Chapter 5
Ovarian Tissue Cryopreservation and Transplantation

Laxmi A. Kondapalli

Introduction

In 2010, there were an estimated 730,000 new cancer cases in females, resulting in approximately 270,000 deaths [1]. Although malignancy remains a critical health concern, significant medical advances in cancer detection and treatment have improved survival rates for patients. Gonadotoxic chemotherapy or radiation for malignant disease may result in altered ovarian function, affecting both hormonal production and reproductive potential (discussed in detail in Chap. 1 of this volume). The most severe consequences are premature ovarian insufficiency and infertility, which patients report as major quality-of-life concerns [2, 3]. Restoration of ovarian function, in terms of not only fertility but also endocrine function, would substantially improve the quality of life for women of reproductive age after they have survived cancer and its treatment [4].

Ovarian Tissue Cryopreservation: An Alternative Option

The American Society of Reproductive Medicine considers embryo cryopreservation (i.e., conventional in vitro fertilization [IVF]) the only "standard" procedure for female fertility preservation that has proven efficacy [5]. However, this option is not appropriate for every patient for several reasons:

1. *Time requirement*: Often, gonadotoxic treatment must be initiated immediately after the cancer diagnosis is made, leaving patients with little time to consider or exercise their options for fertility preservation. Ovulation induction for embryo

L.A. Kondapalli, M.D., M.S.C.E. (✉)
Section of Reproductive Endocrinology and Infertility, University of Colorado Denver, 12631 East 17th Avenue, Mail Stop B198-3, Aurora, CO 80045-0508, USA
e-mail: laxmi.kondapalli@ucdenver.edu

C. Gracia and T.K. Woodruff (eds.), *Oncofertility Medical Practice: Clinical Issues and Implementation*, DOI 10.1007/978-1-4419-9425-7_5,

or oocyte cryopreservation typically requires 2–3 weeks of exogenous hormonal stimulation prior to oocyte retrieval. This delay in cancer treatment may not be acceptable to either the oncologist or the patient.

2. *Lack of partner*: In order to preserve embryos, the female cancer patient must have available sperm from a partner or be willing to utilize donor sperm. Some patients may not have a partner or may find it too burdensome to go through the process of identifying a suitable sperm donor.
3. *Hormone-sensitive malignancies*: Invasive breast cancer is the most common neoplasm in women of reproductive age, with an estimated 190,000 new cases diagnosed in 2009 [6]. These patients are particularly at risk for ovarian dysfunction by virtue of being young at the time of diagnosis and commonly exposed to highly gonadotoxic therapies, such as the alkylating agent cyclophosphamide (see Chap. 1 in this volume for more information on this topic). Given the possible induction of breast cancer cell proliferation and progression of disease [7, 8], traditional controlled ovarian stimulation protocols that result in supraphysiologic estrogen levels should be used with caution in these patients. New stimulation protocols have been developed that include aromatase inhibitors (AIs) to temper the potential impact of elevated serum estradiol levels. Despite the use of AIs, however, some patients and oncologists may still be uncomfortable with fertility preservation options that require controlled ovarian stimulation.
4. *Young cancer patients*: The prospect of offering embryo banking to adolescents poses an ethical dilemma. In fact, many programs have minimum age requirements for ovulation induction and embryo banking. When fertility preservation is considered in minors, assent—meaning that the minor gives permission with a full understanding of their choice—is required in addition to parental consent. Furthermore, ovulation induction is not feasible in sexually immature girls who have not entered puberty. Given the large number of childhood cancer patients, prepubertal girls are a key target group who would benefit from alternative options, such as ovarian tissue cryopreservation (or "banking").
5. *Philosophical objections*: The creation and storage of fertilized embryos may generate mixed feelings for couples. Although patients desire the possibility of future childbearing, they may struggle with the prospect of undergoing assisted reproduction to achieve this end. Some couples may be philosophically opposed to the cryopreservation of embryos, or they may live in countries where the creation of embryos for the purpose of banking is illegal. In Germany, for example, storage of cleavage-stage embryos for any purpose is prohibited [9].

