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Stem cell-based therapies for fertility preservation in males: Current status and future prospects

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Abstract

With the decline in male fertility in recent years, strategies for male fertility preservation have received increasing attention. In this study, by reviewing current treatments and recent publications, we describe research progress in and the future directions of stem cell-based therapies for male fertility preservation, focusing on the use of spermatogonial stem cells (SSCs), SSC niches, SSC-based testicular organoids, other stem cell types such as mesenchymal stem cells, and stem cell-derived extracellular vesicles. In conclusion, a more comprehensive understanding of the germ cell microenvironment, stem cell-derived extracellular vesicles, and testicular organoids will play an important role in achieving male fertility preservation.

Key Words: Fertility preservation; Sperm cryopreservation; Spermatogonial stem cell; Testicular organoids; Stem cell; Extracellular vesicles

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Core Tip: With the decline in male fertility in recent years, strategies for male fertility preservation have received increasing attention. In this study, by reviewing current treatments and recent publications, we describe research progress in and the future directions of stem cell-based therapies for male fertility preservation, focusing on the use of spermatogonial stem cells (SSCs), SSC niches, SSC-based testicular organoids, other stem cell types such as mesenchymal stem cells, and extracellular vesicles derived from stem cells.

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INTRODUCTION

The number of patients with cancer continues to increase due to population ageing and growth, and the age at cancer diagnosis is becoming younger^[1]. Advances in the treatment of cancers prevalent in childhood, adolescence, and young adulthood have increased 5-year survival rates to greater than 80%^[2,3]. Because of these increases in survival and other causes, such as environmental factors^[4], immune diseases^[5], genetic diseases^[6], spermatogenic dysfunction^[7], testicular injury^[8,9], ageing^[10], and delayed childbearing^[11], male fertility preservation has gradually become an important issue in the field of human reproduction^[12,13]. Male fertility preservation and assisted reproductive technology are medical fields that have attracted much attention in recent years^[8,14]. At present, the available methods for preserving male fertility are limited to cryopreservation of sperm and testicular tissue^[15,16]. To address all aspects of male fertility preservation, it is necessary to develop related techniques such as male germ cell transplantation, culture, and differentiation^[6,17,18]. With the identification and characterization of stem cells, male fertility preservation research based on stem cells, especially spermatogonial stem cells (SSCs), has become popular^[7]. This review will elaborate recent research achievements, key areas for the future development of current strategies that can be adopted for fertility preservation, and the role of SSCs, SSC niches, organoids, extracellular vesicles (EVs), and other kinds of stem cells in male fertility preservation (Figure 1).

CURRENT STRATEGIES FOR MALE FERTILITY PRESERVATION

At present, sperm cryopreservation, sperm retrieval surgery, and drug intervention are the most acceptable methods for fertility preservation^[19,20]. The assisted reproductive technology methods of intracytoplasmic sperm injection and sperm cryopreservation have increased the chance of successful conception using stored sperm^[21,22]. Testicular tissue cryopreservation is currently proposed to restore fertility in patients from whom mature sperm cannot be collected before puberty. Although this approach has been successful in mice and rhesus macaques, it remains in the experimental stage in humans^[23-26].

Currently, hormone therapy is one of the most common drug treatments. Hypogonadism may be due to direct damage to testosterone-producing Leydig cells by chemotherapy, radiotherapy, or surgery and destruction of the hypothalamic-pituitary-adrenal axis by tumour invasion, central nervous system surgery, or radiotherapy^[27,28]. Testosterone replacement therapy achieves the best outcomes in inducing or sustaining puberty-related growth, increased bone mass and mental health^[29]. Although testosterone produced by Leydig cells mainly acts on Sertoli cells functioning as vegetative germ cells, it cannot induce sperm production^[30,31]. In patients with hypogonadism, human chorionic gonadotropin or recombinant follicle-stimulating hormone (FSH) can be administered during puberty to stimulate testicular enlargement^[32]. Some researchers have found that FSH use can increase SSC colonization in mammals^[33,34].

