

Short Communication

Fertility Preservation for Cancer Patients

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Abstract

Freezing of eggs or ovarian tissue to preserve fertility for cancer patients has been studied since 1994 with Gosden's original paper in sheep, and before that in smaller mammals for decades prior. Clinically, this tactic has enjoyed great success. Despite the rising popularity of vitrification for egg freezing, it is often done incorrectly, forgetting the original principles Kuwayama established in 2005. So there will be many disappointed patients around the world whose egg freezing was not done well. Greater care to very gradual osmotic shifts and "open" super rapid freeze and thaw will be required to make egg freezing with vitrification more reliable. Many healthy children have been born from the Kuwayama egg vitrification technique for social indications, but until now very few cancer patients have yet to get pregnant from frozen eggs for several reasons. Often, oncologists do not wish to delay cancer treatment while the patient goes through multiple stimulation cycles to retrieve eggs, and the patient can only start using the oocytes after full recovery. Furthermore, egg vitrification is much more sensitive to error than ovary tissue freezing. However, over 40 healthy babies so far have been born from ovary tissue freezing for cancer patients and have had complete return of hormonal function, as well as fertility. Therefore both egg freezing and ovarian tissue freezing seem now to be ready to be applied to preserving fertility for cancer as well as for reasons other than cancer. But you must get the technique right.

INTRODUCTION

In developed societies, characterized by a general trend to postpone childbearing, there is increasing age-related infertility. We therefore see that healthy women foreseeing a pregnancy at a more advanced age turn to cryopreservation techniques to safeguard their future reproductive chances [1,2]. The ESHRE task force on ethics and law recommends that oocyte cryopreservation should be available for the prevention of age related infertility and that fertility specialists should refrain from passing judgment on a woman's motives to do so [3]. However, it should be noted that egg freezing can give terribly disappointing results if one does not pay close attention to very gradual osmotic shifts (Figure 1a-1f). Still, thus far many hundreds of healthy babies have been born from eggs frozen for social reasons and there is no greater incidence of any birth anomalies. Also over 40 healthy babies have been born to otherwise sterile cancer patients, but virtually all of these were from frozen ovary tissue, not eggs [4]. The first successful fresh human ovary transplantation occurred in 2004 between identical twins one of whom had premature ovarian failure (POF) and the other of whom was normal and fertile. Cortical grafting was the technique employed [5]. The first successful human frozen ovary auto-grafts were reported around the same time with tissue cryopreserved for cancer patients prior to their sterilizing bone marrow transplants (BMT) [6,7].

This had been demonstrated in sheep 10 years earlier [8]. The technique was then refined over a remarkably large series of nine fresh ovary transplants in identical twins discordant for POF, all of which were successful, and also two fresh allotransplants, all of which resumed normal function and lead to 14 pregnancies and 11 healthy births from the nine twin recipients [9-12]. This led to refining cryopreservation techniques for cancer patients, with six additional frozen tissue pregnancies with live birth in our center. These patients all preferred spontaneous pregnancy to IVF and egg donation, and wished to accomplish this in a one-time procedure without ovarian hyper stimulation. All studies were carried out with informed consent and IRB approval. Studies do not support a negative effect of unilateral oophorectomy on fertility or age of menopause [13,14]. Thus we felt comfortable in performing the original series of fresh ovary transplants, which led the way toward improving ovarian freezing and frozen ovary transplantation.

Ovarian cryopreservation by original slow freeze technique

Slow freezing was the first approach to ovarian cryopreservation. The cortex is removed from the medulla, divided into multiple strips and transferred to cryovials after incubation in 1.5 mol/L 1,2-propanediol and 0.1 mol/L sucrose at 37°C for 30 minutes, 0.2 mol/L sucrose for 5 minutes, and

