

Time for fertility preservation

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This special issue of five review papers celebrates the 25th anniversary of transplantation of cryopreserved ovarian tissue, a strategy to protect fertility in patients before potentially sterilizing treatment for cancer or other diseases and conditions. Fertility preservation is emerging as a subspecialty across reproductive medicine and oncology, and as a technology, it has parallels in nature where sperm are stored for timely reproduction in species ranging from queen honey bees and ants to chickens and bats (Holt 2011). However, for long-term storage cells must be frozen to low temperatures, and that is usually a lethal state. A brief video in which Professor Gosden provides a short history of fertility preservation is also available (Video 1).

Video 1

A short history of fertility preservation. The video (<http://movie-usa.glencoesoftware.com/video/10.1530/REP-19-0155/video-1>) from the online version of the article is available at <https://doi.org/10.1530/REP-19-0155>.

The first breakthrough in cryobiology arrived 70 years ago when a serendipitous observation in London showed that cockerel sperm could survive freeze-thaw cycles if they were first immersed in a glycerol solution (Polge *et al.* 1949). This was a foundational discovery for semen banking in the cattle industry and human reproductive health care. Four years later, while still a graduate student in Iowa before joining the faculty at the University of Arkansas, Jerome K. Sherman tested glycerol with human sperm and after obtaining positive results he recruited a clinical partner (Bunge & Sherman 1953). Aiming to help men with low sperm counts, they combined sequential ejaculates of thawed semen for inseminating their wives with enriched samples. Pregnancies gave impetus for sperm banking with other applications, although donor insemination was hampered in that era by public hostility to any third-party involvement in conception. Preserving fertility in men ahead of cancer treatment was regarded with greater compassion, although it was seldom considered by clinicians who focused almost exclusively on helping patients to survive when the chances were still dismal, while it was not even an option for prepubertal boys.

Young female patients were occasionally candidates for fertility-sparing surgery by oophorectomy to avoid therapeutic pelvic radiation (Arian *et al.* 2017) or radical trachelectomy instead of hysterectomy for cervical cancer (Mejia-Gomez *et al.* 2012). Most patients, however, had to accept the risk of infertility and early menopause as the costs of overcoming a dreaded disease, and as survival rates improved with higher-dose treatment in the 1990s, those costs also rose.

Embryo cryopreservation was becoming available then, but only for adults with a male partner. And to harvest their oocytes cancer treatment had to be delayed a few weeks for ovarian stimulation with gonadotrophins, and mitigating the aggravating effects of elevated estrogen on breast disease with aromatase inhibitors (Azim *et al.* 2008). Oocyte freezing was needed for single women, but animal studies indicated that slow cooling protocols harmed the spindle apparatus and other parts of the cell (Vincent & Johnson 1992). It did not become widespread until refinements in rapid freezing by vitrification (Kuwayama *et al.* 2005), and in that vacant space ovarian banking found a role.

A 1960 report from the same group in London claimed that mouse ovaries frozen in a glycerol solution could partially restore fertility as isografts (Parrott 1960). A study inspired purely by curiosity, it had no obvious applications at the time, and the method was unlikely to work with large, fibrous organs of farm animals and humans. However, peripheral distribution of primordial follicles offers an opportunity to preserve fecundity by carefully dissecting the ovarian cortex from its underlying stroma. The thin strips of tissue can be safely cooled to liquid nitrogen temperatures using cryoprotective agents that penetrate faster than glycerol. If thawed strips are sutured to the stump from where the organ was removed, there was hope that fertility would recover. The procedures began tests over 25 years ago at a research farm in Roslin, outside Edinburgh. Breeding cycles returned to oophorectomized sheep that received their tissue back as autografts, and mating with rams generated pregnancies with healthy lambs (Gosden *et al.* 1994, Baird *et al.* 1999).

Good results encouraged the freezing of human ovarian biopsies, which thrived as xenografts in

immunodeficient mice (Newton *et al.* 1996). Besides indicating a novel strategy for fertility preservation in patients, the method posed advantages over embryo and oocyte banking. Tissue harvests could be performed without delay, and a graft reinitiating menstrual cycles would reverse menopausal symptoms and restore fertility, either for conception by coitus or IVF if necessary (Gosden & Aubard 1996).

Patients with no alternative for preserving fertility have embraced the opportunity to bank their ovarian tissue, and many thousands are thought to have opted for the procedure worldwide. Few patients have had tissue transplanted back yet, pending a cure of their disease and readiness to start a family. Ten years after the first experiments in sheep, the first babies were announced in Belgium and Israel (Donnez *et al.* 2004, Meirrow *et al.* 2005), and the number now exceeds 100, including a case in which tissue was stored for 14 years from childhood (Matthews *et al.* 2018). Other cases given fresh ovarian grafts have been at least as successful, and some conceived more than one child (Silber *et al.* 2010). Almost all have returned to cycles, although not everyone can have, or wants, a baby. There was a uniform induction period of 4–5 months before the first menses as small follicles grew to maturity. Most grafts function for at least a year or two, and exceptionally up to 10 years, and surplus frozen tissue can replace an exhausted primary graft to prolong the benefit. The method has proved to be so robust that it is proposed as an alternative to egg banking for healthy young women who want to safeguard maternity until late in the fertile lifespan (Stoop *et al.* 2014).

