Review Article

Preservation of fertility in reproductive-age women with the diagnosis of cancer

P. Kovács[1], *M.D.*; S. Mátyás[1], L. Ungár[2], *M.D.*

[1]Kaali Institute IVF Center, [2]Department of Gynecologic Oncology, Szt. István Hospital, Budapest (Hungary)

Summary

Over the past few decades the number of young, reproductive age cancer survivors has increased as a result of improved and less destructive cancer treatments. Certain types of cancers are predominantly diagnosed among reproductive age women and a small proportion of cancers originating in the reproductive tract are also detected in this age group. Treatment in the past used to be definitive and in most cases led to sterility. In recent years, improved medical treatments and more conservative surgical approaches have been introduced increasing the number of young survivors of cancer treatment. These less invasive treatments seem to be associated with similar survival rates and fertility can be preserved in most cases. This has led to studies evaluating the reproductive options of these women. Conservative surgical techniques, the use of chemotherapeutic agents with a reduced gonadotoxic side-effect profile, and the application of more focused radiation therapy are associated with maintenance of fertility. In addition, assisted reproductive technology (ART) has undergone tremendous improvements and now offers several alternatives to those who wish to maintain fertility before or even after cancer therapy. This review summarizes the fertility sparing medical and surgical as well as ART options that reproductive age women desiring to maintain fertility may utilize if they face cancer therapy.

Key words: Cancer; Fertility, surgery; Chemotherapy; Radiation; Assisted reproductive technology; Cryopreservation.

Introduction

Cancer is generally associated with older age. This diagnosis is usually not expected among reproductive age women. Certain cancer types however typically occur among children or young adults and a small proportion of cancers that are otherwise more prevalent among older women are diagnosed in the reproductive age group [1]. In the vast majority of cases, cancer treatment used to result in sterility [2]. Surgery was definitive and chemo- or radiation therapy usually destroyed the ovarian follicles [2].

In recent years, more conservative therapies have been introduced and with the development of new chemotherapeutic agents the salvage of ovarian function has become possible. This not only leads to improved survival rates, but to a need to address the fertility concerns of these patients as well. For example, almost two-thirds of Hodgkin's lymphomas and over 75% of acute lymphocytic leukemias are diagnosed in patients under the age of 44 [1]. Survival rates for Hodgkin's lymphoma improved from 75-88% over the past three decades [1]. Leukemia survival rates improved from 37% to 51% in the same period [1]. This means that there is a large group of reproductive age women whose reproductive options have to be discussed prior to or after chemo- or radiation therapy.

Cancers of the female genital tract may also affect young women. Forty-two percent of cervical cancers, 9% of uterine cancers and 13% of ovarian cancers are diagnosed among women under the age of 45 [1]. Survival rates are excellent for cervical cancer (83% when diagnosed under the age of 45) and endometrial cancer (91% if diagnosed under the age of 45). Overall survival rates have increased for ovarian cancer as well (from 36% to 45% between 1975-2003), and 5-year survival rates are even better (73%) when the diagnosis is made under the age of 45 [1]. A conservative surgical approach and careful selection of chemo- or radiation therapy therefore is important for these women if fertility needs to be maintained.

This review will discuss the surgical, medical and assisted reproductive technology (ART) options of those women who are diagnosed with cancer during the reproductive years. Some of our own cases will be used to illustrate the importance of a conservative approach when it does not compromise the long-term health benefits of the patient.

Surgical options

Cervical cancer

Case 1

NA, a 34-year-old nulliparous woman, was evaluated at our clinic for primary infertility of male origin. She had previously undergone two unsuccessful IVF cycles. Her cervical cytology in 1999 showed atypical cells and a cervical biopsy detected cervical cancer. Her full staging evaluation revealed Stage IB epithelial cervical cancer. Since she had no children at the time of the diagnosis she

Revised manuscript accepted for publication January 21, 2008

opted to undergo fertility sparing radical trachelectomy. The lymph nodes were negative for cancer and the margin of the surgical specimen was also free of disease therefore no adjuvant therapy was administered. Her postoperative follow-up showed no evidence of recurrence. She maintained regular cycles after the procedure and two years after the operation underwent two further IVF cycles. During the second attempt five oocytes were retrieved and three embryos were transferred. This treatment was successful and a singleton gestation was achieved. After an uneventful pregnancy she delivered a healthy girl at term.

Malignant transformation in the cervical epithelium is a slow process and it usually takes several years for invasive cancer to develop. Human papilloma virus infection plays an important role in this process. Well-described precancerous lesions (cervical intraepithelial neoplasia I-III) precede the development of cancer. Effective screening techniques are available that accurately identify precancerous lesions as well as cancer [3].