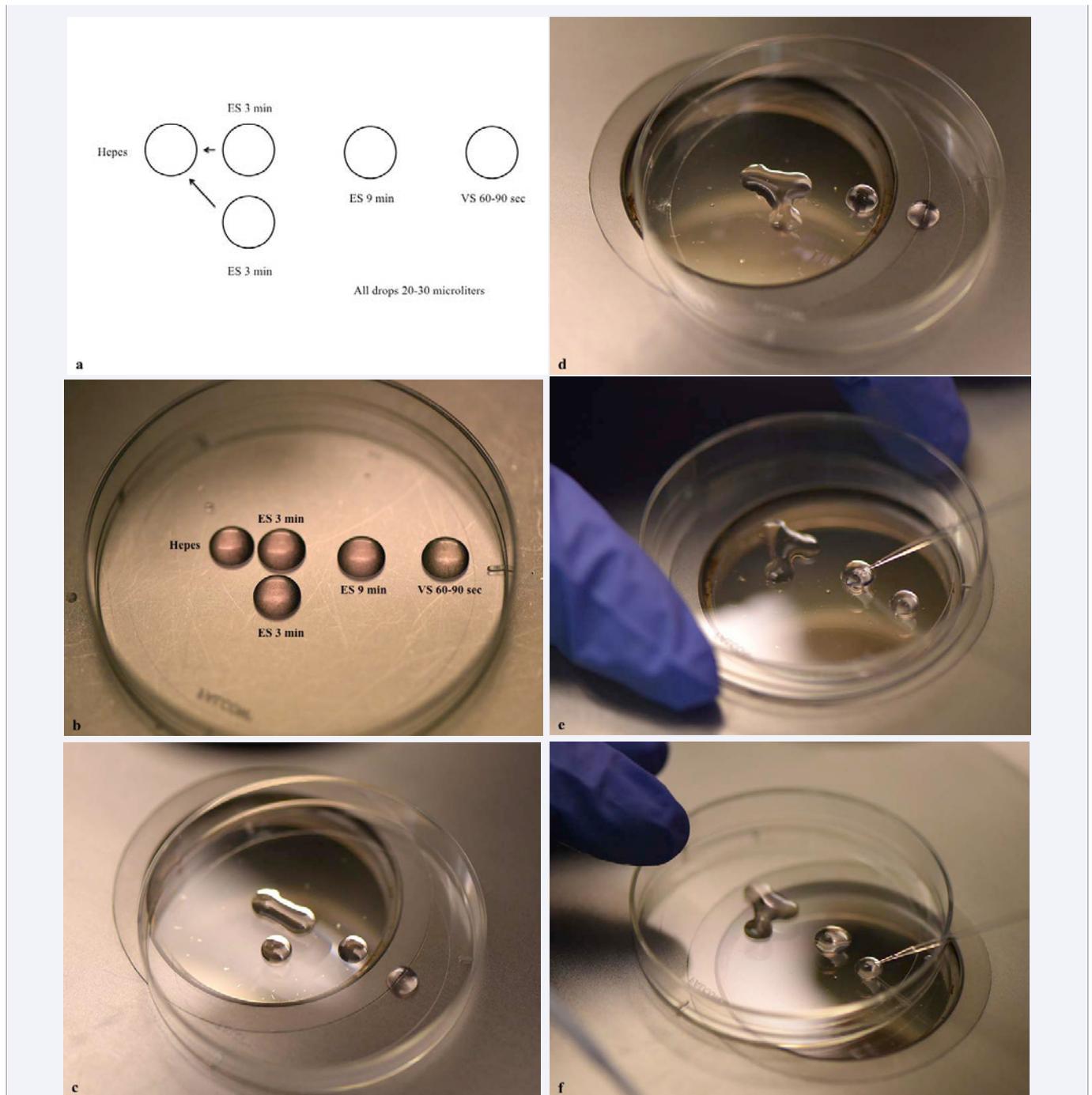


Figure 1 (a) The “bridge” technique for oocyte freezing. (b) Setup for “bridge” equilibration. (c) First “bridge” between ES and isotonic hepes media; three minutes. (d) Second “bridge” equilibration; three minutes (e) Transfer to full concentration ES; nine minutes. (f) Transfer from ES to VS; 60-90 seconds. Caption: High cryoprotectant concentration is not toxic. It is just the rapid osmotic shifts that kill the egg or embryo and give the incorrect impression of toxicity. To avoid over rapid osmotic shifts (that are more poorly tolerated by the egg than the embryo) the original “bridge” technique is best. ES solution droplets are first “bridged” over to the iso-osmotic solution the egg is in, and three minutes later another droplet of ES solution is “bridged” over to the original solution very gradually and continuously raising the osmolality of the solution the oocyte is resting in.

