells allows for the delivery of contents, regulating physiological or pathological processes, and aiding in disease diagnosis and prediction. Due to their potential to traverse biological barriers, EVs stand out as promising clinical drug delivery vehicles,[112] exemplified by their ability to cross the BBB for drug delivery in brain disorders.[113]

Previous studies have demonstrated EVs containing RNA for enhanced green fluorescent protein (EGFP) released into the bloodstream by transplanted human cells. Intriguingly, fluorescent signals observed in mouse epididymal spermatozoa suggest the potential of EVs to deliver somatic cell material to germ cells, possibly crossing blood–blood or blood–epididymal barriers.[114]

Sertoli cells, pivotal for testicular development, hormone secretion, and BTB formation, release exosomes that could be instrumental in treating male infertility. Research indicates that Sertoli cell-derived exosomes facilitate the transfer of miR-486-5p into spermatogonial stem cells (SSCs).[105] Furthermore, these exosomes can cross the BTB, delivering their contents, including CCL20 mRNA, to mesenchymal cells, thereby regulating mesenchymal cell survival through CCL20.[115] In mammalian testicular development, exosomes and their miRNAs play crucial roles in regulating various stages, from SSC proliferation to spermatocyte meiosis, maintaining the testicular immune microenvironment.[106]

Studies on Sertoli cell-derived extracellular vesicles (SC-EVs) loaded with miR-24-3p inhibitors have resulted in a nanomedicine (SCsEV@miR-24-3p inhibitor) capable of crossing the BTB and delivering the inhibitor to germ cells. This nanomedicine proved effective in targeting testicular and germ cells, showcasing its potential in clinical treatments like asthenozoospermia.[116]

Bone marrow mesenchymal stem cell-derived exosomes (BMSC-exos) could reduce the reproductive toxicity of CP by inhibiting

TABLE 2 Application of nanoparticles in testicular-related diseases.

NP type	Drug	Application potential	References
EVs	Nucleic acid drug (miR-486-5p)	Diagnosis of male infertility	105
EVs	Nucleic acid drug (miR-30a-5p)	Male infertility	106
EVs	Nucleic acid drug (miR-24-3p)	Asthenozoospermia	106
LNP	Nucleic acid drug (Dmc1 saRNA)	Male infertility caused by genetic mutations	107
LNP	Nucleic acid drug (PIN1 proteins)	Male infertility	108
LNP	Small-molecular drug (honokiol)	Cisplatin-induced male fertility defects	109
Inorganic NP	Small-molecular drug (MCP I)	Male contraceptive	110
Inorganic NP	Small-molecular drug (curcumin)	Improve spermatogenesis	111
HFn	Small-molecular drug (ATP)	Asthenozoospermia	96

Abbreviations: Dmc1, DNA meiotic recombinase 1; EVs, extracellular vesicles; HFn, human H-ferritin; LNP, lipid nanoparticle; MCP 1, *Carica papaya* seeds extract; miRNA, microRNA; NP, nanoparticle; PIN 1, peptidylprolyl cis/trans isomerase NIMA-interacting 1; saRNA, self-amplifying RNA.

p38MAPK/ERK and AKT signaling pathways and ameliorating CP-induced testicular spermatogenesis dysfunction.[117] Further experiments demonstrated that BMSCs-exos alleviated cell death in CP-exposed spermatogonia GC1 and TM3 mouse Leydig cells and promoted autophagy to improve TD induced by CP by regulating the AMPK-mTOR signaling pathway.[118] A summarized table (Table 2) for the detailed application of nanoparticles in male reproductive treatment is presented. In addition to their loaded drugs (Table 2), these nanoparticles are also functionalized by decorating ligands (e.g., ferritin[96]) for receptor-mediated transcytosis across the BTB, although, many cases are not able to identify the mechanism aiding the nanoparticle transversing the BTB after intravenous application.[107]

While EVs hold promise for drug delivery with attributes like low immunogenicity, low cytotoxicity, and high biocompatibility, challenges remain. Limitations include effective isolation methods, drug loading efficiency, EV uptake by target cells, non-specific biodistribution, and rapid elimination in circulation.[119] Although evidence suggests that EVs can facilitate information exchange between testicular mesenchyme and seminiferous tubules, further experiments are essential to elucidate the precise mechanisms of EVs crossing the BTB in vivo, paving the way for their application in diseases related to the testicles.

