Adult Stem Cells in the Human Testis

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Abstract

The primary function of the mammalian testis is the production of both gametes and hormones over a reproductive lifespan. This production of gametes proceeds in the seminiferous tubules and is supported by a stem cell population, the spermatogonial stem cells (SSCs). Like other tissue-specific stem cells, SSCs are defined by their ability to balance between self-renewal and differentiation. This balance maintains the stem cell pool and guarantees the daily production of spermatozoa from puberty onward. Studying SSCs is difficult because they are very few in number (0.03% of all germ cells), and specific markers have not been identified so far. Most of our knowledge results from experiments in rodent models. Very little is known about human SSCs. This review provides an update on SSCs, on their role in male fertility, and on (future) clinical applications using these fascinating cells.

Keywords
- male infertility
- spermatogenesis
- stem cell
- transplantation

Spermatogonial Stem Cell Progenitors Populate the Testes during Fetal Development

Early in fetal development, the extraembryonic ectoderm expresses bone morphogenetic protein 4 and bone morphogenetic protein 8b, two growth factors indispensable for the development of primordial germ cells (PGCs).[1,2] In mice, PGCs are first observed 1 week postcoitum in the embryonal epiblast as a small cluster of alkaline phosphatase-positive cells.[3] During the formation of the allantois, the PGCs passively leave the embryo and start migrating via the hindgut to the genital ridges. During their journey, PGCs are proliferating. Migration, cell proliferation, and survival of PGCs all depend on the Kit-stem cell factor (SCF) system. PGCs, expressing the c-Kit receptor, are attracted by cells, producing SCF all along the migratory path.[4] Approximately 3000 PGCs will colonize the genital ridges.[5] These germ cells differ morphologically from the migratory PGCs and are therefore called gonocytes.[6] These gonocytes become enclosed in testicular cords formed by Sertoli cell precursors and peritubular myoid cells. Initially, gonocytes are located in the center of the testicular cords, away from the basal membrane. In rats and mice, gonocytes proliferate for a few days and then become quiescent in the G_0/G_1 phase of the cell cycle.[7–9] Shortly after birth, they migrate to the basement membrane and resume their proliferation giving rise to A_s-spermatogonia or spermatogonial stem cells (SSCs).[10,11]

Stem Cell Models Illustrate the Balance between Self-Renewal and Differentiation

SSC proliferation is slightly different for nonprimate and primate mammals. Because most research focuses on rodent spermatogenesis, both proliferation schemes are presented here.

Two models exist about stem cell renewal and spermatogonial multiplication in nonprimate mammals (the A_s model and the A_0/A_1 model), but the prevailing model is the A_s model, as proposed by Huckins and by Oakberg in 1971.[12,13] Spermatogenic proliferation/differentiation is accompanied by incomplete cell division, resulting in daughter cells that remain interconnected by intercellular bridges.[14] The A_s or single, undifferentiated type A spermatogonia, is considered to be the most primitive cell or "true" spermatogonial stem cell. When A_s spermatogonia divide into two A_s cells, they usually migrate separately. If they remain connected to each other by cytoplasmic bridges, they are likely to be A_{pr}. The production of type A_{pr} spermatogonia is the first step toward differentiation. These interconnected spermatogonia gradually lose their stem cell potential (see later). Type A_{pr}

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spermatogonia divide once more to produce groups of four type A_{al} spermatogonia, also connected to one another. The A_{al} cells proliferate resulting in chains of 8, 16, and occasionally 32 cells. Most of the A_{al} spermatogonia undergo a morphological change and transform into type A_1 spermatogonia. These A_1 spermatogonia are the first generation of differentiating A and B spermatogonia (►**Fig. 1**).

However, a revision of this A_s model might be necessary. Recently, several research groups found that the A_s population and spermatogonial chains of the same length are heterogeneous in respect to their gene expression[15–17] (►**Fig. 2**).

Using the transplantation assay developed by the Brinster group,[18,19] it was possible to demonstrate that stem cell activity is limited to the undifferentiated spermatogonia A_s, A_{pr}, and A_{al}.[20,21] Differentiating spermatogonia have a weaker potential to self-renew.[22]

In primates, two morphologically different classes of type A spermatogonia are observed: the dark A_d (or "reserve" stem cells) and the pale A_p spermatogonia (or "renewing" stem cells).[23] The self-renewal of the type A_p spermatogonia is analogous to that in the A_s model because most of the type A_p spermatogonia appear in clones of two, four, and eight cells, but single A_p cells also may exist. Furthermore, the A_d and A_p cells can transform into each other. A_d spermatogonia are often found in clusters. Such a cluster of A_d cells could be the result of a transformation of A_p into A_d at low renewal frequency. Conversely, after cytotoxic injury, the A_d may transform into A_p and start to proliferate.[24] Spermatogenesis is initiated by two divisions of pairs or quadruplets of A_p cells: a first division, after which clones of A_p separate, and a second division, which leads to clones of B_1 spermatogonia as well as pairs or quadruplets of A_p cells. These latter cells are responsible for the maintenance of the original size of the type A population. Because the A_p, which are found in clones of two or four cells, cycle continuously, the so-called true stem cells are probably the rarely dividing single A_p and A_d spermatogonia[25,26] (►**Fig. 3**).

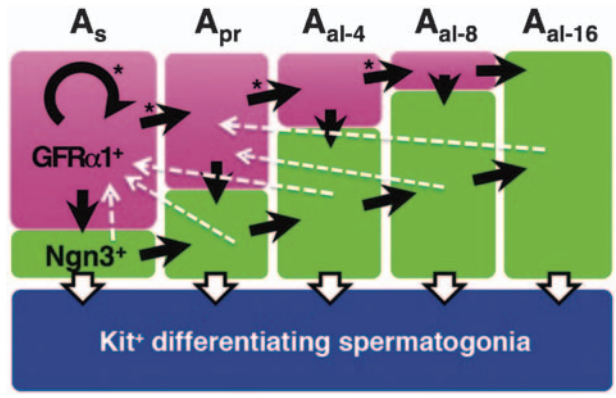

Figure 2 Proposed spermatogonial subpopulations. Black arrows indicate the direction of differentiation of most of the cells. Dashed lines show the ways of "reversion." Arrows without asterisks were actually observed; those with asterisks were not observed but are proposed to occur with high probability. (From Nakagawa T, Sharma M, Nabeshima Y, Braun RE, Yoshida S. Functional hierarchy and reversibility within the murine spermatogenic stem cell compartment. Science 2010;328:62–67. Used with permission.)

