**Ovarian Tissue Freezing For Fertility Preservation In Women Facing A Fertility Threatening Medical Diagnosis Or Treatment Regimen: A Study By The National Physicians Cooperative of the Oncofertility Consortium At Northwestern University**

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National Physicians Cooperative of the Oncofertility Consortium at Northwestern University

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1. **INTRODUCTION – BACKGROUND AND RATIONALE**

Although cancer remains a critical health concern, significant medical advances in cancer detection and treatment have improved survival rates for patients. As patients live longer, the early and late consequences of cancer management are beginning to assume greater importance for survivors, their families and providers. For instance, when considering the long term sequelae of cancer therapy, infertility surfaces as a primary concern, particularly, among female survivors (Zeltzer, 1993). Unlike other late effects of cancer treatment, such as complications in cardiovascular or liver function, female infertility has biological and psychosocial implications that cannot be narrowly defined, nor easily addressed given the number of ethical and legal questions surrounding fertility preservation (Patrizio et al., 2005). The clinical implications can range from acute ovarian failure, to induced premature menopause and ultimately may result in difficulty with getting pregnant (Nieman et al, 2006). Particularly at risk are females who are older at the time of cancer diagnosis, those who have received abdominopelvic radiation or high doses of alkylating agents (particularly cyclophosphamide and procarbazine) and those diagnosed with Hodgkin lymphoma (Chemaitilly et al., 2006; Sklar et al., 2006; Larsen et al., 2003; Chiarelli et al., 1999; Mattison, 1990). These concerns are not limited to cancer patients; treatment for other medical conditions such as rheumatoid arthritis and lupus may also result in infertility.

Traditionally, female cancer patients who wanted to have their own biological children in the future had limited options: protecting the ovaries from radiation and emergency *in vitro* fertilization (IVF) (Meirow & Nugent, 2001; Sonmezer & Oktay, 2004). Shielding a patient’s ovaries during radiation therapy has become common practice (Wallace et al., 2005). However, oocyte or embryo freezing is not always an option for young patients.

Mature oocytes can be retrieved following hormonal stimulation that is identical to that used for in vitro fertilization and cryopreserved (Chen et al, 1986). Until recently, post thaw recovery of cryopreserved oocytes with subsequent fertilization and embryo transfer lead to disappointing results (Tucker et al, 1996; Porcu et al, 1999; Marina and Marina, 2003). However, improvements in both freezing and thawing techniques for human oocytes necessitated by the ban in Italy on freezing embryos (only eggs can be frozen) are currently leading to acceptable pregnancy rates (Coticchio et al., (2006); Paynter et al., (2005); Fabbri R, et al., (2001); Bianchi et al., (2007); Borini A et al., (2004); Borini et al., (2006). Like in vitro fertilization with freezing of embryos, the patient must undergo hormonal therapies to stimulate the growth of multiple follicles, followed by a surgical oocyte retrieval: a process that may take up to 3 weeks and can put the patient at risk of hyperstimulation syndrome. Therefore, the use of emergency IVF with the freezing of embryos or oocytes may delay the start of cancer therapies and may not be feasible for patients who have not yet reached puberty.

Fertility preservation options for female patients who cannot delay treatment or are too young to undergo hormonal stimulation rely upon ovarian tissue cryopreservation with subsequent transplantation and/or *in vitro* follicle maturation. Ovarian tissue containing immature oocytes (primordial follicles, antral and pre-antral follicles) has been successfully cryopreserved in several animal models including rodents, sheep, and non-human primates (Harp et al, 1995; Oktay et al, 1998; Gosden, et al, 1994; Baird et al, 1999). When thawed, this tissue can be grafted into a host with resumption of both endocrine and reproductive function. In 2004, the first successful application of this technology was reported in humans (Donnez, et al.,2004) using autologous orthotopic transplantation of frozen thawed human ovarian tissue. In this study, the patient had return of endocrine function within 3 months of the transplant and achieved a spontaneous pregnancy. As of October 2007, there have been 12 reports of pregnancies (all but one spontaneous) following transplant of frozen/thawed ovarian tissue (Donnez et al.2004; Meirow et al.2005; Meirow et al.2007; Anderson et al.2007; Demeestere et al, 2006; Schmidt et al, 2005; Silber et al.2005, 2007 a,b). At least 13 more have been reported subsequently.

