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Spermatogonial transplantation — an update for the millennium

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Abstract

Spermatogonial transplantation as developed in the laboratory of Ralph Brinster has been a technological breakthrough in the study of Sertoli-germ cell interactions. For the first time, germ cells can be transferred from one animal to another and from one species to another. The transfer technology combined with developments in freezing germ cells, long-term culture of germ cells, and enrichment of stem cell populations portend even more significant breakthroughs in the new millennium. The ultimate application of germ cell transfer would allow the in vitro genetic manipulation of cultured stem cells that could then be transplanted into recipient syngeneic or xenogeneic recipients and give rise to functional male gametes. Clearly, this achievement would have applications in basic science, human medicine, and domestic and wild animal reproduction. While progress in this direction has been significant and swift, significant barriers such as immunological response and mechanisms for introducing genetic material into the stem cells remain to be examined. This report is a chronological review of the technological advances made and conceptual insights gained since the first report of successful transplantation in 1994.

Keywords: Spermatogonia; Transplantation; Spermatogenesis; Mouse; Rat; Hamster

It has been 6 years since the first report of successful spermatogonial transplantation utilizing a microinjection technique, (Brinster and Zimmerman, 1994; Brinster and Avarbock, 1994). Prior to the millennium, less than 30 original papers and reviews had been published directly relating to the topic. Those reviews that are available have generally focused on the initial development and early progress in transplantation technology (Ogawa et al., 1997; Nagano and Brinster, 1998; Nagano et al., 1998; Russell and Brinster, 1998; Russell et al., 1998). This review summarizes both the initial studies and a series of newly published studies relating to spermatogonial transplantation — most or all have appeared before the year 2000. We have chosen to present advances in spermatogonial transplantation technology in almost chronological sequence, making comments/interpretations along the way about the contributions and impact of each study.

In the milestone papers from Brinster's laboratory in 1994 (Brinster and Avarbock, 1994; Brinster and Zimmerman, 1994), the reproductive community was amazed by the initial demonstration of success in spermatogonial transplantation. Brinster and Zimmerman (1994) showed that microinjection of a heterogeneous mouse testis cell mixture into the seminiferous tubules of a genetically sterile mouse recipient would result in donor spermatogenesis in the recipient that could be demonstrated by a specific genetic marker, *LacZ* serving as a reporter gene. LacZ encodes for β-galactosidase, an enzyme that can be demonstrated histochemically as an intracellular blue reaction product. The demonstration that cells introduced into the tubular lumen could initiate and maintain spermatogenesis was counterintuitive to the knowledge that spermatogonia were localized to the basal aspect of the tubules. The spermatogonia, once injected into the lumen of the seminiferous tubules, must have been translocated from the lumen to the base of the seminiferous tubules and, in doing so, had to cross extensive Sertoli–Sertoli junctions in a direction which germ cells normally do not move in the testis.

It is a reasonable assumption that only the stem cell population of spermatogonia is responsible for the initiation and maintenance of continuous spermatogenesis in these transplants although the identity of the stem cell in the testis is sometimes debated (Dym, 1994).

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Current thought is that spermatogenesis must ensue from a single seeded stem cell. The stem cell in the testis is generally thought to be the A_s ($A_{isolated}$) spermatogonium. The division of A_s cells result in not only more A_s cells, but type A spermatogonia committed to the spermatogenic process. It should be mentioned that, to date, only a semipurified heterogeneous population of germ cells and somatic cells has been transplanted into the recipient testes.

The technique of microinjection, although not novel, demonstrated that most of the seminiferous tubules could be filled with donor cells via the connections of the tubules with the rete. In the same year, Brinster and Avarbock (1994) showed in mating experiments that the marker LacZ gene in transplanted cells that developed into sperm was passed to successive generations. In essence, Brinster's laboratory had pioneered another transgenic technology that was capable of modifying the entire genome of the animal. It was also in this report that Brinster showed transplantation would be successful in a recipient if it could be made sterile using the chemotherapeutic agent, busulfan. Busulfan does not kill all endogenous spermatogonia, but leaves some stem cells that will re-initiate spermatogenesis in the recipient; thus a testis will simultaneously develop spermatogenesis via the transplanted spermatogonia and via endogenous stem cells.

The potential uses for and ethical issues involved in spermatogonial transplantation technique were identified (Dym, 1994) and exploited by the popular press (numerous uncited articles). One such potential use mentioned was the development of a new and simpler transgenic technique whereby the germ line is stably modified in culture prior to transplantation.

