Ovarian Tissue Cryopreservation for Fertility Preservation in Young Female Cancer Patients: The First Two Cases in the Philippines*

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Presented are the first two reported cases of patients who underwent ovarian tissue vitrification in the Philippines. One case is a 22 year old nulligravid diagnosed with Invasive Ductal Carcinoma Stage IIIA of the right breast (status post Modified Radical Mastectomy), and advised to undergo systemic chemotherapy with Doxorubicin and Cyclophosphamide. The other case is a 34 year old primary infertility patient, diagnosed with Non-keratinizing Squamous Cell Carcinoma of the Cervix ,Stage 1B2, and was advised neoadjuvant platinum-based chemotherapy with concurrent radiotherapy and brachytherapy. Both women are desirous of pregnancy, and so underwent ovarian tissue cryopreservation prior to medical management of their condition, to preserve future reproduction.

Key words: Ovarian tissue cryopreservation, fertility preservation

Introduction

According to GLOBOCAN 2002, approximately 11,465 new cancer cases were reported in Filipino female patients between 15 to 44 years old.[1] Breast cancer remains to be the leading cause of cancer for both sexes combined (18.7%) and ranks 1st among women (33.2%) in the recent 2012 statistics. The five leading sites among women include breast followed by cervix uteri (12.1%), colorectum (7%), lung (5.9%), and ovary (4.4%).[2]

Advancements in cancer therapies have led to increased long-term survival rates. As the number of young cancer survivors increases, quality of life after cancer treatment is becoming an even more important consideration.[3] Cancer therapies that include chemotherapy, radiotherapy and surgery are known to have adverse effects on ovarian function and reserve and negatively impact a woman's childbearing capacity. In 2006, the American Society of Clinical Oncology released its recommendation on fertility preservation to expand the reproductive options of cancer patients undergoing gonadotoxic therapy or gonadectomy that may compromise future fertility. Now, this is called "Oncofertility" or fertility preservation in the cancer setting.[4] According to Gorman, et al. young adult cancer survivors are concerned about their fertility status and often are uninformed regarding their fertility and fertility preservation options.[5] Current available strategies for female cancer patients include conservative treatments for gynecologic malignancies, ovarian transposition and utilization of assisted reproductive technologies such as embryo and mature oocyte cryopreservation. Recently, a new option for fertility preservation has been made accessible in our country - ovarian tissue cryopreservation, which is still considered experimental but may be the only option in patients who have insufficient time but require immediate treatment, in women with hormone-sensitive malignancies or pre-pubertal girls.[6]

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Case 1

The patient is M.R.I., a 22 year old single, nulligravid diagnosed case of Invasive Ductal Carcinoma Stage IIIA, Right Breast post Modified Radical Mastectomy last July 2015. Her estrogen/ progesterone receptor assays tested positive, HER-2/neu equivocal, and HER2 FISH negative. The patient was offered BRCA 1 and BRCA 2 gene mutation screening but refused. The plan of her oncologist was to initiate systemic chemotherapy with Doxorubicin and Cyclophosphamide four to six weeks after surgery. The patient was however, concerned about her future fertility and was referred to an infertility specialist for "egg freezing" a month after. Since the patient's tumor is hormone-sensitive and there was insufficient time to perform ovarian stimulation, a consensus among infertility specialists was reached to offer ovarian tissue cryopreservation. Because this is not the standard of care and still considered to have controversial results, a thorough counseling and informed consent was secured from the patient and her relatives. The right ovary was surgically removed by laparoscopy with no intraoperative complications. The ovary was placed in saline solution and immediately transported to the embryo lab for specimen processing. Ovarian tissue was also sent for histopathology which revealed cystic follicle and endometrial cyst.

Case 2

The patient is R.M. a 34 year old, nulligravid, married for 1 ½ years, who on infertility work-up was diagnosed to have Non-keratinizing Squamous Cell Carcinoma of the Cervix Stage 1B2. She was advised neoadjuvant platinum-based chemotherapy with concurrent radiotherapy and brachytherapy but has strong desire for a biological offspring. Her husband's semen analysis showed teratozoospermia. Hence, she underwent Emergency In-Vitro Fertilization. Utilizing the Gonadotropin-releasing hormone (GNRH) antagonist protocol, she had controlled ovarian stimulation for 14 days. A total of 13 metaphase II (M2) oocytes were retrieved 36 hours after the GNRH agonist trigger. Intracytoplasmic sperm injection (ICSI) was performed on all the mature oocytes using her husband's sperm. Six eggs fertilized and cleaved into good quality eight-cell embryos that were vitrified as the patient had to complete her therapy. On the day of egg retrieval, the patient also had laparoscopic bilateral oophorectomy for ovarian tissue cryopreservation prior to scheduled chemo-radiation two days postoperatively.

