

RESEARCH HIGHLIGHT

Functional sperm produced after spermatogonial stem cell transplantation into rhesus

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Spermatogonial stem cell (SSC) transplantation is a promising technique to circumvent sterility in prepubertal boys undergoing gonadotoxic treatments. While the cryopreservation of spermatogonial stem cells is being introduced in clinical practices worldwide, a lot of unanswered questions remain regarding their eventual transplantation. In this paper autologous and allogeneic SSC transplantations in the testes of sterilized macaques were performed and spermatogenesis could be restored from donor SSCs. The spermatozoa obtained were competent to fertilize oocytes. This report proves the feasibility of SSC transplantation in a primate model, hence reinforcing the hope that this strategy will eventually find its way into clinical practice.

Spermatogonial stem cells (SSCs) are the founder cells of spermatogenesis and are responsible for the lifelong production of spermatozoa. The cryopreservation and transplantation of these cells has been proposed as a fertility preservation strategy for young boys at risk for stem cell loss, i.e. patients undergoing chemotherapy for cancer or as a conditioning treatment for bone marrow transplantation.¹ Although the feasibility of SSC transplantation has been shown in rodent models, the proof for production of functional sperm in larger animal species was lacking until a recent publication in *Cell Stem Cell*.² In this work, scientists from the University of Pittsburgh School of Medicine performed autologous and allogeneic SSC transplantations into the testes of macaques that were pre-treated with chemotherapy. This animal model, elegantly mimicking the

clinical situation, is of great value for translational research. The observation that spermatozoa could be produced in this animal model and that these spermatozoa were capable of fertilizing oocytes and supporting pre-implantation development, is an important milestone for the introduction of SSC transplantation to a clinical setting.

In contrast to adult men who have the choice to bank a semen sample before their gonadotoxic treatment, young boys cannot benefit from this possibility, because spermatogenesis only starts around puberty. Anticipating that SSCs can eventually restore fertility, more and more worldwide fertility centers started to cryopreserve prepubertal testicular tissue. To prevent lifelong sterility in boys, methods for SSC transplantation have to be developed. Currently, two fertility restoration strategies are being developed and studied: the injection of SSCs and the grafting of testicular tissue containing SSC. Depending on the disease of the patient, one of these two approaches will be applicable. Grafting has the advantage that SSCs can reside within their natural niche, preserving the interactions between germ cells and their supporting cells and may therefore be regarded as the first choice strategy. However, in cases where the risk for malignant contamination of the testicular tissue is real, e.g., leukemia, transplantation of SSCs by injection is preferable over grafting.

In 1994, the transplantation of SSCs from a fertile mouse into the testes of a sterile recipient mouse was introduced by Brinster and colleagues.³ Donor-derived spermatogenesis was observed and viable offspring were obtained. Since then, the technique has been widely used in other animal models and is now considered to be the gold standard bioassay for assessing SSC functionality. However, before this technique can be translated to a clinical application, studies on large animal models are inevitable.

The first SSC transplantations in monkeys were performed in 1999.⁴ In this study, the injection technique was efficiently adapted to larger testes. Whereas, in small animals, the testes have to be exposed before injecting SSCs through the efferent duct in the rete testis, the method developed for larger testes is less invasive. Injections could be performed in the rete testis under ultrasound guidance. SSCs have been injected in X-irradiated monkeys, but evidence for donor-derived spermatogenesis was lacking.⁵ Recently, important progress has been made concerning the translation of autologous testicular tissue grafting. In primates that were rendered infertile by testicular irradiation, testicular fragments were grafted to several locations. Although many fragments were transplanted, a very low efficiency was reported and only fragments grafted in the scrotum revealed spermatogenesis. Unfortunately, as well as in all previous studies on large animals, this study did not include sperm functionality tests.

