Male fertility restoration

in vivo and in vitro stem cell–based strategies using cryopreserved testis tissue: a scoping review

von Rohden, Elena; Jensen, Christian Fuglesang S.; Andersen, Claus Yding; Sønksen, Jens; Fedder, Jens; Thorup, Jørgen; Ohl, Dana A.; Fode, Mikkel; Hoffmann, Eva R.; Mamsen, Linn Salto

Published in:
Fertility and Sterility

DOI:
10.1016/j.fertnstert.2024.07.010

Publication date:
2024

Document version:
Final published version

Male fertility restoration: in vivo and in vitro stem cell–based strategies using cryopreserved testis tissue: a scoping review

Elena von Rohden, M.D.,[a,b,h] Christian Fuglesang S. Jensen, M.D., Ph.D.,[a] Claus Yding Andersen, D.M.Sc.,[b] Jens Sønksen, M.D., Ph.D.,[a,b] Jens Fedder, M.D., Ph.D.,[d,e] Jørgen Thorup, M.D., Ph.D.,[b,f] Dana A. Ohl, M.D.,[g] Mikkel Fode, M.D., Ph.D.,[a,b] Eva R. Hoffmann, Ph.D.,[b,c] and Linn Salto Mamsen, Ph.D.[h]

[a] Department of Urology, Copenhagen University Hospital, Herlev and Gentofte Hospital, Herlev, Denmark; [b] Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [c] Department of Molecular and Cellular Medicine, DNRF Center for Chromosome Stability, University of Copenhagen, Copenhagen, Denmark; [d] Department of Gynecology and Obstetrics, Centre of Andrology & Fertility Clinic, Odense University Hospital, Odense, Denmark; [e] Research Unit of Human Reproduction, Institute of Clinical Research, University of Southern Denmark, Odense, Denmark; [f] Department of Pediatric Surgery, Surgical Clinic, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark; [g] Department of Urology, University of Michigan, Ann Arbor, Michigan; and [h] Laboratory of Reproductive Biology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

Importance: Advances in the treatment of childhood cancer have significantly improved survival rates, with more than 80% of survivors reaching adulthood. However, gonadotoxic cancer treatments endanger future fertility, and prepubertal males have no option to preserve fertility by sperm cryopreservation. In addition, boys with cryptorchidism are at risk of compromised fertility in adulthood.
Objective: To investigate current evidence for male fertility restoration strategies, explore barriers to clinical implementation, and outline potential steps to overcome these barriers, a scoping review was conducted. This knowledge synthesis is particularly relevant for prepubertal male cancer survivors and boys with cryptorchidism.
Evidence Review: The review was conducted after the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews criteria and previously published guidelines and examined studies using human testis tissue of prepubertal boys or healthy male adults. A literature search in PubMed was conducted, and 72 relevant studies were identified, including in vivo and in vitro approaches.
Findings: In vivo strategies, such as testis tissue engraftment and spermatogonial stem cell transplantation, hold promise for promoting cell survival and differentiation. Yet, complete spermatogenesis has not been achieved. In vitro approaches focus on the generation of male germ cells from direct germ cell maturation in various culture systems, alongside human induced pluripotent stem cells and embryonic stem cells. These approaches mark significant advancements in understanding and promoting spermatogenesis, but achieving fully functional spermatozoa in vitro remains a challenge. Barriers to clinical implementation include the risk of reintroducing malignant cells and introduction of epigenetic changes.
Conclusion: Male fertility restoration is an area in rapid development. On the basis of the reviewed studies, the most promising and advanced strategy for restoring male fertility using cryopreserved testis tissue is direct testis tissue transplantation.
Relevance: This review identifies persistent barriers to the clinical implementation of male fertility restoration. However, direct transplantation of frozen-thawed testis tissue remains a promising strategy that is on the verge of clinical application. (Fertil Steril® 2024;122:828–43. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Male fertility restoration, spermatogonial stem cells, autotransplantation, in vitro spermatogenesis

Received April 22, 2024; revised July 4, 2024; accepted July 5, 2024; published online July 9, 2024.
Supported by a grant (2096-00027B) from the Independent Research Fund Denmark, Odense, Denmark (to J.S., covering E.v.R.'s salary during her Ph.D. program).
Correspondence: Elena von Rohden, M.D., Department of Urology, Copenhagen University Hospital, Herlev and Gentofte Hospital, Borgmester Ib Juuls Vej 23A, DK-2730 Herlev, Denmark (E-mail: evrohden@gmail.com).

Fertil Steril® Vol. 122, No. 5, November 2024 0015-0282

https://doi.org/10.1016/j.fertnstert.2024.07.010

Childhood cancer survival rates have increased substantially in the past few decades, with over 80% of survivors reaching adulthood thanks to modern treatment regimens (1, 2). Many prepubertal patients with cancer are facing gonadotoxic treatments such as chemotherapy or gonadal radiation, which poses a significant threat to their future fertility. Prepubertal males have no option to preserve fertility by traditional sperm cryopreservation; for these patients, the only fertility preservation strategy is cryopreservation of immature testis tissue (ITT) (3). Fertility restoration is increasingly becoming a concern, with the focus shifting toward continuation of care after successful cancer treatment and securing quality of life for childhood cancer survivors who wish to become biologic parents in adulthood (4–8). Some centers also offer testis tissue cryopreservation for selected young boys with cryptorchidism who have a high risk of infertility (9–11). A growing number of centers worldwide now offer routine cryopreservation of ITT with the aim of advancing spermatogonial stem cell (SSC)–based treatments to a clinical stage to provide opportunities for fertility restoration (12–14). According to the most recent survey by the ORCHID-NET consortium, 3,118 boys aged <18 years had ITT cryopreserved worldwide (at centers in Europe, the United States, and Australia) by November 2022 (15). Here, it is reported that the indications for fertility preservation are a malignant disease in approximately 60% of cases and a benign condition including Klinefelter syndrome in approximately 40%. Similar numbers had been reported previously (16).

Fertility has a profound impact on our quality of life (17, 18). Male fertility restoration is currently a dynamically evolving field including a broad range of strategies such as surgical and in vitro approaches to achieve restoration of fertility from prepubertally cryopreserved testis tissue. A recent study has mapped how 16 centers currently handle fertility preservation of prepubertal boys using cryopreservation of ITT in clinical practice and has shown that multiple centers are close to clinical trials of transplantation of frozen/thawed testis tissue (15). Meanwhile, the current evidence for fertility restoration strategies has not yet been systematically synthesized, leading to a potential lack of structured research cooperation across different disciplines. Thus, this topic lends itself to a scoping approach to map and assess the extent of the evolving heterogeneous literature and identify gaps in knowledge (19). This review aims to examine the current evidence and clinical applicability of the different strategies for fertility restoration using ITT as well as identify future research questions that will accelerate the expected implementation of therapy options in clinical medicine. Furthermore, potential barriers in the development of clinically relevant therapies are identified. This scoping review was conducted to answer the questions "What is the evidence of in vivo and in vitro stem cell–based strategies for male fertility restoration using prepubertal human cryopreserved testis tissue, and what are the barriers to clinical implementation?"

MATERIALS AND METHODS

The research objectives were assessed using the scoping review framework as previously published by Arksey and O'Malley (20) and the Joanna Briggs Institute (21).

Preregistration

The objective, methods, and eligibility criteria were prespecified and registered online with the Open Science Framework Registry on November 29, 2023 (https://doi.org/10.17605/OSF.IO/8DNRT). The individual criteria were developed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (22). As a scoping review, this study was exempt from applying for institutional review board approval.

Search

An iterative 3-step approach was used to search for relevant literature (21). First, a preliminary search of the National Library of Medicine (PubMed) was conducted to establish the relevant search terms by screening titles and abstracts. Second, the following search string was used to perform the final search in PubMed on November 30, 2023: ((male fertility restoration) OR (spermatogonial stem cell transplantation) OR (testis tissue engraftment OR autotransplantation) OR (in vitro spermatogenesis)) AND (human) AND ((testis tissue) OR (testicular tissue)). Finally, additional records were included from the references of the most recent included articles.

Selection of sources of evidence and eligibility criteria

Original research articles in the English language published between January 1994 and November 2023 were included in the review because SSC transplantation in mice was first reported in 1994 establishing the possibility of SSC-based fertility restoration (23). All available titles and abstracts were screened for eligibility in the review independently by two reviewers (E.v.R., C.F.S.J.) using Rayyan. Any discrepancies were resolved by discussion and subsequent consensus. Selected publications were full-text screened by one reviewer (E.v.R.) and assessed for relevance and congruence with the objective and research question of this review. All full-text articles could be obtained through the institution's library access.

If data were reported in multiple publications, only the publication with the most complete data set was included in the review. The population-concept-context framework was used to select studies for inclusion according to predefined eligibility criteria.

All studies on fertility restoration using human testis tissue of prepubertal boys or healthy male adults (population) with the intention to model male fertility restoration including in vitro strategies, such as SSC culture, organotypic culture, or induced pluripotent stem cell (iPSC)–based therapies, as well as in vivo strategies, plus tissue engraftment and SSC transplantation (concept), were considered for inclusion. Furthermore, strategies to clinically implement transplantation of prepubertally

cryopreserved testis tissue were included (context). Additionally, original research studies that assessed or highlighted barriers to clinical advancement were included.

Data charting process

A form was developed by the reviewing investigators to assess the relevance of the study according to the predefined population, concept, and context and map study characteristics such as year of publication, study center, field of research, methods employed, outcomes reported, and barriers to clinical implementation.

