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The role of stem cell-based extracellular vesicles in male fertility

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Abstract

Fertility tends to decrease over time in physiological or various pathological conditions. Preservation of male and female fertility is of equal importance for successful reproduction. Protection and maintenance of male fertility and infertility treatment are among the medical fields that have attracted attention, especially in recent years. Various therapeutic approaches, especially sperm and testicular tissue cryopreservation, are applied for the preservation and continuity of fertility. In addition to these methods, which are routinely used, there is an increasing interest in stem cell-based therapies, which are not only aimed at preserving fertility but also effective in infertility treatment. Stem cells are therapeutic agents with high differentiation potential found at every stage of life. In the light of the studies, it has been determined that besides all the positive effects of stem cells, they contain nanoparticles known as extracellular vesicles in their structures. This review will discuss the type of extracellular vesicles, their biological functions, the use of stem cell-based extracellular vesicles in men, and current studies.

Keywords: fertility; stem cells; extracellular vesicles; exosome

1. Introduction

In living things, fertility tends to decrease over time under physiological conditions. In addition, it is known that various pathologies (such as spermatogenic insufficiency, severe oligozoospermia), immunological diseases, and various medical procedures applied to patients (such as vasectomy, testicular surgery, chemotherapy) cause fertility to decline or infertility. Therefore, it is possible to say that fertility may decrease due to natural causes, diseases, and treatments applied against diseases. For all these reasons, fertility preservation; has become an increasingly important issue in all living things (1). Preservation of male and female fertility is of equal importance for reproductive success. Preservation of male fertility; is among the essential medical fields that have attracted attention in recent years, and various therapeutic approaches are used for this purpose. Among the main methods available are sperm and testicular tissue cryopreservation. The routinely applied method is sperm cryopreservation (2). However, with these methods, it is impossible to produce new and fertile spermatozoa or increase the fertility of existing spermatozoa; only existing spermatozoa are preserved for future storage. In other words, semen cryopreservation; is not infertility treatment in men; It is only a fertility preservation method. This situation has led researchers to search for alternative approaches for maintaining fertility and treating infertility.

Stem cell therapy; is a relatively new treatment method used in many areas such as healing of degenerated tissues and organs, treatment of immunosuppressive diseases, treatment of congenital anomaly, and cancer treatment. Stem cell therapy today; has also started to take place in the reproductive field, and various studies are being carried out on this subject. Reproductive stem cell studies generally use mesenchymal (MSCs) and spermatogonial stem cells (SSCs) (3). Recent studies are; showed that stem cells can also produce nano-sized cell membrane particles known as extracellular vesicles (EVs) (4). It is known that EVs are produced in the male and female genital tract, as in many paracrine tissues, and there are various studies on the effects of EVs on the reproductive systems. Studies carried out in recent years; EVs derived from stem cells seem to have a regenerative and therapeutic effect like the stem cells themselves (5). In light of all this information, stem cells and EVs obtained from stem cells have become the focus of attention in reproductive fields such as fertility preservation and infertility. This review will discuss the type of extracellular vesicles, their biological functions, the use of stem cell-based extracellular vesicles in men, and current studies.

2. Extracellular Vesicles

Along with releasing secretory vesicles by specialized cells such as hormones or neurotransmitters, various membrane vesicles, known as extracellular vesicles, are also released from all cells. The release of membrane vesicles from the cell surface has been evolutionarily conserved from bacteria to fungi, from parasites to humans (6). Extracellular vesicles (EVs) are nanoparticles released from all cell types with membrane-bound bi-lipid structures containing proteins, lipids, and nucleic acids (microRNAs and mRNAs) involved in cellular communication (7,8). EVs carry a large number of molecules. These molecules include various receptors,

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adhesion molecules, proteins involved in cell trafficking and intracellular signal transduction, cytoskeletal proteins, cytoplasmic enzymes, cytokines, chemokines, and cell-specific antigens (Ags). Moreover, mRNA is enriched with several nucleic acids, including long non-coding RNAs, microRNAs (miRNA), and even extra-chromosomal DNA (9). Lipids enable cells to exchange information because they contain a complex load of signalling proteins, small non-coding RNAs (sncRNAs), and regulatory RNAs (10) (Fig.1).

