Spermatogonial stem cell autotransplantation: towards clinical application

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General Introduction
INTRODUCTION

Over the last three decades effective cancer treatments have improved the survival rates for many types of cancer. In children, the 5-year survival rate for all cancers combined improved from 58% for children diagnosed from 1975 to 1977 to 80% for those diagnosed from 1996 to 2003. Currently, it is estimated that 1 in 250 young adults between 20 and 29 years is a long term survivor of childhood cancer. This implies that adverse side effects including gonadal failure and subsequent poor long-term reproductive outcomes have now become important sequels of previous exposure to chemotherapy and/or radiotherapy. The ensuing sterility can not be prevented or otherwise counteracted, and there are currently no means to preserve fertility prior to treatment in prepubertal boys. This is in contrast to adult men, for whom ejaculated sperm can be cryopreserved and in contrast to women and girls in whom cryopreservation of oocytes or ovarian cortical strips prior to the initiation of cancer treatment can be performed.

Spermatogonial stem cell (SSC) technology may be an avenue to preserve fertility in these prepubertal boys. The quintessence of this new technology would be storage of testicular tissue before chemotherapy and propagation and autotransplantation of SSCs from this frozen/thawed tissue after cure for cancer (Figure 1).

Figure 1. Schematic diagram showing future Spermatogonial Stem Cell (SSC) autotransplantation to restore male fertility.

In 1994, SSC transplantation was performed successfully for the first time in the mouse. Upon transplantation the SSCs migrated to their niche in the testis.
and developed full spermatogenesis. Via this technique two generations of mice were born, which were healthy and showed no differences in the DNA methylation in their spermatozoa or other tissues (liver, kidney and placenta)\textsuperscript{11}.

Long-term-approximately 4 months-maintenance of mouse SSCs in vitro was demonstrated in 1998\textsuperscript{12}, and long term propagation in vitro over a 5-month period using a combination of growth factors and mouse embryonic fibroblasts as feeder cells was reported in 2003\textsuperscript{13}. These long term cultures did not seem to affect (epi)genetic integrity of SSCs\textsuperscript{14} and transplantation of long-term cultured SSCs led to the birth of live and healthy offspring\textsuperscript{13,15}. Thus far, SSC culture and propagation has been successful in rat\textsuperscript{16}, hamster\textsuperscript{17} and bovine\textsuperscript{18}.

These animal data can not be translated to the human directly, because the characteristics of spermatogonia of rodents are different from those in men. It is generally believed that in rodents the A-single (A\textsubscript{s}) spermatogonia are the stem cells, whereas the A-paired (A\textsubscript{pr}) and A-aligned (A\textsubscript{al}) represent the transient amplifying undifferentiated spermatogonial population. The A\textsubscript{1} to A\textsubscript{4}, intermediate, and B spermatogonia are considered to be differentiating spermatogonia\textsuperscript{19,20}. In men, there are two types of A spermatogonia, the A-dark and A-pale spermatogonia. The A-dark spermatogonia are referred to as the reserve spermatogonia, whereas the A-pale are considered the active proliferating spermatogonia including the renewing stem cells\textsuperscript{21-23}. In men, only type B spermatogonia can be recognized as differentiating spermatogonia. Because of these differences between rodent spermatogonia and human spermatogonia, it is essential to test and proof whether propagation of SSCs in vitro and transplantation of SSCs are also possible in men.

SSCs represent a very small population of cells in the testis. In the mouse testis only 0.03\% of germ cells and 1.25\% of spermatogonia are estimated to be SSCs\textsuperscript{20,24}. The efficiency of the SSC transplantation is associated with the number of cells injected\textsuperscript{25}. In future clinical application the number of SSCs in small testicular biopsies may not contain sufficient SSCs to fully repopulate all niches in an entire adult testis after transplantation. Therefore in vitro propagation of SSCs is generally considered to be essential to obtain an adequate number of cells for efficient transplantation\textsuperscript{8,20}.

Another important hurdle in developing human SSC autotransplantation is the avoidance of reintroducing remaining malignant cells in a testis biopsy in case of patients with non-solid tumors\textsuperscript{26}. 
BACKGROUND OF THE THESIS

At the time of starting the studies described in this thesis, there was uncertainty on which adult men and which prepubertal boys should be offered fertility preservation prior to starting gonadotoxic therapy\(^6,27\) as the extent of the effects of gonadotoxic therapy on male fertility were not well known. Moreover, a systematic overview on the success rates of ART with spermatozoa stored prior to gonadotoxic therapy or spermatozoa obtained after gonadotoxic therapy was lacking.

An important issue in fertility preservation is the acceptance of the technique by patients and/or parents. Previous studies showed that parents of boys with cancer eagerly awaited fertility preservation\(^5,28\) but the numbers of parents were low and the attitudes of parents towards fertility preservation in the context of different risk levels of infertility and success rates of fertility restoration were not evaluated.

Finally, some important gaps of knowledge existed on the technique of SSC autotransplantation itself. Although xenotransplantation of human testicular cells to the mouse testis had been shown\(^29\), no one had succeeded in developing an in vitro propagation system for human SSCs. Also, none of the studies on elimination of cancer cells from SSCs had attempted to eliminate malignant cells in the context of a culture system for human SSCs.

OUTLINE OF THE THESIS

We performed a systematic review on 35 years of the literature on semen quality before and after gonadotoxic therapy in adult men and prepubertal boys. We also summarized the available evidence on pregnancy rates after ART using spermatozoa obtained before or after gonadotoxic therapy (Chapter 2). These data may help clinical practice when counseling boys and men with cancer.

We then aimed at exploring the attitudes of parents of boys with cancer towards fertility preservation in the context of different risk levels of infertility and success rates of fertility restoration. We sent out a questionnaire to 465 parents in Iran whose son was treated for cancer as an adolescent or prepubertal boy (Chapter 3).

Thereafter, we embarked on developing a long-term culture system to propa-
gate human adult SSCs following the successful studies in animal models. We cultured adult (mature) human testicular cells from six patients who had undergone bilateral orchiectomy as part of their treatment of prostate cancer (Chapter 4).

We then described the application of the long term culture system from chapter 4 to testis biopsies of two prepubertal boys diagnosed with Hodgkin lymphoma that has been referred for fertility preservation and had donated a small part of their stored testis biopsy for research (Chapter 5).

To understand the fate of remaining malignant cells in testicular biopsy from patients with cancer prior to gonadotoxic therapy, we investigated whether our SSC propagation culture system described in chapters 4 and 5 could eliminate cancer cells from human testicular cells. We mixed acute lymphoblastic leukemia (ALL) cells from three patients with testicular cells at the start of testicular cell culture and we tracked the malignant cells during culture (Chapter 6).

The results of these studies and implications for future research are summarized in Chapter 7.
REFERENCES


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