Oxidative stress is one of the main causes of gonadal toxicity by anticancer therapy^[35]. Oxidative stress also aggravates the effects of cell ageing on the male reproductive system^[36,37]. Therefore, antioxidants are among the popular drugs under study for use in fertility preservation. The specific antioxidant pathway in SSCs or mesenchymal stem cells (MSCs) is not clear but may involve the inhibition of cellular necrosis and apoptosis. Previous studies have shown that necroptosis in the testis promotes the ageing-related deterioration of the male reproductive system in mice^[38]. Our previous study identified a potential method for treating male late-onset hypogonadism by inhibiting the ageing of Leydig cells^[39]. Whether fertility is improved by inhibiting testicular ageing or by promoting and maintaining SSC differentiation and proliferation through improvements in the state of the SSC niche requires further study. The roles of other stem cells in promoting growth and

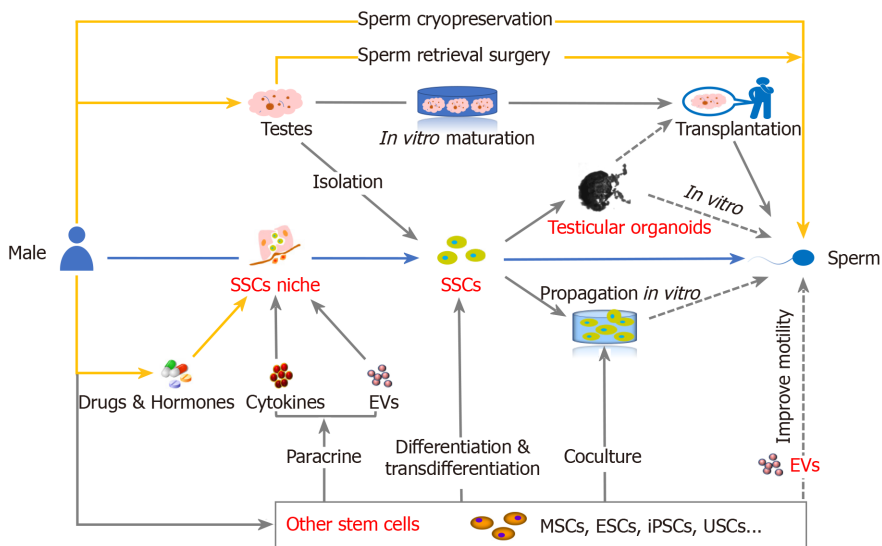


Figure 1 Current and future strategies for male fertility preservation. Blue arrows indicate normal spermatogenesis; orange arrows indicate clinical applications; grey continuous arrows indicate experimental phases; grey dotted arrows indicate possible future developments. SSCs: Spermatogonial stem cells; MSCs: Mesenchymal stem cells; iPSCs: Induced pluripotent stem cells; ESCs: Embryonic stem cells; USCs: Urine-derived stem cells; EVs: Extracellular vesicles.

prolonging the life cycle have been previously reported^[40,41]. Moreover, studies have shown that antioxidants and apoptosis inhibitors affect the enrichment of SSCs in cryopreserved mouse germ cells^[42]. Our previous research suggested that suppressing necrotic apoptosis genes may help preserve SSCs and improve male fertility^[43]. Vitamin E is also utilized to improve human sperm motility and concentration^[44,45]. Levocarnitine is clinically administered to improve asthenospermia and azoospermia^[46-48]. Astaxanthin, ellagic acid, lycopene, vitamin C (ascorbic acid), and other drugs have been proven to preserve fertility in animal experiments^[49-52]. In addition, some cytoprotective agents have been proven to preserve fertility in animals^[53-55].