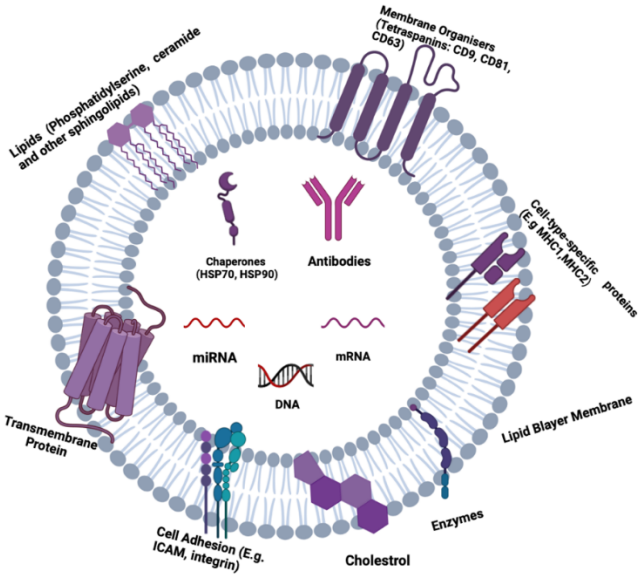

Fig. 1. Cargo contents carried by extracellular vesicles.

EVs; are membrane-packed vesicles secreted by various cell types, including T cells, B cells, dendritic cells, platelets, mast cells, epithelial cells, endothelial cells, neuronal cells, cancerous cells, oligodendrocytes, Schwann cells, embryonic cells, and MSCs (11). It has also been isolated from many biological fluids, including blood, milk, saliva, amniotic fluid, and urine (12, 13). EVs are known to play an important role in cell-to-cell communication. They are involved in physiological and pathological important processes such as immune responses, maintenance of homeostasis, coagulation, inflammation, cancer progression, angiogenesis, and antigen presentation (11, 14). It has been observed that EVs participate in different processes effectively in the physiology of the organism, such as tissue repair (15), preservation of the stem cell status of progenitor cells (4), immunosurveillance (16). They are essential information carriers between cells through the transmission of various proteins, bioactive lipids, and genetic information to alter the phenotype and function of recipient cells. Therefore, extracellular vesicles have been associated with numerous biological and pathological processes (17).

The transported content may vary depending on the cell type and activation state. Once released, EVs can interact with neighboring cells or disperse in the bloodstream and other organic fluids such as semen, saliva, and urine. Their ubiquitination makes EVs critical effectors of cell-to-cell communication, which can occur autocrine, paracrine, exocrine, or endocrine (9). They also trigger cell proliferation, migration, angiogenesis, and apoptosis. It affects altering gene expression, suppresses the immune system, and induces implantation of EVs produced by embryonic tissues (18-20).

Extracellular vesicles can be isolated using differential centrifugation, density gradient centrifugation, ultrafiltration, chromatography, polymeric precipitation, and microfluidic devices. However, there is no single standard applied procedure in isolation methods, and all existing methods have advantages and disadvantages. The chosen method also affects the quality and purity of the vesicles obtained. The most common isolation method used today is the ultracentrifuge method. (21,22). Many isolation methods, including ultracentrifugation, damage the structure of EVs due to the forces involved in isolating these tiny particles. Ultracentrifugal EV isolation methods, ultrafiltration, and immunoaffinity columns can isolate highly pure EVs at the expense of long isolation times that eventually result in few EVs. Polymeric precipitation of EVs, which allows their isolation at average centrifugation speeds, contaminates proteins and other extracellular vesicles (23).