Cervical cancer is staged clinically. During the exam, the size of the primary lesion and its spread to adjacent organs (parametrium, uterus, bladder, rectum) are evaluated. Early stage cervical cancer (Stage I and II) has traditionally been managed surgically. Radical hysterectomy with sampling and if needed removal of the pelvic and paraaortic lymph nodes has been the treatment of choice. Pre- and postoperative radiation, and more recently chemotherapy further improve outcome [4]. While radical surgery is associated with good survival rates, these women are rendered infertile, as the uterus is removed.

In developed countries, the introduction of cervical cytology was associated with early detection of cancer and reduced morbidity and mortality due to treatment and the disease itself [3]. Detection of early-stage cancer, when the disease is localized to the cervix (Stage I-IIB), allows a more conservative surgical approach without compromising survival. In these cases the removal of the cervix, upper vagina and parametrium with adequate lymph node sampling has been associated with similar survival rates to radical hysterectomy [5]. Radical trachelectomy can be carried out through the vagina or by the abdominal route [6, 7].

Vaginal radical trachelectomy requires additional surgical training. It is associated with a higher rate of intraoperative complications (injury to the bladder, ureter, bowel), and less parametrial resection. Recurrence in the parametrium has been described following this procedure [8]. In order to improve outcome and reduce complications, radical trachelectomy via the abdominal route was introduced. The procedure is very similar to radical hysterectomy, but with this procedure the uterus is left in place. Its blood supply is maintained via the vessels that run in the infundibulopelvic ligament.

This conservative approach preserves fertility, as the uterus remains functional. Spontaneous and ART pregnancies have been reported following both the vaginal and abdominal procedure [9, 10].

Some of the patients undergoing radical trachelectomy will require in vitro fertilization (IVF). IVF following the removal of the parametrium and cervix can be particularly challenging. The ovaries lose their support and their position might change. This could interfere with the transvaginal oocyte collection. It is also questionable how well the ovarian vessels can perfuse the uterus, whether the endometrium is able to undergo the necessary changes to allow successful implantation. Earlier studies reported average birthweight among infants born following radical trachelectomy, suggesting that adequate blood supply is maintained [11]. In some cases stenosis or complete obstruction of the remaining "cervical" canal may develop. Such stenosis may cause scarring which might interfere with the embryo transfer. The passage through the canal needs to be evaluated prior to initiating stimulation.

The shortening of the cervix may put the ongoing pregnancy at risk. Preterm delivery and preterm premature rupture of membranes have both been reported to occur with higher frequency. This is most likely due to the lack of protection offered by the cervix and mucous plug [11]. Several groups routinely place a cerclage to add support to the remnants of the cervix [11]. The use of cerclage is controversial even in cases where it is placed to prevent preterm delivery [12]. The potential benefits of cerclage placement under these circumstances require further evaluation.

In order to reduce the load on the lower uterine segment and cervix following radical trachelectomy, a singleton pregnancy is the desired outcome and therefore the number of embryos transferred needs to be limited, preferably to one.

These pregnancies should be followed by a high-risk obstetrician, and delivery by cesarean section should especially be considered in those cases where a cerclage has been placed.

Ovarian tumors

Case 2

AK, a 32-year-old nulliparous woman, at the age of 22 underwent a left salpingo-oophorectomy for an ovarian cyst that was thought to be a mature teratoma prior to the surgery. The final pathologic exam showed papillary cystocarcinoma of the ovary. Following this report she underwent a formal staging procedure with lymph node biopsies, peritoneal washings and omentectomy, and also received six cycles of chemotherapy (cyclophsphamide, cisplatinum, epirubicin). Tumor markers were followed and pelvic ultrasounds (US) were performed at regular intervals. Six years after the initial diagnosis she underwent a right cystectomy for an endometrioma. Eight years after the diagnosis and treatment of ovarian cancer she was having regular cycles, and since her right tube was blocked she underwent IVF treatment. The treatment was successful but she miscarried at eight weeks. Subsequently she underwent two further IVF attempts that were unsuccessful. She always responded well to stimu-

lation and produced 8-11 eggs. During the treatments, follow-up of the tumor markers was continued. She is now scheduled to undergo treatment for the fourth time.

There are various types of ovarian lesions. The appropriate treatment primarily depends on the histology. Benign lesions are generally treated by cystectomy or if it is not possible by oophorectomy. Malignant ovarian tumors have generally been treated by bilateral salpingo-oophorectomy and hysterectomy, and a complete staging procedure with pelvic washings, lymph node biopsies and omentectomy.

In well-selected cases, ovarian cancers however could be managed more conservatively. Ovarian germ cell tumors (2% of ovarian cancers) typically affect young women. For a long time surgery followed by radiation therapy was the standard care. Radiation however destroys ovarian follicles and induces premature ovarian failure. Since most of these patients have not had a chance to start a family, therapy that preserves ovarian activity would be preferable. Therefore chemotherapy has been evaluated instead of radiation for the treatment of germ cell tumors.

