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Review Article

Restoring spermatogenesis through allogeneic Sertoli cell transplantation in cryptorchidism: a systematic review

Zhamynchiev Emir[1]*, Kalmatov Roman[2], Mohd Faizan Siddiqui[2], Yatsyk Sergey[3]

[1]Medical Faculty, Osh State University, Osh City, Kyrgyzstan. [2]International Medical Faculty, Osh State University, Osh City, Kyrgyzstan. [3]National Medical Research Center for Children's Health Federal State, Autonomous Institution of the Ministry of Health, Russian Federation.

Correspondence: Zhamynchiev Emir, Medical Faculty, Osh State University, Osh City, Kyrgyzstan. zhamynchiev@gmail.com

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ABSTRACT

Cryptorchidism, the absence of one or both testes from the scrotum, is a major cause of male infertility due to its negative impact on spermatogenesis. Allogeneic transplantation of undifferentiated Sertoli cells is a potential therapy to restore spermatogenesis in cryptorchid testes. This review evaluates the feasibility and effectiveness of Sertoli cell transplantation as a treatment for cryptorchidism by synthesizing results from experimental models. A systematic search of PubMed and Scopus, following PRISMA 2020 guidelines, yielded 560 articles. We mainly focused on studies published between January 2005 and April 2024 that examined Sertoli cell transplantation in cryptorchidism models. Data on molecular and cellular outcomes, transplantation techniques, and success rates were extracted. The review analyzes the potential of Sertoli cell transplantation to restore spermatogenesis, discussing factors such as immunomodulatory properties, long-term survival, and performance of the transplanted cells. Gaps in research, especially regarding cell delivery optimization, are highlighted. Although encouraging, more study is required to enhance long-term cell viability and functioning. This study underscores the need for forthcoming research, especially in Low- and Middle-Income Countries (LMICs), to evaluate the therapeutic viability of this therapy method.

Keywords: Cryptorchidism, Sertoli cells, Spermatogenesis, Infertility, Transplantation

Introduction

Cryptorchidism, the most prevalent congenital condition involving male genitalia, is characterized by the absence of at least one testicle from the scrotum (undescended testis and maldescended testis). Rather, the undescended testis may be found anywhere along the usual course of testicular descent, including high scrotal regions, the supra scrotal position, or the abdominal cavity or inguinal canal [1]. An ectopic testis, in which the testis is found beyond the typical course of testicular descent, as in the femoral, perineal, or pubic region, is not the same as this condition. Attached to the diaphragm by the craniosuspensory ligament, the growing testis is connected to the inguinal area by a caudal ligament called the gubernaculum. Testis descent occurs in two parts. Between 10 and 23 weeks of gestation, the testis descends into the abdomen to the inguinal area during the first transabdominal phase. Testicular descent is a complex process, and it is often described as two separate phases—the transabdominal phase and the inguinoscrotal phase. Congenital cryptorchidism indicates a prevalent urogenital abnormality observed in male newborns, with reported prevalence rates ranging from 1.6% to 9.0% at birth and 0.9% to 1.8% by the age of 3 months. The condition can be categorized based on the potentially related manifestations into syndromic and non-syndromic types of congenital cryptorchidism. Syndromic cryptorchidism indicates that this condition is linked with a syndrome, meaning there are additional clinical features present alongside cryptorchidism. Conditions of syndromic

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cryptorchidism include cases linked to androgen insensitivity syndrome, anomalies of the sex chromosomes, and Noonan syndrome [2]. Various gene mutations have been linked to syndromic cryptorchidism; however, they are infrequently identified in boys with isolated undescended testis. Despite this, the majority of cryptorchidism cases are non-syndromic (isolated), indicating that this condition does not correlate with other disorders. Acquired cryptorchidism refers to a condition where both testes initially descend properly into the scrotum at birth, but subsequently, one or both testes move away from their normal position. The prevalence reported in various studies ranges from 0.6% to 7% for individuals aged 18 to 36 months, and from 1.1% to 2.2% for those aged 6 to 13 years. The etiology, progression, and treatment strategies for this specific form of cryptorchidism remain ambiguous. The cumulative health influence has been observed to be comparable to that of the congenital form of cryptorchidism; thus, it requires timely suitable intervention [3].

Infertility and testicular germ cell cancers are both 4.8 times more likely in cryptorchidism patients. It is undetermined, however, how cryptorchidism affects testicular hormone production in the long run, especially throughout adolescence. Androgen deficit, pubertal delay, or cessation are common symptoms of syndromic cryptorchidism, congenital hypogonadotropic hypogonadism, and enzyme abnormalities in androgen production. Nonsyndromic cryptorchidism evidence on testosterone levels and pubertal development, on the other hand, is somewhat unclear [4]. Sonography is one of the most important diagnostic methods in the assessment of cryptorchidism, which is a non-invasive, radiation-free, and easily available modality. It is also cheap, easily transportable, and highly accurate hence very useful in the LMICs where other forms of imaging like MRI or CT may not be readily available owing to costs or lack of proper facilities. Sonographic imaging is a useful tool in the evaluation of an undescended testis in the inguinal canal as shown in **Figure 1**. It produces high-quality images that help clinicians measure the size and the morphology of the testes as well as the echotexture. Also, Doppler sonography increases the diagnostic value of the examination as it assesses testicular blood flow which is important in determining the viability of the testes as well as other conditions including hypoplasia or atrophy of the testes as shown in **Figure 2**. These capabilities are demonstrated in **Figures 1 and 2** where one of the images shows the testis in the inguinal canal of a 42-year-old man with cryptorchidism while the other image shows testicular hypoplasia with hypoechoic echotexture and reduced blood flow. In LMICs, the availability of sonography makes it possible for the diagnosis of cryptorchidism to be made early and with great precision thus minimizing delays in management. This is due to it being a non-ionizing form of radiation which makes it safe for use in follow-up imaging to observe the testicular descent or the results of surgeries like orchiopexy. The sonography is also vital in the assessment of associated complications including hernias or epididymal anomalies that are common in patients with cryptorchidism.

