34 Scott, D.A. *et al.* (1999) The Pendred syndrome gene encodes a chloride–iodide transport protein. *Nat. Genet.* 21, 440–443
35 Yoshida, A. *et al.* Pendrin, the protein encoded by the Pendred syndrome gene, is an iodide-specific apical porter which is important for iodide efflux from thyroid cells. In *Proceedings 12th International Thyroid Congress* (in press)
36 Suzuki, K. *et al.* (1999) Follicular thyroglobulin (TG) regulates expression of basal and apical iodide transporters: the sodium–iodide symporter (*NIS*) and pendrin (*PDS*) genes. In *Proceedings of the 81st Annual American Endocrine Meeting,* p. 206
37 Schuppert, F. *et al.* (2000) Increased major histocompatibility complex (MHC) expression in non-toxic goiters is associated with iodide depletion, enhanced ability of the follicular thyroglobulin to increase MHC gene expression, and thyroid autoantibodies. *J. Clin. Endocrinol. Metab.* 85, 858–867
38 Weiss, S.J. *et al.* (1984) Effect of thyrotropin on iodide efflux in FRTL-5 cells mediated by Ca^{2+}. *Endocrinology* 114, 1108–1113
39 Nilson, M. *et al.* (1992) Polarized efflux of iodide in porcine thyrocytes occurs via a cAMP-regulated iodide channel in the apical plasma membrane. *Acta Endocrinol.* 126, 67–74

Germ cell transplantation and the study of testicular function

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Spermatogonial stem cell transplantation is a novel technique in which donor testicular cells are transferred into recipient testes. A population of germ cells from a transgenic or mutant donor is introduced into the seminiferous tubules of recipient testes by microinjection. Following injections, spermatogonial stem cells can colonize the recipient testis, initiate spermatogenesis and produce sperm capable of fertilization. This technique will allow scientists to: (1) investigate fundamental aspects of spermatogenesis; (2) provide a method to regenerate spermatogenesis in infertile individuals; and (3) genetically manipulate spermatogonial stem cells to develop transgenic animals.

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Spermatogenesis is a dynamic, continuous process in which diploid spermatogonia undergo ten mitotic and two meiotic divisions to differentiate into haploid spermatozoa[1]. This process generates a remarkable number of mature sperm – 200 million per day in the human male – and requires an intimate association of germinal cells and somatic Sertoli cells within the seminiferous tubules of the testis. The production of sperm is dependent on the presence of spermatogonial stem cells that reside in the basal compartment of the seminiferous tubules, and which represent a very small proportion of the cells in the testis. Spermatogonial stem cells are capable of both self-renewal and producing cells that commit to spermatogenesis. Germ cells that enter spermatogenesis are supported by Sertoli cells. These germ cells progress apically towards the lumen of the tubule, differentiate into mature sperm and are eventually released into the lumen. Thus, stem cells are essential for the production of gametes and, ultimately, species continuation. Individuals can be sterile owing to a lack of stem cells, the blockage of germ cell development or the production of nonfunctional sperm. A novel transplantation technique has afforded scientists the opportunity to investigate fundamental aspects of spermatogenesis and offer hope for some infertility patients[2].

The technique of testicular germ cell transplantation, pioneered in the laboratory of Ralph Brinster (University of Pennsylvania, Philadelphia, PA, USA), involves the introduction of a mixed population of germ cells from a donor testis into the lumens of the seminiferous tubules of a recipient mouse[2,3]. Brinster and colleagues reported that not only do stem cells colonize the recipient testis, but they also initiate spermatogenesis and produce sperm capable of fertilization[4]. In addition, when germ cells from a rat were introduced into an immunologically-deficient mouse, rat sperm were produced within the mouse testis[5]. These results generated a great deal of interest from reproductive scientists and stimulated research in multiple areas. Experimental approaches utilizing germ cell transplantation have contributed to research in stem cell and male germ cell biology[6,7], the identification of cell types contributing to infertility within specific mutants[8,9], and research of cellular interactions in the testis[10,11]. In addition, germ cell transplantation could potentially be used to treat male infertility, preserve the germ line of valuable or endangered animals and replace the germ line of patients whose endogenous stem cells have been eliminated by chemotherapy.

