

Federal Involvement in Reproductive Tissue Preservation for Women at Risk of Cytotoxic Sterility

**Armand M. Karow, Ph.D.
Xytex Corporation
Augusta, Georgia**

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Young women receiving curative cytotoxic chemo or radiation therapy may consequently endure a lifetime of reproductive sterility. These therapeutic modalities are commonly used to treat more than 650,000 American women annually diagnosed as having cancer. Many women of child-bearing age (15-45 years) diagnosed with cancer must undergo treatments that render them permanently sterile, unable to experience pregnancy and childbirth, a defining event of human life. About 50 percent of these women will not regain their fertility even when the cancer treatment is successful; a majority will survive at least 5 years. The future fertility of prepubertal women undergoing cytotoxic therapy is also at risk. Federal statistics as recently as 2004 from the National Cancer Institute suggest that the fertility of at least 81,000 American women is annually at risk. In addition to treating cancer, cytotoxic therapies are used in preparing patients for bone marrow transplants and in treating a variety of autoimmune diseases, all with similar consequences on reproduction.

Let us look briefly at the effects of cytotoxic therapies on reproduction and then review preventive or prophylactic measures. Our focus will be on preservation of female reproductive tissues prior to cytotoxic therapy. And then I will discuss implementation of tissue preservation strategies in the new regulatory environment of the FDA.

A woman at birth has a fixed number of primordial follicles that will develop into fertilizable oocytes. The number of these follicles decreases so that at menopause she effectively is deprived of fertilizable oocytes. Oocytes and steroid-producing cells of follicles, the granulosa and thecal cells, are exquisitely sensitive to cytotoxic modalities. Women treated and cured by these therapies during prepubertal or subsequent reproductive years, have a 50 to 90 percent chance of premature ovarian failure leading to premature menopause and sterility. The risk of premature ovarian failure is modified by age, pathology and protocol. Treated women under the age of 40 have a 40-55 percent chance of returning to a pre-cytotoxic therapy menstrual pattern, but they will always have a substantial risk of premature menopause. Women not sterilized by cytotoxic therapies face an increased risk of complications during pregnancy such as early pregnancy loss, premature labor and low birth weight. For most of these women this knowledge has a profound negative effect on self-esteem.

A woman's reproductive cells are highly sensitive to alkylating chemotherapeutic agents. Their mechanism of action is so similar to that of ionizing radiation that they have also been known as radiomimetic agents: both produce free radicals that indiscriminately react with proteins and nucleic acids leading to cellular dysfunction and death.

There are two ways of protecting a woman's reproductive capacity prior to cytotoxic therapy. One of course is to physically shield the ovaries using the tools of the radiologist or of the surgeon. When radiation is directed to the pelvic area, it usually diffuses somewhat around shields. Surgical relocation of the ovaries often requires general anesthesia and post-operatively there can be ovarian migration to their orthotopic position. A variation is extirpation and cryopreservation of an entire ovary with subsequent autologous transplantation with the intent of providing physical protection from either therapeutic modality. In contrast to tissue cryopreservation, cryopreservation of entire organs is not likely to be a clinical reality anytime soon. Another interesting course of action is to pharmacologically protect the ovaries. A variety of pharmacological strategies have been attempted but clinical utility remains elusive.

Regardless of these clinical disappointments, physical isolation and storage of female germinal material offers viable approaches to protecting the fertility of women subjected to sterilizing cytotoxic therapy. Similar to extirpation and subsequent transplantation of an entire ovary, these methods involve cryopreservation; but tissue cryopreservation, not organ cryopreservation. For many reasons, thawed tissue is viable as demonstrated for sperm, embryos, ovarian tissue, parathyroid tissue, skin, cornea and bone marrow. There are three effective approaches to isolation and storage of female germinal tissue.

The most successful approach in terms of having a take-home baby is cryopreservation of embryos provided by IVF. Transfer of thawed embryos can yield a 40 percent pregnancy rate. It requires several resources that may not be available to the cancer patient. First is the time, several weeks, necessary to produce 20 or so mature oocytes for fertilization. Second is the need for a sperm donor acceptable to the patient; this almost always means the patient must be married. Of course, the patient must have reached puberty.

The second approach to isolation and storage of female germinal tissue is to cryopreserve fertilizable oocytes obtained through IVF. These frozen oocytes can be stored until fertilization with acceptable sperm is appropriate. Again, the patient must have attained puberty. For physiological and biophysical reasons, many relating to the very large size of oocytes, these cells are readily injured by the freezing process so that the likelihood of a pregnancy with thawed oocytes is only 3 percent. Like embryo storage, obtaining oocytes requires several weeks of hormonal stimulation to produce twenty or so eggs for processing.