Spermatogonial Stem Cell Identity

Spermatogonia are small single cells located on the basement membrane of the seminiferous tubules. They have an ovoid nucleus with the nucleoli close to the nuclear membrane. The dense cytoplasm contains a small Golgi apparatus, few mitochondria, and many free ribosomes. The SSC population is only a very small subpopulation of the spermatogonia. A commonly used method to identify SSCs by their cell surface phenotype is fluorescent-activated cell sorting (FACS) in combination with SSC transplantation. The first results of this approach were reported by Shinohara's group, who found that SSCs were enriched for β_1-integrin (CD29) and α_6-integrin (CD49f) but did not express α_v-integrin (CD51).[27]

During subsequent years, more markers were identified. SSCs are positive for thymus cell antigen 1 (THY1 or CD90),

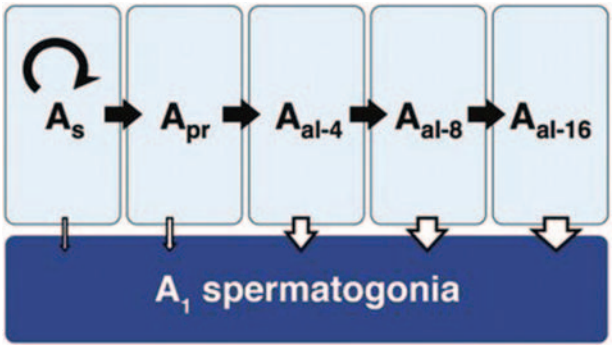

Figure 1 Schematic representation of the A_s model for nonprimate spermatogonial differentiation. Black arrows indicate the direction of differentiation. The width of the white arrows reflects the relative probability of differentiation to A_1 spermatogonia. (From Nakagawa T, Sharma M, Nabeshima Y, Braun RE, Yoshida S. Functional hierarchy and reversibility within the murine spermatogenic stem cell compartment. Science 2010;328:62–67. Used with permission.)

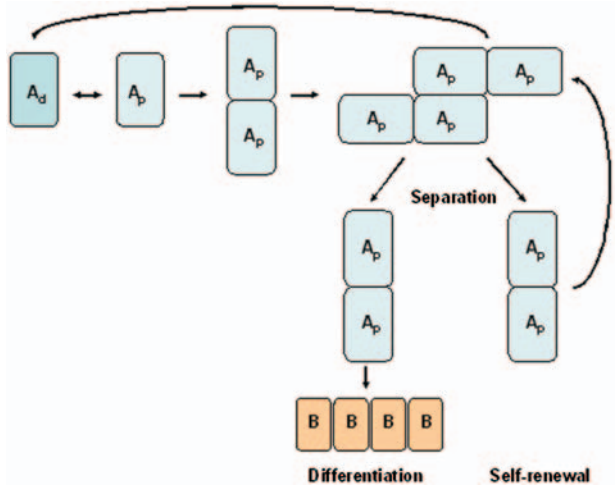

Figure 3 Schematic representation of the A_d/A_p model for primate spermatogonial differentiation.

CD9, glial cell line derived neurotropic factor (GDNF) family receptor α 1 (GFRα1), and E-cadherin,[28–31] and negative for major histocompatibility complex class I, C-Kit, and CD45.[32] Although it is possible to highly enrich cell populations for SSCs (100- to 200-fold) using combinations of positive and negative markers, a pure SSC suspension has not been obtained so far.

Apart from FACS, which is limited to the study of surface markers, other techniques are available to identify both cytoplasmic and nuclear SSC markers. Expression of green fluorescent protein (GFP) under the promoter of a candidate SSC gene is such a tool in mouse models. For example, octamer-binding transcription factor (OCT) 4-GFP mice enabled FACS-based isolation and transplantation of OCT4-expressing germ cells from a heterogeneous testis cell suspension.[21] Cells expressing OCT4 (GFP^+) showed higher stem cell activity compared with the $OCT4^-$ (GFP^-) cells.[33] Alternative approaches to reveal SSC-specific genes or proteins are transgenic, knock-in, or knockout mice. Using these models, investigators determined the expression of GDNF and its receptor GFRα1, promyelocytic leukemia zinc-finger (PLZF), SOX3, neurogenin 3, NANOS2, and stimulated by retinoic acid (STRA) 8 in undifferentiated spermatogonia.[34–40] SOX3 expression co-localizes with neurogenin 3 and is required for spermatogonial differentiation.[37] Because transplantation experiments showed that only 11% of transplantable SSCs were $neurogenin^+$, the idea arose that the SSC population is heterogeneous.[41] This heterogeneity among A_s-spermatogonia was confirmed by a study using whole-mount immunohistochemistry. Undifferentiated spermatogonia were identified by their location at the basement membrane of the seminiferous tubules, by the clone size, and by coexpression of known SSC markers. Although azoospermia-like protein (DAZL) is expressed throughout male germ cell development, its localization depends on the cell type. DAZL is found in spermatogonia in the nucleus but is transferred during meiosis to the cytoplasm.[42] Sal-like protein (SALL) 4, which is important to maintain pluripotency in mouse embryonic stem cells,[43] is specifically expressed in undifferentiated spermatogonia. Although SALL4 expression mostly overlaps with PLZF, its coexpression with GFRα1 revealed heterogeneity in the SSC population. $SALL4^+/GFR\alpha1^-$ and $SALL4^+/GFR\alpha1^+$ populations could be detected, with GFRα1 expression more limited (clones of 1 to 4 cells) than the expression of PLZF and SALL4 (also clones of 8 and 16 cells).[44] The fact that the SSC population does not consist of cells displaying all the same phenotype was also reported by other research groups.[15,16,31,38,45] The implications of this heterogeneity on SSC function remains to be elucidated.

Very recently, Oatley et al proposed a new marker for rodent SSCs.[17] Inhibitor of DNA binding (ID) 4 has the most restricted expression pattern observed to date. However, not all single spermatogonia do express this marker, suggesting heterogeneity among the pool of single spermatogonia. Whether the SSC pool resides entirely in the population of single spermatogonia or extends to pairs and aligned spermatogonia still needs to be clarified. Although the phenotype of rodent SSCs is almost unraveled, the search for markers limited to single A_s spermatogonia is still ongoing.

It has to be mentioned that, in damaged testes, not only A_s spermatogonia have the capacity to self-renew. A_{pr} and A_{al} spermatogonia were able to revert to the A_s state and start spermatogenesis.[41]

Human spermatogonia express many markers equivalent to those of rodent spermatogonia (e.g., α_6-integrin, GFRα1, THY1). However, other markers are not shared. For example, human SSCs do not express β_1-integrin[46] but are positive for testis-specific protein Y-linked (TSPY) 1, CD133, and stage-specific embryonic antigen (SSEA) 4.[47,48] Although during the last few years a lot of progress has been made concerning the characterization of human SSCs, further research is warranted.