Germ cell transplantation and colonization

It is generally believed that donor-derived spermatogenesis in a transplanted testis originates from spermatogonial stem cells. A mixed population of germ cells, which includes a small number of spermatogonial stem cells, can be injected into the testis by several methods[3]. The most widely used method involves inserting a needle through the efferent ductules outside of the testis and passing the needle into the rete testis, a bundle of tubules that connects the seminiferous tubules to the epididymis. The injected cell mixture contains trypan blue so that filling of the seminiferous tubules can be monitored (Fig. 1). Mice that have no endogenous spermatogenesis, such as the W/W^{V} mutant, can be used as germ cell recipients. This mutation affects the gene – in the *White spotting* (*W*) locus of the

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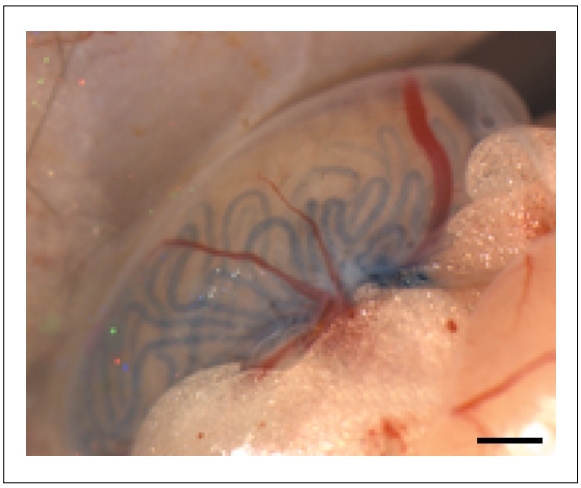

Fig. 1. Testis following germ cell transplantation injection. Germ cells were suspended in a solution containing trypan blue. A glass needle is inserted into the efferent duct, guided to the rete and the suspension transferred into the seminiferous tubules. Seminiferous tubules containing the blue cell suspension can be seen. Scale bar = 0.5 mm.

mouse – encoding the membrane-bound tyrosine kinase, *c-kit*. The c-kit molecule serves as a receptor for Steel factor (kit ligand, Stem cell factor), the gene product of the *Sl* locus. Steel factor is expressed during the migratory pathway of primordial germ cells (PGCs) and in testicular Sertoli cells. The interaction between c-kit and Steel factor is essential for the migration of PGCs to the primitive testis, and for spermatogonial proliferation within the seminiferous tubules. In the testes of mutants homozygous for either c-kit (W/W^v) or Steel factor (Sl/SL^d), the seminiferous tubules are virtually devoid of germ cells and contain few spermatogonia; these mice are infertile[8]. Alternatively, recipient animals can be treated with the chemotherapeutic agent busulfan at a dose that eliminates most germ cells from the testis. Treating with busulfan before transplantation provides a testicular environment that donor cells can colonize. However, a small number of endogenous stem cells persist and can reinitiate spermatogenesis. Therefore, it is helpful to use germ cells from a donor containing a genetic marker[12]. By using transgenically marked donor cells, testicular repopulation and spermatogenesis can be distinguished from endogenous spermatogenesis (Figs 2,3). A comprehensive discussion of the transplantation procedure and the preparation of recipient mice and donor cells can be found in Ogawa *et al.*[3]

