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Spermatogonial stem cell–based cellular therapy for non-obstructive azoospermia: a narrative review

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Review Article

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ABSTRACT

Spermatogonial stem cells (SSCs) constitute the foundation of male sperm production and fertility. SSCs are characterised by their capacity for self-renewal and differentiation into spermatozoa throughout a male's reproductive life, thereby transmitting genetic information to subsequent generations. During embryonic development, SSCs are derived from postnatal testicular gonocytes, which originate from long-lived primordial germ cells. A large proportion of men's infertility is detected by sperm analysis. Unusual sperm parameters include sperm concentration, morphology and motility. Male infertility is mainly due to hormone deficits, physical causes, sexually transmitted diseases, environment, lifestyle and hereditary factors. The most severe form of male infertility is non-obstructive azoospermia (NOA), which is defined as no sperm in the ejaculate because of spermatogenesis failure. The first-line treatment for these individuals has been testicular sperm extraction under an operating microscope (micro-TESE). SSCs are also considered a promising pathway for regenerating impaired or damaged spermatogenesis. This review addresses SSC transplantation as a potential method for treating male infertility and restoring spermatogenesis in patients with degenerative diseases such as cancer, radiotherapy and chemotherapy.

Introduction

In several species, primordial germ cells (PGCs) are the first germline cell population to colonise the developing gonads through active migration (Richardson and Lehmann, 2010; Saitou and Yamaji, 2010). PGCs initiate differentiation in the gonads along a spermatogenic (male) or oogenic (female) pathway. The spermatogenic path is most commonly associated with spermatogonial or male germline stem cells (Spradling *et al.,* 2011). Germ cells are the embryonic progenitor cells that give rise to gametes, which are the reproductive cells that produce offspring. Early in the development of most species, they are separated from the somatic cell lineages (Cinalli *et al.,* 2008). There are two morphologically distinct kinds of spermatogonia: A dark and A pale spermatogonia. A dark has features that reveal it acts as a testicular stem cell. The A pale, on the other hand, has typical progenitor characteristics. Type A pale cells divide and undergo active mitosis to generate Type B cells, which then develop into primary spermatocytes (Niazi Tabar *et al.,* 2021; Tabar *et al.,* 2022). Sertoli and Leydig cells are the two primary somatic cells in the testis that are required for testicular structure and function. Sertoli cells, which act as an anchor for germ cell growth, are found only in the tubular compartment of the testis and contribute to the progression of germ cells to spermatozoa through direct interaction and regulation of the environmental milieu (Griswold, 1998). In contrast, Leydig cells are located in the intertubular or interstitial region and contribute to testosterone production (Krishnamurthy *et al.,* 2001; Chen *et al.,* 2009). In the seminiferous epithelial tissue, spermatogenesis plays an essential role in male fertility (Goh *et al.,* 2015; Gunes *et al.,* 2015; O'Hara and Smith, 2015; Griswold, 2016). Male germ cells, in conjunction with Sertoli cells, are essential contributors to spermatogenesis, the intricate biological process that transforms diploid spermatogonia into spermatozoa (Heller and Clermont, 1963; Auger, 2018). In the last step of spermatogenesis, mature spermatids are discharged into the lumen of the seminiferous tubules from the supporting Sertoli cells (Sharpe, 1987; Phillips *et al.,* 2010). Even slight deviations from the natural spermatogenesis pathway may result in infertility in men (Babakhanzadeh *et al.,* 2020). Infertility is defined as the failure to achieve a clinical pregnancy following 12 months of consistent and unprotected sexual intercourse, which may stem from either female or male factors (Vander Borght and Wyns, 2018; Babakhanzadeh *et al.,* 2020). Men's infertility is mainly diagnosed by semen analysis. Unusual sperm parameters include sperm concentration, morphology and motility (Thonneau *et al.,* 1991; De Kretser and Baker, 1999). The most likely reasons for male infertility include hormonal deficiencies, physical causes, sexually transmitted