While mature oocyte cryopreservation overcomes the need for a partner or sperm donor, this procedure still requires ovarian stimulation that may delay cancer treatment and cannot be performed in prepubertal girls (see Chap. 4 in this volume for further discussion). Ovarian tissue cryopreservation provides a viable alternative option for patients who wish to both preserve fertility with a future male partner and expedite cancer therapy because it requires neither exogenous hormonal stimulation nor a source of sperm. As such, ovarian tissue cryopreservation is the only option that holds promise for fertility preservation for prepubertal girls. Thus, although embryo

banking offers the greatest likelihood of success, ovarian tissue cryopreservation may be the best—and sometimes the only—option for certain patients and thus should be included in any discussion of fertility preservation. Note that ovarian tissue cryopreservation is considered investigational and should therefore be performed under the auspices of an institutional review board (IRB) [5].

Basis for Ovarian Tissue Cryopreservation

The ovary is comprised of two main components: the thin (~1 mm), avascular outer layer known as the cortex and the centrally located, highly vascular medulla. The cortex contains the majority of ovarian follicles in varying stages of maturation, including those comprising the ovarian reserve, the pool of primordial follicles awaiting the signal for further development. By dissecting the thin layer of cortex away from the underlying medulla, follicles within the ovarian tissue can be cryopreserved for future use. Rather than freezing individual oocytes or embryos, ovarian tissue cryopreservation represents a more efficient way of preserving thousands of early-stage (primordial) follicles at one time. In addition, primordial follicles may be less susceptible to the toxic effects of cryoprotectants and the freezing process than mature oocytes. This relative resistance to cryoinjury exhibited by primordial follicles is likely due to their smaller size, slower metabolic rates, and the absence of zona pellucida [10].

Once the patient has survived her cancer and its treatment, the potential uses for the thawed ovarian tissue include in vitro maturation and fertilization of the immature follicles or transplantation of the tissue (Fig. 5.1). While in vitro follicle maturation has not yet been successful in humans, a number of live births have been reported after transplantation of previously frozen ovarian cortical tissue; the techniques and outcomes of ovarian tissue transplantation will be the focus of the remainder of this chapter.

Ovarian Tissue Removal and Cryopreservation

For optimal results, ovarian tissue should be obtained prior to the initiation of cancer treatment, as follicular death and depletion of the ovarian reserve can occur even after a single dose of chemotherapy [11, 12]. Ovarian cortical tissue is typically removed by laparoscopy—a minimally invasive procedure lasting approximately 1 h and requiring general anesthesia. Often, laparoscopic removal of ovarian tissue can be combined with other surgical procedures to avoid additional anesthesia, simplify operating room scheduling, and reduce expense.

The superficial cortical tissue must be sharply dissected from the underlying medullary portion of the ovary [13]. The cortex can be shaved to a thickness of approximately 1 mm to promote early revascularization once the thawed tissue is