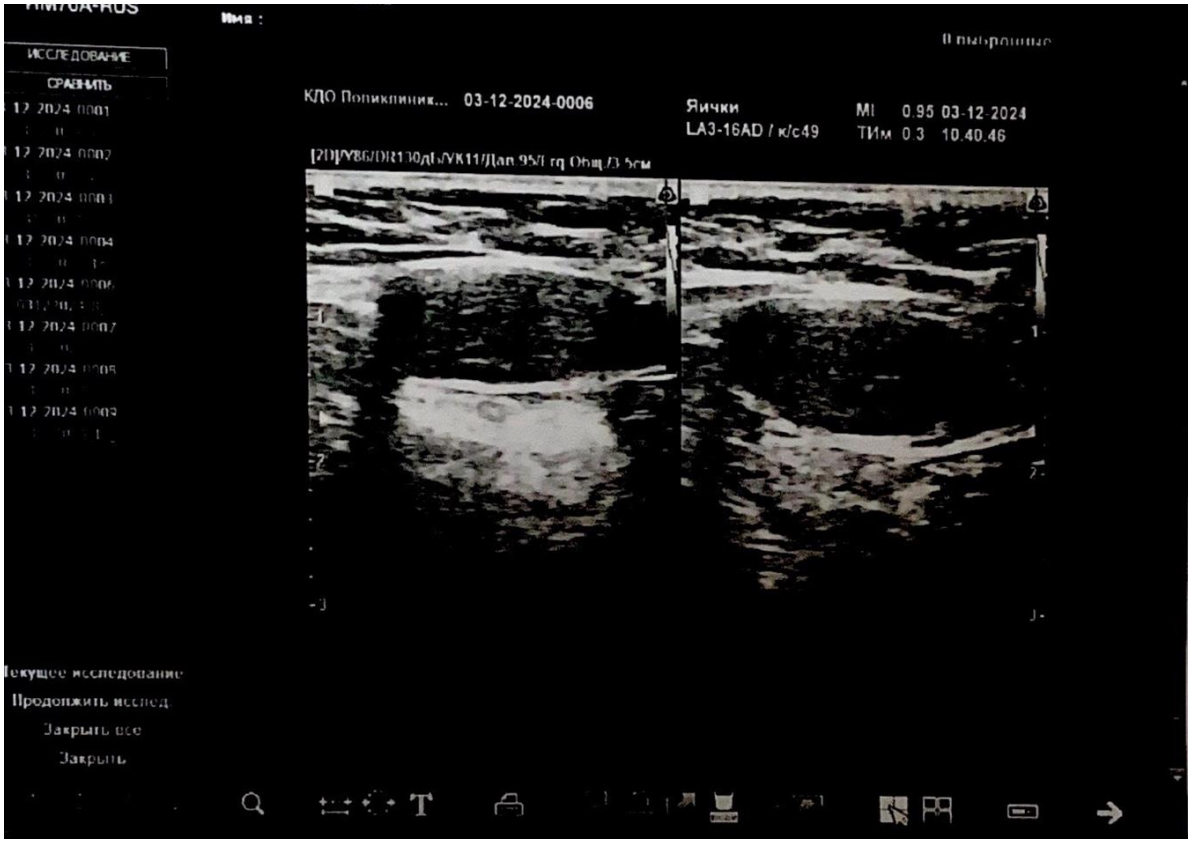
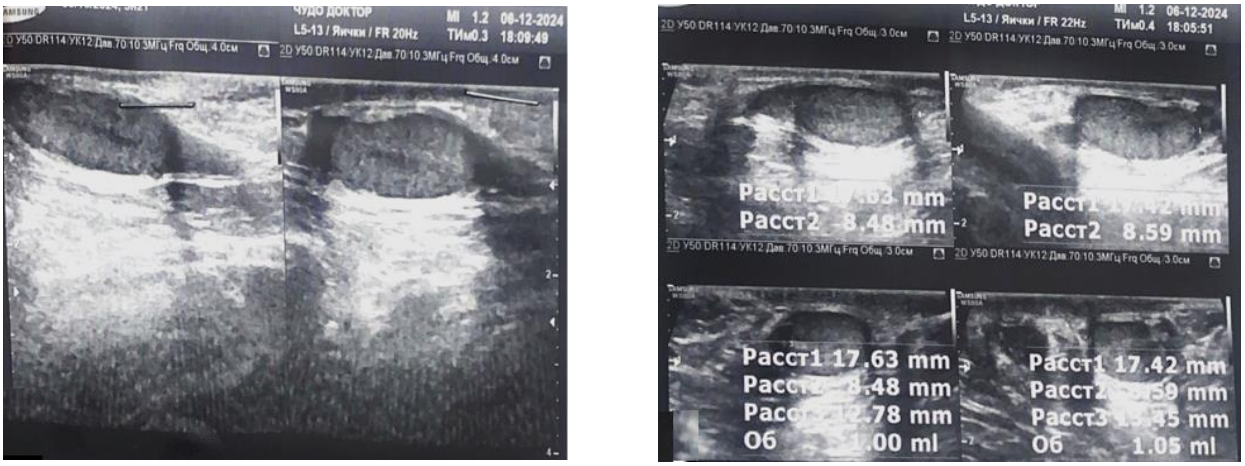