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diseases, environment and lifestyle and genetic factors (Winters and Walsh, 2014; Krausz and Riera-Escamilla, 2018; Lee and Ramasamy, 2018; Ho *et al.,* 2020). Roughly 1% of all men, 10% to 15% of infertile males are diagnosed with Azoospermia, the absence of sperm in the ejaculate (Cocuzza *et al.,* 2013). Obstructive azoospermia (OA) and non-obstructive azoospermia (NOA) represent two classifications of this condition. OA, responsible for 40% of azoospermia cases, results from a physical blockage in the male excurrent ductal system, potentially occurring at any point from the rete testis to the ejaculatory ducts. (Jow *et al*., 1993; Wosnitzer *et al.,* 2014). Non-obstructive azoospermia, however, is responsible for approximately 60% of azoospermic cases (Wosnitzer *et al.,* 2014). NOA is characterised by the lack of sperm in the ejaculate due to a failure in spermatogenesis (Chiba *et al.,* 2016). Men with obstructive azoospermia have two alternatives for fathering children: surgical repair of the blockage and collection of sperm straight from the epididymis or testis, followed by in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) (Jarow *et al.,* 1989, 2010). For men with non-obstructive azoospermia, current management relies primarily on medical therapy and microsurgical testicular sperm extraction (microTESE) (Kumar, 2013; Birowo *et al.,* 2021). Beyond these approaches, stem cell transplantation has emerged as a promising strategy for treating male infertility rooted in impaired spermatogenesis (Ahn *et al.,* 2020). This technique begins with isolating and cryopreserving spermatogonial stem cells (SSCs) obtained through a testicular biopsy, which are later reintroduced into the testis through targeted stem cell transplantation (Abdelaal *et al.,* 2021). Once delivered back into the testicular environment, SSCs have the capacity to regenerate the spermatogonial population and restore spermatogenesis when native cells are damaged or depleted (Kanatsu-Shinohara *et al.,* 2003; Han *et al.,* 2018). Consequently, this approach offers hope not only for cancer survivors exposed to gonadotoxic treatments but also for patients whose fertility is compromised by severe defects in spermatogenesis (Hanna Valli *et al.,* 2015; Forbes *et al.,* 2018).

Spermatogonial stem cell

Mammalian adult stem cells share at least two main features: they can undergo 'indefinite' self-renewal, indicating low mitotic activity throughout the organism's lifetime without undergoing differentiation. They can also generate differentiating daughter cells (Leblond, 1964; Robey, 2000). Germinal stem cells, also known as SSCs, maintain the cell pool while generating sperm through the process of self-renewal. (Aponte, 2015). Antibody-based research on intact (whole-mount) seminiferous tubules has allowed researchers to identify proteins whose expression patterns are exclusive to undifferentiated spermatogonia and, by extension, SSCs. Undifferentiated stem and progenitor spermatogonia, including A single, A paired and A aligned 4–16, have shown the expression of CDH1, GFRA1, LIN28, NANOS2, NANOS3, NEUROG3, ZBTB16 and POU5F1 genes (Azizi *et al*., 2020a, 2020c). Moreover, ID4, PAX7 and BMI1 might be considered marker proteins for ultimate SSCs. However, whether these variables are relevant for SSC function and behaviour in the long run is unclear. This is because the consequences of protein shortage are relatively small (Oatley *et al.,* 2011). Undifferentiated spermatogonia constitute a cohort of early spermatogonia, which includes a subset of SSCs. In rodents, they are composed of spermatogonia that are either single (A s) or paired (A pr) or arranged in chains of four, eight or sixteen cells (A al) (De Rooij, 2017). The daughter cells formed after the division of undifferentiated spermatogonia in rodents may either move apart to facilitate stem cell renewal or remain together as a pair of so-called A pr spermatogonia, which is the first stage in the differentiation process. Then, the pairings may grow to produce clones of 4, 8 or 16, resulting in aligned spermatogonia (A al) cells interconnected by intercellular cytoplasmic bridges (Oatley and Brinster, 2012; De Rooij, 2017). Following their initial development as A al spermatogonia, the A spermatogonia complete six rounds of division to become spermatocytes and, finally, spermatids. At this point, the A spermatogonia have committed permanently to meiosis (De Rooij, 2017). There are several spermatogonial stem cell renewal models. According to the A model (traditional model), the whole A single pool represents the SSCs, and the development of an A-paired syncytium indicates irreversible entrance into a progenitor stage destined for differentiation. This SSC renewal approach has been the most popular for many years. However, two different models have been presented in the recent decade (Lord and Oatley, 2017). All As, Apr and Aal spermatogonia in the newly-emerged 'fragmentation model' possess stem cell potential, and stem cell renewal is promoted by pair and chain fragmentation (Nakagawa *et al.,* 2007, 2010; Klein *et al.,* 2010; Hara *et al.,* 2014). Another model that has been suggested is known as the 'hierarchical model.' According to this model, there is an SSC hierarchy, as certain spermatogonia are capable of long-term self-renewal, while others are not (Oatley *et al.,* 2011; Aloisio *et al.,* 2014; Chan *et al.,* 2014; Komai *et al.,* 2014; Sun *et al.,* 2015 Helsel and Oatley, 2017; Helsel *et al.,* 2017). The undifferentiated spermatogonial population in non-human primates is divided into two types: A dark and A pale. A dark spermatogonia is a regenerative reserve with slow cycling, which cannot participate in spermatogenesis pathways and can only convert to A pale cells. The A pale spermatogonia act as progenitors, dividing mitotically to produce Type B and primary spermatocytes (Clermont, 1963; Ehmcke *et al.,* 2006; Hermann *et al.,* 2010). Before the derivation of spermatocytes, the A pale is followed by just one generation of B spermatogonia. Therefore, technically, only two mitotic stages occur before the generation of spermatocytes from the progenitor cells in the human testis. Only four spermatocytes, leading to the formation of 16 spermatids, are thus derived from each initial division of a germline progenitor (Ehmcke *et al.,* 2006). In humans, the A0/A1 model of SSC self-renewal is an alternative to the A model, which is very similar to the A dark and A pale models used in non-human primates to define stem cell potency (Valli *et al.,* 2015). During all stages, A0 spermatogonia were detected as single cells or pairs of cells, observed in the seminiferous epithelium. Mitotic measures were seldom found in these cells; hence, they were deemed incapable of sustaining spermatogenesis as resource stem cells. Radiation and other potentially hazardous substances are the only factors that can activate these reserve stem cells, which then suppress spermatogenesis. The functional stem cell pool includes A1–A4 spermatogonia. When A4 spermatogonia divide, they always create new A1 spermatogonia (a process called self-renewal) or intermediate spermatogonia (a process called differentiation) (Clermont and Bustos-Obregon, 1968; Dym and Clermont, 1970; Clermont and Antar, 1973). SSCs are essential for maintaining spermatogenesis throughout life; therefore, understanding how they function is crucial for determining the aetiology of specific infertility cases and identifying new avenues for stimulating or inhibiting the spermatogenic process (De Rooij, 2017).