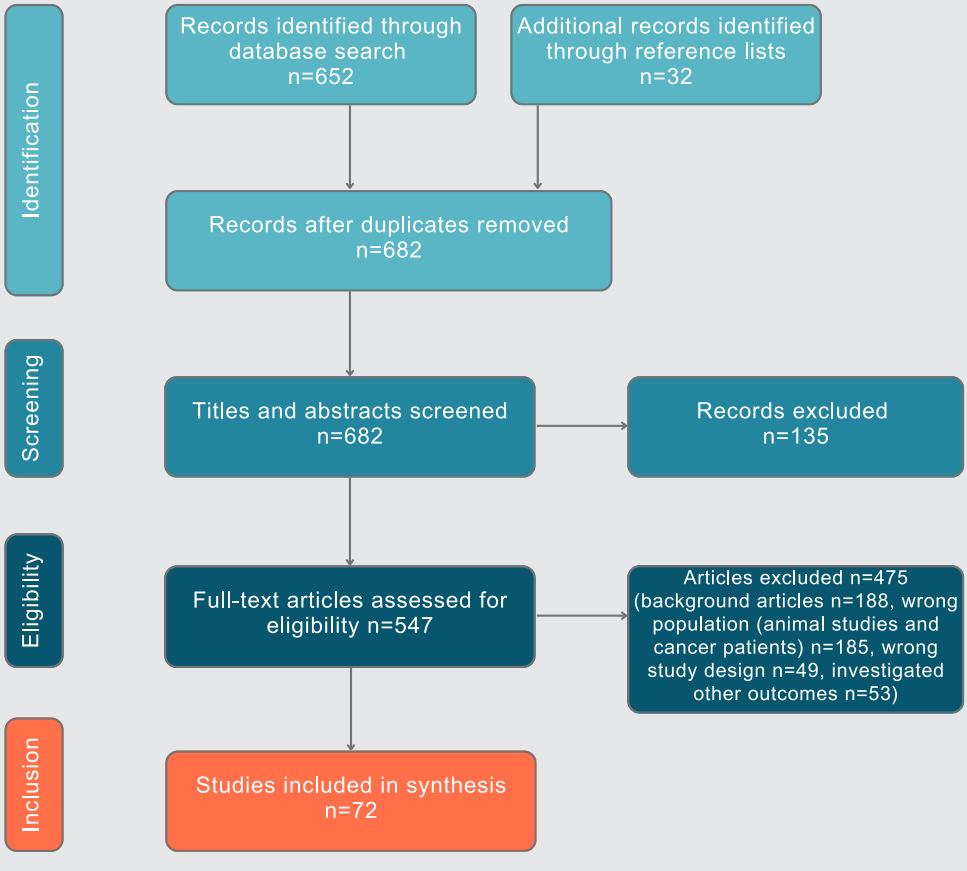
RESULTS

The study selection process is shown in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart (Fig. 1). After abstract and title screening and assessment for eligibility, 72 studies were included in the review—20 and 42 studies investigated in vivo and in vitro approaches to fertility restoration, respectively, and 10 assessed oncofertility programs and barriers to clinical implementation. Of the 20 included studies focusing on in vivo approaches, 14 investigated autologous or xenogeneic engraftment of human testis tissue, and six investigated the xenotransplantation of human SSCs. In vitro strategies were divided into 15 studies researching generation of male germ cells from human iPSCs and embryonic stem cells (ESCs), and 27 studies explored various approaches to direct in vitro maturation of male germ cells. The 27 studies aiming at in vitro spermatogenesis included 2-dimensional, 3-dimensional (3D), and organoid culture systems using SSC suspensions (15 studies) and organotypic cultures on whole tissue pieces (12 studies). The reported themes and distribution of included studies across the range of fertility restoration strategies and methods applied are visualized in Figure 2. Table 1 (4, 24–94) shows an overview of all included studies sorted by fertility restoration strategy and method and listed by year of publication. Additionally, the table has been expanded with country, detailed study design, and results for each included study, as shown in Supplemental Table 1 (available online).

In vivo strategies

Testis tissue engraftment. In the absence of malignant cells, the direct ectopic or homotopic transplantation of frozen-thawed ITT is an option to advance fertility and male germ

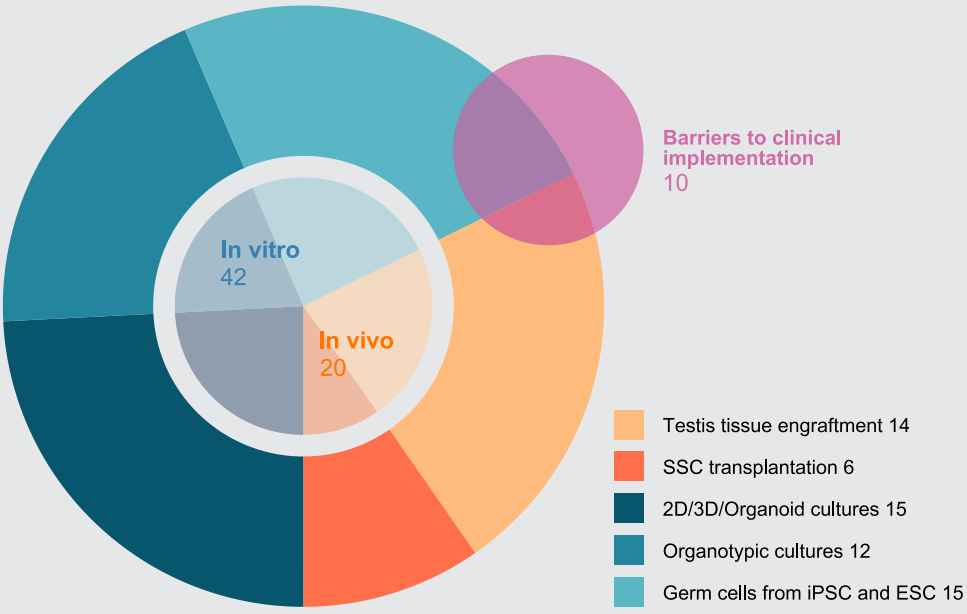
FIGURE 1



Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart of the study selection process.

von Rohden. Restoring male fertility with stem cells. Fertil Steril 2024.

FIGURE 2



Reported themes from scoping review and number of studies. Distribution of included in vivo and in vitro studies sorted by fertility restoration strategy. ESC = embryonic stem cell; iPSC = induced pluripotent stem cell; SSC = spermatogonial stem cell; 3D = 3-dimensional; 2D = 2-dimensional.

von Rohden. Restoring male fertility with stem cells. Fertil Steril 2024.

cell maturation. The SSCs remain in the supporting somatic testis niche, which is presumably the most favorable environment for their proliferation and differentiation. Several promising studies have reported successful long-term xenografting of human ITT into immunodeficient mice demonstrating survival of spermatogonia and differentiation into spermatocytes, but no further advancement of spermatogenesis was observed (24–28). Various graft sizes, locations, surgical techniques, donor material, and observation periods were described. Ntemou et al. (24) used 1.0–1.5-mm^3 fresh human ITT pieces from three prepubertal donors for engraftment, both under the dorsal skin and under the tunica albuginea in the mouse testis for 9 months. Improved spermatogonial survival and seminiferous tubule integrity were demonstrated in tissue pretreated with vascular endothelial growth factor (VEGF) in vitro (24). Sato et al. (26) engrafted 0.5–1.0-mm^3 fresh ITT pieces from a 3-month-old donor under the dorsal skin for 12 months, at which point BOULE- and CDC25A-positive cells suggested the presence of haploid spermatocytes in meiosis. Poels et al. (25) transplanted 1.0-mm^3 fresh and frozen-thawed prepubertal tissue for 6 months into the scrotum of castrated mice. This study revealed good tubule integrity and spermatogonial survival as well as spermatocytes up to the pachytene stage (25). Similarly, Wyns et al. (27) engrafted 1–6-mm^3 frozen-thawed ITT pieces into the peritoneal bursa of the scrotum close to the castration wound and removed the grafts after 6 months. Spermatogonial survival and sustained tubule integrity were reported. Ki67-positive proliferating germ cells and few spermatocytes were observed (27). Van Saen et al. (28) used 1.5–3.0-mm^3 fresh and frozen-thawed ITT pieces for engraftment into the testes of the mice with and without supplemented human recombinant follicle-stimulating hormone (FSH) with removal after 12 months. In both fresh and frozen-thawed tissue with and without FSH application, the tubule integrity was reduced; however, spermatogonial survival was shown, and meiotic germ cells were present (28). Two studies investigated the effect of supplementation of VEGF in tissue cultures before transplantation and demonstrated improved revascularization, tubule integrity, and spermatogonial survival but no influence on cell differentiation compared with culture without VEGF (24, 95).

The first human proof-of-principle study for autologous testis tissue engraftment was recently published. The study cryopreserved testis tissue from a 31-year-old man with nonobstructive azoospermia and later did an autologous engraftment of 3 2 × 4 × 2–mm frozen-thawed testis tissue pieces under the scrotal skin without surgical complications (59). Meanwhile, after successful recovery of the grafted tissue after 6 months, no spermatozoa were found. Two of the grafted tissue pieces with previously dilated tubules (visualized under the operating microscope during a previous microdissection testicular sperm extraction) showed intact tubules, normal cell organization, and Sertoli cells and spermatocytes near the basement membrane on histologic evaluation after extraction. Spermatogonial stem cells expressing MAGEA and VASA were detected in these seminiferous tubules, illustrating that the testis tissue can survive freezing, thawing, and autotransplantation with an intact testis niche (59).

SSC transplantation. Another strategy for male fertility restoration is the propagation and autologous

TABLE 1

Overview of the included studies listed by fertility restoration strategy and publication year.

First author, y	Study description
Engraftment of human TT	
Jensen (60), 2023	Autologous fixation of frozen-thawed TT under the scrotal skin, graft survival after 6 mo, no spermatozoa, n = 1
Hutka (62), 2020	Xenotransplantation of frozen-thawed and fresh prepubertal and peripubertal TT to mice, no progression of spermatogonia after 12 wk
Ntemou et al (24), 2019	Frozen-thawed ITT xenotransplanted to mice after tissue culture, increased vascularization after 9 vs. 4 mo, spermatocytes present
Poels (84), 2014	Xenotransplantation of frozen-thawed ITT into the mouse scrotum without fixation, with spermatogonial survival at day 5
Poels et al (25), 2013	Xenotransplantation of ITT into the mouse scrotum without fixation, integer seminiferous tubules and pachytene spermatocytes after 6 mo
Van Saen et al (28), 2013	Fresh and frozen-thawed ITT xenotransplanted without fixation into the mouse testis, meiotic germ cells at 12 mo, reduced tubule integrity
Van Saen (85), 2011	Prepubertal and postpubertal TT xenografted to mouse testis, SSC survival, secondary spermatocytes at 9 mo in tissue from a 13-y-old
Sato et al (26), 2010	Fresh ITT xenografted under the dorsal skin of mice, pachytene spermatocytes and maturation of Sertoli and Leydig cells at 1 y
Goossens (123), 2008	Fresh ITT xenografted under the dorsal skin of mice, intact tubules and spermatogonia detected at 4 and 9 mo
Wyns et al (27), 2008	Xenotransplantation of frozen-thawed ITT into the mouse scrotum, spermatogonial survival and increased proliferation at 21 d
Wyns (87), 2007	Frozen-thawed ITT xenotransplanted into the scrotum near castration wound, spermatogonia and few pachytene spermatocytes at 6 mo
Geens (88), 2006	Adult healthy TT xenografted under the dorsal skin of mice, spermatogonia maintained >195 d in 22% of grafts
Schlatt (63), 2006	Adult TT from different sources xenografted under the dorsal skin of mice, occasional spermatogonial survival at 2–19 wk
Yu (64), 2006	Fetal ITT from fetuses wk 20 and 26 xenografted under the dorsal skin of mice, germ cells present at BM at 116 and 135 d
SSC transplantation	
Wang (60), 2022	Cell suspension from frozen-thawed ITT transplanted into the seminiferous tubules of mice, prospermatogonia and SSCs present at 6–9 wk
Mohaqiq (65), 2019	2-wk culture of SSCs from men with OA and then transplanted into the mouse testis, homing of SSC-derived cells to BM after 14 d
Mohaqiq (66), 2019	2-wk culture of SSCs from men with OA and then ex vivo transplanted into the testes of mice, SSC-derived cells and round spermatids seen at 8 wk
Izadyar (67), 2011	Injection of SSCs from fresh adult testis (men with OA) into the mouse rete testis, SSEA-4+ cells present at 4 wk
Sadri-Ardekani et al (29), 2009	Germline stem cells from frozen-thawed adult TT cultured for 28 wk and then xenotransplanted to mice, SSC homing to BM
Nagano et al (30), 2002	Cell suspension from adult TT xenotransplanted into the mouse testes, spermatogonial survival at BM after 6 mo
In vitro maturation of germ cells	
Culture from cell suspension (2D/3D/organoid)	
Galdon et al (31), 2022	Cell suspension from pubertal cryopreserved TT cultured as cell monolayer, putative SSCs and spermatogonia present at 110 d
Robinson et al (34), 2022	Cell suspension from a man with NOA cultured for 12 d and 3D bioprinted into tubular structure, improved SSC maintenance
Oliver (68), 2021	Cell suspension from first-trimester embryonic gonads cultured in 3-layer gradient Matrigel system, whole testis organoids at 7 d
Ashouri Movassagh (69), 2020	SSC differentiation culture on DTM vs. 2D culture, using adult whole testes, spermatocytes and spermatids increased at 10 wk on DTM
Dong (70), 2019	Xeno-free culture of cell suspension from ITT in uncoated plates, SSC-like cell clusters and successful homing to BM of the mouse testes
Sakib et al (33), 2019	Cell suspension from frozen-thawed ITT cultured in microwell with centrifugal force aggregation, organoid formed at day 5
Abofoul-Azab et al (37), 2018	Cell suspension from frozen-thawed ITT cultured in methylcellulose, positive postmeiotic germ cell marker in two samples at 15 wk
Mincheva (71), 2018	Fresh TT with spermatogonial arrest from patients with gender dysphoria, cell culture in 24-well plates, cord-like structure formation at 3 wk
Baert et al (38), 2017	Transwell cell culture from adult and pubertal fresh TT, with or without human DTM and agarose gel, spheroid formation at 3 wk
Pendergraft et al (32), 2017	Cell suspension from frozen-thawed adult TT propagated and seeded into hanging drop plates with ECM, 3D organoids at 23 d
Smith (72), 2014	SSCs and somatic cells from adult TT cultured in various model setups, colonies with SSC characteristics up to 3 wk

