WHEN SHOULD WE CRYOPRESERVE TESTICULAR TISSUE?
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The introduction of intracytoplasmic sperm injection in 1992 has revolutionized the treatment of severe male factor infertility. Not only can ejaculated spermatozoa be used successfully with ICSI, also epidymal and testicular spermatozoa can be used with great success. Because ICSI with testicular or epidymal sperm involves a surgical recovery procedure, which cannot always be performed the same day as the ICSI procedure, cryopreservation of testicular spermatozoa is an important part of the treatment of many azoospermic patients.

Apart from freezing testicular tissue for treating couples with azoospermia, testicular tissue can also be frozen to prevent male sterility when a gonadotoxic treatment has to be performed. Although the freezing of testicular tissue can not be viewed as an alternative to cryobanking ejaculated sperm, the importance of freezing testicular tissue is increasing because of new developments within our field.

Cryopreservation of testicular tissue for preventing male sterility: In the 1990’s, the incidence of childhood cancer was 140 per million children aged 0 to 14 years (Steliarova Foucher et al., 2004) 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 (Jemal et al., 2004). Sterility is one of the most important side effects after cancer treatment. About one out of three children treated for leukaemia will become infertile (Leung et al., 2000). 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 (Brinster and Zimmerman, 1994). Subsequently, it was reported that also frozen-thawed testicular cell suspension, including the stem cells could recolonize the seminiferous tubules of recipient mice (Averbook et al., 1996). 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 (Frederickx et al., 2004). Therefore, optimising cryopreservation is a prerequisite for successful clinical implementation. Recent papers have focussed on improved cryopreservation protocols of spermatogonial stem cells (Keros et al., 2005). 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 (Kubota et al., 2004). An optimal cryopreservation protocol for testicular stem cells should ensure both high survival and maintenance of functionality of the stem cells. Whenever possible, it should allow only survival of the spermatogonial stem cells. 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 (Jahnukainen et al., 2001). At present, it is far from clear that testicular cell suspensions from these patients can be decontaminated by cell-sorting techniques (Geens et al., 2007). Possibly, the application with using patient-specific surface markers for cell sorting may be more appropriate (Fujita et al., 2006). Notwithstanding the fact that there are still a lot of questions on both efficiency and safety (Goossens et al., 2003, 2006 a and b), the first phase for clinical implementation, i.e. cryopreservation of stem cells, can be considered (Bahadur, 2004). 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 (Bahadur, 2004).

Testicular tissue banking in adolescents: In most adolescents, spermatogenesis will be active and the indications for testicular tissue banking will be limited. However, when no ejaculated sperm can be obtained, either by masturbation, either by penile vibrostimulation or electroejaculation, surgical sperm recovery and banking of testicular tissue may be an acceptable strategy. After procuring the tissue, a wet preparation before cryobanking should be performed. The freezing protocol should be adapted to every individual situation: when testicular spermatozoa are observed, then testicular tissue should be frozen focussing on maximal survival of the spermatozoa (Crabbé et al., 1999). When spermatogenesis is not yet active, then cryopreservation should be adapted for the testicular stem cells in the testicular tissue.

Testicular tissue banking in adults: Testicular tissue banking in adults can either be performed for treating obstructive or non-obstructive azoospermia or as a preventive measure before any sterilizing treatment. Adult cancer patients, referred for semen banking may be found azoospermic at the time of cancer diagnosis. The prevalence of azoospermia at diagnosing cancer is variable but in the large series up to 17% of patients are found to be azoospermic (Lass et al., 1998). If after extended preparation no spermatozoa are observed and the patient has to undergoing surgery, either for removing his tumor or to place an infusion system for his chemotherapy, a surgical sperm recovery can be proposed. In patients with testicular cancer, testicular tissue from the removed testis has to be explored for mature spermatozoa (Rosenlund et al., 1998; Schrader et al., 2003). About half of patients with testicular germ cell tumours presenting with azoospermia before starting chemotherapy will have mature spermatozoa present in the testicular tissue. In patients with lymphoma (Hodgkin’s and non-Hodgkin’s) again, about 50% will show spermatozoa after wet preparation of testicular biopsy (Schrader et al., 2003). These preliminary results show that cryopreservation of testicular tissue in men presenting with azoospermia before chemotherapy is an important strategy. Banking the testicular tissue aiming for testicular stem cell transplantation has also been proposed (Radford et al., 1999), however, given the uncertainties associated to the clinical implementation of this technique, the rationale for opting for this approach rather then for freezing spermatozoa remains limited.

Cryopreserving testicular tissue in patients undergoing ICSI: Surgical sperm recovery by testicular biopsy has become a routine procedure in assisted reproduction. In patients with obstructive azoospermia a testicular biopsy will reveal a high number of spermatozoa, often with adequate motility. Since surgical retrieval is associated to a variable degree of testicular damage depending on the amount of tissue removed and possible complications, cryopreservation of testicular tissue is an important issue in the treatment. Although the motility and vitality of testicular spermatozoa will decrease substantially after cryopreservation (Verheyen et al., 1997) the results after ICSI with frozen-thawed spermatozoa are similar to those with fresh spermatozoa.

Therefore, in patients with obstructive azoospermia, cryopreservation of testicular tissue is a widely accepted strategy. However, for patients with non-obstructive azoospermia, cryopreservation of testicular tissue in under debate. Few studies in the literature are focussing on this specific subgroup of patients. However, in those studies were ICSI was performed using frozen thawed sperm from non-obstructive azoospermic patients, the cumulative success rates are comparable to those obtained using fresh testicular spermatozoa (Dafopoulos et al., 2005; Giorgetti et al., 2005). When no lower limit for cryopreservation of testicular spermatozoa has been introduced, the risk, however, exist that after thawing no spermatozoa can be retrieved for ICSI (Verheyen et al., 2004). Our data have shown that when no lower limit is introduced, 80% of scheduled ICSI cycles can be performed using the frozen-thawed spermatozoa. However, in 20% of scheduled cycles, spermatozoa could not be obtained after thawing and an alternative approach had to be taken. For most couples, this approach will be a back-up surgical sperm retrieval since such a back-up procedure is associated with very high retrieval rates (Vernaeve et al., 2006). Performing ICSI with frozen-thawed sperm from non-obstructive azoospermic patients also
takes more time than if fresh specimens are used. However, in terms of fertilisation and cleavage rates, no differences were observed. Once embryos are to be transferred, the ongoing clinical pregnancy rate tend to be higher with the frozen-thawed testicular spermatozoa than with fresh spermatozoa. This because the patients with survival of their frozen-thawed testicular spermatozoa are a distinct subgroup with better characteristics in terms of sperm quality (Verheyen et al., 2005).

References