4.2 | Lipid-based nanoparticles

Liposomes and lipid nanoparticles (LNPs) are well-established nanomaterials in drug and gene delivery, many liposome nanomedicine have been instrumental in anti-cancer therapeutic because Doxil was approved by the US Food and Drug Administration (FDA) in 1995, and then LNPs have shown strong potential in gene delivery as applied in some mRNA-based COVID-19 vaccines.[120] Liposomes are self-assembled spheres, composed of amphiphilic molecules with a hydrophilic head facing the outer aqueous environment, exhibit superior biocompatibility and transmembrane transport, resembling cell membranes.[121] Surface-modified liposomes, adorned with specific ligands, can target disease tissues or tumor microenvironments, enabling targeted drug delivery and minimizing the risk of toxic side effects. Their unique structure allows encapsulation of hydrophobic small-molecule drugs, nucleic acids,[122] proteins,[123] and hydrophilic small molecules, offering protection against degradation and prolonging drug half-life in the blood.

Extensively studied for aiding nucleic acid drugs in crossing the BBB, lipid-based nanodelivery vectors have shown promise in helping these drugs traverse the BTB. For instance, studies utilizing cholesterol-amino-phosphate (CAP) lipids, mixed with dioleoyl phosphatidylethanolamine (DOPE) and 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol (DMG-PEG), formed LNPs (CAP2-4 LNP) with a particle size of around 120 nm. These examples successfully aided the delivery of DNA meiotic recombinase 1 (Dmc1) saRNA, demonstrating therapeutic effects in mice with low expression of Dmc1, akin to azoospermic mice.[108]

Lipid-based nanodelivery vectors have also proven effective in assisting the delivery of proteins across the BTB. Studies encapsulating filipin protein nanoparticle complexes in cationic lipids successfully delivered PIN1 proteins to testis cells, restoring protein levels and rescuing mice with testicular immaturity caused by PIN1 deficiency.[109] Additionally, by an yet-to-identify mechanism, liposomes are able to transport across the BTB, release their encapsulated natural polyphenol antioxidants honokiol to reduce reactive oxygen species (ROS) levels, maintaining mitochondrial structure and ATP-producing capacity in testicular cells, thereby compensating for cisplatin-induced defects in male fertility.[107]

While lipid-based nanodelivery vectors stand as primary nanomaterials in drug delivery, their use in crossing the BTB for treating testicular diseases is an emerging area. Surface modifications targeting the BTB could significantly enhance treatment efficiency for testicular-related diseases, overcoming potential limitations such as low drug loading efficiency and systemic drug distribution.

4.3 | Inorganic nanoparticles

Inorganic nanoparticles possess unique physicochemical properties and enhanced bioactivity due to their high surface area-to-volume

ratio. They can be engineered into various sizes, structures, and geometries, and their surfaces can be functionally modified to improve their ability to cross biological barriers.[68] Types of nanoparticles include metal nanoparticles (platinum, gold, silver, and copper), metal oxide nanoparticles (titanium dioxide, zinc oxide, and iron trioxide), and carbon nanomaterials (fullerenes and carbon nanotubes). These nanoparticles have shown promise in drug delivery and imaging.

Gold-based nanoparticles, such as nanospheres, nanorods, and nanocages, have been studied extensively for diagnostic imaging and drug delivery. For instance, gold nanoparticles modified with *Carica papaya* seeds extract MCP I demonstrated enhanced contraceptive effects in rats.[110] The nanoparticles were conjugated with MCP I, overcoming the impediment of the BTB and achieving higher efficacy in albino rats compared with conventional delivery systems.

PEG-modified gold nanoparticles (mPEG@AuNP)[124] demonstrated efficient tissue permeability and low toxicity. These nanoparticles could pass through the BTB and enter germ cells without affecting fertility, making them potential drug delivery systems for treating testicular diseases.

Starfish-like gold–copper alloy nanocrystals[125] showed potential in carrying siRNA, miRNA, DNA, or peptides to male germ cells for treating male infertility. These nanocrystals penetrated male germ cells through the BTB, especially under certain pathological conditions or heat treatment.[126,127]

Iron oxide nanoparticles (IONP) loaded with curcumin[111] demonstrated improved sperm viability in mice subjected to prolonged scrotal thermotherapy. The nanoparticles reduced the required drug dosage and mitigated toxic side effects, enhancing spermatogenesis.

