Oocyte Cryopreservation
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Study Site: Northwestern Medical Faculty Foundation

Introduction

The technical ability to cryopreserve gametes for future use provides important options for patients who wish to preserve their childbearing potential before facing therapy that is anticipated to result in the termination of gonadal function. Most commonly, the desire for fertility preservation is seen in individuals who are about to undergo either chemotherapy or radiation therapy for various malignancies. It has been possible to successfully freeze and thaw semen samples for many years; more recently, it has become feasible for couples to cryopreserve zygotes. Until very recently, however, for a variety of technical reasons, successful cryopreservation of mature oocytes has been problematic.

Mature oocytes can be retrieved following hormonal stimulation identical to that used for in vitro fertilization and cryopreservation (Chen, 1986). Historically, post-thaw recovery of cryopreserved oocytes with subsequent fertilization and embryo transfer led to disappointing results (Porcu et al., 1999; Marina & Marina, 2003). However, due to the ban in Italy on freezing embryos, investigators were compelled to re-explore the practicality of mature oocyte cryopreservation. These political and legal limitations thus facilitated improvements in both freezing and thawing techniques for human oocytes and have led to acceptable pregnancy rates (Coticchio et al., 2006; Paynter et al., 2005; Fabbri et al., 2001; Bianchi et al., 2007; Borini et al., 2004; Borini et al., 2006). Hundreds of babies have now been born worldwide following oocyte cryopreservation. Consequently, it is appropriate to offer this emerging technology to young women at risk for losing ovarian function following cancer treatment.

Purpose & Objective

Purpose: This study will contribute to the knowledge base surrounding oocyte cryopreservation so as to provide cancer patients and women who wish to delay childbearing scientifically sound options for fertility preservation.

Objective: To determine the long term benefits and outcomes associated with the use of oocyte cryopreservation as an option for women who wish to preserve their fertility, regardless of disease status.

Hypothesis: Oocyte cryopreservation will be an effective and safe method of fertility preservation for cancer patients and women who wish to delay childbearing, resulting in future and successful pregnancies.

Rationale: Fertility preservation is an important quality of life issue for cancer patients. 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). Women who wish to delay childbearing may also desire fertility preservation. Finally, patients who are undergoing in vitro fertilization to treat infertility but where a sperm sample is unavailable on the day of their egg retrieval (e.g. partner unexpectedly out of town, unable to collect sample, etc.) can utilize oocyte cryopreservation so that their eggs are not wasted following the egg retrieval.

In 1986, Chen reported the first successful attempt at cryopreserving and thawing a human oocyte – a twin pregnancy resulted 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 & 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. However, these infants were produced with great inefficiency (Stachecki & Cohen, 2004). For instance, Marina & Marina (2003) reported a 4% live birth rate from 99 frozen oocytes. Results with larger sample sizes were even less impressive. In fact, Porcu's (1999) research group reported only 16 pregnancies from 1502 thawed oocytes (slightly more than 1%). Fortunately, 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 et al., 2001; Bianchi et al., 2007; Borini et al., 2004; Borini et al., 2006), yielding pregnancy rates per thaw cycle of 19% and 22% per cycle which compares favorably with the natural fecundity rate of 20% per cycle.

There is little doubt that oocyte cryopreservation is now a reasonable alternative for cancer patients. The procedure is now being offered commercially throughout the United States not only to cancer patients, but also to healthy women who wish to delay childbearing. Nevertheless, little follow-up data regarding the actual efficacy of this approach or the health of infants born following its use are available. It is imperative that such data be prospectively generated, notwithstanding the difficulties inherent in the time frame over which patients might need to be followed.

The Ethics Committee of the American Society for Reproductive Medicine (2005) and Committee on Gynecologic Practice of the American College of Obstetricians and Gynecologists (2008) both stated that while oocyte cryopreservation holds significant promise for fertility preservation, the procedure is still considered investigational and may only be offered with appropriate informed consent in a research setting and with the oversight of an institutional

review board. This practice guideline necessitates that in order to transform oocyte cryopreservation from an experimental to a standard procedure, more rigorous investigation into the methods and consequences of oocyte cryopreservation must be conducted. To this end, this protocol has been designed to identify suitable candidates for oocyte cryopreservation and to systematically follow them in order to assess the long term benefits and outcomes associated with its use.