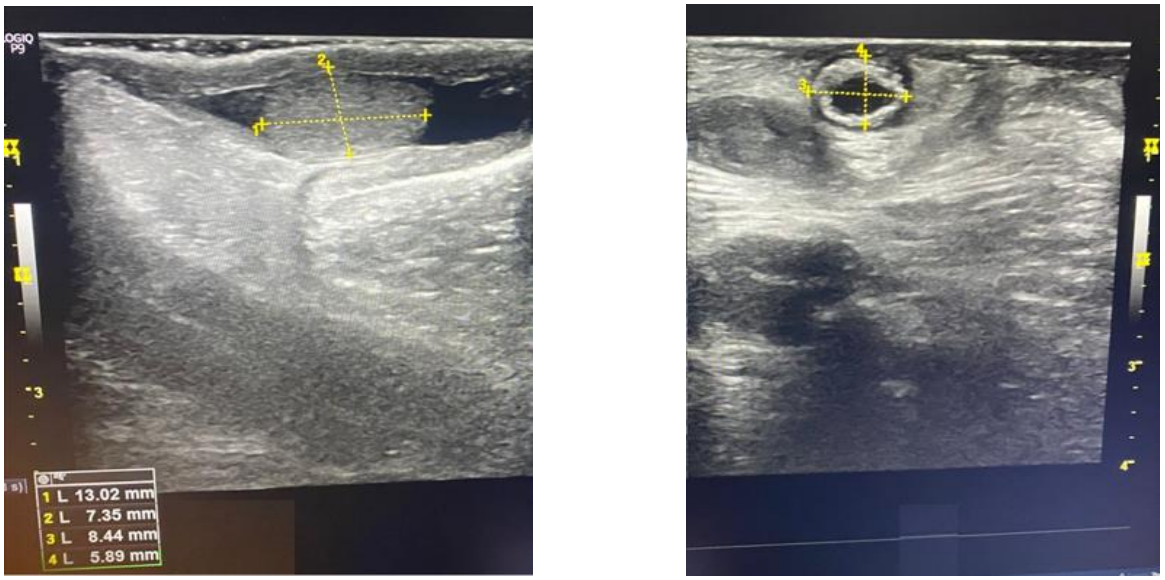
Figure 1. Sonographic imaging of the testis within the inguinal canal in a 42-year-old patient with cryptorchidism.



a) b)

Figure 2. Sonographic evaluation demonstrating cryptorchidism associated with testicular hypoplasia (a) and (b). The affected testis exhibits a hypoechoic texture, reduced dimensions, and diminished vascularity compared to normative testicular parenchyma.

This study reviews the impact of undifferentiated Sertoli cell transplantation in testicular tissue of an animal model with bilateral abdominal cryptorchidism. Partial restoration of seminiferous tubules was found to occur after transplantation. When testis-derived Sertoli cells (SCs) are transplanted, either from the same species or a different one, they can help improve graft survival by acting as "ghost cells" with immune-protective and healing properties. Together, the integration of advanced sonographic diagnostics with newly emerging therapeutic strategies such as SC transplantation provides holistic management of cryptorchidism with testicular hypoplasia as shown in **Figure 3**. This study regards SCs as a promising treatment option for chronic diseases, especially in LMICs and developing countries.



a) b)

Figure 3. Measurement of testicular length, width, and height by ultrasound on a 17-year-old boy with Cryptorchidism with Testicular hypoplasia. The dotted line (1= 13.02 mm), (2= 7.35 mm), (3= 8.44 mm), and (4= 5.89 mm) show measurement of length and height in the longitudinal plane (a) and width in the transverse plain (b)

Materials and Methods

This literature review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [5]. A comprehensive search was conducted across multiple databases, including PubMed and Scopus, to identify relevant studies on “cryptorchidism,” “spermatogenesis,” “Sertoli cell transplantation,” and “allogeneic cell therapy.” The inclusion criteria focused on peer-reviewed studies published in the English language that investigated these topics using experimental models.

The search strategy was built by identifying relevant Medical Subject Headings (MeSH) and keywords. These terms were combined using the “OR” operator within each construct and subsequently connected with the “AND” operator across constructs. The reference period for the search was set from January 1, 2005, to ensure that the most recent advancements in allogeneic transplantation techniques were included. A few additional articles were searched with a time frame of the year

1900s for a historical timeline of the research study. No restrictions on the region of publication were applied. Searches were completed in April 2024, followed by a secondary hand search of the reference lists from the included articles, with the full search strategy detailed in **Table 1**.

This method ensured the inclusion of all relevant studies on restoring spermatogenesis through undifferentiated Sertoli cell transplantation. Data extraction was carried out by two independent reviewers to gather details on study design, animal models, transplantation methods, and outcome measures. Studies were categorized based on transplantation techniques (autologous vs. allogeneic) and experimental outcomes such as the restoration of spermatogenesis, immune response, and behavior in cryptorchidism models. Exclusion criteria include review articles, expert opinions, and studies on unrelated cell therapies. As the purpose of this review was to summarize the methodologies and outcomes used in experimental transplantation studies, a formal quality assessment of the studies was not conducted. Instead, studies with similar designs and outcomes were analyzed collectively to identify key trends, challenges, and potential clinical implications for the use of Sertoli cell transplantation in reproductive medicine.

