Chapter 9
Progress, History and Promise of Ovarian Cryopreservation and Transplantation for Pediatric Cancer Patients

Yasmin Gosiengfiao, MD

Ovarian cryopreservation followed by transplantation is one of the potential ways fertility can be preserved and endocrine function restored in women who are at risk for ovarian failure, early menopause, or loss of fertility.

While much of the progress in this area has occurred in the past couple of decades, the concept of transplantation of reproductive organs has been present since the nineteenth century. The first account of ovarian grafting was published in 1863 in a thesis by Paul Bert. Results were disappointing, hence the loss of interest over the next 30 years. In 1895, New York surgeon Robert Morris performed the first human ovarian implant in a woman with ovarian failure who reportedly became pregnant post-implant, though the pregnancy ended in a spontaneous abortion. Further experiments in ovarian grafting resulted in pregnancy in rabbits, dogs, and sheep from 1895 to 1899. However, because of the low success rate and limited clinical applicability of fresh ovary transplantation at that time, interest in this field waned [see review in 1].

The first attempts to cryopreserve ovarian tissue from rodents in the 1950s were largely unsuccessful, with only a 5% follicle survival rate due to several factors, including lack of effective cryoprotectants, automated cryopreservation machines, and optimized cryopreservation protocols [2–4]. By the 1970s, more effective cryoprotectants such as propanediol, ethylene glycol, and DMSO became available [5]. Slow cooling protocols were subsequently developed and successfully applied to ovarian tissue [6,7]. These developments resulted in restoration of fertility in up to 86% of rodents by the 1990s [8–12]. Despite these advances, doubt remained as to the applicability of these methods in humans since the rodent ovary is different from the bulkier, more fibrous human ovary with characteristically widely dispersed primordial follicles. Encouraging findings came in 1994, when Gosden demonstrated that ovarian cortical strips from sheep were able to survive cryopreservation and revascularize upon transplantation [8,13]. This was a significant step forward, as the sheep ovary is more similar to that of humans. By 1999, Baird showed that ovarian function was maintained for a limited though long-term (nearly 2 years) period in autotransplanted sheep [14]. Transplantation of the whole ovary after cryopreservation resulted in even longer-term function of more than 36 months [15]. In 2004, Lee reported the first live birth after heterotopic transplantation of fresh autologous ovarian tissue in the monkey [16]. There is less

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experience with ovarian transplants in humans than in animals. The ovary can be transplanted orthotopically (to the original site) or heterotopically, to a site other than its original site. Ovarian function has been shown to be similar after either heterotopic or orthotopic transplantation of fresh or frozen ovarian cortex [17].

Radford performed the first orthotopic reimplantation of ovarian cortical strips [18]. Laparoscopic technique for orthotopic transplantation of the ovary has been reported [19]; however, it was not until 2004 when Donnez [20] reported the first live birth after orthotopic autotransplantation of cryopreserved ovarian tissue in the human. Five ovary biopsy samples were obtained and frozen from a 28 year-old female with stage IV Hodgkin lymphoma in 1997 prior to therapy. She received MOPP/ABV chemotherapy and 38 Gy of radiation, after which she became amenorrheic. Hormone replacement therapy (HRT) was instituted from June 1998 to January 2001, at which time she requested to have her ovarian tissue autotransplanted in order to attempt pregnancy. Her follicle-stimulating hormone (FSH), luteinizing hormone (LH) and 17-beta estradiol levels at the time of discontinuation of HRT was consistent with ovarian failure. Yet within 5 months of orthotopic ovarian transplantation, there was evidence of recovery of ovarian function and at 11 months post-transplant, β-hCG and vaginal ultrasound confirmed a viable intrauterine pregnancy. She delivered a healthy baby at term. However, questions have been raised as to the origin of the oocyte that produced the pregnancy, since the patient had ovulated prior to ovarian transplant [21,22].