The next report on transplantation was a paper by Jiang and Short (1995) that reported successful transplantation of rat cells into rat seminiferous tubules that were made sterile with busulfan. There was no positive identification that the donor cells had colonized seminiferous tubules. Recipient spermatogenesis was characterized as intraluminal as it was shown that portions of tubules with characteristic cell associations were noted surrounded by normal spermatogenesis. One characteristic of intraluminal spermatogenesis was the stage-synchronization of the intraluminal cells with those of the seminiferous epithelium. How could intraluminal spermatogenesis occur? To these reviewers, it would seem that intraluminal spermatogenesis could develop if peritubular, Sertoli cells and stem cell fragments were injected as an aggregate. In a follow-up paper, Jiang and Short (1998) indicated that intraluminal spermatogenesis was most likely produced by primordial germ cells whereas postnatal germ cells integrated with the host seminiferous epithelium.

In yet another major development from Brinster's laboratory (Clouthier et al., 1996), it was reported that interspecies (xenogenic) transplantation had been performed. Rat testis cells microinjected into the testes of immuno-compromised animals (nude and SCID mice) could develop and complete spermatogenesis. Rat sperm with the characteristic head shape of rat sperm were found in the epididymis of the mouse months after the transplantation. This was a significant finding from the standpoint that the prevailing paradigm was that the Sertoli cells were highly influential in the spermatogenic process and would not 'support' germ cells which had different developmental timing and which had different morphologies. The success of rat-to-mouse transplants caused the scientific community to re-evaluate the role of the Sertoli cell in terms of the requirements for support of germinal cells. The 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.

Light and electron microscopic studies were undertaken to evaluate the morphology of syngenic and xenogenic germ cell transplants (Russell and Brinster, 1996; Russell et al., 1996). Spermatogenesis with normal cell associations was found in both types of transplants. However, spermatogenesis was not qualitatively or quantitatively complete in transplanted animals, given that elongated spermatids were often missing or deformed. It was clearly documented in rat-to-mouse transplants that rat germ cells developed in association with mouse Sertoli cells.

The culture and storage of spermatogonia are key steps in developing a new transgenic technology. The first culture of 3 month duration was demonstrated by successful transplantation (Brinster and Nagano, 1998). Avarbock et al. (1996) demonstrated that a testis cell mixture could be frozen for extended periods of time prior to transplantation with subsequent development of spermatogenesis. The journal cover labeled the transplanted cells as 'immortal sperm' since it was now possible to store stem cells indefinitely and to produce unlimited sperm from spermatogonial stem cells.

Ogawa et al. (1997) published a thorough and detailed paper on the techniques and methods used in spermatogonial transplantation. They showed that two additional methods: (1) injection through the capsule directly into the rete; and (2) through the bundle of efferent ductules into the rete could be utilized to introduce germ cell mixtures into seminiferous tubules. The last method has become the method of choice to perform transplantations in mouse and rat. Ogawa et al. (1997) provided sufficient information for most individuals to master the spermatogonial transplantation technique.

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) and in sectioned tissue (Parreira et al., 1998).

Whole mounts reveal that some cells, presumably spermatogonia, reach the basal lamina during the first week after transplantation. Colonies can be identified as LacZ positive cells during the first month as chains of cells. These chains occupy about 1 mm of the basal lamina of the seminiferous tubules before spreading of colonies into the adluminal portion of the tubules occurs. In sectioned material, clones of spermatocytes are evident after 1 month of transplantation. By 2 months, sperm are produced and by 3 months an average of 30% of the testis contained donor-derived spermatogenesis. The cumulative lateral spread of spermatogenesis in transplants is rapid, moving at approximately 55–60 μm/day. Macrophages invade the tubule of transplanted testes and phagocytose some sperm. Sertoli cells in other regions of the tubule that lack spermatogenesis also phagocytose sperm (Russell et al., 1996).

To determine the efficiency of transplantation, it is important to establish the optimal number of cells that should be injected. Dobrinski et al. (1999b) set about doing so by first utilizing an image analysis system to quantify the degree of colonization in the testis with any given amount of donor cells. They then showed an almost linear correlation in the number of injected cells with the degree of colonization, recommending the introduction of about 10^7 donor testis cells/recipient testis. Transplant efficiency was shown to increase in conditions of low testosterone (Ogawa et al., 1998).

