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Spermatogonial Transplantation in Mammals

Contents

Abstract

Spermatogonial transplantation from mouse-to-mouse was first reported by Brinster and colleagues in 1994. Since then, many important developments in this fascinating methodology such as interspecies transplants, transplants from

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cryopreserved and cultured spermatogonial stem cells have been made. This technique has been shown a valuable tool to study the biology of spermatogonial stem cells. Also, important functional questions regarding Sertoli-germ cell interactions and the role of the Sertoli cell and germ cells during spermatogenesis have now been answered. Transplantation of cultured spermatogonial stem cells is now opening exciting possibilities for *in vitro* multiplication and manipulation of male germ line cells. Spermatogonial stem cells can be considered "immortal". By freezing and storing testicular tissue, it should be possible to preserve indefinitely the genetic stocks of valuable farm animals, endangered species and unique experimental animals, until a suitable recipient can be found that will maintain the germ line. Transplantation of spermatogonia has also potential clinical application to address human infertility. Overall, spermatogonial transplantation has been proved to be an extraordinary and powerful technique to investigate reproductive biology.

Keywords: testis; spermatogenesis; spermatogonial; stem cells; transplantation; mouse; rat.

Introduction

Spermatogonial transplantation involves the removal of stem cells from a donor and their replacement into a recipient where they grow to form mature sperm with genetic characteristics of the donor. It is a new and exciting technique developed in 1994 by Brinster and colleagues (Brinster & Avarbock, 1994; Brinster & Zimmermann, 1994). The spermatogonial transplantation technique has opened the possibility of the study of both stem cells and Sertoli cell-germ cell interactions, providing many potential opportunities for agricultural, biological, and medical research (Dym, 1994; Hausler & Russell, 1999). It is necessary to understand how sperm are normally produced before we discuss this new techique.

The testis of all mammals functions to support spermatogenesis and produce androgens. This organ is comprised of two major compartments: the intertubular or interstitial compartment (interstitium) and the seminiferous tubule compartment. The interstitial compartment contains capillaries, lymphatic vessels, fibroblastic cells, macrophages, and Leydig cells. The seminiferous tubules are higly convoluted loops that have their two ends connected into the beginning of the excurrent duct system – the rete testis. Besides the peritubular myoid cells, the seminiferous tubules in the adult contains nonproliferating Sertoli cells and the highly proliferating germ cells – the latter in various developmental phases of the process called spermatogenesis. The spermatogenic process is a cyclic and highly organized and coordinated event in which diploid spermatogonia differentiate into mature haploid spermatozoa. Spermatogenesis takes from 30 to 75 days in

mammals (Russell et al., 1990; França & Russell, 1998). During its evolution, spermatogenesis may be divided into three phases based upon functional considerations: (i) the proliferative phase (spermatogonia), in which cells undergo rapid successive divisions; (ii) the meiotic phase (spermatocytes) in which genetic material is duplicated, recombined and segregated; and (iii) the differentiation or spermiogenic phase (spermatids) in which the spermatids transform into cells very specialized and structurally equipped to reach and fertilize the egg (Russell et al., 1990).

Spermatogenesis is initiated via division of spermatogonia stem cell that is located at the base of the seminiferous epithelium. Compared to many other well known self-renewing cell systems in the body, spermatogenesis is considered the one that has the greatest number of cell divisions during its development (Russell et al., 1990; Potten, 1992). In several mammalian species such as rats, mice, hamster, ram and boars, one spermatogonia goes through approximately 10 mitotic divisions before differentiation into spermatocyte which only carries out two meiotic divisions. One spermatogonia is capable of rendering up to 4,096 spermatozoa (De Rooij & Russell, 2000). Thus, from four to thirty millions of spermatozoa are produced daily per gram of testis tissue in mammals.

