Curr Obstet Gynecol Rep (2012) 1:182–189
DOI 10.1007/s13669-012-0025-4

Fertility Preservation

Ayse Seyhan · Baris Ata · Hai Ying Chen · Alex C. Varghese · Alper Mumcu · Seang Lin Tan

Published online: 18 August 2012

Abstract Advances in early diagnosis and cancer treatment have allowed the quality of life postcancer treatment as a recognized issue, and efforts have been made to preserve the fertility potential. Radiotherapy and chemotherapy have detrimental effects on ovarian reserve. Well-established methods against radiotherapy-induced gonadotoxicity are pelvic shielding or removal of the ovaries from the radiation field. Gonadal toxicity of chemotherapy depends mainly on the type of treatment and patient's age. In vitro fertilization (IVF) and embryo cryopreservation are the most commonly used procedures for females undergoing chemotherapy. Oocyte cryopreservation is a promising method and has the advantage of eliminating the contribution of a partner. Ovarian tissue cryopreservation is experimental and the only method for prepubertal girls. The trend toward delaying child-bearing results in increased prevalence of age-related infertility and has raised debates on social egg freezing in many countries. Fertility preservations options for different indications are discussed in the light of recent literature.

Keywords Cancer · Fertility preservation · Chemotherapy · Radiotherapy · Social fertility preservation · Oocyte cryopreservation

A. Seyhan (✉)
Clinical and Research Fellow,
Division of Reproductive Endocrinology and Infertility,
Department of Obstetrics and Gynecology, McGill University,
Montreal, Canada
e-mail: aaseyhan@hotmail.com

B. Ata
Division of Reproductive Endocrinology and Infertility,
Department of Obstetrics and Gynecology, Uludag University,
Bursa, Turkey

H. Y. Chen · A. C. Varghese · A. Mumcu · S. L. Tan
Montreal Reproductive Centre,
Montreal, Canada

S. L. Tan
Department of Obstetrics and Gynecology, McGill University,
Montreal, Canada

S. L. Tan
McGill Reproductive Centre, MUHC,
Montreal, Canada

Introduction

According to the American Cancer Society, 790,740 new cancer cases are estimated to occur in females in 2012 within a significant subset of them presenting in the reproductive age [1]. Increased awareness of possible warning signs and regular screening examinations result in the early detection of cancer and improvement in survival rates. Quality of life (QoL) issues in cancer survivors has gained increasing attention following progress in successfully treating the disease. The World Health Organization Quality of Life group defines quality of life as individuals' perception of their position in life in the context of culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns [2]. Preserving fertility is fundamental component of QoL, and many cancer patients will choose a less effective treatment if they believed it will reduce the adverse impact on fertility.

Fertility is age-dependent and declines progressively after age 35 years [3]. Today, in vitro fertilization (IVF) is increasingly used for age-related infertility; even with IVF, success rate for women aged 40 years and older is low [4]. Fertility preservation technologies also can be recommended to fertile women to stop the effects of biological clock and preserve women's reproductive

autonomy. The purpose of this paper was to review the current fertility preservation options for cancer patients and to discuss developments in social fertility preservation.

Fertility Preservation for Female Patients with Cancer: Who is at Risk?

Breast cancer is the most frequent cancer in women followed by digestive and respiratory cancers. Based on rates from 2007–2009, 12.38 % of women born today will be diagnosed with cancer of the breast at some time during their lifetime and 12 % of them will be diagnosed before age 44 years [5]. The overall 5-year relative survival is reported as 89 % [5]. Breast cancer in the reproductive age patients tends to be more aggressive and mostly requires gonadotoxic adjuvant chemotherapy [6]. Furthermore, women with estrogen receptor-positive tumors are usually given tamoxifen or aromatase inhibitors to block the effects of estrogen on the growth of breast cancer cells and they have to postpone pregnancy for 5 years.

Pediatric and young adult cancer patients may experience treatment-related side effects years after cancer treatment. An estimated 12,060 new cases are expected to occur among children 0 to 14 years of age in 2012. Overall survival rate of childhood cancers is 83 % [1]. Dramatic improvements in cancer treatment have exerted more pronounced considerations on quality of life of cancer survivors in terms of future fertility and maintenance of ovarian endocrine functions. Increased awareness of the gonadal insult after chemotherapy influenced the chemotherapeutic regimens in the pediatric Hodgkin lymphoma population. Traditional treatment with alkylating agents has been modified to less gonadotoxic regimen, and pelvic shielding for abdominal irradiation has been introduced to management [7]. The 5-year survival among children 0–14 years of age for Hodgkin lymphoma is 95 % with the improvement in reproductive capacity [1]. Traditional treatment modality for non-Hodgkin lymphoma includes alkylating agents, which produce high survival rates (86 %) but comes at a cost of increased risk of gonadal failure [1, 8]. Furthermore, the prebone marrow transplantation conditioning with high-dose chemotherapy and radiation is the mainstay treatment for many onco-hematological malignancies and is associated with a high incidence of sterility.

An estimated 81,580 new cases of gynecological cancers are expected in the United States in 2012 [1]. According to SEER data, approximately 40.1 % of cervical cancer patients, 12.3 % of ovarian cancer patients, and 7.5 % of corpus and uterine cancers will be diagnosed before age 44 years [9–11]. The increased screening and earlier diagnosis of gynecological cancers in women of reproductive age has heightened the awareness of need for fertility preservation (FP) as well. In selected patients, fertility-sparing surgery can be recommended.

