# Experimental methods to preserve male fertility and treat male factor infertility

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Infertility is a prevalent condition that has insidious impacts on the infertile individuals, their families, and society, which extend far beyond the inability to have a biological child. Lifestyle changes, fertility treatments, and assisted reproductive technology (ART) are available to help many infertile couples achieve their reproductive goals. All of these technologies require that the infertile individual is able to produce at least a small number of functional gametes (eggs or sperm). It is not possible for a person who does not produce gametes to have a biological child. This review focuses on the infertile man and describes several stem cell-based methods and gene therapy approaches that are in the research pipeline and may lead to new fertility treatment options for men with azoospermia. (Fertil Steril® 2016;105:256–66. ©2016 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).)

**Key Words:** Fertility, infertility, azoospermia, stem cells, transplantation, grafting, culture, de novo testicular morphogenesis, gene therapy

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In vitro fertilization was pioneered in the United Kingdom by Drs. Patrick Steptoe (physician) and Robert Edwards (researcher) (1) and led to the birth of Louise Brown (born July 25, 1978), the world's first baby conceived in a Petri dish. This technology has now led to the birth of nearly five million babies worldwide and the 2010 Nobel Prize in Medicine for Dr. Edwards. Despite this progress in treating infertile couples, many still remain beyond the reach of current assisted reproductive technology (ART) because they are not able to produce mature sperm or eggs. For those couples, there are several methods in the research pipeline that may expand fertility options and lead to the next renaissance in the field of assisted reproduction. This review focuses on experimental options to preserve male fertility and/or treat male factor infertility.

Spermatogenesis is an extraordinarily productive process that yields millions of sperm each day throughout the postpubertal life of men (2). Spermatogenesis arises from a relatively small pool of spermatogonial stem cells (SSCs) that are located in the seminiferous tubules of the testis (3–5). These adult tissue stem cells (designated Adark and Apale spermatogonia in humans) balance self-renewing divisions that maintain the stem cell pool with differentiating divisions that insure continuous sperm production (Fig. 1) (6–8). Therefore, SSCs are essential for spermatogenesis and male fertility. Conditions that compromise the stem cell pool, the differentiation process, or the testicular environment (e.g., genetic, environmental, medical, age, injury) can lead to subfertility or infertility. Refinements in ART, including testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI), now allow many men with azoospermia (no sperm in their ejaculate) to father biological children from rare sperm that are biopsied directly from the testes (Fig. 2A) (10–12). At present, there are no options for men with azoospermia and failed TESE to have biological children.

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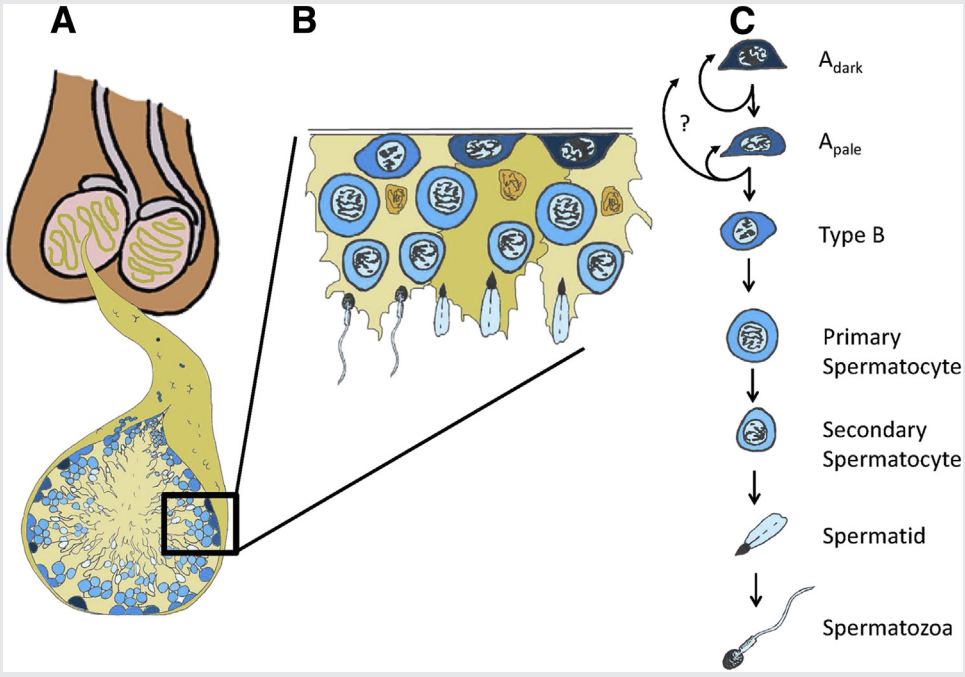
## FIGURE 1