The development and function of the germinal epithelium is closely linked to the development of the somatic elements of the testis (Hochereau-de Reviers et al., 1987). Somatic cells of the male are key to the normal functioning of the male reproductive system (Russell et al., 1994). The Sertoli cell, or nurse cell of the testis, is considered the most important somatic cell to guide germ cell development (Russell & Griswold, 1993).

Spermatogonial stem cells initiate the development of other germ cells committed to form sperm, being also able to give rise to more stem cells. Besides this functional definition (differentiation and self renewal), in the mammalian testis the spermatogonia stem cells are morphologically characterized as the only type of spermatogonia without intercellular bridges; most people believe that they are the type A_s spermatogonia (De Rooij & Russell, 2000).

Spermatogonial Transplant Technique

Herein we describe only the basics of the spermatogonial transplantation technique; for more details see the report by Ogawa et al. (1997) or several other reviews that has been published in this field in the last couple years (Brinster & Nagano, 1998; Russell & Brinster, 1998; Russell et al., 1998; Hausler & Russell, 1999; Johnston et al., 2000; Ogawa, 2000; Russell & Griswold, 2000; Schlatt et al., 2000). Isolation of testes cells were performed by Brinster et al. (1994) utilizing a

modified procedure reported by Bellvé et al. (1977). With this procedure, in which germ cells of a donor mouse were collected using a two-step enzymatic (trypsin, collagenase) digestion protocol to prepare the cell suspension, Brinster's laboratory was able to obtain sufficient quantities of isolated cells to perform transplantation studies. As this procedure did not purify spermatogonia, the pool of testicular cells obtained contained all kind of cells including germ cells, Sertoli cells and Leydig cells.

Germ cells can be isolated from donor mice ranging in development from the late neonatal period through adulthood (Ogawa et al., 1997; Russell et al., 1998). The donor animals contain a reporter transgene composed of a zinc finger (ZF) promoter fused to the *E. coli* LacZ structural gene (LacZ), that results in the production of β-galactosidase (Brinster & Avarbock, 1994). This enzyme is expressed in all male germ cells and can be easily detected histochemically by incubation of whole testes or histological sections of testes in transplanted animals through specific staining techniques (X-gal) (Brinster & Avarbock, 1994). Donor spermatogenesis can also be demonstrated in breeding studies when the LacZ gene is propagated to all cells of the progeny and identified as described before (Brinster & Avarbock, 1994). Also, donor germ cells can be isolated from the Rosa 26 (B6,129TgR) and express the *E. coli* LacZ structural gene in nearly all cell types, including cells at all stages of spermatogenesis. In this way, analysis of the recipient testis for the transgene can be performed at any time after transplantation (Johnston et al., 2000; Shinohara et al., 2000).

The recipients utilized must have depleted spermatogenesis. W/W (W-locus) mice (Brinster & Avarbock, 1994; Brinster & Zimmermann, 1994) fit this criterion since they lack functional c-kit receptor essential for spermatogonial proliferation (Mintz, 1957; De Rooij & Russell, 2000). The ideal recipient for transplantation of spermatogonia is a male whose seminiferous tubules contain only normal Sertoli cells. The Sertoli cells of W/W mice are fully differentiated and apparently normal (Kurohmaru et al., 1992). Also, because Sertoli cells secrete fluid, an usually evident lumen in the seminiferous tubules is observed, which is likely important for the development of the germ cell transplants.

If a genetic model is not used as a recipient, the recipient animals must first be largely deprived of endogenous spermatogenesis so that transplanted stem cells do not compete with endogenous cells. In this regard, mice treated with busulfan were also utilized as recipients (Brinster & Zimmermann, 1994). Busulfan is a chemoterapeutic alkylating drug that induces chromosomal aberrations and results in loss of proliferative tissues. When used in a dose of 40 mg per kg of body weight for at least one month before transplantation, this drug kills all spermatogenic cells with the exception of a small number of the highly resistant stem cells (Bucci

& Meistrich, 1987). Therefore, a busulfan-treated recipient testis simultaneously develop spermatogenesis via the transplanted spermatogonia and also via endogenous stem cell, requiring a genetic (LacZ gene) or morphological marker to identify the sperm arising from the transplanted stem cell.