Effects of Chemotherapy and Radiotherapy

Primordial follicles make up the ovarian reserve, and they are gradually depleted during the female life. Chemotherapy targets the primordial follicles and compromises the reproductive function. Extent of damage depends on the chemotherapeutic agent received, patient's age, and dose of drug. Younger patients have better ovarian reserve, and the effect gonadal damage on the reproductive outcome is less severe than in older patients [12]. Alkylating agents, such as cyclophosphamide, ifosfamide, chlorambucil, melphalan, procarbazine, and chlormethine, have high gonadotoxic impact, whereas cisplatin and adriamycin are in the medium-risk group and bleomycin, vincristine, methotrexate, dactinomycin, mercaptopurine, and vinblastine are in the low-risk group [12].

Radiation therapy has effects on primordial follicles, uterine vasculature, uterine elasticity and volume, and endometrium depending on the radiation dose, radiation field, fractionation schedule, and age at the time of treatment. Lethal dose to destroy half the total number of oocytes is less than 2 Gy [13]. The effect of radiotherapy on ovarian follicular pool is age-dependent. The radiation dose required for complete ovarian failure at birth is 20.3 Gy, at 10 years 18.4 Gy, at 20 years 16.5 Gy, and 30 years 14.3 Gy [14]. As a result of changes in the uterine myometrium, endometrium, and vasculature secondary to radiation, there is an increased risk for spontaneous miscarriage, intrauterine growth retardation, preterm delivery, and placenta accretae [13, 15, 16]. Lastly, cranial irradiation used for some childhood leukemias and cranial tumors can disrupt the hypothalamic-pituitary-gonadal axis resulting in delayed puberty, amenorrhea, and infertility [7].

Fertility Preservation Options to Protect Ovarian Functions

1. Ovarian suppression to prevent follicle damage

   The number of follicles is estimated to be approximately 7 million at 20 weeks, and the stock of primordial follicles is depleted starting from intrauterine life with a steep decline beginning after age 35 years, resulting in sharp decline of pregnancy and live birth rate even with IVF [17–20]. Although the dynamics of primordial follicles from resting to growing stage is not fully understood, the primordial follicles lack follicle-stimulating hormone

(FSH) receptor and initial recruitment is not dependent on gonadotropins [21]. Gonadal protection from chemotherapy with creating prepubertal hormonal milieu was thought to help or work.

Gonadotropin-releasing hormone (GnRH) agonists downregulate GnRH receptors, which results in profound hypogonadal effect. In theory, suppressing gonadotropins were supposed to prevent the primordial follicles' recruitment; however, primordial follicles are not sensitive to gonadotropins. Although the main proposed mechanism has lost its validity, many trials are still ongoing due to its easy availability.

Resumption of menses and amenorrhoea were the most common outcome measures among the studies evaluating the effect of GnRH agonist on ovarian function [22–28]. Resumption of menstruation following treatment is not a reliable indicator of fertility. Some patients may retain ovarian function despite lack of menstrual activity, and some patients may preserve menstruation while ovarian reserve is very poor. Resumption of menses is expected to be higher in hormone receptor-negative patients who do not receive tamoxifen and in younger patients [26, 29]. Hormone receptor status, age, and treatment type should be considered while interpreting the results of the studies. There is only one such trial so far in which amenorrhea rates on triptorelin were comparable to those seen in control group [27]. Antimüllerian hormone as an ovarian reserve marker was evaluated for the benefit of GnRH agonist cotreatment [24, 30••]. No significant difference was detected in both studies. A recent trial was closed prematurely for futility, because the AMH level was reduced in all patients with or without GnRHa cotreatment [30••]. To date, studies investigating effect of GnRH agonist on ovarian function are inconclusive and have shown no protection for follicle damage.

2. Fertility preservation for patients receiving radiotherapy

Pelvic or abdominal shielding is required for some cervical and vaginal cancers, Hodgkin's disease, and sarcomas. Pelvic shielding to reduce the radiation to ovaries and uterus or removal of the ovaries from the radiation field are the two standard procedures for female patients receiving radiotherapy. The objective is generally transposition of the ovaries at least 3 cm above the upper border of radiation field [31]. The classical laparoscopic approach includes dissection of peritoneum and ovarian vessels up to the level of aortic bifurcation to preserve vascularization, mobilization of both ovaries by dissecting utero-ovarian ligament, transposition of the ovaries to paracolic gutter, fixation lateral and above the psoas muscle, and marking the upper and lower parts of ovaries with hemoclips to allow the localization during radiotherapy [32]. The overall success rate defined as preservation of menstrual function is 50 % [33]. Ovarian repositioning may be needed for IVF when there is no spontaneous pregnancy. Complications of ovarian transpositions are pain due to ovarian cyst, chronic abdominal pain, and difficulty diagnosing metastasis in the ovary [34].

3. Fertility-sparing surgery

Radical vaginal trachelectomy (RVT) with laparoscopic lymphadenectomy is a viable option for women with early-stage cervical cancer [35]. Criteria for patient selection are squamous cell carcinoma, adenocarcinoma, adenosquamous, with exclusion of unfavorable histologic stage, stage 1A1, 1A2, or 1B1 <2 cm, tumor limited to cervix, and no evidence of lymphatic spread [32]. A growing body of evidence in the literature shows that RVT is a well-established FP option without compromising oncologic outcomes [35–41].