after that cooled as described previously [8,15]. Thawing is performed rapidly in a warmed water bath and tissue is trimmed under an operating microscope before transplantation [16]. This slow freeze method has worked quite well, and most of the over 20 pregnancies and live births achieved so far have been with slow freeze. However, we now use vitrification exclusively

for cryopreservation in humans because of our in vitro viability analysis studies as well as in vivo transplant studies in the bovine showing no egg loss with vitrification of ovarian tissue [17]. There was no significant difference between fresh and vitrified tissue, but the viability of slow freeze-cryopreserved tissue was less than one-half that of fresh (42%; $p < 0.01$) [9]. Transmission

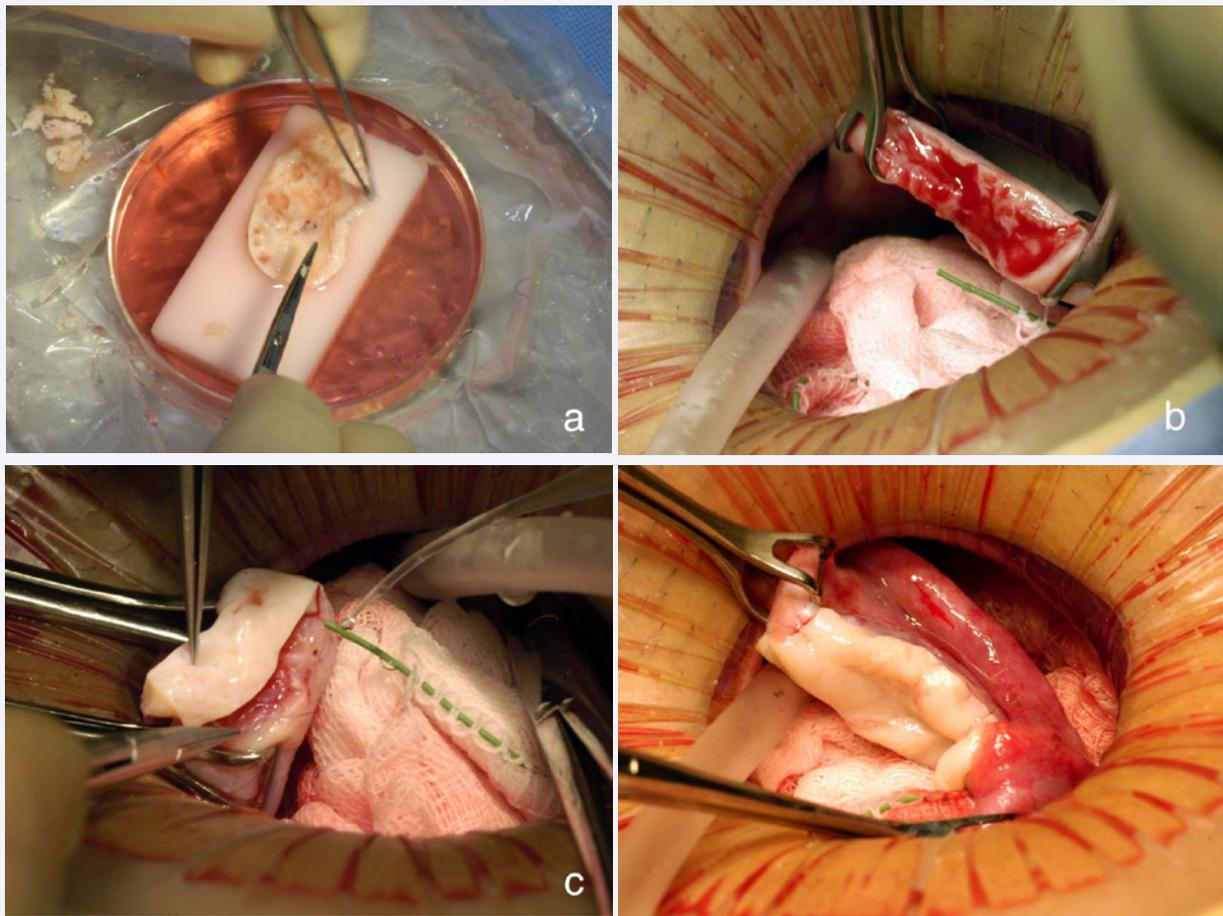


Figure 2 Steps in the procedure of ovarian transplantation between MZ twin sisters: (a) preparation of donor ovarian cortex by dissection in a Petri dish on ice; (b) preparation of recipient ovarian medulla; (c) attaching donor cortical tissue to recipient ovarian medulla; (d) attaching thawed donor cortical tissue for re-transplant to the recipient medulla.

electron microscopy also has shown vitrification to produce less damage than slow freeze [18].