Specialized Niches for Spermatogonial Stem Cells

Spermatogonial stem cells can develop in three different ways: They can renew themselves, they can differentiate, or they can go into apoptosis. The mechanism determining which pathway will be followed is the subject of a great deal of investigation. The discovery in 2000 of the stem cell niche in *Drosophila* has accelerated the study of this regulatory system.[49]

Niches are specialized microenvironments regulating tissue homeostasis by controlling stem cell self-renewal and differentiation. The SSC niche in the mammalian testis is located on the basal membrane of the seminiferous tubules and comprises Sertoli cells, peritubular myoid cells, and extracellular matrix components. Each niche houses one stem cell that is connected to the basement membrane through adhesion molecules (integrins). The fate of the stem cells is regulated by paracrine factors secreted by the niche. A key regulator of SSC self-renewal is glial cell line–derived neurotrophic factor (GDNF), which is secreted by Sertoli cells and acts on A_s, A_{pr}, and A_{al} spermatogonia through the RET/GFRα1 receptor complex (►**Fig. 4**).[34]

Sertoli cells are polarized columnar epithelial cells dividing the seminiferous tubule into a basal and an adlumenal compartment. The basal compartment comprises mainly spermatogonia, whereas the adlumenal compartment houses

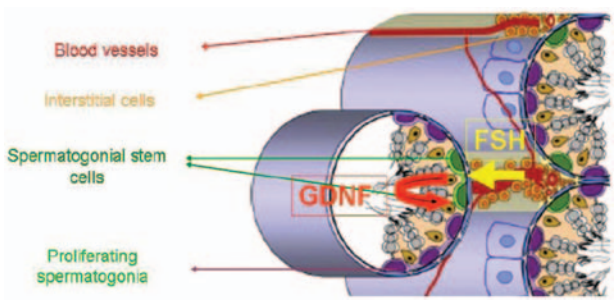

Figure 4 The spermatogonial stem cell (SSC) niche. Under the stimulation of follicle-stimulating hormone (FSH), Sertoli cells secrete glial cell–derived neurotrophic factor (GDNF). GDNF binds to SSCs and induces self-renewal.

the more advanced germ cells. The two compartments are separated by tight junctions between Sertoli cells, the so-called blood–testis barrier (BTB). Germ cells have to cross the BTB during germ cell differentiation. The opening of the BTB regulates germ cell development by permitting the passage of preleptotene and leptotene spermatocytes. In this way, the differentiation process from leptotene spermatocytes up to mature sperm is separated from the systemic circulation. As such, Sertoli cells can supply developing germ cells with the necessary nutrients and establish an immune-privileged environment for haploid germ cells.[50]

Only recently, the influence of peritubular myoid cells on germ cell regulation was revealed. Because colony-stimulating factor 1 expression was detected in Leydig and peritubular myoid cells, and its receptor was highly enriched in $THY1^+$ cells, a role on SSC maintenance was suggested.[51]

Not every germ cell located at the basal membrane is a SSC. Stem cell niches are not distributed randomly along the tubule but are thought to be localized in areas near the vasculature, implying a regulatory function for specific factors produced by the vascular endothelial cells.[52,53]

The cell density in the seminiferous tubules is kept constant by density-dependent degeneration of differentiating spermatogonia.[54] Because of these observations, it was suggested that there is no need for a precise regulatory mechanism to maintain the number of the stem cells in the normal testis. However, when the seminiferous epithelium is depleted by cytotoxic agents or irradiation, restoration of spermatogenesis must occur from stem cells. SSCs are less sensitive than differentiating spermatogonia but can still be lost. It was observed that, in this situation, the percentage of A_{pr} daughter cells was much lower than in the normal testis, which indicates that stem cells prefer self-renewal to differentiation.[55,56]

Spermatogonial Stem Cell Culture

In a first attempt to maintain SSCs in vitro, testicular cells were cultured on embryonic fibroblasts in a medium containing fetal bovine serum.[57] The efficacy of SSC culture could be improved by enriching the cell suspension for SSCs or by using pup testicular cells.[58] The addition of GDNF to the culture increased SSC self-renewal[34] and was a great step forward in the establishment of long-term mouse SSC culture systems.[59,60] Whereas fibroblast growth factor 2 was indispensable, feeder layers and serum could be omitted.[61,62] The exclusion of feeder cells and serum is a remarkable leap forward in the establishment of any clinical application.

The culture of SSCs from other mammalian species has been reported too,[63–66] but it was not until very recently that human SSC culture was realized. Sadri-Ardekani et al isolated and cultured testicular cells from six adult men[67] and two prepubertal boys.[68] Frozen-thawed human SSCs could be proliferated in vitro in the presence of epidermal growth factor, GDNF, and leukemia inhibitory factor without losing the expression of spermatogonial markers.

The Pluripotent Character of Spermatogonial Stem Cells

The pluripotent character of mouse SSCs has been reported by different research teams.[69–71] Kanatsu-Shinohara et al were able to derive cells from neonatal mouse testes in vitro, displaying phenotypical similarities to embryonic stem cells (ESCs). These ESC-like cells showed the potency to differentiate into various types of somatic cells in vitro and to induce teratoma formation after injection into nude mice. Furthermore, germline chimeras could be produced after injecting ESC-like cells into blastocysts.[69] Guan et al performed similar experiments with SSCs from adult mice. Adult SSCs generated cells that were able to differentiate in vitro into cell types of all three germ layers and to form teratomas once transplanted in immunodeficient mice. These cells also contributed to the development of various organs after injection into blastocysts.[70]

Similar findings were reported for human SSCs. ESC-like cell lines could be successfully established from adult human SSCs. The cells displayed cellular and molecular characteristics of human ESCs and differentiated into various cell types of the three germ layers.[47,72] However, these results have been put into question. When comparing human adult germline stem cells (GSCs) with human ESCs and human testicular fibroblasts, Ko et al found that the "pluripotent" adult GSCs had a similar gene expression profile to human testicular fibroblasts but different from ESCs.[73] It was reported before that some of the testis-derived cells shared more characteristics with epiblast stem cells than with ESCs.[69,74] This variety in pluripotent states might originate from differences in the derivation protocol.[75]

The debate on the plasticity of SSCs is still ongoing and indicates a need for further characterization of the testis-derived cells before we may have a thorough understanding of their pluripotent potential.

Fertility Preservation and Restoration

About 1 in every 600 children develops cancer before the age of 15 years. Thanks to the remarkable progress that has been made in the treatment of cancer in infants and children, today >80% of them can be cured. It has been estimated that, by now, 1 in 250 adults in the age group of 20 to 30 years is a childhood cancer survivor.[76,77] Besides cancer, other diseases requiring gonadotoxic treatments (e.g., sickle cell disease) or genetic diseases (e.g., Klinefelter syndrome, AZF deletions) may lead to spermatogonial stem cell loss.[78,79] It is unquestionable that the prevention of sterility needs special attention in both oncology and reproductive medicine. The inability to father his genetically own children can have a significant impact on the psychological well-being of the patient in later adulthood. Whereas adult patients can be offered sperm banking before SSC loss, no such option exists to preserve the fertility in prepubertal boys. The cryopreservation of spermatogonial stem cells before gonadotoxic therapy followed by autologous intratesticular transplantation of these stem cells after cure is possibly the only option (►**Fig. 5**).