Nagano and co-workers[13] elegantly described the pattern and kinetics of donor-derived spermatogonial stem cell colonization in transplanted testes. These researchers described the colonization in three continuous phases. First, transplanted cells were randomly distributed throughout the seminiferous tubules during the first week after transplantation. By this time, a small number of transplanted stem cells have migrated to the basement membrane of the seminiferous tubules. This finding is remarkable and surprising for the following reasons. Sertoli cells within the seminiferous tubules form tight junctions with adjacent Sertoli cells[14]. These junctions constitute the major component of the blood–testis barrier, and this barrier creates an immunoprivileged environment within the seminiferous tubules. When spermatogonia enter meiosis and become spermatocytes, the tight junctions are broken down and reformed to allow spermatocytes to translocate apically, complete meiosis and differentiate into spermatids in the immunoprivileged adluminal portion of the seminiferous tubules[1]. Consequently, when germ cells are introduced into recipient seminiferous tubules during the germ cell transplantation procedure, spermatogonial stem cells translocate in a basal direction through the tight junctions, towards the basement membrane of the seminiferous tubule. This surprising result means that Sertoli cells recognize stem cells and direct germ cell translocation in a retrograde manner.

The second phase of spermatogonial transplantation described by Nagano *et al.*[13] occurred one week to one month after transplantation. During this phase, stem cells and primitive spermatogonia proliferated longitudinally in the seminiferous tubule, forming a network or chain of donor cells. The differentiation of donor cells occurred during the third phase, which began one month after transplantation. Donor cells established a colony of spermatogonia and continued to expand laterally along the tubules. Spermatozoon production began two months after transplantation. Colony expansion continued throughout the study, and the number of colonized sites did not change between one and four months after transplantation[1,13]. The length of donor-derived colonies increased during the third phase, and, after three months, ~30% of the testis contained donor-derived spermatogonia[15]. It is important for this discussion to note the relative inefficiency of this process. Nagano *et al.*[13] reported that, typically, 19 donor-derived colonies are produced when 10^6 cells are introduced into a testis. Most cells remained in the intraluminal compartment and were phagocytosed by Sertoli cells[15]. Therefore, approximately 20 stem cells survived isolation, colonized the recipient testis and initiated spermatogenesis.

Application of germ cell transplantation

Several approaches have been used to improve the efficiency of colonization of recipient testes after germ cell transplantation. The first study used the gonadotropin-releasing hormone (GnRH) agonist, leuprolide, to enhance colonization after transplantation[16]. Mice injected with leuprolide had markedly enhanced donor cell colonization, both in the efficiency of colonization and in the lateral expansion of donor-derived colonies. Leuprolide might have acted directly on germ cells or reduced the concentration of luteinizing hormone (LH) or testosterone. However, no GnRH receptors have been found on germ cells or cells associated with the seminiferous tubules. Therefore, it is unlikely that leuprolide had a direct effect on spermatogonial

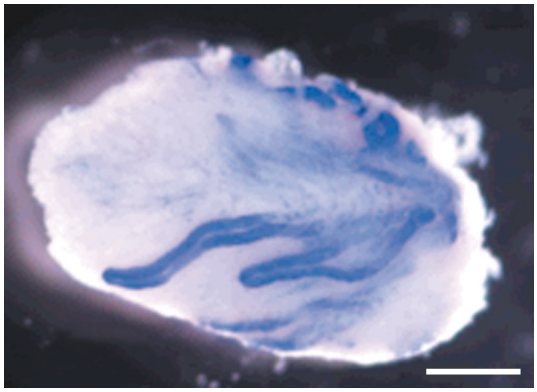

Fig. 2. Colonization of recipient testis by Rosa26 donor cells that were collected from day 5 mice. Rosa26 mice are transgenic for the bacterial gene encoding β-galactosidase, which is expressed by all cells of the testis. Blue tubules indicate areas of spermatogenesis generated from donor testicular cells. The testis was fixed in 4% paraformaldehyde for 1 h, stained with X-gal, embedded in paraffin wax, sectioned and counterstained with fast red. Scale bar = 1 mm.

colony formation and proliferation. The authors note that the suppression of testosterone by GnRH agonists or by the negative feedback initiated by exogenous testosterone might stimulate spermatogenesis after irradiation damage, and that high levels of testosterone appear to inhibit spermatogenesis[17]. These data suggest that leuprolide might have made conditions in the testis more permissive of spermatogenesis after transplantation by reducing the intratesticular testosterone concentration. Thus, the mode of action of leuprolide is probably indirect, and a study to investigate the effect of testosterone antagonists on germ cell transplantation seems warranted. Lastly, it is important to note that the observed effects in rodents might not be applicable to humans.