Human spermatogonial stem cells and spermatogenesis. (**A**) Spermatogenesis occurs inside the seminiferous tubules of the testis. (**B**) Cut-out of the basement membrane of the seminiferous tubule. (**B** and **C**) The basement membrane of the seminiferous epithelium contains undifferentiated (Adark and Apale) spermatogonia and differentiating type B spermatogonia. Type B spermatogonia give rise to primary spermatocytes that enter meiosis and migrate off the basement membrane. Subsequent meiotic divisions and spermiogenesis give rise to secondary spermatocytes, spermatids, and the terminally differentiated spermatozoa, which are released into the lumen of the seminiferous tubules. (Figure reprinted from Valli et al.[9] with permission from Elsevier. Artwork is by Dr. Bart Phillips, National Institute of Environmental Health Sciences.)

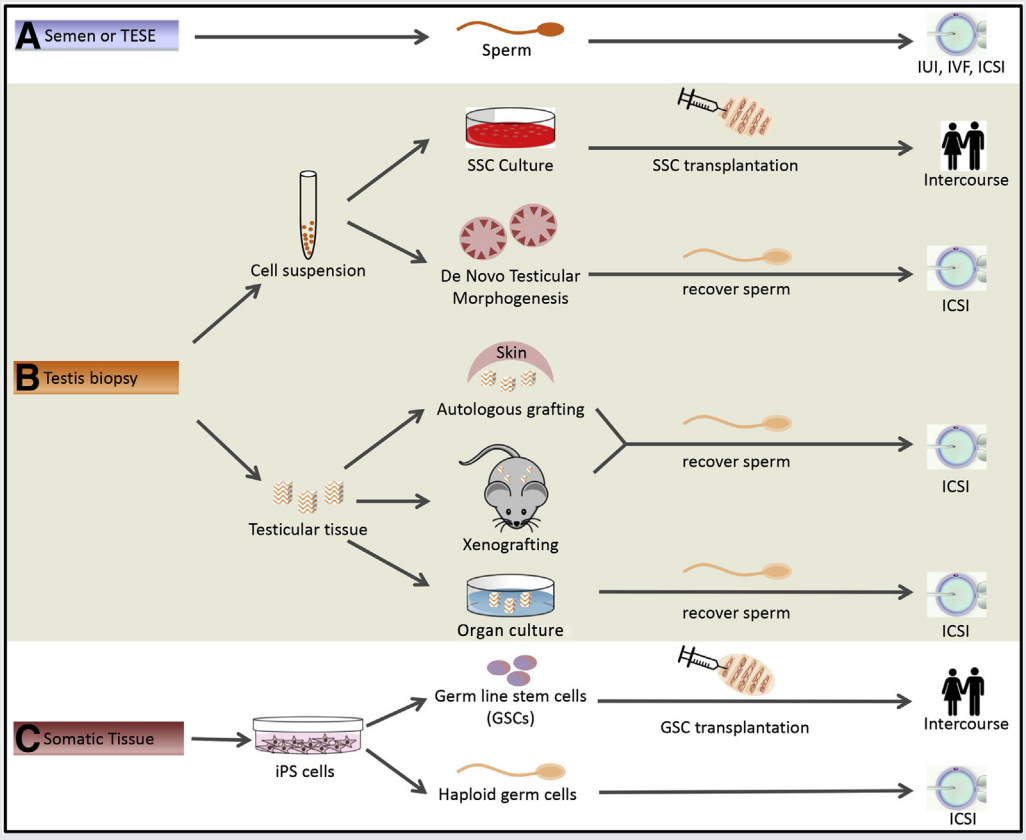
*Gassei. Experimental therapies for male factor infertility. Fertil Steril 2016.*