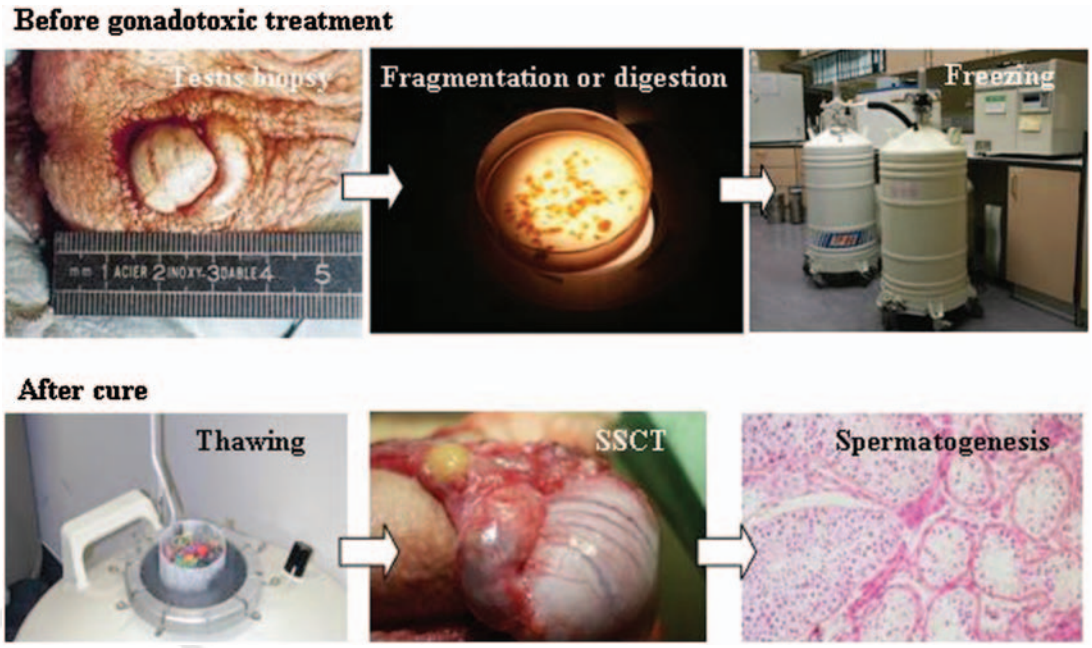

Figure 5 Spermatogonial stem cell transplantation as a method for fertility restoration. Testicular tissue is removed and cryopreserved before the onset of the cancer treatment. After the patient has been cured, the thawed tissue can be transplanted into the remaining testis. When the boy reaches puberty, spermatogenesis may be established.

Cryopreservation of Stem Cells

To safeguard the reproductive potential of young cancer patients, cryopreservation of testicular tissue containing SSCs is preferred above cryopreservation of SSC suspensions. Indeed, the presence of the extracellular matrix and supporting cells is critical to germ cell survival and germ cell function.[80] Any cryopreservation protocol should thus aim at preserving both the stem cells and their niche cells. Undoubtedly, cryopreservation of testicular tissue is a challenging task. The complexity of the tissue architecture demands optimal conditions for each cellular type. Controlled slow freezing with dimethyl sulphoxide is routinely used to cryopreserve immature testicular tissue.[81–84] In rodents, controlled slow freezing of prepubertal testicular tissue fragments has already led to the birth of healthy offspring.[85] Two teams have published freezing protocols for human testicular tissue using controlled rate freezing. Kvist et al reported the cryopreservation of testicular tissue in boys with cryptorchidism.[86] Later, Keros et al proposed a protocol for prepubertal testicular tissue.[87] Nevertheless, the drawback of controlled slow freezing is the need for expensive computerized equipment. Moreover, this freezing process consumes a lot of time and resources. Therefore, uncontrolled slow freezing has been explored. Like controlled freezing, uncontrolled freezing of prepubertal testicular tissue has been successfully used in different animal species, and it has been fully validated in mice as a means to preserve reproductive potential.[88–91] Recently, in piglets and mice, vitrification was shown to yield similar results compared with slow freezing.[92–94] Because both uncontrolled freezing and vitrification are inexpensive, convenient, and fast executable protocols, these methods might be considered for human testicular tissue too.[95]

Spermatogonial Stem Cell Transplantation

The technique of spermatogonial stem cell transplantation was first reported by Brinster and Zimmermann in 1994. It involves the introduction of a germ cell suspension from a fertile donor testis into the seminiferous tubules of an infertile recipient mouse.[18] Transplanted spermatogonial stem cells were able to relocate onto the basement membrane and colonize the tubules during the first month after transplantation. From that moment on, SSCs started to proliferate and initiated spermatogenesis. The first meiotic germ cells appear after 1 month, and their number gradually increases thereafter.[96] It has been shown that the recipient mice could reproduce in vivo after transplantation and produce transgenic offspring.[19] Similar experiments were performed using SSCs that had been frozen and thawed.[97] Shortly after, this technology was performed in other mammalian species including primates.[98–102] Even the transplantation between different species with close phylogeny was proven successful.[103,104] These encouraging results, especially those from primate studies, suggest a possibility of banking and subsequently transplanting human spermatogonial stem cells to prevent sterility caused by SSC loss.

Before this application can be introduced in a clinical setting, it is important to evaluate the efficiency and the safety of the procedure. In the mouse, it was shown that sperm cells obtained after spermatogonial stem cell transplantation were able to fertilize and produce normal embryos after assisted reproduction, although litter sizes were smaller compared with normal fertile control mice.[105] A detailed analysis of the motility kinematics and concentrations of spermatozoa showed a lower sperm concentration

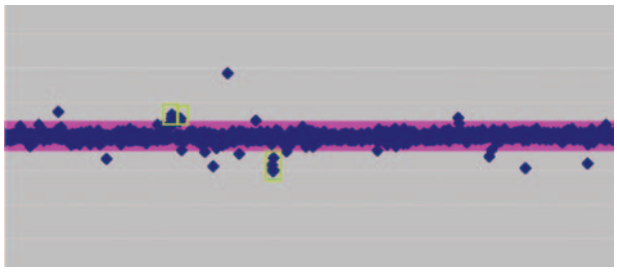

Figure 6 Array comparative genomic hybridization on offspring after spermatogonial stem cell transplantation. A detailed graph of chromosome 17 of first-generation offspring. Every genomic gain or loss, detected in the offspring (green box), were found to be polymorphisms.

and sperm motility after transplantation.[106] In contrast, when donor and recipient were genetically related, the offspring showed normal genetic and epigenetic characteristics for most of the investigated modifications (►**Figs. 6** and **7**).[107–109] Only histone 4 lysines 5 and 8 acetylation, which is important in spermatids for the histone-to-protamine exchange, was impaired in spermatogonia and spermatocytes. The function of H4K5ac and H4K8ac in these cell types still has to be explored.