An important advancement in the process of germ cell transplantation was the regeneration of spermatogenesis from cryopreserved cells. Avarbock *et al.*[7] demonstrated that cryopreserved testicular cells, from both prepubertal and adult mice, established spermatogenesis after transplantation. These cells were frozen for between four and 156 days with the use of techniques similar to those generally used for somatic cell cryopreservation. The application of this technique is potentially important because: (1) cryopreservation of spermatogonial stem cells from valuable or endangered animals would allow regeneration of the germ line at any time in the appropriate host; and (2) in a clinical setting, an individual undergoing chemotherapy or radiation treatment, which often produces prolonged and sometimes irreversible depression of sperm counts in humans, could have a testicular biopsy cryopreserved before treatment and transplanted at a later date to regenerate spermatogenesis.

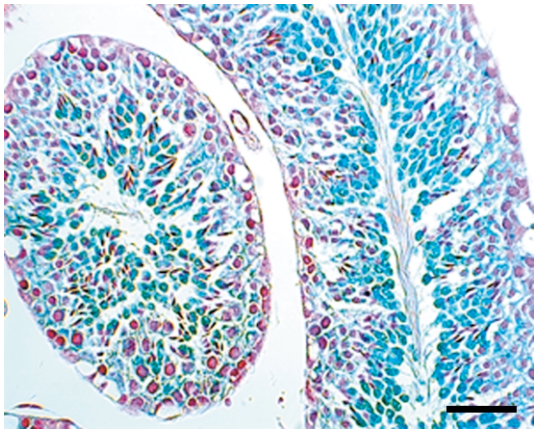

Fig. 3. Microscopic appearance of spermatogenesis in testis from Fig. 2. Note morphology and cellular associations indicating that donor-derived spermatogenesis has occurred. The testis was fixed in 4% paraformaldehyde for 1 h, stained with X-gal, embedded in paraffin wax, sectioned and counterstained with fast red. Scale bar = 50 μm.

The development of germ cell transplantation has provided a functional system to evaluate the success of the long-term culture of germ cells. Two reports from Brinster's laboratory described the culture of testicular cells on STO cell feeder layers. The STO cell line is derived from mouse embryonic fibroblasts, and feeder layers are generated by treating dividing STO cells with either mitomycin C or irradiation to halt cell division. STO cell feeder layers provide an environment that supports spermatogonial stem cell survival *in vitro*. Testicular cells maintained in culture from 19 to 132 days successfully generated donor-derived spermatogenesis after transplantation[12,19]. This is a remarkable finding considering it was generally believed that germ cells do not survive in culture for more than a few weeks[20].

Spermatogonial stem cells have been successfully transfected with the use of a retroviral vector. Nagano *et al.*[21] used several approaches to expose stem cells to the retroviral particles. Germ cells from the testes of neonatal animals, and from cryptorchid testes of adult animals, were used. Adult mice were made cryptorchid by securing the testes within the body cavity. At normal body temperature, differentiating germ cells die, whereas somatic and stem cells of the testis are unaffected. Thus, cryptorchid testes represent an enriched source of spermatogonial stem cells because of the higher proportion of these cells that they contain compared with wild-type controls. Germ cells isolated from neonatal and cryptorchid testes were incubated with: (1) retroviral-producing cells alone; (2) an STO feeder layer and retroviral producing cells; and (3) an STO feeder layer with periodic exposure to retroviral particles. After incubation, cultured cells were injected into recipient animals. All methods of culture and infection generated stably transfected stem cells that colonized recipient testes, with the periodic infection system producing the highest level of infection[21]. Donor-derived spermatogenesis was permanent and produced sperm positive for the transfected gene – the gene encoding β-galactosidase. In addition, co-injection of retroviral particles and fresh germ cells into recipient testes also resulted in incorporation of the reporter gene. These results demonstrate that spermatogonial stem cells are dividing in culture and that genes can be introduced directly into the male germ line[21]. This remarkable result has multiple applications, including somatic cell therapy, the introduction of genes into multiple animal species and the genetic manipulation of stem cells to answer basic questions about spermatogenesis.

