Middle East Fertility Society Journal (2012) 17, 1–7

Middle East Fertility Society

Middle East Fertility Society Journal

www.mefsjournal.com
www.sciencedirect.com

REVIEW

Spermatogonial stem cells as a source for regenerative medicine

Liang Ning [a,b,*], Ellen Goossens [a], Mieke Geens [a], Dorien Van Saen [a], Herman Tournaye [a,c]

[a] *Research Group Biology of the Testis (BITE), Department of Embryology and Genetics (EMGE), Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium*
[b] *Department of Urology, The First Affiliated Hospital of Medical School, Xi'an Jiaotong University, 710061 Xi'an, China*
[c] *Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium*

Received 3 April 2011; accepted 9 June 2011
Available online 25 August 2011

KEYWORDS

Differentiation;
Regenerative medicine;
Spermatogonial stem cell;
Transplantation

Abstract Several researchers have reported the derivation of multipotent cells from both mouse and human spermatogonial stem cells. These spermatogonia-derived stem cells show similarities with embryonic stem cells both for phenotype and functionality, indicating that these cells may be a promising alternative source for stem-cell based therapies in regenerative medicine.

Contents

* Corresponding author at: Research Group Biology of the Testis (BITE), Department of Embryology and Genetics (EMGE), Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium. Tel.: +32 2477 4644; fax: +32 2477 4632.
E-mail address: lianning@vub.ac.be (L. Ning).

Peer review under responsibility of Middle East Fertility Society.
doi:10.1016/j.mefs.2011.06.002

Production and hosting by Elsevier

1. Spermatogonial stem cells

Germ cell development starts with the specification of the primordial germ cells (PGCs) early in human fetal life. PGCs arise from the proximal epiblast and will translocate to the extra-embryonic mesoderm during the fourth and fifth week of embryonic development. By the end of the fifth week to early sixth week, PGCs will embark for a second migration via the dorsal mesenterium of the gut to the gonadal ridge. During their migration, PGCs proliferate but once they have reached the gonadal ridge, they enter into a mitotic arrest while differentiating into gonocytes. Shortly after birth, these gonocytes locate on the basal membrane of the seminiferous tubules where mitosis is reinitiated. From that moment on, they are called spermatogonial stem cells or SSCs (1). In the adult testis, only 0.03% of all germ cells are spermatogonial stem cells. They have the potential to self-renew and to differentiate in order to produce spermatozoa (2,3).

Different markers can be used to characterize spermatogonial stem cells: glial cell-line derived neurotrophic factor family receptor alpha 1 (GFRα1) (4), melanoma associated antigen family-A4 (MAGE-A4) (5), stimulated by retinoic acid gene 8 (Stra8) (6), α6 integrin (CD49f) (7), β1 integrin(7), Thy-1 (CD90) (8), CD9 (9), RET (10), stage-specific embryonic antigen-4 (SSEA-4) (11,12), ID4 (13) and for positive selection and c-kit (CD117) and MHC-1 (H-2K^b in mouse) for negative selection (8,14). Because no specific SSCs markers could be identified yet, the presence of SSCs can only be proven by a functional assay. For SSCs the only functional assay is the re-establishment of spermatogenesis in infertility recipients after spermatogonial stem cell transplantation (SSCT) (15) or by clonal proliferation in culture (16).

The most important role of SSCs is the unlimited production of sperm. In mammals, spermatogenesis is a strictly regulated and continuous process, during which the spermatogonial stem cells undergo three distinct phases: mitosis, meiosis and spermiogenesis followed by spermiation. Every day, 45–207 million spermatids are produced in the normal adult testes. This multiplication of germ cells is not regulated by the stem cells themselves but by the microenvironment surrounding the stem cells, i.e. the stem cell ''niche''.

The niche is defined as ''the microenvironment around stem cells that provides support and produces signals regulating self-renewal and differentiation'' (17). The niche can act on a stem cell through different mechanisms: niche cells can make direct contact with the stem cell, niche cells can secrete paracrine factors acting on the stem cell or intermediate cells can ''communicate'' between the niche and the stem cells.

In testes, Sertoli cells, peritubular myoid cells, Leydig cells and other interstitial cells contribute to the spermatogonial stem cell niche. Under the stimulation of follicle stimulating hormone (FSH), Sertoli cells produce and secrete glial cell-line derived neurotrophic factor (GDNF) which is an important self-renewal factor for spermatogonial stem cells. GDNF binds to the RET and the GFRα1 co-receptor mediating intracellular protein kinase signaling through the Src family kinase (SFK) and 3-kinase/serine–threonine kinase AKT family (PI3K/AKT) pathways. The latter regulate the expression of specific genes, such as Etv5 and Bcl6b, which are involved in SSC self-renewal. However, other regulatory genes, not regulated by GDNF, e.g. Zbtb16, Taf4b, and Lin28, are likely to be controlled by different signals and may block SSC differentiation (18).