Several large studies have reported similarly excellent survival rates with chemo- and radiation therapy. Brewer *et al.*, followed 26 women who were treated by surgery and bleomycin, cisplatin, and etoposide chemotherapy for ovarian dysgerminoma (16 patients underwent conservative surgery [unilateral salpingo-oophorectomy] and ten had hysterectomy and bilateral salpingo-oophorectomy). After a median follow-up of 89 months 96% of these women remained disease free. Among those who had conservative surgery, 14/16 maintained regular menstrual cycles post chemotherapy. Five pregnancies were reported in this group without any birth defects [13]. Gershenson *et al.* followed 132 survivors of ovarian germ cell cancer; 71 of these women had fertility sparing surgery, 87.3% of them reported regular menstrual cycles post treatment (surgery plus chemotherapy), and 24 of the survivors had 37 children during the follow-up period [14]. Gershenson in an earlier report evaluated reproductive outcome among 40 germ cell cancer survivors following fertility sparing surgery and chemotherapy (most commonly vincristine, dactinomycin, cyclophosphamide). Twenty-seven of 40 women maintained regular menstrual cycles, 13 had menstrual difficulties of which three were considered severe; 11/16 patients who wished to have children were successful and delivered 22 children altogether [15]. These reports provide good evidence for the use of conservative surgery in combination with chemotherapy for those young women who are diagnosed with early or even advanced stage ovarian germ cell tumor and desire to maintain fertility.

The answer is not this straight forward when epithelial ovarian cancer is diagnosed in reproductive age women. This type of cancer has traditionally been treated by hysterectomy, bilateral salpingo-oophorectomy and formal staging with the aim of maximal tumor debulking as most of them are diagnosed in an advanced stage. Some of these cancers are however diagnosed when they are still localized to the ovary. Unilateral salpingo-oophorectomy with staging followed by chemotherapy could be a conservative and fertility sparing option if it did not affect survival.

Schilder *et al.*, studied 52 reproductive-age women (mean age 26 years) who were diagnosed with Stage IA-IC epithelial ovarian cancer and were managed by unilateral salpingo-oophorectomy with or without adjuvant chemotherapy. Cisplatin/taxol or carboplatin/taxol were given to 19/52 of these women. Five patients were diagnosed with recurrence and had to be treated again. Fifty of 52 women were alive and well after a median of 68 months follow-up. 5-year survival was estimated to be 98%. Seventeen of 24 women attempting pregnancy conceived and have delivered 26 children [16]. It appears that in well-selected cases unilateral salpingo-oophorectomy with appropriate staging could be offered to those young women who desire future fertility. It is important that these women be followed by tumor markers and regular pelvic US for evidence of recurrence. They also need to be made aware of the excess risk that they take even if it appears to be small.

Borderline ovarian tumors (15% of ovarian tumors) are histologically a special group of ovarian tumors. In these cases the epithelium undergoes atypical proliferation but the stroma is not infiltrated. If fertility is desired, conservative surgery with or without adjuvant therapy can be offered. Wong *et al.*, reviewed the outcome of 247 cases of ovarian borderline tumors; 33% of the women were managed by unilateral salpingo-oophorectomy, 15% by simple cystectomy, and the rest by bilateral salpingo-oophorectomy and hysterectomy. After a mean follow-up of 59 months six recurrences were found and the overall survival was 98% [17]. In another large series of borderline ovarian tumors, the potential benefit of adjuvant therapy was evaluated. Of the 370 cases, 77 women were treated with fertility sparing surgery. This study found no evidence for adjuvant chemotherapy to improve long-term survival [18]. Crispens *et al.* in their series of low malignant potential ovarian tumors showed that survival depended on optimal cytoreduction and also reported no benefit of non-surgical treatment alternatives [19]. These reports suggest that borderline ovarian tumors can be successfully managed by surgery alone. Conservative surgery is an alternative in those cases where fertility maintenance is desired. The ideal surgical approach has not been determined yet. Tinelli *et al.* evaluated recurrence and pregnancy rates in 43 women treated by conservative laparoscopic surgery for borderline ovarian tumors. The recurrence rate was 7%. Half of the patients successfully achieved pregnancy and over half of these pregnancies were spontaneous conceptions [20]. Maneo *et al.* compared long-term outcome in 62 women with borderline ovarian tumors following conservative surgical treatment either by laparoscopy (N = 30) or laparotomy (N = 32). Recurrences were detected in 11/30 and 7/32 cases. In the laparoscopic surgery group relapses were found more often when the initial cyst was greater than 5 cm [21]. Conservative laparoscopic management

of borderline ovarian tumors should be reserved for experienced surgeons who are able to perform appropriate staging during laparoscopic exploration. In addition, cases where the cyst is large (> 5 cm) seem to have a better prognosis when managed by laparotomy.