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Male infertility and azoospermia

Infertility – whether in a woman or a man – is defined as the inability to achieve a clinical pregnancy after twelve months of regular, unprotected intercourse (Vander Borght and Wyns, 2018; Babakhanzadeh *et al.,* 2020). In men, infertility specifically refers to the inability to impregnate a fertile female partner after at least one year of unprotected sexual activity (Leslie *et al.,* 2026). This challenge affects roughly 16% of couples worldwide, with male factors contributing to nearly half of all infertility cases (Tharakan *et al.,* 2021). A wide range of conditions can underlie male infertility, some reversible and others permanent; factors such as age, medications, past surgeries, environmental exposures, genetic abnormalities and systemic illnesses can influence reproductive potential in either partner (Shih *et al.,* 2019). Therefore, evaluating male fertility centres on identifying the causes of infertility, treating those that are reversible, determining suitability for assisted reproductive technologies (ART) and providing counselling when the condition is irreversible (Cocuzza *et al.,* 2013) (Figure 1). Among men, about 1% – and 10% to 15% of those who are infertile – are diagnosed with azoospermia, a condition characterised by the complete absence of sperm in the ejaculate (Safran *et al.,* 1998). Azoospermia itself can arise from diverse causes, including obstruction of the genital tract or intrinsic failure of testicular function (Tharakan *et al.,* 2021). Although azoospermia once implied absolute infertility, advances in testicular sperm extraction and ART now allow many affected men to biologically father children (Jarow *et al.,* 1989). Clinically, azoospermia is classified as either OA or NOA, with OA resulting from blockages within the testicular or ductal systems and NOA caused by impaired spermatogenesis (Roshan *et al.,* 2024).

The aetiology of azoospermia is conventionally classified according to the anatomical position of the cause: pre-testicular, testicular and post-testicular (Jarvi *et al.,* 2015; Tharakan *et al.,* 2021). Pre-testicular azoospermia, which accounts for 2% of all azoospermic males, is caused by a hypothalamic or pituitary abnormality. Testicular failure or non-obstructive azoospermia affects between 49% and 93% of males with azoospermia. Spermatogenesis in men with testicular dysfunction may be impaired, halted in its maturation process or completely absent, as shown in Sertoli-cell-only syndrome. Men struggling with post-testicular or obstructive azoospermia (7–51% of azoospermic cases) have normal spermatogenesis but suffer from obstruction in the ductal system or ejaculatory dysfunction (Wang *et al.*, 1987; Jarow *et al.,* 1989; Matsumiya *et al.,* 1994; Fedder *et al.,* 2004; Fogle *et al.,* 2006). Other groups of men with the inability to ejaculate are men with spinal cord injuries, psychogenic failure to ejaculate or neurological damage (Jarvi *et al.,* 2015). It's noteworthy that the absence of sperm should be determined by centrifuging the semen sample; for an appropriate evaluation, at least two semen samples should be assessed (Schlegel, 2004). If sperm analysis confirms azoospermia, the physician must clarify whether the patient has OA or NOA. A thorough medical history, physical examination, hormonal assessment and genetic testing are used to verify the diagnosis. The diagnosis of spermatogenesis failure is further supported by medical history of variables such as anticancer therapy or an undescended testis. Furthermore, identifying the patient's medicines is crucial, since some drugs, including steroids, can impede spermatogenesis (Ramasamy *et al.,* 2015). Additional tests, such as karyotyping and genetic analysis, should be conducted if NOA has been diagnosed. It has been shown that 13.7% of azoospermia patients have an aberrant karyotype. Tests for azoospermia factor (AZF) – which includes three sub-regions, AZFa, AZFb and AZFc – are the most prevalent and critical genetic tests for NOA diagnosis. AZF is located on the long arm of the Y-chromosome (Yq). (Van Assche *et al.,* 1996; Ferlin *et al.,* 2007).