2.1. Biogenesis of Extracellular Vesicles

EVs consist of 3 different groups according to their biogenetic pathways and sizes; exosomes, microvesicles (MVs), and apoptotic bodies. MVs are formed by budding directly from the cell membrane and are reported to have sizes between 100 nm and 1000 nm. They carry a cargo of proteins, lipids, mRNAs, and microRNAs and interact with recipient cells with specific receptor-ligand complexes (11). They are rich in selectins, integrins, CD-40, phosphatidylserine and metalloproteinase. Apoptotic bodies are fragments of dying cells formed and secreted in the extracellular space by the budding of the plasma membrane during the apoptotic process. They are irregular in shape and range in size from 500 to 4000 nm. They are rich in DNA and histones (24). Exosomes are 30-100 nm vesicles in size of endosomal origin and are found in multidimensional bodies that fuse with the cell membrane and are then released into the extracellular space (9) (Fig. 2).

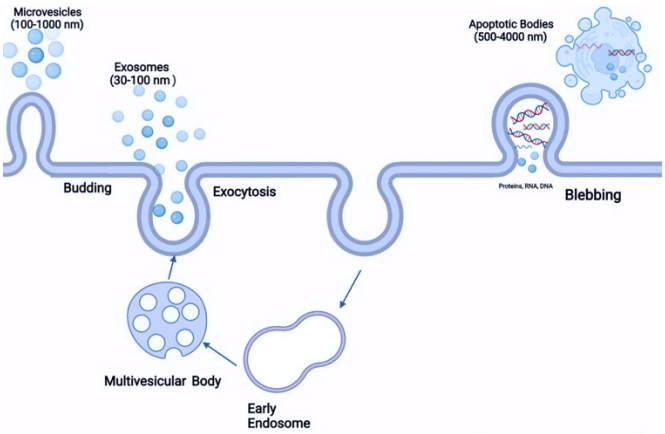

Fig. 2. Schematic representation of EVs biogenesis.

Exosomes are enclosed in a bilayer membrane that protects their contents and allows them to travel long distances in tissues. While this bilayer membrane contains a small amount of phosphatidylserine, it contains more cholesterol, ceramide, and sphingolipid (12,14). Exosomes carry membrane proteins, cytosolic proteins, transcription factors, DNA, mRNA, rRNA, miRNA, and various signal transduction molecules. They are rich in heat shock proteins (HSPs), annexins, cytoskeletal proteins, signal transduction proteins, and multidimensional body synthesis proteins (9). The most important evolutionarily conserved proteins in exosomes; are heat shock proteins (HSP), CD63, and tetraspanins (25). Exosomes carry free fatty acids and bioactive lipids derived from arachidonic acid. Signalling mediators such as prostaglandins, arachidonic acid, phospholipase A2, phospholipase C, and phospholipase D are also components of the exosome lipid pool (26). Exosomes are small particles with major functions, and they stand out thanks to their big role in intercellular and intracellular communication (27). It is well known that cholesterol and phospholipids are essential components of mammalian cell membranes, including exosomes, and cellular membrane integrity can be maintained by balancing the physiological cholesterol/phospholipid ratio. Growth factors associated with exosomes have also been found to play an essential role in the repair and healing of damaged tissue (28).