These reports suggest that in well-selected cases when future fertility is desired ovarian tumors (even epithelial cancer) can be managed by conservative, fertility-sparing surgery in combination with chemotherapy (when needed). In most cases spontaneous pregnancy may follow. Ovulation induction has not been shown to increase recurrence rates in these selected cases [22]. Appropriate patient counseling is very important and close follow-up with tumor markers and US is also a crucial part of the care of these women.

Ovarian lateral position prior to radiation

Radiation therapy alone or in combination with surgery might be required to treat certain cancers (e.g., cervical cancer, Hodgkin's lymphoma). Radiation is known to have a dose-dependent toxic effect on ovarian follicles. Radiation in doses over 800 cGy will always result in ovarian failure, but lower doses could also be associated with sterilization [23]. The full effect of lower doses depends on the patient's age and the cumulative dose delivered directly to the ovaries. When pelvic or lower abdominal radiation is planned the position of the ovaries out of the radiation field may preserve ovarian function. By displacing the ovaries out of the field of radiation the dose delivered to them can be significantly reduced. Transposition can be performed at the time of laparotomy or laparoscopy and typically the ovaries are removed laterally out of the radiation field. For best results the ovaries should be placed at least 3 cm out of the field [24]. If the transposition is closer to the field or if the ovaries migrate back to their original position ovarian failure may develop. Ovarian function could also be affected by vascular compromise as a result of the procedure [25]. Those ovaries that maintain their cyclic function at the completion of therapy will produce a sufficient amount of estradiol and progesterone needed for an endometrial cycle. The unusual position of the ovaries however may lead to infertility. Further surgeries might be needed if ART is required to achieve a pregnancy.

Medical options

Progestin therapy of endometrial cancer

Case 3

HMB, a nulliparous woman, was seen in our clinic following years of infertility evaluation and treatment. She had been diagnosed with polycystic ovary syndrome (PCOS) based on irregular cycles, hirsutism and elevated testosterone levels. Her body mass index (BMI) was 33.8 kg/m^2. Initially, she had been treated with clomiphene citrate and intrauterine inseminations. She had six treatment cycles that were unsuccessful. Following the inseminations she was scheduled to undergo IVF when an endometrial polyp was detected on US. Hysteroscopic polypectomy was performed. Histologic evaluation showed a well-differentiated carcinoma and areas of atypical hyperplasia. At this point she was counseled to have definitive therapy by removal of the uterus. Since she wished to have children she chose conservative management with high-dose gestagen (medroxyprogesterone acetate (MPA), 100 mg daily). Over the six-month gestagen therapy she underwent repeat biopsies, which showed that the disease had regressed. On completion of her treatment she underwent IVF in our center. Clomiphene citrate in combination with gonadotropins was used. Four eggs were retrieved and two embryos were transferred. Her treatment was successful and after an uneventful pregnancy she gave birth to a healthy baby boy. A year after delivery she returned to discuss a possible second treatment. She was advised again about the potential risks that she would take by delaying definitive therapy but she wanted to undergo a second IVF treatment. Her endometrial biopsy at this point showed complex hyperplasia without atypia. She was given 100 mg of MPA for three months and the histology regressed to simple hyperplasia. She underwent IVF using the same stimulation protocol. This time two eggs were retrieved and one embryo was transferred. Her treatment was successful again and she gave birth to another healthy male at term. She did not desire any more children at this point and is discussing definitive treatment options with her oncologist.

About 10% of endometrial cancers are diagnosed in premenopausal women [1]. Most of these cases are diagnosed among women with PCOS. Anovulation and extended periods of unopposed estrogen exposure are common with this syndrome and are linked to endometrial cancer. Most of these cancers are endometrioid type and are well differentiated. Endometrial cancer is generally treated surgically with the removal of the uterus which would render women desiring to maintain fertility infertile. As these tumors are mostly hormone-dependent and express receptors for both estrogen and progesterone, medical therapy using high-dose progestins could be offered if fertility maintenance is desired. Randall *et al.* managed 29 women with well-differentiated endometrial carcinoma or atypical endometrial hyperplasia using high-dose progestins (megestrol acetate or medroxyprogesterone acetate [26]). The disease has not progressed in any of the cases. Regression was seen in all cases except for four where the disease persisted. Twenty-five of these women were managed for infertility later on and five of them conceived successfully [26]. Gotlieb *et al.* followed 13 women with a diagnosis of endometrial cancer who chose medical therapy. Treatment with high-dose megestrol acetate or MPA was administered for a minimum of three months. Five women developed local recurrence over a mean follow-up of 82 months. Six out of 13 women successfully conceived either on their own or using ART [27]. Further case reports/case series lend support for the use of high-dose progestin for the conservative treatment of well-differentiated endometrial cancer