Conservative management of endometrial carcinomas in young women is an unsolved issue. Younger age at diagnosis corresponds to favorable prognostic factors, such as low-grade histology, focal well-differentiated lesion, estrogen-progesterone receptor-positive tumor, and better overall survival [42]. However, the risk of occult ovarian tumor in young patients with endometrial carcinoma is approximately fivefold higher than in postmenopausal women [43]. Early onset of endometrial carcinoma also has a higher risk of Lynch syndrome [44, 45]. Genetic counselling and screening may be recommended.

Only young patients with grade I endometrial adenocarcinoma without suspicion of myometrial, lymph node, or ovarian involvement may be considered as candidates for conservative treatment. Progestins are the most frequently used for conservative treatment [46–52]. Although the survival and fertility outcomes are promising, there is no consensus on the type of progestin, dose, duration, or mode of administration. Some investigators propose combination of hysteroscopic excision of the tumor with the underlying myometrium and progestins [53, 54]. Results need to be validated with further studies.

According to the guidelines of ACOG and European Society of Medical Oncology, fertility-sparing surgery, which corresponds to unilateral salpingo-oophorectomy (USO), is recommended for patients with early epithelial ovarian cancer (EOC) [55, 56]. Conservative surgery does not seem to compromise the survival outcome in young patients with unilateral

stage IA, grade 1 or 2, non-clear cell histology EOC (tumor growth limited to one ovary; no ascites present containing malignant cells; no tumor on the external surface; capsule intact) [55, 56]. Malignant ovarian germ-cell tumors, except for dysgerminomas, are in most cases unilateral. Conservative surgery with USO can be performed and in case of bilateral dysgerminomas bilateral cystectomy is the treatment of choice [57]. Borderline ovarian tumors usually occur during childbearing age. Standard surgery is USO or cystectomy with surgical staging. Even though the recurrence is higher compared with radical surgery, patient survival is not affected [32, 57].

With appropriate patient selection, preoperative counseling, postoperative follow-up, and multidisciplinary care, fertility-sparing surgery is a realistic option for FP.

Ovarian Tissue Cryopreservation

Ovarian tissue cryopreservation is the only option for prepubertal patients undergoing gonadotoxic chemotherapy or whole body irradiation and for sexually mature patients who are reluctant to ovarian stimulation. Immature oocytes within the antral follicles also can be collected from ovarian biopsy specimens and can be vitrified following IVM, because cryopreserving ovarian tissue saves the primordial and primary follicles [58]. This combination of ovarian tissue cryobanking and IVM improves the services provided by a FP program.

Autotransplantation of cryopreserved ovarian tissue aims to maintain the ovarian endocrine function: puberty, fertility, and protection against bone loss. Ovarian cortex contains the primordial follicles, which can be cryopreserved with great efficiency [59]. Thin fragments of ovarian cortex (1- x 1- x 3-cm) are harvested by laparoscopy and cryopreserved. The size and number of ovarian biopsies depends on the patient's age, because the main determinant of mean concentration of the primordial follicles in the specimen is age [59]. Frozen-thawed ovarian tissue can be transplanted to near the infundibulopelvic ligament /ovary (orthotopic) or extrapelvic sites, such as forearm and suprapubic area (heterotropic).

Main concern in autotransplantation of the cyropreserved ovarian tissue is reintroduction of malignant cells. Ovarian tissue should be screened carefully to detect metastasis, especially in leukemias. To date, there is no technology to mature oocytes from cryopreserved ovarian tissue in humans, so there is no feasible FP option for patients with confirmed tumor cells in ovarian tissue biopsy. In the literature, there are more than 15 human live births and one girl had an induction of puberty following orthotopic transplantation of the ovarian graft [60–69].

According to American Society of Clinical Oncology (ASCO) recommendations, ovarian cryopreservation should only be performed in centers with necessary expertise under institutional review board-approved protocols that include follow-up for recurrent cancer [33].

Ovarian Stimulation Protocols and Cryopreservation of Oocytes/Embryos

IVF and embryo cryopreservation is the only method of female FP approved by the American Society of Clinical Oncology and American Society of Reproductive Medicine. Controlled ovarian hyperstimulation and embryo cryopreservation seem to be the most suitable approach for patients who have a male partner and sufficient time. Oocyte cryopreservation is another option that overcomes the limitation of the need for a contribution of a male partner. Oocyte and embryo cryopreservation are reported to be safe in terms of risk of congenital abnormalities [70, 71].

There are particular concerns regarding ovarian stimulation, especially for breast cancer patients. Circulating estradiol levels are elevated during conventional ovulation induction for IVF. To avoid the potential risks of high E2 levels in breast cancer patients with estrogen receptor-positive disease, a stimulation protocol with aromatase inhibitor (letrozole) in combination with gonadotropins was developed [72]. Letrozole is started on the second or third day of the cycle in a dose of 5 mg/day, and 2 days after letrozole administration gonadotropins are started. GnRH antagonist is added when the leading follicle is 14 mm or E2 level exceeds 918 pmol/l. Final oocyte maturation is triggered by human chorionic gonadotropin (HCG). Letrozole is reinitiated after oocyte collection to decrease the E2 levels further. Letrozole is continued until E2 levels fall below 183.5 pmol/l [72, 73]. This protocol resulted in 44 % reduction in gonadotropin requirement, similar oocyte and embryo yield, and significantly lower peak E2 levels [72, 74]. HCG has a long half-life and prolongs estrogen production. The original protocol was modified with GnRH agonist triggering (1 mg leuprolide acetate) for final oocyte maturation instead of HCG [75]. Following trigger, estradiol levels dropped significantly in the luteal phase and moderate-severe OHSS was completely eliminated [75]. GnRH seems to be a safer approach compared with HCG in terms of less exposure to estradiol.