von Rohden. Restoring male fertility with stem cells. Fertil Steril 2024.

TABLE 1

Continued.

First author, y	Study description
Yang et al (36), 2014	Cell suspension from fresh adult TT, cultured with RA, haploid spermatids after 7–10 d, fertilized murine oocyte
Piravar (73), 2013	Cell suspension from fresh adult TT, floating germ cells transferred to culture in laminin-coated dishes, germ cells maintained for 2 mo
Chen (89), 2009	Cell suspension from fetal ITT cultured xeno-free on hESC-derived fibroblast-like cells, maintained germ cells for 2 mo
Lee et al (57), 2006	Cell suspension cultured from fresh adult TT, encapsulated in calcium alginate, some haploid cells with oocyte cleaving ability at 6 wk
Organotypic tissue culture	
Aden (74), 2023	2–4-mm^3 ITT pieces cultured on agarose gel, gas-liquid interface, germ cell survival for 3 wk, spermatogonia most advanced cell type
Younis et al (43), 2023	1-mm-diameter prepubertal and peripubertal ITT cultured at gas-liquid interface, maintained for 32 d, spermatocytes detected
Wang (61), 2022	1–1.5-mm^3 frozen-thawed ITT cultured in xeno-free medium with or without added RA, spermatocytes at 60 d
Kurek (75), 2021	A third of first-trimester gonads and 1-mm^3 tissue pieces cultured on agarose gel in an air-liquid interface, germ cells present at 14 d
Yuan et al (42), 2020	3-mm tissue fragment fetal male gonads cultured, fertilization-competent spermatids at days 30 and 50, blastocyst formation
Portela (76), 2019	1–2-mm^3 fresh or frozen-thawed TT pieces cultured at gas-liquid interphase, spermatogonial survival for 5 wk
de Michele et al (41), 2018	1-mm^3 frozen-thawed ITT pieces cultured in wells inside Millicell inserts, spermatogonia at 139 d, individual round spermatids from day 16
Medrano (91), 2018	1–2-mm^3 frozen-thawed ITT cultured on agarose gel under different conditions, individual spermatogonia at day 70
de Michele et al (39), 2017	1-mm^3 frozen-thawed ITT pieces cultured in wells inside Millicell inserts, preserved tubules at 139 d, loss but presence of spermatogonia
Perrard et al (35), 2016	20–50-mm^3 seminiferous tubule pieces, adult TT, cultured in a chitosan hydrogel cylinder, haploid spermatozoa with flagella at 34 d
Jørgensen et al (40), 2015	Hanging drop tissue culture of first-trimester whole fetal gonads with and without RA, reduced gonocytes at 2 wk with RA
Sá (77), 2012	1-mm^3 seminiferous tubule segments from fresh adult TT cultured for 28 d, maintenance of tubule structure, round spermatids present
Germ cells from iPSCs and ESCs	
Hwang et al (58), 2020	Inducing of hiPSCs into hPGC-like cells, xenogeneic mouse testis culture, primary transitional prospermatogonia-like cells present
Xu et al (47), 2020	3-step protocol to differentiate an hESC line and hiPSC into SSC-like cells, homing ability in mice, spermatocytes and haploid cells
Chen et al (46), 2015	HUMSCs cultured and sorted by pluripotent stem cell markers, survival at 120 d, homing in mouse testes and differentiation
Irie (78), 2015	hESC and iPSC lines were grown on irradiated mouse embryonic fibroblasts and cultured, resulted in hPGC-like cells
Ramathal (90), 2015	iPSC cell lines transplanted into the seminiferous tubules of mice, formation of GCLCs 8 wk after transplantation
Sasaki (92), 2015	iPSCs from men with azoospermia and fertile men cultured and differentiated on laminin-coated plates, hPGC-like cells formed from aggregates
Durruthy (93), 2014	mRNA-reprogrammed iPSCs transplanted into the seminiferous tubules of mice, GCLCs generated in vivo after 8 wk, tumor formation in the testes
Ramathal (94), 2014	iPSC cell lines derived from the human skin transplanted into mice, differentiation into GCLCs, tumor formation in the testes
Easley et al (45), 2012	hESCs and hiPSCs cultured in mouse SSC culture conditions, few acrosin-positive haploid spermatid-like cells at 10 d
Eguizabal et al (44), 2011	hiPSC lines from keratinocytes and cord blood, cultured on human foreskin fibroblasts, haploid germ cell-like cells of both genetic sexes
Panula (79), 2011	hiPSCs from adult and fetal somatic cells and hESCs, cultured on Matrigel-based feeder cell-free system, postmeiotic haploid cells produced
Aflatoonian (80), 2009	Induction of spontaneous differentiation of human ESCs in culture medium, postmeiotic spermatids at 14 d
Hua (81), 2009	Human mesenchymal stem cells from the fetal bone marrow induced for 10–15 d, differentiation into male GCLCs
Kee (119), 2009	hESCs cultured on Matrigel with conditioned medium, haploid PGC-like cells produced after 7 and 14 d
Park (83), 2009	hESCs and hiPSCs from human blastocysts and skin fibroblasts codifferentiated with human fetal placenta into PGC-like cells
Barriers to clinical implementation	
El Alaoui-Lasmaili et al (51), 2023	Surveys and qualitative data from patients, parents, and healthcare professionals identifying factors affecting oncofertility discussions

von Rohden. Restoring male fertility with stem cells. Fertil Steril 2024.

TABLE 1

Continued.

First author, y	Study description
Duffin et al (50), 2023	20 centers collected data on pediatric oncofertility cases, fertility preservation was offered to 20.5% of patients, 67.9% of those accepted
Salama et al (49), 2023	Oncofertility care in 39 centers in limited vs. optimum resource settings, presents oncofertility best practice model to optimize care
Salama et al (48), 2021	25 leading oncofertility centers surveyed, reintroduction of malignant cells because of contamination identified as the main safety concern
Hildorf et al (4), 2020	Assessment of parental approval rate for ITT cryopreservation in prepubertal boys, 90% acceptance rate found
Shabani et al (52), 2018	Effect of cisplatin loaded nanoparticles vs. free drug on cocultures of SSCs and a mouse leukemia cell line as a safety assessment
Sadri-Ardekani et al (55), 2014	Coculture of SSCs and leukemia cells to test for malignant cell survival, leukemia cells undetectable in the cell cultures after 26 d
Dovey et al (56), 2013	Feasibility of isolation of germ cells from leukemia cell line by FACS, SSC-like homing ability and no tumors after xenotransplantation
Geens et al (53), 2011	In vitro cell culture of artificially contaminated SSCs, B cell presence tested by FACS, MACS, and PCR, malignant cells were detected
Fujita et al (54), 2006	Isolation of germ cells from malignant cells by FACS; apart from one malignant cell line, no malignant cells found in isolated germ cell fraction

Note: BM = basal membrane; DTM = decellularized testicular matrix; ECM = extracellular matrix; FACS = fluorescence-activated cell sorting; GCLC = germ cell-like cell; hESC = human embryonic stem cell; hiPSC = human induced pluripotent stem cell; hPGC = human primordial germ cell; HUMSC = human umbilical mesenchymal stem cell; ITT = immature testis tissue; MACS = magnetic-activated cell sorting; mRNA = messenger ribonucleic acid; NOA = nonobstructive azoospermia; OA = obstructive azoospermia; PCR = polymerase chain reaction; PGC = primordial germ cell; RA = retinoic acid; SSC = spermatogonial stem cell; 3D = 3-dimensional; TT = testis tissue; 2D = 2-dimensional.

von Rohden. Restoring male fertility with stem cells. Fertil Steril 2024.

transplantation of SSCs into the testes of the patient with subsequent regeneration of spermatogenesis in situ. In the case of former patients with cancer, there is a risk that cryostored tissue may contain malignant cells, which can be reintroduced into the patient on transplantation. To avoid this, it is hypothesized that the SSC suspension could be cleared of malignant cells by cell sorting or continued culturing beforehand. There are no trials involving human autologous transplantation but the homing and long-term survival of human SSCs in mouse seminiferous tubules have been successfully demonstrated (29, 30, 60, 96). Nagano et al. (30) demonstrated successful homing and survival of adult testis-derived SSCs in busulfan-treated mouse testis 6 months after grafting. No differentiation of spermatogonia occurred, and no difference between the uses of fresh and frozen-thawed cell suspensions was observed (30). Sadri-Ardekani et al. (29) maintained germline stem cell clusters from an adult testis cell suspension in a long-term culture for up to 28 weeks and showed their migration and homing capabilities into the seminiferous tubules of busulfan-treated immunodeficient mice. Most recently, Wang et al. (60) explored the direct xenotransplantation of a single cell suspension from fresh infant human testis tissue from boys with cryptorchidism into busulfan-treated immunodeficient mouse testes without previous cell proliferation in vitro. Six to 9 weeks after transplantation, no donor-derived somatic cells were detected in the seminiferous tubules, but human SSC clusters were found at the basal membrane, demonstrating that xenotransplantation and correct homing of SSCs are feasible from ITT (60).

In vitro strategies

Culture from cell suspension (2-dimensional/3D/organoid). For patients with malignant diseases, a fertility restoration option that avoids reintroduction of malignant cells is full in vitro spermatogenesis for later use of spermatozoa in connection with intracytoplasmic sperm injection (ICSI). Furthermore, effective SSC culture systems are the basis for successful propagation of human SSCs for autotransplantation.