Table 1. Two reviewers independently screened the titles and abstracts of articles identified through the electronic database search to determine their relevance based on the following criteria: (1) the study involved cryptorchidism or undescended testis, (2) spermatogenesis was evaluated, and (3) Sertoli cell transplantation or allogeneic cell therapy was part of the study's methodology. Full-text manuscripts of citations potentially meeting these criteria were obtained for further evaluation. The reviewers independently assessed the full texts to identify eligible studies that investigated the restoration of spermatogenesis through Sertoli cell transplantation. Inclusion criteria for the final selection were: (1) studies using animal models, (2) first-hand data on Sertoli cell transplantation, and (3) experimental models of cryptorchidism. Studies were excluded if they were review articles, expert opinions, or focused on unrelated cell therapies. Any disagreements were resolved by consensus among the reviewers.

Database	Search Terms
PubMed	(("cryptorchidism"[MeSH Terms] OR "cryptorchidism"[All Fields] OR "undescended testis"[All Fields]) AND ("spermatogenesis"[MeSH Terms] OR "spermatogenesis"[All Fields] OR "sperm production"[All Fields]) AND ("Sertoli cells"[MeSH Terms] OR "Sertoli cells"[All Fields] OR "Sertoli cell transplantation"[All Fields] OR "undifferentiated Sertoli cells"[All Fields]) AND ("cell transplantation"[MeSH Terms] OR "allogeneic"[All Fields] AND "transplantation"[All Fields] OR "allogeneic cell therapy"[All Fields]))
Scopus	(TITLE-ABS-KEY("cryptorchidism" OR "undescended testis") AND TITLE-ABS-KEY("spermatogenesis" OR "sperm production") AND TITLE-ABS-KEY("Sertoli cells" OR "Sertoli cell transplantation" OR "undifferentiated Sertoli cells") AND TITLE-ABS-KEY("cell transplantation" OR "allogeneic cell therapy")) AND PUBYEAR > 2005 AND PUBYEAR < 2024

Fundamental characteristics and role of sertoli cells

The specialized organs known as seminiferous tubules found in mammal testes allow the process of spermatogenesis. Surrounded by an interstitial area packed with fibroblasts, blood arteries, leukocytes, and Leydig cells, these tubules include germ and Sertoli cells. As well as providing immunological protection for these highly antigenic cells, Sertoli cells (SCs) are essential in enabling germ cells to grow into fully formed spermatids. Most autoantigenic germ cells are protected by a barrier formed by somatic cells, which also release immunomodulatory chemicals [6].

Up until the 1950s, SCs were mainly regarded as mechanical support cells; barely 25 studies on this kind of cell have been published. Over the past 20 years, advances in electron microscopy, biochemistry, histochemistry, and molecular biology have led to a surge in Sertoli cell-related publications, now more than 500. Up to 1955, when cellular membranes and junctional complexes were revealed by electron microscopy, the exact structure of SCs remained unknown. Moreover, electron microscopy and other imaging techniques have helped to define the complex interactions among Sertoli cells (SCs), germinal cells, adjacent SCs, and cells of the seminiferous tube wall. Large, elongated cells (75–100 μm in diameter), spermatogonial cells include multiple projections and complex folds or crevices that house growing germ cells [7].

Strong connections between Sertoli cells on their basolateral surfaces create a division in the seminiferous epithelium, separating it into basal and adluminal compartments. As puberty starts, Sertoli cells (SCs) develop basolateral strong connections and transform immature germinal cells into fully developed spermatids. Only those antigens produced during fetal development are recognized by the immune system as "self." 'Non-self' antigens are those generated after puberty from freshly arrived post-meiotic or meiotic germ cells. To prevent the immune system from classifying these high antigenic developing germ cells as "foreign," the 'blood-testis barrier' (BTB) and the release of immunoregulatory molecules originating from Sertoli cells must be preserved structurally. Germ cells may persist in an environment created by the special capacity of Sertoli cells to regulate the immune system. Apart from immunomodulatory substances, SC releases trophic, anti-inflammatory, and anti-apoptotic molecules among other substances. Both cellular survival and germ cell differentiation are greatly aided by these additional substances [8].

Role of sertoli cells in immunomodulation

Biologically, meiotic and post-meiotic germ cells initially express up during adolescence and release fresh antigens the developing

immune system marks as "foreign". The testis has a special microenvironment that inhibits immunity to these alien invaders. Everybody knows that the testis is an immune-privileged location. Sertoli cells (SCs) mostly sustain the distinctive immune-protective state of the testes. Research suggests that while the blood-testis barrier (BTB) contributes to the protection of germ cells, it is not the only mechanism of their safeguarding. Although data indicates that the blood-testis barrier (BTB) aids in preventing immune responses to germ cells inside the adluminal compartment of the seminiferous epithelium, other mechanisms also play a role in this protection. Tissue transplanted beyond the blood-testis barrier into the interstitial compartment of the testis—such as skin fragments, islets, or parathyroid tissue—exhibited prolonged viability compared to tissue transplanted into non-immunoprivileged regions [9]. The observation that most transplanted cells were located near, but not encircled by, SCs indicates that the protection against allografts and/or xenografts likely resulted via SC-mediated modulation of the immune response rather than only from the physical blood-tissue barrier (BTB). Allogeneic islets transplanted in the contralateral kidney were safeguarded in NOD mice by Sertoli cells injected underneath the right kidney capsule. These studies taken together show that SCs alter the immune response to foreign antigens, not the BTB, which is the main barrier to immunological detection of these antigens [10]. Additional studies indicate that Sertoli cells secrete immune-suppressive chemicals to inhibit the immunological response during spermatogenesis, thereby playing a crucial role in maintaining testis immune privilege. It is still unknown, nevertheless, exactly which immunosuppressive biomolecules these allogeneic and xenogeneic transplantation investigations identified [10]. Among them are cytokines (such as interleukins and interferons) and many prostaglandins, proteins, and peptides derived from SC [11].