SPERMATOGENIAL STEM CELLS AND THEIR NICHE

SSCs are adult stem cells in the testis and the foundation of spermatogenesis; thus, they are essential for male fertility^[56]. SSCs are located in the base of seminiferous tubules and are very rare among all male germ cells. They have the ability to differentiate and self-renew in the testis, but the development of appropriate methods for the stable culture and transplantation of isolated SSCs will be an important step in fertility preservation^[57,58]. Spermatogenesis in the human testis occurs in a complex microenvironment in which various cells interact through different cytokines. SSCs and spermatogonial cells reside in the base of the seminiferous tubules, where they contact Sertoli cells and peritubular cells (PTCs). Sertoli cells are columnar cells extending from the basement membrane to the lumen of the seminiferous tubules that play a supporting role^[59]; considered the most basic components of the testicular microenvironment, these cells secrete important cytokines such as fibroblast growth factor 2, glial cell line-derived neurotrophic factor, activin A, bone morphogenetic factor 4, and stem cell factor^[60]. Among these cytokines, glial cell line-derived neurotrophic factor and fibroblast growth factor 2 are considered necessary for maintaining SSC proliferation and differentiation^[61,62]. The basement membrane is composed of extracellular matrix proteins, which not only provide structural support but also regulate niches and mediate local cellular signaling by binding and releasing growth factors^[63]. Mesenchymal tissue is another key component of the SSC niche, contributing various growth factors and signaling molecules. The stroma is composed of many cell types, including vascular cells, interstitial cells, macrophages, and PTCs. Periductal muscle-like cells are arranged on the outer side of the seminiferous basement membrane, providing structural support, mediating peristalsis, and secreting a variety of cytokines^[64,65]. The blood-testis barrier, established by various cell contacts, creates a unique microenvironment and regulates SSC proliferation or differentiation by regulating access to secretions from testicular endothelial cells and interstitial cells^[66,67]. Leydig cells and other interstitial cells are also involved in cellular

communication within the niche^[68-70]. This cell network responds to hormone signals and other signaling in the niche to drive spermatogenesis and testosterone production^[71,72]. The differences in cellular interactions in the testicular microenvironments of different mammals have not yet been clarified^[73], but the testicular microenvironment is essential for spermatogenesis^[74].

Brinster *et al*^[75] were the first to successfully use SSCs to restore fertility in mice with busulfan-induced infertility. Through our research, we found that busulfan can be used to generate an ideal animal model of azoospermia^[76]. Wyns *et al*^[77] proposed that spermatogenesis recovery can be achieved by injecting isolated SSCs into germ cell-free testes and transplanting testicle fragments containing SSCs. Germ cell-knockout models established with busulfan in bovines, pigs, and dogs show the ability to undergo complete spermatogenesis, but the function of the produced sperm needs to be further evaluated^[78-81]. Studies have confirmed that SSC transplantation does not increase the risk of tumor formation or reduce longevity in mice and that the genome of offspring is unchanged^[82,83]. Future studies should consider strategies to achieve further improvements in the efficiency of SSC transplantation and reductions in SSC loss^[84]. Increasing research results support the potential use of SSCs to restore fertility in clinical applications^[85]. At present, it is generally believed that providing a good microenvironment for SSCs can achieve the goal of using these cells for fertility preservation.

The successful and efficient culture of SSCs requires the accurate selection of cells and the implementation of conditions required for SSC growth, including the use of the appropriate medium containing factors such as cytokines and the inclusion of supporting cells^[86,87]. In 2003, Kanatsu-Shinohara *et al*^[88] first reported the long-term expansion (more than 5 mo) of mouse SSCs in specific medium and indicated that various specific cytokines were necessary for SSC proliferation and passaging *in vitro*. Related experiments have since proven that cytokines such as growth factors are indeed necessary to maintain SSC proliferation *in vitro*^[89]. Although mouse SSCs have been successfully cultured, it has been difficult to achieve similar success with human SSCs^[90]. Some researchers believe that the coculture of SSCs with isolated testicular cell suspensions enables human SSC proliferation *in vitro*. This culture method depends on the ability of somatic cells to adhere to the plate while some germ cells remain in suspension, allowing SSC enrichment after differentiation and culture^[91,92]. Only a few studies have demonstrated the enrichment of SSCs by quantification in seminiferous tubules after the allotransplantation of *in vitro*-expanded SSCs^[86]. In coculture, the ratio of somatic cells to SSCs affects the proliferation of SSCs^[93-95]. Related studies have shown that Dulbecco's modified Eagle's medium/F12 is a better culture medium^[96]. Other researchers who studied the efficiency of selecting germ cells from testicular cells cultured under different conditions did not find any differences in the number of germ cells recovered^[90]. Many phenotypic markers have been used to isolate SSCs, such as GPR125^[97], CD9^[98], SSEA-4^[99], and ITGa6^[100]. No long-term amplification system for human SSCs has been established; in related research, human SSCs have been cultured and propagated *in vitro* for just 4 mo^[91].