Testicular Tissue Grafting

Testicular tissue grafting has been suggested as an alternative to SSC transplantation. Testis tissue has been grafted under the back skin, in the scrotum, or in the testis. Mature spermatozoa could be obtained from ectopic grafts,[110] and progeny were born using intracytoplasmic sperm injection.[111] Full spermatogenesis was obtained in grafts using immature testis tissue from different species.[110,112–114] Ectopic grafting was also performed using human testicular tissue. The first reports using adult testicular tissue showed only limited spermatogonial survival, with most of the tubules completely regressed.[115,116] Using prepubertal and neonatal tissue, spermatogonial survival[91] and differentiation up to primary spermatocytes[117] were reported in ectopic grafts. In an attempt to improve the results after grafting, immature testicular tissue was placed in the peritoneal bursa inside the scrotum. Long-term survival of spermatogonia and differentiation up to pachytene spermatocytes was observed.[118]

Intratesticular tissue transplantation was first reported in 2002. Sperm was produced after grafting cryopreserved immature testicular tissue from mice and rabbits into the testicular parenchyma. Progeny were born when rabbit sperm from the xenograft was used for microinsemination.[119] A comparison of intratesticular grafting and SSC transplantation (SSCT) in a mouse model showed a better reestablishment of spermatogenesis after grafting. Whereas some epigenetic modifications were found to be altered after SSCT, this was not the case after grafting.[109]

Cryopreservation of the grafts did not adversely affect the colonization efficiency and restoration of spermatogenesis.[120] Although intratesticular grafting seems to be more efficient, it can only be offered to patients with nonmalignant diseases or nonmetastasizing tumors.

Removal of Contaminating Malignant Cells

Many pediatric malignancies are capable of metastasizing through the blood, causing a potential risk of contamination of the collected testicular tissue. The transplantation of as few as 20 leukemic cells could cause malignant recurrence in rats.[121] In the human, the threshold number of malignant cells able to cause malignant relapse when transplanted to the testis is unknown. Therefore, it is of utmost importance to detect even the slightest contamination of the testicular tissue. In the case of contamination, the isolation of SSCs from malignant cells before transplantation is necessary. Apart from ours, another research group studied the use of magnetic-activated cell sorting and/or FACS for depleting cancer cells from mouse and human testicular cell suspensions. However, both reported insufficient depletion.[122–124] Also cell selection by selective matrix adhesion was not efficient enough.[125]

Cell Therapy

Regenerative Medicine

Methods for stem cell therapy based on ESC or induced pluripotent stem cells have important disadvantages because of their tumorigenesis or ethical controversies. Transdifferentiation from (autologous) adult stem cells could be a more feasible and easier method. Moreover, compared with

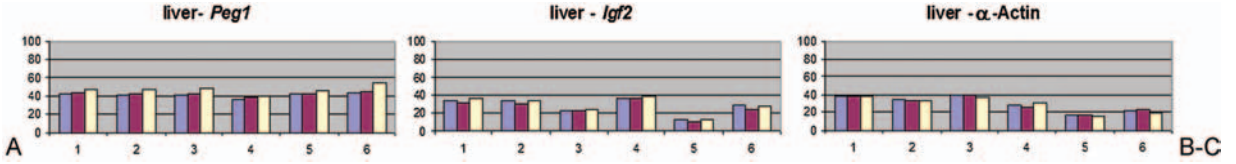

Figure 7 Methylation status of six cytosine phosphate guanine (CpG) sites of (A) *Peg*1, (B) *Igf*2, and (C) α-Actin in the liver of posttransplantation offspring. The X-axis represents the analyzed CpG sites (1 to 6); the Y-axis shows the methylation percentage. Light violet boxes represent the data obtained from first-generation offspring; dark violet boxes show the data obtained from second-generation offspring, and light yellow boxes show the control data. The methylation status of control and first- or second-generation offspring did not show significant differences.

ESC-based research, there are fewer ethical constraints. SSCs share many molecular characteristics with ESC, providing new and unique opportunities for the therapeutic use of SSCs for regenerative medicine. The banking and transplantation of human SSCs may become a clinical routine for the preservation of male fertility in the future, so there could be a clinical future for SSC-based therapies as well.

Studies in mice showed the potential of SSCs to generate tissues of all three germ layers (functional neurons, glia, cardiomyocytes, and other somatic cell types) without first converting into a less differentiated state.[126–129] Our research group was able to transdifferentiate SSCs into hematopoietic cells in vivo. The donor-derived cells presented phenotypical and functional characteristics of hematopoietic cells in vitro and in vivo.[130] However, the mechanism of transdifferentiation is still unclear. Some researchers believe in cell reprogramming.[131,132] Others suggest that SSCs first become pluripotent before differentiating into another cell type.[133] It is possible that a certain subpopulation of SSCs is pluripotent (Pou5f1$^+$/C-kit$^+$), whereas the Pou5f1$^+$/C-kit$^-$ SSCs are committed to the germ line. The pluripotent SSCs may transdifferentiate when exposed to a different microenvironment.[134]

Transgenerational Therapy

Spermatogonial stem cells are the only stem cells in humans that can transmit parental genetic information to the offspring, making them an attractive target cell population for transgenerational gene therapy. Mouse SSCs have been successfully transfected with the use of a retroviral vector. The co-injection of retroviral particles and germ cells into recipient testes also results in incorporation of the reporter gene. It is now possible to transfect both adult and immature stem cells by retroviral-mediated gene delivery in vitro[135] and in vivo by using a retrovirus vector.[136]

Concluding Thoughts

Banking and transplantation of SSCs may become a promising method to preserve the fertility of prepubertal patients. According to recent discoveries, the potential of SSCs to become pluripotent or to transdifferentiate into other cell types is interesting and may create an additional role for SSCs as a source for stem cell therapy. Nevertheless, the methods established in mice still need to be tested and adapted to human applications.

Acknowledgments

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References

1 Lawson KA, Dunn NR, Roelen BA, et al. Bmp4 is required for the generation of primordial germ cells in the mouse embryo. Genes Dev 1999;13(4):424–436
2 Ying Y, Qi X, Zhao GQ. Induction of primordial germ cells from murine epiblasts by synergistic action of BMP4 and BMP8B signaling pathways. Proc Natl Acad Sci U S A 2001;98(14): 7858–7862
3 Ginsburg M, Snow MH, McLaren A. Primordial germ cells in the mouse embryo during gastrulation. Development 1990;110 (2):521–528
4 Fleischman RA. From white spots to stem cells: the role of the Kit receptor in mammalian development. Trends Genet 1993;9 (8):285–290
5 Bendel-Stenzel M, Anderson R, Heasman J, Wylie C. The origin and migration of primordial germ cells in the mouse. Semin Cell Dev Biol 1998;9(4):393–400
6 Sapsford CS. Changes in the cells of the sex cords and the seminiferous tubules during development of the testis of the rat and the mouse. Aust J Zool 1962;10:178–192
7 Tam PP, Snow MHL. Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. J Embryol Exp Morphol 1981;64:133–147
8 McLaren A. Primordial germ cells in the mouse. Dev Biol 2003;262(1):1–15
9 Töhönen V, Ritzén EM, Nordqvist K, Wedell A. Male sex determination and prenatal differentiation of the testis. Endocr Dev 2003;5:1–23
10 Huckins C, Clermont Y. Evolution of gonocytes in the rat testis during late embryonic and early post-natal life. Arch Anat Histol Embryol 1968;51(1):341–354
11 Vergouwen RPFA, Jacobs SGPM, Huiskamp R, Davids JAG, de Rooij DG. Proliferative activity of interstitial cells, Sertoli cells and gonocytes during testicular development in the mouse. J Reprod Fertil 1991;93:233–243
12 Huckins C. The spermatogonial stem cell population in adult rats. I. Their morphology, proliferation and maturation. Anat Rec 1971;169(3):533–557
13 Oakberg EF. Spermatogonial stem-cell renewal in the mouse. Anat Rec 1971;169(3):515–531
14 de Rooij DG, Russell LD. All you wanted to know about spermatogonia but were afraid to ask. J Androl 2000;21(6):776–798
15 Suzuki H, Sada A, Yoshida S, Saga Y. The heterogeneity of spermatogonia is revealed by their topology and expression of marker proteins including the germ cell-specific proteins Nanos2 and Nanos3. Dev Biol 2009;336(2):222–231
16 Zheng K, Wu X, Kaestner KH, Wang PJ. The pluripotency factor LIN28 marks undifferentiated spermatogonia in mouse. BMC Dev Biol 2009;9:38
17 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;85(2): 347–356
18 Brinster RL, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. Proc Natl Acad Sci U S A 1994;91(24): 11298–11302
19 Brinster RL, Avarbock MR. Germline transmission of donor haplotype following spermatogonial transplantation. Proc Natl Acad Sci U S A 1994;91(24):11303–11307
20 Shinohara T, Orwig KE, Avarbock MR, Brinster RL. Spermatogonial stem cell enrichment by multiparameter selection of mouse testis cells. Proc Natl Acad Sci U S A 2000;97(15):8346–8351
21 Ohbo K, Yoshida S, Ohmura M, et al. Identification and characterization of stem cells in prepubertal spermatogenesis in mice small star, filled. Dev Biol 2003;258(1):209–225