Moreover, SC Toll-like receptors mediate the production of antiviral and antibacterial proteins like defensins and interferons. All these results taken together demonstrate that the seminiferous epithelium contains unique antiviral, antibacterial, and immunosuppressive defense systems that are mostly mediated by SCs rather than the BTB. It's the Sertoli cells (SCs), more than the blood-testis barrier (BTB), that offer immunological protection to antigen-bearing germ cells in the seminiferous epithelium and help keep them viable [12].

Several immunoprotective substances released by SCs prevent the growth of B- and T-lymphocytes, which reduces the synthesis of IL-2. Main members of the interstitial cell population, macrophages, and lymphocytes, are essential for testis-related immunoprivilege. About 80% of macrophages lean mostly toward the M2 phenotype; about 20% are M1, which is more widespread in other organs. Still not entirely clear are the processes controlling this equilibrium between M1 and M2 macrophages. Sertoli cells and Leydig cells, however, most certainly may generate and release molecules that encourage the change from M1 to M2. The only generally acknowledged idea is that the testis regulates this delicate balance differently than other tissues, which has resulted in the discovery of some local mechanisms connected to the SCs [13]. Winnall *et al*. showed that the differentiation toward the M2 macrophage phenotype might be driven in part by TGF-β, IL-10, and activin-A. These drugs could improve the expression of CD 163 and boost IL-10 generation, both indicators of M2 macrophages, hence transforming the M2 subtype. Winnall Wr *et al*. (2013) also discovered that producing TGF-β is necessary for Sertoli cells (SCs) to efficiently preserve islet grafts after co-transplantation in NOD animals [14].

The immune system's regulation largely depends on a specific subgroup of regulatory T cells (Tregs) characterized by CD4-/CD25-/Foxp3-positive markers [15]. Recent research has revealed that SC plays a crucial role in enhancing the Treg phenotype, primarily through pathways involving indoleamine 2,3-dioxygenase (IDO). This process has been shown to significantly improve autoimmune conditions. Indeed, diabetes was cured via a TGF-β-IDO-dependent mechanism when porcine SC (PSC) was injected into NOD mice, an animal model of spontaneous autoimmune diabetes. Along with other immunomodulating agents secreted by Sertoli cells, TGF-β activates certain immune cell subsets that help control Th2 cells, Tregs, and M2 macrophages. More recently, research revealed a tight relationship between Tregs and M2 macrophages. Tregs secrete IL-10 and TGF-β, which help to cause the M1 to M2 cell phenotypic transition. These are the same substances that Tregs are activated by and that M2 macrophages produce. It seems then that the testicular defense against autoimmune damage is partially ascribed to the SC-Treg-macrophage cascade. By two main mechanisms—inhibiting complement activation and encouraging the death of CD8+ cytotoxic T cells and natural killer cells—Sertoli cells help to immunologically defend [16].

Moreover, lymphocyte-mediated cytotoxicity may be opposed by SCs. CD8 T cells and natural killer cells are the main cytotoxic lymphocytes that induce apoptosis in transplanted cells through the Fas–Fas ligand pathway or by releasing cytolytic granules. To the target cells, lymphocytes carry cytolytic granules containing granulysin, granzymes, and perforin. Additionally found in murine SC conditioned media by Jensen CF *et al*. (2024) is SERPINA3N, a granzyme B inhibitor. Further protease inhibitors expressed by SCs include P19 and SERPINB9. The latter prevents apoptosis produced by FAS–FASL. Apoptosis of the lymphocytes results from the caspase cascade activation initiated by the FASL binding to FAS [17, 18].

Apart from the potential of SCs to shield germ cells from infection, an efficient response to bacterial and viral pathogens demonstrates their innate immune responsiveness. Campese AF *et al*. (2014) showed the ability of Toll-like receptors on the SC membrane to distinguish between a wide range of bacteria and viruses. Through the Notch/Jagged1 pathway, controlled by TGF-β, a soluble version of JAGGED1 (JAG1) identified in a conditioned medium from murine stem cells may induce the de novo generation of Tregs [19].

Results and Discussion

A total of 560 records with titles and abstracts were retrieved from the database search, along with an additional 19 studies identified through hand-searching reference lists and 26 studies from pre-prints. After removing 73 duplicates, 532 records were screened, and 296 irrelevant articles were excluded. The full texts of 236 studies were then reviewed, and 27 met the eligibility criteria for inclusion in the qualitative synthesis. The study selection process is illustrated in **Figure 4**. Several research conducted in laboratory settings and living organisms have shown the strong potential of Sertoli cells (SCs) to modulate the immune system, particularly in relation to protecting against cells from other individuals or species. These remarkable cells have the potential to eliminate the need for persistent general/systemic immune suppression, which is now required for successful transplantation procedures.