Germ cell transplantation and testicular stem cells

Despite the importance of spermatogonial stem cells for continuous sperm production, there is a paucity of information regarding these cells. It is generally accepted that it is the spermatogonial stem cells that colonize the recipient testis and initiate donor-derived spermatogenesis after germ cell transplantation. The age of animals used as germ cell donors for transplantation has varied. Donor-derived testicular colonization and spermatogenesis have been reported after the injection of germ cells from animals at day 4 through to the adult. However, the sophistication of the retrieval and identification of cells selected for injection has increased dramatically. These techniques will probably lead to accurate methods of identifying testicular stem cells. The development of the germ cell transplantation technique has established a functional assay for testicular stem cells. This allows conclusive verification of the presence and quantification of stem cells within a sample. The research involving germ cell transplantation to identify testicular stem cells is described in this section.

It has been estimated that of the 10^8 cells in a testis, 2×10^4 are stem cells; in other words, a testis contains two stem cells per 10^4 cells[18]. As mentioned earlier, 10^6 donor cells are injected into a testis; therefore, ~200 stem cells are injected into the recipient testis. A process to select these cells, thereby enriching the proportion of stem cells injected, would help to improve colonization efficiency. Shinohara *et al.*[6] hypothesized that testicular stem cells might be associated with components of the basement membrane. They selected testicular cells based on the ability of the cells to adhere to molecules that make up the extracellular matrix. Spermatogonial stem cells preferentially adhered to laminin but not to collagen IV or fibronectin, based on the ability of these cells to colonize recipient testes. In addition, by exploiting the abilities of β_1-integrin to adhere to laminin and of α_6-integrin to form dimers with β_1-integrin, testicular cells were isolated with magnetic beads and anti-β_1- and anti-α_6-integrin antibodies. The colonization of recipient testes by cells selected with the aid of anti-β_1- and anti-α_6-integrin antibodies was significantly enhanced compared with controls. By contrast, cells selected with an anti-c-kit antibody failed to improve recipient colonization[6].

Multiple selection techniques have been utilized for hematopoietic stem cell purification[22]. Shinohara *et al.* hypothesized that a multiparameter approach could similarly be used to enrich for testicular stem cells[23]. The significant increase of colonization by testicular cells that had been selected by their ability to bind laminin was considered as the first step in this process[6]. Second, the colonizing ability following transplantation of a source of testicular cells enriched for stem cells was investigated[23]. The previously described *Kitl*^{Sl} mutant contains stem cells but, owing to the lack of Steel ligand, these cells do not proliferate and produce mature sperm. Thus, in this mutant, the stem cells constitute a higher proportion of the total cell number than they do in wild-type mice. Likewise, the cryptorchid model provides another source of germ cells in which stem cells represent a higher proportion of the total cells when compared with a wild-type adult. Using germ cell transplantation as a functional assay for the presence of spermatogonial stem cells, cells recovered from cryptorchid tesis were enriched for stem cells 25- and 50-fold based on the number of colonies and on the area the seminiferous tubule colonized, respectively[23]. Surprisingly, cells from the *Kitl*^{Sl} mutant increased the number of colonies and area colonization to a much lower extent than did cryptorchid cells. Further enrichment of stem cells was attempted by incubating cells from cryptorchid testes with laminin. Laminin selection failed to increase stem cell enrichment when compared with the unselected cryptorchid control[23].

Building upon the information obtained with the cryptorchid testis model and laminin selection, Shinohara *et al.*[24] further characterized testicular stem cells with the use of fluorescence-activated cell sorting (FACS). Cryptorchid mouse testicular cells were fractionated by FACS as determined by their light-scattering properties and the expression of cell-surface molecules. Selection for cells based upon light-scattering properties demonstrated that spermatogonial stem cells are characterized by low side scatter, a measure of intracellular complexity. In addition, testicular stem cells have little or no c-kit or α_v-integrin, but are positive for α_6-integrin. Selecting testicular cells for low side scatter and expression of α_6-integrin resulted in a 166-fold enrichment of spermatogonial stem cells based on recipient testicular colonization following transplantation[24]. These reports represent significant advances in the characterization of testicular stem cells. Thus, the spermatogonial transplantation technique has proved valuable in the investigation of the molecular characteristics of spermatogonial stem cells.