The Process of Ovarian Tissue Vitrification

The primary, developing and primordial follicles are abundantly located in the ovarian cortex, thereby obtaining a portion of the ovarian cortical tissue enables cryopreservation of oocytes. It is ideal that ovarian tissue be harvested before initiation of gonadotoxic treatment either laparoscopically or by mini laparotomy. It is not dictated by a woman's menstrual cycle and can be done at any time. Removal of an ovary for fertility preservation differs from a simple oophorectomy in that the tissue must be protected from necrosis by minimizing the time that the blood supply is clamped. The tissue must be transported to the lab and processed within an hour of ?collection to maintain the viability of the tissue and the oocytes within.[7]

In there two cases, once the ovaries were obtained they were immediately transported to the embryo lab immersed in saline solution. Transport time was less than 10 minutes. Upon receipt, stromal tissue was removed to reduce the thickness of the ovarian tissue (Figure 1) prior to slicing it into 10mm x 10mm x 1mm slivers of tissue (Figure 2) that will allow permeation of the cryoprotectant. The procedure was completed on a warm plate working at a constant temperature of 37°C. The sliced ovarian tissue was then washed with human tubal fluid (HTF) culture medium supplemented with human serum albumin (HSA) and HEPES-buffer. At room temperature, the cryoprotective medium was then introduced to the ovarian tissue slices. At SLMC,

cryopreservation protocol by Kitazato Biopharma Co. Ltd Ova Cryo Kit Type M was observed. The cortex fragments were sequentially submerged in Cryo1 for 5 minutes, Cryo2 for another 5 minutes and Cryo3 for 15 minutes. Excess medium was removed by placing the ovarian tissue on a piece of gauze. Then, the ovarian tissues were placed onto thin metal strips (Figure 3) (Ova Cryo Device Type M; Kitazato Biopharma Co. Ltd) with its superficial dimension spread out as much as possible and plunged directly into sterile liquid nitrogen. Following which, the strip was inserted into a protective container and placed into a liquid nitrogen storage tank.

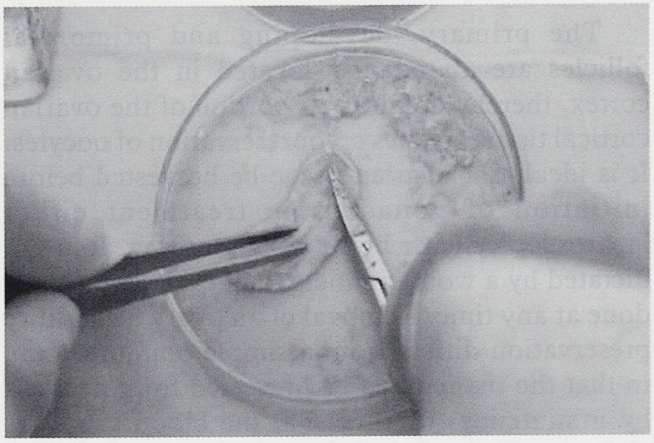

Figure 1. Excess stromal tissue was removed.

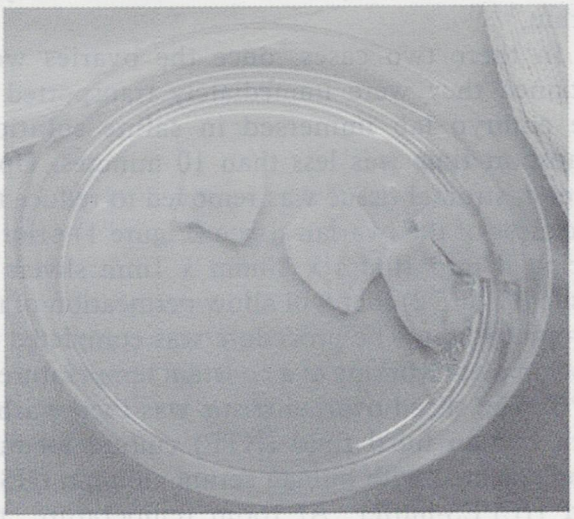

Figure 2. The ovarian tissue was cut into 1mm × 10mm × 10mm slices.