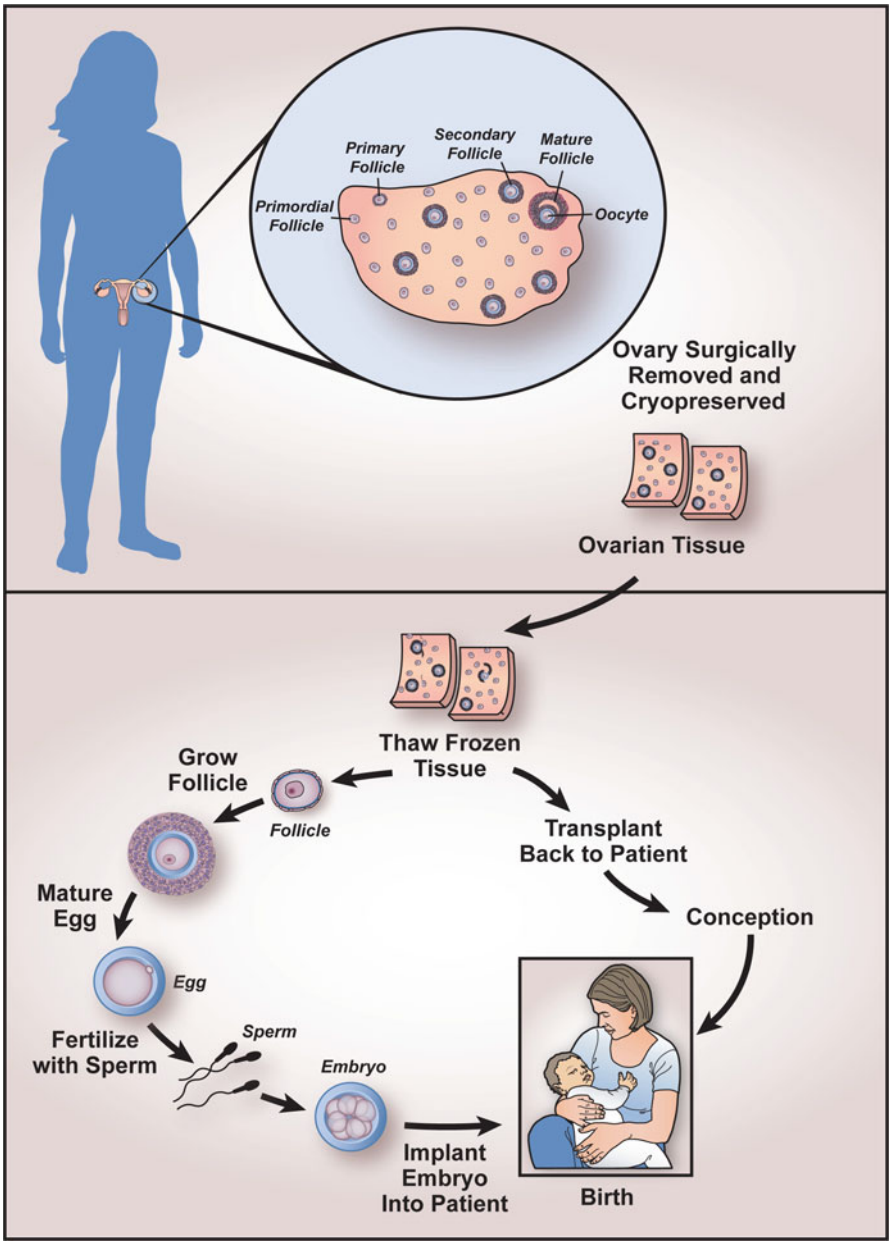

Fig. 5.1 Uses of cryopreserved ovarian tissues include transplantation and in vitro *maturation* of immature follicles and fertilization

transplanted [14]. If the whole ovary or a large portion is removed, the cortex can be further sectioned into 5 × 5-mm segments and cryopreserved using slow-freeze techniques. New vitrification protocols involving rapid cooling of ovarian tissue segments are also being explored [15].

Ovarian Tissue Transplantation

Given the limited lifespan of ovarian tissue grafts, transplantation should be postponed until the patient is ready to conceive or begins to experience symptoms of ovarian insufficiency. The patient should be in remission from disease, should have support from oncology to proceed with the transplantation and pregnancy, and may benefit from consultation with a perinatologist or high-risk obstetrics specialist to discuss the potential perinatal complications unique to cancer survivors.

Ovarian tissue can be transplanted orthotopically to the pelvis [16, 17] or heterotopically to subcutaneous areas such as the forearm or abdomen [18, 19]. Restoration of ovarian function has been reported from both transplantation sites. It appears that peritoneal tissue is superior to subcutaneous tissue as a site of transplantation, likely due to improved revascularization of the transplanted tissue in the peritoneum and subsequent loss of fewer primordial follicles [20, 21]. Regardless of the site of transplantation, the ovarian graft will undergo ischemia and potential follicular atresia during the period of time after transplantation but before tissue revascularization; this remains a challenge to the success of this technique. Depending on the amount of tissue that has been cryopreserved, it may be possible to reserve a portion of the tissue for future transplants in the event that the primary graft fails.

Orthotopic Ovarian Transplantation

Orthotopic transplantation involves the grafting of ovarian cortex near the primary blood supply of the ovary—the ovarian vessels contained within the infundibulopelvic ligaments—or to the exposed medulla of the denuded ovary. Orthotopic transplantation exploits the existing ovarian vascular system to restore perfusion and function to the grafted tissue. In theory, orthotopic transplantation of an ovarian autograft might allow natural pregnancy if the fallopian tubes are intact. Transplantation can be performed using laparoscopy or minilaparotomy approaches. Autologous orthotopic transplantation of previously frozen cortical tissue has led to a series of live births (Table 5.1) and is currently the most effective technique for transplantation.