After preparation, the concentration of donor cells is adjusted to about 1 to 5 x 10^7 cells per mililiter and cooled to 5°C where the temperature is held constant until injection, usually 1-4 h (Ogawa et al., 1997). The range of injection volume required for a mouse recipient is usually less than 100 µl, of which about 10 µl enter the tubules (Dobrinski et al., 1999[b]; Johnston et al., 2000). As the volume injected is more than can be handled by a single seminiferous tubule, the injected material enters the rete testis and flows into many seminiferous tubules. Mixed germ cells, including an unknown number of spermatogonial stem cells, are introduced into the lumen of seminiferous tubules. The concentration of testicular stem cells is believed to be very small. According to Meistrich and van Beek (1993) and Tegelenbosh and De Rooij (1993), approximately 20,000 stem cells are found in an adult mouse testis containing 10^8 cells; which means one in every 5,000 cells. To initiate spermatogenesis, some of the stem cells introduced into the tubular lumen had to pass in a retrograde fashion through Sertoli-Sertoli junctions to the basal compartment, which was not predictable before the transplantation technique was reported.

In the first reports, spermatogonial transplantation was performed via a microinjection technique where cells were introduced directly into seminiferous tubules (Brinster & Avarbock, 1994; Brinster & Zimmermann, 1994; Clouthier et al., 1996). Although not novel, this technique demonstrated that most of the seminiferous tubules (about 20 in mice; Bascom & Osterud, 1925) could be filled with donor cells via the connections of the tubules with the rete. Filling of seminiferous tubules may also be obtained by microinjection of germ cells into the rete or the efferent ductules (Ogawa et al., 1997). Wheter injected into seminiferous tubules, the rete, or the efferent ductules, the flow of the injection fluid fills the majority of seminiferous tubules with about equal transplantation success (Ogawa et al., 1997). Although the last method has become the method of choice to perform transplantation in mouse and rats, each approach is likely to be useful for different experimental purposes in a variety of species (Ogawa et al., 1997).

Hand held micropipettes can be used to inject into the rete; a micromanipulator is used to position a micropipette for microinjection directly into seminiferous tubules. Micropipettes with a tip diameter of about 50 µm are used to inject cell suspensions. For seminiferous tubule injections, the tunica albuginea is cut and the micropipette needle aligned in parallel with one of the protuding seminiferous tubules. Adding a small amount of trypan blue to the injection medium

allows monitoring of the filling of tubules. Generally, less than three minutes is required to fill from 50%-100% of surface seminiferous tubules (Ogawa et al., 1997).

Mouse to Mouse Transplants and Development of Transplanted Germ Cells

In the first reports on spermatogonial transplantation (Brinster & Avarbock, 1994; Brinster & Zimmermann, 1994) it was shown that the injection of mice germ cells into the testis of sterile recipient mice gives rise to functional sperm in the recipient testis. Normal donor spermatogenesis has been observed for several months post-transplantation and probably remains active during the entire reproductive life of the animal. So, it was assumed that donor-derived spermatogenesis was initiated by spermatogonial stem cells; otherwise the germ cell population would be depleted from the seminiferous epithelium after about five to six weeks due to failure of self-renew of stem cells. Another important aspect in spermatogonial transplants technique is the fact that spermatogonial stem cells injected and present in the seminiferous tubule lumen must traverse extensive Sertoli cells tight junctions toward the basal compartment. This mechanism of germ cell migration in the seminiferous epithelium was thought not to be possible before transplantation was performed.

Mating experiments performed by Brinster and Avarbock (1994) showed that the bacterial marker LacZ gene in transplanted cells that developed into sperm was passed to successive generations. Thus, spermatogonial transplantation is another transgenic technique. This finding has great potential applicability in transgenic technology once the entire genome of the animal can be substituted/ modified.