Management of non-obstructive azoospermia

NOA is characterised by a complete lack of sperm in the ejaculate resulting from intrinsic failures in spermatogenesis and is regarded as the most severe type of male infertility (Azizi *et al.,* 2021a, 2021b, 2021c). Patients with NOA were unable to conceive children of their own earlier, and their only alternatives were donor sperm or adoption. The first successful live birth using IVF was reported in 1978, while the first successful live birth with the ICSI method was reported in 1992 (Steptoe and Edwards, 1978; Palermo *et al.,* 1992). After that, pregnancy was documented in NOA patients after testicular sperm extraction (TESE) and ICSI, allowing them to father their children [82]. Recent advancements in assisted reproductive technology (ART) have profoundly transformed the treatment of NOA. Furthermore, recent biotechnology developments have increased the possibility of employing germ cells derived from stem cells in the future (Chiba *et al.,* 2016). A five-step approach is advised to handle patients with NOA. To confirm or demonstrate that NOA is associated with spermatogenic failure, a differential diagnosis of azoospermia must first be administered. Second, genetic testing is performed to identify men who have NOA due to microdeletions in the long arm of the Y chromosome and to inform affected patients about their chances of sperm retrieval success. Third, it is investigated whether any intervention may stimulate sperm production before a surgical retrieval attempt. Fourth, the most effective and efficient retrieval technique for testicular sperm is adopted. Finally, cutting-edge laboratory methods are used to manage retrieved gametes and cultivate embryos produced by sperm injections (Esteves, 2015). For the majority of patients with NOA, there is yet no treatment that can restore spermatogenesis, except for those who develop testicular failure subsequently. So, for impacted couples to become pregnant without a donor, ICSI using spermatozoa extracted from the testes is their only choice (Donoso *et al.,* 2007). Techniques for sperm retrieval have been developed, including percutaneous, incisional and microsurgically aided methods. Testicular sperm aspiration (TESA) is an example of the percutaneous approach, in which testicular tissue is aspirated using small or large-bore needles. The needle is usually attached to a syringe, creating suction as the needle tip is moved around within each testis to sample the seminiferous tubular tissue. Incisional techniques are conventional testicular sperm extraction (cTESE) or microdissection testicular sperm extraction (mTESE). cTESE involves extracting seminiferous tube tissue through one or more testicular incisions (Medicine, 2018). Numerous randomly chosen testicular samples are often collected and analysed, since it is unfeasible to predict the presence of sperm or the location of islets of normal spermatogenesis, assuming they exist, before doing cTESE. A drawback of cTESE is that removing huge portions of testicular tissue may imperil the already reduced androgen production transiently or permanently, resulting in severe hypogonadism (Esteves, 2015). The technique of microdissection in instances of NOA, TESE (micro-TESE), has been suggested as an ideal replacement for TESE. In addition to reducing the amount of testicular tissue removed and increasing the spermatozoa yield per biopsy, this approach also has the added

