J. Mamm. Ova Res. Vol. 30 (3), 101–108, 2013

—Review—

# Fertility Preservation

Koichi Kyono*

*Kyono ART Clinic, 1-1-1, Miyagi, 980-0014, Japan*

***Abstract:*** Oocyte, embryo, ovarian tissue, and sperm cryopreservation before cancer treatments (aggressive chemotherapy/radiotherapy and bone marrow transplantation) are useful for the fertility preservation of cancer patients.This paper discusses the importance of the available treatment options including the risks, advantages and disadvantages of fertility preservation options. The reimplantation of contaminated ovarian tissue could be a life-threatening event. Therefore, the detection of minimal residual disease (MRD) is also discussed.

***Key words:*** Fertility preservation, Cryopreservation, Cancer, Pregnancy, Minimal residual disease (MRD)

## Introduction

While it is important for us to promote positive lifestyle habits in order to reduce the risk of cancer, at the moment, two percent of people in the developed world are diagnosed with cancer before the age of 40. Detecting cancer before the cancer metastasizes greatly increases the possibility of controlling and eradicating the disease.

If a cancer is detected, health-care professionals must ensure that while saving the patient, they are careful to maintain the patient's quality of life. Cancer survivors can be physically and mentally scarred by various forms of cancer treatment. The negative impact of the treatment must be minimized. To this end, when a cancer is detected in a woman under 40 years of age, that is, in her child-bearing years, she must be offered the opportunity to preserve her fertility. Unfortunately, cancer and cancer treatments such as chemotherapy can destroy a woman's fertility. However, cryopreservation of ovaries, oocytes, and embryos can help to preserve a patient's fertility [1–5]. After treatment and recovery, the autotransplantation of thawed ovaries or the transfer of the embryos following ICSI with warmed oocytes and the partner's sperm or the transfer of warmed embryos can lead to pregnancy and ultimately birth, thus contributing greatly to the cancer survivor's quality of life [4,6–12]. In the same way, a man with testicular cancer must be encouraged to have his sperm cryopreserved in order to maintain the possibility of his having children. When considering autoimplantation of ovarian tissue, the utmost care must be taken to ensure the absence of residual signs of the cancer. When ovarian tissue is grafted into the abdomen, residual cancer cells could lead to a redevelopment of the cancer [13]. A variety of tests and analyses must be performed in order to be certain that this is averted. Current analyses may not be enough and further methods of analysis need to be developed.

## Fertility Preservation

In Japan, an estimated 1 out of 62.5 women will be diagnosed with some type of invasive cancer by the age of 40, and approximately 448.3 women per 100,000 were diagnosed with cancer in 2007 [14]. The most common cancers in women under the age of 40 are uterine cancer (including cervical cancer and corpus cancer), breast cancer, thyroid cancer, and ovarian cancer [14] (Table 1).

In the United States, an estimated 1 out of 46 women will be diagnosed with some type of invasive cancer by the age of 40, and approximately 805,500 women will be diagnosed with cancer in 2013 [15].

The most common cancers in women of reproductive age are breast cancer, melanoma, cervical cancer, leukemia, and non-Hodgkin's lymphoma [16]. Fortunately, the 5 year relative survival rate for all cancers is up from 49% (1975–1977) to 67% (2001–2007), reflecting the improved diagnosis and treatment. The 5 year female cancer survival rate is dependent on the stage of cancer at diagnosis, but is currently 90% for breast cancer, 93% for melanoma, 69% for cervical cancer, 70% for non-Hodgkin's lymphoma, and 57% for leukemia.

Given the relatively high incidence of cancer in women of reproductive age and improvements in 5 year survival rate, an increasing number of women are presenting for discussion of fertility preservation and pregnancy after cancer treatment.


Received: June 19, 2013
Accepted: July 24, 2013
*To whom correspondence should be addressed.
e-mail: info@ivf-kyono.or.jp

**Table 1.** Cancer incidences by age and site (2007)

| | 0–4 | 5–9 | 10–14 | 15–19 | 20–24 | 25–29 | 30–34 | 35–39 | 0–39 |
|---|---|---|---|---|---|---|---|---|---|
| Uterine cancer*1 | 0 | 0 | 0 | 30 | 455 | 1,852 | 3,861 | 4,482 | 10,680 |
| Breast cancer | 0 | 0 | 0 | 2 | 61 | 249 | 1,152 | 2,804 | 4,268 |
| Thyroid cancer | 0 | 0 | 6 | 41 | 97 | 138 | 318 | 499 | 1,099 |
| Ovarian cancer | 0 | 11 | 15 | 70 | 72 | 135 | 208 | 362 | 873 |
| Leukemia | 138 | 64 | 75 | 60 | 65 | 55 | 71 | 131 | 659 |
| Gastric cancer | 0 | 0 | 0 | 0 | 24 | 51 | 163 | 400 | 638 |
| Colon cancer | 0 | 0 | 0 | 8 | 12 | 54 | 158 | 354 | 586 |
| Malignant lymphoma | 29 | 16 | 42 | 44 | 70 | 126 | 97 | 119 | 543 |