In the present study, Hermann and colleagues² did address this important aspect and assessed the regenerative capacity of primate SSCs by performing autologous SSC injections in busulfan-treated macaques.⁶ To be able to distinguish donor from endogenous spermatogenesis, donor cells were treated with lentiviral vectors containing GFP or mCherry inserts. After transplantation, donor-derived spermatozoa were detected in the ejaculate of 70% of the recipients. Unfortunately, because the efficiency of SSC-marking was very low, the authors were not able to conduct fertilization experiments after autologous SSC transplantation. Therefore, they conducted a series of allogeneic transplantations in which the donor spermatozoa had unique DNA microsatellite allele profiles that could be discriminated from those of endogenous spermatozoa. Donor-derived spermatozoa were found in 33% of the recipients. Spermatozoa from

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one rhesus were used to fertilize rhesus oocytes by intracytoplasmic sperm injection and embryos exhibiting donor DNA advanced to the morula stage. This is the first time that sperm obtained from transplanted primate SSCs are shown to be capable of fertilization and preimplantation embryo development. Furthermore, the study showed that spermatozoa could be obtained both after autologous transplantation of SSC in either prepubertal or adult recipients. This is an important finding for any future clinical application, because it implies that SSC transplantation may be postponed for several years until it becomes clear whether the patient is devoid of any residual spermatogenesis after being cured from cancer.

A lot of progress has been made since the first paper on SSC transplantation almost two decades ago. Clinical application has come into sight, yet additional research needs to be done. As the authors acknowledge in their paper, full term development of embryos and production of viable offspring needs still to be demonstrated.

Future studies on nonhuman primates should therefore focus on the improvement of fertility restoration efficiency. So far, efficiency is rather low, but it might be enhanced by optimizing recipient preparation and by increasing the SSC population through enrichment strategies^{7,8} or *in vitro* propagation.⁹ These adaptations might increase the proportion of donor sperm and facilitate the production of donor offspring.

There is an urgent need to conduct these studies and to translate SSC transplantation to a clinical application as, in the near future, patients will be referred back for transplanting their banked SSCs. Thanks to the latest paper of Hermann and colleagues,² oncologists, hematologists and fertility specialists are able to better counsel their patients and their parents.

1 Bahadur G, Ralph D. Gonadal tissue cryopreservation in boys with paediatric cancers. *Hum Reprod* 1999; **14**: 11–7.

- 2 Hermann BP, Sukhwani M, Winkler F, Pascarella JN, Peters KA *et al.* Spermatogonial stem cell transplantation into rhesus testes regenerates spermatogenesis producing functional sperm. *Cell Stem Cell* 2012; **11**: 715–26.
- 3 Brinster RL, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. *Proc Natl Acad Sci U S A* 1994; **91**: 11298–302.
- 4 Schlatt S, Rosiepen G, Weinbauer GF, Rolf C, Brook PF *et al.* Germ cell transfer into rat, bovine, monkey and human testes. *Hum Reprod* 1999; **14**: 144–50.
- 5 Schlatt S, Foppiani L, Rolf C, Weinbauer GF, Nieschlag E. Germ cell transplantation into X-irradiated monkey testes. *Hum Reprod* 2002; **17**: 55–62.
- 6 Jahnukainen K, Ehmcke J, Nurmio M, Schlatt S. Autologous ectopic grafting of cryopreserved testicular tissue preserves the fertility of prepubescent monkeys which receive a sterilizing cytotoxic therapy. *Cancer Res* 2012; **72**: 5174–8.
- 7 Gassei K, Ehmcke J, Dhir R, Schlatt S. Magnetic activated cell sorting allows isolation of spermatogonia from adult primate testes and reveals distinct GFRa1-positive subpopulations in men. *J Med Primatol* 2010; **39**: 83–91.
- 8 Hermann BP, Sukhwani M, Salati J, Sheng Y, Chu T *et al.* Separating spermatogonia from cancer cells in contaminated prepubertal primate testis cell suspensions. *Hum Reprod* 2011; **26**: 3222–31.
- 9 Sadri-Ardekani H, Mizrak SC, van Daalen SK, Korver CM, Roepers-Gajadien HL *et al.* Propagation of human SSCs in-vitro. *JAMA* 2009; **302**: 2127–34.