Xenogeneic germ cell transplantation

One of the most remarkable aspects of this research is the ability of the testis of one species to support spermatogenesis of germ cells from another species. Xenogeneic (cross-species) germ cell transplantation provides an opportunity to investigate fundamental aspects of spermatogenesis. Clouthier *et al.*[5] reported that rat testis cells will colonize and complete spermatogenesis when transplanted into immunocompromised mouse testes. Sperm with the characteristic head shape of rat sperm were found in the epididymides of recipient mice. Likewise, this approach allowed Franca *et al.*[25] to determine whether the timing of spermatogenesis was controlled by the germ cell or Sertoli cell in xenogeneic transplants. It takes ~35 days for a spermatogonium to complete spermatogenesis in the mouse, whereas this process takes 52–53 days in the rat[1].

Franca *et al.*[25] demonstrated that rat germ cells developed at their characteristic rate of 52–53 days in the mouse. In the same testis, mouse sperm developed at their characteristic rate of 35 days. Therefore, germ cells control the rate of spermatogenesis and a testis can maintain spermatogenesis at two different rates simultaneously.

Additional xenogeneic germ cell transplants have demonstrated some of the limitations of this procedure. Hamster germ cells injected into immunocompromised mouse testes will colonize and hamster spermatogenesis develops[26]. However, it was clear that both qualitative and quantitative hamster spermatogenesis in mouse testes was inferior to spermatogenesis seen in mouse-to-mouse and rat-to-mouse transplants. Abnormally developing germ cells were observed in the seminiferous tubules, and hamster sperm present in the epididymis lacked acrosomes, heads and tails[26].

Transplantation of rabbit and dog testicular cells into immunocompromised mouse testes has also been demonstrated[27]. In these experiments, the injected spermatogonial stem cells translocated to the basal compartment of the seminiferous tubules and, in the case of the rabbit cells, divided and formed chains of cells. When dog cells were transplanted, donor-derived cells were present in pairs and short chains, but colonization was less extensive than that following transplantation of rabbit cells. However, cells from either species did not progress into later stages of spermatogenesis and no mature sperm were observed[27]. From these results, it was hypothesized that the success of donor-derived colonization was correlated to the degree of evolutionary relatedness of the species.

Germ cell transplantation has also been performed in bull, monkey and human testes[28]. Ultrasound-guided intratesticular rete testis injections were performed on surgically removed bull, monkey and human testes. All procedures involved the injection of cells from the same species into the recipient testis. Injected cells were observed mainly in the rete testis and some seminiferous tubule filling was reported. Two cynomolgus monkeys were treated with a GnRH antagonist before *in vivo* germ cell injection. One animal was injected with testicular cells labeled with bromodeoxyuridine (BrdU). Labeled cells were identified four weeks after transplantation in the testicular interstitium and in some seminiferous tubules of the recipient. The tubule cells were identified as B-spermatogonia and appeared as single cells and small clusters of cells[28]. This was the first report of injection into human and primate testes, and described a novel ultrasound-guided method for the direct injection of the rete.

Xenogeneic transplantation of human spermatogonia into mice has been reported[29]. Spermatogonia from azoospermic men undergoing infertility treatment were injected into Kit^{W/W_v} and severe combined immunodeficient (SCID) mice. Successful injection of cells into the seminiferous tubules was reported. However, at 150 days after injection no donor germ cells were found in the mouse testes. The authors speculate that the donor cells did not implant and colonize the mouse testes because of non-compatible cellular interactions and immunological rejection resulting from interspecies differences[29].