*1: including uterine cervical cancer and uterine corpus cancer

Cancer statistics in Japan-2012 by Foundation for Promotion of Cancer Research

## At Our Clinic

At our clinic, following the Mayo Clinic system, patients are informed of their treatment options, regarding fertility preservation, before they receive cancer treatment [17]. Certain cancer treatments, including the removal of reproductive organs, chemotherapy and radiation, affect reproductive organs being harmful to fertility or can cause sterility, depending on the type of cancer and its treatment. Such treatments can also cause male fertility problems such as ejaculation or hormonal problems. Also, surgical removal of the testicles, chemotherapy or radiation can be harmful because these will damage sperm quantity, structure, and motility, or DNA. Female fertility can be compromised by cancer treatments that involve the surgical removal of the uterus or ovaries. Treatment can also affect the development of eggs, hormone levels, or the functioning of the ovaries, Fallopian tubes, uterus or cervix. Women who initially have regular menstrual cycles after cancer treatment may experience premature menopause, and the risk of developing premature menopause increases with age. The older women become, the more likely permanent ovarian damage is to occur. The influence of chemotherapy and radiation therapy varies depending on the drug or size and location of the radiation field, etc. It's said that when radiation is applied to the ovaries or testicles, the most severe damage is caused.

Patients who are planning to receive cancer treatment and want to take steps to preserve their fertility should discuss suitable options with their doctor, an oncologist or a reproductive specialist as soon as possible. Fertility can be damaged by one cancer therapy session and, for women, some methods of fertility preservation can only be done during certain phases of the menstrual cycle. To improve coping and prevent emotional distress, taking steps to protect fertility is strongly recommended.

There are various methods of preserving patients' fertility before cancer treatments.

Embryo cryopreservation is one of these options. For embryo cryopreservation, patients receive injections to stimulate their ovaries for the collection of oocytes. These eggs are then fertilized with sperm from their partner or a donor and then cryopreserved. Embryo cryopreservation has the highest potential to preserve patients' fertility. However, it takes a number of weeks (2–6 weeks) to retrieve oocytes. Oocyte cryopreservation also needs ovarian stimulation before egg collection. After that, unfertilized oocytes are cryopreserved. We have previously reported some pregnancies and births following oocyte cryopreservation. We achieved the first pregnancy and delivery of a healthy female infant after slow freezing of oocytes in Japan [18], a healthy male infant after vitrification of mature human oocytes [19], and a healthy male infant after transfer of vitrified-warmed blastocysts derived from intracytoplasmic sperm injection of vitrified-warmed oocytes and frozen-thawed spermatozoa [20]. Today, oocyte cryopreservation should no longer be considered experimental [21]. Unfertilized eggs survive the process more than 90 percent of the time. When possible, women are often encouraged to use the more effective embryo cryopreservation instead of oocyte cryopreservation–even if it requires using donor sperm, which our ethical committee has permitted. Another option is gonadal shielding. When shields can be placed in the right place, they can reduce the reproductive organs' exposure to radiation. If patients receive radiation to the pelvis but no chemotherapy, they can choose ovarian transposition. The ovaries need to be placed far from the radiation field. After chemotherapy, they must be returned to their natural position to enable a natural pregnancy or IVF treatment. Fertility can be preserved in patients with early-

stage cervical cancer by surgical removal of the cervix. Other fertility preservation methods, such as ovarian cryopreservation and ovarian suppression before cancer therapy, are being investigated.

Men also have some fertility preservation options before cancer treatment. Patients can cryopreserve their sperm which are collected by masturbation or another method, such as testicular sperm extraction. The sperm can be cryopreserved for years. Another choice is gonadal shielding, which can reduce the testicles' exposure to radiation. There are other fertility preservation methods which are being researched, such as testicular tissue cryopreservation and testicular suppression before cancer therapy.

## Our Data for Men

*Sperm cryopreservation for intracytoplasmic sperm injection (ICSI) and intrauterine insemination (IUI) in cases of cancer and collagen diseases [22]*

To compare the effectiveness of ICSI with IUI using frozen sperm of cancer and collagen disease patients, the clinical records of 84 cases from January 1997 to April 2012 were retrospectively reviewed. The patients' average age was 30.3 (16–57) years old; 34 patients were married, 47 were single, and 3 were engaged; 40 patients had had testicular cancer, 25 hematological diseases, 17 solid tumors, and 2 collagen diseases. Semen analysis was normal in 25 patients and abnormal in 59. IUI was performed in 20 cycles of 5 cases, and ICSI in 28 cycles of 17 cases. Fifteen couples achieved pregnancy following ICSI (8 births, 4 ongoing, 1 miscarriage, 2 ectopic pregnancies). None of the patients' partners became pregnant after IUI.

According to this data, ICSI is effective, but IUI is not effective when using the frozen sperm of cancer patients. Testicular cancer patients have a high possibility of fertility after recovery; however, most hematological disease patients lose most of their fertility and need to cryopreserve sperm, especially before bone marrow transplantation.