2. Spermatogonial stem cells as a source for fertility restoration

Spermatogonial stem cell loss is an important cause of male infertility. Stem cell loss can occur after chemo- and radiotherapy (19–22) or due to a genetic disease, e.g. 47, XXY Klinefelter's syndrome or AZF deletion (23,24). Because children do not have the possibility to bank spermatozoa, the preservation and transplantation of SSCs may become an important strategy to treat reproductive stem cell loss disorders. In the last decade, a lot of research has been done on human SSC preservation and transplantation (25).

Spermatogonial stem cell transplantation was first reported in the mouse in 1994 by Brinster and Zimmermann (26). Spermatogenesis could be reinitiated in infertile recipient mice after transplantation of testicular cell suspensions from fertile donors. Shortly after, this technology was performed in other mammalian species, including primates (27–31). Even the transplantation between different species was proven successful (32,33). These encouraging results, especially those from primate studies, suggest a possibility of banking and transplanting human spermatogonial stem cells to prevent sterility caused by SSC loss.

However, several aspects must be investigated before any clinically applicable protocol can be developed. First, a successful protocol for long term storage of human SSCs or testicular tissue is mandatory. Because invariably there will be a long time period between the removal and the reintroduction of the SSCs to the patient, it is of utterly importance to keep the cells or tissue alive and functional during preservation. Avarbock and colleagues showed the reinitiation of spermatogenesis from SSC that had been frozen for 156 days (34). Six years later, a protocol for cryopreservation of bovine type-A spermatogonia, the cell population containing the spermatogonial stem cells, was reported by Izadyar and colleagues (35). However, as both development and structure of the testis are not similar between prepubertal boys and adults, it might also be important to preserve not only the spermatogonia but also the niche cells conserving all the cell–cell contacts within the

tissue. Several protocols have been studied for preserving human prepubertal tissue (36–38). Kvist et al. used a slow freezing protocol to freeze testicular tissue from cryptorchid prepubertal boys. They used 1.5 M ethylene glycol and 0.1 M sucrose as cryoprotectants. Keros et al. applied programmed slow freezing with 0.7 M Dimethylsulfoxide (DMSO) as cryoprotectant. Wyns et al. used a modified protocol developed from Keros' protocol in which DMSO 0.7 M was supplemented with 0.1 M sucrose and 10 mg/ml human serum albumin (HSA). They evaluated the functional capacity of the spermatogonia and Sertoli cells after thawing. Histology showed that 15% of the initial spermatogonial population survived and that 32% of these cells showed proliferative activity, compared to 18% before cryopreservation and grafting. The number of Sertoli cells remained unchanged and 5% were proliferating, compared to none before freezing and grafting. Vitrification, being an efficient method for preserving oocytes and ovarian tissue, was also employed for the preservation of human testicular tissue. Preliminary results show that this technique of ultra-rapid freezing has the potential to become an alternative strategy to controlled slow-freezing (39).

As an alternative to cryopreservation, in vitro culture of SSCs could be proposed. Nagano et al. reported the long term culture of SSCs without SSCs losing their proliferation and potential to differentiate (40). Kanatsu-Shinohara et al. reported SSC expansion in vitro in a complete absence of serum or somatic feeder cells allowing the production of higher numbers of SSCs which may be needed for a successful transplantation protocol in the clinic (41).

One of the major risks associated with autologous transplantation in cancer patients is the possibility of reintroducing malignant cells to the patient. Isolation of the spermatogonial stem cells from malignant cells before transplantation will be necessary. In 2005, Fujita et al. reported the use of fluorescence-activated cell sorting (FACS) for depleting cancer cells from murine testicular cell suspensions. They claimed that no tumor contamination was observed after transplantation into recipient mice (42). However, the results from us and other groups show that, after magnetic-activated cell sorting and/or FACS deletion, malignant cells still could be detected both by PCR and by a mouse transplantation assay (43,44). Recently, differential plating using a selective matrix adhesion was employed to enrich germ cells and deplete cancer cells from contaminated human testicular cell suspensions, but again the results were not satisfying (45). Further research is thus still needed before SSCT can become a clinical routine for cancer patients at risk of having malignant contamination of their testes.

