RECONSTITUTION OF SPERMATOGENESIS FROM TESTIS FAILURE AFTER SPERMATOGONIAL STEM CELLS (SSC) TRANSPLANTATION FOR MALE FERTILITY PRESERVATION-A TRANSGENIC MOUSE MODEL

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Purpose: Fertility preservation for prepubertal male mouse is feasible by the reconstruction of spermatogenesis after spermatogonial stem cells transplantation.

Materials and methods: A. Donor cell preparation. Cells for transplantation are obtained from testes of FVB/N-Tg (PolII-luc) Ltc transgenic mouse 5 to 60 days after birth by a two-step enzymatic digestion protocol. The cell volume needed to inject both testes of a mouse ranges from 0.1 to 0.5 ml depending on the injection method. Cell concentrations of up to about 300x10⁶ cells per ml can be used. Concentrations above 10⁶ cells/ml are associated with high levels of clumping and plugging of pipettes, which may be reduced by the addition of DNase at the time of injection. The cells are maintained at SSC until the time of loading into an injection pipette, usually 1 to 4 h. B. Transplantation of SSC into recipient mouse. In this procedure, donor cells are harvested from the testes of fertile donor mice that express a reporter transgene, and suspension of the cells are microinjected into seminiferous tubules of FVB/NJNarl wild type recipient infertile adult mice at 6-week-old after complete busulfan-induced testis failure. C. In vivo tracking of grafted SSC by bioluminescence imaging (BLI). Reporter genes can be used to assay for the activity of a particular RNA Polymerase II promoter (PolII) in each viable cell. Reporter gene products are luciferase (enzymes). The enzyme luciferase catalyzes a reaction with a luciferin to produce light which is quantified as quantum. The reporter gene is simply placed under the control of the target promoter (PolII) and the reporter gene product's activity is quantitatively measured. After gonadal tissues or germ cells transplantation, each mouse was imaged every other day for 2 weeks and subsequent every weeks for 2 months. For imaging of mice with gonadal tissue transplants. Mice were anesthetized with isoflurane. A saturating concentration of the substrate D-luciferin was injected intraperitoneally (150 mg/kg). Bioluminescence was quantified the quantum by summing pixel intensities within equal area ROI. D. Fertilization assay. Mating the recipient male to a wild-type female, then progeny is produced by nature fertility.

Results: Live birth pup of FVB/N-Tg (PolII-luc) Ltc transgenic mouse were born and imaged by bioluminescence after mating FVB/NJNarl female wild type and male wild type recipient 3-4 month after FVB/N-Tg (PolII-luc) SSC transplantation.

Conclusions: Spermatogonial stem cell transplantation could be used to restore fertility in men following chemotherapy or radiation treatment. Development of techniques for the in vitro differentiation of spermatogonial stem cells to functional spermatozoa is a crucial step for the treatment of infertility or germine gene therapy.