TESTICULAR ORGANIDS

The research and application of organoids remain at initial stages. Organoids have great potential in the study of a wide range of fields, including developmental biology, disease pathology, cell biology, regeneration, precision medicine, drug toxicity, and drug efficacy. These organoids, a kind of *in vitro* culture system, contain self-renewing stem cells that differentiate into various organ-specific cell types and tissues similar to those in the original organ and can recapitulate some organ functions^[101,102]. The testicular microenvironment was originally reconstructed by culturing SSCs in two dimensions (2D) *in vitro*. Interactions between different cells are necessary to support germ cell development and achieve the culture of testicular structures and tissue. In 1980, Tung *et al*^[103] promoted the reorganization of Sertoli cells and seminiferous tubule-like structures *in vitro* through the 2D coculture of Sertoli cells and myoid cells in rodents. Tubule formation in these cultures is driven by fibronectin, a component of the basement membrane synthesized by myoid cells that promotes Sertoli cell migration^[104]. Some studies have shown that in coculture systems with Vero or Sertoli cells as a feeder layer, round spermatid cells can produce sperm cells with prolonged fertilization ability, and the production efficiency is increased when the cultures are supplemented with FSH^[105-107]. Tanaka *et al*^[108] confirmed the process of producing round spermatids from primary spermatocytes cultured with Vero cells. Although

these findings indicate the achievement of basic structural reorganization, the development of germ cells is limited. In other words, the progression of spermatogenesis requires a complete system of testicular somatic cells. In 2018, von Kopylow *et al*^[109] cultured various testicular somatic cells for more than 12 wk, but the structure was limited in its ability to support germ cells. Yang *et al*^[110] produced haploid sperm cells in conventional single-cell cultures by stimulating isolated human SSCs with retinoic acid and stem cell factor. Through multidisciplinary cooperation, male reproductive biology research groups have begun to establish and characterize testicular organs. Such multidisciplinary research will be helpful for studying complex cell interactions, growth, preservation, and tissue development and generating drug and toxicity screening models^[111].

Compared with 2D culture, 3D culture can meet the requirement of recapitulating the natural physical structure of the human body and the microenvironment with its network of cell-cell interactions^[112]. In mammals, 3D culture models have been more effective than 2D culture models^[113,114]. In 1954, Trowell *et al*^[115] first immersed semisolid-supported tissue fragments in culture medium to balance nutrient transport with effective gas exchange. Based on this approach, Sato *et al*^[116] achieved complete mouse spermatogenesis *in vitro*, and sperm with fertilization ability were differentiated from immature mouse testicle fragments and cultured, which proved that spermatozoa can be fully matured by maintaining the natural testicular microenvironment. Lambrot *et al*^[117] also used the gas-liquid interface method to culture human fetal testes with membranes instead of an agar block. After treatment with retinoic acid, the number of germ cells in cultured human fetal testes decreased. In other studies, this semipermeable membrane has been used as a scaffold for the culture of human prepubertal testicular tissue. Although this approach can maintain the somatic microenvironment for testicular fragments, it leads to a decrease in the germ cell population after mitosis and meiosis^[118,119].

Another culture method is the hanging drop technique, in which testicular tissue fragments are cultured in a small volume of medium placed on the lid of a culture plate. This method has been used to explore the effects of chemical treatments on human and mouse testes and to study fetal vascularization, morphogenesis, and organogenesis^[120,121]. Pendergraft *et al*^[122] used a hanging drop approach to produce a functional testicular organ system by coculture with adult SSCs. Some researchers used this method to observe the mechanism of Zika virus infection in the testis and showed that organ vitality and the expression of spermatogonial and somatic cell markers decreased after infection^[123,124].