However, an important concern with transplanting ovarian tissue is the potential reintroduction of cancerous cells into a patient in remission, as seen in rodent lymphoma. Ovarian tissue transplant using this leukocyte rich tissue may be contraindicated in patients with certain types of cancer (Shaw et al., 1996 a, b).

An alternative to transplantation of the ovarian tissue is isolation of the immature oocytes from the cryopreserved ovarian tissue and maturation *in vitro* with subsequent in vitro fertilization (Oktay, et al.1997). Traditional in vitro maturation systems make use of a 2-dimensional culture (Cortvrindt et al., 1996) that yields few oocytes that are viable, mature or fertilizable. Researchers at Northwestern University (Xu et al., 2006) have demonstrated that a 3-dimensional scaffold system using alginate, which may more closely mimic the *in vivo* ovarian physiologic conditions, produces a high follicle survival rate (93%) and oocyte maturation success (73%) in mice. Embryos derived from these oocytes which were successfully fertilized *in vitro* and transferred to pseudopregnant female mice were able to produce live young. Both male and female offspring were also fertile. Parallel studies have been performed in non-human primates. Ovarian follicles are contained in the cortex of the ovary. Large numbers of follicles can be obtained from strips of the ovarian cortex thus eliminating the need for hormonal stimulation of the ovary. Restoration of fertility and endocrine function made possible by maintaining the structural integrity of follicle using in vitro follicle maturation could substantially improve the quality of life for women of reproductive age receiving cancer therapies which may otherwise impair ovarian function. Patient age, marital status, time available before treatment and specific diagnosis are just a few of the factors that affect the choice of fertility preservation options in each individual patient.

**2.0 SPECIFIC AIMS**

The primary objective of this study is to establish technologies that will enable long-term preservation of ovarian function and allow the production of viable oocytes in females undergoing chemotherapy, radiation or treatment that is expected to reduce their fertility. This study will provide research tissue to a Northwestern University research laboratory that will be used to:

* Optimize techniques for freezing and thawing of ovarian tissue for use in transplant or in vitro follicle maturation (IFM).
* Investigate factors affecting successful maturation and quality of immature oocytes and follicles obtained from ovarian tissue including the use of biomaterial scaffolds, growth factors, hormones and other culture conditions.

In addition to research tissue, this study will:

* Determine the efficacy of ovarian cryopreservation techniques.
* Provide participants with a substantial portion of their own tissue to cryopreserve and reserve for future use.
* Provide long-term follow up for patients who have undergone ovarian tissue frozen for their own use.

**3.0 SIGNIFICANCE**

Fertility preservation is an important quality of life issue for cancer survivors. While cancer treatments have been become more efficacious, some can be expected to reduce fertility. This study will provide ovarian tissue for research to develop and test methods to expand the range of fertility preservation options available to female cancer patients. At the same time, most of the patient’s tissue will be cryopreserved and reserved for her own future use.

**4.0 PRIMARY STUDIES**

The investigators of the proposed study have extensive clinical or laboratory experience in the utilization of Assisted Reproductive Technologies for infertile patients. The clinical investigators are fully trained and highly skilled in all the surgical procedures and clinical counseling needed in this study. The laboratory investigators of the study have extensive training in the physiology of oocyte and embryo development and have all the laboratory skills required for this study, including cell/tissue culture, culture and manipulation of gametes and embryos in vitro, and gamete/embryo cryopreservation. They also have extensive knowledge of the federal regulations governing reproductive tissue banking, tissue preparation and patient screening and testing for long term tissue banking.