Study Design

Research Design & Methodology: We will conduct a prospective study to assess the long term benefits and outcomes of the existing oocyte cryopreservation methods for fertility preservation in women with cancer diagnoses.

Duration: Until the American Society of Reproductive Medicine determines that this procedure is no longer experimental and need not be done under IRB supervision.

Location: Female patients will be identified and consented and all study procedures will be conducted through the Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Northwestern Medical Faculty Foundation.

Participant Selection

This study will enroll women of reproductive age in three different categories:

1. Women of reproductive age who are diagnosed with cancer or any disease whose treatment or its progression may impair their reproductive potential.
2. Women undergoing standard In Vitro Fertilization to treat infertility whose partner is unable to provide a semen sample on the day of the egg retrieval so that the eggs can be cryopreserved and not lost for use. (For example, failed testicular sperm aspirations, inability to collect or unexpected need to be out of town).
3. Women seeking oocyte cryopreservation for social (lifestyle) issues such as delaying childbearing

Women seeking fertility preservation are referred for a comprehensive consultation with a Reproductive Endocrinologist to discuss the range of treatment options available to her. Only patients who choose oocyte cryopreservation as their method of fertility preservation will be enrolled in the study.

Informed Consent Procedure: Participation in this study is entirely voluntary. Choosing to not participate will not impact the care received at this institution. Following selection and counseling as outlined above, all subjects will be required to provide informed consent for participation in the study. All potential subjects will be informed of the risks of the procedure. Information about oocyte retrieval and cryopreservation will be provided and the experimental nature of oocyte cryopreservation will be emphasized. They will be informed of the extent to which they may benefit from the study. The subject will be granted time to read the informed consent document, and all questions will be answered to her satisfaction.

Inclusion Criteria:

- Women of reproductive age who are diagnosed with cancer or any disease whose treatment or its progression may impair their reproductive potential (this would include but not be limited to cancer patients requiring treatment with chemotherapy or radiation, patients with rheumatologic diseases such as lupus, rheumatoid arthritis and ulcerative colitis and patients with genetic predisposition to cancers.
    - Age 16-41
    - Girls under the age of 18 must have reached menarche and must, in the estimation of their physician and parents (and with their assent) be able to tolerate the entire procedure including vaginal ultrasounds and retrieval.
- Women over the age of 18, undergoing standard In Vitro Fertilization to treat infertility whose partner is unable to provide a semen sample on the day of the egg retrieval or who are unable to consent to freezing of embryos so that the eggs can be cryopreserved and not lost to use. (For example, failed testicular sperm aspirations, inability to collect or unexpected need to be out of town or patients with religious objects to freezing of embryos).
- Women seeking oocyte cryopreservation for social (lifestyle) issues such as delaying childbearing.
    - Age 18-39
- Patients who are carriers of BRCA Mutations predisposing them to cancer.
    - Age 18-39
- Otherwise healthy females
- Ability and willingness to comply with study protocol
- Informed written consent, prior to any study-related procedure not part of normal care, with the understanding that the subject may withdraw consent at any time without prejudice to their future medical care

Exclusion Criteria:

- Current pregnancy
- Serum FSH > 15 for patients having egg freezing for a medical indication
- Serum FSH >11 for patients having egg freezing for social reasons

Compensation to Subjects for Participation:

Subjects will not receive compensation for participation in the study. No direct reimbursement will be made to the subjects or to their families. All costs will be billed to the subject or subject's insurance. All non-covered services are the subject's responsibility.

In the event of injury or illness resulting from the research procedures, medical treatment for injuries or illness is available through the Northwestern Medical Faculty Foundation. Payment for this treatment will be the subject's responsibility.

Study Procedures

After selecting and counseling potential subjects, and obtaining informed consent, enrolled subjects will participate in the following:

Oocyte Harvesting and Cryopreservation: Subjects will undergo controlled ovarian hyperstimulation according to established clinical protocols utilized in the In Vitro Fertilization Program at NMFF. Briefly, they will be treated with variable dosages of injectable gonadotrophins over a period of 8 to 12 days. Response will be monitored using vaginal ultrasound and serum estradiol levels. When appropriate follicle maturation has been achieved, a single dose of human chorionic gonadotropin (hCG) will be administered to induce final oocyte maturation. Thirty-six hours after hCG administration, the subject will undergo standard transvaginal oocyte retrieval under ultrasound guidance. The procedure takes approximately 15 minutes and is carried out under conscious sedation with Fentanyl and Versed. The oocytes are immediately handed off to the embryology technicians in the IVF laboratory.