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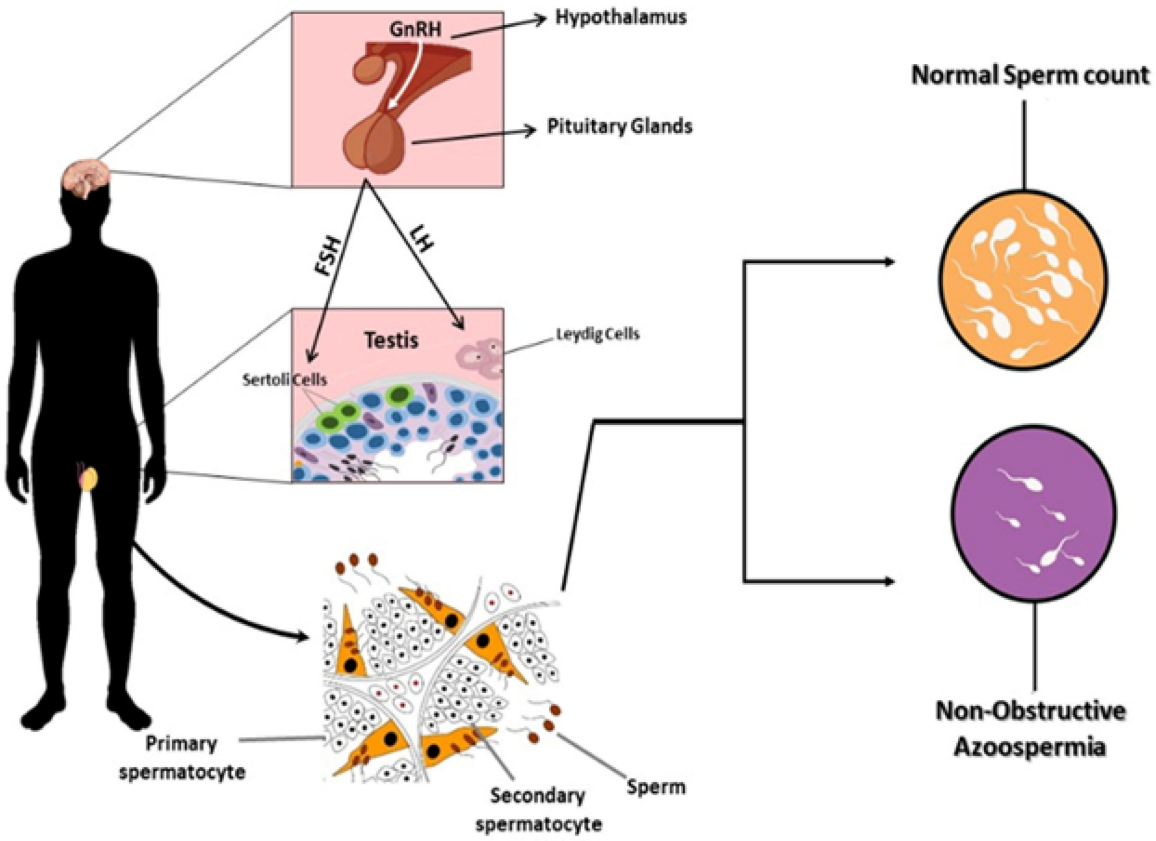


Figure 1. Indicates the impact of two hormones, LH and FSH, on Leydig and Sertoli cells. LH induces testosterone production in the testes' interstitial cells (Leydig cells). FSH stimulates testicular growth and increases the synthesis of an androgen-binding protein by Sertoli cells, a component of the testicular tubule required for sustaining the maturing sperm cell.

benefit of identifying blood arteries, which helps to limit vascular damage. (Schlegel, 1999). The technique of microdissection for TESE involves making a large incision in the testis. Then, under the optical magnification of an operating microscope, the largest-diameter seminiferous tubules are selected (Medicine, 2018). One other method to consider is FNA. It was first used for diagnostic reasons and is a less intrusive technique for sperm retrieval compared to TESE. Yet, compared to TESE, FNA has a much lower sperm retrieval rate (SRR), according to the majority of research. (Tournaye *et al.,* 1995; Hauser *et al.,* 1998). The timing of sperm extraction is a crucial element in the therapy of NOA. Surgical sperm extraction may be performed concurrently with oocyte retrieval during an IVF cycle, using fresh sperm for ICSI if available. If viable sperm are retrieved before ovarian stimulation, they may be cryopreserved for use in future in vitro fertilisation cycles. Each method has theoretical advantages. Using freshly retrieved sperm keeps the sperm from the stress of cryopreservation. Conversely, cryopreserving the collected sperm for future use decouples the time of in vitro fertilisation from sperm extraction, enabling the female partner to circumvent superfluous ovarian stimulation in the absence of identified sperm. (Medicine, 2018). The next step after SR is to transport the sample of testicular tissue to an in vitro fertilisation facility for analysis and possible sperm retrieval. There has been evidence of poorer fertilisation rates, embryo development retardation and pregnancy rates when using spermatozoa collected from males with NOA in ICSI. This is likely because these gametes are often of lower quality and more delicate compared to ejaculated gametes (Ubaldi *et al.,* 1999; Verza Jr and Esteves, 2008; Meseguer *et al.,* 2009; Vozdova *et al.,* 2012; Esteves and Agarwal, 2013; Esteves *et al.,* 2014). The lower the tissue is extracted, the easier it is to analyse and locate the sperm. Micro-TESE can enhance laboratory process efficiency, reduce testicular damage and increase SR success (Esteves and Varghese, 2012; Ashraf *et al.,* 2013; Esteves, 2013). Micromanipulation can only take place in an environment that adheres to good laboratory practices, which include maintaining a sterile environment, controlling the temperature and pH of the working solution and ensuring optimal air quality. (Esteves and Verza, 2012; Esteves and Bento, 2013).