High E2 levels during stimulation and concerns about delay of the initiation of treatment are the main reasons why FP is not accepted among many patients [76]. Another strategy is in vitro maturation of oocytes (IVM), which expands the FP options for women who are not suitable for stimulation. These patients may undergo immature oocyte collection regardless of the day of cycle and cryopreserve resultant oocytes/embryos without any gonadotropin

stimulation [77]. Completion of the FP procedure is between 2 to 10 days, preventing a delay in treatment of the primary disease [78].

The interval from surgery to the start of chemotherapy is usually 4 to 12 weeks. Two weeks of ovarian stimulation from the onset of menses is required, but delays with the referral and insufficient time to wait for menstrual period necessitate emergency FP methods. Growing evidence suggests that multiple waves of antral follicles are recruited in each cycle instead of a single cohort of follicles growing during the follicular phase [79]. Luteal phase immature and mature oocyte collection has been reported to be successful in terms of oocyte yield [77, 80–82]. Oktay et al. reported immature oocyte collection after premature LH surge in a breast cancer patient undergoing letrozole stimulation [80]. We reported three cases between days 15 to 30 of the cycle with insufficient time for conventional stimulation. Five to seven immature oocytes were recovered, and following IVM three to five oocytes matured and were cryopreserved [77]. Based on the theory of multiple waves of antral follicular recruitment, random start controlled ovarian stimulation approach was developed [81]. Random start of letrozole with combination of r-FSH between days 11 to 17 of the cycle resulted in mature oocyte yield and satisfactory numbers of cryopreserved embryos as well [82].

Social Fertility Preservation

To date, oocyte cryopreservation was reserved for FP in patients with cancer or autoimmune disease. The average age for women having their first child is climbing, and IVF is increasingly applied for age-related infertility [83]. Fertility declines sharply with age; women older than age 35 years who have delayed childbearing may face difficulty conceiving and increased risk of miscarriage and delivering a child with genetic/congenital abnormalities [3]. In a recent study, 328 university students were asked how they would feel and react if hypothetically presented with unfavorable ovarian reserve test results. Only 29 % of the women agreed to take action regarding their education and work despite the bad news. Participants were reluctant to postpone or stop their education/career, but 53 % of them said they would agree to freeze oocytes [84].

With the introduction of vitrification technique for oocytes, reproductive outcomes dramatically increased compared with the slow-cooling technique. Survival rate after vitrification and warming was reported to be 90 % to 99.4 % [85–89]. Fertilization rates, embryo development, and implantation rates were shown to be similar to those from the fresh oocytes [85–89]. Vitrified oocytes retain their reproductive potential and are not affected by the vitrification procedure in young women.

Cryopreservation of the oocytes gives women the opportunity to become pregnant at their discretion and release the pressure about biological clock. In a cost-effectiveness study on oocyte freezing, a decision-analytical model for 35-year-old women who want to postpone pregnancy until age 40 years was used. If patients freeze oocytes at age 35 and use them at age 40, the cumulative live birth would be 84 % with miscarriage rate of 26 %. Oocyte freezing is cost-effective if at least 61 % of the women return to collect their oocytes, and if there is a willingness to pay €19,560 extra per additional live birth [90].

Data indicate that approximately 20–25 frozen oocytes are needed to achieve a satisfactory clinical pregnancy rate in patients younger than age 35 years [71, 91, 92••]. Multiple stimulated IVF cycles are needed to achieve this number of oocytes; however, natural cycle IVF, IVM can be offered to these healthy patients with the advantages of lower cost, less-medication, simplicity, and total elimination of the risk of ovarian hyperstimulation syndrome (OHSS).

Conclusions

Cryopreservation of embryos is the standard practice. Conservative surgeries and transposition of the ovaries or gonadal shielding before radiotherapy are other options for selected patients [33]. Cryopreservation of oocytes and ovarian tissue were pronounced as experimental options to preserve reproductive function in women in the 2006 recommendations of ASCO; however, continued research in oocyte vitrification made FP possible for patients without partners or who do not want to preserve embryos [33]. Given that reproductive outcomes are similar in vitrified and fresh oocytes, oocyte freezing should no longer be considered experimental.

Preserving fertility is a major issue, especially for young cancer patients with high survival rates. Seventy-three percent of young breast cancer patients reported concerns about their fertility, and 29 % of the women indicated that concern about fertility impacted their treatment decision [93]. ASCO recommends that the oncologist should address the possibility of infertility following cancer treatment, discuss FP options, and refer interested patients to reproductive specialists [33]. Unfortunately, 50 % of patients received little information about fertility options from the oncology team and were referred to a reproductive endocrinologist [94]. Recent study found out that only 7.6 % of the breast cancer patients underwent FP following FP consultation [95]. Another recent study found that after a single FP consultation, only 50 % of the information was understood correctly. Additional contact with a fertility specialist and discussing FP with someone else increased the post-visit knowledge [96••]. There is too much information to process for a patient undergoing the stress of a newly diagnosed cancer; additional

care and support can increase the comprehension of FP options. Oncologists' prompt referral and additional support from the fertility clinic may help these patients to make a healthier decision regarding their future fertility.