MgH_2, a nanomaterial used for hydrogen storage, positively affected male fertility by inhibiting oxidative stress.[128] Additionally, intravenously injected amorphous silica nanoparticles (MSN) accumulated in supporting cells and germ cells, penetrating the BTB without causing significant damage to the testes. This opens avenues for targeted drug delivery through an applied magnetic field.[129]

Fluorescent europium-doped zinc oxide nanoparticles (ZnO: Eu NPs) accumulated in the testis without adverse effects, showcasing their potential for diagnostics.[130] Physical methods, such as pulsed unfocused ultrasound (PuFUS)[127] and EMP[131] exposure, were also explored for enhancing the permeability of the BTB to drugs.

Inorganic nanoparticles with magnetic, radioactive, or plasmonic properties find applications in diagnostics, imaging, and photothermal therapy. Targeting moieties,[132] such as human h-ferritin (HFn), can be modified on the surface of nanoparticles to achieve specific targeting of elevated spermatozoa in the testis, improving sperm viability.[96]

While inorganic nanoparticles offer diverse possibilities in drug delivery and diagnostics, challenges include potential toxicity, low solubility, and non-specific distribution. Surface modifications with targeting moieties aim to address these challenges, emphasizing the importance of continued research for clinical applications.

The use of inorganic nanoparticles for male contraception is also a topic that is currently attracting considerable interest. In light of the superior physicochemical properties of IONP, researchers have identified a method of controlled male contraception. This involves the injection of PEG-coated iron oxide nanoparticles (PEG@Fe_3O_4-50), with a diameter of 50 nm, into the testes under the action of an alternating magnetic field (AMF).[133] A further study investigated the potential of human heavy chain ferritin (HFn) nanocarriers loaded with aggregation-induced emission luminogens (AIEgens) for non-invasive and controlled male contraception, guided by Near-Infrared-II (NIR-II) fluorescence imaging.[134] Nevertheless, these methodologies are not without inherent constraints. For instance, testicular administration and infrared lasers may cause discomfort, while the non-readily degradable nature of nanoparticles may give rise to certain safety concerns. These methods are acceptable for patients with diseases but less convenient for healthy men who require contraception. Consequently, the utilization of nanoparticles for male contraception necessitates the collective endeavors of numerous researchers.

5 | CONCLUSION AND PERSPECTIVES

The presence of the BTB limits the bioavailability of drugs in the testis, which complicates the therapeutic regimen for testis-related diseases. There is an urgent need for a safe and effective drug delivery strategy to address this limitation. Nanotechnology enables potential drug delivery systems across the BTB to effective targets. Adjustable size, morphology, and especially modifiable surfaces enable nanocarriers to passively or actively target specific areas.

Ongoing research is developing new testicular delivery strategies that will safely, accurately, and efficiently penetrate the BTB through various non-invasive routes to deliver drugs to the target site, ultimately enhancing drug efficacy. These strategies include (1) Surface modification with antibodies, peptides, and other ligands to achieve active targeting of the desired site and improve in vivo distribution and accumulation by specific coupling with receptors on the membrane. This approach ensures more effective and precise delivery of the therapeutic agents; (2) Biomimetic nanoparticles can acquire the traits of the original cells, overcome multiple biological barriers in vivo, achieve homologous targeting, and reduce immune rejection; (3) Coupling the delivery vehicle with bioactive decorations enhances the efficiency of drug penetration into the BTB. Moreover, the limited availability and side effects of current male contraceptives highlight the need for further research. The use of nanoparticle loading to enhance the contraceptive efficacy and biosafety of hormonal male contraceptives is thus a highly promising avenue. Yet, despite the emerging studies for the exciting avenue shipping across the BTB, current research still lacks a suitable model, in vivo or in vitro, to assess BTB permeability. As for future translation, data must be obtained to address safety concerns, such as potential reproductive toxicity, in vivo metabolic changes organ accumulation, and so forth before proceeding with clinical translation.

AUTHOR CONTRIBUTIONS

Feifei Zhao, Mengyu Fan, Zhiyang Jing and Yanxu Zhang contributed equally to this work. Feifei Zhao, Xue Xia, and Yang Liu contributed to the conception design. Zhiyang Jing, Mengyu Fan, and Yanxu Zhang contributed to literature search and scheme inter-

pretation. Robert John Aitken and Congli Zhou contributed to the drafting and discussion; Feifei Zhao, Xue Xia, Zhiyang Jing, Mengyu Fan, and Yanxu Zhang wrote the manuscript draft. Xue Xia, Yang Liu, Zhiyang Jing, Mengyu Fan, Yanxu Zhang, Yanlin Wang, and Congli Zhou revised the manuscript. All authors were involved in this manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this paper as no datasets were generated.

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