Several technologies have emerged during the past two decades that may substantially increase the number of reproductive options available to men who do not produce any sperm and desire to have biological children: SSC transplantation; SSC culture; testicular tissue grafting; testicular tissue organ culture; induced pluripotent stem cells; the $1,000 genome; and gene therapy (Fig. 2). This review describes two patient scenarios to illustrate how these technologies could be used to preserve fertility and generate or regenerate spermatogenesis in men with azoospermia. The first scenario is the prepubertal cancer patient who cannot preserve a semen sample before initiation of treatment and who grows up to become an infertile adult survivor of childhood cancer. The second scenario is a man with idiopathic nonobstructive azoospermia and no previous comobidities.

## PATIENT SCENARIO 1: MEDICALLY INDUCED (IATROGENIC) AZOOSPERMIA

Chemotherapy and radiation treatments for cancer and other conditions can cause permanent infertility. Adult men have the option to cryopreserve a semen sample before the initiation of treatment and use this sample in the future to achieve a pregnancy with their partner using ART (Fig. 2A) (1, 13, 14). This option is not available to prepubertal boys who are not making sperm or to adult survivors who did not preserve sperm before treatment. This is a significant human health problem because we estimate that each year in the United States there are >4,000 male patients who will receive treatments that put them at risk of permanent azoospermia and did not or could not save a semen sample (Valli et al. [8, 9]). Testicular sperm extraction may be an option for azoospermic adult survivors of childhood cancers who did not save semen or testicular tissue. This is possible because a few SSCs may survive the gonadotoxic therapy and produce focal areas of spermatogenesis in the seminiferous tubules, which can be retrieved by biopsy. Picton and colleagues (20) surveyed results from five centers and reported an overall sperm recovery rate of 44% in patients with azoospermia undergoing TESE after chemotherapy (15–20). Prepubertal boys cannot save a semen sample before therapy, but they do have Adark and Apale SSCs in their testes (Fig. 1) (21) that are poised to initiate sperm production during puberty. Several centers in the United States and around the world are collecting (through biopsy) and cryopreserving testicular tissue or cells with anticipation that experimental SSC-based therapies will be available in the future (experimental options are reviewed later and in Fig. 2) (20, 22–27).

## FIGURE 2




Standard and experimental options to treat male factor infertility. (**A**) Sperm obtained from ejaculated semen or by testicular sperm extraction (TESE) of infertile men can be used to achieve pregnancy by intrauterine insemination (IUI), in vitro fertilization (IVF), or IVF with intracytoplasmic sperm injection (ICSI). (**B**) When it is not possible to obtain sperm, testicular tissue containing spermatogonial stem cells (SSCs) can be obtained by biopsy. Testicular tissue can be digested with enzymes to produce a cell suspension from which spermatogonial stem cells can be expanded in culture and/or transplanted into the testes of the patient. This method has the potential to regenerate spermatogenesis and possibly natural fertility. Heterogeneous testicular cell suspensions also have the potential to undergo de novo testicular morphogenesis to produce seminiferous tubules with a polarized epithelium surrounded by a basement membrane with germ cells inside and interstitial cells outside the tubules. Sperm generated in the "rebuilt" testes can be used to fertilize eggs by ICSI. Intact testicular tissues from prepubertal males can be grafted or xenografted under the skin or in the scrotum and produce mature sperm that can be used to fertilize eggs by ICSI. Sperm can also be generated when immature testicular tissues are maintained in organ culture and used to fertilize eggs by ICSI. (**C**) Patient-specific induced pluripotent stem (iPS) cells can be derived from patient somatic tissues (e.g., skin or blood) and differentiated into germ line stem cells (GSCs) to be transplanted into patient testes. This method may have the potential to regenerate spermatogenesis and natural fertility. It may also be possible to differentiate induced pluripotent stem cells into sperm that can be used to fertilize eggs by ICSI.