Oocyte Cryopreservation:

Oocyte cryopreservation will be carried out using one of the following two techniques:

Slow freezing: This slow freezing protocol will be a modification of the techniques of Fabbri et al, 2001and Porcu et al., 2003; 2004) t. The technique may be modified as needed.

Vitrification: This method that used will b that of Bagchi et al, 2008, Chian et al, 2009 and Kuwuyama, et al. 2007). These methods will be modified as needed as new studies are published in peer reviewed literature.

Oocyte Storage:

For cancer patients seeking fertility preservation and for women pursuing oocyte cryopreservation for lifestyle choices:
All cryopreserved oocytes 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. Patients can store their oocytes 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 oocytes 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 enter a separate storage agreement with Reprotech, Ltd., which defines the length of storage, shipping requirements, infectious disease, screening, and disposition of the oocytes in the event of their death.

Patients and/or their insurance carrier will be responsible for costs associated with the shipping of their oocytes (approximately $195) and an annual storage fee ($300). There is financial assistance available for those who qualify. Patients will enter into a separate storage agreement with Reprotech, Ltd. Patient samples will be stored at an NMFF laboratory for a short time prior to shipping to Reprotech and the storage agreement that is signed with Reprotech will determine the disposition of the oocytes if the patient dies while they are still in storage at NMFF (the oocytes will be shipped to Reprotech).

For IVF patients whose partner is unable to provide a semen sample on the day of egg retrieval:
Oocytes will be stored at NMFF and patients will be charged an annual storage fee (approximately $500) (this is the same fee currently charged infertility patients for embryo storage). Maximum storage period at Northwestern: 3 years. Patients have the option, at any time, to transfer their oocytes to another facility for storage or use or to request that they be discarded.

Utilization and Final Disposition of Stored Oocytes: Subjects will have access to their oocytes for the purpose of initiating a pregnancy at any point in time following their initial storage. Subjects will also have the option of having their oocytes discarded or earmarked for any appropriate research studies at any point. If the subject chooses to donate their oocytes for research studies, no such studies would involve a fertilization step. All stored oocytes will be considered the property of the subjects and will not be made available to anyone other than the subject without her approval. If the oocytes are stored at Reprotech, the separate Reprotech storage agreement signed by the patient will determine the disposition of the oocytes if the patient fails to pay the annual

storage fee or dies while they are still in storage. If the oocytes are stored at NMFF, the patient will designate in her signed consent the disposition of the oocytes if she dies, abandons them, or fails to pay her storage fee. Oocytes will be stored at NMFF for a maximum of three years. At the end of three years, if oocytes are still available, subjects will be asked to choose between utilizing the oocytes, disposing of them, or transferring the oocytes to a commercial long term storage facility.

Establishment and Maintenance of the Outcome Data: Subjects in the study will agree to maintain contact with the investigators, as outlined in the consent form. Specifically, they will agree to notify the investigators of any changes in their contact information. Any subsequent attempts to utilize the stored oocytes for the purpose of initiating a pregnancy will be recorded, along with the outcome of the attempt. All other dispositions of the oocytes will also be recorded. Individual files will be kept active until all oocytes have been utilized, disposed of in some other way, or ten years following storage, whichever comes first. Patients will be assigned a research number and data follow-up will include the use of the research number. All research data will be stored in a locked file cabinet and only the research coordinator and the PI will have access to the database.

The subject's participation in this study may involve the following risks.

Ovarian stimulation: The ovarian stimulation step often causes a sense of fullness or bloating, which usually goes away within a few days after the retrieval. In about 1% cases, patients will develop ovarian hyperstimulation syndrome (OHSS) a serious complication resulting in the accumulation of fluid in the abdominal cavity. This complication is self-limited, but severe cases may require several days of hospitalization for fluid management.

Oocyte retrieval: Risks of oocyte retrieval include infection, damage to internal organs, or bleeding problems as a result of the insertion or manipulation of the needle used to recover the oocytes. The chance hospitalization or more extensive surgery for the management of such complications is about 1/1000. Such complication(s) may necessitate a delay in further chemotherapy or radiation therapy treatments for the subject's disease. Minor complications, such as temporary abdominal pain or cramping, are common.