3. The Role of Extracellular Vesicles in the Male Reproductive System

Studies have shown that EVs and their cargoes have influential roles in male reproduction. From puberty to adulthood, the spermatogonial stem cells in the testis differentiate in the seminiferous tubules and mature into spermatozoa. Spermatozoa undergo many changes as they leave the testicles and pass into the epididymis (29). Especially, EVs coming from the epididymis and prostate gland interact with spermatozoa during these transitions, giving them many changes and functions. EVs; are secreted in different parts of the reproductive tract (male and female), epididymosomes, prostasomes, uterosomes, and oviductosomes (30). Although isolated from all tissues found in the male reproductive tract, they are best characterized by the epididymis (epididymosomes) and seminal fluid (prostasomes). Although first documented in hamsters, they have been identified in all mammals studied, including rats, bulls, rams, and humans. In general, EVs support the development and function of spermatozoa and support reproductive success by affecting the physiology of female reproductive system cells. Through cargoes carried by EVs to spermatozoa, spermatozoa gain motility, attachment of the zona pellucida, and the ability to fertilize the oocyte (31,32). Membrane vesicles derived from seminal plasma were first described in humans and named prostasomes because they are secreted by the acinar epithelial cells of the prostate gland (33). Prostasomes are the most abundant extracellular vesicles in seminal plasma. These vesicles have also been isolated in animal seminal plasma after human. The seminal fluid inhibits sperm capacitation mainly due to its high cholesterol content. Prostasomes are also the primary sources of cholesterol found in seminal fluid. It is known that they have many protective properties and can transfer proteins and lipids to spermatozoa. It is accepted that the primary function of prostasomes is to interact with sperm and prepare them to encounter oocyte after ejaculation while maintaining and maintaining their fertilization capacity (34). Human prostasomes, including exosomes, have played essential roles in increasing sperm motility, influencing tyrosine phosphorylation of sperm proteins, delaying the acrosomal reaction, and influencing sperm-oocyte interaction by transferring related proteins to spermatozoa. It has been determined that prostasomes play a role in sperm maturation, capacitation, and acrosome reaction and have antioxidant and antibacterial properties (35-37). Prostasomes are classified as exosomes because of their size. At the same time, epididymosomes are larger and are more commonly referred to as microvesicles because they are shed from the plasma membrane of epididymis epithelial cells. Prostasomes have many important functions, including stimulation of immune activity, sperm motility, and capacitation in the female reproductive tract. Epididymosomes transfer proteins to spermatozoa that pass through the epididymis epithelium (38). These are surface proteins required for male gamete function (39). The epididymosomes of mice, humans, and bulls contain abundant antioxidant enzymes. Thus, they have a protective effect on spermatozoa that are sensitive to oxidative stress and play a role in eliminating damaged spermatozoa. In other words, thanks to the sperm quality control mechanism, protection against quality spermatozoa is formed (40). miRNAs in proximal epididymosomes are known to alter the gene expression of epididymal cells. Thanks to these properties, it has been seen that it can change the essential functions that ensure the maturation of spermatozoa and regulate fertilization ability and can be used as a biomarker (41). Seminal EVs transfer different molecules involved in $Ca2+$ signalling, including receptors and enzymes that maintain sperm motility. One such molecule, CRISP1, was also more abundant in seminal extracellular vesicles from normozoospermic men than in asthenozoospermic men. EVs derived from normozoospermic males have been shown to improve sperm motility and induce capacitation (42).

After combining with target cells, these functional molecules cause different phenotypic and functional modifications and ultimately affect adhesion, regeneration, resistance to external factors, and viability. Spermatozoa interact with these vesicles during their passage through the reproductive system. This spermatozoa-vesicle interaction is crucial for the proper functioning of spermatozoa (critical biological events such as maturation and capacitation). These vesicles contribute molecules required for different physiological events such as maturation, motility, activation, protection, capacitation, acrosomal reaction, and fertilization

(30). In human and bull studies on EVs, it has been shown to prevent early acrosome reaction and premature capacitation (34,43) and is important for capacitation, acrosome reaction, and fertilization in mice and pigs (44) has been reported to inhibit polyspermia in mice (45). Due to these features, limited studies have been conducted on whether it has a curative effect during freezing and storage in semen cryopreservation. As a result of the studies, it was observed that sperm motility, viability, membrane integrity, and percentage of acrosome integrity were improved in spermatozoa after thawing (46-49). Vesicles, including exosomes, are thought to prevent premature acrosome reaction by early attachment to or fusion with the sperm membrane during capacitation (50) and it has been found that exosomes have important bioactive functions such as maturation, capacitation, acrosome reaction and fertilization of spermatozoa. It has been observed that spermatozoa improve the integrity of the plasma membrane and protect their function by binding to the membrane of spermatozoa and transferring proteins (such as AWN and PSP1) to spermatozoa. EVs can transfer Spermadezines to the sperm membrane, which can help maintain sperm function by inhibiting premature capacitation during long-term storage. It has been reported that there is a positive correlation between increasing exosomal concentration and improving both motility and vitality (51). Many studies have been conducted on their use in physiological events and as disease biomarkers. Especially in the diagnosis of prostate cancer, PCA-3 and TMPRSS2: ERG, which is prostate cancer biomarkers, have been detected in exosomes obtained from urine (52).