Heterotopic Ovarian Transplantation

Common sites for heterotopic transplantation are subcutaneous tissues such as the forearm and abdomen. This approach is practical for patients who will receive pelvic radiation because the ovarian tissue will be removed and protected from the radiation field. Although orthotopic transplantation replaces the ovarian tissue back to its anatomically correct location, heterotopic transplantation presents some significant advantages: (1) it avoids major abdominal surgery and general anesthesia, (2) follicular development can easily be monitored by transdermal ultrasound or even visually as the graft site expands with follicular growth, and (3) the transplanted tissue can easily be removed and/or replaced when necessary [2]. One disadvantage of this

Table 5.1 Summary of live births after autologous transplantation of cryopreserved-thawed ovarian tissue

Diagnosis	Age at cryo (years)	Prior chemotherapy	Surgical method	Reimplantation site	Conception	Reference
Hodgkin's lymphoma	25	No	Ovarian biopsies	Orthotopic	Spontaneous singleton	[32]
Non-Hodgkin's lymphoma	28	Yes	Ovarian biopsies	Orthotopic	IVF singleton	[31]
Hodgkin's lymphoma	24	Yes	Unilateral oophorectomy	Orthotopic and heterotopic (abdominal wall)	Spontaneous singleton Spontaneous singleton	[33, 34]
Hodgkin's lymphoma	26	Yes	Unilateral oophorectomy	Orthotopic	IVF singleton	[35]
Ewing's sarcoma	27	No	Ovarian biopsies	Orthotopic	IVF singleton Spontaneous singleton	[35, 36]
Sickle cell anemia	20	No	Unilateral oophorectomy	Orthotopic	Spontaneous singleton	[37]
Breast cancer	36	No	Ovarian biopsies	Orthotopic	IVF twins	[38]
Metastatic neuroectodermic tumor	17	No	Ovarian biopsies	Orthotopic	Spontaneous singleton	[39]
Hodgkin's lymphoma	20	No	Ovarian biopsies	Orthotopic	Spontaneous singleton	[40]
Microscopic polyangiitis	27	Yes	Unilateral oophorectomy	Orthotopic	IVF singleton	[40]

technique is that IVF is required for pregnancy to be achieved using oocytes from the transplanted tissue. While a live birth in a primate after heterotopic ovarian transplantation has been reported [22], no human births have occurred to date. Nonetheless, a chemical pregnancy following IVF and embryo transfer has been reported after heterotopic ovarian transplantation [23, 24].

Whole Ovary Transplantation

In an effort to minimize follicle loss due to the initial ischemia that occurs after transplantation, some investigators have explored the possibility of cryopreservation and transplantation of the entire ovary combined with its vascular supply, though with limited success [25, 26]. Whole ovary transplantation requires specialized expertise with microsurgical methods and vascular reanastomosis, as the vascular pedicles of the grafted tissue must be attached to the ovarian vessels in situ. No pregnancies have been reported using this technique. Given the lack of an efficient technique to preserve the whole ovary and its vascular pedicle, the transplantation of ovarian cortical tissue grafts remains the preferred method. Furthermore, cortical grafting is less invasive, bears minimal operative risk, and has a shorter recovery time [27].

Xenotransplantation

Xenotransplantation is an approach in which human ovarian tissue is transplanted into immunodeficient mice. Severe combined immunodeficiency disease (SCID) mice can maintain tissues from foreign species without demonstrating a graft-versus-host response [28], and thus serve as an ideal model for ovarian transplantation studies. Ovarian tissue can be grafted subcutaneously, intramuscularly, or placed under the kidney capsule to promote neovascularization [2]. Xenotransplantation has been explored as a method to eliminate the risk of cancer transmission and recurrence (from residual malignant cells in the transplanted tissue) and as a possible application for women with hormone-sensitive malignancies. Moreover, follicular development can be easily monitored and the tissue is readily accessible for oocyte retrieval. Unfortunately, possible transmission of infectious diseases from animal to human via the ovarian tissue remains a serious concern [10]. For now, xenotransplantation remains a scientific challenge at the bench and will not be translated into clinical applications until the safety issues can be resolved.