Human SSCs have been successfully cultured and maintained in monolayer cell cultures (31) and 3D scaffold cultures (32), via a testicular organoid formation (33), and with the use of novel bioprinting techniques (34). Elongated spermatids are, to date, the most advanced germ cell differentiation detected in in vitro cultures but with one report of spermatozoa formed in a patented bioreactor culture technology (35). In 2016, Perrard et al. (35) cultured seminiferous tubule segments from adult human testis tissue in a chitosan-based cylindric hydrogel reactor for 60 days and detected morphologically mature spermatozoa after 34 days. The fresh and frozen-thawed adult testis tissue was obtained from patients undergoing gender confirmation surgery and was in an induced state of spermatogenic arrest because of the treatment with antiandrogens (35). In another study, functional haploid spermatids were differentiated from adult patients with cryptorchidism with a mean age of 29 years in a monolayer cell culture sustained for up to 10 days with added retinoic acid and stem cell factor (36). In 2017, Pendergraft et al. (32) equally generated elongated spermatids from an SSC suspension from human adult testis tissue, cultured on hanging drop plates with human decellularized testicular matrix. Abofoul-Azab et al. (37) detected postmeiotic germ cells after culturing a cell suspension from prepubertal frozen-thawed ITT in a 42% methylcellulose scaffold for 15 weeks.

To advance spermatogenesis in vitro, various studies have explored the formation of testicular organoids as a scaffold for an in vitro niche germ cell maturation. Using a cell

suspension from frozen-thawed prepubertal ITT, Sakib et al. (33) demonstrated organoid formation after 5 days. The organoids were generated by centrifugal force aggregation and culture in microwell plates and showed an inside-out arrangement of somatic and germ cells (33). By culturing cell suspensions from adult and pubertal fresh testis tissue with or without human decellularized testicular matrix in a permeable cell culture insert, Baert et al. (38) demonstrated the spheroid formation of a testicular organoid after 3 weeks of culture. Normal seminiferous epithelial cell architecture and function and spermatogonial survival were shown. A recent feasibility study by Robinson et al. (34) explored the possibility of bioprinting a testis cell suspension using a bioink containing collagen, alginate, and testis cell fractions into tubular structures mimicking the testis environment. There were viable Sertoli, Leydig, peritubular myoid, and meiotic germ cells present after 12 days in culture, and an upregulation of spermatogenic genes was shown (34).

Organotypic tissue culture. In organotypic tissue culture systems, whole testis tissue fragments are cultured to maintain the cell-cell interaction and paracrine communication between somatic cells and germ cells within the 3D architecture of the seminiferous epithelium (61). It is hypothesized that SSCs remaining in their natural niche have a better ability to self-renew and differentiate (39). Organotypic cultures can maintain SSC populations successfully using either hanging drop (40), transwell (41), or agarose gel (42) culture setups. Yuan et al. (42) cultured human fresh fetal male gonads aged 12–19 weeks after conception on agarose gel for 50 days, which remarkably showed differentiation into fertilization-competent spermatids. The embryo formed after round spermatid injection developed to the blastocyst stage with correct chromosome content and proven genetic paternal contribution (42). de Michele et al. (41) previously achieved the differentiation into round spermatids and showed an increase in haploid germ cells from frozen-thawed prepubertal ITT (boys aged 2–12 years). The tissue was cultured in a permeable cell culture insert for 139 days (41). Younis et al. (43) cultured frozen-thawed ITT from prepubertal boys aged 1–3 years for 32 days in a transwell air-liquid interface system, which resulted in primary spermatocytes but no complete spermatogenesis. Wang et al. (61) maintained frozen-thawed ITT (derived from boys with cryptorchidism aged 6 months to 1.5 years) in a xeno-free culturing system on agarose gel for 60 days, and spermatocytes could be detected in the tissue pieces cultured in medium enriched with retinoic acid.

Germ cells from iPSCs and ESCs. The generation of male germ cells from iPSCs and ESCs would both avoid the challenge of malignant cell contamination and provide an option of fertility restoration to men who faced gonadotoxic therapies because children but did not have ITT cryopreserved. Despite a growing number of studies on this subject, it is still a challenge to advance the iPSCs or ESCs further than primordial germ cell-like cells. Eguizabal et al. (44) and Easley et al. (45) both succeeded at generating postmeiotic haploid germ cells from human ESCs and iPSCs in 2011 and 2012, respectively. Eguizabal et al. (44) cultured iPSCs and ESCs from keratocytes and umbilical cord blood on human foreskin fibroblasts and used a timed supplementation of retinoic acid, human recombinant leukemia inhibitory factor, and basic fibroblast growth factor. Easley et al. (45) cultured human ESCs and human foreskin fibroblast-derived iPSCs directly in standardized mouse SSC conditions and produced postmeiotic spermatid-like cells expressing unique spermatid/sperm proteins. Chen et al. (46) investigated the capacity of human umbilical cord mesenchymal stem cells to survive and differentiate in busulfan-treated mouse testes. These cells exhibited migration patterns of differentiating germ cells in the seminiferous tubules and expressed germ cell markers. The germ cell-like cells survived for 120 days, and the histologic architecture showed signs of recovery from busulfan treatment in human umbilical cord mesenchymal stem cell–transplanted tubules (46). Most recently, Xu et al. (47) followed a 3-step protocol to differentiate human ESCs and iPSCs into SSC-like cells. Spermatogonial stem cell-like cells could be propagated in vitro for 4 months and showed a similar gene expression pattern as human GPR125+ spermatogonia from testis tissue. A small amount of SSC-like cells could be differentiated into acrosin+ haploid germ cells and showed homing capability to the basal membrane when transplanted into seminiferous tubules of busulfan-treated immunodeficient mice (47).

Barriers to clinical implementation

In 10 original studies, barriers to clinical implementation of male fertility restoration strategies from ITT were evaluated. The Repro-Can-OPEN II study reports on oncofertility programs in optimal healthcare settings globally with a focus on rating the implementation of fertility-protective measures and the use of cryopreservation of testis tissue (48). The Repro-Can-OPEN I and II study recently surveyed 39 oncofertility centers in limited and optimal resource settings and found a frequent use of fertility-protective measures during treatment but an insufficient use of ITT cryopreservation (49). An ongoing national audit by the Children's Cancer and Leukaemia Group found that across six centers with 273 male patients with childhood cancer across the United Kingdom, oncofertility care was discussed only in 66% of cases and fertility preservation was offered to 21% of the patients (50). The acceptance rate for ITT cryopreservation, however, was 68% (50). Another study among Danish boys with cryptorchidism and a high risk of infertility showed a 90% parental acceptance rate of ITT cryopreservation offered before the planned orchidopexy procedure (4). The lack of robust clinical systems for oncofertility discussions and ITT cryopreservation appears to be an additional barrier to clinical implementation despite evidence for a high parental acceptance rate when offered. With lack of time due to severity of disease or religious beliefs identified as inflexible barriers, both the implementation of professional training for healthcare providers and the systematic psychological support of the families have been identified as potential strategies to better facilitate oncofertility care discussions (51).

The Repro-Can-OPEN studies characterized the reintroduction of malignant cells from the cryopreserved ITT as the main barrier to clinical implementation. Six original articles

were identified investigating this challenge (51–56). The studies were mostly using different cell markers and matrix adhesion in combination with advanced fluorescence-activated cell sorting or magnetic-activated cell sorting techniques. However, these techniques are not successful at removing malignant cells entirely. An SSC culturing protocol has been piloted that could potentially produce a cancer-free SSC suspension with viable SSCs, sorting leukemia cells from healthy testis cells within 20–26 days (55). The use of nanoparticles to deliver targeted chemotherapy to leukemia cells may be a promising approach for decontamination of cell suspensions; however, the study failed at fully removing malignant cells from an SSC culture (52).

DISCUSSION

In this scoping review, 62 studies investigating stem cell–based strategies for male fertility restoration and 10 studies evaluating barriers to clinical implementation were identified. From animal models, we have learned that fertility can be restored after autologous engraftment of testis tissue, with successful engraftment of cryopreserved and thawed ITT resulting in live-born offspring in mice (97) Also in nonhuman primates, Fayomi et al. (98) provided the proof-of-principle for complete spermatogenesis after autologous engraftment of cryopreserved ITT resulting in healthy offspring after ICSI. A first-in-human feasibility study showed successful graft survival (59); however, it remains to be shown whether autologous engraftment supporting full spermatogenesis is translatable to a clinical setting (49). Studies investigating xenografting of adult human testis tissue and ITT into mice have shown heterogeneous surgical techniques and different methods of reporting on both spermatogonial survival and tubule integrity, which makes it difficult to compare results. The optimal graft size, graft location, surgical technique, hormonal environment, and grafting time remain unknown and are continuously being discussed (16). We found from studies in primates that grafting ITT subcutaneously under the back skin or the skin of the shoulder or forearm, as well as under the scrotal skin, can support successful graft survival (99–101). The surgical fixation of the testis tissue pieces may trigger local angiogenesis (59, 98). However, this has been difficult to test in mice because of size but could be shown in one human case (59) and a study performed in rhesus macaques (98) resulting in mature spermatozoa and healthy offspring. Foresta et al. (102) demonstrated the improvement of Sertoli cell function by suppression of high endogenous FSH levels in men with azoospermia; therefore, it could be hypothesized that the mimicking of a pubertal hormonal environment leading to maturation of Sertoli cells may support spermatogenesis after engraftment (16). Lastly, the time needed for maturation of germ cells after engraftment of ITT cryopreserved in different stages of immaturity is unknown, with studies in primates explanting tissue between 6 and 17 months after engraftment with promising results (98–101). There may only be a narrow window for extraction of spermatozoa successfully formed in engrafted tissue pieces before reabsorption because of a lack of connection to the natural ejaculatory system.

The autologous transplantation of propagated SSCs into the rete testis is the only strategy that may permanently restore fertility and facilitate natural conception (103). However, the possibility for reintroduction of malignant cells (16, 104) and the lack of knowledge about potential genetic and epigenetic changes because of the cell culturing process pose a challenge that still needs to be addressed (105–107). Because of the small numbers of SSCs present in ITT, this strategy relies on the development of safe and effective SSC propagation systems (96). Meanwhile, because of the large regenerative potential of SSCs from ITT, it has been suggested that a relatively low number of SSCs are sufficient to repopulate the niche in the seminiferous tubules when using this tissue (108).

Culture of SSCs with full in vitro maturation of human SSCs could provide functional spermatozoa for ICSI and would have the benefit of avoiding potential reintroduction of malignant cells (104). It would also avoid additional surgical procedures and, thereby, potential surgical complications. Spermatogonial stem cell cultures may also provide an effective way to increase SSC numbers from small ITT biopsies for potential autotransplantation (96).

In 2011, Sato et al. (109) succeeded with the first complete in vitro spermatogenesis from prepubertal mouse testis tissue leading to healthy offspring, providing proof-of-concept of this approach. Meanwhile, in vitro strategies still face a number of challenges—including the lack of knowledge about genetic and epigenetic changes and how to successfully recreate the testicular microenvironment and keep tissue viable in organotypic culture (110)—that need to be addressed before the clinical advancement to human trials is safe and feasible. In 2016, Perrard et al. (35) reported the full differentiation of human male germ cells. These results could not be reproduced to date; thus, despite singular evidence of full in vitro spermatogenesis, we are still lacking robust and reproducible conditions for successful full germ cell differentiation to mature, functional spermatozoa in vitro. Few studies have been able to advance in vitro spermatogenesis to postmeiotic spermatids (32, 36, 42, 57); however, because of a lack of proven genomic and epigenetic stability and fertilization trials in a xeno-free setup, it is difficult to translate the current methods into a clinical setting. In addition, despite recent efforts to characterize human SSCs more effectively (111, 112), to date, neither molecular markers on protein level nor specific genetic profiles are identified that can specifically determine all human SSCs (103). New technologies are emerging using microfluidic tissue culture systems, mimicking the physiological conditions with a continuous flow of culture medium and applying them to various setups, such as the testis-on-chip platform (113, 114). Studies using this technology have been able to support SSCs and spermatogenesis up to an elongated spermatid stage from mouse testis tissue (113, 114).