In mouse and rat, the efferent duct has been shown to be an efficient site for reintroducing SSCs by injection. However, compared to mouse and rat testes, human testes are bigger and more fibrous. Therefore, the injection technique has to be modified. By using excised human testes donated by orchidectomy patients, several injection sites have been investigated: the seminiferous tubules, the rete testis, the epididymis and the deferent duct. Schlatt et al. have shown that ultrasound-guided intratesticular rete testis injection was the best and least invasive injection technique with maximal infusion efficiency for larger testes (monkey, bovine and human) (28). Labelled cells could be found in tubules close to the rete testis but not in tubules far from the rete testes. Brook et al. evaluated the efficiency of single and multiple injections through the rete testis of isolated human testes (46). However, the puncture into the testis was not monitored and it was unclear whether the injected dye had been injected in the seminiferous tubules or in the interstitial tissue or both.

Even if spermatogonial stem cell transplantation has been demonstrated to be a successful method to produce live offspring in a mouse model (47), genetic and epigenetic changes due to the procedure should be taken into consideration. Goossens et al. observed that after in vivo and in vitro conception, transplanted males produced smaller litter sizes compared to normal fertile control mice (48,49). However, the offspring showed normal karyotypes and methylation patterns (50,51). In another study, a detailed analysis of the motility kinematics and concentrations of spermatozoa obtained after transplantation was performed showing a lower sperm concentration and sperm motility after transplantation (52). The latter findings may explain the reduced litter size as observed in the in vivo mating studies.

Taking into account the importance of the spermatogonial stem niche in spermatogenesis, grafting testicular tissue may be an alternative to acquire functional sperm that is able to fertilize oocytes. In rodents and rabbits, complete spermatogenesis has been observed and fertile offspring could be obtained through assisted reproductive techniques after grafting immature testicular tissue (53,54). Grafting testicular tissue of human, however, did not yield spermatozoa. Our group found that after grafting adult human testicular tissue to immunodeficient recipient mice, adult spermatogonia could be maintained over a period of 6 months (55). In prepubertal xenografts, this period was even longer (9 months) (56). Recently, differentiation up to primary spermatocytes and the presence of a few secondary spermatocytes was reported in testicular tissue from a peripubertal boy (13 years old) after xenotransplantation to mouse (57).

Another potential risk in SSC transplantation is zoonosis. Animal-derived material such as serum and feeder layers are often employed in the preservation and banking of human testicular tissue and cells. Animal-derived viruses or bacteria may be transmitted to human cells, and eventually be introduced into the human body by transplantation, potentially impairing the health of both the recipients and the offspring. To avoid this risk, alternative non-animal derived material needs to be tested before any clinical application.

3. Spermatogonial stem cells as pluripotent cells

Recently, the pluripotency of SSCs from neonatal and adult mouse testes has been reported by different research teams. Kanatsu-Shinohara et al. reported the derivation of ES-like cells from neonatal mouse testes in culture. In their experiments, ES-like cells were shown to be phenotypically similar to embryonic stem cells and to have the ability to differentiate in vitro into various types of somatic cells and to produce teratomas after injection into nude mice. Furthermore, these ES-like cells formed germline chimeras when injected into blastocysts, which proves the pluripotency of the spermatogonial stem cells derived from neonatal testis (58). Subsequently, Guan et al. showed that SSCs from adult mouse testes could generate multipotent cells in vitro that were able to differentiate into different cell types of all three germ layers and to generate teratoma in immune-deficient mice. These multipotent

adult germline stem cells contributed to the development of various organs after injection into blastocysts (59).

Similar phenomena could be observed in human spermatogonial stem cells. Conrad et al. established an ES-like cell line derived from spermatogonial stem cells of adult human testis. The cells obtained cellular and molecular characteristics of human embryonic stem cells, but additionally these germline derived stem cells differentiated into various types of somatic cells of all three germ layers when grown under conditions used to induce the differentiation of human embryonic stem cells (60). Similar results were reported by other research groups (61,62). However, these findings should be interpreted with caution. Ko et al. compared adult germline stem cells (haGSCs) generated from adult human testis tissue with human embryonic stem cells (hESCs) and human testicular fibroblasts using global gene expression analysis. Surprisingly, they found that the "pluripotent" haGSCs had a similar gene expression profile to human testicular fibroblasts, but different from hESCs. These results put the conclusion of Conrad et al. into question (63). Geijsen et al. reviewed human and mouse pluripotent stem cells derived by different research groups, and found that these pluripotent stem cells can exist in a variety of distinct pluripotent states depending on the stage of the donor embryo, the strain background and the derivation conditions (64). For example, some of these testis-derived multipotent cells share more features with epiblast stem cells than with ESCs (58,65). This debate indicates the need for further characterization of these testis-derived multipotent cells before we may have a thoroughly understanding of their pluripotent potential.