Management of different abnormalities in men with NOA

Treatment of azoospermia depends on the cause, and each cause has a different prognosis. A varicocele is a disorder in which the scrotal venous pampiniform plexus drains blood from each testicle, dilates and enlarges abnormally. Being the most often documented cause of abnormalities in sperm analysis, lower sperm count, poor sperm motility and abnormal morphology, varicoceles are clinically significant (Yetkin and Ozturk, 2018; Paick and Choi, 2019). Surgical varicocelectomy is a crucial therapy for reestablishing fertility, since varicocele is linked to NOA in 5–10% of individuals. (Chiba *et al.,* 2016). Recent investigations, however, have revealed that even if spermatogenesis improves, postoperative sperm concentration is still poor and ART such as ICSI will be needed. Consequently, it is essential to evaluate the feasibility of doing varicocelectomy or sperm retrieval without concomitant varicocele

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repair for these patients (Berookhim and Schlegel, 2014). Undescended testis is a common congenital condition generally noticed and treated during infancy. There is a risk of testicular cancer and spermatogenesis impairment when the testis is improperly located (e.g., abdominal or inguinal) (Hutson *et al.,* 1997; Barthold and González, 2003; Murphy *et al.,* 2007; Virtanen *et al.,* 2007). The best way to treat undescended testicles (UDT) remains a topic of debate. There are, however, several guidelines for the treatment of individuals with UDT: (1) Hormonal therapy is not suggested due to the short-term and potential long-term spermatogenesis consequences. (2) Surgery is the preferred therapy; it is effective in roughly 95% of UDT cases, with a low complication rate (about 1 percent). (3) Orchiopexy should be performed between the ages of 12 and 18 months or at the time of initial contact if the disease is diagnosed later (Niedzielski *et al.*,) Many infertile men have had NOA due to a genetic disorder in the last twenty years. These include Y-chromosomal microdeletions, karyotype abnormalities and missense mutations in genes crucial for reproduction (Cerván-Martín *et al.*, 2020). The most common chromosomal defect among NOA patients is Klinefelter syndrome. In men, small testes, less muscle, less body hair, poor sex desire and gynaecomastia are all symptoms of Klinefelter syndrome. The diagnosis is usually made during a male infertility assessment. A 47, XXY chromosomal complement is seen in approximately 95 percent of men with Klinefelter syndrome (Oates, 2008). Micro-TESE coupled with ICSI is the only treatment option available for NOA patients with Klinefelter syndrome (Ando *et al.,* 2013). An increased incidence of sex and autosomal chromosomal aneuploidies may be seen in spermatozoa from individuals with Klinefelter syndrome (Morel *et al.,* 2003; Staessen *et al.,* 2003). Secondary testicular dysfunction induced by hypothalamic or pituitary disorders is known as hypogonadotropic hypogonadism (HH). The hyposecretion of gonadotropins leads to low testosterone production by the testes and impaired spermatogenesis. There are two types of HH: congenital and acquired. Although HH is a rare condition, medical treatment can improve fertility in patients with this condition. The use of gonadotropins is a conventional medical treatment when fertility is a problem (Chiba *et al.,* 2016).

Future perspectives and cellular therapy for management of non-obstructive azoospermia