when fertility is desired [28, 29]. Fukuda *et al.* have shown that endometrial cancers that are well differentiated are more likely to express steroid receptors and positive immunohistochemistry for the progesterone receptor was associated with less invasion, longer disease-free and overall survival [30]. It is also important to mention that not all cases end positively. Vinker *et al.* reported a case where a well-differentiated endometrial carcinoma was initially managed medically. Due to the lack of response definitive surgical treatment was performed and even adjuvant radiation had to be used for advanced stage disease [31]. At this point several issues need to be answered. Are all patients with localized well-differentiated endometrial cancer candidates for medical therapy? Which preparation should be used, at what dose, and for how long? Based on published reports it appears that a minimum of three months is required to have a positive effect. If the disease persists continuation with a higher dose could be attempted but definitive therapy should seriously be considered. It is also not clear how these patients should be evaluated ("staged") pretherapy. Should US or MRI findings influence the decision? On one issue most experts seem to agree. Once childbearing has been completed definitive therapy by removal of the uterus should follow.

Prevention of chemotherapy related toxic effects

Chemotherapy works by destroying rapidly dividing cancer cells, but it also destroys other sensitive cells as well. Chemotherapy often negatively influences gonadal function. Chemotherapy induces loss of follicles and leads to ovarian fibrosis. The full effect depends on the patient's age, gonadal function prior to such therapy and most importantly on the agent itself and duration of treatment. Certain agents are well known for toxic ovarian effects (e.g., cyclophosphamide, busulfan, phenylalanine mustard) while others have no significant ovarian impact (e.g., methotrexate, 5-fluorouracil) [32]. Depending on the exact drug regimen fertility may be compromised to different degrees. Byrne *et al.* assessed the effect of childhood chemotherapy on fertility among survivors and compared it to controls. Cancer survivors were less likely to achieve pregnancy (RR: 0.85 [95% CI: 0.78-0.92]). In this group of patients alkylating agent use alone had no significant effect but when combined with radiation reduced fertility was observed [33]. Byrne *et al.* in a different paper assessed the risk of premature ovarian failure among adolescent cancer treatment survivors. When treatment was administered between ages 13-19 the risk of premature menopause was increased fourfold when compared to age-matched controls. In this group of patients the risk of ovarian failure was increased 9-fold with the use of alkylating agents and 27-fold if alkylating agents were combined with radiation therapy [34].

Since the use of chemotherapy cannot be avoided in all cases efforts have been made to reduce the toxic reproductive effects. It is presumed that dividing cells are more sensitive to chemotherapy and therefore several drugs that "suspend" ovarian activity have been evaluated in combination with chemotherapy.

Whitehead *et al.*, followed nine women on oral contraceptives (OC) undergoing chemotherapy. Seven of the nine women reported some degree of menstrual irregularity following chemotherapy, therefore OCs alone did not seem to provide the needed protection [35].

Blumenfeld *et al.* evaluated the effect of depot gonadotropin releasing hormone agonist (GnRHa) in combination with chemotherapy for Hodgkin's lymphoma (n = 65). Post therapy ovarian function was compared with a control group of women undergoing the same chemotherapy for the same disease. GnRHa was not administered in the control group. Ninety-seven percent of women in the GnRHa group and 63% of the control women resumed regular menses upon discontinuation of chemotherapy [36]. Potolog-Nahari *et al.* evaluated the combination of GnRHa and GnRH antagonist in nine women undergoing chemotherapy. In 89% of them gonadotropin levels returned to baseline and they reported regular menstruation [37]. Cyclophosphamide is often used in women with lupus. Somers *et al.* found that in women receiving monthly cyclophosphamide for severe lupus GnRHa administration was associated with reduced risk of ovarian failure when compared to age-matched controls undergoing the same therapy without GnRHa (5% vs 30%) [38]. Castelo-Branco *et al.* reported significantly fewer cases of premature ovarian failure in patients undergoing chemotherapy for Hodgkin's disease when they also received GnRHa in combination with tibolone (10% vs 23%) [39]. These reports are very encouraging and suggest that by rendering the ovary inactive follicles may be saved and ovarian function could be maintained when chemotherapy with known ovarian toxicity has to be used.

To be complete we also mention that animal experiments show promising results with apoptosis inhibitors (e.g., shingosine-1 phosphate) but it is beyond the scope of this paper to enter the details of these experiments [40].

Assisted reproductive technology (ART): options prior to chemotherapy

Embryo cryopreservation

Case 4

JJ, a 30-year old nulliparous woman, was referred to us by her oncologist. Prior to seeing us she had had a tumor removed from her right inguinal region. Histopathologic exam showed a synovial sarcoma. She was scheduled to undergo chemo- and radiation therapy. She came to us for a consultation to review her options to maintain fertility in light of the therapy she was scheduled to undergo. After discussing her options (egg, ovarian tissue or embryo freezing), we decided to proceed with IVF and elective embryo cryopreservation. The oncologist was not aware of any adverse effect of stimulation on the course of her disease. Ten eggs were retrieved after stimulation

using an antagonist protocol. Seven eggs were successfully fertilized using ICSI, and all seven pronuclear stage embryos were frozen. She has completed her treatment and is currently well two years after the initial diagnosis. If she remains disease free she plans to undergo frozen embryo transfer in the future.