4. Transdifferentiation of spermatogonial stem cells into other cell types

Transdifferentiation is defined as "the conversion of a differentiated cell of one developmental commitment into a differentiated cell of another lineage without first reverting to a more primitive stem cell or progenitor, with concomitant loss of tissue-specific markers and function of the original cell type, and acquisition of markers and function of the transdifferentiated cell type" (66). In human development, transdifferentiation is observed during embryogenesis, growth and regeneration (67).

Previous studies in mice showed the potential of SSCs to generate tissues of all three germ layers without first converting into a more de-differentiated state: functional neurons, glia (68,69), cardiomyocytes (70) and other somatic cell types (71).

Based on these experiments, but also on the common embryonic origin of SSCs and haematopoietic cells, we hypothesized that mouse SSCs may have the capability to transdifferentiate into haematopoietic cells. SSCs of GFP^+ male donor mice were isolated and injected into the bone marrow of Busulfan-treated GFP^- female mice. Twelve weeks post-transplantation, the recipients were sacrificed and their bone marrow, peripheral blood and spleen cells were collected and evaluated by phenotypical methods and functional assays. Donor cells could be observed in the bone marrow and the peripheral blood of recipient mice. These cells presented phenotypical and functional characteristics of haematopoietic cells in vitro and in vivo (72).

The mechanism of transdifferentiation is still unclear. Some researchers assume that cell reprogramming is a possible mechanism for cell transdifferentiation (73,74). Others suggest that SSCs might de-differentiate to pluripotency before differentiating into another tissue (75). Izadyar et al. put forward the idea that a subpopulation of pluripotent $Pou5f1^+$/$C\text{-}kit^+$ SSCs, different from the $Pou5f1^+$/$C\text{-}kit^-$ SSCs committed to the germ line, are transdifferentiating when exposed to a different microenvironment (62). In our research, the SSCs which were transplanted into the bone marrow of recipients might have undergone a similar procedure under the "pressure" of the bone marrow environment, i.e. their new stem cell "niche". They may have been induced into "bone-marrow-like cells" by paracrine factors secreted by the niche cells.

Previous studies indicate the plasticity of mouse SSCs and their potential for stem-cell based therapies. Some stem cell therapy methods, such as ESC or induced pluripotent stem cell (iPS) based therapies, have important disadvantages because of ethical controversies or oncogenic concerns. Compared to the generation of differentiated cells or tissues from allogeneic ESCs, transdifferentiation from adult stem cells could be a more feasible and easier method. Moreover, compared to ESC based research, there are less ethical constraints. Since the banking and transplantation of human SSCs may become a clinical routine for the preservation of male fertility in the future, there could be a clinical future for SSC based therapies as well.

5. Conclusion and future directions

Banking and transplantation of SSCs may become a promising method to preserve the fertility of prepubertal patients. Their testicular tissue could be removed and cryopreserved before therapy. After recovery, the tissue/cells could be grafted or transplanted into the patient's own testis and hopefully spermatogenesis will be reinitiated. For cancer patients, additional procedures would be necessary for the isolation of SSCs and the removal of malignant cells.

Another future option would be in vitro maturation of SSCs after isolation and malignant cell removal. By this means, functional spermatozoa could be derived from mouse SSCs (76). These in vitro produced spermatozoa could be used to fertilize oocytes by assisted reproductive techniques.

These above mentioned strategies could also be suitable for adult patients with non-obstructive azoospermia. After isolation and in vitro maturation, SSCs from these donors may have the capacity to produce functional spermatozoa. Although, before using these strategies in patients, more research is needed.

For prepubertal girls facing fertility loss, strategies have been developed to bank immature oocytes, ovarian tissue or the entire ovary. Banking and in vitro maturation of immature oocytes is a favorable strategy because of its feasibility in clinical applications (77). However, this strategy could be impossible due to the young age of the patients or the risks caused by hormonal stimulation (78). In these cases, the removal and cryopreservation of ovarian cortex (which contains thousands of immature follicles) or whole ovary freezing might become a reasonable alternative strategy. Grafting human ovarian cortex has been reported to be successfully leading to the resumption of ovarian function and live birth (79,80). The whole ovary freezing has been started by several groups as well, but as it may have higher chances of ischemia and only one chance

to be transplanted (81). A combination of ovarian cortical strip cryopreservation, follicle culture and oocyte maturation in vitro seems to be a promising method to protect the fertility of many women in the future.