*Gassei. Experimental therapies for male factor infertility. Fertil Steril 2016.*

## PATIENT SCENARIO 2: IDIOPATHIC NONOBSTRUCTIVE AZOOSPERMIA

About 1% of men in the general population and 10%–15% of infertile men are azoospermic (no sperm in the ejaculate) (28–31). Azoospermia can be classified as obstructive (15%–20% of cases) or nonobstructive (80%–85% of cases), which indicates a problem with spermatogenesis (30). Nonobstructive azoospermia is characterized by spermatogenic failure and can be subclassified as Sertoli cell only, early or late maturation arrest, mixed atrophy, or complete hyalinization of the seminiferous tubules. The chances of sperm recovery by TESE from the testes of men with "true" Sertoli cell only or complete hyalinization phenotypes are very low. Sperm recovery rates from men with uniform early maturation arrest (spermatogonia or spermatocytes) are considerably lower (23%–41%) than patients with late maturation arrest and/or mixed atrophy (54%–90%) (29, 32, 33). Review of our own records and four retrospective studies (29, 32–34) revealed that approximately 15% of patients with nonobstructive azoospermia and 2% of all infertile men have a uniform maturation arrest phenotype. A diagnosis of nonobstructive azoospermia with maturation arrest suggests that germ cells are present, but fail to progress through meiosis and/or spermiogenesis. Maturation arrest could be due to intrinsic germ cell defects or extrinsic somatic/endocrine environment defects, or both. If an underlying genetic cause of a somatic defect can be identified, a male factor

infertility diagnosis of nonobstructive azoospermia with maturation arrest might be amenable to gene therapy using methods that have already been described in mice (discussed later). Improved knowledge about the genetic basis of infertility will aid in the counseling of infertile men; justify the development of diagnostic screens; and may lead to the development of patient-specific treatment options.

## STEM CELL THERAPIES FOR MALE FACTOR INFERTILITY

### SSC Transplantation

Spermatogonial stem cell transplantation was described in 1994 by Ralph Brinster and colleagues (35, 36). In the initial reports, testicular tissue was obtained from the testes of transgenic mice with ubiquitous expression of the lacZ transgene. The tissue was digested with enzymes and the resulting cell suspension was transplanted into the seminiferous tubules of infertile recipient mice. A few months after transplantation, donor-derived spermatogenesis could be recognized in the testes of recipient animals by X-gal staining, which generated a blue color due to the activity of the lacZ encoded $\beta$-galactosidase activity. One of those classic studies reported that fertility was restored and the lacZ transgene was transmitted to progeny, providing unequivocal evidence that SSCs could engraft the basement membrane of recipient seminiferous tubules and regenerate spermatogenesis with functional sperm (35). This approach for homologous species SSC transplantation has now been recapitulated in rats, pigs, goats, bulls, sheep, dogs, and monkeys with the production of donor-derived embryos or offspring in mice, rats, goats, sheep, and monkeys (37–48). Functional SSCs can be obtained from the testes of all ages from newborn to adult (39, 48, 49) and SSCs retain their biological function after freezing and thawing (48, 50–55). Wu and colleagues (55) demonstrated that mouse SSCs were competent to regenerate spermatogenesis and produce offspring after 14 years of cryostorage. Based on these observations, it should be possible to cryopreserve testicular tissue or cells for prepubertal boys before they initiate cancer treatment and thaw those cells years later for transplantation and regeneration of spermatogenesis (Fig. 2B).