Infertility and knockout studies

Recently, several reports have demonstrated the utility of germ cell transplantation for the investigation of cases of infertility occurring as a result of a natural mutation or targeted gene deletion[8,9]. It is relatively common that gene knockout experiments lead to male infertility. Stem cells might not be present, germ cell development might be blocked at any of several stages, or functional sperm might not be produced. In most cases, the identity of the testicular cell type(s) where the disrupted gene is phenotypically important is not readily apparent. Transplantation of germ cells carrying the disrupted gene into wild-type recipients can provide this information. Ogawa *et al.*[8] applied this type of approach by transplanting germ cells from infertile mice carrying the *Sl* mutation into infertile, white-spotting (W/W^V or W^V/W^{54}) mutant mice: the recipient mice were shown to be fertile. Thus, fertility was restored after transplantation of spermatogonial stem cells from an infertile donor into an infertile recipient that had a permissive testicular somatic cell environment.

Another naturally occurring mutation in mice that affects spermatogenesis is the juvenile spermatogonial depletion (*jsd*) mutation[30]. This mutation results in mice undergoing a single wave of spermatogenesis, followed by a failure of type A spermatogonial stem cells to repopulate the testis. When germ cells from *jsd* animals were injected into W/W^V or busulfan-treated recipients, no donor-derived spermatogenesis was observed. By contrast, the injection of non-*jsd* germ cells into *jsd* recipients demonstrated that *jsd* animals can support donor-derived spermatogenesis for up to seven months[30]. Thus, these data indicate that the *jsd* phenotype results from a defect in the germ cells, and not in the somatic cells, of the testis.

The first report of germ cell transplantation correcting infertility in a knockout was accomplished in animals in which estrogen receptor α (ERα) had been deleted[9]. Male ER deficient (αERKO) mice are infertile; however, when germ cells from αERKO mice were transplanted into wild-type males, qualitatively normal donor-derived spermatogenesis developed and functional sperm were produced that carried a normal copy of the gene encoding αERKO. Thus, these results demonstrated that a gene knockout that disrupts spermatogenesis has no deleterious effects on germ cells.

Conclusions

Germ cell transplantation has multiple applications that will continue to contribute to a better understanding of spermatogenesis. The clinical use of this technique might one day become a viable therapy for infertility. Germ cell transplantation will allow scientists to combine various germ cell and Sertoli cell mutants so that somatic and germ cell function can be differentiated experimentally. Animal scientists can apply this technique for preservation of the germ line of economically valuable animals, older animals unable to breed naturally and endangered species[31]. Furthermore, this technique provides an avenue to the investigation of stem cell biology in a unique system in which genetic material can be passed to future generations.