Ovarian Transplantation Outcomes

Autotransplantation of ovarian tissue, either fresh or cryopreserved, has the potential benefit of restoring temporary endocrine function to cancer survivors who develop premature ovarian failure [19]. Donnez et al. reported restoration of ovarian

function after orthotopic transplantation of fresh ovarian cortex between genetically nonidentical sisters [29]. Similarly, transplantation of cryopreserved tissue after ovarian failure has led to a return in ovarian function [30, 31].

In humans, there have been several case reports of ovarian tissue autotransplantation for restoring fertility (Table 5.1) [2, 31–40]. In 2004, Donnez et al. reported the first live birth from orthotopically grafted ovarian tissue fragments to a woman 3 years following chemotherapy and radiotherapy for stage IV Hodgkin's lymphoma [32]. This natural conception was a breakthrough and has invigorated the oncofertility field. There has been substantial controversy regarding the origin of the oocyte responsible for this pregnancy, and whether it originated from the remaining ovaries or the autotransplants [41]. Meirow et al. described a case of a 28-year-old woman with non-Hodgkin's lymphoma who developed premature ovarian failure following high-dose chemotherapy [31]. Midway through treatment, she underwent ovarian tissue cryopreservation and returned for transplantation 2 years after cessation of menses. She subsequently resumed ovarian hormone production and spontaneous menstruation, and delivered a live infant after IVF.

Bedaiwy conducted a systematic review of case reports and case series of women at high risk of premature ovarian insufficiency and summarized the reproductive outcomes after fresh and cryopreserved ovarian tissue transplantation [42]. Women with follicle-stimulating hormone (FSH) greater than 30 IU/I at the time of transplantation were included in the review ($n=23$). In these women, ovarian function resumed within a median of 120 days, ranging from 60 to 244 days; however, four women developed recurrent ovarian failure within 6 months of their procedures. Multivariate analysis revealed that fresh grafts were less likely to be associated with recurrent ovarian failure compared with cryopreserved grafts, with an adjusted HR of 0.47 (95% CI 0.18, 1.12; $P=0.09$).

Clinical Concerns and Considerations

Duration of Graft

Accumulated clinical experience suggests that ovarian cortical transplants have short-lived hormonal function, between 9 months and 3 years [43]. Oktay et al. observed resumption of menses and spontaneous ovulation within 3 months of transplanting fresh ovarian tissue into the forearm of a 37-year-old woman who underwent oophorectomy for benign cysts [19]. Unfortunately, the graft lost function after 3 years; the age of the patient and the size of the grafted tissue likely contributed to the overall duration of graft function.

While the loss of follicles from ovarian tissue due to the freezing and thawing process is relatively small, up to two-thirds of follicles are lost during a period of ischemia just after transplantation, resulting in a significant reduction in the size of the follicle pool or ovarian reserve [2]. Some patients may even require repeat transplantation to maintain ovarian hormonal function [44]. Because of the limited

duration of graft function, ovarian tissue transplantation is not recommended as a strategy for long-term hormone replacement.

Reseeding Malignant Cells

One serious and legitimate concern is the theoretical possibility of reimplanting malignant cells from the primary tumor back into women who have overcome their cancer and are in remission. Clinically, the risk of transferring malignant cells depends on the cancer type, stage, activity, and volume of cancer cells that are transferred [45]. Fortunately, the common malignancies faced by women of reproductive age do not metastasize to the ovaries, with the exception of some leukemias, Burkitt's lymphoma, neuroblastoma, and some forms of advanced breast and colon cancers [13, 46]. There is particular concern about reseeding leukemic cells through ovarian tissue transplantation. A recent study of 18 patients with chronic myelogenous leukemia (CML) or acute lymphoblastic leukemia (ALL) showed that leukemic tumors occurred when thawed ovarian cortical tissue from four of these patients was xenografted to mice [47]. Although this phenomenon was not seen when ovarian tissue from lymphoma patients was grafted to immunodeficient mice [48], the risk is not yet clear in humans, and so it must be factored into patient counseling.

