Editorial comment to: Recruiting Testicular Torsion Introduces an Azoospermic Mouse Model for Spermatogonial Stem Cell Transplantation

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In present study the authors aimed to transplant spermatogonial stem cell (SSC) after testicular torsion and ischemia. The rational for the study has not been mentioned clearly. In mammalian testes, SSCs are located at the basement membrane of seminiferous tubules. They produce the spermatogenic family, guaranteeing lifelong fertility. An important concern in young prepubertal boys at risk for stem cell loss, i.e. Patients undergoing radiotherapy and/or chemotherapy for malignancy, is fertility preservation in the future. The cryopreservation and transplantation of SSC has been proposed as a fertility preservation strategy for these patients.[1] Like other stem cells, SSCs are undifferentiated and have the capacity of self-renewal. The studies of SSCs are complicated because these cells are rare and no distinctive recognizing characteristics have been described to date. Spermatogonial stem cells contribute only 0.03% of the total germ cell population in rodent and human testis.[2] Thus, their few numbers and the absence of specific markers are the main problems to their determination characterization. Careful isolation of germ cells, including stem cells, is mandatory prior to transplantation. Therefore the validation of surface markers for the isolation of germ cells is required. To address this issue surface markers should be used. Isolated germ cells must contain spermatogonial stem cells that can undertake spermatogenesis after transplantation. mhc class i, c-kit, and thy-1.2 are surface markers for spermatogonial stem cells. In addition, fluorescence-activated cell sorting (FACS), combined with SSC transplantation is a prevailing modality that can help researchers to scientifically detect cell surface molecules of SSCs. It is not clear how SSCs have been isolated from other cells in present study. This step is vital. What if there is a risk that the biopsy contains malignant cells? It is clear that this is an experimental study, but the main implication is generalization the results for humans. In addition,

due to very scarce number of SSCs, accurate isolation of SSCs without using specific markers or other related tools, are impossible. Jahnukainen and colleagues reported that transplantation of testicular cells from leukemic rats induces transmission of leukemia.[3] Therefore, it has outmost importance to detect even the slightest contamination of the testicular tissue.

Another drawback of present study is defining the successfulness of SSC transplantation procedure. The best method to confirm that transplanted SSCs yield functional sperm is to show their ability to fertilize oocytes. The autologous transplant approach used in this study is not amenable to fertilization studies. Therefore, an allogeneic transplant model where donor testis cells from unrelated individual animals are transplanted into recipient testes is needed to draw accurate conclusion. It is important to document that transplanted SSCs can produce sperm in higher primate prototypes that have the greatest applicability to human testis anatomy and physiology. Likewise it is important to document in primates that the testicular environment is perfect to maintain spermatogenesis from transplanted SSCs following depletion.

Another potential source of bias in SSC investigation is the differentiation between proliferation and self-renewal. The differentiation between SSCs and progenitor cells is especially vital in the analysis of in vitro data. Because the definition of a stem cell is based on functional criteria, it is awkward to define cultured cells as stem cells merely because they proliferate. Detecting the number of colonies in a recipient testis seems easy, but there are some potential problems. When SSCs are transplanted, donor cells yield colonies of various sizes. Because a single SSC results in a single colony, this difference in size demonstrates that the proliferative potential of individual SSCs vary. On the contrary, individual stem cells might not result in single colonies under other experimental conditions. The clonal origin of a germ cell colony can be documented only via transplantation experiments and cannot be pragmatic to other cases. Although germ cell transplantation has also been tried in large animal species and higher primates, low transplantation competence and complicated recipient preparation make it improbable for practical and clinical applications. The best-case scenario would be to recover SSCs from a piece of testis for germ line stem (GS) cell establishment and direct the cells in vitro for sperm production after required genetic manipulations.

It would be worthwhile to quote the clinical implication of this study. Steady progress regarding SSC transplantation techniques is continuing and this is why many investigators and clinicians are becoming gradually assured that SSC transplantation is applicable method to restore fertility in young boy with malignancy. Currently, SSC transplantation is considered the most encouraging modality for fertility restoration in prepubertal cancer patients. This technique comprises the injection of a testicular cell suspension from a fertile donor into the testis of an infertile recipient. Before this technique can be bring into a clinical scenery, it is imperative to assess the effectiveness and the safety of the procedure.

