

## **FERTILITY PRESERVATION IN PREPUBERTAL MALES**

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In the 1990's, the incidence of childhood cancer was 140 per million children aged 0 to 14 years and more alarming, there is a significant annual increase in its incidence by 1%. Thanks to the development of efficient treatment protocols including chemotherapy, radiation and bone marrow transplantation, more than 70% of children will survive. Sterility is one of the most important side effects after cancer treatment. About one out of three children treated for leukaemia will become infertile. Currently, the only strategy to prevent sterility is cryobanking ejaculated spermatozoa. However, before puberty, this is not a valid option because spermatogenesis is not yet active. The prepubertal testis only contains the testicular stem cells in their gametogenic cell line.

In 1994, Brinster and Zimmerman published a research paper in which they reported the successful transplantation of male germ cells in a rodent model. Using a testicular suspension from a transgenic mouse, they could prove that spermatogenesis can be induced from the transplanted stem cells in a busulfan-treated recipient mouse. Subsequently, it was reported that also frozen-thawed testicular cell suspension, including the stem cells could recolonize the seminiferous tubules of recipient mice. This experimental model has been successfully reproduced in primates too. This method of spermatogonial stem cell transplantation into the testis is thus a potential strategy in order to prevent sterility in prepubertal boys undergoing a sterilizing treatment (for review: Tournaye et al., 2004). Before any clinical application, this strategy has to be evaluated. The technical feasibility has to be explored, the reproductive efficiency has to be established and the safety issues have to be scrutinized. An important aspect for establishing this technique successfully is an optimal cryopreservation of the testicular tissue. In a mouse model, we have shown that, although survival of spermatogonial stem cells is adequate, their functionality decreases after cryopreservation. Therefore, optimising cryopreservation is a prerequisite for successful clinical implementation. Another strategy could be to expand the number of spermatogonial stem cells, either before cryobanking or after thawing. In certain mouse strains this approach has proven successful. Many children of the target population may have malignant cells in their testis, e.g. leukaemia patients. It has been shown previously in a rat model, that when transplanting testicular cell suspensions from leukaemic rats, even after cryopreservation, the recipient animal will be contaminated with the malignant cells after transplantation. At present, it is far from clear that testicular cell suspensions from these patients can be decontaminated by cell-sorting techniques. Possibly, the application with using patient-specific surface markers for cell sorting may be more appropriate. Notwithstanding the fact that there are still a lot of questions on both efficiency and safety, the first phase for clinical implementation, i.e. cryopreservation of stem cells, can be considered. Ovarian tissue banking has also been introduced about 10 years ago and is now applied worldwide. Yet, the first two pregnancies have only been obtained recently. It is important to stress to the patient and his parents that banking prepubertal testicular tissue has to be considered as an experimental preventive strategy with currently no proof of either efficiency or safety. Cryobanking prepubertal tissue can be offered only after informed consent of both parents and (whenever possible) the child and without any financial implications.