INFERTILITY

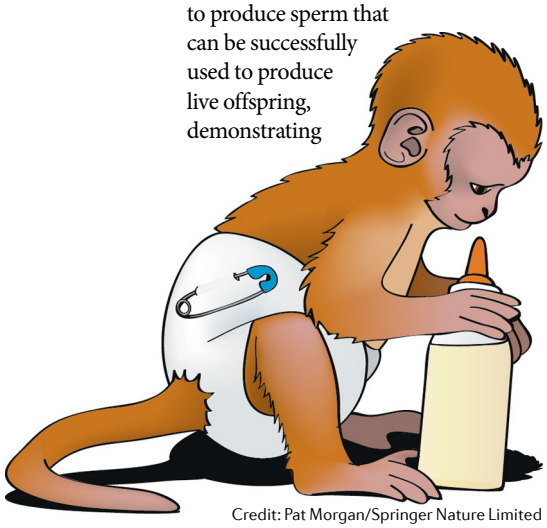
Grady — the miracle monkey born from cryopreserved prepubertal testis graft

> frozen testicular tissue from young patients can be matured to produce sperm that can be used to fertilize eggs and lead to the birth of healthy offspring

A study in *Science* has reported the birth of live offspring following autologous grafting of prepubertal testicular tissue, raising hope for preserving fertility in patients treated for childhood cancers.

Many therapies for cancer and other conditions are gonadotoxic, causing depletion of spermatogonial stem cells and resulting in permanent infertility. Thus, cryopreservation of sperm is a well-established technique in adult men with cancer; however, this approach is not an option in boys diagnosed with cancer before they begin puberty, as spermatogenesis has not begun in these patients. "The good news is that with improved cancer therapies, most children will survive their cancers and can look forward to a full and productive life after cure," corresponding author Kyle Orwig tells *Nature Reviews Urology*. "The bad news is that some adult survivors of childhood cancers discover that their life-saving cancer treatment had an unintended side effect — infertility".

Previous studies have shown that testicular tissues can be obtained from one species and grafted into nude mice to produce sperm that can be successfully used to produce live offspring, demonstrating that xenografted testicular tissue from a human could produce sperm in an animal host. However, this technique raises concerns that xenobiotics could be transferred from the host and, therefore, requires caution. However, in their recent paper, a team of researchers led by Orwig have described a next-generation technology that can produce sperm from immature testes via autologous grafting, circumventing concerns associated with animal hosts.



Credit: Pat Morgan/Springer Nature Limited

In their proof-of-principle study, five prepubertal rhesus monkeys were hemicastrated, and the removed testicular tissue was prepared and cryopreserved. At ~5–7 months after castration, the remaining testis was also removed. Histological analysis of the tissues confirmed that they were immature and that spermatogenesis was not yet occurring. The fresh and cryopreserved testicular fragments were then immediately implanted back into the animals, either under the skin of the back or the scrotum, and, in some, Matrigel was added to stimulate angiogenesis.

The grafts increased in size and as the animals reached maturity a functional hypothalamic–pituitary–gonadal axis was confirmed by the presence of circulating testosterone and levels of follicle-stimulating hormone that were in the normal range.

At ~8–12 months after implantation, grafts were harvested from all sites. The grafted tissue had grown approximately fivefold from the implanted weight and the individual tissue implants had fused into one large tissue mass. The presence of seminiferous tubules was evident in all grafts, and in most cases sperm was retrievable by dissection or collagenase digestion for use in fertilization studies. Complete spermatogenesis (with elongated spermatids and/or sperm) was demonstrated in ≥70% of seminiferous tubules and was confirmed in tissues from all graft sites regardless of whether the implanted tissue was fresh or preserved and independent of the addition of Matrigel and graft location.

Harvested sperm from one animal was used for intracytoplasmic sperm injection (ICSI). Of a total of 138 eggs fertilized by ICSI, 39 (28%) progressed to the two-cell stage and 16 of the resulting 39 (41%) developed into blastocysts.

Blastocyst-stage embryos (10 fresh and 1 frozen) were then transferred to female macaques. One of the females that had been implanted with a fresh blastocyst was confirmed pregnant and, after a normal pregnancy, the offspring was delivered by Caesarean section. The resulting graft-derived young — named Grady — went on to develop normally in terms of behavioural assessments and play activities.

This paper provides the first evidence that frozen testicular tissue from young patients can be matured to produce sperm that can be used to fertilize eggs and lead to the birth of healthy offspring. This result offers considerable hope for men who are treated for childhood cancers.

"We believe that our results provide important preclinical safety and feasibility data to justify translating this technique to the human fertility clinic," Orwig comments. "I believe we owe this to our patients who have already accepted the risk of testicular biopsy surgery and trusted us to develop next-generation reproductive therapies".

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ORIGINAL ARTICLE Fayomi, A. P. et al. Autologous grafting of cryopreserved prepubertal rhesus testis produces sperm and offspring. *Science* **363**, 1314–1319 (2019)
FURTHER READING Esteves, S. C. et al. Intracytoplasmic sperm injection for male infertility and consequences for offspring. *Nat. Rev. Urol.* **15**, 535–562 (2018)