In 2005, Meirow reported the first live birth after in vitro fertilization (IVF) following transplantation of thawed cryopreserved ovarian cortical tissue [23]. A 28 year-old female with non-Hodgkin lymphoma received VACOP-B chemotherapy then relapsed. She was subsequently treated with MINE-ESHAP chemotherapy and ovarian cortical tissue was harvested and frozen before she underwent high-dose chemotherapy and stem cell transplantation. Soon after stem cell transplantation, she became amenorrheic and experienced ovarian failure for 24 months. The cryopreserved ovarian tissue was thawed and transplanted into her atrophic left ovary. She menstruated 8 months post-ovarian transplant, with an ultrasound confirming the presence of a preovulatory follicle in the left ovary. At 9 months post-transplant, IVF was performed successfully.

The first heterotopic reimplantation of fresh ovarian tissue in humans was reported by Leporrier in 1987 in a patient with Hodgkin lymphoma [24]. The patient developed regular ovarian cycles and the ovary reportedly remained functional for 16 years [25]. In 1997, Marconi accidentally autografted an ovary [26]. Since then, heterotopic transplantation of the ovary to varying sites, including the peritoneum, arm, and forearm have been reported with restoration of ovarian function [27,28]. The first embryo development after heterotopic transplant of the ovary was reported in 2004 [29]. Conventional thought is that heterotopic ovarian transplantation cannot result in pregnancy without IVF. However, in 2005, Oktay reported two spontaneous conceptions in a 32 year-old woman who had her ovarian tissue heterotopically transplanted [30]. At age 28, this patient was diagnosed with Hodgkin lymphoma. She underwent six cycles of ABVD and radiation to the chest and spleen and did not develop ovarian failure. She relapsed a year later. Her left

ovary was harvested and cryopreserved before she received further treatment with ICE and Rituximab, followed by high-dose chemotherapy and hematopoietic stem cell transplant. Post-ovarian transplant, she became menopausal for 2 1/2 years without hormone replacement. Her ovarian tissue was transplanted back subcutaneously to the suprapubic area. She conceived twice, one resulting in a spontaneous abortion and the other resulting in the birth of a healthy female at 40 weeks gestation. These cases have raised the question as to the origin of spontaneous pregnancies after ovarian transplantation, and alternative hypotheses have since been brought forth on the possibility for germ cell renewal and migration [31,32].

While there has been limited success with a few live births after transplantation of cryopreserved ovaries, concern remains that malignancy or infection may be reintroduced when transplanting tissue that is potentially involved, as demonstrated by Shaw [33]. This issue may be circumvented by harvesting oocytes from the cryopreserved ovarian tissue, then performing in vitro oocyte maturation followed by assisted reproduction. This would potentially expand the utility of ovarian cryopreservation to childhood cancer patients whose ovarian follicles are immature at the time that they receive fertililty-threatening treatments. Several murine models for in vitro oocyte maturation have been reported but with limited success [34,35]. The use of a three-dimensional alginate hydrogel matrix to mimic the in vivo follicle architecture has recently been used to successfully mature immature follicles in mice, resulting in IVF and embryo implantation birth rates that are improved over those of a conventional two-dimensional system [36]. Though encouraging, further studies are necessary to determine if these advances are applicable to human oocytes as well.

With the advances in treatment of childhood cancer, survival has increased to greater than 75% [37]. Yet, many treatment regimens are potentially gonadotoxic and potential effects on future fertility is becoming of great concern for patients with cancer and their families. Due to legal and ethical issues, few studies in fertility preservation have been conducted in children. However, a small number of children in France have undergone laparoscopic harvest and cryopreservation of ovarian cortical strips since 1998. The procedure has been noted to be feasible and safe, even in prepubertal girls, with the higher mean number of primordial follicles per mm^3 in younger girls [38,39].

Advances in ovarian cryopreservation and transplantation have been encouraging, with restoration of endocrine function and a few live births demonstrated. Further studies are needed to optimize the procedures and extend their applicability to prepubertal girls with cancer.

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