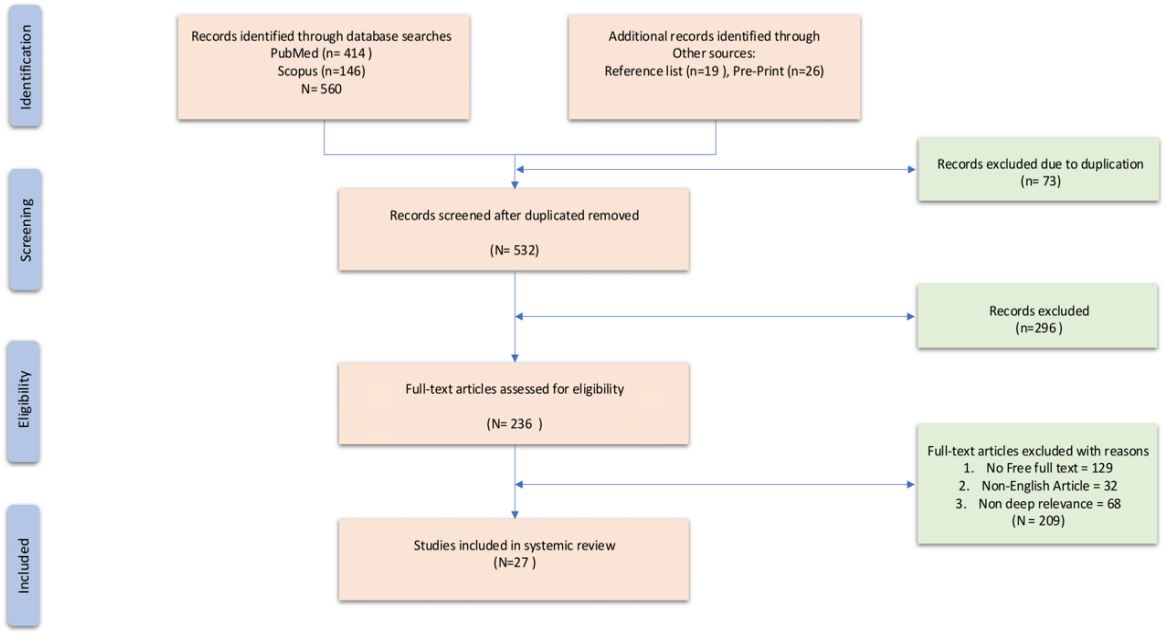


Figure 4. Flowchart illustrating the study review and inclusion process, detailing the steps taken to identify, screen, and select articles for the qualitative synthesis.

Reviving spermatogenesis: from animal models to human fertility

In 1994 when Brinster and Zimmermann engrafted spermatogonial stem cells into the seminiferous tubules of busulfan-treated mice, many approaches and techniques for the restoration of fertility in different species were discovered [20]. In bigger animals, the most commonly used method of SSCs transplantation is the ultrasound-guided injection into the rete testis because it is easily reachable and it is surrounded by the seminiferous tubules. Kanatsu-Shinohara *et al*. presented the first mouse spermatogonial stem cell (SSC) culture in 2003 when testicular cells were plated on gelatin-coated surfaces [21]. Somatic cells could adhere due to this differential plating technique, which also helped to isolate floating germ cells for further culture. This approach enhanced SSCs and reduced somatic cell overgrowth. Following two rounds of differential plating, the germ cells were grown on mouse embryonic fibroblast feeder cells in a media supplemented with glial cell line-derived neurotrophic factor (GDNF), fibroblast growth factor 2 (FGF2), epidermal growth factor (EGF), and leukemia inhibitory factor (LIF). Transplantation of functioning SSCs into infertile patients validated their proliferation since the cells recovered spermatogenesis and created functional sperm capable of fertilization and offspring formation. Later developed for human SSCs by Sadri-Ardekani *et al.*, using differential plating to lower somatic cell contamination, was a similar protocol as Kanatsu-Shiohara *et al*. [22]. On plates covered with human placental laminin in StemPro media supplemented with GDNF, FGF2, EGF, and LIF, floating cells were grown. Over 64 days, the procedure produced an 18,000-fold rise in SSCs—enough for a successful transplantation. Especially, this approach helped SSC cultures from prepubertal human testes to be effectively propagated.

Although more than 20 research on human SSC culture has been reviewed by Murdock *et al*. (2019) many employing techniques similar to those detailed by Kanatsu-Shinohara and Sadri-Ardekani have produced inconsistent outcomes [23]. While some research revealed a rapid decline in germ cell count, others showed a notable increase in SSCs. Variations in beginning cell populations, culture conditions, and approaches to outcome analysis could lead to these variances. Notwithstanding these difficulties, new developments including single-cell sequencing are clarifying human SSC characteristics, which should help to maximize culture systems and increase the repeatability and efficacy of SSC transplantation techniques.

Successful fertilization of the first nonhuman primate offspring came from sperm taken from testicular transplants showed in a study by A.P. Fayomi *et al.* (2019). In their work, prepubertal rhesus monkeys underwent castration, and cryopreservation of their testicular tissue was performed [24]. The tissue was thawed and reimplanted—either under the scrotal skin or on the back—when the girl entered puberty. Even with just transplanted testicular tissue present, the monkeys started making testosterone and showed a functioning hypothalamic-pituitary-gonadal axis. Whether on the rear or in the scrotum, all of the graft sites developed and generated sperm. Following intracytoplasmic sperm injection (ICSI), these sperm were then used to produce a healthy female child. Although autologous testicular grafting has not been investigated in people, this work provides important safety and feasibility information that might guide the next therapeutic uses in human fertility.