References

1 Russell, L.D. *et al.* (1990) *Histological and Histopathological Evaluation of the Testis*, Cache River Press
2 Brinster, R.L. and Zimmermann, J.W. (1994) Spermatogenesis following male germ-cell transplantation. *Proc. Natl. Acad. Sci. U. S. A.* 91, 11298–11302
3 Ogawa, T. *et al.* (1997) Transplantation of testis germinal cells into mouse seminiferous tubules. *Int. J. Dev. Biol.* 41, 111–122
4 Brinster, R.L. and Avarbock, M.R. (1994) Germline transmission of donor haplotype following spermatogonial transplantation. *Proc. Natl. Acad. Sci. U. S. A.* 91, 11303–11307
5 Clouthier, D.E. *et al.* (1996) Rat spermatogenesis in mouse testes. *Nature* 381, 418–421
6 Shinohara, T. *et al.* (1999) β1 and α6-integrin are surface markers on mouse spermatogonial stem cells. *Proc. Natl. Acad. Sci. U. S. A.* 96, 5504–5509
7 Avarbock, M.R. *et al.* (1996) Reconstitution of spermatogenesis from frozen spermatogonial stem cells. *Nat. Med.* 2, 693–696
8 Ogawa, T. *et al.* (2000) Transplantation of male germ line stem cells restores fertility in infertile mice. *Nat. Med.* 6, 29–34
9 Mahato, D. *et al.* (2000) Spermatogenic cells do not require estrogen receptor-α for development or function. *Endocrinology* 141, 1273–1276
10 Russell, L.D. *et al.* (1996) Ultrastructural observations of spermatogenesis in mice resulting from transplantation of mouse spermatogonia. *J. Androl.* 17, 603–614
11 Russell, L.D. and Brinster, R.L. (1996) Ultrastructural observations of spermatogenesis following transplantation of rat testis cells into mouse seminiferous tubules. *J. Androl.* 17, 615–627
12 Nagano, M. *et al.* (1998) Culture of mouse spermatogonial stem cells. *Tissue Cell* 30, 389–397
13 Nagano, M. *et al.* (1999) Pattern and kinetics of mouse donor spermatogonial stem cell colonization in recipient testes. *Biol. Reprod.* 60, 1429–1436
14 Russell, L.D. (1993) Form, dimensions, and cytology of mammalian Sertoli cells. In *The Sertoli Cell* (Russell, L.D. and Griswold, M.D., eds), pp. 1–38, Cache River Press
15 Parreira, G.G. *et al.* (1998) Development of germ cell transplants in mice. *Biol. Reprod.* 59, 1360–1370
16 Ogawa, T. *et al.* (1998) Leuprolide, a gonadotropin-releasing hormone agonist, enhances colonization after spermatogonial transplantation into mouse testes. *Tissue Cell* 30, 583–588
17 Meistrich, M.L. and Kangasniemi, M. (1997) Hormone treatment after irradiation stimulates recovery of rat spermatogenesis from surviving spermatogonia. *J. Androl.* 18, 80–87
18 Meistrich, M.L. and Van Beek, M.E.A.B. (1993) Spermatogonial stem cells. In *Cell and Molecular Biology of the Testis* (Desjardins, C. and Ewing, L.L., eds), pp. 266–295, Oxford University Press
19 Brinster, R.L. and Nagano, M. (1998) Spermatogonial stem cell transplantation, cryopreservation and culture. *Semin. Cell Dev. Biol.* 9, 401–409
20 Kierszenbaum, A.L. (1994) Mammalian spermatogenesis *in vivo* and *in vitro*: a partnership of spermatogenic and somatic cell lineages. *Endocr. Rev.* 15, 116–134
21 Nagano, M. *et al.* (2000) Retrovirus-mediated gene delivery into male germ line stem cells. *FEBS Lett.* 475, 7–10
22 Osawa, M. *et al.* (1996) Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* 273, 242–245
23 Shinohara, T. *et al.* (2000) Functional analysis of spermatogonial stem cells in steel and cryptorchid infertile mouse models. *Dev. Biol.* 220, 401–411
24 Shinohara, T. *et al.* (2000) Spermatogonial stem cell enrichment by multiparameter selection of mouse testis cells. *Proc. Natl. Acad. Sci. U. S. A.* 97, 8346–8351
25 Franca, L. *et al.* (1998) Germ cell genotype controls cell cycle during spermatogenesis in the rat. *Biol. Reprod.* 59, 1371–1377
26 Ogawa, T. *et al.* (1999) Xenogeneic spermatogenesis following transplantation of hamster germ cells to mouse testes. *Biol. Reprod.* 60, 515–521
27 Dobrinski, I. *et al.* (1999) Transplantation of germ cells from rabbits and dogs into mouse testes. *Biol. Reprod.* 61, 1331–1339
28 Schlatt, S. *et al.* (1999) Germ cell transfer into rat, bovine, monkey, and human testes. *Hum. Reprod.* 14, 144–150
29 Reis, M.M. *et al.* (2000) Xenogeneic transplantation of human spermatozoa. *Zygote* 8, 97–105
30 Boettger-Tong, H.L. *et al.* Jsd (juvenile spermatogonial depletion) mutant seminiferous tubules are capable of supporting transplanted spermatogenesis. *Biol. Reprod.* (in press)
31 Hausler, C.L. and Russell, L.D. (1999) Prospects for spermatogonial transplantation in livestock and endangered species. In *The Male Gamete: From Basic Science to Clinical Applications* (Gagnon, C., ed.), pp. 37–45, Cache River Press