E EVs are vital structures for basic biological processes in reproduction. Assisted reproductive methods are a promising therapeutic tool in many fields, such as diagnosis and treatment of reproductive diseases and fertility preservation.

4. Stem Cell Based Extracellular Vesicles

Stem cells (SC) are reserve cells that can self-renew and differentiate into various cell types. Stem cells can divide indefinitely, regenerate damaged cells and produce differentiated progeny, and they also have vital roles in maintaining cellular homeostasis and repairing tissue damage. Studies have shown that stem cells also secrete small vesicles known as extracellular vesicles into the extracellular environment (4,21). During the regeneration of tissues, stem cells have to reach target cells at a distance or near. They communicate with target cells via long thin tubular processes such as soluble factors, cytonema, cilia, or extracellular vesicles (53). EVs are thought to play a role in morphogen, nucleic acid release and distribution, establishment and maintenance of cell-tissue polarity during embryonic development, and adult tissue regeneration. Stem cells are controlled by specific microenvironments known as niches. Niche-emitted Wnt signals are self-renewal factors for stem cells in many mammalian tissues. In other words, extracellular developmental signalling proteins known as Wnt signals provide lifelong renewal and protection of tissues by feeding stem cell activity. These Wnt signalling ligands are located across the plasma membrane, between cells, and on the surface of EVs. Thus, it also contributes to the tasks of EVs in cellular homeostasis and tissue repair after damage (54,55). At the same time, it has been found that stem cell-derived EVs stimulate tissue regeneration in studies conducted on terms of injuries in the kidney, heart, liver, and nerve tissue (56).

Stem cells with differentiation and self-renewal gain importance in many areas, such as male fertility preservation and male infertility treatment. Stem cell-based extracellular vesicles have extraordinary potential for regenerative and therapeutic medicine.

4.1. Use of Stem Cell-Based Extracellular Vesicles in Male Fertility

Today, the most commonly used stem cell types to be used in the protection of male fertility; mainly mesenchymal stem cells and spermatogonial stem cells (57). Mesenchymal stem cells (MSCs) are a type of cell commonly used in clinical studies. Clinical and animal studies have shown that mesenchymal stem cells exert their therapeutic effects, not through their differentiation potential but through extracellular vesicles and paracrine factors (58). EVs obtained from MSCs can reprogram by stimulating damaged areas in target cells (59). Mesenchymal stem cells can be isolated from almost all body tissues. However, bone marrow and adipose tissue (adipose tissue) are the most common and essential sources due to easy stem cell healing and minimal donor site morbidity (60). Adipose tissue offers some advantages over other stem cell sources under investigation. Because the tissue is easy to collect, the number of cells obtained from the isolation is very high, and a small piece of fat is sufficient for isolation. In addition, adipose tissue has high initial cell yields, robust in vitro proliferative capacities, and higher immunomodulatory properties (61). Adipose tissue mesenchymal stem cells have specific therapeutic mechanisms of action such as anti-inflammatory, antibacterial, antiviral, tissue regeneration, and extracellular vesicle production (62). Microvesicles derived from bone marrow mesenchymal stem cells improved the quality of mouse spermatozoa after freezing and thawing. It has also been suggested to enhance the properties of surface adhesion molecules (CD29, CD44, ICAM-I, and VCAM-I), which are involved in spermatozoa's fusogenic and signalling properties. Decreased levels of necrosis and apoptosis were detected in spermatozoa. This study also shows that supplementation with microvesicles derived from mesenchymal stem cells improves semen quality (46). The presence of exosomes obtained from adipose tissue mesenchymal stem cells in dogs during cryopreservation has been reported to improve post-thaw motility, acrosome integrity, and plasma membrane integrity. It has been reported that exosome-treated spermatozoa show an increased lateral head displacement (ALH) amplitude compared to untreated spermatozoa (47). Conversely, no effect was found on parameters related to semen quality after thawing in a study