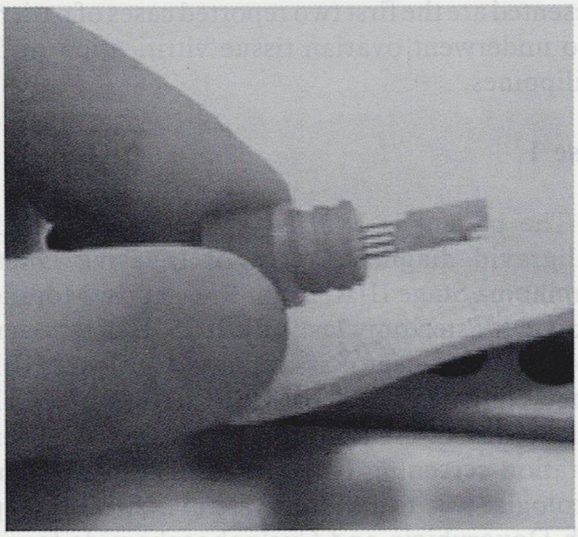

Figure 3. Ovarian tissue was then placed in a minimum volume of solution onto thin metal strips prior to vitrification procedure.

Discussion

Fertility preservation is an emerging field in medicine that enables maintenance of reproductive health when it is threatened by gonadotoxic treatment.[8] Chemoradiotherapy-induced gonadotoxicity is almost always irreversible. Premature ovarian insufficiency (POI) is a well-known consequence of exposure of the female gonad to chemotherapeutic drugs and radiotherapy. Histologic sections of the ovary after treatment with cytotoxic drugs known to cause ovarian failure show a spectrum of changes ranging from depletion of primordial follicles to absent follicles to fibrosis. The dogma of ovarian physiology is that germline stem cells do not occur in the postnatal ovary. If primordial cells are depleted like in menopause or destroyed through therapy, no new oocytes are generated.

The most important predictive parameters of degree and persistence of ovarian damage from chemotherapy and radiotherapy are the age of the patient, the class of drug, the cumulative dose of the drug and radiation and the number of episodes needed to deliver the dose.

The risk of gonadal damage increases as the age of the woman increases and is most likely

caused by the presence of a lower number of remaining oocytes compared with younger patient.[9] In our cases, our patients were diagnosed with the disease at ages 22 and 34. Serum anti-mullerian hormone were checked pre-operatively which revealed results of 3.43 ng/mL and 10.7 ng/mL respectively for case 1 and 2 signifying an adequate ovarian reserve.

Cytotoxic chemotherapeutic agents are not equally gonadotoxic. Table 1 summarizes the relative gonadotoxicity of different chemotherapeutic agents. The most significant agent in terms of loss of ovarian function is cyclophosphamide. M.R.I. (Case 1) was advised to undergo chemotherapy protocol that included Cyclophosphamide 500-600 mg/m^2 per cycle, Doxorubicin 50-60 mg/m^2 per cycle and Paclitaxel 80 mg/m^2 weekly to be instituted after four cycles of the two previous agents. Cyclophosphamide is an alkylating agent with demonstrated efficacy as adjuvant treatment for breast cancer. It has a strong impact in primordial follicle reserve: a reduction higher than 90% in follicle density has been reported 48 hours after administration. The effect on reproductive life span is equally strong: one single cycle at a standard dose can accelerate ovarian aging by up to three years in terms of reproductive function, while a whole regimen administered over 12-16 weeks can increase aging by ten years. This means that a woman who starts at 30 will have the equivalent ovarian age of 40 by the end of the treatment.[10] With this gonadotoxic threat, the patient was then advised fertility preservation.

Table 1.Gonadotoxicity of different chemotherapeutic agents.[10,21]

High risk	Alkylating agents, Cyclophosphamide, Busulfan, Melphalan, Nitrogen mustard
Intermediate risk	Cisplatin, Adriamycin
Low risk or no risk	Methotrexate, 5-Fluorouracil, Actinomycin D, Bleomycin, Vincristine, 6-Mercaptopurine

For the second case, since the patient was diagnosed to have Cervical Cancer Stage IB2, the tretament plan for her was concurrent platinum-based chemotherapy and radiotherapy and brachytherapy. The possible exposure to ionizing radiation during pelvic external beam radiotherapy poses adverse effects to ovarian function. The ovarian follicles are remarkably vulnerable to DNA damage from ionizing radiation, resulting in a significant reduction in the ovarian follicle pool. Oocytes show a rapid onset of pyknosis, chromosome condensation, disruption of the nuclear envelope and cytoplasmic vacuolization. Serum levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) increase progressively within 4-8 weeks after radiation exposure along with a decline in serum estradiol levels. A dose-dependent reduction in the primordial follicle pool was noted upon exposing the ovary to radiotherapy. It is estimated that less than 2 Gy is enough to destroy 50% of the oocyte population. Studies indicated that the breakpoint for radiation-induced ovarian failure is approximately 300 cGy to the ovaries and that radiation is more toxic when given in a single dose compared with fractionated doses.[9] In cervical cancer, 50.4 Gy is usually given in 28 fractions depending on the internal examination findings with possible boost to the parametria and paraaortic. Ovarian transposition or oophorexy can also be offered when pelvic irradiation will be performed as part of cancer treatment. However, this technique is not always succesful because of possible radiation scatter.[12] Hence, in this case, the patient was offered ovarian tissue preservation and both ovaries were removed laparoscopically for vitrification.