Ethical considerations and barriers to clinical implementation

Despite continuous dissemination and discussion of research and publication of clinical guidelines, the clinical practice within male fertility preservation and restoration is

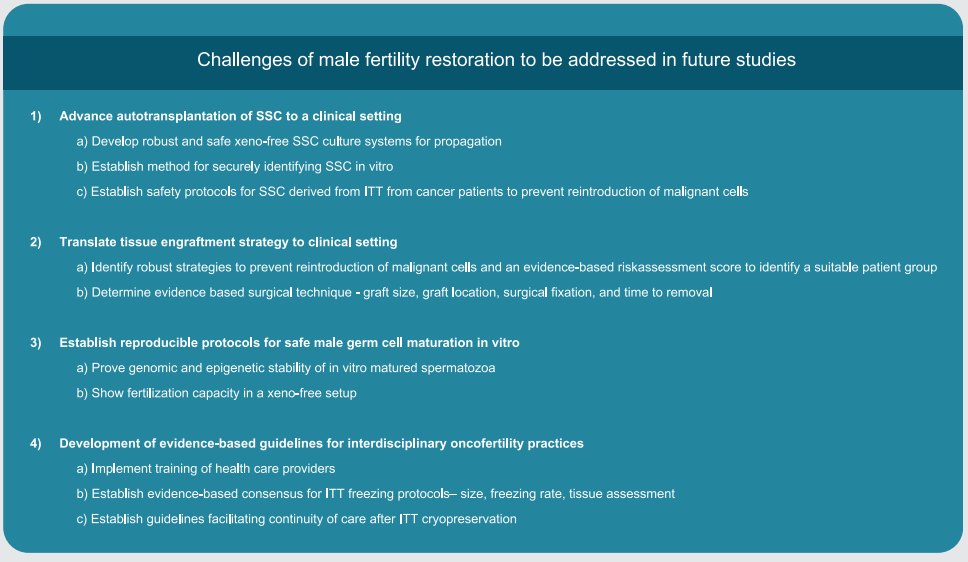
heterogeneous even among centers that provide cryopreservation of ITT, and this poses a barrier to a consistent and ubiquitous implementation of the available strategies for fertility preservation and restoration (49, 115, 116). Although ITT cryopreservation is being increasingly implemented, a recent review highlighted the heterogeneity of freezing protocols used, size of tissue fragments frozen, cryoprotectant used, freezing rate, and tissue assessment (117), emphasizing that fertility preservation in males is still experimental and there are not yet sufficient data to establish consensus (107). Another step forward is the wide-ranging agreement on and dissemination of guidelines across pediatric oncology departments and collaborating reproductive laboratories. A multidisciplinary team approach to ensure both continuity of care and access to testis tissue for vital research has been previously suggested (118). The collaboration within groups such as the PanCareLIFE Consortium (5), International Late Effects of Childhood Cancer Guideline Harmonization Group (119), and ORCHID-NET (https://www.orchid-net.com/, accessed November 23, 2023) is essential for reaching a scientific consensus and for publishing, implementing, and evaluating robust clinical guidelines for male fertility preservation and restoration.

The main safety concern in patients with cancer and, thereby, the barrier to advancing autologous engraftment of cryopreserved ITT to clinical trials is the risk of reintroducing malignant cells because of contaminated tissue leading to recurrence of cancer (103, 120–122). Hematologic cancers such as leukemia and lymphomas as well as testis cancer appear to be posing the highest risk (123–127), whereas solid tumors are rarely suspected to cause testicular malignant cell infiltration in a pediatric population. However, there were several reported cases of testicular involvement of pediatric neuroblastoma (127), rhabdomyosarcoma (128), and Wilm tumors (nephroblastoma) (129, 130). Kourta et al. (120) reported a high variability in testicular malignant cell infiltration in pediatric patients with leukemia and lymphoma (21%–100% and 15%–50%, respectively), which can be attributed to the extremely heterogeneous populations and detection methods of the 15 included studies. Here, the use of decontaminated human SSC suspensions may be a promising approach but is still showing an insufficiently robust safety profile (53, 54, 56). All identified studies on removing malignant cells from SSC cultures were performed on artificially contaminated cell lines; thus, the clinical applicability remains to be shown, especially due to vastly different cell-cell interactions with malignant cells in vivo (120). No strategy for decontaminating cryopreserved ITT for autotransplantation has been suggested yet. Figure 3 shows an overview of the current challenges of male fertility restoration and suggested focus in future studies.

Advancing in vitro spermatogenesis from iPSCs or ESCs could be another safe option for fertility restoration in men with a previous cancer disease with a high risk of malignant cell contamination of the ITT as soon as the technology has advanced further and proven safe regarding genetic and epigenetic aberration risks (46, 47, 58).

Autotransplantation of testis tissue or testis cell suspensions from patients with cancer should be considered carefully because of the risk of reintroducing malignant cells. Meanwhile, the first human male has, with no previous history of malignant disease, recently been successfully autotransplanted with frozen-thawed testis tissue (59). This is a

FIGURE 3



Overview of the current challenges of male fertility restoration and suggested focus in future studies. ITT = immature testis tissue; SSC = spermatogonial stem cell.

von Rohden. Restoring male fertility with stem cells. Fertil Steril 2024.

milestone that has been reached, proving that adult testis tissue can survive freezing, thawing, and autotransplantation, suggesting a great potential for transplantation of ITT. From the different approaches reviewed here, autotransplantation of ITT is the most promising next step in male fertility restoration. The global male fertility preservation network, ORCHID-NET, has published that 11 centers worldwide are planning to transplant ITT, four of these already have the ethical approvals in place, and several others plan to request ethical approval (15), suggesting that we will soon see the first cases of human ITT transplantations (59).

The lack of additional databases and sources for evidence in the search strategy poses a potential limitation to this study. Because of the wide scope of sources of evidence, we decided to limit the included studies to publication in English. Additionally, the full-text screening and assessment for relevance were not conducted by two investigators independently, which would strengthen this study when reproduced.

CONCLUSION

Male fertility restoration is a rapidly transforming field with new evolving technologies and a recent substantial advancement with the first proof-of-principle study of testis tissue engraftment in a human male. A broad range of studies on in vitro and in vivo stem cell–based strategies for male fertility restoration demonstrated significant advancements. However, lack of standardized outcomes and reporting tools, heterogeneous source tissue material, risk of reintroduction of malignant cells, and unresolved challenge of definite identification of SSCs in vitro are still challenging clinical implementation. After assessment of the currently available reports, autologous engraftment of cryopreserved and thawed testis tissue appears to be the most promising strategy for male fertility restoration and is likely close to the first human experimental clinical trials. Testis tissue has been cryopreserved from >3,000 boys aged <18 years worldwide (15), and it is now a collective task to team up, improve interdisciplinary efforts, and share knowledge across countries for establishing evidence-based robust guidelines for clinical male fertility preservation and restoration.

CRediT Authorship Contribution Statement

Elena von Rohden: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Visualization. Christian Fuglesang S. Jensen: Conceptualization, Investigation, Methodology, Project administration, Writing – review & editing. Claus Yding Andersen: Supervision, Writing – review & editing. Jens Sønksen: Funding acquisition, Supervision, Writing – review & editing. Jens Fedder: Supervision, Writing – review & editing. Jørgen Thorup: Writing – review & editing. Dana A. Ohl: Writing – review & editing. Mikkel Fode: Supervision, Writing – review & editing. Eva R. Hoffmann: Writing – review & editing. Linn Salto Mamsen: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of Interests

E.v.R. reports travel grant for the European Testis Workshop 2023 from the EU COST Action CA20119. C.F.S.J. has nothing to disclose. C.Y.A. reports consulting fees from Ferring Pharmaceuticals; honoraria from IBSA Pharma and Ferring Pharmaceuticals; and stock options from ARTSMedia Denmark, outside the submitted work. J.O.R.S. reports a grant from the Independent Research Fund Denmark (grant number 2096-00027B) for the submitted work. J.F. has nothing to disclose. J.T. reports royalties from Springer-Verlag – Textbook and institutional support for attending European Society for Paediatric Urology congress and European Paediatric Surgeons' Association congress, outside the submitted work. D.A.O. has nothing to disclose. M.F. has nothing to disclose. E.R.H. reports funding from Greater Copenhagen Health Science Partners and Danish National Science Foundation (DNRF115) for the submitted work; funding from Novo Nordisk Foundation (NNF0C0074308 and NNF21OC0066487); honoraria for lectures from Ferring Pharmaceuticals (October 29–30, 2022, Copenhagen, Denmark), Takeda Science Foundation (Osaka, Japan), and Merck (October 25, 2022, Rungstedlund, Copenhagen, Denmark), and for Doctor of Philosophy examination from Crick Institute; travel support for the 2024 Gordon Research Conference on Meiosis (invited speaker); and waiver for conference fees by the Gordon Research Conference; support for conference attendance (room and board) for the Memorial Sloan Kettering Cancer Center–Crick–University of Copenhagen Conference (invited speaker; May 9–11, 2024) from the organizer; travel and hotel fees for the Wellcome Trust interview (January 23, 2024) at the Wellcome Trust, Novo Nordisk Foundation; support for travel and conference attendance for the Protein Signaling Conference (invited speaker; November 27, 2023, to December 1, 2023) from the organizer; waiver for conference fees and travel support for the European Society of Human Reproduction and Embryology (ESHRE) Campus Course, Florence, Italy (invited speaker; November 9–11, 2023) from ESHRE; travel and subsistence fees for the Danish National Research Foundation – Early Career Research Conference (invited speaker; November 11, 2023) from the organizers; conference and subsistence fees for the Aging Research and Drug Discovery Conference, Copenhagen (invited speaker; August 28, 2023, to September 1, 2023), from the organizers; conference, subsistence, room, and travel fees for the Gordon Conference on Aging 2023 (invited speaker; July 3–9, 2023), from the organizers; conference, room, and subsistence fees for the ESHRE Conference (invited speaker; June 26–28, 2023), from the organizers; travel, room, and subsistence fees for the Takeda Conference (invited speaker; January 24–27, 2023), from the Takeda Foundation; travel, room, and subsistence fees for the Origin of Aneuploidy (invited speaker; November 12–13, 2022), from the Origins of Aneuploidy Research Consortium; and travel, room, and subsistence fees for the ReproYoung Conference (invited Board Member of ReproUnion; May 28–29, 2022, Saltsjøbadet, Ystad, Sweden), from ReproUnion and is Chair of the Committee for Disputas, University of Lund, Sweden; Scientific Advisory Board of Institut Curie; Member of the Independent Research

Fund Denmark–Committee for Health and Disease, National Granting Committee (January 1, 2021, to December 31, 2023); Steering Group Member of the Danish Korean Rights Group; and Member of the National Committee for Infrastructure, Ministry of Higher Education and Research, Denmark, outside the submitted work. L.S.M. has nothing to disclose.