Spermatogenesis with normal cell associations was found in mouse-to-mouse transplants. However, spermatogenesis was not allways qualitatively or quantitatively normal in transplanted animals. Germ cell degeneration, phogocytosis of released sperm and elongated spermatids were often missing or deformed (Russell et al., 1996; Parreira et al., 1998).

The growth of colonies from stem cells from mouse donors transplanted to mouse recipients has been tracked in whole-mounted tissue (Nagano et al., 1999), in sectioned tissue (Parreira et al., 1998), or histologically and *in vivo* under ultraviolet light, following transplantation of cells labeled with enhanced green fluorescent protein (Ohta et al., 2000). Whole mounts reveal that some cells, presumably stem cells, reach the basal lamina within a few days after transplantation. Colonies can be identified as chains of LacZ positive cells within one week to one month, increasing 24 µm/day. After this period, germ cells chains grow in length along the basal aspect of the tubule and then develop toward the adluminal portion

of the seminiferous epithelium. From two to four months, the lateral spread of spermatogenesis in transplants is rapid, reaching up to 69 μm per day (Nagano et al., 1999). In sectioned material, clones of spermatocytes are observed after one month following transplantation. By two months, sperm are produced and by three months an average of 1/3 of seminiferous tubules cross-sections analysed contained donor-derived spermatogenesis (Parreira et al., 1998). Similar results were found at one week, one month and two months, in the studies involving germ cell producing green fluorescent protein (Ohta et al., 2000).

Interspecies and Rat to Rat Spermatogonial Transplants

The success of interspecies transplants is probably the most fascinating and surprising finding that has emerged from stem-cell transplantation studies (Clouthier et al., 1996). Even though the testis is considered as an immune privileged organ, in xenogeneic transplant donor and host strains must be selected to be immunologically compatible. Thus, rat spermatogenesis occurred and rat sperm with apparently normal morphology were produced over several months following transplantation, when immunodeficient nude mice (lacking T cells) or SCID (lacking both B and T cells) mice treated with busulfan were used as hosts (Clouthier et al., 1996).

Recipient mice treated with busulfan also show endogenous spermatogenesis meaning that in a xenogenic transplantation, both rat and mouse spermatogenesis are conducted simultaneously within the same mouse recipient. Since the donor rat strain carried a LacZ transgene, rat spermatogenesis could be distinguished by staining for β-galactosidase. Also, rat and mouse spermatozoa could promptly be distinguished in the epididymis by their morphological characteristics. When donor spermatogonia are injected into host seminiferous tubules, some donor Sertoli cells are transferred along with germ cells. In the rat into mouse transplantation experiments, the donor rat carried a LacZ transgene that were expressed in Sertoli cells. All the Sertoli cells lining the seminiferous tubules did not show LacZ staining and were thus of host origin (Clouthier et al., 1996). The shape of the spermatid head, the degree of condensation of spermatid head, and the positioning of organelles within germ cells provided unequivocal identification of the species origin of cell types (Russell & Brinster, 1996). Since mouse and rat Sertoli cell can be distinguished at the ultrastructural level by the appearance of the mitochondria, ultrastructural observations showed that no rat Sertoli cell was found lining the seminiferous tubules (Russell & Brinster, 1996).

Similar to what was observed in mouse-to-mouse transplants, spermatogenesis with normal rat cell associations was found in rat-to-mouse

transplants (Russell & Brinster, 1996). Both rat and mouse spermatogenesis occurs simultaneously in the mouse testis after rat cells are transplanted into the busulfan-treated mouse testes. However, there appears to be no intermixing of rat and mouse cells within a given region of the tubule. Therefore, it appears that the ability to form cell associations characteristic of the donor species is an endogenous property of germ cells.

