Germline stem cells: toward the regeneration of spermatogenesis

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Improved therapies for cancer and other conditions have resulted in a growing population of long-term survivors. Infertility is an unfortunate side effect of some cancer therapies that impacts the quality of life of survivors who are in their reproductive or prereproductive years. Some of these patients have the opportunity to preserve their fertility using standard technologies that include sperm, egg, or embryo banking, followed by IVF and/or ET. However, these options are not available to all patients, especially the prepubertal patients who are not yet producing mature gametes. For these patients, there are several stem cell technologies in the research pipeline that may give rise to new fertility options and allow infertile patients to have their own biological children. We will review the role of stem cells in normal spermatogenesis as well as experimental stem cell–based techniques that may have potential to generate or regenerate spermatogenesis and sperm. We will present these technologies in the context of the fertility preservation paradigm, but we anticipate that they will have broad implications for the assisted reproduction field. (Fertil Steril® 2014;101:3–13. ©2014 by American Society for Reproductive Medicine.)

Key Words: Male fertility, male infertility, regenerative medicine, spermatogonial stem cells, stem cells

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High-dose chemotherapy, whole-body radiation, or radiation to the gonads can cause permanent infertility (1). This is a significant human health concern because over 75,000 people under the age of 40 in the United States are diagnosed with cancer each year and most are cured (2). Thus, cancer patients can look beyond their diagnosis and treatment to quality of life after cancer. Parenthood is important to cancer survivors, and distress over infertility can have long-term psychological and relationship implications (3). Therefore, the American Society for Clinical Oncology (ASCO) (4) and the American Society for Reproductive Medicine Ethics Committee (5) recommend that the reproductive risks of gonadotoxic therapies and options for preserving fertility be discussed with patients before initiating treatment. While adoption and third-party reproduction provide alternative family-building options, the available data indicate that most cancer survivors prefer to have their own biological children (4).

Postpubertal adolescent and adult males have the option to cryopreserve sperm before oncological treatment (Fig. 1, *top*). This is a simple and established method for preserving fertility potential and allows men to father their own genetic children. Nearly 17,000 men between the ages of 15 and 44 are diagnosed with cancer each year in the United States, and nearly 2,385 survivors will receive a treatment that puts them at high risk of azoospermia (2, 6). Unfortunately, only about 24% of men in this age range cryopreserve semen before their oncological treatment (7). Therefore, we calculate that each year in the United States, over 1,800 adult cancer survivors will be infertile with azoospermia and have limited options to have their

Received September 19, 2013; revised October 22, 2013; accepted October 28, 2013; published online December 5, 2013.
H.V. has nothing to disclose. B.T.P. has nothing to disclose. G.S. has nothing to disclose. J.A.B. has nothing to disclose. A.T.C. has nothing to disclose. M.L.M. has nothing to disclose. K.E.O. reports compensation for service on National Institutes of Health study section and honorarium as Keynote Speaker, American Association of Tissue Banks Annual Conference 2013.
Supported by National Institutes of Health Eunice Kennedy Shriver National Institute of Child Health and Human Development grant nos. HD055475 and HD061289; Magee-Womens Research Institute and Foundation; Richard King Mellon Foundation; and the United States–Israel Binational Science Foundation. (to K.E.O.).
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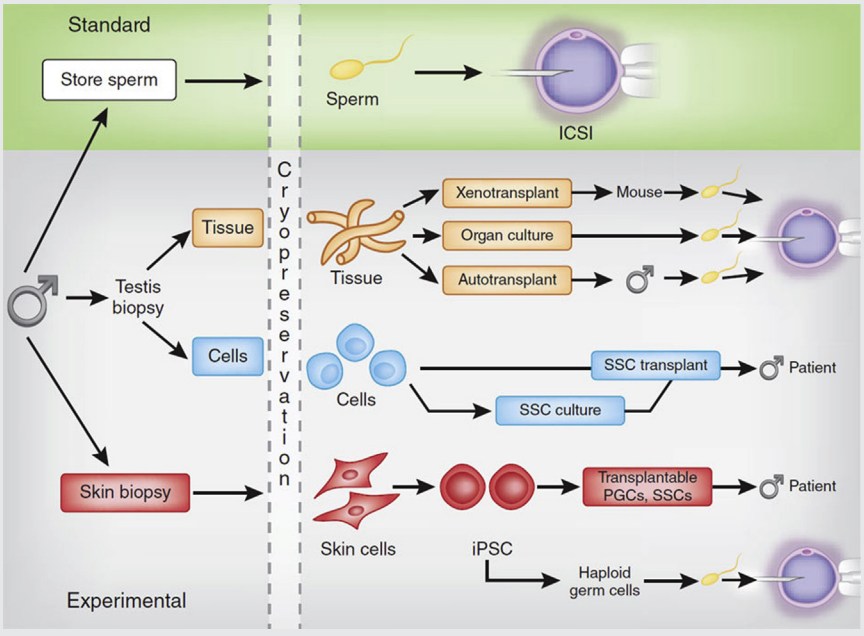
Fertility and Sterility® Vol. 101, No. 1, January 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.10.052