### Translating SSC Transplantation to the Clinic

It is not widely known that Radford and colleagues (56) had already biopsied and cryopreserved testicular cell suspensions for 12 adult patients with non-Hodgkin's lymphoma in 1999. This was before the method of SSC transplantation had been translated to any large animal species. Testicular cells were later reintroduced into the testes of seven of those patients (57, 58) after completion of their cancer treatments, but their fertility status has not been reported. Nonetheless, those reports demonstrate that patients are willing to enroll in experimental stem cell protocols to preserve and potentially restore their fertility. As indicated, homologous species stem cell transplants have now been performed in several large animal models (42–47), including our report that nonhuman primate SSCs could be frozen, thawed, and transplanted to regenerate spermatogenesis with functional sperm (48). Furthermore, we estimate from published reports and personal communications that testicular tissues or cells have been cryopreserved for several hundred patients worldwide (9, 20, 59). Therefore, translation of the SSC transplantation technique to the clinic appears imminent.

Although some centers are freezing testicular cell suspensions (57, 60–63), most are freezing intact pieces of testicular tissue for patients because this preserves the option for both tissue-based and cell-based therapies in the future (see Fig. 2) (20, 22, 23, 25, 26, 59). Biopsied testicular tissues are typically cut into small pieces (1–9 $mm^3$), suspended in a dimethyl sulfoxide (DMSO)-based freezing medium and frozen at a controlled slow rate using a programmable freezing machine (20, 22, 23, 25, 27, 59, 61, 64). Some centers have reported using an ethylene glycol-based freezing medium instead of DMSO (57, 60, 65) and some centers have reported that viability of vitrified testicular tissue is similar to tissue frozen at a controlled slow rate (66–69).

### SSC Culture

The testicular biopsy that can be obtained from the testes of prepubertal boys is relatively small in size and may contain a few SSCs. The number of SSCs that will be required to regenerate spermatogenesis and fertility in humans is not known, but it is reasonable to assume that SSC numbers will need to be expanded in culture before transplantation to ensure robust engraftment and spermatogenesis. Methods for maintaining mouse SSCs in culture were established in 2003/2004 and these methods were translated to rats in 2005. Cultured rodent SSCs can be maintained in long-term culture with exponential expansion in numbers and they can retain their biological potential to produce spermatogenesis and restore fertility when transplanted into the testes of infertile recipient mice (70–73). Langenstroth and colleagues (74) reported maintaining nonhuman primate SSCs in short-term culture and several groups of investigators (24, 75–83) reported culturing human SSCs for short term or long term. Each group reporting human SSC culture used different methods to isolate cells for culture, different feeder or matrix substrates, different growth factor cocktails, and different methods to assess progress. At present, no human SSC culture method has be independently replicated by another group and this needs to happen to move the field forward (Valli et al. [8, 59]). Furthermore, although transplantation to regenerate spermatogenesis with functional sperm and offspring is the gold standard assay for rodent SSCs, there is not equivalent assay of human SSCs. Molecular markers and human-to-mouse xenotransplantation may be reasonable surrogate assays, but there is no gold standard that is universally agreed and deployed for human SSC experimentation. Perhaps de novo testicular morphogenesis and/or decellularized testes can be developed into tools to assay complete human spermatogenesis (discussed later).

## De Novo Testicular Morphogenesis

Heterogeneous testicular cell suspensions (including germ cells, Sertoli cells, peritubular myoid cells, Leydig cells, and other interstitial cells) have the remarkable ability to reorganize into normal-looking seminiferous tubules when grafted under the skin of immune-deficient recipient mice (84–88). Dobrinski's laboratory (86, 88) reported complete spermatogenesis when neonatal pig and sheep testis cells were pelleted and grafted under the skin of SCID mice. Kita and colleagues (87) obtained similar results with fetal or neonatal mouse, rat, and pig testis cells. Furthermore, this group of investigators mixed cultured green fluorescent protein (GFP) positive mouse SSCs with GFP negative neonatal mouse or rat testis cells. Complete spermatogenesis was observed in reorganized seminiferous tubules 7–10 weeks after grafting, including GFP positive round spermatids that were recovered and used to fertilize mouse eggs by round spermatid injection. The resulting embryos were transferred to pseudopregnant females and resulted in live offspring (87). In principle a similar experimental design could be used to assay cultured human SSCs by mixing them with human testis cells (e.g., obtained from organ donors) and grafting them under the skin of immune deficient mice. The method could also be used to generate sperm for clinical application. However, to our knowledge de novo testicular morphogenesis with human testis cells has not been reported. Furthermore, de novo testicular morphogenesis has not been achieved using adult cells from any species and access to human fetal or neonatal cells is likely to be very limited. Baert and colleagues (89) recently reported decellularizing human testes and observed that human testis cells could infiltrate the three-dimensional scaffold. Perhaps in future studies human testis cells can be infused into decellularized human testis scaffold and grafted under the skin of mice to facilitate de novo testicular morphogenesis.