REFERENCES

1. Winther JF, Kenborg L, Byrne J, Hjorth L, Kaatsch P, Kremer LC, et al. Childhood cancer survivor cohorts in Europe. Acta Oncol 2015;54:655–68.
2. Gatta G, Botta L, Rossi S, Aareleid T, Bielska-Lasota M, Clavel J, et al. Childhood cancer survival in Europe 1999-2007: results of EUROCARE-5-a population-based study. Lancet Oncol 2014;15:35–47.
3. Jensen CFS, Dong L, Gul M, Fode M, Hildorf S, Thorup J, et al. Fertility preservation in boys facing gonadotoxic cancer therapy. Nat Rev Urol 2022;19:71–83.
4. Hildorf S, Cortes D, Gül M, Dong L, Kristensen SG, Jensen CFS, et al. Parental acceptance rate of testicular tissue cryopreservation in Danish boys with cryptorchidism. Sex Dev 2020;13:246–57.
5. Byrne J, Grabow D, Campbell H, O'Brien K, Bielack S, Am Zehnhoff-Dinnesen A, et al. PanCareLIFE: the scientific basis for a European project to improve long-term care regarding fertility, ototoxicity and health-related quality of life after cancer occurring among children and adolescents. Eur J Cancer 2018;103:227–37.
6. Nahata L, Caltabellotta NM, Yeager ND, Lehmann V, Whiteside SL, O'Brien SH, et al. Fertility perspectives and priorities among male adolescents and young adults in cancer survivorship. Pediatr Blood Cancer 2018;65:e27019.
7. Kinahan KE, Didwania A, Nieman CL. Childhood cancer: fertility and psychosocial implications. Cancer Treat Res 2007;138:191–200.
8. Zebrack BJ, Casillas J, Nohr L, Adams H, Zeltzer LK. Fertility issues for young adult survivors of childhood cancer. Psychooncology 2004;13:689–99.
9. Kvist K, Thorup J, Byskov AG, Høyer PE, Møllgård K, Yding Andersen C. Cryopreservation of intact testicular tissue from boys with cryptorchidism. Hum Reprod 2006;21:484–91.
10. Thorup J, Clasen-Linde E, Dong L, Hildorf S, Kristensen SG, Andersen CY, et al. Selecting infants with cryptorchidism and high risk of infertility for optional adjuvant hormonal therapy and cryopreservation of germ cells: experience from a pilot study. Front Endocrinol (Lausanne) 2018;9:299.
11. Thorup J, Hildorf S, Hildorf AE, Baastrup JM, Mamsen LS, Andersen CY, et al. The fate of germ cells in cryptorchid testis. Front Endocrinol (Lausanne) 2024;14:1305428.
12. Goossens E, Van Saen D, Tournaye H. Spermatogonial stem cell preservation and transplantation: from research to clinic. Hum Reprod 2013;28:897–907.
13. Onofre J, Baert Y, Faes K, Goossens E. Cryopreservation of testicular tissue or testicular cell suspensions: a pivotal step in fertility preservation. Hum Reprod Update 2016;22:744–61.
14. Valli-Pulaski H, Peters KA, Gassei K, Steimer SR, Sukhwani M, Hermann BP, et al. Testicular tissue cryopreservation: 8 years of experience from a coordinated network of academic centers. Hum Reprod 2019;34:966–77.
15. Duffin K, Neuhaus N, Andersen CY, Barraud-Lange V, Braye A, Eguizabal C, et al. A 20-year overview of fertility preservation in boys: new insights gained through a comprehensive international survey. Hum Reprod Open 2024;2024:hoae010.
16. Kanbar M, Delwiche G, Wyns C. Fertility preservation for prepubertal boys: are we ready for autologous grafting of cryopreserved immature testicular tissue? Ann Endocrinol (Paris) 2022;83:210–7.
17. Asazawa K, Jitsuzaki M, Mori A, Ichikawa T, Shinozaki K, Porter SE. Quality-of-life predictors for men undergoing infertility treatment in Japan. Jpn J Nurs Sci 2019;16:329–41.
18. Makara-Studzińska M, Limanin A, Anusiewicz A, Janczyk P, Raczkiewicz D, Wdowiak-Filip A, et al. Assessment of quality of life in men treated for infertility in Poland. Int J Environ Res Public Health 2022;19:2950.
19. Peters MDJ, Marnie C, Tricco AC, Pollock D, Munn Z, Alexander L, et al. Updated methodological guidance for the conduct of scoping reviews. JBI Evid Synth 2020;18:2119–26.
20. Arksey H, O'Malley L. Scoping studies: towards a methodological framework. Int J Soc Res Methodol 2005;8:19–32.
21. Peters MD, Godfrey C, McInerney P, Munn Z, Tricco AC, Khalil H. JBI Manual for Evidence Synthesis. Scoping reviews. Available at: https://synthesismanual.jbi.global. Accessed November 23, 2023.
22. Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): checklist and explanation. Ann Intern Med 2018;169:467–73.
23. Brinster RL, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. Proc Natl Acad Sci U S A 1994;91:11298–302.
24. Ntemou E, Kadam P, Van Laere S, Van Saen D, Vicini E, Goossens E. Effect of recombinant human vascular endothelial growth factor on testis tissue xenotransplants from prepubertal boys: a three-case study. Reprod Biomed Online 2019;39:119–33.
25. Poels J, Van Langendonckt A, Many MC, Wese FX, Wyns C. Vitrification preserves proliferation capacity in human spermatogonia. Hum Reprod 2013;28:578–89.
26. Sato Y, Nozawa S, Yoshiike M, Arai M, Sasaki C, Iwamoto T. Xenografting of testicular tissue from an infant human donor results in accelerated testicular maturation. Hum Reprod 2010;25:1113–22.
27. Wyns C, Van Langendonckt A, Wese FX, Donnez J, Curaba M. Long-term spermatogonial survival in cryopreserved and xenografted immature human testicular tissue. Hum Reprod 2008;23:2402–14.
28. Van Saen D, Goossens E, Haentjens P, Baert Y, Tournaye H. Exogenous administration of recombinant human FSH does not improve germ cell survival in human prepubertal xenografts. Reprod Biomed Online 2013;26:286–98.
29. Sadri-Ardekani H, Mizrak SC, van Daalen SK, Korver CM, Roepers-Gajadien HL, Koruji M, et al. Propagation of human spermatogonial stem cells in vitro. J Am Med Assoc 2009;302:2127–34.
30. Nagano M, Patrizio P, Brinster RL. Long-term survival of human spermatogonial stem cells in mouse testes. Fertil Steril 2002;78:1225–33.
31. Galdon G, Deebel NA, Zarandi NP, Teramoto D, Lue Y, Wang C, et al. In vitro propagation of XXY human Klinefelter spermatogonial stem cells: a step towards new fertility opportunities. Front Endocrinol 2022;13:1002279.
32. Pendergraft SS, Sadri-Ardekani H, Atala A, Bishop CE. Three-dimensional testicular organoid: a novel tool for the study of human spermatogenesis and gonadotoxicity in vitro. Biol Reprod 2017;96:720–32.
33. Sakib S, Uchida A, Valenzuela-Leon P, Yu Y, Valli-Pulaski H, Orwig K, et al. Formation of organotypic testicular organoids in microwell culture†. Biol Reprod 2019;100:1648–60.
34. Robinson M, Bedford E, Witherspoon L, Willerth SM, Flannigan R. Using clinically derived human tissue to 3-dimensionally bioprint personalized testicular tubules for in vitro culturing: first report. F S Sci 2022;3:130–9.
35. Perrard MH, Sereni N, Schluth-Bolard C, Blondet A, D Estaing SG, Plotton I, et al. Complete human and rat ex vivo spermatogenesis from fresh or frozen testicular tissue. Biol Reprod 2016;95:89.
36. Yang S, Ping P, Ma M, Li P, Tian R, Yang H, et al. Generation of haploid spermatids with fertilization and development capacity from human spermatogonial stem cells of cryptorchid patients. Stem Cell Rep 2014;3:663–75.
37. Abofoul-Azab M, Abumadighem A, Lunenfeld E, Kapelushnik J, Shi Q, Pinkas H, et al. Development of postmeiotic cells in vitro from spermatogonial cells of prepubertal cancer patients. Stem Cells Dev 2018;27:1007–20.
38. Baert Y, De Kock J, Alves-Lopes JP, Söder O, Stukenborg JB, Goossens E. Primary human testicular cells self-organize into organoids with testicular properties. Stem Cell Rep 2017;8:30–8.
39. de Michele F, Poels J, Weerens L, Petit C, Evrard Z, Ambroise J, et al. Preserved seminiferous tubule integrity with spermatogonial survival and induction of Sertoli and Leydig cell maturation after long-term organotypic culture of prepubertal human testicular tissue. Hum Reprod 2017;32:32–45.
40. Jørgensen A, Nielsen JE, Perlman S, Lundvall L, Mitchell RT, Juul A, et al. Ex vivo culture of human fetal gonads: manipulation of meiosis signalling