Embryo cryopreservation is a well established technique in IVF centers. In humans the first pregnancy was reported in 1983 and the first delivery in 1985 [41, 42]. Embryo cryopreservation is primarily used to store the surplus embryos created during the fresh cycle but in some cases all embryos are frozen electively. Freezing can be performed at different developmental stages (two pronucleus, 2-8 cells, blastocyst). Freezing techniques have undergone a lot of changes and today most IVF centers use slow freezing protocols. Permeable cryoprotectants like 1,2-propanediol (PROH), dimethyl-sulfoxide (DMSO) or glycerin are combined with non-permeable agents such as sucrose in most protocols. First, the cells are dehydrated and after equilibration, the embryos are loaded into plastic straws or cryovials. Computer-assisted freezers are used to achieve adequate cooling. Vitrification (ultra-rapid freezing) is an alternative to slow cooling. Cooling rates and the cryoprotectants used differ between vitrification and slow freezing. During vitrification, a glass-like solidification of the freezing solution is achieved by using a high concentration of the cryoprotectants. Vitrification was first used to cryopreserve murine embryos by Rall and colleagues [43]. Today it is used for both embryo cryopreservation and oocyte freezing [44, 45].

Beyond the different technical approaches, the developmental stage of frozen embryos also has great impact on survival [46]. The highest survival rate was observed for zygotes (86.5%), followed by day 2 (61.7%) and day 3 (43.1%) embryos. In women under the age of 36, cryopreservation at the 4-cell stage was associated with the best outcome (implantation rate of 30.9%) following the transfer of a single frozen-thawed embryo [47].

The main benefit of embryo cryopreservation is its well established nature. Thousands of children born following the transfer of frozen-thawed embryos prove its efficacy and safety. High survival rates of the cryopreserved embryos usually allow several transfer attempts and therefore offer the possibility of having more children as a result of a single stimulation. In those cases where stimulation is not allowed embryo cryopreservation cannot be offered unless immature oocytes are retrieved and matured in the laboratory for IVF. Embryo freezing is also not the ideal option for those who are not in a stable relationship at the time of treatment unless they agree to fertilization with donor sperm.

Oocyte freezing

Oocyte freezing would be a good option for those who are single at the time of cancer therapy and do not want to use donor sperm to fertilize their eggs. Stimulation is not always important prior to obtaining eggs for freezing, therefore patients with hormone-sensitive tumors could utilize this method as well. Oocyte cryopreservation is a relatively new technique, and its results have not been as consistent as results with embryo freezing [48, 49]. It however has additional benefits when compared to embryo cryopreservation [50]. First, women who lose ovarian function due to surgery, chemotherapy, or radiotherapy could maintain their fertility via oocyte cryopreservation. Second, ethical, religious and legal issues surrounding embryo cryopreservation can be avoided if oocytes are frozen.

Unfortunately, mature human oocytes have lower survival rates than do embryos when similar freezing protocols are used [51]. The cytoplasmic membrane of the oocytes has fewer submembranous actin microfilaments and therefore is more fragile during cryopreservation [52]. Furthermore, the volume to surface ratio in the oocytes is greater, making the dehydration process more difficult. Freezing and thawing may result in the disturbance of the meiotic spindle, which could lead to chromosomal dispersion, failure of normal fertilization, and failure to develop [53]. The meiotic spindle is crucial for the events following fertilization, including completion of meiosis, second polar body formation, migration of the pronuclei, and formation of the first mitotic spindle [54]. Another problem with frozen-thawed oocytes is the hardening of the zona pellucida, caused by premature release of cortical granules. It can prohibit the entry of the spermatozoon [55]. To prevent this, intracytoplasmic sperm injection (ICSI) has been used to assist fertilization two to three hours after thawing [56].

Oocyte freezing is routinely performed two to three hours postretrieval [57]. For slow freezing, Porcu *et al.* used 1.5 mol/l 1,2-propanediol (PROH) with a sucrose concentration of 0.2 mol/l and obtained a 59% post-thaw survival rate [56]. Fabbri *et al.* showed that increasing the sucrose concentration to 0.3 mol/l and exposing oocytes for 15 min to cryoprotectants yielded higher oocyte survival rates (82%) [58]. Borini and colleagues achieved a 17.2% pregnancy rate per embryo transfer (ET) with embryos obtained from frozen oocytes [59]. If vitrification is used, ethylene glycol is the primary choice of cryoprotectant [60].

Oocyte survival rates depend on the type and concentration of cryoprotectant used, on the freezing protocol (slow vs vitrification) and on the stage of development. Cryopreservation of immature (GV-stage) oocytes is also on the horizon [61]. Freezing of immature oocytes (primordial follicle level) could lead to even better results since the metabolic rate in these eggs is lower, there is no zona pellucida, and the meiotic spindle has not formed yet [62].

