Storage of cryopreserved reproductive tissues: evidence that cross-contamination of infectious agents is a negligible risk

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A misconception in the field of reproductive medicine is that there is a significant risk of cross-contamination during gamete or embryo cryostorage. This article is a review of the available literature on animal models and human IVF and it suggests otherwise. There is a negligible risk of cross-contamination in IVF working conditions. (Fertil Steril® 2010;94:1181–8. ©2010 by American Society for Reproductive Medicine.)

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Increasing regulatory oversight of reproductive medicine by agencies like the United States Food and Drug Administration (FDA) is apparent with the regulations for the testing and screening of tissue donors entitled *Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)* found in 21 CFR part 1271, subpart C (1). For advanced reproductive technology (ART) programs, these regulations focus on the policies and methods attendant to identifying eligible gamete or embryo donors based on direct testing and identification of risk factors for relevant communicable disease. Additional regulations exist that focus on the laboratory practices for the preparation and storage of tissues, found in Subpart D of 21 CFR Part 1271, *Current Good Tissue Practice for Human Cell, Tissue, and Cellular and Tissue-Based Product Establishments; Inspection and Enforcement* (herein called, Good Tissue Practice). Although these latter regulations are not, for the most part, currently being applied to clinical ART laboratories, the FDA could attach these regulations to the field of ART in the future.

One of the prominent features of the FDA Good Tissue Practice regulations is how the regulation relates to tissue cryopreservation and storage. The intent of the regulation is to prevent the transmission or cross-contamination of samples by infectious disease agents during the processes of preparation, culture, and storage. For several years, there have been anecdotal discussions at various meetings and on e-mail list servers about the potential for cross-contamination in liquid nitrogen storage tanks. These discussions have been fueled by incidents of contamination in two reports: one reporting contamination under entirely unrelated conditions (e.g., blood banking) (2), and the other reporting contamination from contrived scenarios that bear no relation to embryo culture or embryo cryopreservation conditions (3, 4). The intent of this article is to explore the risks that oocytes and embryos pose as vectors for disease and to discuss the relevant evidence regarding cross-contamination in liquid nitrogen storage systems and hopefully dispel any potential myths about the risks of embryo culture and embryo cryogenic storage.

FDA REGULATIONS ON DONOR TISSUE

In 1997, the FDA proposed the regulation of all donor tissue in the United States (5). These regulations grouped all donor tissue together—reproductive tissue (oocytes, embryos, and semen) as well as nonreproductive tissue (e.g., bone marrow, skeletal muscle, ocular tissue). The regulation became effective on May 25, 2005. At present, reproductive tissue is exempted from the portion of the regulations related to Good Tissue Practices, except for donor eligibility determination. The purpose of these rules, as stated in the FDA regulations, is to "prevent the introduction, transmission, and spread of communicable diseases by HCT/P's [human cells, tissues, and cellular products]." (1).

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EMBRYOS AS UNIQUE TISSUE

When one is considering embryos as vectors for pathogens, it is important to remember that reproductive tissues are quite different from the other tissues regulated by the FDA. Differences between reproductive and nonreproductive tissues center on the embryo's sensitivity to toxicants, the lack of any blood supply, and the small number of cells contained in an embryo. Another major difference is that, except for blood and bone marrow, most nonreproductive tissues are harvested from cadavers. These differences affect the potential risk of these tissues acting as pathogen vectors. The potential result of a general regulation like 21 CFR 1271, and especially the Good Tissue Practice, is that time and resources will likely be spent on meeting requirements and performing procedures that might have no effect on increasing the safe use of donated reproductive tissues. In addition, this approach may even damage reproductive tissues because of this overgeneralization.