Unlike SSCs, the existing of female germ line stem cells is still unidentified. Since ovaries only produce a finite number of eggs during post-pubertal life, it was generally believed that no germline stem cells were present in adult mammalian ovaries. Recently, this dogma was challenged by several discoveries. In 2004, Johnson et al. reported that the ovarian surface epithelium of adult mice is a source of proliferating female germ line stem cells (82). In the following studies, Johnson et al. and other research groups demonstrated that the bone marrow/peripheral blood could be served as a "pool" of putative stem cells for female germ cells regeneration (83,84). Furthermore, Bukovsky et al. proposed the derivation of primitive granulosa cells and germ cells from the mesenchymal cells (85). Although above results revealed the possibility of the presence of an extra/intra ovarian source of female germ cells, this new view already faced many criticisms. Further research is still needed to define the real "female germ stem cells".

The potential of human spermatogonial stem cells to de-differentiate into pluripotent cells or transdifferentiate into other cell types is interesting and may create an additional role for spermatogonial stem cell banking. Apart from fertility preservation, the banked cells could potentially be used as a source for stem cell therapy targeting other diseased organs. SSCs could be induced into multi- or pluripotent cells in vitro, by adding certain growth factors or inductive mesenchymes before their induction into the target cell type. Another strategy may be the direct injection into the human body at the site of injury or disease, e.g. bone marrow, myocardium. However, a number of problems remain before SSCs can be used as an alternative source for pluripotent stem cells. The major challenge is to obtain sufficient numbers of purified SSCs from a small piece of testicular biopsy. A large piece (about 1 g) of testicular biopsy contains less than 5 million cells with only 1500 being true stem cells. This is far from the number needed for cell therapy besides mentioning the very low de- and transdifferentiation rates. Therefore, an efficient method for SSC isolation and in vitro amplification has to be developed. Some research on the in vitro amplification of human adult SSCs has been reported (86), however, the propagation efficiency should be improved substantially.

An important challenge for any clinical application is to avoid the risk of teratoma formation after transplantation. The development of an efficient induction protocol and the regulation of the recipient's immunology system may be indispensable to eliminate the risk of teratoma formation.

Although at present there is not yet a clear clinical prospect for SSC-based cell therapy in the clinic, SSC banking may become a promising and practical strategy in the future.

References

(1) Ohta H, Wakayama T, Nishimune Y. Commitment of fetal male germ cells to spermatogonial stem cells during mouse embryonic development. Biol Reprod 2004;70:1286–91.

(2) Kubota H, Brinster RL. Technology insight: In vitro culture of spermatogonial stem cells and their potential therapeutic uses. Nat Clin Pract Endocrinol Metab 2006;2:99–108.

(3) de Rooij DG, Russell LD. All you wanted to know about spermatogonia but were afraid to ask. J Androl 2000;21: 776–98.

(4) Meng X, Lindahl M, Hyvonen ME, Parvinen M, de Rooij DG, Hess MW, et al. Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. Science 2000;287:1489–93.

(5) Aubry F, Satie AP, Rioux-Leclercq N, Rajpert-De Meyts E, Spagnoli GC, Chomez P, et al. MAGE-A4, a germ cell specific marker, is expressed differentially in testicular tumors. Cancer 2001;92:2778–85.

(6) Giuili G, Tomljenovic A, Labrecque N, Oulad-Abdelghani M, Rassoulzadegan M, Cuzin F. Murine spermatogonial stem cells: targeted transgene expression and purification in an active state. EMBO Rep 2002;3:753–9.

(7) Shinohara T, Avarbock MR, Brinster RL. Beta1- and alpha6-integrin are surface markers on mouse spermatogonial stem cells. Proc Natl Acad Sci U S A 1999;96:5504–9.

(8) Kubota H, Avarbock MR, Brinster RL. Spermatogonial stem cells share some, but not all, phenotypic and functional characteristics with other stem cells. Proc Natl Acad Sci U S A 2003;100:6487–92.

(9) Kanatsu-Shinohara M, Toyokuni S, Shinohara T. CD9 is a surface marker on mouse and rat male germline stem cells. Biol Reprod 2004;70:70–5.

(10) Naughton CK, Jain S, Strickland AM, Gupta A, Milbrandt J. Glial cell-line derived neurotrophic factor-mediated RET signaling regulates spermatogonial stem cell fate. Biol Reprod 2006;74:314–21.

(11) 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. Hum Reprod 2011.

(12) Maki CB, Pacchiarotti J, Ramos T, Pascual M, Pham J, Kinjo J, et al. Phenotypic and molecular characterization of spermatogonial stem cells in adult primate testes. Hum Reprod 2009;24:1480–91.

(13) Oatley MJ, Kaucher AV, Racicot KE, Oatley JM. Inhibitor of DNA binding 4 is expressed selectively by single spermatogonia in the male germline and regulates the self-renewal of spermatogonial stem cells in mice. Biol Reprod 2011.

(14) Yoshinaga K, Nishikawa S, Ogawa M, Hayashi S, Kunisada T, Fujimoto T. Role of c-kit in mouse spermatogenesis: identification of spermatogonia as a specific site of c-kit expression and function. Development 1991;113:689–99.