Several steps can be implemented to minimize the potential risks associated with banking and transplanting ovarian tissue that may contain metastatic disease. First, it is prudent to perform histological evaluation of segments of the ovarian tissue samples, particularly if multiple samples are obtained from different areas of the ovary. In addition, consultation with medical oncology prior to ovarian tissue cryopreservation and transplantation is always appropriate. Finally, a discussion with pathology to convey the special circumstances of the pathologic review is recommended. Ovarian transplantation should be avoided in patients who are at high risk of ovarian metastasis. For these patients, in vitro follicle growth and oocyte maturation, though still experimental, may be a preferred alternative approach to restoring fertility after cancer.

Oocyte and Embryo Quality

There are limited data regarding the effect of cancer therapies on oocyte and embryo quality. Chemotherapeutic agents are known to cause genetic mutations, structural breaks, DNA adducts, and oxidative damage to somatic and germ cells [13]. In addition, higher pregnancy losses and fetal malformations have been observed in mice exposed to cyclophosphamide around the time of conception [49]. However, studies of cancer survivors who conceive years after exposure to chemotherapeutic agents demonstrate no significant increase in the rate of congenital malformations, genetic abnormalities, or malignant neoplasms in the resulting offspring [50, 51]. The effect of cancer therapies given prior to ovarian tissue harvesting and cryopreservation on

the future performance of the transplanted tissue is unknown at present. Ideally, given the loss of follicles observed after exposure to chemotherapeutic agents and radiation therapy, every attempt should be made to harvest ovarian tissue prior to the start of cancer treatment.

Reproductive Outcomes

An important element of counseling patients who are considering ovarian transplantation is a discussion of the realistic chance for pregnancy by this technique. Data on pregnancy outcomes of patients undergoing ovarian transplantation is limited simply because there are so few pregnancies reported in the literature. In a pooled analysis of reproductive outcomes in 25 women, a total of 11 pregnancies were identified in nine women [42]. One of these women was excluded because she had two natural conceptions that were ectopic pregnancies. Eight women had nine pregnancies in five reports. Half of these women (4/8) underwent autologous transplantation using cryopreserved cortical tissue, and the remaining four women had heterologous transplants using fresh tissue donated by their monozygotic twin. The 12-month cumulative pregnancy rate was 37% (95% CI 19–60%) with the first pregnancy achieved after a median duration of 9 months after transplantation. Six pregnancies occurred naturally, whereas three pregnancies were achieved by IVF. Of note, five of the live births occurred in patients who cryopreserved tissue after the initiation of chemotherapy. Unfortunately, there are no published reports of the short- and long-term outcomes of the children conceived by these methods.

Conclusions

Ovarian tissue cryopreservation prior to cancer treatment and subsequent transplantation after cancer is an exciting, yet investigational, option for fertility preservation. Although ovarian transplantation began in the late nineteenth century, many questions regarding patient selection, safety, and optimal technique remain unanswered. For this reason, ovarian cryopreservation and transplantation should be offered as an experimental procedure to appropriately selected patients in centers with IRB approval. Given the limited success and resumption of ovarian function, it is essential that clinicians critically evaluate the risk of ovarian dysfunction after cancer treatment in their patients. A multidisciplinary team of experts including oncologists and reproductive endocrinologists may best achieve this assessment. Although the impact of specific cancer treatment regimens on ovarian function may be uncertain, patients should be given reasonable estimates of risk with the best available evidence as they make informed decisions about undertaking investigational procedures to preserve their fertility and/or restore hormonal function.

Aside from the need to improve current ovarian tissue cryopreservation and thawing protocols, further research is needed to identify the optimal site for transplantation, create strategies that reduce follicular loss, and develop methods to enhance revascularization of the transplanted tissue. An encouraging area of investigation is in vitro follicle growth and oocyte maturation, using immature follicles recovered from fresh or cryopreserved ovarian cortical tissue samples, conducted by Woodruff and others [52], which would eliminate the risk of reseeding malignant cells and would be suitable for patients for whom controlled ovarian stimulation is contraindicated. While live births using this method in the mouse model have been reported in the literature, little is known about the offspring conceived. As no single institution may have large enough patient volume to assess the reproductive outcomes of in vitro follicle growth/oocyte maturation, this work would greatly benefit from the development of an international outcomes registry of the shared data and experiences of pioneers in this field. The collective evidence can then be evaluated in a rigorous scientific manner to offer patients realistic expectations of success and outcomes. Nevertheless, maintaining the option for parenthood in the future conveys an important message of hope for young cancer patients, and researchers are actively investigating new approaches to fertility preservation.