FIGURE 1



Standard and experimental options for preserving male fertility. *Top*, sperm obtained by ejaculation or surgical retrieval from the testes or epididymides are competent to fertilize oocytes using assisted reproductive techniques including IUI, IVF, or IVF ICSI that are standard in most fertility clinics. These options are not available to prepubertal boys who are not producing sperm or to adult azoospermic men. *Bottom*, testis tissue obtained via biopsy from prepubertal boys contains spermatogonial stem cells (SSCs) that can produce sperm in the context of the intact tissue by xenotransplant, organ culture, or autologous transplantation back into the individual (*orange boxes*). Sperm retrieved from cultured or transplanted tissue can be used for ICSI. Cells in suspension obtained from biopsied testicular tissue can be transplanted back into the endogenous seminiferous tubules of the patient (*blue boxes*). SSCs in the suspension can regenerate spermatogenesis and, in some cases, fertility. For infertile individuals who did not preserve germ cells before gonadotoxic therapy, iPSCs may be produced from their somatic cells (e.g., skin or blood) to differentiate into transplantable germ cells (primordial germ cells [PGCs] or SSCs) or haploid germ cells that can be used for ICSI (*red boxes*). Excerpted with permission from Clark AT, Phillips BT, Orwig KE, Nat Med 2000;1564–5.

Valli. Stem cell therapies for male factor infertility. Fertil Steril 2014.

own biological children because they did not save a semen sample. In some cases, sperm can be recovered surgically from small focal areas of spermatogenesis in the testes using the testicular sperm extraction method and can be used to fertilize oocytes by intracytoplasmic sperm injection (ICSI) (8).

There are no options to preserve the fertility of prepubertal boys, who are not yet making sperm. This is a significant problem because about 5,131 boys under the age of 15 in the United States are expected to develop cancer each year and 83% are expected to survive (2). A report from the Childhood Cancer Survivor Study indicates that the cytotoxic therapies for cancer reduce the number of young men subsequently able to have children by 44% (6, 9). Based on these statistics, we calculate that each year in the United States, 1,874 young male cancer patients will become sterile owing to their treatment. In addition to cancer survivors, over 500 patients under the age of 20 receive hematopoietic stem cell transplants each year in the United States for nonmalignant conditions (e.g., bone marrow failure, blood and immune deficiencies, autoimmune disorders) (10). Myeloablative conditioning therapy before bone marrow transplantation is associated with a high risk of infertility (4, 9, 11, 12). The ASCO report notes that "Impaired future fertility is difficult for children to understand, but potentially traumatic to them as adults" (4). The available data indicate that greater than 80% of parents consented to fertility preservation procedures on behalf of their children before initiation of gonadotoxic therapies (13, 14).

The summed incidence of chemotherapy or radiation-induced male factor infertility that cannot be treated with existing reproductive therapies is approximately 4,000 individuals each year in the United States. Therefore, responsible development of novel therapies to help these patients have biological children has a significant potential impact.

Promising results in animal models and human cell lines (Fig. 1, *bottom*) have generated enthusiasm that stem cells might be used or manipulated to preserve and/or restore the fertility of patients who are not producing sperm and have no other options to protect their future fertility before receiving gonadotoxic chemotherapy or radiation treatments (14–34). We will review the methods of spermatogonial stem cell (SSC) transplantation (Fig. 1, *blue boxes*), testicular tissue grafting, testicular tissue organ culture (Fig. 1, *orange boxes*), and induced pluripotent stem cell differentiation into gametes or transplantable male germ line stem cells (Fig. 1, *red boxes*). Table 1 summarizes published reports detailing the progress of each method. Enthusiasm for these experimental stem cell technologies is tempered by concerns about feasibility and safety, particularly for the vulnerable prepubertal