## Our Data for Women

*1) Pregnancies following assisted reproductive technology (ART) for cancer survivors [23]*

We evaluated whether cancer patients can become pregnant and have healthy babies after recovery.

Fifty-six patients received cancer treatment, and 53.6% (30/56) of them opted for assisted reproductive technology (ART) treatment. The diseases of the 30 patients who received ART were as follows: breast cancer (16), uterine cancer (4), leukemia (4), aplastic anemia (2), ovarian cancer (2), thyroid cancer (1), and pelvic sarcoma (1).

Oocyte vitrification was performed for three single women. 48.0% (13/27) became pregnant following ART including intrauterine insemination (IUI), which has resulted in 9 healthy births, 1 abnormal birth (muscular dystrophy), 1 miscarriage (13 trisomy), and 2 ongoing pregnancies.

According to these data, more than 50% of cancer survivors were fertile. It is crucial to cooperate and communicate with oncologists, nurses and counselors about a patient's age, ovarian reserve, cause of infertility (male factor: yes or no), influence of chemotherapy and irradiation, pregnancy rate and miscarriage rate. We hope to create a situation which enables cancer patients to consult medical professionals about fertility preservation in relation to their treatment for malignant diseases.

*2) A live birth with vitrified-warmed oocytes by an acute lymphoblastic leukemia patient five years after an allogenic bone marrow transplantation [11,12].*

We reported a successful ongoing pregnancy with vitrified-warmed oocytes stored for around 5 years, following ICSI and embryo transfer to the patient's own uterus after bone marrow transplantation (BMT) for acute lymphoblastic leukemia (ALL) conditioned with a high dose of cyclophosphamide and total body irradiation (TBI) of 12 Gy.

Ten mature oocytes were vitrified using the Cryotop method in December 2006 and January 2007. The serum LH, FSH, and E2 levels of the patient were 23.8 mIU/ml, 40.8 mIU/ml and <5 pg/ml respectively, in October 2011. Six oocytes were warmed, and in November 2011, ICSI was performed on the six mature oocytes. Four of the six were fertilized. Two embryos (eight-cell and seven-cell, grade 1 by Veeck's criteria) were transferred. One of the remaining embryos (eight-cell) was vitrified on day 3 and the other embryo was cultured for two more days and vitrified on day 5.

A healthy girl weighing 3008 g was born at 40 weeks 0 days of gestation.

According to these data, vitrification of mature oocytes before BMT for fertility preservation is an attractive strategy for leukemia patients. We hope to maintain patients' quality of life and confirm the safety of this technique.

## Ovarian Cryopreservation, Birth, and Minimal Residual Disease (Mrd)

Sixty cases of orthotopic reimplantation of cryopreserved ovarian tissue have so far been performed by three teams. Among these eleven conceived (pregnancy rate: 18.3%) [24], and 24 live births have been reported in

**Table 2.** Series of pregnancies and live births after orthotopic autotransplantation of frozen-thawed ovarian tissue

| Disease | Age at cryo (year) | Time interval between reimplantation and pregnancy (months) | Natural/ IVF | Sex/weight (g) | Author (country, year) |
|---|---|---|---|---|---|
| Hodgkin's lymphoma | 25 | 11 | natural | ♀/3720 | Donnez (Belgium, 2004) |
| Neurecto-dermic tumor | 19 | 9 | natural | ♂/2830 | Donnez (Belgium, 2011) |
| Hodgkin's lymphoma | 20 | 8 | natural | ♂/3089 | Donnez (Belgium, 2011) |
| Pelvic inflammatory disease | 18 or 19 | 13 | IVF | ♂/2370 | Donnez (Belgium, 2012) |
| no information | no information | no information | natural | no information | Donnez (Belgium, 2013) |
| no information | no information | no information | IVF | no information | Donnez (Belgium, 2013) |
| Non-Hodgkin's lymphoma | 28 | 11 | IVF | ♀/3000 | Meirow (Israel, 2005) |
| Hodgkin's lymphoma | 24 | 8 | natural | ♀/3130 | Demeestere (Belgium, 2007) |
| | | 48 | natural | ♀/2870 | |
| Ewing sarcoma | 27 | 6 | IVF | ♀/3204 | Andersen (Denmark, 2008) |
| | | 25 | natural | ♀/3828 | Ernst (Denmark, 2010) |
| | | 72 | natural | ♂/4015 | Andersen (Denmark, 2012) |
| Hodgkin's lymphoma | 25 | 10 | IVF | ♂/2600 | Andersen (Denmark, 2008) |
| POF | 24 | no information | natural | no information | Silber (U.S, 2012) |
| Hodgkin's lymphoma | 31 | no information | natural | no information | Silber (U.S, 2012) |
| Hodgkin's lymphoma | 33 | no information | natural | no information | Silber (U.S, 2012) |
| Systemic necrotizing vasculitides | 27 | 11 | IVF | ♀/2030 | Piver (France, 2009) |
| Breast cancer | 36 | 10 | IVF | ♂/1650 | S-Serrano (Spain, 2010) |
| | | | | ♂/1830 | |
| Sickle cell anemia | 20 | 6 | natural | ♀/3700 | Roux (France, 2010) |
| Thalassemia | 19 | 10 (from 3rd transplantation) | IVF | ♂/3026 | Revel (Israel, 2011) |
| Hodgkin's lymphoma | 25 | 3+5 (5th cycle from 3 months after reimplantation) | IVF | ♂/3360 | Dittrich (Germany, 2012) |
| Intermediate thalassemia | 21 | 15 | natural | ♀/3670 | Revelli (Italy, 2012) |
| Bilateral mature teratoma | 31 | 4 | IVF | no information | Garcia Rada (Spain, 2012) |