Ovarian tissue freezing

Cryopreservation of ovarian tissue has several advantages over cryopreservation of oocytes or embryos. The premenopausal human ovarian cortex contains large numbers of primordial follicles and by cryopreserving

ovarian tissue its endocrine function can also be preserved. Furthermore, ovarian tissue can be collected relatively easily by laparoscopy or laparotomy at any time during the menstrual cycle. Since stimulation is not required prior to surgery it can also be performed in those cancer cases where stimulation would otherwise not be allowed. And finally, primordial follicles appear to be less sensitive to cryopreservation when compared to mature oocytes. This is most likely associated with the low metabolic rate of tissues. It also has to be pointed out that in the case of cancers that may also involve the ovary reintroduction of cancer is a potential risk after transplantation.

Ovarian tissue needs to be prepared for the freezing process. First the ovarian medullary tissue is removed, and then the remaining tissue is cut into strips (1-5 mm×1 mm×1 mm) using optical tweezers. Usually, ovarian tissue is cryopreserved by slow freezing, which leads to excellent results [63-65]. During the slow protocol DMSO or DMSO/PROH is used as cryoprotectant with or without adding sucrose as a non-permeable cryoprotectant. Similarly to embryo or oocyte cryopreservation, vitrification with a higher concentration of cryoprotectants has been evaluated with ovarian tissue as well. It is however still difficult to adopt a vitrification protocol to ovarian tissue because of the different physical structures and cell types in human ovarian tissue [66]. Ovarian fragments contain various types of cells, have high cell density, and also have an intact vascular system. Improvements in the freezing technology have led to improved survival rates of follicles and recent studies report morphologically intact follicles in excess of 80% [62, 66-68]. Success rates depend on the cryoprotectant, its concentration, the freezing protocol (slow vs vitrification) and the transplantation or in vitro maturation technique. Smaller or larger pieces can be frozen. Cryodamage is less likely with small cortical fragments but since they lack adequate blood supply ischemia is a major problem following transplantation. Blood supply can be maintained when larger pieces are frozen, but follicle loss due to insufficient freezing becomes an issue in this case [69].

After thawing the tissue can either be transferred to its original site (orthotopic transplantation) or to a different anatomic location (heterotopic transplantation). Successful ortho- and heterotopic transplantations have both been reported. Oktay *et al.* described two cases where ovarian tissue was transplanted to the forearm following pelvic radiation or chemotherapy. Follicular activity with cyclic changes in estradiol and progesterone hormones was documented in these cases [70]. In 2004 the same group published results with ovarian tissue transplantation into the subcutaneous layer of the lower abdomen. Three months after the surgery, follicular and hormonal activity could be demonstrated. In repeat cycles of stimulations altogether 20 eggs were retrieved. Immature eggs had to be matured in vitro, but fertilization and embryo development were achieved [65]. Donnez and colleagues reported a successful spontaneous pregnancy following the transplantation of ovarian tissue to its original anatomic location [63].

Work is still in progress to refine techniques of in vitro maturation of frozen-thawed immature oocytes, and the frozen-thawed ovarian cortical tissue slices. Mikkelsen and colleagues achieved a 24% clinical pregnancy rate per oocyte collection with in vitro matured fresh GV oocytes [71]. Children born after IVM appear to be healthy. These data, taken together, suggest that in the future, immature oocyte retrieval combined with freezing and IVM could replace conventional IVF in selected patients and could play a particularly important role among cancer patients.

Prechemotherapy stimulation

Steroid hormones negatively affect the course of certain types of cancer, while such an association is not evident with others. Estradiol levels significantly increase during stimulation, therefore in the case of hormone sensitive cancers (e.g., breast cancer) ovarian stimulation prior to cancer treatment is not recommended. If a cancer with steroid hormone sensitivity is detected the patient may still undergo natural cycle IVF when stimulation is not used, but even if everything goes well a single embryo can be stored frozen. Without stimulation immature eggs can be retrieved and cryopreserved for later use. The freezing-thawing and the need for in vitro maturation limits its clinical use.

In cases of estrogen-sensitive tumors ovarian stimulation with the combination of an aromatase inhibitor was evaluated in a prospective cohort study by Azim *et al.* Patients with invasive breast cancer desiring to preserve fertility by embryo cryopreservation prior to initiating chemotherapy were enrolled. They were assigned to either letrozole plus follicle stimulating hormone (FSH) or anastrazole plus FSH. Peak estradiol level was significantly lower in the letrozole (5 mg) group (427.78 ± 278.24 pg/ml vs 1325.89 ± 833.17 pg/ml) [72]. Oktay *et al.* prospectively evaluated stimulation outcome with tamoxifen, tamoxifen plus FSH or letrozole plus FSH among women with invasive breast cancer prior to starting chemotherapy. Significantly more oocytes were retrieved and more embryos were frozen in the groups where FSH was administered as well. Peak estradiol levels were lower in the tamoxifen only and letrozole plus FSH groups when compared to the tamoxifen plus FSH group. Recurrence rates were similar in the three stimulation groups when compared to a control group of women not undergoing stimulation prior to chemotherapy [73]. In a different study, Oktay *et al.* compared stimulation outcome with letrozole plus FSH in women with breast cancer to an age-matched control group of women free of breast disease undergoing IVF using standard stimulation protocols. Despite a significantly lower peak, estradiol level stimulation outcome and the number of available embryos were comparable [74].