The fact that mouse Sertoli cells can fully support rat spermatogenesis, in spite of approximately 12-16 million year of evolution that separate these two species, is an extraordinary finding. Germ cells from different species usually present different developmental timing and morphologies. Thus, Sertoli cells of one species must have considerable capability to support germ cells of another. It is also suggested that germ cells apparently do not have rigid time requirements for Sertoli cell secreted products and/or Sertoli cells have more flexibility to respond to germ cell requirements (Russell & Griswold, 2000). Although the rat and mouse are closely related species, their spermatogenic cycle length differ by approximately 50%, being the length of the spermatogenic cycle in mice approximately 35 days while in rats it lasts about 52 days.

Male germ cell transplantation between the testes of two immunologically diverse strains of rats has also been successful in supporting spermatogenesis within the lumen of the host seminiferous tubules that were made sterile with busulfan (Jiang & Short, 1995). There was no positive identification that the donor cells had colonized seminiferous tubules. The stage of spermatogenesis in donor and adjacent host seminiferous epithelium was closely synchronized. According to the same authors in another report (Jiang and Short, 1998) intraluminal development of spermatogenesis was most likely produced when primordial germ cells were injected; whereas, injected postnatal germ cells probably integrated with the host seminiferous epithelium. Preparation of the recipient is also very important for transplant success. Recent investigation by Ogawa et al. (1999) showed that, although depletion of endogenous spermatogenesis before donor cell transplantation was more difficult in rat than reported for mouse recipients, rat-to-rat and mouse-to-rat transplantation were most successful in recipients made cryptorchid and treated with busulfan and leuprolide (GnRH antagonist). Several techniques to improve transplant efficiency has been utilized lately; these techniques will be discussed in more detail below.

Hamster sperm was shown to be produced into a nude mice testis following transplantation of spermatogonial cells (Ogawa et al., 1999). However, the efficiency of colonization was lower and many abnormal spermatids were observed than in other xenogenic transplants conducted to date.

Germ cells from rabbits, dogs and large domestic animals (boar, bull and horse) were transplanted into nude mice testes. Although different degrees of

colonization were observed comparing the various donors investigated, no spermatogenesis of the donors ocurred (Dobrinski et al., 1999[a]; Dobrinski et al., 2000). Also, Reis and collaborators (2000) have shown that human spermatogonia did not survive when transplanted into the testes of mutant (W-locus) and immunodeficient (SCID) mice. Therefore, xenogeneic spermatogonial transplantation has shown some aspects of the limitations of the technology and the similarities and differences in the testicular environment among different mammalian species (Clouthier et al., 1996; Dobrinski et al., 1999[a]; Ogawa et al., 1999; Dobrinski et al., 2000). Although it is believed that the success of transplantation is positively related with the degree of phylogenetical proximity of species during evolution, the investigation by Dobrinski et al. (2000) with large domestic animals suggests that other components than phylogenetical distance might play a role in transplant success. Since spermatogonia are present in most xenogeneic transplants of immunodeficient recipients, the immunological incompatibility may not be the only problem associated with failure of transplant success. Taking these considerations into account, an understanding of host rejection with respect to stem cell transplantation will be important before this technology can be used for agricultural, zoological and clinical purposes (Johnston et al., 2000). Another important aspect is to optimize the injection procedure. In a comparative investigation, Schlatt et al. (1999) injected germ cells into the testes of rats, bulls, monkeys and humans that had been surgically removed. Because seminiferous tubules from larger animals have a resistent lamina propria and highly convoluted tubular mass, greater success was achieved using an ultrasound-guided rete testis injection.

Transplants from Cryopreserved and Cultured Germ Cells

Another breakthrough development employing the spermatogonial transplant technique was the successful transplant of cryopreserved germ cells (Avarbock et al., 1996). Mouse spermatogonial stem cells were cryopreserved at -196°C. After thawing, mouse stem cells that have been frozen for months were able to generate spermatogenesis in mouse testis. By freezing and storing testicular tissue, it should be possible to preserve indefinitevely the germ cell lines of unique experimental animals, endangered species or valuable farm animals, each of which would be capable of passing on the genes of the donor. In this regard, spermatogonial stem cells can be considered "immortal" (Avarbock et al., 1996).