by retinoic acid treatment disrupts testis development. Hum Reprod 2015; 30:2351–63.
41. de Michele F, Poels J, Vermeulen M, Ambroise J, Gruson D, Guiot Y, et al. Haploid germ cells generated in organotypic culture of testicular tissue from prepubertal boys. Front Physiol 2018;9:1413.
42. Yuan Y, Li L, Cheng Q, Diao F, Zeng Q, Yang X, et al. In vitro testicular organogenesis from human fetal gonads produces fertilization-competent spermatids. Cell Res 2020;30:244–55.
43. Younis N, Caldeira-Brant AL, Chu T, Abdalla S, Orwig KE. Human immature testicular tissue organ culture: a step towards fertility preservation and restoration. Front Endocrinol (Lausanne) 2023;14:1242263.
44. Eguizabal C, Montserrat N, Vassena R, Barragan M, Garreta E, Garcia-Quevedo L, et al. Complete meiosis from human induced pluripotent stem cells. Stem Cells 2011;29:1186–95.
45. Easley CA 4th, Phillips BT, McGuire MM, Barringer JM, Valli H, Hermann BP, et al. Direct differentiation of human pluripotent stem cells into haploid spermatogenic cells. Cell Rep 2012;2:440–6.
46. Chen H, Tang QL, Wu XY, Xie LC, Lin LM, Ho GY, et al. Differentiation of human umbilical cord mesenchymal stem cells into germ-like cells in mouse seminiferous tubules. Mol Med Rep 2015;12:819–28.
47. Xu H, Yang M, Tian R, Wang Y, Liu L, Zhu Z, et al. Derivation and propagation of spermatogonial stem cells from human pluripotent cells. Stem Cell Res Ther 2020;11:408.
48. Practice Committee of the Oncofertility Consortium. Installing oncofertility programs for common cancers in optimum resource settings (Repro-Can-OPEN Study Part II): a committee opinion. J Assist Reprod Genet 2021; 38:163–76.
49. Salama M, Nahata L, Jayasinghe Y, Gomez-Lobo V, Laronda MM, Moravek MB, et al. Pediatric oncofertility care in limited versus optimum resource settings: results from 39 surveyed centers in Repro-Can-OPEN Study Part I & II. J Assist Reprod Genet 2023;40:443–54.
50. Duffin K, Seneviratne N, Paccagnella D, Friend A, Ratnasingham D, Ismail R, et al. 899 fertility discussions with childhood cancer patients: initial results from a CCLG national audit. Arch Dis Child 2023;108:A291, 1.
51. El Alaoui-Lasmaili K, Nguyen-Thi PL, Demogeot N, Lighezzolo-Alnot J, Gross MJ, Mansuy L, et al. Fertility discussions and concerns in childhood cancer survivors, a systematic review for updated practice. Cancer Med 2023;12:6023–39.
52. Shabani R, Ashjari M, Ashtari K, Izadyar F, Behnam B, Khoei S, et al. Elimination of mouse tumor cells from neonate spermatogonial cells utilizing cisplatin-entrapped folic acid-conjugated poly(lactic-co-glycolic acid) nanoparticles in vitro. Int J Nanomedicine 2018;13:2943–54.
53. Geens M, Goossens E, Tournaye H. Cell selection by selective matrix adhesion is not sufficiently efficient for complete malignant cell depletion from contaminated human testicular cell suspensions. Fertil Steril 2011;95:787–91.
54. Fujita K, Tsujimura A, Miyagawa Y, Kiuchi H, Matsuoka Y, Takao T, et al. Isolation of germ cells from leukemia and lymphoma cells in a human in vitro model: potential clinical application for restoring human fertility after anticancer therapy. Cancer Res 2006;66:11166–71.
55. Sadri-Ardekani H, Homburg CH, van Capel TM, van den Berg H, van der Veen F, van der Schoot CE, et al. Eliminating acute lymphoblastic leukemia cells from human testicular cell cultures: a pilot study. Fertil Steril 2014;101: 1072–8.e1.
56. Dovey SL, Valli H, Hermann BP, Sukhwani M, Donohue J, Castro CA, et al. Eliminating malignant contamination from therapeutic human spermatogonial stem cells. J Clin Invest 2013;123:1833–43.
57. Lee DR, Kim KS, Yang YH, Oh HS, Lee SH, Chung TG, et al. Isolation of male germ stem cell-like cells from testicular tissue of non-obstructive azoospermic patients and differentiation into haploid male germ cells in vitro. Hum Reprod 2006;21:471–6.
58. Hwang YS, Suzuki S, Seita Y, Ito J, Sakata Y, Aso H, et al. Reconstitution of prospermatogonial specification in vitro from human induced pluripotent stem cells. Nat Commun 2020;11:5656.
59. Jensen CFS, Mamsen LS, Wang D, Fode M, Giwercman A, Jørgensen N, et al. Results from the first autologous grafting of adult human testis tissue: a case report. Hum Reprod 2024;39:303–9.
60. Wang D, Hildorf S, Ntemou E, Dong L, Pors SE, Mamsen LS, et al. Characterization and survival of human infant testicular cells after direct xenotransplantation. Front Endocrinol (Lausanne) 2022;13:853482.
61. Wang D, Hildorf S, Ntemou E, Mamsen LS, Dong L, Pors SE, et al. Organotypic culture of testicular tissue from infant boys with cryptorchidism. Int J Mol Sci 2022;23:7975.
62. Hutka M, Kadam P, Van Saen D, Homer NZM, Onofre J, Wallace WHB, et al. Fertility preservation in childhood cancer: Endocrine activity in prepubertal human testis xenografts exposed to a pubertal hormone environment. Cancers (Basel) 2020;12:1–21.
63. Schlatt S, Honaramooz A, Ehmcke J, Goebell PJ, Rübben H, Dhir R, et al. Limited survival of adult human testicular tissue as ectopic xenograft. Human Reproduction 2006;21:384–9.
64. Yu J, Cai ZM, Wan HJ, Zhang FT, Ye J, Fang JZ, et al. Development of neonatal mouse and fetal human testicular tissue as ectopic grafts in immunodeficient mice. Asian J Androl 2006;8:393–403.
65. Mohaqiq M, Movahedin M, Mazaheri Z, Amirjannati N. Successful human spermatogonial stem cells homing in recipient mouse testis after in vitro transplantation and organ culture. Cell J 2019;20:513–20.
66. Mohaqiq M, Movahedin M, Mazaheri Z, Amirjannati N. In vitro transplantation of spermatogonial stem cells isolated from human frozen-thawed testis tissue can induce spermatogenesis under 3-dimensional tissue culture conditions. Biol Res 2019;52:16.
67. Izadyar F, Wong J, Maki C, Pacchiarotti J, Ramos T, Howerton K, et al. Identification and characterization of repopulating spermatogonial stem cells from the adult human testis. Human Reproduction 2011;26:1296–306.
68. Oliver E, Alves-Lopes JP, Harteveld F, Mitchell RT, Åkesson E, Söder O, et al. Self-organising human gonads generated by a Matrigel-based gradient system. BMC Biol 2021;19.
69. Ashouri Movassagh S, Ashouri Movassagh S, Banitalebi Dehkordi M, Pourmand G, Gholami K, Talebi A, et al. Isolation, identification and differentiation of human spermatogonial cells on three-dimensional decellularized sheep testis. Acta Histochem 2020;122:151623.
70. Dong L, Gul M, Hildorf S, Pors SE, Kristensen SG, Hoffmann ER, et al. Xeno-free propagation of spermatogonial stem cells from infant boys. Int J Mol Sci 2019;20:1–14.
71. Mincheva M, Sandhowe-Klaverkamp R, Wistuba J, Redmann K, Stukenborg JB, Kliesch S, et al. Reassembly of adult human testicular cells: Can testis cord-like structures be created in vitro? Mol Hum Reprod 2018; 24:55–63.
72. Smith JF, Yango P, Altman E, Choudhry S, Poelzl A, Zamah AM, et al. Testicular niche required for human spermatogonial stem cell expansion. Stem Cells Transl Med 2014;3:1043–54.
73. Piravar Z, Jeddi-Tehrani M, Sadeghi MR, Mohazzab A, Eidi A, Akhondi MM. In vitro culture of human testicular stem cells on feeder-free condition. J Reprod Infertil 2013;14:17–22.
74. Aden NL, Bleeke M, Kordes UR, Brunne B, Holstermann B, Biemann R, et al. Germ cell maintenance and sustained testosterone and precursor hormone production in human prepubertal testis organ culture with tissues from boys 7 years+ under conditions from adult testicular tissue. Cells 2023;12.
75. Kurek M, Åkesson E, Yoshihara M, Oliver E, Cui Y, Becker M, et al. Spermatogonia loss correlates with LAMA 1 expression in human prepubertal testes stored for fertility preservation. Cells 2021;10:1–18.
76. Portela JMD, De Winter-Korver CM, Van Daalen SKM, Meißner A, De Melker AA, Repping S, et al. Assessment of fresh and cryopreserved testicular tissues from (pre)pubertal boys during organ culture as a strategy for in vitro spermatogenesis. Human Reproduction 2019;34:2443–55.
77. Sá R, Graça I, Silva J, Malheiro I, Carvalho F, Barros A, et al. Quantitative analysis of cellular proliferation and differentiation of the human seminiferous epithelium in vitro. Reproductive Sciences 2012;19:1063–74.
78. Irie N, Weinberger L, Tang WWC, Kobayashi T, Viukov S, Manor YS, et al. SOX17 is a critical specifier of human primordial germ cell fate. Cell 2015; 160:253–68.
79. Panula S, Medrano JV, Kee K, Bergström R, Nguyen HN, Byers B, et al. Human germ cell differentiation from fetal- and adult-derived induced pluripotent stem cells. Hum Mol Genet 2011;20:752–62.