In conjunction with tissue engineering science, Perrard *et al*^[125] reported a bioreactor system using chitosan water gel tubes in 2016. The system enabled the differentiation of germ cells into morphologically mature sperm. Komeya *et al*^[126] developed a microfluidic device that can maintain mouse testicular tissue and complete spermatogenesis for 6 mo. The steady-state balance between tissue fragments and the culture medium may be a necessary condition for the mature somatic cell microenvironment to promote germ cell differentiation^[112]. Alternatively, the cell aggregates themselves can function as a 3D scaffold to support the reorganization of cells into testicular-like structures. In 1981, Zenzes *et al*^[127] cultured isolated rat testicular cells *via* a rotational culture method to explore the effects of specific cell populations and testicular maturation stages on new tissue formation. In 2013, Yokonishi *et al*^[128] maintained germ cells *in vitro* and promoted their initial differentiation by using cellular pellets in an air-liquid interface method.

With the 3D culture approach, different support matrices have yielded different effects in testicular culture and organoid research. In 1985, Hadley *et al*^[129] embedded testicular cells in a Matrigel matrix to explore the potential for cell recombination and germ cell differentiation in this 3D scaffold. Thereafter, a calcium alginate matrix^[130], collagen matrix^[131], poly (D, L-lactic-co-glycolic acid) matrix^[132], methylcellulose culture system^[133], soft agar culture system, and other soft matrices were used to culture isolated testicular cells^[113,134]. In 2017, Alves-Lopes *et al*^[135] used a three-layer gradient system based on the Matrigel matrix as a new platform for studying the microenvironment of SSCs *in vitro* and looked for novel factors related to germ cell proliferation and differentiation. In 2014, Reuter cultured cells with a collagen sponge and found that the cells colonized the whole scaffold for as long as 35 d, with signs of tubule formation. The cell mass was mainly composed of Sertoli cells and PTCs surrounded by undifferentiated spermatogonia, but no haploid cells were detected, confirming the lack of differentiation^[136]. In 2017, Baert *et al*^[137] cultured rat and human testicular cells using an acellular testicular matrix and found that primary human testicular cells formed organ-like organoids in a manner independent of the presence of scaffolding that did not recapitulate the testicular-specific cellular structure in

organs.

ROLE OF OTHER STEM CELLS IN MALE FERTILITY PRESERVATION

MSCs, induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), and other stem cells have been intensely studied in the field of male fertility preservation. MSCs can be isolated and identified from rodent and human tissues and are considered a potential source of Sertoli cells, Leydig cells, and PTCs in the developing adult testis^[138]. Clinical studies on MSCs are relatively extensive and have proven better safety profiles with these cells^[139]. MSCs derived from bone marrow, adipose tissue, the umbilical cord, and other fetal sources have been widely accepted as candidate cells for clinical cell therapy^[140,141]. Stem cells from different sources are considered to differentiate into embryonic cell lines. In 2006, Nayernia *et al*^[142] first reported that bone marrow-derived MSCs can differentiate towards germ cells *in vitro*. The injection of MSCs into the testes of busulfan-treated mice led to the production of more seminiferous tubules, which is helpful for rebuilding the SSC niche^[143,144]. In 2018, Kadam *et al*^[144] proved that co-transplantation with MSCs improves the transplantation efficiency of SSCs. Leydig cells and Sertoli cells in the testes of cancer patients are damaged to varying degrees after chemotherapy, so the application of MSCs as supporting cells and interstitial cells can improve the effects of SSC transplantation^[145-148]. Therefore, it must be determined whether stem cells cocultured with SSCs produce functional sperm. The high self-renewal capacity, multilineage differentiation potential, and immunomodulatory properties of MSCs make them an attractive tool for research and clinical applications^[149]. Other stem cells may also have great application potential, but this hypothesis needs to be supported by further experimental evidence. The paracrine effects of MSCs on cell survival, immune regulation, cell migration, angiogenesis, cell proliferation, and antioxidant effects are key issues related to the use of stem cells for the preservation and promotion of male fertility^[150,151]. An article reviewing the role of human umbilical cord perivascular cells (HUCPVCs) in male fertility preservation suggests that HUCPVCs have broad development prospects^[7]. First, the umbilical cord source is associated with high immunity^[147,152,153]. These cells share characteristics with MSCs, including cell surface markers related to MSCs and pericytes; have the ability to differentiate into cartilage and undergo adipogenesis and osteogenesis *in vitro*; and show multidirectional differentiation potential^[154]. Similar to other MSCs, HUCPVCs express and secrete many cytokines related to cell proliferation, survival, chemotaxis, angiogenesis, immune regulation, and the beneficial modulation of the local microenvironment^[155-157].