Exciting new studies have demonstrated the great transformation potential of germline cells in recent years (Hübner *et al.*, 2003). Primordial germ cells extracted from the embryonic epiblast or teratocarcinoma can colonise the testis and differentiate into male germ cells (Nayernia *et al.,* 2004). Approximately 24 days after fertilisation, they become visible in human embryos within the yolk sac wall, near the allantois. During early embryonic development, SSCs emerge from postnatal testicular gonocytes, which in turn come from long-lived PGCs. (Freeman, 2003). SSCs are seen as a potential option for regenerating deficient or impaired spermatogenesis. SSC transplantation is a prospective approach for treating male infertility and restoring spermatogenesis in patients suffering from degenerative conditions such as cancer, radiation and chemotherapy (Abdelaal *et al.,* 2021). The most delicate premeiotic germ cells are destroyed after cytotoxic treatment; therefore, the remaining spermatogonial stem cells must restart the spermatogenesis process from the beginning (Ehmcke *et al.,* 2006). Before beginning cancer treatment, SSCs are isolated and cryopreserved from a testicular biopsy, followed by intra-testicular stem cell transplantation (Abdelaal *et al.,* 2021). Before starting treatment, a sterile biopsy is performed on the testis of children with cancer or adults with azoospermia, whatever the cause may be. Extracting testicular tissue in humans is an alternative way for biopsy. To obtain a single-cell suspension, the testicular tissue or biopsy is digested using enzymes such as trypsin and type I collagenase (Amirinezhadfard *et al.,* 2025). The process of isolating stem cells from spermatozoa begins with this stage. One effective method that utilises density gradient centrifugation is Percoll isolation (Liu *et al.,* 2011; Oatley, 2017). Propagation of SSCs requires cultivation and growth after isolation or preservation. The main culture of SSCs is established by first making mitomycin-treated STO feeders, followed by washing them with HBSS and then adding the SSCs, whether freshly isolated or cryopreserved (Takahashi and Yamanaka, 2006). To improve SCC differentiation into spermatocytes and spermatids, a 3D culture technique was employed in humans that utilised agarose gel (Mohaqiq *et al.,* 2019). Human GPR125+ spermatogonia were propagated on hydrogel-coated or gelatin-coated plates with growth factors (GDNG, bFGF, GFRA1-Fc fusion protein, NUDT6, TGFB, EGF and LIF) (Azizi *et al.,* 2017). SSC transplantation involves recipient preparation. The preceding protocols focused on preparing SSCs from donors (Azizi *et al.*, 2020b). There are two methods for SSC transplantation: the first involves the retrieval of complete testis or testicular tissue, which requires a specific preparation protocol, and the second involves the extraction and culture of cells. In the case of whole testis tissues, the tunica albuginea should be removed. The testes are then immersed in HBSS, and collagenase is added to facilitate the separation of the seminiferous tubules. Subsequently, HBSS with trypsin-EDTA is added for digestion and cell dispersion. Following this, centrifugation, washing and resuspension in SFM are performed. In the second case of culture, a cell suspension is performed directly in SFM. In both cases, the same procedure is followed, which involves microinjecting the cells into the lumen of the seminiferous tubules with the aid of trypan blue for easier gross monitoring during the injection process. After that, the recipient males are kept under observation for an extra eight weeks to ensure strong donor-derived spermatogenesis (Goodyear and Brinster, 2017). SSC transplantation as a treatment for male infertility due to impaired spermatogenesis is promising. However, it has not yet reached its full potential or been well implemented and still faces several challenges. The whole SSC transplantation process requires more evaluation. The cell and tissue cryopreservation efficiency must be pursued with extreme caution, as this stage is crucial, and the efficacy and effectiveness of cryopreserved SSCs will determine the subsequent treatment approach for infertility. Preserving SSCs before they are depleted and degraded by radiation and chemotherapy is crucial for the future fertility of children impacted by cancer before therapies. Another major and urgent problem is the possibility of cancerous contamination of cancer-stricken children's testes, which might undermine cryopreservation efforts. There are significant concerns about improving protocol efficiency without compromising the testicular niche (Geens *et al.,* 2008). A community's socio-cultural attitude and viewpoint remain the primary barrier to the widespread implementation of the various SSCs being considered. The reason for this is that stem cell treatment is not an option for adult patients who can preserve sperm obtained from ejaculated sperm or TESE. There is a lack of expertise and information regarding the cryopreservation of testicular tissue from prepubertal patients. Furthermore, only a limited number of institutions have the requisite expertise, infrastructure or regulatory authorisation to treat and maintain testicular tissue (Hanna Valli *et al.,* 2015). Other

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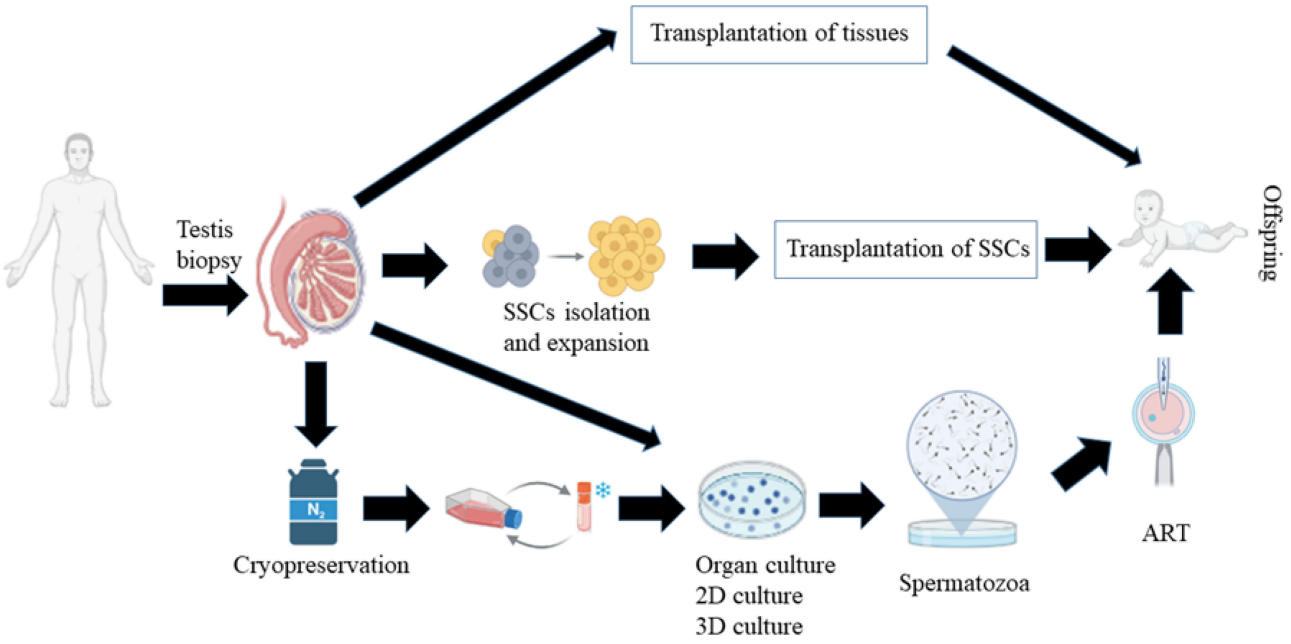