with mesenchymal stem cells derived from the amniotic membrane. However, it has been said that the reason for this may be the low concentration of the added vesicles, and different concentrations should be tried (63). Four miRNAs were detected in a study with EVs derived from adipose tissue mesenchymal stem cells in pigs. Compared to stem cells, EVs were found to have richer miRNAs. Among them, genes involved in cellular pathways such as angiogenesis, cellular transport, apoptosis, and proteolysis have been identified. Thus, tissue repair potentials have been identified (64). Mesenchymal stem cells are also promising cells for the treatment of diseases. In a study on diabetes mellitus-associated erectile dysfunction in mice, dysfunction treatment was tried using exosomes derived from adipose tissue-derived stem cells. As a result of the study, it was observed that fibrosis decreased, the number of endothelial cells increased, and erectile function was restored when treated with exosomes obtained from adipose tissue-derived stem cells. At the same time, it has been determined that miRNAs (miR-126, miR-130a, miR-132, miR-let7b, miR-let7c) play a crucial role in healing and repair and are transported to target cells by exosomes (65). A previous study observed that exosomes obtained from mesenchymal stem cells decreased corpus cavernosum apoptosis and increased the amount of both smooth muscle and endothelium in mice with erectile dysfunction (66).

The stem cells found at the origin of spermatogenesis in mammalian testicles are called spermatogonial stem cells (SSCs). Spermatogonial stem cells are found in the basal part of the seminiferous epithelium. These cells are the only stem cells in the body that transmit genetic information and self-renewal to offspring throughout life (67). There needs to be a balance between self-renewal and differentiation of SSCs. Excessive self-renewal or differentiation can negatively affect spermatogenesis and cause infertility by causing impaired fertility. Some niches provide endocrine and paracrine signals to regulate these mechanisms. In mammalian testicles and factors released from these cells, Sertoli and Leydig cells are primary contributors to SSC niches (68). Spermatogonial stem cell development and maturation of spermatozoa occur in waves in the highly regulated environment of the seminiferous tubules and spermatogonial stem cell niche (29). However, the molecular mechanisms in the differentiation of SSCs are still not fully known. In a study, it was observed that Sertoli cell-derived exosomes favored differentiation more than Leydig cell-derived exosomes. In order to better understand how it works, miRNA analysis was also performed, and it was found that miR-486-rich exosomes were transferred from Sertoli cells to SSCs. miR-486 is important in the regulation of male germ cell differentiation. Thus, the effect of exosomes on differentiation has been contributed, and it has been thought that it can be used as a biomarker in diagnosing male infertility (69). Although many studies have been conducted on spermatogonial stem cells and their importance in male fertility, there are not many studies in terms of extracellular vesicles. In a recent study, extracellular vesicles were detected in the basal part of the seminiferous epithelium in mice, rats, rabbits, and humans at different stages of spermatogenesis (70).

The number of studies on bioactive vesicles has increased significantly in recent years. EVs are emerging as critical players in both normal physiology and pathophysiology. Stem cell EVs exert their therapeutic and regenerative effects by transferring biologically active molecules in their vesicular cargo containing proteins, lipids, mRNA, and microRNA. EVs and their secretions act as regulators of both reproductive physiology and reproductive pathology processes. Spermatozoa interact with extracellular vesicles passing through the male and female reproductive tracts. It affects male gamete quality by acting as a regulator of reproductive success in males. The importance of the intercellular communication provided by EVs in the male reproductive system is becoming more and more a matter of curiosity. With many new dark spots, EVs and their tasks are increasingly being understood and discovered more and more.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: M.D.T., M.Ç., Design: M.D.T., Data Collection or Processing: M.D.T., Analysis or Interpretation: M.D.T., Literature Search: M.D.T., M.Ç., Writing: M.D.T.

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