the literature to date. All pregnancies obtained after human ovarian tissue reimplantation have so far been from tissue frozen according to the slow-freezing technique. Graft sites were 7 in the peritoneal window (or pocket), 13 in the ovarian medulla, and 4 in both peritoneal window and ovarian medulla. Ten of 24 patients required IVF to become pregnant [4, 6, 7, 8, 9, 24–37] (Table 2). Our objective is to offer safe fertility preservation options to young patients at risk of premature ovarian failure after treatment.

However, storing ovarian tissue of cancer patients for fertility preservation carries the risk of the presence of malignant cells that could lead to the recurrence of cancer after reimplantation. Research in this field has to continue in order to develop different possibilities for fertility preservation that would allow us to propose the most appropriate option to patients, according to their disease [38–47].

In the future we hope to use new technologies such as boron neutron capture therapy (BNCT) to eliminate cancer cells from ovarian tissue before reimplantation. BNCT can kill cancer cells without harming healthy neighbouring tissue. The first accelerator-based BNCT system was recently ordered by the Tohoku Research Institute for Neuroscience, and clinical trials are planned to commence in 2015. At the moment there are three methods to detect MRD: immunohistochemistry, histology, reverse transcription-polymerase chain reaction (RT-PCR)

and analysis of xenografts. Unfortunately, the tissue analyzed with these procedures cannot then be used for grafting. The analyzed tissue is, in essence, a sample of the tissue. The sample tissue being free of cancer cells does not guarantee that the rest of the tissue, including the tissue which will be grafted, is cancer-free. There is inevitably a risk of reimplantation of tissue with MRD. With BNCT, however, we will be able to ensure reimplantation without this anxiety. [48–49]

Dittrich et al. (2012) reported the first live birth after transplantation of ovarian tissue following overnight (>20 h) transplantation of frozen-thawed tissue [36]. Prolonged transportation did not impact the viability of either vitrified-warmed primordial or primary follicles as determined by analyses using fluorescent vital staining and oxygen consumption rates (OCR). The prolongation of transport would make it possible to accept patients from all areas of Japan, and also from neighboring countries in East Asia (Kyoya et al., 2013) [50].

## Safety of Ovarian Tissue Transplantation for Cancer Patients

*1) Analysis of Ovarian Tissue by Histology and Immunohistochemistry*

We examined the percentage of ovarian metastasis in 5,571 cancer patients under 40 years old by reviewing the national autopsy files of Japan, collected by the Japanese Society of Pathology from 1981 to 2005 [38]. The percentage of cancer patients with ovarian metastasis was 22.4% (1250/5571). The percentage of ovarian metastasis was highest in those with gastric carcinoma (55.8%), followed by colon carcinoma (26.6%), breast cancer (24.2%), pulmonary carcinoma (23.4%), lymphoma (13.3%), uterine cancer (13.1%), and leukemia (8.4%). However, these data are only histological findings. In recent reports, histology and immunohistochemistry did not reveal malignant cell infiltration in the ovarian cortex; however, PCR has detected potentially malignant cells in leukemia patients [39–41] and advanced breast cancer patients [42].

This illustrates the limitations of histology and immunohistochemistry in terms of sensitivity of detection. Moreover, these methodologies only examine a small part of the tissue and therefore, while necessary, are not sufficient to establish the safety of ovarian autotransplantation in cancer patients.

*2) Molecular Analysis of Ovarian Tissue*

Quantitative RT-PCR has a high sensitivity and specificity in disseminated cancer cell detection [43–47], one cancer cell in up to 107 normal cells, and can be applied to virtually all types of cancer if adequate tissue or cancer-specific molecular markers are available. In Dolman's 2010 study, no malignant cells were detected by histology in the ovarian tissue of six patients with chronic myelocytic leukemia (CML) and 12 patients with ALL, whereas ovarian tissue in 33% of CML patients and 70% of ALL patients was found to be positive by quantitative RT-PCR. Moreover, xenograft experiments showed leukemia invasion of grafts originating from 5/12 ALL patients [40].

Similar observations were made in another study in which histology and multimarker immunohistochemical analyses were both negative for the presence of malignant cells, whereas disease-specific genetic markers were detected by quantitative RT-PCR in 6 of 8 patients with CML or ALL [41]. Surprisingly, Dolman later reported that for mice grafted with ovarian tissue from patients with advanced breast cancer, PCR and MGB2-gene sequencing were positive for the ovarian tissue of 5 out of 10 patients, but none of the xenografted mice developed tumor masses during the 6-month grafting period [41].