80. Aflatoonian B, Ruban L, Jones M, Aflatoonian R, Fazeli A, Moore HD. In vitro post-meiotic germ cell development from human embryonic stem cells. Human Reproduction 2009;24:3150–9.
81. Hua J, Pan S, Yang C, Dong W, Dou Z, Sidhu KS. Derivation of male germ cell-like lineage from human fetal bone marrow stem cells. Reprod Biomed Online 2009;19:99–105.
82. Kee K, Angeles VT, Flores M, Nguyen HN, Reijo Pera RA. Human DAZL, DAZ and BOULE genes modulate primordial germ-cell and haploid gamete formation. Nature 2009;462:222–5.
83. Park TS, Galic Z, Conway AE, Lindgren A, Van Handel BJ, Magnusson M, et al. Derivation of primordial germ cells from human embryonic and induced pluripotent stem cells is significantly improved by coculture with human fetal gonadal cells. Stem Cells 2009;27:783–95.
84. Poels J, Abou-Ghannam G, Herman S, Van Langendonckt A, Wese FX, Wyns C. In search of better spermatogonial preservation by supplementation of cryopreserved human immature testicular tissue xenografts with N-acetylcysteine and testosterone. Front Surg 2014;1:47.
85. Van Saen D, Goossens E, Bourgain C, Ferster A, Tournaye H. Meiotic activity in orthotopic xenografts derived from human postpubertal testicular tissue. Human Reproduction 2011;26:282–93.
86. Goossens E, Geens M, De Block G, Tournaye H. Spermatogonial survival in long-term human prepubertal xenografts. Fertil Steril 2008;90:2019–22.
87. Wyns C, Curaba M, Martinez-Madrid B, Van Langendonckt A, François-Xavier W, Donnez J. Spermatogonial survival after cryopreservation and short-term orthotopic immature human cryptorchid testicular tissue grafting to immunodeficient mice. Human Reproduction 2007;22:1603–11.
88. Geens M, De Block G, Goossens E, Frederickx V, Van Steirteghem A, Tournaye H. Spermatogonial survival after grafting human testicular tissue to immunodeficient mice. Human Reproduction 2006;21:390–6.
89. Chen B, Wang Y Bin, Zhang ZL, Xia WL, Wang HX, Xiang ZQ, et al. Xeno-free culture of human spermatogonial stem cells supported by human embryonic stem cell-derived fibroblast-like cells. Asian J Androl 2009;11:557–65.
90. Ramathal C, Angulo B, Sukhwani M, Cui J, Durruthy-Durruthy J, Fang F, et al. DDX3Y gene rescue of a y chromosome AZFa deletion restores germ cell formation and transcriptional programs. Sci Rep 2015;5.
91. Medrano JV, Vilanova-Pérez T, Fornés-Ferrer V, Navarro-Gomezlechon A, Martínez-Triguero ML, García S, et al. Influence of temperature, serum, and gonadotropin supplementation in short- and long-term organotypic culture of human immature testicular tissue. Fertil Steril 2018;110:1045–57.e3.
92. Sasaki K, Yokobayashi S, Nakamura T, Okamoto I, Yabuta Y, Kurimoto K, et al. Robust in vitro induction of human germ cell fate from pluripotent stem cells. Cell Stem Cell 2015;17:178–94.
93. Durruthy JD, Ramathal C, Sukhwani M, Fang F, Cui J, Orwig KE, et al. Fate of induced pluripotent stem cells following transplantation to murine seminiferous tubules. Hum Mol Genet 2014;23:3071–84.
94. Ramathal C, Durruthy-Durruthy J, Sukhwani M, Arakaki JE, Turek PJ, Orwig KE, et al. Fate of iPSCs derived from azoospermic and fertile men following Xenotransplantation to murine seminiferous tubules. Cell Rep 2014;7:1284–97.
95. Poels J, Abou-Ghannam G, Decamps A, Leyman M, Rieux Ad, Wyns C. Transplantation of testicular tissue in alginate hydrogel loaded with VEGF nanoparticles improves spermatogonial recovery. J Control Release 2016;234:79–89.
96. Sadri-Ardekani H, Akhondi MA, van der Veen F, Repping S, van Pelt AM. In vitro propagation of human prepubertal spermatogonial stem cells. J Am Med Assoc 2011;305:2416–8.
97. Shinohara T, Inoue K, Ogonuki N, Kanatsu-Shinohara M, Miki H, Nakata K, et al. Birth of offspring following transplantation of cryopreserved immature testicular pieces and in-vitro microinsemination. Hum Reprod 2002;17:3039–45.
98. Fayomi AP, Peters K, Sukhwani M, Valli-Pulaski H, Shetty G, Meistrich ML, et al. Autologous grafting of cryopreserved prepubertal rhesus testis produces sperm and offspring. Science 2019;363:1314–9.
99. Wistuba J, Luetjens CM, Wesselmann R, Nieschlag E, Simoni M, Schlatt S. Meiosis in autologous ectopic transplants of immature testicular tissue grafted to Callithrix jacchus. Biol Reprod 2006;74:706–13.
100. Luetjens CM, Stukenborg JB, Nieschlag E, Simoni M, Wistuba J. Complete spermatogenesis in orthotopic but not in ectopic transplants of autologously grafted marmoset testicular tissue. Endocrinology 2008;149:1736–47.
101. Jahnukainen K, Ehmcke J, Nurmio M, Schlatt S. Autologous ectopic grafting of cryopreserved testicular tissue preserves the fertility of prepubescent monkeys that receive sterilizing cytotoxic therapy. Cancer Res 2012;72:5174–8.
102. Foresta C, Bettella A, Spolaore D, Merico M, Rossato M, Ferlin A. Suppression of the high endogenous levels of plasma FSH in infertile men are associated with improved Sertoli cell function as reflected by elevated levels of plasma inhibin B. Hum Reprod 2004;19:1431–7.
103. Goossens E, Jahnukainen K, Mitchell RT, van Pelt A, Pennings G, Rives N, et al. Fertility preservation in boys: recent developments and new insights†. Hum Reprod Open 2020;2020:hoaa016.
104. Bashiri Z, Hosseini SJ, Salem M, Koruji M. In vivo and in vitro sperm production: an overview of the challenges and advances in male fertility restoration. Clin Exp Reprod Med 2024. https://doi.org/10.5653/cerm.2023.06569.
105. Goossens E, De Vos P, Tournaye H. Array comparative genomic hybridization analysis does not show genetic alterations in spermatozoa and offspring generated after spermatogonial stem cell transplantation in the mouse. Hum Reprod 2010;25:1836–42.
106. Goossens E, De Rycke M, Haentjens P, Tournaye H. DNA methylation patterns of spermatozoa and two generations of offspring obtained after murine spermatogonial stem cell transplantation. Hum Reprod 2009;24:2255–63.
107. Kanatsu-Shinohara M, Ogonuki N, Iwano T, Lee J, Kazuki Y, Inoue K, et al. Genetic and epigenetic properties of mouse male germline stem cells during long-term culture. Development 2005;132:4155–63.
108. Nagano M, Avarbock MR, Brinster RL. Pattern and kinetics of mouse donor spermatogonial stem cell colonization in recipient testes. Biol Reprod 1999;60:1429–36.
109. Sato T, Katagiri K, Gohbara A, Inoue K, Ogonuki N, Ogura A, et al. In vitro production of functional sperm in cultured neonatal mouse testes. Nature 2011;471:504–7.
110. Robinson M, Sparanese S, Witherspoon L, Flannigan R. Human in vitro spermatogenesis as a regenerative therapy - where do we stand? Nat Rev Urol 2023;20:461–79.
111. von Kopylow K, Spiess AN. Human spermatogonial markers. Stem Cell Res 2017;25:300–9.
112. Sohni A, Tan K, Song HW, Burow D, de Rooij DG, Laurent L, et al. The neonatal and adult human testis defined at the single-cell level. Cell Rep 2019;26:1501–17.e4.
113. Önen S, Atik AC, Gizer M, Köse S, Yaman Ö, Külah H, et al. A pumpless monolayer microfluidic device based on mesenchymal stem cell-conditioned medium promotes neonatal mouse in vitro spermatogenesis. Stem Cell Res Ther 2023;14:127.
114. Komeya M, Hayashi K, Nakamura H, Yamanaka H, Sanjo H, Kojima K, et al. Pumpless microfluidic system driven by hydrostatic pressure induces and maintains mouse spermatogenesis in vitro. Sci Rep 2017;7:15459.
115. Mulder RL, Font-Gonzalez A, Hudson MM, van Santen HM, Loeffen EAH, Burns KC, et al. Fertility preservation for female patients with childhood, adolescent, and young adult cancer: recommendations from the PanCareLIFE Consortium and the International Late Effects of Childhood Cancer Guideline Harmonization Group. Lancet Oncol 2021;22:e45–56.
116. Font-Gonzalez A, Mulder RL, Loeffen EA, Byrne J, van Dulmen-den Broeder E, van den Heuvel-Eibrink MM, et al. Fertility preservation in children, adolescents, and young adults with cancer: quality of clinical practice guidelines and variations in recommendations. Cancer 2016;122:2216–23.
117. Dhonnabháin BN, Getreu N. Freezing protocols for the cryopreservation of immature testicular tissue - a systematic review. Cryo Letters 2021;42:188–201.
118. Sadri-Ardekani H, McLean TW, Kogan S, Sirintrapun J, Crowell K, Yousif MQ, et al. Experimental testicular tissue banking to generate spermatogenesis in the future: a multidisciplinary team approach. Methods 2016;99:120–7.

119. Kremer LC, Mulder RL, Oeffinger KC, Bhatia S, Landier W, Levitt G, et al. A worldwide collaboration to harmonize guidelines for the long-term follow-up of childhood and young adult cancer survivors: a report from the international late effects of Childhood Cancer Guideline Harmonization Group. Pediatr Blood Cancer 2013;60:543–9.
120. Kourta D, Kanbar M, Amorim CA, Wyns C. Cancer cell contamination and decontamination methods for ovaries and testes: special focus on prepubertal gonads with a view to safe fertility restoration. Hum Reprod 2023;38:780–98.
121. Picton HM, Wyns C, Anderson RA, Goossens E, Jahnukainen K, Kliesch S, et al. A European perspective on testicular tissue cryopreservation for fertility preservation in prepubertal and adolescent boys. Hum Reprod 2015;30:2463–75.
122. Nguyen HTK, Terao MA, Green DM, Pui CH, Inaba H. Testicular involvement of acute lymphoblastic leukemia in children and adolescents: diagnosis, biology, and management. Cancer 2021;127:3067–81.
123. de Jesus LE, Dekermacher S, Resende GC, Justiniano RR. Testicular involvement in pediatric acute lymphocytic leukemia: what to do about it? Int Braz J Urol 2022;48:981–7.
124. Aldrink JH, Glick RD, Baertschiger RM, Kulaylat AN, Lautz TB, Christison-Lagay E, et al. Update on pediatric testicular germ cell tumors. J Pediatr Surg 2022;57:690–9.
125. Akhtar M, Ali MA, Burgess A, Aur RJ. Fine-needle aspiration biopsy (FNAB) diagnosis of testicular involvement in acute lymphoblastic leukemia in children. Diagn Cytopathol 1991;7:504–7.
126. Kim TH, Hargreaves HK, Brynes RK, Hawkins HK, Lui VK, Woodard J, et al. Pretreatment testicular biopsy in childhood acute lymphocytic leukaemia. Lancet 1981;2:657–8.
127. Simon T, Hero B, Berthold F. Testicular and paratesticular involvement by metastatic neuroblastoma. Cancer 2000;88:2636–41.
128. Gow KW, Murphy 3rd JJ, Wu JK, Desa DJ. Metastatic testicular rhabdomyosarcoma–a report of two cases. J Pediatr Surg 2003;38:E1–3.
129. Dhal S, Mehta MJ, Parikh A, Bhagat M, Banidutta A. Testicular metastasis in Wilms' tumor. South Asian J Cancer 2017;6:101.
130. Palmer BW, Xiong Y, Gherezghiher A, Buethe D, Metwalli A, Frimberger D, et al. Wilms' tumor metastatic to bilateral testes at presentation: case and review of the literature. Urology 2012;79:899–901.

Restauración de la fertilidad masculina: estrategias in vivo e in vitro basadas en células madre usando tejido testicular criopreservado: una revisión exploratoria

Importancia: Los avances en el tratamiento del cáncer infantil han mejorado significativamente las tasas de supervivencia, con más del 80% de los supervivientes alcanzando la edad adulta. Sin embargo, los tratamientos oncológicos gonadotóxicos ponen en peligro la fertilidad posterior y los varones prepúberes no tienen la opción de preservar su fertilidad mediante criopreservación de semen. Además, los niños con criptorquidismo corren el riesgo de ver comprometida su fertilidad en la edad adulta.

Objetivo: Se realizó una revisión exploratoria para investigar la evidencia actual sobre estrategias de restauración de fertilidad masculina, explorar barreras para su implementación clínica y detallar los posibles pasos para superar dichas barreras. Esta síntesis del conocimiento es particularmente relevante para varones prepúberes supervivientes de cáncer y para niños con criptorquidismo.

Revisión de la evidencia: La revisión se llevó a cabo según los criterios de la *Preferred Reporting Items for Systematic Reviews and Meta-Analyses extensión for Scoping Reviews* y directrices publicadas anteriormente y evaluó estudios que usaron tejido testicular humano de niños prepúberes o de varones adultos sanos. Se realizó una búsqueda de literatura en PubMed y fueron identificados 72 estudios relevantes, incluyendo enfoques in vivo e in vitro.

Hallazgos: Las estrategias in vivo, tales como el injerto de tejido testicular y el trasplante de células madre espermatogoniales, son prometedoras como promotoras de la supervivencia y diferenciación celular. Sin embargo, la espermatogénesis completa no ha sido lograda hasta ahora. Las estrategias in vitro están enfocadas en la generación de células germinales masculinas a partir de una maduración directa de células madre en diversos sistemas de cultivo, acompañadas de células madre pluripotenciales inducidas humanas y células madre embrionarias. Estos enfoques representan avances significativos en el entendimiento y la promoción de la espermatogénesis, pero lograr espermatozoides plenamente funcionales in vitro continúa siendo un desafío. Las barreras a la implementación clínica incluyen el riesgo de reintroducción de células malignas y al introducción de cambios epigenéticos.

Conclusión: La restauración de la fertilidad masculina es un área en rápido desarrollo. Sobre la base de los estudios revisados, la estrategia más prometedora para restaurar la fertilidad masculina empleando tejido testicular criopreservado es el trasplante directo de tejido testicular.

Relevancia: Esta revisión identifica barreras persistentes a la implementación clínica de la restauración de la fertilidad masculina. Sin embargo, el trasplante directo de tejido testicular crioconservado continúa representando una estrategia prometedora al borde de su aplicación clínica.