Studies to date have been performed on mouse ovarian follicles to investigate the feasibility of using biomaterials and additional factors to construct an optimal environment for supporting long-term culture of ovarian follicles in vitro. Such results are currently being translated into both nonhuman primate and human models. Advances obtained using primate ovarian tissue to date include: isolating and culturing primordial follicles from ovarian tissue, optimizing cryopreservation protocols of human secondary follicles and developing a noninvasive index for later growth potential, developing human ovarian follicles from the early secondary to antral stage in vitro with healthy oocytes, investigating age-related chromosomal abnormalities in in vitro matured eggs, and maximizing the use of human ovarian tissue through optimized transport of research tissue. These studies were performed in collaboration with scientists in the Departments of Chemical Engineering (Dr. Lonnie Shea) and Molecular Biosciences (Dr. Teresa Woodruff), Northwestern University.

**5.0 PATIENT ELIGIBILITY**

5.1 Inclusion criteria:

5.1.1 Be female, 18-41 years of age.

5.1.2 Will undergo surgery, chemotherapy, drug treatment and/or radiation for the treatment or prevention of a medical condition or malignancy expected to result in permanent and complete loss of subsequent ovarian function.

5.1.3 Or, have a medical condition or malignancy that requires removal of all or part of one or both ovaries.

5.1.4 Patients may have newly diagnosed or recurrent disease. Those who were not enrolled at the time of initial diagnosis are eligible if they have not received therapy that is viewed as likely to result in complete and permanent loss of ovarian function.

5.1.5 Patients undergoing elective removal of an ovary for fertility preservation only must have two ovaries.

5.1.6 Patients who already have stored cryopreserved ovarian tissue in a frozen state prior to undergoing cancer treatments (surgery, chemotherapy or radiation) will be eligible for enrollment with informed consent.

* + 1. Signed an approved informed consent and authorization permitting the release of personal health information. The patient and/or the patient’s legally authorized guardian must acknowledge in writing that consent for specimen collection has been obtained, in accordance with institutional policies approved by the U.S. Department of Health and Human Services.

5.1.8 Are not a candidate for or choose not to utilize embryo or oocyte banking, or are having this procedure done in addition to embryo or oocyte banking

5.2 Exclusion criteria:

5.2.1 Women with psychological, psychiatric, or other conditions which prevent giving fully informed consent.

5.2.2 Women whose underlying medical condition significantly increases their risk of complications from anesthesia and surgery.

5.2.3  Women who have a large cancerous mass in the ovary that is being removed will not be enrolled in the study. That is, ovarian tissue cryopreservation will not be performed on portions of an ovary that contained a large cancerous mass since experience in this study indicates that this tissue is not suitable for patient use in the future (contains limited or no follicles).

5.2.4 Serum FSH levels above 20 mIU/ml when no chemotherapy has been administered.

**6.0 METHODS**

6.1 Informed Consent and Survey

All potential participants will be informed of the risks of the planned treatment (surgery/chemotherapy/radiation) on subsequent fertility. Information about ovarian tissue preparation, freezing and cryopreservation will be provided and the experimental nature of ovarian tissue cryopreservation will be emphasized. They will be informed of the extent to which participating in this study might be of benefit to them. In addition, they will be counseled prior to study entry for the unknown risk of possible genetic damage/fetal defects from cryopreservation and subsequent in vitro handling of the gametes and embryos. Clinical psychologists with extensive experience in issues related to Assisted Reproduction will be made available for counseling. A consent form for the study is attached.

6.2 Collection of Ovarian Tissue

If the patient chooses to participate and provides informed consent, she will be screened to determine eligibility and to determine if her medical condition significantly increases her risk of complications from surgery and/or anesthesia. The patient will also have a serum FSH and estradiol drawn, on cycle day 3 when possible, as well as an AMH, as markers of ovarian reserve to use in counseling her on her options.