A third approach to isolation and storage of female germinal tissue is to cryopreserve ovarian cortex. This experimental approach has been proven many times in mice, sheep, monkeys and even elephants. Pioneering work in humans has occurred at

Cornell University Medical Center and at the Jones Institute. The first baby from this procedure was born last September in Belgium and was normal. An Israeli mother treated with autologous transplantation of cryopreserved ovarian tissue is near parturition at this time. The preferred procedure is cryopreservation of ovarian biopsy material. The ovarian tissue can be taken on a day's notice using an ultrasound-guided procedure even from pre-pubertal women. The ovarian tissue survives cryopreservation quite nicely because, relative to oocytes, the cells in the tissue are small. When thawed, it has been autologously re-implanted orthotopically or in the forearm or in the abdominal wall. The development of follicles is under normal endocrinologic control. For tissue implanted orthotopically, as in the Belgian and Israeli women, fertilization can take place naturally. When the tissue is in a subcutaneous site, follicular development can be palpated and visualized. In this instance mature follicles are aspirated and fertilized in vitro. The greatest risk to the tissue is the ischemic interval after transplantation. A second risk is the transfer of neoplastic cells that have metastasized to the tissue; diagnostic laboratory tests are being developed for this.

Any of these three procedures for isolation and storage of female germinal tissue can be implemented by most IVF laboratories since their components are routinely practiced. In fact, the ovarian tissue procedure can be implemented in most Gyn practices since the physician needs only to acquire the tissue, and then send it to a cryopreservation laboratory. Although clinically simple, the ovarian tissue approach should currently be performed with IRB approval since it is technically experimental. This is also true of oocyte cryopreservation.

Regardless of the clinical simplicity of preserving female germinal tissue, a layer of complexity is being introduced by the US Food and Drug Administration. On 25 May 2005 the FDA will for the first time begin regulating human reproduction tissue banks serving American physicians and their patients. These regulations, officially Part 1271 of title 21 of the Code of Federal Regulation (21 CFR Part 1271), directly affect IVF laboratories and other tissue processors. In fact, it is important to note that it is the processor that is being regulated, not the tissue nor the process. In reality the regulations are minimal for those programs that isolate, store and autologously transfer female germinal tissue, in other words, returning the tissue to the donor herself; the donor is the recipient.

Let us first look at how 1271 is put together and then look at its requirements for processors of autologous female germinal tissue.

Part 1271 is organized into five subparts. Subpart A is entitled "General Provisions" and gives an overview of the purpose and scope of the Part. In short, the intent is to prevent transmission of infectious diseases through transplanted tissues. Of course, there is a major emphasis on sexually transmitted infections. However, since the process in question deals with autologous tissue, or tissue involving sexually intimate partners, the FDA recognizes that the risk of transmitting infection is essentially nil. The FDA also recognizes that some of the ex vivo processing could possibly alter the tissue, but that in reality, the processing could be characterized as "minimal manipulation," to

use their terminology. Processors that minimally manipulate tissue must be registered with the FDA and potentially are subjected to all of 1271.

Subpart B gives the “Procedures for Registration and Listing”; this lets the FDA know who is processing and where it is being done. Subpart C deals with “Donor Eligibility”; Subpart D, “Current Good Tissue Practices”; and Subpart E, “Additional Requirements.” The FDA has exempted processors of autologous tissue from Subpart C, donor eligibility, presumably because of its low risk of disease transmission. The FDA has also exempted processors of reproductive tissues from Subparts D and E. So this leaves processors of autologous human female germinal tissue responsible for complying with Subparts A, B and F. Subpart F grants FDA with the authority to inspect and to enforce part 1271. Federal inspections, therefore, are a realistic possibility; but the substance of inspections, Subparts C, D and E are not included in the inspections!

In summary, a processor of reproductive tissues is regulated by 21 CFR 1271, subparts A, B and F, if the tissue is minimally manipulated. IVF programs minimally manipulate reproductive tissues and therefore are subject to FDA inspections.

The FDA offers an alternative for physicians using ovarian tissue that obviate his or her compliance with 1271 entirely. Even when autologous ovarian tissue is minimally manipulated, the clinical practice does not have to be involved in that process; it can be contracted to an independent program that *is* prepared to be regulated under 1271 by the FDA. The FDA clearly states that “you are not required to comply with the requirements of this part [1271] if you are a [program] that does not recover, screen, test, process, label, package or distribute, but only receives or stores [tissue] solely for implantation...within your facility.” The reference program regulated by the FDA recovering and processing the tissue can be off-site or even co-located within your facility. The important distinction is that the reference program be operated independently of your practice and that it is recognized by the FDA as being responsible for tissue recovery and processing. Such a reference program may be your key to enabling women to avoid sterility regardless of their need for cytotoxic therapy.