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Reply by Author

Male germ cell (GC) transplantation via injection from a fertile donor to the testis seminiferous tubules of an infertile recipient is unique approach that can result in donor-derived sperm production by recipient animals. Transplantation of spermatogonial stem cells (SSCs) has been widely used to investigate the spermatogenesis recovery in various species.[1]

Testicular torsion is a serious urological emergency in newborn and adolescent males that can lead to potential serious infertility and sub-fertility in affected testis. To date, various drugs and chemicals have been used to protect testes against ischemic reperfusion injury[2] in torsion status but SSCs transplantation into testicular Torsion/detorsion has not been performed for functional assessment of SSCs yet.

An increase in the efficiency of donor engraftment has been shown following ablative treatments that remove endogenous stem cells and increase niche accessibility. An alternative as a recipient model is removal of endogenous germ cells by torsion/detorsion approach, so we investigated long term assessment of the testes for providing a new azoospermic recipient model. Our purpose was to investigate long-term effect of testicular torsion and detorsion on sperm parameters and testis structure in order to introduce a novel mice azoospermic model for spermatogonial stem cell transplantation.

As you mentioned, SSCs similar to other stem cells are generally rare. It has been demonstrated that the approximate number of SSCs in mice and rats (but not human!) is 0.03% of all germ cells, therefore, we predict that human SSCs may be rare and similar to rodent SSCs. Proliferation of SSCs in vitro enhances SSC numbers and successful transplantation. In addition, it provides large numbers of stem cells for biochemical or molecular analysis.

SSCs functionality can be evaluated by two procedures: 1) cluster-forming assay in vitro[3] and 2) SSCs transplantation into a recipient testis.[4] Recent studies have definitely shown that each

colony arises from a single stem cell. Since germ cell clusters have a distinct three-dimensional structure, SSCs might be possibly analyzed quantitatively in vitro by counting clusters, a procedure analogous to the neurosphere assay for neuronal stem cells. Therefore, by counting the number of colonies, the number of functional SSCs can be determined. The cluster-forming assay based on two important stem cell characteristics including clonogenicity and long-term self-renewal ability. As there are no specific biochemicals or morphological markers for SSCs in clusters and only the stem cells are able to colonize and repopulate in testes 4, transplantation is performed as a functional assay to determine the presence of SSCs in a cell population. There is no doubt that contamination of donor samples by leukemic cells may result in relapse, which is an unsolved problem for patient with cancer by now. However various studies have showed donor germ cells (including spermatogonial stem cells without sorting), can be injected into the seminiferous tubules of recipient testis. Then these transplanted stem cells undergo spermatogenesis,[1] and recipient testis transmit the donor haplotype to their progeny. Transplantation of germ cells has been successful even in primates. In our study, we transplanted cultured-germ cells from neonate 3-6-day-old mice into adult testicular ischemia 2 weeks after reperfusion via rete testis (homologous transplantation).

Also, to date, no SSC-specific marker has been identified for any species but the combination of expression of multiple markers can provide important information about spermatogonial cell types in rodents and other species. Although molecular characterizations of spermatogonial cells in culture have not been mentioned in this project, we have checked them here and before. We have recently reported isolated and cultured spermatogonial cells and the presence of spermatogonial cells in the culture. We determined spermatogonial markers [Oct4, GFRα-1, PLZF, Mvh (VASA),Itgα6, and Itgβ1] by a reverse transcriptase polymerase chain reaction (RT_PCR) followed by a confirmation via immunocytochemistry (for Itgα6, Itgβ1, Oct4 and thy-1 markers). We have also reported the results of our ultrastructural study of cell clusters and SSCs transplantation to a recipient azoospermic mouse. Our finding showed that testicular and cultured cells expressed spermatogonial cells-specific genes (including PLZF, Oct4, GFRα-1, VASA, Itgα6, and Itgβ1) and c-Kit as a differentiated germ cell gene.[5] The present study mainly demonstrated that Torsion/detorsion may result in an irreversible azoospermia in mouse, and emphasized on the possibility of recruiting this technique in recipient preparation for SSCs transplantation in mouse. To investigate and assess the exact efficiency of GC transplantation following this particular technique is recommended.

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