Patients in ***four categories*** may participate in this study:

1. Patients who are having one or both ovaries removed for the treatment or prevention of a disease.
2. Patients who are having surgery to remove all or part of one or both ovaries for medical reasons where cryopreservation of the remaining limited portions of normal ovarian cortex is the only option for fertility preservation at the time (except that ovarian cortex from the ovary that contains the cancerous mass will not be cryopreserved).
3. Patients who are having surgery to remove all or part of one or both ovaries for medical reasons where cryopreservation of the remaining limited portions of normal ovarian cortex is the only option for fertility preservation at the time but who cannot or will not provide tissue to the research pool (except that ovarian cortex from the ovary that contains the cancerous mass will not be cryopreserved). These patients are willing to participate in the long term follow-up described in this study.
4. Patients having one ovary removed electively, solely for the purpose of fertility preservation because they are not candidates for or choose not use more mature fertility preservation technologies, or who wish to have this procedure done in addition to the use of more mature techniques

Patients in Categories 1-3 will have surgical removal of their ovarian tissue using the methods determined by their surgeon based on their medical/surgical diagnosis or treatment.

Patients undergoing elective removal of an ovary (Category 4 above) will undergo a procedure called laparoscopy to remove the ovary. This surgery will be performed under general anesthesia and one ovary will be removed in total through an instrument channel employing standard techniques of operative laparoscopy. This surgical procedure is performed solely for fertility preservation but can potentially be coordinated with another procedure such as placement of a central line for future chemotherapy or laparotomy for another purpose. Although only one ovary will be removed, both of the patient's ovaries must appear normal for the procedure to be completed. The ovary to be removed will be chosen at the time of surgery based on appearance and ease of removal. After the surgery is complete, the patient will not have any further procedures except for a routine post-operative visit. A 0.5 cm cross section from the short axis of the ovary will be provided to the Department of Pathology for routine histological evaluation, and the remaining tissue will be processed for cryopreservation. If pathology finds evidence of cancer in the ovarian tissue provided at surgery, they may request that all of the patient tissue (even tissue that has been frozen for patient use) be returned to pathology for a more detailed examination. In this case, no tissue is available for the patient to use for fertility preservation purposes.

Despite a pre-operative treatment plan to remove and cryopreserve ovarian tissue for fertility preservation, the surgeon or pathologist may determine intraoperatively that the entire ovary or a significant portion of the cortical tissue is needed for diagnostic purposes. Therefore, there may be no tissue available for cryopreservation for either patient use or research use. If only a small portion of the ovary is available after surgery or pathological sampling then a determination will be made whether there is sufficient tissue available to freeze (see below).

6.3. Cryopreservation:

Ovarian tissues will be cryopreserved using modifications of the techniques described by Gosden et al. (1994) or will be vitrified using a modification of the techniques of Kuwayama et al., 2007. The ovary will be transported from the operating room to the designated laboratory for cryopreservation.

If the patient chooses to enroll in the study, a small fraction of the ovarian tissue, not to exceed 20% of the ovary remaining after a portion has been provided to pathology will be provided to a Northwestern University research laboratory for research purposes. In addition, the medulla and wash media that would typically be discarded following processing of tissue into cortical strips for cryopreservation may be used for research purposes. The research tissue may be used fresh or may be frozen as described above. The remainder (majority (80%) of the participant’s ovarian tissue will be cryopreserved for her own potential use in the future. She may utilize that tissue at any institution for any treatment modality she chooses. The 20% of the ovary designated for research will go to a Northwestern University research laboratory and will be used for the research aims described above. None of the research tissue will be used for experiments that involve fertilization.

The ovarian cortex will be dissected from the medulla and cut into strips in culture/holding media, washed to remove blood cells, and passed through a series of cryopreservation solutions with increasing concentrations of cryoprotectants (including but not limited to propanediol, glycerol or ethylene glycol, 0 - 15%; sucrose, 0 - 0.3M) (freezing media). The tissues will be placed in cryovials or straws containing freezing media and frozen using a programmable freezer to cool tissues from 20o to -40o C using defined ramps or vitrified directly or will be vitrified by direct plunge into liquid nitrogen. The vials or straws will be placed in liquid nitrogen for storage. The procedure for cryopreservation/vitrification may be modified as improvements become available.