Although women's request for FP to protect reproductive potential against the effect of time is not generally classified as a disease-related reason, age-related infertility is large part of the current need for IVF. More enlightened thinking will make FP for age-related declining fertility considered as preventive medicine and offered as an option to the general population. The European Society of Human Reproduction and Embryology recommends promoting efforts designed to raise awareness of age-related female fertility and to refrain from passing judgement on a woman's motives for postponing childbearing [97]. Women interested in oocyte cryopreservation for age-related infertility should be informed carefully with the target of 20–25 vitrified oocytes for reasonable live birth rates [71, 92••].

Disclosure No potential conflicts of interest relevant to this article were reported.

References

Papers of particular interest, published recently, have been highlighted as:
•• Of major importance

1. Society AC. Cancer Facts and Figures. 2012 [updated 2012; cited 2012]; Available from: http://www.cancer.org/Research/CancerFactsFigures/index.
2. WHOQOL. The World Health Organization Quality of Life assessment (WHOQOL): position paper from the World Health Organization. Soc Sci Med. 1995;41(10):1403–9.
3. Baird DT, Collins J, Egozcue J, Evers LH, Gianaroli L, Leridon H, et al. Fertility and ageing. Hum Reprod Update. 2005;11(3):261–76.
4. SART. IVF Success Rate National Data Summary2010: Available from: https://www.sartcorsonline.com/rptCSR_PublicMultYear.aspx?ClinicPKID=0.
5. National Cancer Institute. SEER Stat Fact Sheets: Breast 2011. Available from: http://seer.cancer.gov/statfacts/html/breast.html.
6. Anders CK, Hsu DS, Broadwater G, Acharya CR, Foekens JA, Zhang Y, et al. Young age at diagnosis correlates with worse prognosis and defines a subset of breast cancers with shared patterns of gene expression. J Clin Oncol. 2008;26(20):3324–30.
7. Knopman JM, Papadopoulos EB, Grifo JA, Fino ME, Noyes N. Surviving childhood and reproductive-age malignancy: effects on fertility and future parenthood. Lancet Oncol. 2010;11(5):490–8.
8. Meirow D. Ovarian injury and modern options to preserve fertility in female cancer patients treated with high dose radio-chemotherapy for hemato-oncological neoplasias and other cancers. Leuk Lymphoma. 1999;33(1–2):65–76.
9. National Cancer Institute. SEER Stat fact sheet: cervix uteri cancer. 2011. Available from: http://seer.cancer.gov/statfacts/html/cervix.html.
10. National Cancer Institute. SEER Stat fact sheet: cervix uteri: ovary. 2011. Available from: http://seer.cancer.gov/statfacts/html/ovary.html.
11. National Cancer Institute. SEER Stat fact sheet: corpus and uterine cancer. 2011. Available from: http://seer.cancer.gov/statfacts/html/corp.html.
12. Wallace WH, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol. 2005;6(4):209–18.
13. Wallace WH, Thomson AB, Kelsey TW. The radiosensitivity of the human oocyte. Hum Reprod. 2003;18(1):117–21.
14. Wallace WHB, Thomson AB, Saran F, Kelsey TW. Predicting age of ovarian failure after radiation to a field that includes the ovaries. Int J Radiat Oncol Biol Phys. 2005;62(3):738–44.
15. Critchley HO, Wallace WH, Shalet SM, Mamtora H, Higginson J, Anderson DC. Abdominal irradiation in childhood; the potential for pregnancy. Br J Obstet Gynaecol. 1992;99(5):392–4.
16. Hawkins MM, Smith RA. Pregnancy outcomes in childhood cancer survivors: probable effects of abdominal irradiation. Int J Cancer. 1989;43(3):399–402.
17. Baker TG. A quantitative and cytological study of germ cells in human ovaries. Proc R Soc Lond B Biol Sci. 1963;158:417–33.
18. Hansen KR, Knowlton NS, Thyer AC, Charleston JS, Soules MR, Klein NA. A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. Hum Reprod. 2008;23(3):699–708.
19. Menken J, Trussell J, Larsen U. Age and infertility. Science. 1986;233(4771):1389–94.
20. Tan SL, Royston P, Campbell S, Jacobs HS, Betts J, Mason B, et al. Cumulative conception and livebirth rates after in-vitro fertilisation. Lancet. 1992;339(8806):1390–4.
21. Oktay K, Briggs D, Gosden RG. Ontogeny of follicle-stimulating hormone receptor gene expression in isolated human ovarian follicles. J Clin Endocrinol Metab. 1997;82(11):3748–51.
22. Waxman JH, Ahmed R, Smith D, Wrigley PF, Gregory W, Shalet S, et al. Failure to preserve fertility in patients with Hodgkin's disease. Cancer Chemother Pharmacol. 1987;19(2):159–62.
23. Badawy A, Elnashar A, El-Ashry M, Shahat M. Gonadotropin-releasing hormone agonists for prevention of chemotherapy-induced ovarian damage: prospective randomized study. Fertil Steril. 2009;91(3):694–7.
24. Giuseppe L, Attilio G, Edoardo DN, Loredana G, Cristina L, Vincenzo L. Ovarian function after cancer treatment in young women affected by Hodgkin disease (HD). Hematology. 2007;12(2):141–7.
25. Gerber B, von Minckwitz G, Stehle H, Reimer T, Felberbaum R, Maass N, et al. Effect of luteinizing hormone-releasing hormone agonist on ovarian function after modern adjuvant breast cancer chemotherapy: the GBG 37 ZORO study. J Clin Oncol. 2011;29(17):2334–41.
26. Del Mastro L, Boni L, Michelotti A, Gamucci T, Olmeo N, Gori S, et al. Effect of the gonadotropin-releasing hormone analogue triptorelin on the occurrence of chemotherapy-induced early menopause in premenopausal women with breast cancer: a randomized trial. JAMA. 2011;306(3):269–76.
27. Munster PN, Moore AP, Ismail-Khan R, Cox CE, Lacevic M, Gross-King M, et al. Randomized trial using gonadotropin-releasing hormone agonist triptorelin for the preservation of ovarian function during (neo)adjuvant chemotherapy for breast cancer. J Clin Oncol. 2012;30(5):533–8.
28. Gilani MM, Hasanzadeh M, Ghaemmaghami F, Ramazanzadeh F. Ovarian preservation with gonadotropin–releasing hormone analog during chemotherapy. Asia Pac J Clin Oncol. 2007;3(2):79–83.
29. Petrek JA, Naughton MJ, Case LD, Paskett ED, Naftalis EZ, Singletary SE, et al. Incidence, time course, and determinants of menstrual bleeding after breast cancer treatment: a prospective study. J Clin Oncol. 2006;24(7):1045–51.
30. •• Behringer K, Wildt L, Mueller H, Mattle V, Ganitis P, van den Hoonaard B, et al. No protection of the ovarian follicle pool with