*3) Xenotransplantation, a Tool to Evaluate the Safety of Ovarian Tissue Autotransplantation in Cancer Patients*

Ovarian tissue xenotransplantation to immunodeficient mice was used to evaluate the risk of reintroducing leukemia, in parallel with histology and quantitative RT-PCR. In fact, as leukemia is considered a systemic cancer, malignant cells may be present in the bloodstream, and can thus easily migrate to the ovary. After long-term xenografting (6 months) of frozen-thawed ovarian tissue from patients with CML and ALL into severe combined immunodeficiency (SCID) mice, one third of the mice grafted with tissue from ALL patients [40] showed massive macroscopic peritoneal invasion. No malignant cells were microscopically identified in grafts retrieved from the mice transplanted with ovarian tissue from CML patients; however, obvious invasion of lymphoblasts was observed in 5 of the 12 mice grafted with ovarian tissue from ALL patients. These results are quite alarming considering all ovarian tissues were determined to be healthy and disease-free following histological analysis preceding the xenograft.

We can select oocytes, embryos, and ovarian tissue for cryopreservation for fertility preservation according to age, marital status, stage and type of cancer. It is clear that at present, autotransplantation of ovarian tissue cannot be proposed for leukemia patients. It would be interesting to develop a multicentric and multidisciplinary approach combining molecular and xenograft analyses

for pathologies with low-to-moderate risk of ovarian invasion. Alternative approaches such as *in vitro* follicle culture [51] and transplantation of isolated follicles [52] are promising; however, these methods are still highly experimental.

The decision to graft a patient or not must involve a multidisciplinary discussion including oncologists, gynecologists, anatomopathologists, and molecular biologists. It is essential to balance the risks and benefits for each patient, and to be extremely cautious regarding ovarian cortex autotransplantation [53].

## Risks Of Pregnancy after Cancer and the Risks Of Treatment

In a study of 2000 women treated with pelvic radiotherapy, 95% had permanent ovarian failure following radiotherapy of 5–105 Gy [54]. Exposure to 20–30 Gy of abdominal or pelvic radiation has been shown to increase the future risk of miscarriage, preterm labor, and low birth weight [55]. The primary impact of chemotherapy on fertility is related directly to the loss of ovarian function secondary to the gonadotoxicity of many chemotherapeutic agents. The greatest risk is in women over 40 years of age receiving alkylating agents with up to 80% of patients having permanent amenorrhea after treatment. However, in women under 30, the risk of permanent amenorrhea is substantially decreased to less than 20% [56]. Therefore, we recommend female patients under the age of 40 to have their oocytes, embryos, or ovarian tissue cryopreserved, and male patients over the age of 12 to have their sperm cryopreserved before cancer treatment. For example, for breast cancer patients, the goal of IVF before cancer treatment is to decrease the time to oocyte retrieval and achieve a reasonable ovarian response with 7–10 embryos cryopreserved per patient [57]. With six vitrified-warmed oocytes, we enabled an ALL patient to give birth five years after an allogenic bone marrow transplantation [11, 12].

The likelihood of conceiving after cancer treatments is dependent on the type of cancer, age at diagnosis, treatments with gonadotoxic agents including type and duration, and various other fertility factors. In our data, more than 50% of cancer patients who recovered after cancer treatment were fertile, and more than 20% of the female patients became pregnant [23]. It has been recommended to delay pregnancy for at least 6 months after treatment with chemotherapy [58], and 12 months following completion of radiotherapy to minimize risks to offspring [59]. It would appear prudent to advise waiting a minimum of 6 months after diagnosis before attempting pregnancy [60], and more than 2 years is perhaps advisable, but this will depend on individual patient characteristics. It has been found that parous women have changes in expression of markers of disease recurrence, including estrogen receptor alpha and beta and human epidermal growth factor receptor 2 (HER 2), for up to 10 years after pregnancy, which may provide protection from cancer recurrence [61].

Overall, pregnancy appears safe for most patients after cancer treatment, but it will depend on individual patient characteristics [62].

## Conclusions

Cancer prevention and early detection of cancer are very important for enjoying a long healthy life. Also, fertility preservation of cancer patients before cancer treatment is important for maintaining a high quality of life, including a future child. We should keep in mind that although recovering fertility is very important to some patients, reimplantation of contaminated ovarian tissue could be a life-threatening event. With cancer prevention, fertility preservation and appropriate attention to possible risks of reimplantation of contaminated tissue, couples can enjoy normal healthy lives with children.

## Acknowledgement

I would like to thank Chiharu Onuma, Tomomi Kuzuno, Yuko Nakano, and Steve Beacall for assistance with English.