Conscious sedation: The sedation step is very safe and rarely results in complications. In unusual cases, sedation may result in cessation of breathing efforts (apnea), and medications to counteract the sedating drugs may need to be administered. This complication occurs in about 1/1000 cases.

Cryopreservation: Although care will be taken, damage to the removed oocytes may occur during any part of the cryopreservation (freezing) or storage process. The effects of cryopreservation and long term storage on human oocytes are not

known and possible damage to the oocytes 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. Thousands of children have been born worldwide from frozen embryos and there has been no report of increased risk of birth defects in these children. Hundreds of children have been born worldwide from the use of frozen eggs and there does not appear to be an increased incidence of birth defects but more data must be collected. The oocytes removed may not yield usable eggs, or pregnancy may not result when the eggs are ultimately used. Oocytes could be lost or made unusable due to equipment failure, or unforeseeable natural disasters beyond the control of this program.

Emotional Risks: Participation in this study may subject the participant to additional emotional risks beyond those directly related to her planned treatment.

Risk/Benefit Ratio: The oocytes stored for the patients own use can eventually be used successfully to initiate a pregnancy. Participation in this research study may indirectly help other women who could benefit from information about the efficacy and safety of oocyte cryopreservation, long term oocyte storage and use of frozen oocytes. The risks are small in comparison to the benefit and are the same as those encountered by all infertility patients undergoing routine in vitro fertilization (IVF) procedures in our Division.

Alternatives: The subject has the alternative to choose not to participate in this study.

If the subject is undergoing medical treatments (such as for cancer) she may receive chemotherapy or radiation therapy without undergoing retrieval and storage of oocytes. If she has a partner, she has the option of undergoing treatment with *in vitro* fertilization in order to cryopreserve (freeze) embryos for future use. She also has the alternative of undergoing therapy GnRH agonists prior to your cancer treatment. There is some evidence that such treatment reduces the risk of damage to the ovaries by either chemotherapy or radiation therapy. This treatment is still considered experimental. It is not approved for this use at this time. She also has the option of undergoing ovarian tissue cryopreservation which is also an experimental procedure.

If the subject is undergoing IVF, has had eggs retrieved but the sperm sample needed to fertilize the oocytes is not available or she is unable to give consent for freezing of embryos, the eggs can be discarded.

If the subject is freezing eggs for social reasons, she can decide not to do that.

Protection of Subjects: The principal investigator, co-investigators, and other members of the investigative team will only access data collected as part of this study. All information will be kept confidential and will not be shared except as

may be required by law. No information by which the subject can be identified will be released or published in connection with this study. All data will be stored in a confidential database.

Information will only be used for the purpose of this study. The following groups of people may have access to the research information: the research team, the Hospital's ethics committee (Institutional Review Board), and the Food and Drug Administration and the Center for Disease Control as required by federal law.

Data Safety & Monitoring: The principal investigator and clinical coordinator will monitor the subject records periodically for completeness.

Criteria for Terminating the Study: Once the risk/benefit ratio of the protocol becomes unfavorable, where the subject is exposed to greater harm than potential benefit, the study will be terminated. Notice of study termination will be submitted to Northwestern University's IRB. If ASRM determines that oocyte cryopreservation is no longer investigational, the study will be terminated.

Procedures for Reporting Deviations from the Original Plan: Any deviations from the original protocol that take place during the course of the study will be reported in a timely fashion to Northwestern University's IRB in the form of a revision or safety/other submission.

Conclusion

Fertility preservation ranks as one of the greatest concerns for women diagnosed with cancer. Therefore, establishing the effectiveness and safety of oocyte cryopreservation as a fertility preservation option would greatly impact the reproductive destinies of women undergoing fertility-threatening treatment. At the conclusion of the study, we hope to have sufficient data to assess the long term benefits and outcomes of oocyte cryopreservation so as to confidently offer the procedure as a safe and effective method of fertility preservation.

Anticipated Results & Potential Pitfalls: It is anticipated that oocyte cryopreservation will provide a scientifically sound fertility preservation option for cancer patients and for women who wish to delay child bearing. While the cryopreservation procedure is still experimental, early findings have demonstrated promising results. The findings from this study will contribute to the existing knowledge base by providing valuable evidence about how to perfect the cryopreservation process and increase pregnancy rates in women whose oocytes have been frozen and thawed and to document the long term outcomes from the use of these oocytes (pregnancy rates and rate of birth defects).

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