Quality Control procedures for the freezing and storage processes will be according to FDA regulations for reproductive tissues (Federal Register 21 CFR Part 1271 and Part 1270), guidelines of the American Association of Tissue Banks and any other applicable federal, state and local regulations. The designated Laboratory Director, John Zhang, Ph.D., is responsible for the overall quality control of all of these clinical laboratory activities, including ovarian tissue cryopreservation.

**Minimum Amount of Ovarian Tissue:** In order to ensure that the patient will have adequate tissue available for her own fertility preservation efforts, we define a minimum amount of tissue that must be available before the research portion can be obtained. This minimum is based on published information regarding amount of ovarian tissue required for two transplants of ovarian tissue (as the most conservative estimate of tissue needed to restore fertility). For this study, research tissue will not be removed unless there are at least 6 strips of cortical tissue measuring 2cm X 0.5cm. If less than 6 strips (worth of tissue) are available then the patient will indicate in advance, in her informed consent, if she wants all of the tissue frozen for her own use or if she wants it all to be used for research.

6.4. Tissue storage and infectious disease testing:

6.4.1 Ovarian Tissue Storage: Cryopreserved patient tissues will be transferred to Reprotech, Ltd. (RTL) in Roseville, MN for storage and subsequent release. Reprotech, Ltd. is an FDA compliant and American Association of Tissue Banks accredited long term storage facility for reproductive tissues. Based on the extended periods of time that these tissues are likely to be stored (patients may wait for five years from cancer treatment to be considered cancer free and begin a family; some may wait longer based on age), Reprotech, Ltd. provides maximum flexibility for the patients involved. In this way, they can store tissues as long as they wish and ship them to a fertility treatment center of their choice at the time of use. The patient can determine how her own tissue will be used as technology changes and based on her unique circumstances. Reprotech, Ltd. does not perform fertility treatments and is not affiliated with any fertility center so there is no potential conflict of interest. Patients will execute a separate storage agreement with Reprotech, Ltd which defines the length of storage, shipping requirements, infectious disease, screening and disposition of the tissues in the event of their death.

Tissues designated for research will be provided to a research laboratory at Northwestern University via the National Physicians Cooperative of the Oncofertility Consortium either fresh or frozen.

6.4.2 Infectious Disease Screening and Testing: Banking and subsequent use of ovarian tissue is regulated by the Food and Drug Administration (FDA). In order to comply with current tissue banking regulations and to be prepared for any future changes in regulations while these ovarian tissues are in storage, patients will be tested and screened for a number of infectious diseases prior to banking ovarian tissue. All infectious disease testing will be performed at Memorial Blood Center in St. Paul, MN. The testing will include but not be limited to testing for Hepatitis B and C and HIV. The screening and testing that will be performed are the same as would be performed on an anonymous reproductive tissue donor and include a physical examination and questions regarding potential high risk behaviors. The testing that will be performed will be testing that is mandated for donors of leukocyte rich tissues and must be performed within 7 days of tissue procurement. In this way, the tissue could potentially be used by the patient herself and it could also be suitable for use in another individual (such as a gestational surrogate) in the future if indicated by the patient’s medical diagnosis. (For example, use of in vitro maturation of follicles followed by IVF with embryo transfer to a gestational carrier for a patient without a uterus). In addition, a sample of the patient’s blood plasma will be stored with the ovarian tissue to permit any future testing required under federal tissue banking regulations. In spite of storing blood plasma, it is still possible that federal regulations may change and therefore, it may not be possible to perform the appropriate testing to permit heterologous use of the tissue in the future. Infectious disease testing is performed in this study to permit patient use of her own tissue and not for the purposes of research tissue or research study.

6.5. Clinical Data to be Collected

6.5.1. Safety data

6.5.1.1. Adverse events after an elective laparoscopic oophorectomy (Category 3 Patients, see 6.2) will be identified using the Common Toxicity Criteria for Adverse Events (CTCAE). A copy of the CTC version 3.0 can be downloaded from the CTEP home page (<http://ctep.info.nih.gov)>. All appropriate treatment areas should have access to a copy of the CTCAE version 3.0. The severity of the event should then be graded using the CTCAE criteria. Determination of whether the event was related to the surgical procedure and whether the adverse event was expected or unexpected will be made. Data on the time from surgery to start of definitive therapy (chemotherapy or radiation) will also be collected.