## Testicular Tissue Grafting and Xenografting

Honaramooz and colleagues (90) reported that testicular tissue from newborn mice, rats, pigs, and goats, in which spermatogenesis was not yet established, could mature and produce complete spermatogenesis when grafted under the skin of immune-deficient nude mice. The same group of investigators (91) later reported the production of live offspring from sperm obtained from mouse testicular tissue grafts. Fertilization-competent sperm was also produced from xenografts of prepubertal nonhuman primate testicular tissue transplanted into mice (92). These results suggest that it may be possible to obtain fertilization-competent sperm by xenografting small pieces of testicular tissue from a prepubertal cancer patient under the skin of mice or other animal recipients, such as pigs, that are already an established source for human food consumption, replacement heart valves (93, 94), and potentially other organs (95). Xenografting would also circumvent the issue of malignant contamination in cases such as leukemia where it would be unsafe to return testicular tissue or cells back into the body of a cancer survivor. However, the xenografting approach raises concerns about transmission of viruses from mice, pigs, and other species to human cells (96, 97). Also, at present there is no evidence that xenografted human testicular tissue can produce spermatogenesis or sperm in mice (98–103).

If malignant contamination of the testicular tissue is not a concern, autologous testicular tissue grafting can be considered. Luetjens and colleagues (104) demonstrated that fresh autologous testicular tissue grafts from prepubertal marmosets could produce complete spermatogenesis when transplanted into the scrotum, but not under the skin. Frozen and thawed grafts did not produce complete spermatogenesis in that study, but those grafts were only transplanted under the skin. Therefore, additional experimentation is merited. Testicular tissue grafting or xenografting will not restore natural fertility but could generate haploid sperm that can be used to fertilize oocytes by ICSI (Fig. 2B).

## Testicular Tissue Organ Culture

There is some limited evidence that haploid germ cells can be produced in culture without the supporting structure of the seminiferous tubules (105–107). However, fertilization has never been reported with those putative haploid cells. In contrast, Sato and colleagues (108, 109) reported that intact testicular tissue pieces from newborn mice could be maintained in organ culture and matured to produce complete spermatogenesis with fertilization-competent haploid germ cells. Testicular tissues were minced into pieces (1–3 $mm^3$) and placed in culture at the gas-to-liquid interface on a slab of agarose that was soaked in medium. Haploid round spermatids and sperm were recovered from the tissue after 3–6 weeks in culture and used to fertilize mouse eggs by ICSI. The resulting embryos were transferred to pseudopregnant females and gave rise to healthy offspring that matured to adulthood and were fertile. If testicular tissue organ culture can be translated to humans, it will provide an alternative to autologous SSC transplantation or autologous grafting in cases where there is concern about malignant contamination of the testicular tissue. Sato et al. (108) were also successful in producing haploid germ cells in organ culture of frozen and thawed testicular tissues, which is particularly relevant to the cancer survivor paradigm. However, the fertilization potential of those sperm was not tested (108). To our knowledge, human testicular tissue organ culture with production of haploid gametes has not been reported. As with de novo testicular morphogenesis, one of the challenges to developing human testicular tissue organ culture is the limited access to fetal, newborn, and/or prepubertal tissues.