From the original studies described by Brinster and Avarbock (1994), it was not clear if purification of stem cells would enhance transplant success, in fact there was data to the contrary. The question was settled in 1999 when Shinohara et al. (1999) used antibodies against proteins known to be associated with the surface of stem cells on a basal lamina to enrich the spermatogonial cells. A ten-fold enrichment of spermatogonial stem cells produced up to a ten-fold increase in the number of colonies in recipients. Ideally, isolation of pure stem cells would be the most effective means to increase transplantation efficiency.

Since transplantation of spermatogonia had been successful into species other than the mouse (xenogenic) in instances when immuno-deficient recipients were used (Clouthier et al., 1996), it was unclear if spermatogenesis could be successfully initiated after allogenic transplantation. Personal communication from Brinster's laboratory and from M. Griswold's laboratory suggested that spermatogonia would seed in allogenic mouse transplants but not go forward to produced spermatogenesis.

It was reported by Ogawa et al. (1999b), that hamster spermatogenesis could be produced in immuno-deficient recipients. It was also clear that the success of hamster spermatogenesis did not match that of the mouse-to-mouse or rat-to-mouse transplants. Transplantations from rabbit to mouse and from dog to mouse (xenogenic) into genetically immuno-deficient animals were not successful in producing sperm. The transplanted cells, although translocated to the basal compartment remained there and did not progress. There were likely cell divisions but they did not produce more mature cell types (Dobrinski et al., 1999b). It was suggested that the success of transplants related positively to the degree of evolutionary relatedness of species. Since spermatogonia are present in xenogenic transplants of immuno-deficient recipients, the immunological incompatibility may not be the only problem associated with failure of allogenic transplant success. Brinster has found additional ways to suppress the immune system to increase the success rate of mouse-to-rat transplantation (Ogawa et al., 1999a).

Spermatogonial transplantation recently solved a major question regarding germ cell developmental timing (França et al., 1998). It is well known that the timing of germ cell development, from spermatogonia to sperm, is rigidly regulated for each species (Russell et al., 1990). For example, about 35 days are necessary in the mouse and 52–53 days in the rat. It was not known if the developmental timing was inherent to the germ cell or was influenced or even guided by the Sertoli cell. 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. Thus, the somatic cells had no influence over the rate of germ cell development. This finding serves as a paradigm for other self-renewing systems of the body.

Logical practical extensions of the spermatogonial transplantation are in basic science, medical science and animal sciences. Spermatogonial transplantation seems to be an ideal technique to pinpoint the problematic cell type in a genetic condition affecting the testis. It answers the question: 'Is the primary problem a somatic cell problem (e.g. the Sertoli cell) or is it a germ cell problem?'. No doubt numerous experiments will be undertaken along these lines.

Experimental procedures are underway to develop the technique in humans (Schlatt et al., 1999). Currently envisioned is the use of auto-transplantation for prepubertal boys undergoing chemotherapy such they may regain fertility after a chemotherapy or radiotherapy. 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.

Finally, there are potential improvements in livestock breeding where the genetic stock of superior males may be maintained indefinitely in surrogate recipients (Hausler et al., 1999). Likewise, preservation of endangered species may occur through freezing of gametes until a suitable recipient can be found that will maintain and spread the germ line.

References

Avarbock, M.R., Brinster, C.J., Brinster, R.L., 1996. Reconstitution of spermatogenesis from frozen spermatogonial stem cells. Nat. Med. 2, 693–696.

Brinster, R.L., Avarbock, M.R., 1994. Germline transmission of donor haplotype following spermatogonial transplantation. Proc. Natl. Acad. Sci. 91, 11303–11307.

Brinster, R.L., Nagano, M., 1998. Spermatogonial transplantation, cryopreservation and culture. Cell Dev. Biol. 9, 401–409.

Brinster, R.L., Zimmerman, J.W., 1994. Spermatogenesis following male germ-cell transplantation. Proc. Natl. Acad. Sci. 91, 11298–11302.

Clouthier, D.E., Avarbock, M.R., Maika, S.D., Hammer, R.E., Brinster, R.L., 1996. Rat spermatogenesis in mouse testes following spermatogonial stem cell transplantation. Nature 381, 418–421.

Dobrinski, I., Avarbock, M., Brinster, R., 1999a. Transplantation of germ cells from rabbits and dogs into mouse testes. Biol. Reprod., 61, 1331–1339.

Dobrinski, I., Ogawa, T., Avarbock, M., Brinster, R., 1999b. Computer assisted image analysis to assess colonization of recipient seminiferous tubules by spermatogonial stem cells from transgenic donor mice. Mol. Reprod. Dev. 53, 142–148.