6.5.2. Other data to be collected:

Age

Diagnosis

Treatment sequence associated with high risk of sterility

Method of ovarian collection (laparotomy vs. laparoscopy)

Time from surgery to start of definitive therapy (chemotherapy or radiation)

Outcome: remission, relapse or death

Reproductive history before, during and after cancer diagnosis with long term follow up

Choices regarding use of cryopreserved stored tissues for fertility restoration

Telephone script for long term follow-up is attached

6.6 Efficacy of Cryopreservation

In 1986, Chen reported the first successful attempt at cryopreserving and thawing a human oocyte resulting in a twin pregnancy after *in vitro* fertilization and embryo transfer. Progress in the field moved slowly for the next decade after murine data suggested that cryopreservation showed higher levels of chromosomal anomalies when compared with fresh eggs (Johnson and Pickering, 1987). However work in the early 1990s by Gook et al. demonstrated that cryopreservation was not as detrimental as originally thought, leading to renewed research interest in human oocyte cryopreservation (Gook et al, 1994).

By 2004, 100 human babies had been born from cryopreserved oocytes. These infants were produced with great inefficiency (Stachecki and Cohen, 2004). For instance, Marina and Marina (2003) reported a 4% live birth rate from 99 oocytes frozen. Results with larger sample sizes were even less impressive. In fact, Porcu’s research group (1999) reported only 16 pregnancies from 1502 thawed oocytes (slightly more than 1%). Breakthroughs made by Italian researchers in optimizing freezing and thawing methods greatly improved pregnancy rates with mature human oocytes (Coticchio et al., (2006); Paynter et al., (2005); Fabbri R, et al., (2001); Bianchi et al., (2007); Borini A et al., (2004); Borini et al., (2006)) yielding pregnancy rates per thaw cycle of 19% and 22% per patient. However, these techniques make use of oocytes retrieved after hormonal stimulation utilized for in vitro fertilization which requires several weeks of treatment.

Cryopreservation of human ovarian tissue containing oocytes poses additional challenges. Oocytes have larger cell volumes, different freezing characteristics and varying membrane permeability to cryoprotectants compared to ovarian tissue. Developing a method for tissue cryopreservation that permits recovery of a maximum number of mature follicles is one aim of this study. Tissue donated to the Northwestern University research laboratory via the National Physicians Cooperative will be frozen using varying cryopreservation methods and thawed to determine the efficacy of the freeze/thaw techniques. Follicles may be isolated from the thawed tissue and matured in vitro. The number of viable follicles isolated and their performance in culture will be recorded for each tissue specimen as indices of the efficacy of the cryopreservation process. Maturation of at least 50% of the oocytes/follicles recovered from the cryopreserved tissue is expected based on the literature. Data gathered from this research will assist in identifying and overcoming some of the challenges to successful freezing and thawing of tissue with subsequent *in vitro* maturation of follicles or oocytes.

6.7. Feasibility of 3-Dimensional Systems for *In Vitro* Maturation of Immature Ovarian Follicles

Tissue provided to the research laboratory (no more than 20% of total tissue obtained from each patient) will be thawed as described by Gosden et al.(1994). Preantral and antral follicles (if available) will be isolated from the tissues and placed in culture. Factors that affect in vitro maturation of human oocytes will be investigated using the research tissue including but not limited to:

(1) **Growth factors** such IGF-1, IGF-2, EGF, GDF-9 and activin have been implicated in promoting follicular growth and oocyte maturation. Such growth factors and others will be tested individually and in combination.

(2) **Hormones** such as recombinant human FSH and LH will be included in the culture medium at different concentrations.

(3) **Co-culture** of follicles will be performed with certain somatic cells, cell lines, or other follicles that may improve *in vitro* maturation.

