

## Newsletter 2024 – March

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### Prepubertal testicular tissue freezing and grafting – are we there yet?

Fayomi et al., Science. 2019 Mar 22;363(6433):1314-1319.

One of the most significant advancements in the area of fertility preservation in the last 10 years was the birth of a monkey following grafting of frozen /thawed prepubertal testicular tissue (Fayomi *et al.*, 2019). Prepubertal tissue from Rhesus macaques was slow frozen and subsequently autografted under the skin on both the back and scrotum. After 5 months graft development was obvious and animals had entered puberty. At 8-12 months grafts were removed and histological evaluation showed plump seminiferous tubules and the presence of sperm. Grafts were manually dissected and sperm was isolated and used for intra cytoplasmic sperm injection. Eleven blastocysts were transferred and 1 resulted in a live birth, named Grady (graft-derived-baby). A second birth has since been reported (Oncofertility Meeting, Pittsburgh 2023). Graft function appeared to be similar to fresh tissue with a high proportion of seminiferous tubules containing sperm/spermatids, and was independent of graft site. One point to note was that grafts were difficult to dissect and collagenase IV was used to assist dissection. More abnormal sperm were recovered with the enzyme digested tissue and all the resultant blastocysts were from sperm collected from manually dissected tubules.

Although live births have been reported previously with prepubertal mouse testicular tissue, this primate birth is more clinically relevant to the human situation. This work is significant in providing proof of concept that function of spermatogonial stem cells (SSCs) can be preserved with slow freezing of tissue using the cryoprotectant DMSO and that the sperm produced from these SSCs are fully competent. Also, significant numbers of sperm can be recovered from these grafts (1000-21 million/graft) demonstrating preservation of large numbers of SSCs within the tissue. The study sets the scene for the introduction of human trials.

The relevance of this study to the field of fertility preservation is that there are now numerous centres worldwide preserving testicular tissue from young boys with malignant disease and other non-malignant conditions, who are at high risk of imminent infertility. A survey, about to be published in Human Reproduction Open (Duffin *et al.*, 2024), of 16 centres through the ORCHID-NET group, reported over 3000 males under the age of 18 years have frozen testicular tissue; triple the number previously reported in 2019 (Goossens *et al.*, 2020). While still classified as experimental, these groups are conducting their programs under a clinical



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trial with an IRB approved protocol and governance pathway. With some of the early established programs now twenty years on, there will be some of these males now in relationships and potentially looking towards using their stored testicular tissue. Experimental data from the above primate study will help to inform the design of clinical autografting trials for these males.

Further important information has come from the recent report of autologous grafting of frozen/thawed adult human testicular tissue from a male with non-obstructive azoospermia (Jensen *et al.*, 2024). Tissue with dilated tubules was sutured to a subcutaneous scrotal skin pocket on one side of the scrotum and tissue with undilated tubules to the other side. After 6 months, grafts were removed and examined but no sperm were found. Despite this, and the fact that only limited evaluation was possible due to the priority to attempt to obtain sperm, Doppler ultrasound showed blood flow to the grafted tissue and immunohistochemistry of the dilated tubules showed preservation of some SSCs and structural organisation after the 6 months. At this point we do not know if a longer period after grafting would result in development of sperm from the SSCs. So, now the conversation begins regarding the many questions associated with starting clinical trials of grafting human peri/ prepubertal testicular tissue. Interesting times are ahead for this area of fertility preservation.

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