Germ cell cryopreservation and transplantation technique has also clinical application, being a potential method to preserve future male fertility. Men likely to lose spermatogenic function after chemotherapy could have a testicular biopsy

taken and cryopreserved for later reintroduction into their testes to restore spermatogenesis. In lymphoma and leukemia patients the testis is a likely organ for the settlement of metastasing cells. One must be careful not to reintroduce malignant cells into a patient who was previously cured.

The successful transplantation of cultured stem cells into a recipient was a crutial advance in spermatogonial transplant technique. The first culture of approximately four months of duration was demonstrated by Brinster and colleagues (Nagano & Brinster, 1998; Nagano et al., 1998) using a modified Eagle's medium. In some experiments, STO feeder layers similar to those employed for embryonic stem cell culture were co-cultured with the donor germ cells. Although it could not be determined whether the stem cells divided during the culture period, these studies have shown that stem cells is able to survive in culture. The small number and confined location of spermatogonial stem cells within the testis make them relatively inaccesible to genetic manipulation. Thus, transplantation of cultured spermatogonial stem cells opens exciting possibilities in the near future for *in vitro* multiplication and manipulation of male germ line cells (Nagano et al., 1998).

Techniques to Improve Transplant Efficiency

Although spermatogonial transplantation technique has proved to be an excellent methodology to study reproductive biology, its efficiency is still relatively poor. In this regard, new methods have been developed by Brinster and colleagues. Ogawa et al. (1998) have shown that treatment with an GnRH antagonist (leuprolide) increased markedly the transplant efficiency in recipient mice probably due to the low intratesticular testosterone concentration secondary to gonadotropin deficiency.

Transplantation of spermatogonial stem cells provides a unique system to study the biology of spermatogonial stem cells. Utilizing an image analysis system to quantify the degree of colonization, Dobrinski et al. (1999[b]) showed that there is an almost linear correlation in the number of injected stem cells with the degree of colonization of the recipient testis. In their study, these authors recommended the introduction of approximately 10^7 donor testis cells per recipient testis to optimize transplant efficiency.

Another potential strategy for increasing the transplantation efficiency is to develop methods for enriching stem cell population. Using antibodies against β1 or α6 integrins, known to be associated with the surface of many stem cell types residing on a basal lamina, Shinohara et al. (1999) showed an increase of up to ten times in the number of colonies in recipients, when a ten-fold enrichment of spermatogonial stem cell was obtained. Although both steel factor and its surface tyrosine kinase receptor (c-kit) are critical for development and maintenance of spermatogenesis,

and c-kit appears in early stages of spermatogenesis, no improvement in the number of colonies was obtained when c-kit antibodies were utilized as a marker for isolation of stem cells (Shinohara et al., 1999).

Germ Cell Transplants as a Tool to Understand Spermatogenesis

Spermatogonial transplantation can answer important functional questions, being particularly useful in discriminating the role of the Sertoli cell from that of the germ cell during spermatogenesis. The time required for spermatogonia to develop into sperm is rigidly regulated for each species (Russell et al., 1990; França & Russell, 1998). For instance, the length of spermatogenesis in mice is approximately 35 days while in rats it lasts about 52 days. Until recently, it was not known if the developmental timing was intrinsic to the germ cell or was influenced or even guided by the Sertoli cell or other somatic elements. Rat-to-mouse transplantation showed that rat germ cells developed at the speed they would have normally developed in the rat and that mouse germ cells in the same testis developed at the speed characteristic of the mouse (França et al., 1998). Thus, somatic cells, particularly the Sertoli cell, had no influence over the rate of germ cell development; the regulation being inherent to the germ cells. This finding may serves as a paradigm for other self-renewing systems of the body.