In 2006, researchers discovered that pluripotent stem cells can be isolated and expanded *via* somatic cell reprogramming^[158]. In the last decade, pluripotent stem cells have become the focus of medical research^[159]. iPSCs and ESCs have shown great clinical potential^[160]. In 2004, Clark *et al*^[161] observed the expression of Ribonucleic Acid (RNAs) and proteins indicative of mature germ cells, providing evidence that ESCs can be transformed into germ cells. In 2006, Nayernia *et al*^[162] bred offspring using spermatozoa differentiated from mouse ESCs. In 2016, Zhou *et al*^[163] completely reproduced meiosis *in vitro* and produced euploid fertile offspring. Although these cells show good applicability, ethical and other considerations limit the further clinical development and application of ESCs^[164]. Currently, some researchers believe that very small embryonic-like stem cells can undergo *in vitro* differentiation and assist in fertility preservation, but this has not been studied^[165,166]. The methods for fertility promotion and preservation involving iPSCs include obtaining primordial germ cells from somatic cells of a patient and differentiating these cells into Sertoli cells and Leydig cells, as testicular microenvironment support, to promote the proliferation and differentiation of SSCs^[167-169] into gametes *in vitro*^[170,171]. In 2011, Eguizabal *et al*^[172] obtained haploid cells that completed meiosis from iPSCs. In recent years, some researchers have differentiated human germ cells from iPSCs and ESCs, but the differentiation efficiency is low, and the resultant differentiation is still insufficient^[173-175]. In addition, genetic and epigenetic mutations in iPSCs that arise during reprogramming and proliferation must be further explored^[176].