the use of GnRH-analogues or oral contraceptives in young women treated with escalated BEACOPP for advanced-stage Hodgkin lymphoma. Final results of a phase II trial from the German Hodgkin Study Group. Ann Oncol. 2010;21(10):2052–60. *This trial investigated the use of gonadotropin-releasing hormone-analogues (GnRH-a) and oral contraceptives (OC) in young women with advanced-stage Hodgkin lymphoma (HL) for the reduction of treatment-related toxic effects. The anti-Mullerian hormone level after at least 12 months was reduced in all patients with ovarian follicle preservation rate of 0 %. Trial was closed prematurely.*

31. Rasool N, Rose PG. Fertility-preserving surgical procedures for patients with gynecologic malignancies. Clin Obstet Gynecol. 2010;53(4):804–14.
32. Martinez A, Poilblanc M, Ferron G, De Cuypere M, Jouve E, Querleu D. Fertility-preserving surgical procedures, techniques. Best Pract Res Clin Obstet Gynaecol. 2012;26(3):407–24.
33. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006;24(18):2917–31.
34. Morice P, Juncker L, Rey A, El-Hassan J, Haie-Meder C, Castaigne D. Ovarian transposition for patients with cervical carcinoma treated by radiosurgical combination. Fertil Steril. 2000;74(4):743–8.
35. Sonoda Y, Chi DS, Carter J, Barakat RR, Abu-Rustum NR. Initial experience with Dargent's operation: the radical vaginal trachelectomy. Gynecol Oncol. 2008;108(1):214–9.
36. Mathevet P, Laszlo de Kaszon E, Dargent D. Fertility preservation in early cervical cancer. Gynecol Obstet Fertil. 2003;31(9):706–12.
37. Plante M, Renaud MC, Francois H, Roy M. Vaginal radical trachelectomy: an oncologically safe fertility-preserving surgery. An updated series of 72 cases and review of the literature. Gynecol Oncol. 2004;94(3):614–23.
38. Schlaerth JB, Spirtos NM, Schlaerth AC. Radical trachelectomy and pelvic lymphadenectomy with uterine preservation in the treatment of cervical cancer. Am J Obstet Gynecol. 2003;188 (1):29–34.
39. Burnett AF, Roman LD, O'Meara AT, Morrow CP. Radical vaginal trachelectomy and pelvic lymphadenectomy for preservation of fertility in early cervical carcinoma. Gynecol Oncol. 2003;88 (3):419–23.
40. Covens A, Shaw P, Murphy J, DePetrillo D, Lickrish G, Laframboise S, et al. Is radical trachelectomy a safe alternative to radical hysterectomy for patients with stage IA-B carcinoma of the cervix? Cancer. 1999;86(11):2273–9.
41. Gien LT, Covens A. Fertility-sparing options for early stage cervical cancer. Gynecol Oncol. 2010;117(2):350–7.
42. Lee NK, Cheung MK, Shin JY, Husain A, Teng NN, Berek JS, et al. Prognostic factors for uterine cancer in reproductive-aged women. Obstet Gynecol. 2007;109(3):655–62.
43. Gitsch G, Hanzal E, Jensen D, Hacker NF. Endometrial cancer in premenopausal women 45 years and younger. Obstet Gynecol. 1995;85(4):504–8.
44. Lu KH, Schorge JO, Rodabaugh KJ, Daniels MS, Sun CC, Soliman PT, et al. Prospective determination of prevalence of lynch syndrome in young women with endometrial cancer. J Clin Oncol. 2007;25(33):5158–64.
45. Walsh CS, Blum A, Walts A, Alsabeh R, Tran H, Koeffler HP, et al. Lynch syndrome among gynecologic oncology patients meeting Bethesda guidelines for screening. Gynecol Oncol. 2010;116 (3):516–21.
46. Kaku T, Yoshikawa H, Tsuda H, Sakamoto A, Fukunaga M, Kuwabara Y, et al. Conservative therapy for adenocarcinoma and atypical endometrial hyperplasia of the endometrium in young women: central pathologic review and treatment outcome. Cancer Lett. 2001;167(1):39–48.