Ovarian failure is diagnosed by at least two measurements of FSH above 40 mIU/mL, regardless of menstrual bleeding. Continuation of menstruation is not a reliable indication of ovarian function and fertility, as pregnancy rates are extremely low when FSH measurements on the second or third day of the menstrual period exceed 12 mIU/mL. Likewise, elevation of estradiol levels above 75 pg/mL on the second or third day of the menstrual period is also associated with compromised fertility.[11]

Fertility Preserving Techniques in the Female

This paper is limited to the discussion of fertility preservation options in the female.

Conservative Gynecologic Surgery

In the treatment of other gynecologic malignancies, interventions to spare fertility have generally centered on doing less radical surgery with the intent of sparing the reproductive organs as much as possible. Ovarian cystectomy can be performed for early-stage ovarian cancer.[12] Patients with a well-differentiated, grade 1, Endometrial Endometriod Adenocarcinoma with no myometrial invasion and no extrauterine involvement may be candidates for fertility-sparing treatment. Progestins have been the mainstay of conservative hormonal treatment for endometrial cancer in the young woman who wants to preserve fertility. It has been suggested that radical trachelectomy (surgical removal of the uterine cervix) should be restricted to stage IA2 to IB cervical cancer with diameter 2 cm and invasion 10 mm. The patient R.M. presented with a 4 cm mass and was clinically staged to have cervical cancer 1B2. Recommendations for management from the Society of Gynecologic Oncologists of the Philippines for this stage include concurrent chemo-radiotherapy with or without radical hysterectomy with lymph node dissection.[13] With her strong desire for future fertility, definitive surgery with radical hysterectomy was not entertained and proceeded with chemotherapy and radiotherapy.

Ovarian Transposition

The surgical procedure of ovarian transposition intends to move the ovaries outside the irradiation field. It consists of releasing the ovary from its pelvic attachments and placing it behind the uterus or in the paracolic gutter. This strategy is useful in patients who require only radiotherapy for cancer treatment. In our patient R. M. with cervical cancer, this could have been performed prior to pelvic external beam radiotherapy but its effectivity is not always certain due to possibility of radiation spread.

Assisted Reproductive Technology

Patients also have the option of preserving gamete function ex vivo with assisted reproductive technology (ART). Embryo preservation is currently the most established procedure of fertility preservation in female cancer patients according to the American Society of Clinical Oncology and the Ethics Committee of the American Society for Reproductive Medicine. This is a feasible option for postpubertal women who have a committed male partner or who are prepared to use a donor sperm such as in the case of R.M. who has been married for more than a year and is desirous of starting their own family. Embryo cryopreservation involves ovarian stimulation for multifollicular development, oocyte retrieval, embryo generation through in vitro fertilization, and freezing of embryos for future implantation. Survival rates for thawed embryos range from 35 to 90%, implantation rates from 8 to 30%, and cumulative pregnancy rates can exceed 60%.[14]

In women without a partner, freezing mature or immature oocytes may be the only practical option. Four randomized controlled trials of fresh vs. vitrified/warmed oocytes indicate that implantation and clinical pregnancy rates are similar.[15] M.R.I. is still single with no sexual exposure, however, with an advanced stage of her breast malignancy coupled with hormone sensitive tumor, ovarian stimulation with high dose gonadotropins to achieve multifollicular development for oocyte preservation was risky. Other protocols may be employed to obviate need for gonadotropin-use such as utilization of an aromatase inhibitor but this will need a prolonged stimulation time which the patient did not have during the time she consulted the reproductive medicine specialist. She was advised to immediately undergo chemotherapy by her oncologist hence this was not a very appealing option for her.

Ovarian Tissue Cryopreservation

Once experimental, this technique has nowadays been demonstrated to be useful as reports of the achievement of several pregnancies after ovarian transplantation is being published.