(15) Nagano M, Brinster RL. Spermatogonial transplantation and reconstitution of donor cell spermatogenesis in recipient mice. APMIS 1998;106:47–55 [discussion 6–7].

(16) Yeh JR, Zhang X, Nagano MC. Establishment of a short-term in vitro assay for mouse spermatogonial stem cells. Biol Reprod 2007;77:897–904.

(17) Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells 1978;4: 7–25.

(18) Wu X, Schmidt JA, Avarbock MR, Tobias JW, Carlson CA, Kolon TF, et al. Prepubertal human spermatogonia and mouse gonocytes share conserved gene expression of germline stem cell regulatory molecules. Proc Natl Acad Sci U S A 2009;106:21672–7.

(19) Wallace WH, Shalet SM, Crowne EC, Morris-Jones PH, Gattamaneni HR, Price DA. Gonadal dysfunction due to cisplatinum. Med Pediatr Oncol 1989;17:409–13.

(20) Wallace WH, Shalet SM, Lendon M, Morris-Jones PH. Male fertility in long-term survivors of childhood acute lymphoblastic leukaemia. Int J Androl 1991;14:312–9.

(21) Radford JA, Lieberman BA, Brison DR, Smith AR, Critchlow JD, Russell SA, et al. Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma. Lancet 2001;357:1172–5.