## Pluripotent Stem Cell Technology

Induced pluripotent stem cells can be derived from any tissue of the body using a cocktail of reprogramming factors (110, 111). Several groups of investigators (105, 107, 112–123) have now reported that rodent, monkey, and human pluripotent embryonic stem cells or induced pluripotent stem cells can be differentiated into germ cells. Hayashi and co-workers (116) reported that it is possible to differentiate

embryonic stem cells or induced pluripotent stem cells into epiblast-like cells that then give rise to primordial germ cell-like cells when cultured in the presence of Bone Morphogenic Protein-4 (MBP4). The resulting germ cells were transplanted into the seminiferous tubules of infertile recipient mice where they regenerated spermatogenesis and haploid gametes that were used to fertilize mouse oocytes by ICSI. The embryos were transferred to recipient females and live offspring were obtained. However, some of the offspring developed tumors in the neck area and died prematurely, suggesting that further optimization of the culture and differentiation protocols will be required (116). The same groups of investigators also reported generation of epiblast-like cells and primordial germ cell-like cells from female-induced pluripotent stem cells. The resulting primordial germ cell-like cells were transplanted into recipients and functional eggs and live offspring were obtained (124). Two groups of investigators (121, 123) recently reported the differentiation of human pluripotent stem cells into putative human primordial germ cell-like cells, exhibiting gene expression patterns similar to bona fide human primordial germ cells. Unfortunately, the human studies cannot be validated by transplantation or the production of offspring. As mentioned previously, a surrogate assay of human spermatogenic potential is needed.

At present the recommended and best approach to preserve fertility is to obtain and freeze gametes or tissue before the initiation of therapy that can damage or eliminate germ cells (125, 126). However, if the induced pluripotent stem cell to germ cell differentiation technology is responsibly developed and translated to the human clinic, this fertility preservation paradigm could change. An adult survivor of a childhood cancer who desires to start his family and discovers that he is infertile could theoretically produce sperm and biological offspring from his own skin, blood, or other somatic cell type (Fig. 2C). This scenario applies not only to childhood cancer survivors, but all survivors or other infertile patients who cannot preserve or produce functional gametes.

## GENE THERAPIES FOR MALE FACTOR INFERTILITY

### The $1,000 Genome and Gene Therapy

It took 11 years, more than 200 scientists, and 3 billion dollars to sequence and publish the first draft of the human genome in 2001 (127, 128). This was a monumental achievement, but the exorbitant cost precluded sequencing of thousands or millions of genomes that would be necessary to uncover the genetic basis of many human diseases. In 2004, the National Human Genome Research Institute initiated the $1,000 genome project to stimulate unprecedented academic/industrial collaboration to improve speed and reduce cost of human genome sequencing (128). In 2015, the program has nearly achieved its goal of sequencing the whole human genome at the cost of about $5,000 and the whole exome sequencing at considerably less than that cost. This progress has accelerated the discovery of genes associated with human male factor infertility, which may in turn lead to the development of diagnostic screens and personalized treatment plans, perhaps including gene therapy.

### Germ Line Gene Therapy

Methods for genetic modification of germ line stem cells and Sertoli cells of the testis are well-established and used routinely in the basic research laboratory (38, 129–138). Germ cell gene therapy is technically feasible, but mired in ethical concerns that the genetic modification would be passed to progeny, thereby treating not only the infertile patient, but all subsequent generations. This subject matter is actively debated (139–144) and was a key topic for discussion at an International Summit on Human Gene Therapy that was jointly sponsored by the National Academies of Science, the National Academy of Medicine, the Chinese National Academy, and the Royal Society in December 2015 (http://www.nationalacademies.org/gene-editing/index.htm).