## References

1) Oktay, K. (2001): Ovarian tissue cryopreservation and transplantation: preliminary findings and implications for cancer patients. Hum. Reprod. Update, 7, 526–534.
2) Porcu, E., Bazzocchi, A., Notarangelo, L., Paradisi, R., Landolfo, C. and Venturoli, S. (2008): Human oocyte cryopreservation in fertility and oncology. Curr. Opin. Endocrinol. Diabetes. Obes., 15, 529–535.
3) Cobo, A. and Diaz, C. (2011): Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials. Fertil. Steril., 96, 277–285.
4) Demeestere I., Simon P., Emiliani S., Delbaere A. and Englert Y. (2010): Birth of a second healthy girl more than 3 years after cryopreserved ovarian graft, Hum. Reprod., 25, 1590–1591.
5) Donnez J., Squifflet J., Van Eyck A.S., Demylle D., Jadoul P., Van Langendonckt A. and Dolman M.M. (2008): Restoration of ovarian function in orthotopically transplanted cryopreserved ovarian tissue: a pilot experience, RBM Online, 16, 694–704.
6) Donnez, J., Dolman, M.M., Demylle, D., Jadoul, P., Pirard,

C., Squifflet, J., Martinez-Madrid, B. and van Langendonckt, A. (2004): Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet, 364, 1405–1410.
7) Meirow, D., Levron, J., Eldar-Geva, T., Hardan, I., Fridman, E., Zalel, Y., Schiff, E. and Dor, J. (2005): Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N. Engl. J. Med., 353, 318–321.
8) Demeestere, I., Simon, P., Emiliani, S., Delbaere, A. and Englert, Y. (2007): Fertility preservation: successful transplantation of cryopreserved ovarian tissue in a young patient previously treated for Hodgkin's disease. Oncologist, 12, 1437–1442.
9) Andersen, C.Y., Rosendahl, M., Byskov, A.G., Loft, A., Ottosen, C., Dueholm, M., Schmidt, M.D.K., Andersen, A.N. and Ernst, E. (2008): Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. Hum. Reprod., 23, 2266–2272.
10) Kim, M.K., Lee, D.R., Han, J.E., Kim, Y.S., Lee, W.S., Won, H.J., Kim J.W. and Yoon, T.K. (2011): Live birth with vitrified-warmed oocytes of a chronic myeloid leukemia patient nine years after allogenic bone marrow transplantation. J. Assist. Reprod. Genet., 28, 1167–1170.
11) Sakai, H., Sakamoto, E., Kuchiki, M., Doshida, M., Toya, M. and Kyono, K. (2012): Ongoing pregnancy with vitrified-warmed oocytes of an acute lymphoblastic leukemia patient five years after an allogenic bone marrow transplantation. Hum. Reprod., 27, ii256.
12) Doshida, M., Nakajo, Y., Toya, M. and Kyono, K. (2013): A live birth from vitrified-warmed oocytes in a Philadelphia chromosome-positive acute lymphoid leukemia patient 5 years following allogenic bone marrow transplantation and after a magnitude 9.0 earthquake in Japan. Reprod. Med. Biol. (in press).
13) Shaw, J.M., Bowles, J., Koopman, E.C. and Trounson, A.O. (1996): Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit the cancer to graft recipients. Hum. Reprod., 11, 1668–1673.
14) Foundation for Promotion of Cancer Research (2012): Cancer Statistics in Japan -2012
15) Cancer Facts and Figures. (2013): American Cancer Society, Atlanta, Ga, USA.
16) Siegel, R., Naishadham, D. and Jemal, A. (2012): Cancer statistics, 2012. CA Cancer J. Clin., 62, 10–29.
17) Mayo Clinic Staff (accessed May 17, 2013): Fertility preservation: Understand your options before cancer treatment. MAYO Clinic, AZ, US, http://www.mayoclinic.com/health/fertility-preservation/MY01616.