(22) Thomson AB, Campbell AJ, Irvine DC, Anderson RA, Kelnar CJ, Wallace WH. Semen quality and spermatozoal DNA integrity in survivors of childhood cancer: a case-control study. Lancet 2002;360:361–7.
(23) Navarro-Costa P, Plancha CE, Goncalves J. Genetic dissection of the AZF regions of the human Y chromosome: thriller or filler for male (in)fertility? J Biomed Biotechnol 2010;2010:936569.
(24) Giltay JC, Maiburg MC. Klinefelter syndrome: clinical and molecular aspects. Expert Rev Mol Diagn 2010;10:765–76.
(25) Geens M, Goossens E, De Block G, Ning L, Van Saen D, Tournaye H. Autologous spermatogonial stem cell transplantation in man: current obstacles for a future clinical application. Hum Reprod Update 2008;14:121–30.
(26) Brinster RL, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. Proc Natl Acad Sci U S A 1994;91:11298–302.
(27) Honaramooz A, Behboodi E, Megee SO, Overton SA, Galantino-Homer H, Echelard Y, et al. Fertility and germline transmission of donor haplotype following germ cell transplantation in immunocompetent goats. Biol Reprod 2003;69:1260–4.
(28) Schlatt S, Rosiepen G, Weinbauer GF, Rolf C, Brook PF, Nieschlag E. Germ cell transfer into rat, bovine, monkey and human testes. Hum Reprod 1999;14:144–50.
(29) Mikkola M, Sironen A, Kopp C, Taponen J, Sukura A, Vilkki J, et al.. Transplantation of normal boar testicular cells resulted in complete focal spermatogenesis in a boar affected by the immotile short-tail sperm defect. Reprod Domest Anim 2006;41:124–8.
(30) Kim Y, Turner D, Nelson J, Dobrinski I, McEntee M, Travis AJ. Production of donor-derived sperm after spermatogonial stem cell transplantation in the dog. Reproduction 2008;136:823–31.
(31) Hermann BP, Sukhwani M, Hansel MC, Orwig KE. Spermatogonial stem cells in higher primates: are there differences from those in rodents? Reproduction 2010;139:479–93.
(32) Dobrinski I, Avarbock MR, Brinster RL. Transplantation of germ cells from rabbits and dogs into mouse testes. Biol Reprod 1999;61:1331–9.
(33) Ogawa T, Dobrinski I, Avarbock MR, Brinster RL. Xenogeneic spermatogenesis following transplantation of hamster germ cells to mouse testes. Biol Reprod 1999;60:515–21.
(34) Avarbock MR, Brinster CJ, Brinster RL. Reconstitution of spermatogenesis from frozen spermatogonial stem cells. Nat Med 1996;2:693–6.
(35) Izadyar F, Matthijs-Rijsenbilt JJ, den Ouden K, Creemers LB, Woelders H, de Rooij DG. Development of a cryopreservation protocol for type A spermatogonia. J Androl 2002;23:537–45.
(36) Kvist K, Thorup J, Byskov AG, Hoyer PE, Mollgard K, Yding Andersen C. Cryopreservation of intact testicular tissue from boys with cryptorchidism. Hum Reprod 2006;21:484–91.
(37) Keros V, Hultenby K, Borgstrom B, Fridstrom M, Jahnukainen K, Hovatta O. Methods of cryopreservation of testicular tissue with viable spermatogonia in pre-pubertal boys undergoing gonadotoxic cancer treatment. Hum Reprod 2007;22:1384–95.
(38) Wyns C, Curaba M, Martinez-Madrid B, Van Langendonckt A, Francois-Xavier W, Donnez J. Spermatogonial survival after cryopreservation and short-term orthotopic immature human cryptorchid testicular tissue grafting to immunodeficient mice. Hum Reprod 2007;22:1603–11.
(39) Curaba M, Poels J, van Langendonckt A, Donnez J, Wyns C. Can prepubertal human testicular tissue be cryopreserved by vitrification? Fertil Steril 2011.
(40) Nagano M, Avarbock MR, Leonida EB, Brinster CJ, Brinster RL. Culture of mouse spermatogonial stem cells. Tissue Cell 1998;30:389–97.
(41) Kanatsu-Shinohara M, Miki H, Inoue K, Ogonuki N, Toyokuni S, Ogura A, et al. Long-term culture of mouse male germline stem cells under serum-or feeder-free conditions. Biol Reprod 2005;72:985–91.
(42) Fujita K, Ohta H, Tsujimura A, Takao T, Miyagawa Y, Takada S, et al. Transplantation of spermatogonial stem cells isolated from leukemic mice restores fertility without inducing leukemia. J Clin Invest 2005;115:1855–61.
(43) Geens M, Van de Velde H, De Block G, Goossens E, Van Steirteghem A, Tournaye H. The efficiency of magnetic-activated cell sorting and fluorescence-activated cell sorting in the decontamination of testicular cell suspensions in cancer patients. Hum Reprod 2007;22:733–42.
(44) Hou M, Andersson M, Zheng C, Sundblad A, Soder O, Jahnukainen K. Decontamination of leukemic cells and enrichment of germ cells from testicular samples from rats with Roser's T-cell leukemia by flow cytometric sorting. Reproduction 2007;134:767–79.
(45) 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.
(46) Brook PF, Radford JA, Shalet SM, Joyce AD, Gosden RG. Isolation of germ cells from human testicular tissue for low temperature storage and autotransplantation. Fertil Steril 2001;75:269–74.
(47) Brinster RL, Avarbock MR. Germline transmission of donor haplotype following spermatogonial transplantation. Proc Natl Acad Sci U S A 1994;91:11303–7.
(48) Goossens E, Frederickx V, De Block G, Van Steirteghem AC, Tournaye H. Reproductive capacity of sperm obtained after germ cell transplantation in a mouse model. Hum Reprod 2003;18:1874–80.
(49) Goossens E, Frederickx V, de Block G, van Steirteghem A, Tournaye H. Evaluation of in vivo conception after testicular stem cell transplantation in a mouse model shows altered post-implantation development. Hum Reprod 2006;21:2057–60.
(50) 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.
(51) 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.
(52) Goossens E, De Block G, Tournaye H. Computer-assisted motility analysis of spermatozoa obtained after spermatogonial stem cell transplantation in the mouse. Fertil Steril 2008;90:1411–6.
(53) Honaramooz A, Snedaker A, Boiani M, Scholer H, Dobrinski I, Schlatt S. Sperm from neonatal mammalian testes grafted in mice. Nature 2002;418:778–81.
(54) Schlatt S, Honaramooz A, Boiani M, Scholer HR, Dobrinski I. Progeny from sperm obtained after ectopic grafting of neonatal mouse testes. Biol Reprod 2003;68:2331–5.
(55) Geens M, De Block G, Goossens E, Frederickx V, Van Steirteghem A, Tournaye H. Spermatogonial survival after grafting human testicular tissue to immunodeficient mice. Hum Reprod 2006;21:390–6.
(56) Goossens E, Geens M, De Block G, Tournaye H. Spermatogonial survival in long-term human prepubertal xenografts. Fertil Steril 2008;90:2019–22.
(57) Van Saen D, Goossens E, Bourgain C, Ferster A, Tournaye H. Meiotic activity in orthotopic xenografts derived from human postpubertal testicular tissue. Hum Reprod 2011;26:282–93.
(58) Kanatsu-Shinohara M, Inoue K, Lee J, Yoshimoto M, Ogonuki N, Miki H, et al. Generation of pluripotent stem cells from neonatal mouse testis. Cell 2004;119:1001–12.
(59) Guan K, Nayernia K, Maier LS, Wagner S, Dressel R, Lee JH, et al.. Pluripotency of spermatogonial stem cells from adult mouse testis. Nature 2006;440:1199–203.