Potential concerns regarding the contamination of embryos with pathogens have been voiced (6), but caution must also be used to ensure that proposed methods and new regulations do not just reduce a hypothetical risk while limiting the ability of infertile couples to have children. The introduction of technologies suitable for protecting nonreproductive tissue but ill-suited for embryos could increase the risks that embryos are damaged. This would result in either reduced viability of embryos, destruction of sensitive embryos, or even the induction of developmental anomalies in resulting offspring. For example, although formaldehyde could be used for sterilization of some surfaces in the manufacture of skeletal tissue, it would certainly be inappropriate to use it in the vicinity of human embryos. Formaldehyde, as a volatile genotoxic substance could, when used near embryos, produce mutagenic effects that could result in chromosomal aberrations and malformations in children (7). In many laboratories, isopropyl alcohol and other cleaning agents are not allowed due to concerns that these volatile substances, even in low concentrations, could damage embryos. In addition, all items that contact embryos during the IVF process are subjected to bioassays to ensure that embryo toxins do not enter the laboratories and damage human embryos.

RISKS OF USING UNPROVEN METHODS

One problem in grouping reproductive tissue with nonreproductive tissue is that one may potentially do irreparable damage to someone's embryos by not taking into account such differences as the increased sensitivity of reproductive tissue to toxicants. Little is known regarding the optimum in vitro conditions for culturing and preserving human embryos as research on them is fraught with social issues. Consequently, most of our knowledge is derived from research on embryos from either rodents or livestock species. This does not leave much leeway for modification of current methods, as any modification may result in damage or destruction of the embryos being studied. An illuminating example where a change in method can have dire consequences is a study that showed how a culture medium used with mouse embryos resulted in the improper expression of imprinted genes when compared with another medium (8). In cattle and other livestock, it appears that some in vitro culture conditions may cause what has been termed abnormal offspring syndrome. These animals are not only unusually large, but they also exhibit organ defects (9, 10). Thus, any modification to current protocols should be studied before implementation to ensure that changes do not harm embryos or cause abnormalities in resulting children.

There are five major types of donor reproductive tissue, sperm (or semen), testicular tissue, ovarian tissue, oocytes, and embryos. Among these types, semen, testicular tissue, and ovarian tissue should be considered separately from embryos and oocytes. The potential for semen to act as a vector and to infect a recipient is real. The mass of semen is large when compared with oocytes and embryos. It is not collected as a sterile tissue and often contains other blood components that can become infected with pathogens. Although cryopreserved ovarian and testicular tissues for fertility preservation are still experimental and are performed only in few ART programs, these tissues also contain blood components. Thus the storage of these tissues may need to be considered differently than oocytes or embryos.

Although embryos and oocytes are some of the largest cells in the body, they are only about 200 μm in diameter, about the size of a speck of dust. Most donor tissue regulated by the FDA is large relative to the size of oocytes, sperm, and embryos. Compared with skin or even a cornea, the embryo is miniscule. Another characteristic of reproductive tissue that differs from other donor tissue is that these reproductive tissues are usually completely free of blood, one of the most common carriers of pathogens. In addition, unlike nonreproductive tissue, reproductive tissue remains in the body for a relatively short time and is placed into areas where the blood supply is not disrupted. For example, with nonreproductive tissue such as bone marrow, large numbers of cells that might contain potential pathogens are placed in contact with the recipient's blood supply. This increases the possibility for infection as some of the usual body defenses are bypassed, especially in the cases where allografts are used and the recipient's immune system is impaired to minimize donor tissue rejection. The microscopic embryo, on the other hand, is transferred atraumatically (usually without blood) into an immune-competent recipient.

For a pathogen from a gamete to infect a donor it must [1] contaminate the gamete or embryo and then [2] the pathogen must be able to infect the recipient's tissue. It should be pointed out that even if a donor has a disease, the pathogen must become attached to the donor tissue for it to be passed to a recipient. Many pathogens are tissue specific and will only bind tissue for which the pathogen has receptors. The outer covering of eggs and embryos, the zona pellucida (ZP), has been shown to act as barriers to many pathogens. If a pathogen has somehow contaminated a donor tissue like an embryo, it must then either be passed along in the medium that envelops the embryo or be attached to the outside of the