18) Kyono, K., Fukunaga, N., Haigo, K., Chiba, S. and Sato, T. (2001): Pregnancy and delivery of a healthy female infant after intracytoplasmic sperm injection into cryopreserved human oocytes. Jpn. J. Fertil. Steril., 46, 171–177 (in Japanese).
19) Kyono, K., Fuchinoue, K., Yagi, A., Nakajo, Y., Yamashita, A. and Kumagai, S. (2005): Successful pregnancy and delivery after transfer of a single blastocyst derived from a vitrified mature human oocyte. Fertil. Steril., 84, 1017.e5–1017.e6.
20) Kyono, K., Nakajo, Y., Doshida, M., Toya, M. and Araki, Y. (2009): Birth of a healthy male infant after transfer of vitrified-warmed blastocysts derived from intracytoplasmic sperm injection with vitrified-warmed oocytes and frozen-thawed spermatozoa. 26, 451–453.
21) Practice Committees of the American Society for Reproductive Medicine and The Society for Assisted Reproductive Technology (2013): Mature oocyte cryopreservation: a guideline. Fertil. Steril., 99, 37–43.
22) Sato, Y., Hattori, M., Nakajo, Y., Doshida, M., Toya, M. and Kyono, K. (2012): The effectiveness and problems of sperm cryopreservation with ICSI and IUI in cases of cancer and collagen diseases. Jpn Society Reprod. Med., 57, 196 (in Japanese).
23) Sakamoto, E., Sakai, H., Kuchiki, M., Sato, K., Nakajo, Y. and Kyono, K. (2012): Successful pregnancies following assisted reproductive technology (ART) for cancer survivors. Hum. Reprod., 27, ii257.
24) Donnez, J., Dolman, M.M., Pellicer, A., Diaz-Garcia, C., Serrano, M.S., Schmidt, K.T., Ernst, E., Luyckx, V. and Andersen, C.Y. (2013). Restoration of ovarian activity and pregnancy after transplantation of cryopreserved ovarian tissue: a review of 60 cases of reimplantation. Fertil. Steril. 99, 1503–1513.
25) Donnez J., Silber S., Andersen C.Y., Demeestere I., Piver P., Meirow D., Pellicer A. and Dolman M.M. (2011): Children born after autotransplantation of cryopreserved ovarian tissue. A review of 13 live births. Ann. Med., 43, 437–450.
26) Donnez, J., Jadoul, P., Pirard, C., Hutchings, G., Demylle, D., Squifflet, J., Smithz, J. and Dolman, M.M. (2012): Live birth after transplantation of frozen-thawed ovarian tissue after bilateral oophorectomy for benign disease. Fertil. Steril., 98, 720–725.
27) Donnez, J., Squifflet, P., Jadoul, P., Demylle, D., Cheron, A.C., Van Langendonckt, A. and Dolmans, M.M. (2011): 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., 95, 1787.e1-e4.
28) Ernst, E., Bergholdt, S, Jorgensen, J.S. and Andersen, C.Y. (2010): The first woman to give birth to two children following transplantation of frozen/thawed ovarian tissue. Hum Reprod., 25, 1280–1281.
29) Silber, S., Kagawa, N., Kuwayama, M. and Gosden, R. (2010): Duration of fertility after fresh and frozen ovary transplantation. Fertil. Steril., 94, 2191–2196.
30) Piver, P., Amiot, C., Agnani, G., Pech, J., Rohrlich, PS., Vidal, E., Aubard, Y. and Roux, C. (2009): Two pregnancies obtained after a new technique of autotransplantation of cryopreserved ovarian tissue. Hum. Reprod., 24, i15.
31) Sánchez-Serrano, M., Crespo, J., Mirabet, V., Cobo, A.C., Escribá, M.J., Simón, C. and Pellicer, A. (2010): Twins born after transplantation of ovarian cortical tissue and oocyte vitrification. Fertil. Steril., 93, 268.e1–e13.
32) Roux, C., Smiot, C., Agnani, G., Aubard, Y., Rohrlich, P.S. and Piver, P. (2010): Live birth after ovarian tissue autograft in a patient with sickle cell disease treated by allogeneic bone marrow transplantation. Fertil. Steril., 93, 2413.e15–e19.