Acknowledgment This work was supported by the Oncofertility Consortium NIH/NICHD 5UL1DE019587.

References

1. National Center for Health Statistics. 2010. http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-026210.pdf. Accessed 10 Nov 2011.
2. Sonmezer M, Oktay K. Fertility preservation in female patients. Hum Reprod Update. 2004;10:251–66.
3. Partridge AH, et al. Web-based survey of fertility issues in young women with breast cancer. J Clin Oncol. 2004;22:4174–83.
4. Zeltzer L. Cancer in adolescents and young adults psychosocial aspects. Long-term survivors. Cancer. 1993;71:3463–8.
5. Ethics Committee of the American Society for Reproductive Medicine. Fertility preservation and reproduction in cancer patients. Fertil Steril. 2005;83:1622–8.
6. Jemal A, et al. Cancer statistics, 2009. CA Cancer J Clin. 2009;59:225–49.
7. Prest SJ, May FE, Westley BR. The estrogen-regulated protein, TFF-1, stimulates migration of human breast cancer cells. FASEB J. 2002;1(6):592–4.
8. Allerd CD, et al. Soy diets containing varying amounts of genistein stimulate growth of estrogen-dependent (MCF-7) tumors in a dose-dependent manner. Cancer Res. 2001;61:5045–50.
9. Embryo Bank. 2011. http://www.ivf-worldwide.com/Education/embryo-bank.html. Accessed 10 Nov 2011.
10. Seli E, Tangir J. Fertility preservation options for female patients with malignancies. Curr Opin Obstet Gynecol. 2005;17:299–308.
11. Fauser B. Follicle pool depletion: factors involved and implications. Fertil Steril. 2000;74:629–30.
12. Oktem O, Oktay K. A novel ovarian xenografting model to characterize the impact of chemotherapy agent on human primordial follicle reserve. Cancer Res. 2007;67:10159–62.