22 Barroca V, Lassalle B, Coureuil M, et al. Mouse differentiating spermatogonia can generate germinal stem cells in vivo. Nat Cell Biol 2009;11(2):190–196
23 Clermont Y, Hermo L. Spermatogonial stem cells and their behavior in the seminiferous epithelium of rats and monkeys. In: Cairnie AB, Lala PK, Osmond DG, eds. Stem Cells of Renewing Cell Populations. New York, NY: Academic Press; 1976:273–286
24 van Alphen MMA, van de Kant HJG, de Rooij DG. Depletion of the spermatogonia from the seminiferous epithelium of the rhesus monkey after X irradiation. Radiat Res 1988;113(3):473–486
25 Ehmcke J, Luetjens CM, Schlatt S. Clonal organization of proliferating spermatogonial stem cells in adult males of two species of non-human primates, *Macaca mulatta* and *Callithrix jacchus*. Biol Reprod 2005;72(2):293–300
26 Ehmcke J, Simorangkir DR, Schlatt S. Identification of the starting point for spermatogenesis and characterization of the testicular stem cell in adult male rhesus monkeys. Hum Reprod 2005;20 (5):1185–1193
27 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(10):5504–5509
28 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 (11):6487–6492
29 Kanatsu-Shinohara M, Toyokuni S, Shinohara T. CD9 is a surface marker on mouse and rat male germline stem cells. Biol Reprod 2004;70(1):70–75
30 Buageaw A, Sukhwani M, Ben-Yehudah A, et al. GDNF family receptor alpha1 phenotype of spermatogonial stem cells in immature mouse testes. Biol Reprod 2005;73(5):1011–1016
31 Tokuda M, Kadokawa Y, Kurahashi H, Marunouchi T. CDH1 is a specific marker for undifferentiated spermatogonia in mouse testes. Biol Reprod 2007;76(1):130–141
32 Fujita K, Ohta H, Tsujimura A, et al. Transplantation of spermatogonial stem cells isolated from leukemic mice restores fertility without inducing leukemia. J Clin Invest 2005;115(7): 1855–1861
33 Ohmura M, Yoshida S, Ide Y, Nagamatsu G, Suda T, Ohbo K. Spatial analysis of germ stem cell development in Oct-4/EGFP transgenic mice. Arch Histol Cytol 2004;67(4):285–296
34 Meng X, Lindahl M, Hyvönen ME, et al. Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. Science 2000;287(5457):1489–1493
35 Costoya JA, Hobbs RM, Barna M, et al. Essential role of Plzf in maintenance of spermatogonial stem cells. Nat Genet 2004;36 (6):653–659
36 Yoshida S, Takakura A, Ohbo K, et al. Neurogenin3 delineates the earliest stages of spermatogenesis in the mouse testis. Dev Biol 2004;269(2):447–458
37 Raverot G, Weiss J, Park SY, Hurley L, Jameson JL. Sox3 expression in undifferentiated spermatogonia is required for the progression of spermatogenesis. Dev Biol 2005;283(1):215–225
38 Sada A, Suzuki A, Suzuki H, Saga Y. The RNA-binding protein NANOS2 is required to maintain murine spermatogonial stem cells. Science 2009;325(5946):1394–1398
39 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(8):753–759
40 Sadate-Ngatchou PI, Payne CJ, Dearth AT, Braun RE. Cre recombinase activity specific to postnatal, premeiotic male germ cells in transgenic mice. Genesis 2008;46(12):738–742
41 Nakagawa T, Nabeshima Y, Yoshida S. Functional identification of the actual and potential stem cell compartments in mouse spermatogenesis. Dev Cell 2007;12(2):195–206
42 Reijo RA, Dorfman DM, Slee R, et al. DAZ family proteins exist throughout male germ cell development and transit from nucleus to cytoplasm at meiosis in humans and mice. Biol Reprod 2000;63 (5):1490–1496
43 Yang J, Chai L, Fowles TC, et al. Genome-wide analysis reveals Sall4 to be a major regulator of pluripotency in murine-embryonic stem cells. Proc Natl Acad Sci U S A 2008;105(50):19756–19761
44 Phillips BT, Gassei K, Orwig KE. Spermatogonial stem cell regulation and spermatogenesis. Philos Trans R Soc Lond B Biol Sci 2010;365(1546):1663–1678
45 Grisanti L, Falciatori I, Grasso M, et al. Identification of spermatogonial stem cell subsets by morphological analysis and prospective isolation. Stem Cells 2009;27(12):3043–3052
46 Dym M, He Z, Jiang J, Pant D, Kokkinaki M. Spermatogonial stem cells: unlimited potential. Reprod Fertil Dev 2009;21(1):15–21
47 Conrad S, Renninger M, Hennenlotter J, et al. Generation of pluripotent stem cells from adult human testis. Nature 2008;456(7220):344–349
48 Izadyar F, Wong J, Maki C, et al. Identification and characterization of repopulating spermatogonial stem cells from the adult human testis. Hum Reprod 2011;26(6):1296–1306
49 Tran J, Brenner TJ, DiNardo S. Somatic control over the germline stem cell lineage during *Drosophila* spermatogenesis. Nature 2000;407(6805):754–757
50 Cheng CY, Mruk DD. Cell junction dynamics in the testis: Sertoli-germ cell interactions and male contraceptive development. Physiol Rev 2002;82(4):825–874
51 Oatley JM, Oatley MJ, Avarbock MR, Tobias JW, Brinster RL. Colony stimulating factor 1 is an extrinsic stimulator of mouse spermatogonial stem cell self-renewal. Development 2009;136(7): 1191–1199
52 Yoshida S, Sukeno M, Nabeshima Y. A vasculature-associated niche for undifferentiated spermatogonia in the mouse testis. Science 2007;317(5845):1722–1726
53 Ogawa T, Ohmura M, Ohbo K. The niche for spermatogonial stem cells in the mammalian testis. Int J Hematol 2005;82(5): 381–388
54 De Rooij DG, Lok D. Regulation of the density of spermatogonia in the seminiferous epithelium of the Chinese hamster: II. Differentiating spermatogonia. Anat Rec 1987;217(2):131–136
55 Van Keulen CJG, de Rooij DG. Spermatogenic clones developing from repopulating stem cells surviving a high dose of an alkylating agent. I. First 15 days after injury. Cell Tissue Kinet 1975;8:543–551
56 van Beek MEAB, Meistrich ML, de Rooij DG. Probability of self-renewing divisions of spermatogonial stem cells in colonies, formed after fission neutron irradiation. Cell Tissue Kinet 1990;23(1):1–16
57 Nagano M, Avarbock MR, Leonida EB, Brinster CJ, Brinster RL. Culture of mouse spermatogonial stem cells. Tissue Cell 1998;30 (4):389–397
58 Nagano M, Shinohara T, Avarbock MR, Brinster RL. Retrovirus-mediated gene delivery into male germ line stem cells. FEBS Lett 2000;475(1):7–10
59 Kanatsu-Shinohara M, Ogonuki N, Inoue K, et al. Long-term proliferation in culture and germline transmission of mouse male germline stem cells. Biol Reprod 2003;69(2):612–616
60 Kubota H, Avarbock MR, Brinster RL. Growth factors essential for self-renewal and expansion of mouse spermatogonial stem cells. Proc Natl Acad Sci U S A 2004;101(47):16489–16494
61 Kanatsu-Shinohara M, Ogonuki N, Iwano T, et al. Genetic and epigenetic properties of mouse male germline stem cells during long-term culture. Development 2005;132(18):4155–4163
62 Kanatsu-Shinohara M, Inoue K, Ogonuki N, Morimoto H, Ogura A, Shinohara T. Serum- and feeder-free culture of mouse germline stem cells. Biol Reprod 2011;84(1):97–105
63 Ryu BY, Kubota H, Avarbock MR, Brinster RL. Conservation of spermatogonial stem cell self-renewal signaling between mouse and rat. Proc Natl Acad Sci U S A 2005;102(40):14302–14307