Ovarian banking has become standard practice in some countries. Andersen and Kristensen report in this issue a central laboratory service in Denmark which produces excellent outcomes by uniform processing of specimens and enabling patients to be treated locally and avoid travel (Andersen *et al.* 2019, *this issue*). Research continues to strive for improvements in graft longevity, which is limited by age, biopsy size and the fraction of follicles lost, which is mainly due to transplantation as opposed to cryopreservation. Follicle wastage during ischemia is compounded by super-recruitment that is reflected by a temporary surge of serum AMH levels from growing follicles. In this journal issue, Roness and Meirrow (2019) discuss how this wastage might be reduced by speeding up revascularization and retarding the recruitment process using extracellular matrix, hormones or drugs. Progress can be anticipated since the signaling pathways responsible for progression of primordial follicles to the growing pool are known, involving PI3K-Akt antagonized by PTEN, as well as KIT-ligand and the protein kinase HIPPO.

Success with ovarian tissue has encouraged a mirror technology for the unsolved problem in boys. Since experimental evidence exists for testicular tissue survival after cryopreservation and transplantation in animals

(Honaramooz *et al.* 2002, Schlatt *et al.* 2002), we have greater confidence in banking testicular tissue for children, which Kilcoyne and Mitchell (2019) describe in another accompanying paper. Testicular biopsy is a less invasive procedure than harvesting ovarian tissue, although great care is needed to minimize damage to small, delicate organs. For young patients, some of them still infants, fatherhood is a remote, even unimaginable prospect, and storage will be required for two decades or more, laying heavy responsibility on the security of tissue banks. There is reassurance that the investment is worthwhile after news of a macaque monkey conceived by ICSI with sperm extracted from an autologous testicular graft cryopreserved before puberty (Fayomi *et al.* 2019). Former patients now unable to preserve fertility, and those sterile from birth, have had to resort to donor gametes or even surrogacy, if available, but advances in developmental biology and genetics point to radical help in future. When mouse fibroblasts were engineered through IPS cell intermediaries they could produce viable gametes *de novo*, breaching what was long regarded as an unfathomable chasm between somatic and germ cell lineages. Such striking discoveries could lead to a profound revolution in reproduction if this technology can ever be proved safe (Hayashi *et al.* 2018).

One of the most urgent agendas is to find ways of helping patients to realize their reproductive goals without the risk of returning malignant cells in tissue harvested before it is free of disease. The risks are most serious with leukemia, some lymphomas, neuroblastoma and any disease that sends metastases to the gonads. Dolmans and Amorim (2019) review in this issue progress for constructing artificial ovaries to avoid this hazard. The ovary is a plastic organ that can be disaggregated to single cells and isolated follicles for eliminating malignancy before recombining the healthy components in molecular scaffolds for transplantation.

Alternatively, follicles isolated from the ovarian stroma can be cultured individually to produce mature oocytes. This feat was first achieved many years ago by Eppig and O'Brien (1996), and the mouse is still the only species in which complete development has been confirmed with healthy offspring. Techniques continue to roll forward with greater sophistication from better knowledge of the growth factors and extracellular matrix involved in development (Shea *et al.* 2014). A technology for human oocytes seems a titanic challenge by comparison with mice because the follicles grow more slowly and attain sizes *in vivo* that exceed our ability to mimic in culture systems that we currently envision. In the final paper of this issue, Telfer (2019) holds an optimistic view based on a multi-stage system that enables oocytes from primordial follicles to grow and become competent for meiosis. Provided they remain within a nest of granulosa cells, other cellular components of a Graafian follicle are not required, and accelerated development *in vitro*

facilitates the process. Unfortunately, few research centers have access to such precious research material, and although progress is slower and more laborious than we like it is surely edging toward a technology that will be routine one day. Oocytes grown in culture for IVF avoid the risk of transmitting disease back to patients and will render ovarian transplantation less necessary or even redundant, but isn't all technology interim?

The former drought of options for fertility preservation is turning into a flood as novel techniques progress from experimental stages to testing in patients and clinical trials where sufficient cases exist. Cryopreservation of gonadal tissue and gametes will remain a cornerstone for a long time while the search continues for chemoprotection to preserve germ cells in situ, avoiding the burden of surgery and frozen banking. Co-treatment of GnRH agonists with gonadotoxic drugs for cancer is claimed to reduce follicle wastage, but the evidence is mixed and any benefits appear to be slight. A menu of candidate gonadoprotective molecules has emerged for fresh avenues of investigation that [Fisch and Abir \(2018\)](#) discuss in their admirable review published in the last year's anniversary issue.

New techniques take hold more rapidly now than in the past, and this impression is especially welcome for a subject where timeliness is imperative for personalized treatment. Cells can be preserved indefinitely in the deep cold, but in the human lifespan, the reproductive window is open for a limited time. Uterine transplants were unthinkable a few years ago, and yet are already enabling women to conceive healthy babies, and the matter is now leaping forward toward bioengineering prostheses to treat uterine infertility and agenesis ([Brännström 2017](#)). But nothing written here about research compares with harnessing the revolutionary potential of IPS cells to create gametes. Fertility preservation has traveled far from its origin in cryopreservation and holds even greater expectations for the future.

Declaration of interest

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