Through several pre-clinical and lab experiments, it has become evident that isolated Sertoli cells, whether placed in a culture or transplanted along with other cells, may effectively extend the lifespan of various cell types. This is achieved by the Sertoli cells producing both immunomodulatory and trophic substances. The cells mentioned in the study by Kaur G *et al.* (2012) are very valuable for cell transplantation treatment due to their ability to protect themselves from immunological rejection, even when transplanted into a different species (xenografts) [25]. Due to significant practical and ethical constraints, it is necessary to use cells obtained from an alternative animal source, such as pre-pubertal pigs, as isolating Sertoli cells from human testes is challenging. The current recommendations for animal cell, tissue, or organ transplantation focus on reducing the risk of transmitting zoonotic illnesses to transplant recipients.

The use of Sertoli cells (SCs) in transplantation presents a novel technique in the treatment for the restoration of spermatogenesis and has great potential to influence the management of reproductive health disorders. The capabilities of SCs in regulating the immune response coupled with their ability to establish an immune-protected microenvironment provide solutions to the longstanding hurdles in cell therapy. Patient supervision depends on the inclusion of non-ionizing imaging modalities since SCs are intrinsically sensitive and rely on a suitable microenvironment for survival and functioning. Non-invasive methods of assessing transplanted cells abound from ultrasound to magnetic resonance imaging (MRI). By allowing real-time monitoring of SC viability, integration within host tissues, and the identification of any potential issues, these imaging instruments help to reduce ionizing radiation-related risks. Moreover, developments in functional MRI might provide insights into the metabolic and physiological dynamics of the transplanted cells, thereby improving the accuracy of post-transplantation assessments.

Although porcine endogenous retroviruses (PERVs) cannot be eradicated from porcine donors due to their integration into the swine genome, there is growing evidence that PERVs do not pose an infectious risk in living organisms, despite their ability to infect human cells in laboratory conditions [26]. Although the cloning of these cells into pigs has not yet been accomplished, many firms provide this service for suitable animals. Fujita H *et al.* (2010) successfully acquired pure, living, and fully functioning swine Sertoli cells utilizing a safe pig colony that is free from particular pathogens (SPF). The viability of SCs has not been diminished after 24 hours of the microencapsulation process, which aligns with the duration required for long-distance transportation of transplants to remote regions [27]. The combination of these two approaches has the potential to create a feasible SC product.

Particularly during adolescence, a major event in the beginning of in vivo spermatogenesis is the development of the blood-testis barrier (BTB), which is necessary for the correct spermatogenesis progression [28]. Studies in mice with genetic changes or mutations in BTB proteins frequently indicate abnormalities in spermatogenesis; it is therefore quite likely that similar abnormalities in humans would cause subfertility. In vitro testicular cultures have also revealed BTB structural proteins like connexin 43 and claudin 11, suggesting that BTB generation can take place outside of the normal testicular surroundings as observed by De Michele *et al.* (2018) [29]. Still, the whole consequences of this observation are not yet understood.

Apart from imaging, strict follow-up procedures have to be provided to ensure the long-term efficiency of transplanted SCs and reliable spermatogenesis restoration. These procedures have to cover clinical follow-ups, endocrine checks, and frequent imaging tests. Such steps would enable quick therapeutic responses and help to identify complications such as insufficient cell performance or graft rejection. SC-based treatments must be clinically translated using a multidisciplinary approach combining modern imaging technologies with thorough follow-up plans. Particularly in tackling the worldwide issue of infertility, these elements will be essential in closing the gap between preclinical results and clinical implementation. Future research should give top priority to assessing the long-term effectiveness and safety of these treatments in clinical trial environments, especially with regard to non-invasive monitoring techniques and their contribution to maximizing patient outcomes. A key limitation of this study is the reliance on experimental animal models, which may not fully replicate the complexities of human cryptorchidism and its response to Sertoli cell transplantation. Another limitation is the lack of long-term follow-up data in the reviewed studies, making it difficult to assess the sustained functionality and viability of transplanted cells over extended periods. Particularly in Low- and Middle-Income Countries (LMICs), where the impact of infertility is great and access to sophisticated reproductive technologies is restricted, future studies should concentrate on turning these discoveries into practical clinical trials. Encouragement of such research in LMICs might help to improve knowledge of the viability, economy, and wider influence of Sertoli cell transplantation in many different hospitals and medical centers.

Sertoli cells transplantation: current application and pre-clinical studies

Pre-clinical research has made tremendous progress in using Sertoli cells for transplantation. In the past thirty years, Sertoli cells transitioned from a purely structural role to an active functional system with significant immunomodulatory capabilities. These cells are used in experimental treatments like cell transplantation and co-transplantation for degenerative and chronic inflammatory diseases [30]. For their possible use in cell transplantation studies for chronic or autoimmune diseases, such as diabetes mellitus, Laron dwarfism, Duchenne muscular dystrophy, and efforts to lessen skin allograft rejection, both naked and microencapsulated Sertoli cells have been studied. Furthermore, the possible medicinal applications of prolonged transplantations of human Sertoli-like cells with suitable cell types, like skin grafts and pancreatic islets, for the treatment of skin burns and diabetes have been investigated. Immunoprotective characteristics of Sertoli cells help to create an ectopic immune-privileged milieu [31]. Co-transplanted xenogeneic and allogeneic cells, like neurons and pancreatic islets, are successfully extended in a lifetime by this environment. These developments point to promise for transplantation therapies in the future for many clinical disorders.