64 Wu Z, Falciatori I, Molyneux LA, Richardson TE, Chapman KM, Hamra FK. Spermatogonial culture medium: an effective and efficient nutrient mixture for culturing rat spermatogonial stem cells. Biol Reprod 2009;81(1):77–86

65 Kanatsu-Shinohara M, Muneto T, Lee J, et al. Long-term culture of male germline stem cells from hamster testes. Biol Reprod 2008;78(4):611–617

66 Aponte PM, Soda T, Teerds KJ, Mizrak SC, van de Kant HJ, de Rooij DG. Propagation of bovine spermatogonial stem cells in vitro. Reproduction 2008;136(5):543–557

67 Sadri-Ardekani H, Mizrak SC, van Daalen SK, et al. Propagation of human spermatogonial stem cells in vitro. JAMA 2009;302 (19):2127–2134

68 Sadri-Ardekani H, Akhondi MA, van der Veen F, Repping S, van Pelt AM. In vitro propagation of human prepubertal spermatogonial stem cells. JAMA 2011;305(23):2416–2418

69 Kanatsu-Shinohara M, Inoue K, Lee J, et al. Generation of pluripotent stem cells from neonatal mouse testis. Cell 2004;119 (7):1001–1012

70 Guan K, Nayernia K, Maier LS, et al. Pluripotency of spermatogonial stem cells from adult mouse testis. Nature 2006;440 (7088):1199–1203

71 Izadyar F, Pau F, Marh J, et al. Generation of multipotent cell lines from a distinct population of male germ line stem cells. Reproduction 2008;135(6):771–784

72 Kossack N, Meneses J, Shefi S, et al. Isolation and characterization of pluripotent human spermatogonial stem cell-derived cells. Stem Cells 2009;27(1):138–149

73 Ko K, Araúzo-Bravo MJ, Tapia N, et al. Human adult germline stem cells in question. Nature 2010;465(7301):E1; discussion E3

74 Seandel M, James D, Shmelkov SV, et al. Generation of functional multipotent adult stem cells from GPR125+ germline progenitors. Nature 2007;449(7160):346–350

75 Geijsen N, Hochedlinger K. gPS navigates germ cells to pluripotency. Cell Stem Cell 2009;5(1):3–4

76 Hawkins MM, Stevens MC. The long-term survivors. Br Med Bull 1996;52(4):898–923

77 Bleyer WA. The impact of childhood cancer on the United States and the world. CA Cancer J Clin 1990;40(6):355–367

78 Aksglaede L, Wikström AM, Rajpert-De Meyts E, Dunkel L, Skakkebaek NE, Juul A. Natural history of seminiferous tubule degeneration in Klinefelter syndrome. Hum Reprod Update 2006;12(1):39–48

79 Yamada K, Fujita K, Quan J, et al. Increased apoptosis of germ cells in patients with AZFc deletions. J Assist Reprod Genet 2010;27 (6):293–297

80 de Rooij DG. The spermatogonial stem cell niche. Microsc Res Tech 2009;72(8):580–585

81 Schlatt S. Spermatogonial stem cell preservation and transplantation. Mol Cell Endocrinol 2002;187(1-2):107–111

82 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(4):1736–1747

83 Milazzo JP, Vaudreuil L, Cauliez B, et al. Comparison of conditions for cryopreservation of testicular tissue from immature mice. Hum Reprod 2008;23(1):17–28

84 Milazzo JP, Travers A, Bironneau A, et al. Rapid screening of cryopreservation protocols for murine prepubertal testicular tissue by histology and PCNA immunostaining. J Androl 2010;31(6):617–630

85 Shinohara T, Inoue K, Ogonuki N, et al. Birth of offspring following transplantation of cryopreserved immature testicular pieces and in-vitro microinsemination. Hum Reprod 2002;17(12):3039–3045

86 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(2):484–491

87 Keros V, Hultenby K, Borgström B, Fridström 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(5):1384–1395

88 Honaramooz A, Snedaker A, Boiani M, Schöler H, Dobrinski I, Schlatt S. Sperm from neonatal mammalian testes grafted in mice. Nature 2002;418(6899):778–781

89 Ohta H, Sakaide Y, Wakayama T. The birth of mice from testicular spermatozoa retrieved from frozen testicular sections. Biol Reprod 2008;78(5):807–811

90 Jahnukainen K, Ehmcke J, Hergenrother SD, Schlatt S. Effect of cold storage and cryopreservation of immature non-human primate testicular tissue on spermatogonial stem cell potential in xenografts. Hum Reprod 2007;22(4):1060–1067

91 Goossens E, Frederickx V, Geens M, De Block G, Tournaye H. Cryosurvival and spermatogenesis after allografting prepubertal mouse tissue: comparison of two cryopreservation protocols. Fertil Steril 2008;89(3):725–727