The technique consists of the surgical retrieval of ovarian tissue including ovarian cortical tissue that has hundreds of primordial and growing follicles. The age of the patient should be considered as the follicle reserve declines with age. Follicles undergo accelerated atresia in women after the age of 36,[16,17] with early-growing and non-growing follicles disappearing rapidly in aging women. Hence, ovarian tissue cryopreservation in aged patients may not be fruitful as their ovary may have very few follicles for cryopreservation.

Once this ovarian tissue is obtained it can either be used for post-chemotherapy transplantation. When the patient is in remission, it is transplanted back to the patient in 2 ways: orthotopic (into the pelvic cavity) and heterotopic (outside the peritoneal cavity).

It is logical that there is a risk of ovarian metastasis if the patient survives a malignant tumor. Hematologic diseases such as leukemias and Burkitt's lymphoma are high risk in ovarian involvement, some advanced stage solid tumors such as breast and colon cancers are moderate risk. Most other cancers in reproductive age women have low risk of ovarian metastasis, including breast cancer in stage I-III, squamous cell carcinoma of the cervix, Non-Hodgkin's lymphoma, Hodgkin's lymphoma, osteogenic sarcoma, Ewing's sarcoma, etc. However, in spite of the risk of metastatic disease, histopathological should be performed to determine the presence of cancer cells in the ovarian tissue before and after cryopreservation of ovaries.[18] For both patients, after a multidisciplinary meeting, this was the chosen option for fertility preservation primarily because of time constraint in the first case in a background of a hormone-sensitive malignancy and in the second case due to the uncertainty of ovarian transposition to prevent effects of irradiation, ovarian tissue vitrification was suggested.

Resumption of normal ovarian tissue has been reported to occur in an average of 4-5 months after transplantation and ovarian function can persist for 7 years after transplantation of fresh or cryopreserved ovarian tissue with a mean duration of approximately 4 to 5 years if the follicular density is well preserved. Live births after the ovarian tissue transplantation were reported as well. The pregnancy occurred as either natural (13) or IVF (5).[19] However, data regarding pregnancy are confounded by the fact that most of the initial surgical procedures did not involve removal of both ovaries, and the site of ovulation (native ovary versus transplanted ovarian tissue) was not confirmed.[6]

Currently, M.R.I. has been having her regular menstrual periods. She completed four cylces of Cyclophosphamide and Doxorubicin and 12 cycles of Paclitaxel with 35 days of pelvic external beam radiotherapy. Repeat ovarian testing was not performed.

R.M., on the other hand, was given Drospirenone 2 mg and estradiol hemihydrate 1 mg (Angeliq) for hormonal replacement after surgery. She completed concurrent chemotherapy with Cisplatin and Paclitaxel, 28 cycles of radiotherapy and brachytherapy. Frozen embryo transfer was deferred due to sonographic findings suggestive of intrauterine adhesions on endometrial surveillance after priming and is still for further work-up and possible lysis. The patient also expressed desire to have her vitirified ovarian tissue be transplanted back to her. The attending reproductive medicine specialist and her team is preparing for the first possible autotransplantation of vitrified ovarian tissue if all work-up results will be favorable.

Conclusion

The American Society of Clinical Oncology (ASCO) Fertility Preservation Guidelines highlighted the need for the following: having frank discussions of fertility preservation, early referral to reproductive specialists, addressing fertility preservation as early as possible before starting cancer treatment, referring for psychosocial specialists if distress is present, and encouraging participation in clinical studies and registries when

appropriate. Receiving a diagnosis of cancer may be devastating for young women. This is especially true if they have not completed childbearing plans. It is the responsibility of the health care team to explain prognosis, treatment options, and potential toxicities and adverse effects of chemotherapy, radiotherapy, and endocrine treatments, educate about fertility issues, and refer them early to appropriate specialist as requested. An interdisciplinary approach including those with medical oncologists, reproductive specialists, obstetrician and gynecologists, primary care physicians, nurses, psychologists, and other allied health professionals is ideally used throughout each young breast cancer survivors' journey. This discussion should be taken as soon as possible to provide wider options for these patients.[12]

Receiving specialized counseling about reproductive loss and pursuing fertility preservation is associated with less regret and greater quality of life for survivors, yet few patients are exposed to this potential benefit. Reproductive aged women should have expert counseling and be given the opportunity to make active decisions about preserving fertility.[20]

Making fertility preservation a reality involves a multidisciplinary field that requires the collaboration and coordination of professionals from different subspecialties. Individuals under threat of fertility loss should be offered appropriate fertility preservation options. Young survivors of cancer do not only want to preserve their lives, but also their quality of life.[10]

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