Transplantation of germ cells from infertile mice carrying the steel (Sl) mutation to infertile white spotting (W/W^v or W^v/W^{54}) mutant male mice showed that the recipient developed qualitatively normal spermatogenesis (Ogawa et al., 2000). This result shows that complete spermatogenesis can be restored when testicular environment is permissive.

Transplantation of wild-type germ cells into recipients carrying the knockout and transplantation of germ cells carrying the disrupted gene into wild-type recipients is a powerful strategy to provide information to whether somatic or germ cells are responsible for a particular disruption on the development of spermatogenesis. In a report by Mahato et al. (2000) with a comment by Griswold (2000), germ cells from the testis of the estrogen receptor α (ERα) knockout mouse were transplanted into the seminiferous tubules of germ cell-depleted wild-type mice. The germ cell carrying the knockout mutation underwent qualitatively normal spermatogenesis, and the recipients were fertile. Offspring derived from some of the recipient mice were shown by coat color and PCR to be derived from sperm carrying the disrupted gene for the ER. The authors proved that not only was spermatogenesis qualitatively normal but functional fertile sperm were produced that carried the ERα knockout gene. Also, the successful transplantation of normal spermatogonia into juvenile spermatogonial depletion (*jsd*) seminiferous tubules provided clear evidence that the *jsd* defect lies in the germ cells rather than in the somatic cells of the testis (Boettger-Tong et al., 2000).

Concluding Remarks and Future Research

Male and female germ cell lines are developmentally very different. The male continues to produce gametes after puberty while the female germ cells arreste in meiosis prior to birth and complete meiosis only at fertilization. In this regard, the presence of highly proliferative spermatogonial stem cells in the adult testis offers a potentially simpler and cheaper route for genetic engineering and to germline modification by homologous recombination than the existing use of embryonic stem cells (McLaren, 1998). Also, transfecting the desired gene into spermatogonia followed by successful transplantation would facilitate the production of transgenics.

The horizon offers many opportunities for spermatogonial transplantation. There are potential improvements in livestock breeding where the genetic stock of superior males may be maintained indefinitevely in surrogate recipients (Hausler & Russell, 1999). Likewise, preservation of endangered species may occur through freezing of gametes until a suitable recipient can be found that will maintain the germ line. Also, if an adequate culture system for spermatogonia could be devised, transplantation of genetically modified cells to a host testis would ensure germline transmission; especially now that *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) does not require that large numbers of spermatozoa to be produced to achieve pregnancies (McLaren, 1998). Although the ethical aspects of this strategy remains to be clarified, spermatogonial transplantation could also be beneficial for human reproduction using surrogate donors. Usually, many cases of male infertility has proved intractable to therapy. If the testis in these infertile individuals contains at least spermatogonial stem cells, it might be possible to transplant these cells into a host testis of the same or different species to obtain sufficient sperm of donor origin to achieve a pregnancy using ICSI.

Another potential clinical use of spermatogonial technique is the replacement of the germ line in patients whose endogenous stem cells had been eliminated (or damaged) as a result of gonadotoxic chemo-or radiotherapeutic treatment (Bahadur et al., 2000). Transplant success in humans could involve only a single seminiferous tubule since retrieval of haploid cells or single sperm followed by in vitro fertilization could be utilized to obtain a pregnancy. Also, the replacement of a defective germ line or the surrogate production of spermatozoa in the case of a Somatic cell defect could be considered.

Spermatogonial transplantation has been proven to be an extraordinary and powerful technique to investigate reproductive biology and many important advances has been made utilizing this methodology, since it was first reported in 1994 by Brinster and colleagues. While progress in this direction has been significant and swift, significant barriers such as immunological response and mechanisms for

introducing stable genetic material into stem cells remain to be examined (Johnston et al., 2000; Russell & Griswold, 2000). Allogenic and xenogenic transplants make it possible to study the immune system and its suppression could improve the transplantation efficiency, allowing this technique to be applicable to virtually all species.

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