47. Wang CB, Wang CJ, Huang HJ, Hsueh S, Chou HH, Soong YK, et al. Fertility-preserving treatment in young patients with endometrial adenocarcinoma. Cancer. 2002;94(8):2192–8.
48. Yamazawa K, Hirai M, Fujito A, Nishi H, Terauchi F, Ishikura H, et al. Fertility-preserving treatment with progestin, and pathological criteria to predict responses, in young women with endometrial cancer. Hum Reprod. 2007;22(7):1953–8.
49. Gotlieb WH, Beiner ME, Shalmon B, Korach Y, Segal Y, Zmira N, et al. Outcome of fertility-sparing treatment with progestins in young patients with endometrial cancer. Obstet Gynecol. 2003;102(4):718–25.
50. Signorelli M, Caspani G, Bonazzi C, Chiappa V, Perego P, Mangioni C. Fertility-sparing treatment in young women with endometrial cancer or atypical complex hyperplasia: a prospective single-institution experience of 21 cases. BJOG. 2009;116(1):114–8.
51. Hahn HS, Yoon SG, Hong JS, Hong SR, Park SJ, Lim JY, et al. Conservative treatment with progestin and pregnancy outcomes in endometrial cancer. Int J Gynecol Cancer. 2009;19(6):1068–73.
52. Minig L, Franchi D, Boveri S, Casadio C, Bocciolone L, Sideri M. Progestin intrauterine device and GnRH analogue for uterus-sparing treatment of endometrial precancers and well-differentiated early endometrial carcinoma in young women. Ann Oncol. 2011;22(3):643–9.
53. Mazzon I, Corrado G, Masciullo V, Morricone D, Ferrandina G, Scambia G. Conservative surgical management of stage IA endometrial carcinoma for fertility preservation. Fertil Steril. 2010;93(4):1286–9.
54. Laurelli G, Di Vagno G, Scaffa C, Losito S, Del Giudice M, Greggi S. Conservative treatment of early endometrial cancer: preliminary results of a pilot study. Gynecol Oncol. 2011;120(1):43–6.
55. ACOG. ACOG Practice Bulletin. Management of adnexal masses. Obstet Gynecol. 2007;110(1):201–14.
56. Aebi S, Castiglione M. Epithelial ovarian carcinoma: ESMO clinical recommendations for diagnosis, treatment and follow-up. Ann Oncol. 2008;19 Suppl 2:ii14–6.
57. Vitobello D, Siesto G, Bulletti C, Accardi A, Levi Setti PE. Gynecological fertility-sparing surgery. Placenta. 2011;32 Suppl 3:S224–31.
58. Huang JY, Tulandi T, Holzer H, Tan SL, Chian RC. Combining ovarian tissue cryobanking with retrieval of immature oocytes followed by in vitro maturation and vitrification: an additional strategy of fertility preservation. Fertil Steril. 2008;89(3):567–72.
59. Poirot C, Vacher-Lavenu MC, Helardot P, Guibert J, Brugieres L, Jouannet P. Human ovarian tissue cryopreservation: indications and feasibility. Hum Reprod. 2002;17(6):1447–52.
60. Poirot C, Abirached F, Prades M, Coussieu C, Bernaudin F, Piver P. Induction of puberty by autograft of cryopreserved ovarian tissue. Lancet. 2012;379(9815):588.
61. Donnez J, Silber S, Andersen CY, Demeestere I, Piver P, Meirow D, et al. Children born after autotransplantation of cryopreserved ovarian tissue. a review of 13 live births. Ann Med. 2011;43 (6):437–50.
62. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004;364(9443):1405–10.
63. Donnez J, Squifflet J, Jadoul P, Demylle D, Cheron AC, Van Langendonckt A, 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(5):1787e1–4.
64. Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, et al. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med. 2005;353(3):318–21.
65. Ernst E, Bergholdt S, Jorgensen JS, Andersen CY. The first woman to give birth to two children following transplantation of frozen/thawed ovarian tissue. Hum Reprod. 2010;25(5):1280–1.