33) Revel, A., Laufer, N., Ben Meir, A., Lebovich, M. and Mitrani, E. (2011): Micro-organ ovarian transplantation enables pregnancy: a case report. Hum. Reprod., 26, 1097.
34) Revelli, A., Marchino, G., Dolfin, E., Molinari, E., Delle Piane, L., Salvagno, F. and Benedetto, C. (2013): Live birth after orthotopic grafting of autologous cryopreserved ovarian tissue and spontaneous conception in Italy. Fertil. Steril., 99, 227–230.
35) Claus, Yding., Andesen., Sherman, J., Silber, Stinne, Holm., Berghold, Jan, Stener., Jorgensen, Erik, Ernst. (2012) Long-term duration of function of ovarian tissue trasplants: case reports (2012). RBM online, 25, 128–132.
36) Dittrich, R., Lotz, L., Keck, G., Hoffmann, I., Muller, A. and Beckmann, M.W. (2012): Live birth after ovarian tissue autotransplantaion following overnight transportation before cryopreservation. Fertil Steril., 97, 387–390
37) García Rada, A. Spanish woman becomes pregnant through ovarian tissue transplantation (2012). BMJ, 344: d8350.
38) Kyono, K., Doshida, M., Toya, M., Sato, Y., Akahira, J., Sasano, H. (2010): Potential indications for ovarian autotransplantation based on the analysis of 5,571 autopsy findings of females under the age of 40 in Japan. Fertil. Steril., 93, 2429–2430.
39) Meirow, D., Hardan, I., Dor, J., Fridman, E., Elizur, S., Ra'anani, H., Slyusarevsky, E., Amariglio, N., Schiff, E., Rechavi, G., Nagler, A. and Yehuda, D.B. (2008): Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients. Hum. Reprod., 23, 1007–1013.
40) Dolmans, M.M., Marinescu, P., Saussoy, A., Van Langendonckt, A., Amorim, C. and Donnez, J. (2010): Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe. Blood, 116, 2908–2914.
41) Rosendahl, M., Andersen, M.T., Ralfkioer, E., Ljeldsen, L., Andersen, M.K. and Andersen, C.Y. (2010): Evidence of residual disease in cryopreserved ovarian cortex from female patients with leukemia. Fertil Steril., 94, 2186–2190.
42) Dolman, M.M. (2012): Risk of reimplanting malignant cells after transplantation of cryopreserved ovarian tissue in cancer women. The International Ovarian Conference 2012, 32–33.
43) Campana, D. (2004): Minimal residual disease studies in acute leukemia. Am. J. Clin. Pathol., 122, S47–S57.
44) Paietta, E. (2002): Assessing minimal residual disease (MRD) in leukemia: a changing definition and concept? Bone Marrow Transplant. 29, 459–465.
45) Ring, A.E., Zabaglo, L., Ormerod, M.G., Smith, I.E. and Dowsett, M. (2005): Detection of circulating epithelial cells in the blood of patients with breast cancer: comparison of three techniques. Br. J. Cancer, 92, 906–912.
46) Lacroix, M. (2006): Significance, detection and markers of disseminated breast cancer cells. Endocr. Relat. Cancer, 13, 1033–1067.
47) Lu, J., Getz, G., Miska, E.A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B.L., Mak, R.H., Ferrando, A.A., Downing, J.R., Jacks, T., Horvitz, H.R. and Golub, T.R. (2005): MicroRNA expression profiles classify human cancers. Nature, 435, 834–838.
48) Sumitomo Heavy Industories, Ltd. (2012): World's First Accelerator Based Boron Neurtron Caoture Therapy(BNCT) System Ordered for a Hospital. Fiscal 2012 News.
49) Barth, R.F., Coderre, J.A., Vicente, M.G. and Blue, T.E. (2005): Boron Neutron Capture Therapy of Cancer: Current Status and Future Prospects. Clin. Cancer. Res., 11, 3987.
50) Kyoya, T., Nakamura, Y., Miyatani, S., Miyagawa, T., Tomiyama, T. and Kyono, K. (2013): Evaluation of oxygen consumption in human vitrified and wormed pre-antral follicles after prolonged low temperatures REMB (in press).
51) Xu, M., Barrett, S.L., West-Farrell, E., Kondapalli, L.A., Kiesewetter, S.E., Shea, L.D. and Woodruff, T.K. (2009): In vitro grown human ovarian follicles from cancer patients support oocyte growth. Hum Reprod., 24, 2531–2540.
52) Dolmans, M.M., Martinez-Madrid B, Gadisseux E. Guiot, Y., Yuan, WY., Torre, A., Camboni, A., Langendonckt, A.V. and Donnez, J. (2007): Short-term transplantation of isolated human ovarian follicles and cortical tissue into nude mice. Reproduction, 134, 253–262.
53) Bockstaele, L., Tsepelidis, S., Dechene, J., Englert, Y. and Demeestere, I. (2012): Safety of ovarian autotransplantation for cancer patients. Obstet. Gynecol. Int., 2012, 495142.
54) Bianchi, M. (1983): Cytotoxic insult to germinal tissue-part 2: the ovary in Cytotoxic Insult to Tissue: Effects on Cell Lineages (Potten, C.S. and Hendry, J.H., eds.), pp. 309–328, Churchill-Livingstone, Edinburgh, UK.
55) Critchley, H.O. and Wallace, W.H. (2005): Impact of cancer treatment on uterine function. J. Natl. Cancer Inst. Monogr., 2005, 64–68.
56) Lee, S.J., Schover, L.R., Partridge, A.H., Patrizio, P., Wallace, W.H., Hagerty, K., Beck, L.N., Brennan, L.V. and Oktay, K. (2006): American Society of Clinical Oncology Recommendations on Fertility Preservation in Cancer Patients. J. Clin. Oncol., 24, 2917–2931.
57) Sönmezer, M., Türkçüoğlu, I., Coşkun, U. and Oktay, K. (2011): Random-start controlled ovarian hyperstimulation for emergency fertility preservation in letrozole cycles. Fertil. Steril., 95, 2125.e9–e11.
58) de Bree, E., Makrigiannakis, A., Askoxylakis, J., Melissas, J. and Tsiftsis, D.D. (2010): Pregnancy after breast cancer. A comprehensive review. J. Surg. Oncol., 101, 534–542.
59) Fenig, E., Mishaeli, M., Kalish, Y. and Lishner, M. (2001): Pregnancy and radiation. Cancer Treat Rev., 27, 1–7.
60) Azim, H.A., Santoro, L., Pavlidis, N., Gelber, S., Kroman, N., Azim, H. and Peccatori, F.A. (2011): Safety of pregnancy following breast cancer diagnosis: a meta-analysis of 14 studies. Eur. J. Cancer, 47, 74–83.
61) Pagani, O., Price, K.N., Gelber, R.D., Castiglione-Gertsch, M., Holmberg, S.B., Lindtner, J., Thürlimann, B., Collins, J., Fey, M.F., Coates, A.S. and Goldhirsch, A. (2009): Patterns of recurrence of early breast cancer according to estrogen receptor status: a therapeutic target for a quarter of a century. Breast Cancer Res. Treat., 117, 319–324.
62) Matthews, M.L., Hurst, B.S., Marshburn, P.B., Usadi, R.S, Papadakis, M.A. and Sarantou, T. (2012): Cancer, fertility preservation, and future pregnancy: A comprehensive review. Obstet. Gynecol. Int., 2012, 953937.