(60) Conrad S, Renninger M, Hennenlotter J, Wiesner T, Just L, Bonin M, et al. Generation of pluripotent stem cells from adult human testis. Nature 2008;456:344–9.
(61) Kossack N, Meneses J, Shefi S, Nguyen HN, Chavez S, Nicholas C, et al. Isolation and characterization of pluripotent human spermatogonial stem cell-derived cells. Stem Cells 2009;27:138–49.
(62) Izadyar F, Pau F, Marh J, Slepko N, Wang T, Gonzalez R, et al. Generation of multipotent cell lines from a distinct population of male germ line stem cells. Reproduction 2008;135:771–84.
(63) Ko K, Arauzo-Bravo MJ, Tapia N, Kim J, Lin Q, Bernemann C, et al. Human adult germline stem cells in question. Nature 2010;465:E1 [discussion E3].
(64) Geijsen N, Hochedlinger K. GPS navigates germ cells to pluripotency. Cell Stem Cell 2009;5:3–4.
(65) Seandel M, James D, Shmelkov SV, Falciatori I, Kim J, Chavala S, et al. Generation of functional multipotent adult stem cells from GPR125+ germline progenitors. Nature 2007;449:346–50.
(66) Wagers AJ, Weissman IL. Plasticity of adult stem cells. Cell 2004;116:639–48.
(67) Thowfeequ S, Myatt EJ, Tosh D. Transdifferentiation in developmental biology, disease, and in therapy. Dev Dyn 2007;236:3208–17.
(68) Glaser T, Opitz T, Kischlat T, Konang R, Sasse P, Fleischmann BK, et al. Adult germ line stem cells as a source of functional neurons and glia. Stem Cells 2008;26:2434–43.
(69) Streckfuss-Bomeke K, Vlasov A, Hulsmann S, Yin D, Nayernia K, Engel W, et al. Generation of functional neurons and glia from multipotent adult mouse germ-line stem cells. Stem Cell Res 2009;2:139–54.
(70) Guan K, Wagner S, Unsold B, Maier LS, Kaiser D, Hemmerlein B, et al. Generation of functional cardiomyocytes from adult mouse spermatogonial stem cells. Circ Res 2007;100: 1615–25.
(71) Simon L, Ekman GC, Kostereva N, Zhang Z, Hess RA, Hofmann MC, et al. Direct transdifferentiation of stem/progenitor spermatogonia into reproductive and nonreproductive tissues of all germ layers. Stem Cells 2009;27:1666–75.
(72) Ning L, Goossens E, Geens M, Van Saen D, Van Riet I, He D, et al. Mouse spermatogonial stem cells obtain morphologic and functional characteristics of hematopoietic cells in vivo. Hum Reprod 2010;25:3101–9.
(73) Baeyens L, Bouwens L. Can beta-cells be derived from exocrine pancreas? Diabetes Obes Metab 2008;10(4 Suppl):170–8.
(74) Eberhard D, Tosh D. Transdifferentiation and metaplasia as a paradigm for understanding development and disease. Cell Mol Life Sci 2008;65:33–40.
(75) Kanatsu-Shinohara M, Shinohara T. The germ of pluripotency. Nat Biotechnol 2006;24:663–4.
(76) 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.
(77) Saragusty J, Arav A. Current progress in oocyte and embryo cryopreservation by slow freezing and vitrification. Reproduction 2011;141:1–19.
(78) Rodriguez-Wallberg KA, Oktay K. Fertility preservation in women with breast cancer. Clin Obstet Gynecol 2010;53:753–62.
(79) Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 2004;364:1405–10.
(80) Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Restoration of ovarian function after orthotopic (intraovarian and periovarian) transplantation of cryopreserved ovarian tissue in a woman treated by bone marrow transplantation for sickle cell anaemia: case report. Hum Reprod 2006;21:183–8.
(81) Bromer JG, Patrizio P. Fertility preservation: the rationale for cryopreservation of the whole ovary. Semin Reprod Med 2009;27:465–71.
(82) Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004;428:145–50.
(83) Johnson J, Bagley J, Skaznik-Wikiel M, Lee HJ, Adams GB, Niikura Y, et al. Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. Cell 2005;122:303–15.
(84) Lee HJ, Selesniemi K, Niikura Y, Niikura T, Klein R, Dombkowski DM, et al. Bone marrow transplantation generates immature oocytes and rescues long-term fertility in a preclinical mouse model of chemotherapy-induced premature ovarian failure. J Clin Oncol 2007;25:3198–204.
(85) Bukovsky A, Caudle MR, Svetlikova M, Upadhyaya NB. Origin of germ cells and formation of new primary follicles in adult human ovaries. Reprod Biol Endocrinol 2004;2:20.
(86) 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. JAMA 2009;302:2127–34.