(4**) Three-dimensional culture environment**. Under conventional culture conditions using plastic culture dishes, most types of cells will attach to the bottom of culture dish and spread. When follicular cells attach and spread on culture dishes, the 3-D structure of a follicle is lost and cellular contact between oocytes and surrounding follicular cells is disrupted, which may be detrimental to oocyte growth and maturation. In this study, preantral follicles will be encapsulated in biomaterials to prevent attachment and spreading of the follicular cells and to maintain the three dimensional structure of the follicle. Agar has been used successfully to culture hamster and human antral follicles (Roy and Greenwald, 1989; Roy and Treacy, 1993) but it has a relatively high gel temperature (45 degree C), which may damage the cells during encapsulation. In our research studies, we will use biomaterials including but not limited to: alginate, fibrin-alginate, matrigel, and polyethylene-glycol gels. These biomaterials have been used successfully in IFM of follicles from numerous species including mouse, nonhuman primate, and human. Such biomaterials permit ready diffusion of growth factors, hormones and other factors in and out of the follicle. The 3-D culture conditions needed to maximize in follicle maturation will be examined. Endpoints studied will include hormone production, follicle size and growth rate, oocyte maturity and quality, structural changes using light and electron microscopy and gene expression. None of the endpoints will involve fertilization of the oocytes.

**7.0 STATISTICAL CONSIDERATIONS AND DATA MANAGEMENT**

7.1 Accrual

We estimate to accrue one patient per month.

7.2 Data Collection and Access

Participation in this research is confidential. All research tissues will be de-identified; participants will be identified by number, not name. No information by which the patient can be identified will be released or published in connection with this study. Only the PI and co-investigators will have access to files matching the patient with tissue specimen numbers. All data will be stored in a confidential database including the Cancer Central Clinical Database (C3D) provided by NIH/NCI for the Oncofertility Consortium. Data will be permanently stored by the PI and co-investigators.

1. **RISKS**

The patient's participation in this study may involve the following risks. Most of these are related to the general risks of surgery.

* FOR PATIENTS UNDERGOING ELECTIVE LAPAROSCOPIC OOPHRECTOMY that was required for fertility preservation only (Category 4 patients, see 6.2):
  + 1. Risks of laparoscopy include infection, damage to the patient's internal organs or bleeding problems as a result of insertion or manipulation of the laparoscopic instruments. The chance of the patient requiring hospitalization for complication(s) is about 1%. The patient's chance of dying as a result of such complication(s) is less than 1 in 10,000.
    2. Risks of general anesthesia: The patient's risk of death from anesthesia is less than 1 in 10,000.
* REMOVAL OF THE OVARY: There is a theoretical risk that the patient may experience a loss of fertility due to the removal of an ovary and/or may experience an early menopause caused by the loss of hormones produced by ovaries. The patient may regain spontaneous ovarian function. The surgery to remove tissue would then have been unnecessary. In addition, it is possible that the surgery itself could cause scar tissue or damage to the remaining ovarian tissue, so that chances for pregnancy could be reduced. These circumstances are very unlikely and much less likely than the patient’s chance of losing ovarian function as a consequence of her medical, surgical or radiation treatment of her cancer or medical condition. She may also be at risk for the psychological consequences, including emotional upset, of having one ovary removed
* CRYOPRESERVATION: Although care will be taken, damage to the removed ovarian tissue may occur during any part of the cryopreservation (freezing), shipping or storage process. The effects of cryopreservation and long term storage on human ovarian tissues are not known and possible damage to the tissues may occur. The risk of birth defect(s) and/or genetic damage to any child who may be born following such a procedure is also unknown. However, thousands of children have been born worldwide from frozen embryos and eggs and there has been no report of increased risk of birth defects in these children. The ovarian tissue removed may not yield usable eggs, or pregnancy may not result when the eggs are ultimately used. Some patients may have particular risks associated with their underlying disease. If a cancer or other disease already affects the ovarian tissue, then it may never be possible to use the tissue in the future. This may not be known until the patient wishes to use her ovarian tissue. Tissue could be lost or made unusable due to equipment failure, or unforeseeable natural disasters beyond the control of this program.
* INFECTIOUS DISEASE TESTING. Infectious disease testing may indicate that the patient has an infectious disease of which she was previously unaware and which may require treatment or may delay the start of her cancer or medical treatment. Although infectious disease testing will be performed within 7 days of removal of the ovary and additional plasma samples from the patient will be stored with her ovarian tissue, a future change in federal regulations for testing prior to use of this tissue may render the tissue unusable. Infectious disease testing and screening performed around the time of surgery may be inadequate to permit safe use of your tissue after the long term storage of the tissue. While a sample of blood plasma will be stored to minimize this risk, the tests required in the future may require a sample other than plasma, the plasma sample may be inadequate or it may be lost or damaged during cryopreservation or storage. Infectious disease testing is only required for the patient to make use of her own tissue and is not required for use of the research tissue or the research study.