Ovarian tissue vitrification technique

Cortex tissue is cut into slices 1 x 10 x 10 mm [17]. The precise 1 mm tissue thickness was guaranteed with a tissue slicer on the surface of the ovary. The cortical ovarian tissue is thus cut into 1 x 10 x 10 mm pieces. The ultra-thinness of the tissue is crucial for cryopreservation by vitrification. It is very similar to egg or embryo vitrification. Ovarian tissues are initially equilibrated in 7.5% ethylene glycol (EG) and 7.5% dimethyl sulphoxide (DMSO) for 20 minutes followed by a second equilibration in 20% EG and 20% DMSO with 0.5 mol/l sucrose for 15 minutes. Ovarian tissue slices are then placed virtually “dry” onto a thin metal strip, and submerged directly into sterile liquid nitrogen. For thaw, the metal strip is immersed directly into 40 ml of 37°C solution of 1.0 mol/l sucrose for 1 minute, and then transferred into 15 ml of 0.5 mol/l sucrose solution for 5 minutes at room temperature. No ice crystal formation occurs during any of these vitrification procedures [17].

Cortical ovarian tissue transplantation technique

The recipients of fresh or frozen ovary tissue were prepared

by mini laparotomy via a 3.5 cm incision above the pubis. The recipient ovarian cortex was resected to expose medullary tissue (Figures 2a & 2b) and hemostasis was obtained with micro bipolar forceps, and pulsatile irrigation with heparinized saline to avoid adhesion formation or micro hematomas between transplanted cortex and underlying medulla. The ovarian cortical graft was attached using 9-0 nylon interrupted sutures under and operating microscope (Figure 2c). The medullary bed was also sutured to the under surface of the cortical graft to maintain tight tissue approximation (Figure 2d). The post-operative recovery in all cases was swift and easy [5].

DISCUSSION AND CONCLUSION

All nine fresh identical transplants were orthotropic and all were successful. The recipients continued to cycle from two years in two patients whose donor had low ovarian reserve to over six or seven years in most cases. Even those few with low ovarian reserve got pregnant from the fresh transplant within those two years, and later again got pregnant from transplantation of residual frozen tissue. Menstrual cycles began within 3 months and Day 3 FSH levels returned to normal by 4.5 months in all cases (Figure 3). A total of 14 healthy babies resulted from the 12 ovary transplants, 11 from the 9 fresh transplants, and 6 from

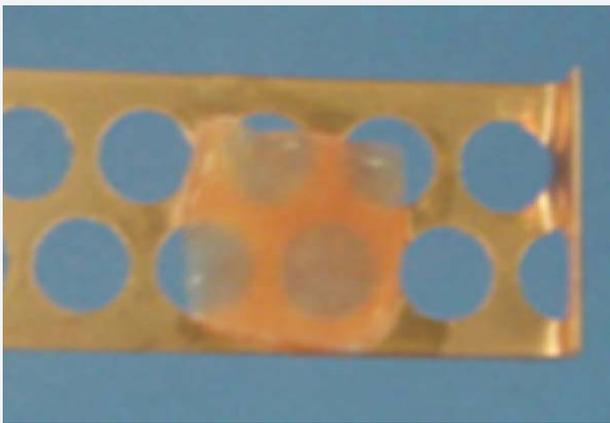


Figure 3 Ovarian tissue slice.

the 8 frozen transplants. This newly favorable experience with ovarian cortex grafting is not limited just to our center [19]. Robust results are being experienced in Brussels, Paris, Spain, Denmark, and Israel. Frozen ovarian grafts (even with the slow freeze technique) in Denmark are lasting over 5 years and many spontaneous pregnancies have been reported with no need for IVF or other ancillary treatment. At the time of this writing, over 37 healthy babies have been born from frozen ovarian tissue grafting, and most from just regular intercourse with no other treatment. With vitrification of ovarian tissue there is no difference clinically between fresh unfrozen controls and frozen tissue [9,15-17]. It seems likely, therefore, that vitrified ovarian tissue would give better results after transplantation than tissue cryopreserved by slow-freeze, but nonetheless many healthy babies have been born from slow freeze. Whether by vitrification (our preference) or slow freeze (the preference or many others), cryopreservation of ovarian tissue may not only extend the reproductive lifespan, but even delay menopause.

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