Dym, M., 1994. Spermatogonial stem cells of the testis. Proc. Natl. Acad. Sci. 91, 11287–11289.

França, L., Ogawa, T., Avarbock, M., Brinster, R.L., Russell, L., 1998. Germ cell genotype controls cell cycle during spermatogenesis. Biol. Reprod. 59, 1371–1377.

Hausler, C., Xiong, C., Russell, L.D., 1999. Prospects for developing spermatogonial transplantation in domestic animals. In: The Male Gamete, C. Gagnon, ed. Cache River Press, Vienna, IL.

Jiang, F.-U., Short, R.V., 1998. Different fate of primordial germ cells and gonocytes following transplantation. APMIS 106, 53–63.

Jiang, F.-X., Short, R.V., 1995. Male germ cell transplantation in rats: apparent synchronization of spermatogenesis between host and donor somniferous epithelia. Int. J. Androl. 18, 326–330.

Nagano, M., Brinster, R., 1998. Spermatogonial transplantation and reconstitution of donor cell spermatogenesis in recipient males. APMIS 106, 47–57.

Nagano, M., Avarbock, M., Leonida, E., Brinster, C., Brinster, R.L., 1998. Culture of mouse spermatogonial stem cells. Tissue Cell 30, 389–397.

Nagano, M., Avarbock, M., Brinster, R., 1999. Pattern and kinetics of mouse donor spematogonial stem cell colonization in recipient testes. Biol. Reprod. 60, 1429–1436.

Ogawa, T., Arechaga, J., Avarbock, M., Brinster, R., 1997. Transplantation of testis germinal cells into mouse seminiferous tubules. Int. J. Dev. Biol. 41, 111–121.

Ogawa, T., Dobrinski, I., Avarbock, M.R., Brinster, R.L., 1998. Leuprolide, a gonadotropin-releasing hormone agonist, enhances colonization after spermatogonial transplantation into mouse testes. Tissue Cell 30, 583–588.

Ogawa, T., Dobrinski, I., Brinster, R., 1999a. Recipient preparation is critical for spermatogonial transplantation in the rat. Tissue Cell, 31, 461–472.

Ogawa, T., Dobrinski, M., Brinster, R., 1999b. Xenogeneic spermatogenesis following transplantation of hamster germ cells to mouse testes. Biol. Reprod. 60, 515–521.

Parreira, G., Ogawa, T., Avarbock, M., Brinster, R., Russell, L., 1998. Development of testis cell transplants. Biol. Reprod. 59, 1360–1370.

Parreira, G.G., Ogawa, T., Avarbock, M.R., França, L.R., Brinster, R.L., Russell, L.D., 1999. Development of germ cell transplants: morphometric and ultrastructural studies. Tissue Cell 31, 241–254.

Russell, L.D., Brinster, R.L., 1996. Ultrastructural observations of spermatogenesis following transplantation of rat testis cells into mouse seminiferous tubules. J. Androl. 17, 615–627.

Russell, L.D., Brinster, R.L., 1998. Spermatogonial transplantation. In: Zirkin, B.R. (Ed.), Germ Cell Development, Division, Disruption and Death. Springer, Serono Symposium USA, New York, pp. 19–27.

Russell, L.D., Ettlin, R.A., Sinha-Hikim, A.P., Clegg, E.D., 1990. Histological and Histopathological Evaluation of the Testis. Cache River Press, Clearwater, FL.

Russell, L.D., França, L.R., Brinster, R.L., 1996. Ultrastructural observations of spermatogenesis in mice resulting from transplantation of mouse spermatogonia. J. Androl. 117, 603–614.

Russell, L.D., Nagano, M., Brinster, R.L., 1998. Spermatogonial transplantation. In: Stefanini , M., Boitani, C., Galdiere, M., Geremia, R., Patombi, F. (Eds.), Testicular Function: From Gene Expression to Genetic Manipulation. Springer, Berlin, pp. 41–57.

Schlatt, S., Rosiepen, G., Weinbauer, G., Rolf, C., Brook, P., Nieschlag, E., 1999. Germ cell transfer into rat, bovine, monkey, and human testes. Hum. Reprod. 14, 144–150.

Shinohara, T., Avarbock, M.R., Brinster, R.L., 1999. B1- and A6-integrin are surface markers on mouse spermatogonial stem cells. Proc. Natl. Acad. Sci. 96, 5504–5509.