Here is a list of various studies that have explored the use of Sertoli cells (SC) as a treatment in different experimental models of diseases **(Figure 5)**.

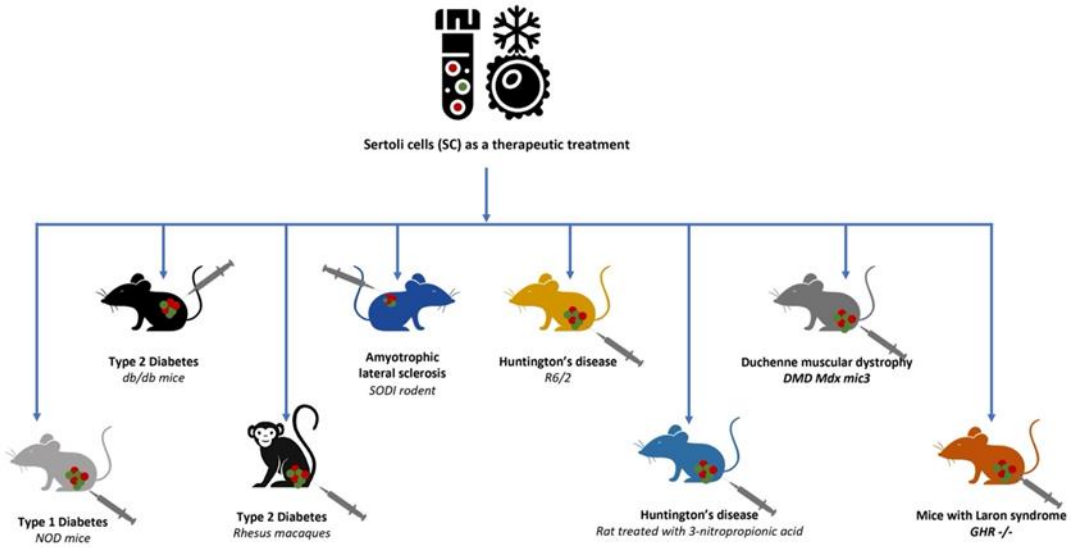


Figure 5. Therapeutic application of Sertoli cells (SC) in experimental disease models. This illustration depicts the use of SC in various experimental setups, highlighting their potential therapeutic role in treating different diseases.

- *In the case of Type 1 diabetes (NOD mice):* Intraperitoneal (i.p.) injection of Microencapsulated Sertoli cells (MC-SC) without additional therapy was found to prevent and reverse diabetes. The restoration of systemic immune tolerance and the development of Treg cells were reliant on TGF- and IDO [32].
- *In the case of Type 2 diabetes (db/db mice)*: Subcutaneous (s.c.) administration of MC-SC induced improvements in glucose tolerance and restore glucose homeostasis. Additionally, it enhanced GLUT-4 expression in skeletal muscle tissue while reducing inflammation in macrophages [11].
- *In the case of Type 2 diabetes in spontaneous rhesus macaques*: The intraperitoneal injection of MC-SC led to decreased daily insulin dose requirements, as well as lower levels of plasma glucose and glycated hemoglobin. Furthermore, there was a noted reduction in CD8+ T cells, B cells, and Treg cells [33].
- *In the case of amyotrophic lateral sclerosis (SOD1 rodents)*: It was discovered that motor neuron local survival was enhanced via spinal cord injection of mouse SC (mSC) [34].
- *In the case of Huntington's disease (R6/2 mice):* Intraperitoneal injection of MC-SC prolonged lifespan, maintained motor function, and prevented motor deterioration, according to a study by. In the brain, it also exhibited neuroprotective and anti-inflammatory properties [35].
- *In the case of Huntington's disease in rats treated with 3-nitro propionic acid:* Injection of rat SC (rSC) into the striatum decreased locomotor hyperactivity, with some behavior returning to baseline [36].
- *In the case of Duchenne muscular dystrophy, DMD (mdx mice):* It was shown that administering MC-SC intraperitoneally to pre-symptomatic, symptomatic, and chronic animals improved muscle morphology and performance. This enhancement was linked to a reduction in muscle inflammation and an increase in utrophin expression stimulated by heregulin β1 released from Sertoli cells [37].
- *In case of mice with Laron syndrome (GHR−/−):* Intraperitoneal administration of MC-SC rescued body growth mediated by SC-derived IGF-1 [38].

Conclusion

This study emphasizes the groundbreaking idea that Sertoli cells (SCs) serve as a sophisticated and distinct 'microlaboratory' capable of producing and releasing a combination of immunomodulatory and trophic substances. These substances have shown the capacity to work together to shield both foreign and genetically similar cells from immune responses, as well as improve and sustain their survival and preserve the specialized function of cells transplanted together. The use of isolated Sertoli cells in cell transplantation aims to replace or enhance ineffective treatments for severe human diseases. This approach represents a unique and potentially significant step toward the straightforward, yet still unachieved, idea that effective therapy should involve replacing dysfunctional cells with functional ones. There is undoubtedly considerable work ahead to achieve this therapeutic goal. However, the substantial amount of promising laboratory and pre-clinical research, along with advancements in cell transplantation techniques discussed in this review, offers compelling evidence for the practical use of these specialized cells—whether in their natural form or encased in a protective layer—in treating complex and chronic diseases that are difficult to manage.

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