Figure 2. Types of methods of maintaining fertility in in vitro and in vivo conditions. Obtaining testicular tissue samples from azoospermic individuals or individuals exposed to chemotherapy.

stem cell types can also be used in this process, particularly in patients who lack SSCs. As a result, these stem cells give individuals the opportunity to have children in the future. ESCs, pluripotent cells that can differentiate into various cell lineages, are isolated from the inner blastocyst cell mass. They can produce germ cell lines in vitro; however, using embryonic stem cells in humans is fraught with ethical issues. Furthermore, ESCs lose their plasticity during differentiation (Pourmoghadam *et al.,* 2018). In vitro, ESCs differentiate into male germ-like cells; however, this approach has the drawback of being genetically unrelated to patients (Devolder, 2010). VSELs are another type of stem cell that can be utilised in this process. These cells are pluripotent in humans and mice. They possess a self-renewal ability and can regenerate. Since VSELs are not damaged by chemotherapy, they can contribute to the development of germ cells in men who underwent chemotherapy as infants (Kurkure *et al.,* 2015; Wyns *et al.,* 2021). iPSCs, which can be reprogrammed, can be utilised to replace ESCs, avoiding ethical controversy. The benefits of iPSCs include a straightforward sample approach, in which somatic cells, such as skin cells, can be acquired and reprogrammed to form germ cells (Takahashi and Yamanaka, 2006; Forbes *et al.,* 2018; Pourmoghadam *et al.,* 2018). Due to their versatility, immunomodulatory capabilities, excellent safety profile and ease of isolation and culture, mesenchymal stem cells (MSCs) have found applications in a wide range of fields and medical conditions. After injecting MSCs into a busulfan-induced azoospermic rat, researchers found that the animals resumed normal spermatogenesis, indicating that MSCs may be a promising treatment for male infertility (Cakici *et al.,* 2013; Zhang *et al.,* 2014). Remarkably, even after transplanting half as many SSCs, the reproductive efficiency of SSC transplantation was improved when co-transplanted with TGF1-treated MSCs (Kadam *et al.,* 2019) (Figure 2).

Conclusion

NOA represents the most severe form of male infertility, and current clinical management remains limited. Although micro-TESE enables identification and use of rare spermatozoa in nearly half of affected men, it does not restore endogenous spermatogenesis, and it offers no reproductive options for patients in whom sperm cannot be retrieved. The high prevalence of chromosomal and genetic aberrations in NOA underscores the need for comprehensive diagnostic testing and careful counselling; however, these measures do not alter the fundamental therapeutic gap. In this context, spermatogonial stem cell (SSC)–based interventions emerge as a critical frontier. SSC isolation, expansion and transplantation hold unique potential to reestablish sustained spermatogenesis and thereby offer a biological cure – rather than a procedural workaround – for NOA. Yet, despite decades of progress in experimental models, several translational challenges remain unresolved, including optimisation of SSC preservation, validation of transplantation protocols and assurance of genetic and epigenetic safety. Addressing these challenges is particularly urgent for prepubertal boys undergoing gonadotoxic treatments, whose only opportunity to preserve future fertility may lie in banking and later autologous transplantation of SSCs. Ensuring the viability and functionality of cryopreserved SSCs is therefore not merely a technical consideration but a decisive step that will determine the feasibility of SSC-based therapies as the next generation of fertility restoration. Ultimately, the successful clinical deployment of SSC therapy could transform the landscape of NOA management by restoring natural spermatogenesis and expanding reproductive options for patients who currently have none. Continued foundational research, rigorous translational testing and the development of standardised clinical frameworks will be essential to realise this therapeutic promise.

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