### Sertoli Cell Gene Therapy

In 2002, three groups independently demonstrated that in vivo Sertoli cell gene therapy could reverse the infertile phenotype in "Steel" mice that lack the Kit ligand in Sertoli cells (129, 130, 132). Steel mice are infertile with small testes that are completely devoid of spermatogenesis. The testes of Steel mice do contain rare undifferentiated spermatogonia that fail to differentiate in the absence of the Kit ligand, similar to the human phenotype of azoospermia with early maturation arrest. Reciprocal transplantation experiments revealed that the residual spermatogonia in Steel mouse testes are competent to produce complete spermatogenesis when transplanted into a permissive (Kit ligand positive) environment (37). Adenovirus (130), lentivirus (129), and electroporation (132) were used, respectively, to introduce a functional Kit ligand gene into the Sertoli cells of Steel mice. In all cases, spermatogenesis was partially restored and in two cases (adenovirus and lentivirus) sperm were recovered and used to produce offspring by ICSI and ET (129, 130). Important, the corrective transgene was not transmitted to offspring in either of those studies, suggesting that it may be possible to implement Sertoli cell gene therapy without the risk of germ line modification. However, a combined total of only 33 pups were evaluated in those two studies, therefore more rigorous assessments of germ line transmission risk are needed.

In humans, mutations in the Kit signaling pathway lead to the Piebald condition (145), which is characterized by patches of pale hair or skin, but is not associated with infertility. However, with the increasing accessibility of whole genome and whole exome sequencing technology, genes associated with human male factor infertility are being revealed at an increasingly rapid pace. Some examples include the germ cell genes SOHLH1 (146, 147) and TEX11 (148, 149) and somatic cell genes, androgen receptor (150–153), and NR5A1 (154, 155). The successes in the Steel mouse model may suggest that in vivo Sertoli cell gene therapies could be developed to treat infertility of men with somatic defects,

including androgen receptor and NR5A1. These examples are complicated because they have multiple endocrine phenotypes. However, Bashamboo and colleagues (154) identified NR5A1 mutations in men with spermatogenic failure, but who were otherwise healthy and these investigators commented that this broadens the range of NR5A1 phenotypes, which had previously been reported in more severe forms of gonadal dysgenesis. Similarly, androgen receptor mutations generate a range of phenotypes from complete sex reversal to mild phenotypes characterized primarily by male factor infertility (150–153). Compelling progress in basic research investigations may justify comprehensive genetic screening of patients with infertility to identify causative genes to facilitate counseling and possibly develop individualized treatments.

Safety and feasibility studies will be needed in rodents, nonhuman primates, and human cells to confirm that Sertoli cell gene therapy can be achieved without risk of germ line transmission and to carefully map genomic integrations of the therapeutic transgene. The risks of insertional mutagenesis (156, 157) may be reduced by using nonintegrating adenoviral vectors (158) or integration-deficient lentiviral vectors (159). Safety and feasibility studies will be particularly important for the human gene therapy field, which has already suffered serious setbacks due to unexpected adverse outcomes in previous trials (156, 160, 161).

In conclusion, 10%–15% of couples and 12% of men in the United States are subfertile or infertile (162, 163). Infertility is an insidious condition that impacts not only the ability to have biological children, but has broader implications for psychological well-being, relationships, finances (assisted reproduction and adoption can be expensive), general health, and life expectancy (163–167). Assisted reproductive technologies are available for men who produce even a small number of sperm in their ejaculates or testes. It is not possible for men who do not have cryopreserved or endogenous sperm to have biological children. This review describes several technologies that are currently in the research pipeline and may expand fertility options for men in the future. Each technology described in this review has produced functional sperm and progeny in at least one animal model, but none except SSC transplantation has been deployed in the human fertility clinic as of yet. In all cases, fundamental translational and preclinical studies of safety and feasibility are still needed. Nonhuman primates are expensive, but are amenable to transplantation/grafting studies that produce spermatogenesis and ART to produce progeny. Furthermore, stem cell dynamics, spermatogenic lineage development, and testis anatomy in nonhuman primates are similar to human (8, 168). Human tissue/cell studies are equally important, but challenged by limited availability of tissues and biological assays of spermatogenic potential and sperm function. With responsible basic, translational, and preclinical development, we believe that it is reasonable to expect that one or more of the experimental reproductive technologies described in this review will impact the male fertility clinic in the next decade.

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