ROLE OF STEM CELL-DERIVED EXTRACELLULAR VESICLES IN MALE FERTILITY PRESERVATION

Originally, terms such as EVs, exosomes, and microvesicles were not strictly defined in the literature and were often used as synonyms^[177,178]. As important components of the paracrine system, these structures carry cargo such as microRNAs, mRNA, proteins, and lipids to target cells^[179]. Subsequent publications established three categories of EVs: Apoptotic bodies (1–5 µm), shedding microvesicles (200–1000 nm), and exosomes (30–200 nm)^[180]. Exosomes, with a diameter of approximately 30 nm to 200 nm, can more readily pass through the blood-brain barrier to play a therapeutic role^[181]. Human semen contains a high concentration of EVs that promote sperm function in various ways, such as improving sperm motility, regulating acrosome activity, and affecting the fertilization process^[182]. Related studies have proven that exosomes can affect semen quality and enter sperm, thereby enhancing sperm motor ability and capacitation after ejaculation^[183]. Studies have shown that seminal plasma exosomes regulate sperm motility and mitochondrial metabolism in mammals^[184]. The epididymis plays an important role in sperm maturation in the male reproductive tract, and male sperm rely heavily on interactions with epididymal epithelial secretions^[185]. Epididymal and prostate exosomes are thought to be closely related to the later stages of sperm maturation^[186–188]. Maturing sperm mix with the fluid secreted by each gonad throughout the ejaculation process^[189]. Exosomes are also very important for cellular communication^[190]. In 2014, Vojtech *et al.*^[191] demonstrated that small RNAs carried by exosomes in semen exhibit signal-regulating function. Related studies have shown that in genitourinary diseases, the biological information carried by exosomes can function as a biomarker of prostate cancer, bladder cancer, kidney cancer, and other diseases^[192–195]. The relationship between sperm RNA and male infertility has been studied and proven to play a role in predicting health and individual outcomes of different fertility treatments^[196,197]. Therefore, it is reasonable to consider that a patient's semen quality can be determined by exosome analysis or that treatments for male infertility can involve exosomes^[198,199]. Exosomes in the vascular, skeletal, and nervous systems have received widespread attention in relation to tissue reconstruction and regeneration, but there have been fewer studies on the preservation of male fertility^[200]. Therapeutic drug delivery by MSC exosomes is an emerging research direction^[201]. As the stem cell type with the most abundant in-depth research and most extensive sources, MSCs are widely used in many kinds of experiments^[202]. MSC-derived exosomes have been shown to improve various diseases^[203], for example, to protect against renal injury^[204], repair the cornea^[205], and improve some inflammatory diseases^[206]. Exosomes derived from other types of stem cells, such as oral, adipose, and urine-derived stem cells, are increasingly being used in the study of diseases^[207–209]. Our recent study showed that urine-derived stem cells can restore spermatogenesis in busulfan-induced nonobstructive azoospermic mouse models through paracrine exosomes^[210]. Therefore, the role of exosomes secreted by various types of stem cells in preserving male fertility requires more in-depth study. With the rapid development of tissue engineering technology, one future focus is to improve sperm function and promote male fertility preservation through the use of exosomes^[198].

FUTURE DIRECTION

The efficiency of SSC proliferation and culture remains low. The ability to rebuild the microenvironment, especially the SSC niche, will be key to promoting sustained SSC proliferation and differentiation into sperm. Further research is needed to ascertain how to promote SSC proliferation and maintain their original function after transplantation. There has been recent progress in understanding the testicular tissue microenvironment and recapitulating this microenvironment by growing different cells in a supporting matrix. Although considerable progress has been made, achieving full human spermatogenesis and mature sperm production with fertilization capability will require the creation of a complete testicular microenvironment in which immature testicular tissues can mature and SSCs can proliferate and differentiate into mature sperm. With the combination of testicular organoids and tissue engineering, the use of emerging technologies such as 3D printing to create functional human testicular substitutes is another potential direction of future development. A new type of micromodel, the “organoid-on-a-chip” concept, has been recently proposed^[211]. This chip is used to simulate the smallest functional, structural unit of an organ and has

potential use in drug screening and disease modelling^[212,213]. Recent *in vitro* cell experiments have proven that the toxicity of various drugs to the testis imposes obvious limitations^[122,214]. Further scientific efforts are needed to determine whether it is possible to simulate spermatogenic functional units *in vivo* by building a “testicular chip” to conduct drug screening and build disease models. The powerful role of stem cell differentiation and paracrine function in organoid formation and maintenance needs to be further explored. Paracrine exosomes have been proven to have beneficial therapeutic and diagnostic effects in other areas, but research on their use for male fertility preservation remains in the earliest stage. For this reason, it is necessary to further explore the supportive role of exosomes in the SSC niche, especially regarding whether exosomes can improve sperm motility and maturation in oligospermia or asthenospermia. Multidisciplinary cooperation will result in more diverse, stem cell-based experiments and provide strong support for future medical development.

CONCLUSION

The issues with fertility preservation based on stem cells have been widely studied. Research in this field has resulted in great achievements in testicular tissue cryopreservation and transplantation, SSC culture and transplantation, *in vitro* sperm production, and organoid generation. However, the efficiency, final outcomes, and safety of each experimental method need to be further evaluated. A more comprehensive understanding of the regulation of the germ cell microenvironment will play an important role in culturing SSCs and inducing their proliferation and differentiation *in vitro*. At the same time, the role of exosomes in sperm maturation and the testicular microenvironment is receiving increasing attention. Sperm maturation based on exosome approaches, the differentiation and proliferation of SSCs, and other fertility preservation-related topics may become popular in future research.

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