There may be no direct benefit to the patient from participating in this research study, but, as described above, there is a possibility that the portion of the tissue stored for her own use may eventually be used successfully to initiate a pregnancy. Participation in this research study may indirectly help other patients, who could benefit from ovarian tissue storage by helping to advance understanding of how to successfully freeze and thaw ovarian tissue in a manner that permits future use.

The use of ovarian transplant, in vitro maturation or other new technology that cannot be envisioned now may allow the patient to use some of her cryopreserved and stored ovarian tissue in the future. However this possibility cannot be assured and the patient will be thoroughly counseled about the experimental nature of this protocol.

The participant has the alternative to choose not to participate in this study. Instead she may receive cancer treatment, chemotherapy or radiation therapy, without undergoing surgery for the removal of one of her ovaries. If she is already undergoing surgery to remove all or part of her ovary (ovaries) she can choose not to have the tissue cryopreserved. The patient may delay her treatment and undergo in vitro fertilization with freezing of embryos or oocytes. There are no other alternative methods for preserving ovarian tissue available to the participant at this time.

**9.0 REIMBURSEMENTS AND RESEARCH COST**

9.1. Reimbursement

No direct reimbursement will be made to patients or their families.

9.2. Research costs

Patients in ***four categories*** will participate in this study:

1. Patients who are having one or both ovaries removed for the treatment or prevention of a disease.
2. Patients who are having surgery to remove all or part of one or both ovaries for medical reasons where cryopreservation of the remaining limited portions of normal ovarian cortex is the only option for fertility preservation at the time (except that ovarian cortex from the ovary that contains the mass will not be cryopreserved)..
3. Patients who are having surgery to remove all or part of one or both ovaries for medical reasons where cryopreservation of the remaining limited portions of normal ovarian cortex is the only option for fertility preservation at the time but who cannot or will not provide tissue to the research pool (except that ovarian cortex from the ovary that contains the mass will not be cryopreserved).. These patients are willing to participate in the long term follow-up described in this study.
4. Patients having one ovary removed electively, solely for the purpose of fertility preservation because they are not candidates for or choose not use more mature fertility preservation technologies.

If the tissue does not meet the minimum quantity criteria (see 6.3), the patient will indicate in her consent form if the tissue is to be used entirely for research or reserved exclusively for her own use.

9.2.1 Hospital, Surgical and Physician Costs:

All costs will be billed to the patient or patient’s insurance. All non-covered services are the patient’s responsibility.

9.2.2 Patient Tissue Storage

All fees for the storage of the patient’s own tissue will be the patient’s responsibility (approximately $300 per year). Reprotech, Ltd has a financial assistance program in place to reduce this annual fee for those who can demonstrate financial need based on income.

9.2.3 Infectious Disease Testing

Infectious disease testing is required for storage and use of the patient’s own tissue and is not part of the research study. Infectious disease testing will be billed to the patient or patient’s insurance. Non-covered services are the patient’s responsibility.

9.3. Provisions for dealing with research-related injury

In the event of injury or illness resulting from the research procedures, medical treatment for injuries or illness is available through the Northwestern Memorial Hospital.

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