92 Zeng W, Snedaker AK, Megee S, et al. Preservation and transplantation of porcine testis tissue. Reprod Fertil Dev 2009;21(3):489–497

93 Abrishami M, Anzar M, Yang Y, Honaramooz A. Cryopreservation of immature porcine testis tissue to maintain its developmental potential after xenografting into recipient mice. Theriogenology 2010;73(1):86–96

94 Curaba M, Verleysen M, Amorim CA, et al. Cryopreservation of prepubertal mouse testicular tissue by vitrification. Fertil Steril 2011;95(4):1229–1234, e1

95 Baert Y, Goossens E, Van Saen D, et al. Orthotopic grafting of cryopreserved prepubertal testicular tissue: in search of a simple yet effective cryopreservation protocol. Fertil Steril 2012;97 (5):1152–1157

96 Parreira GG, Ogawa T, Avarbock MR, França LR, Brinster RL, Russell LD. Development of germ cell transplants in mice. Biol Reprod 1998;59(6):1360–1370

97 Avarbock MR, Brinster CJ, Brinster RL. Reconstitution of spermatogenesis from frozen spermatogonial stem cells. Nat Med 1996;2(6):693–696

98 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(1):144–150

99 Honaramooz A, Behboodi E, Blash S, Megee SO, Dobrinski I. Germ cell transplantation in goats. Mol Reprod Dev 2003;64(4):422–428

100 Mikkola M, Sironen A, Kopp C, 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(2):124–128

101 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(6): 823–831

102 Hermann BP, Sukhwani M, Hansel MC, Orwig KE. Spermatogonial stem cells in higher primates: are there differences from those in rodents? Reproduction 2010;139(3):479–493

103 Dobrinski I, Avarbock MR, Brinster RL. Transplantation of germ cells from rabbits and dogs into mouse testes. Biol Reprod 1999;61(5):1331–1339

104 Ogawa T, Dobrinski I, Avarbock MR, Brinster RL. Xenogeneic spermatogenesis following transplantation of hamster germ cells to mouse testes. Biol Reprod 1999;60(2):515–521

105 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(9):1874–1880

106 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(4, Suppl): 1411–1416

107 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(9):2255–2263

108 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 (7):1836–1842

109 Goossens E, Bilgec T, Van Saen D, Tournaye H. Mouse germ cells go through typical epigenetic modifications after intratesticular tissue grafting. Hum Reprod 2011;26(12):3388–3400

110 Honaramooz A, Snedaker A, Boiani M, Schöler H, Dobrinski I, Schlatt S. Sperm from neonatal mammalian testes grafted in mice. Nature 2002;418(6899):778–781

111 Schlatt S, Honaramooz A, Boiani M, Schöler HR, Dobrinski I. Progeny from sperm obtained after ectopic grafting of neonatal mouse testes. Biol Reprod 2003;68(6):2331–2335

112 Honaramooz A, Li MW, Penedo MC, Meyers S, Dobrinski I. Accelerated maturation of primate testis by xenografting into mice. Biol Reprod 2004;70(5):1500–1503

113 Schlatt S, Kim S, Gosden R. Spermatogenesis and steroidogenesis in mouse, hamster and monkey testicular tissue after cryopreservation and grafting. Reproduction 2002;124:323–329

114 Oatley JM, de Avila DM, Reeves JJ, McLean DJ. Spermatogenesis and germ cell transgene expression in xenografted bovine testicular tissue. Biol Reprod 2004;71(2):494–501

115 Geens M, De Block G, Goossens E, Frederickx V, Van Steirteghem AC, Tournaye H. Spermatogonial survival after grafting human testicular tissue to immunodeficient mice. Hum Reprod 2006;21 (2):390–396

116 Schlatt S, Honaramooz A, Ehmcke J, et al. Limited survival of adult human testicular tissue as ectopic xenograft. Hum Reprod 2006;21(2):384–389

117 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 (5):1113–1122

118 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 (11):2402–2414

119 Shinohara T, Inoue K, Ogonuki N, et al. Birth of offspring following transplantation of cryopreserved immature testicular pieces and in-vitro microinsemination. Hum Reprod 2002;17(12):3039–3045

120 Van Saen D, Goossens E, De Block G, Tournaye H. Regeneration of spermatogenesis by grafting testicular tissue or injecting testicular cells into the testes of sterile mice: a comparative study. Fertil Steril 2009;91(5, Suppl):2264–2272

121 Jahnukainen K, Hou M, Petersen C, Setchell B, Söder O. Intratesticular transplantation of testicular cells from leukemic rats causes transmission of leukemia. Cancer Res 2001;61(2): 706–710

122 Fujita K, Ohta H, Tsujimura A, et al. Transplantation of spermatogonial stem cells isolated from leukemic mice restores fertility without inducing leukemia. J Clin Invest 2005;115(7): 1855–1861

123 Fujita K, Tsujimura A, Miyagawa Y, 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(23):11166–11171

124 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(3):733–742

125 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(2):787–791

126 Glaser T, Opitz T, Kischlat T, et al. Adult germ line stem cells as a source of functional neurons and glia. Stem Cells 2008;26 (9):2434–2443

127 Streckfuss-Bömeke K, Vlasov A, Hülsmann S, et al. Generation of functional neurons and glia from multipotent adult mouse germline stem cells. Stem Cell Res (Amst) 2009;2(2):139–154

128 Guan K, Wagner S, Unsöld B, et al. Generation of functional cardiomyocytes from adult mouse spermatogonial stem cells. Circ Res 2007;100(11):1615–1625

129 Simon L, Ekman GC, Kostereva N, et al. Direct transdifferentiation of stem/progenitor spermatogonia into reproductive and nonreproductive tissues of all germ layers. Stem Cells 2009;27 (7):1666–1675

130 Ning L, Goossens E, Geens M, et al. Mouse spermatogonial stem cells obtain morphologic and functional characteristics of hematopoietic cells in vivo. Hum Reprod 2010;25(12): 3101–3109

131 Baeyens L, Bouwens L. Can beta-cells be derived from exocrine pancreas? Diabetes Obes Metab 2008;10(Suppl 4):170–178

132 Eberhard D, Tosh D. Transdifferentiation and metaplasia as a paradigm for understanding development and disease. Cell Mol Life Sci 2008;65(1):33–40

133 Kanatsu-Shinohara M, Shinohara T. The germ of pluripotency. Nat Biotechnol 2006;24(6):663–664

134 Izadyar F, Pau F, Marh J, et al. Generation of multipotent cell lines from a distinct population of male germ line stem cells. Reproduction 2008;135(6):771–784

135 Nagano M, Shinohara T, Avarbock MR, Brinster RL. Retrovirus-mediated gene delivery into male germ line stem cells. FEBS Lett 2000;475(1):7–10

136 Kanatsu-Shinohara M, Toyokuni S, Shinohara T. Transgenic mice produced by retroviral transduction of male germ line stem cells in vivo. Biol Reprod 2004;71(4):1202–1207