66. Sanchez-Serrano M, Crespo J, Mirabet V, Cobo AC, Escriba MJ, Simon C, et al. Twins born after transplantation of ovarian cortical tissue and oocyte vitrification. Fertil Steril. 2010;93(1):268e11–3.
67. Andersen CY, Rosendahl M, Byskov AG, Loft A, Ottosen C, Dueholm M, et al. Two successful pregnancies following auto-transplantation of frozen/thawed ovarian tissue. Hum Reprod. 2008;23(10):2266–72.
68. Roux C, Amiot C, Agnani G, Aubard Y, Rohrlich PS, Piver P. Live birth after ovarian tissue autograft in a patient with sickle cell disease treated by allogeneic bone marrow transplantation. Fertil Steril. 2010;93(7):2413e15–9.
69. Dittrich R, Lotz L, Keck G, Hoffmann I, Mueller A, Beckmann MW, et al. Live birth after ovarian tissue autotransplantation following overnight transportation before cryopreservation. Fertil Steril. 2012;97(2):387–90.
70. Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, et al. Reproductive technologies and the risk of birth defects. N Engl J Med. 2012;366(19):1803–13.
71. Oktay K, Cil AP, Bang H. Efficiency of oocyte cryopreservation: a meta-analysis. Fertil Steril. 2006;86(1):70–80.
72. Oktay K, Buyuk E, Libertella N, Akar M, Rosenwaks Z. Fertility preservation in breast cancer patients: a prospective controlled comparison of ovarian stimulation with tamoxifen and letrozole for embryo cryopreservation. J Clin Oncol. 2005;23(19):4347–53.
73. Rodriguez-Wallberg KA, Oktay K. Fertility preservation in women with breast cancer. Clin Obstet Gynecol. 2010;53(4):753–62.
74. Oktay K, Hourvitz A, Sahin G, Oktem O, Safro B, Cil A, et al. Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. J Clin Endocrinol Metab. 2006;91(10):3885–90.
75. Oktay K, Turkcuoglu I, Rodriguez-Wallberg KA. GnRH agonist trigger for women with breast cancer undergoing fertility preservation by aromatase inhibitor/FSH stimulation. Reprod Biomed Online. 2010;20(6):783–8.
76. Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. J Clin Oncol. 2008;26(16):2630–5.
77. Demirtas E, Elizur SE, Holzer H, Gidoni Y, Son WY, Chian RC, et al. Immature oocyte retrieval in the luteal phase to preserve fertility in cancer patients. Reprod Biomed Online. 2008;17(4):520–3.
78. Rao GD, Chian RC, Son WS, Gilbert L, Tan SL. Fertility preservation in women undergoing cancer treatment. Lancet. 2004;363 (9423):1829–30.
79. Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle: a review. Hum Reprod Update. 2012;18(1):73–91.
80. Oktay K, Demirtas E, Son WY, Lostritto K, Chian RC, Tan SL. In vitro maturation of germinal vesicle oocytes recovered after premature luteinizing hormone surge: description of a novel approach to fertility preservation. Fertil Steril. 2008;89(1):228e19–22.
81. Sonmezer M, Turkcuoglu I, Coskun U, Oktay K. Random-start controlled ovarian hyperstimulation for emergency fertility preservation in letrozole cycles. Fertil Steril. 2011;95 (6):2125e9–2125e11.
82. Ozkaya E, San Roman G, Oktay K. Luteal phase GnRHa trigger in random start fertility preservation cycles. J Assist Reprod Genet. 2012;29(6):503–5.
83. UNECE. Many women in Europe tend to postpone child birth. United Nations Economic Commission for Europe 2007. Accessed 23 Aug 2011; http://w3.unece.org/pxweb/Dialog/Weekly/2007/08/Default.asp]. Available from: http://w3.unece.org/pxweb/Dialog/Weekly/2007/08/Default.asp.
84. Bavan B, Porzig E, Baker VL. An assessment of female university students' attitudes toward screening technologies for ovarian reserve. Fertil Steril. 2011;96(5):1195–9.
85. Antinori M, Licata E, Dani G, Cerusico F, Versaci C, Antinori S. Cryotop vitrification of human oocytes results in high survival rate and healthy deliveries. Reprod Biomed Online. 2007;14(1):72–9.
86. Cobo A, Kuwayama M, Perez S, Ruiz A, Pellicer A, Remohi J. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. Fertil Steril. 2008;89(6):1657–64.
87. Cobo A, Meseguer M, Remohi J, Pellicer A. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. Hum Reprod. 2010;25 (9):2239–46.
88. Nagy ZP, Chang CC, Shapiro DB, Bernal DP, Elsner CW, Mitchell-Leef D, et al. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. Fertil Steril. 2009;92(2):520–6.
89. Garcia JI, Noriega-Portella L, Noriega-Hoces L. Efficacy of oocyte vitrification combined with blastocyst stage transfer in an egg donation program. Hum Reprod. 2011;26(4):782–90.
90. van Loendersloot LL, Moolenaar LM, Mol BW, Repping S, van der Veen F, Goddijn M. Expanding reproductive lifespan: a cost-effectiveness study on oocyte freezing. Hum Reprod. 2011;26 (11):3054–60.
91. Jain JK, Paulson RJ. Oocyte cryopreservation. Fertil Steril. 2006;86(4 Suppl):1037–46.
92. •• Ata B, Kaplan B, Danzer H, Glassner M, Opsahl M, Tan SL, et al. Array CGH analysis shows that aneuploidy is not related to the number of embryos generated. Reprod Biomed Online. 2012;24 (6):614–20. *This study retrospectively analyzed the array of comparative genomic hybridization (CGH) results of 7,753 embryos from 990 patients to determine the frequency of embryonic euploidy. Increasing female age was associated with a significant decrease in euploidy rate of day 3 and day 5 embryos.*
93. Partridge AH, Gelber S, Peppercorn J, Sampson E, Knudsen K, Laufer M, et al. Web-based survey of fertility issues in young women with breast cancer. J Clin Oncol. 2004;22(20):4174–83.
94. Meneses K, McNees P, Azuero A, Jukkala A. Development of the Fertility and Cancer Project: an Internet approach to help young cancer survivors. Oncol Nurs Forum. 2010;37(2):191–7.
95. Lawrenz B, Jauckus J, Kupka MS, Strowitzki T, von Wolff M. Fertility preservation in >1,000 patients: patient's characteristics, spectrum, efficacy and risks of applied preservation techniques. Arch Gynecol Obstet. 2011;283(3):651–6.
96. •• Balthazar U, Deal AM, Fritz MA, Kondapalli LA, Kim JY, Mersereau JE. The current fertility preservation consultation model: are we adequately informing cancer patients of their options? Hum Reprod. 2012;27(8):2413–9. *This study's objective was to assesspatients' knowledge following fertility preservation consultation and to examine predictors of increased knowledge. Following a single FP consultation, only 50 % of the information was understood correctly. Higher knowledge scores were associated with an additional contact with the FP specialist following the initial FPC and use of specific reference websites.*
97. Dondorp W, de Wert G, Pennings G, Shenfield F, Devroey P, Tarlatzis B, et al. Oocyte cryopreservation for age-related fertility loss. Hum Reprod. 2012;27(5):1231–7.