13. Practice Committee of American Society for Reproductive Medicine; Practice Committee of Society for Assisted Reproductive Technology. Ovarian tissue and oocyte cryopreservation. Fertil Steril. 2008;90:S241–6.
14. Silber S, Gosden RG. Ovarian transplantation in a series of monozygotic twins discordant for ovarian failure. N Engl J Med. 2007;356:1382–4.
15. Silber S, et al. Duration of fertility after fresh and frozen ovary transplantation. Fertil Steril. 2010;94:2191–6.
16. Radford JA, et al. Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma. Lancet. 2001;357:1172–5.
17. Tryde Schmidt KL, et al. Orthotopic autotransplantation of cryopreserved ovarian tissue to a woman cured of cancer—follicular growth, steroid production and oocyte retrieval. Reprod Biomed Online. 2004;8:448–53.
18. Oktay K, et al. A technique for transplantation of ovarian cortical strips to the forearm. Fertil Steril. 2003;80:193–8.
19. Oktay K, et al. Endocrine function and oocyte retrieval after autologous transplantation of ovarian cortical strips to the forearm. JAMA. 2001;286:1490–3.
20. Donnez J. Laparascopic ovarian tissue transplantation in humans. Gunaikeia. 2003;8:168.
21. Marhhom E, Cohen I. Fertility preservation options for women with malignancies. Obstet Gynecol Surv. 2006;62:58–72.
22. Lee DM, et al. Live birth after ovarian tissue transplant. Nature. 2004;428:137–8.
23. Rosendahl M, et al. Biochemical pregnancy after fertilization of an oocyte aspirated from a heterotopic autotransplant of cryopreserved ovarian tissue: a case report. Hum Reprod. 2006;21:2006–9.
24. Oktay K. Spontaneous conceptions and live birth after heterotopic ovarian transplantation: is there a germline cell connection? Hum Reprod. 2006;21:1345–8.
25. Imhof M, et al. Cryopreservation of a whole ovary as a strategy for restoring ovarian function. J Assist Reprod Gen. 2004;21:459–65.
26. Bedaiwy MA, et al. Cryopreservation of intact human ovary with its vascular pedicle. Hum Reprod. 2006;21:3258–69.
27. Silber SJ, et al. A series of monozygotic twins discordant for ovarian failure: ovary transplantation (cortical versus microvascular) and cryopreservation. Hum Reprod. 2008;23:1531–7.
28. Bosma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. Nature. 1983;301:527–30.
29. Donnez J, et al. Restoration of ovarian function after allografting of ovarian cortex between genetically non-identical sisters. Hum Reprod. 2010;25:2489–95.
30. Oktay K, Karlikaya G. Ovarian function after transplantation of frozen, banked autologous ovarian tissue. N Engl J Med. 2000;342:1919.
31. Meirow D, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med. 2005;353:318–21.
32. Donnez J, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004;364:1405–10.
33. Demeestere I, et al. Fertility preservation: successful transplantation of cryopreserved ovarian tissue in a young patient previously treated for Hodgkin's disease. Oncologist. 2007;12:1437–42.
34. Demeestere I, et al. Birth of a second healthy girl more than 3 years after cryopreserved ovarian graft. Hum Reprod. 2010;25:1590–1.
35. Andersen CY, et al. Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. Hum Reprod. 2008;23:2266–72.
36. Ernst E, et al. The first woman to give birth to two children following transplantation of frozen/thawed ovarian tissue. Hum Reprod. 2010;25:1280–1.
37. Roux C, et al. Live birth after ovarian tissue autograft in a patient with sickle cell disease treated by allogeneic bone marrow transplantation. Fertil Steril. 2008;93:2413.e15–e19.
38. Sánchez-Serrano M, et al. Twins born after transplantation of ovarian cortical tissue and oocyte vitrification. Hum Reprod. 2010;93:e11–3.

39. Donnez J, et al. Pregnancy and live birth after autotransplantation of frozen-thawed ovarian tissue in a patient with metastatic disease undergoing chemotherapy and hematopoietic stem cell transplantation. Fertil Steril. 2011;95:e1–4.
40. Donnez J, et al. Children born after autotransplantation of cryopreserved ovarian tissue. A review of 13 live births. Ann Med. 2011;43:437–50.
41. Oktay K, Tilly J. Livebirth after cryopreserved ovarian tissue autotransplantation. Lancet. 2004;364:2091–2. author reply 2092–2093.
42. Bedaiwy MA, et al. Reproductive outcome after transplantation of ovarian tissue: a systematic review. Hum Reprod. 2008;23:2709–17.
43. Oktay K, Sonmezer M. Ovarian tissue banking for cancer patients: fertility preservation, not just ovarian cryopreservation. Hum Reprod. 2004;19:477–80.
44. Kim S, et al. Long-term ovarian function and fertility after heterotopic autotransplantation of cryobanked human ovarian tissue: 8-year experience in cancer patients. Fertil Steril. 2009;91:2349–54.
45. Donnez J, et al. The role of cryopreservation for women prior to treatment of malignancy. Curr Opin Obstet Gynecol. 2005;17:333–8.
46. Yada-Hashimoto N, et al. Metastatic ovarian tumors: a review of 64 cases. Gynecol Oncol. 2003;89:314–7.
47. Dolmans MM, et al. Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe. Blood. 2010;116:2908–14.
48. Kim SS, et al. Ovarian tissue harvested from lymphoma patients to preserve fertility may be safe for autotransplantation. Hum Reprod. 2001;16:2056–60.
49. Meirow D, et al. Administration of cyclophosphamide at different stages of follicular maturation in mice: effects on reproductive performance and fetal malformations. Hum Reprod. 2001;16:632–7.
50. Hawkins MM. Pregnancy outcome and offspring after childhood cancer. BMJ. 1994;309:1034.
51. Sanders JE, et al. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. Blood. 1996;87:3045–52.
52. Smitz J, et al. Current achievements and future research directions in ovarian culture, in vitro follicle development and transplantation: implications for fertility preservation. Hum Reprod Update. 2010;16:395–414.