Based on still small numbers of cases it appears that aromatase inhibitors in combination with FSH can be used in women desiring fertility preservation who are scheduled to start chemotherapy of a hormone sensitive tumor.

Ovarian stimulation has no known negative effect on the course of other types of cancers. If a short delay with chemo- or radiation therapy does not compromise cancer treatment ovarian stimulation, retrieval of mature eggs and the cryopreservation of oocytes, fertilized oocytes or embryos can be offered. Currently, embryo cryopreservation is considered to be the most effective among these options [69].

Options after chemotherapy: Post chemotherapy stimulation

Case 5

DBM, a 32-year old nulliparous woman, was diagnosed with gastric cancer at the age of 24. She had had part of her stomach resected and received chemotherapy for six months. Post-therapy she had transient amenorrhea, but has reported regular 28-day cycles over the past three years. She came to us for infertility evaluation. During the evaluation normal semen parameters were found and an HSG revealed blocked tubes. Her early cycle FSH was 12.6 IU/l. She underwent ovarian stimulation with daily 300 IU gonadotropins for IVF. This cycle was cancelled due to the presence of a dominant follicle. In a repeat cycle, stimulation with higher dose gonadotropins was initiated. After 18 days of stimulation (total of 5,275 IU gonadotropins) two eggs were retrieved and fertilized successfully. She had two embryos transferred but the treatment was not successful. The use of donor oocytes as an alternative to further stimulations was discussed with her due to the poor response during stimulation. The patient agreed to the use of donor eggs. She had two blastocyst transferred in an artificially supported cycle. The treatment resulted in a singleton intrauterine pregnancy that was unfortunately miscarried at seven weeks. She is now scheduled to undergo a frozen embryo transfer cycle.

Regular ovarian activity may be preserved after chemo- or radiation therapy. Prior to any ART treatment ovarian reserve is assessed by various studies to help in choosing the optimal stimulation. Various hormone measurements (early cycle FSH, inhibin B, anti-Mullerian hormone), dynamic tests, and ultrasound findings (ovarian volume and antral follicle count) can be used as ovarian reserve tests. Cutoff levels were established in the general infertile population but we might not be able to apply these cutoff values in cancer survivals. Ovarian reserve tests do need to be evaluated among cancer survivors too [75]. Similarly to stimulation options prior to cancer treatment, a decision about stimulation needs to be made based on the hormone sensitivity of the primary tumor.

Donor oocyte use/adoption

When cancer treatment results in ovarian failure and none of the pretreatment options to maintain fertility could be or were utilized the patient is left with two options. If the uterus is preserved she can undergo IVF treatment with donor oocytes. In most countries donor cycles are regulated. The donor has to be a healthy, fertile young woman (< 35 years) who is willing to undergo treatment and offers her oocytes for use by someone else. Simultaneously with the donor's cycle the recipient's endometrium also has to be prepared for implantation. Oral, transdermal or injectable estradiol and vaginal or muscular progesterone preparations are used for this purpose. If the uterus is intact excellent pregnancy rates can be achieved. Radiation therapy however may compromise uterine function. Radiation may damage the uterine vessels and therefore could compromise endometrial development. Pelvic radiation has been associated with increased miscarriage, preterm delivery rates, and low birthweight. This effect is also dose dependent [76].

As a last resort adoption is also an option for cancer survivors if they have no other solutions to start a family.

Summary

While cancer is rare among young women, when the diagnosis is made it can be devastating for several reasons. Even with improved treatment options the outcome is not always positive. Some of these young reproductive-age women have not had a chance to start a family prior to the diagnosis and treatment in a lot of the cases will induce sterility. Over the past decades emphasis was placed on developing surgical and medical methods that could maintain fertility in these women. Rapidly improving ART also provides hope for them. Therefore, it is very important for those involved in taking care of young cancer patients to be familiar with the available options. This often requires a multi-disciplinary approach by internists, surgeons, oncologists and infertility experts. Future medical and ART improvements could provide even safer and more effective fertility solutions for the survivors of cancer therapy during the reproductive years.

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Address reprint requests to:
P. KOVÁCS, M.D.
Kaali Institute IVF Center
Istenhegyi u 54/a
1125 Budapest (Hungary)
e-mail: peterkovacs1970@hotmail.com