Chapter 24
What is sperm banking? When and how is it (or should it be) used in humans? Animals?

Sperm banking, consequences of its use in clinical and animal practice

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Definition and history

Sperm banking, more formally referred to as sperm cryopreservation, is a process intended to preserve sperm function by freezing and storage at ultra-low temperature. Upon thawing, sperm are introduced into a suitable recipient female by insemination into either the endocervical canal or the intrauterine cavity, or are used to inseminate oocytes during in vitro fertilization. Sperm freezing originated in the late eighteenth century. However, the development of many sperm banking applications did not begin until after 1950, following the discoveries that glycerol can act as a cryoprotectant and ultra-low temperature liquid gases, especially liquid nitrogen, were suitable for freezing and long-term storage.

The benefits of sperm cryopreservation include:

- almost indefinite storage (at least multiple decades), allowing preservation of fertility potential that would be lost due to onset of disease, infertility, gonadotoxic exposures, surgery, or death;
- ability to "quarantine'' the sperm while the human or animal donor can be tested for semen-borne infections or genetic anomalies;
- acquisition of sperm in advance for subsequent use;
- shipping to distant locations.

The process of sperm cryopreservation

In spite of the important uses of cryopreserved sperm, little is known about the physical and biochemical events that occur during sperm freezing, storage, and thawing, or about how to adequately detect cryogenic damage. Sperm from most species survive current cryopreservation protocols very poorly, and best efforts usually result in recovery of only about half of the original sperm motility. Sperm function is also impaired, as manifested after thawing by shortened longevity, poor cervical mucus penetration and reduced membrane stability.

The goals of sperm cryopreservation methods are to prevent lethal intracellular ice crystal formation, to control wide fluctuations in cell volume, and to reduce membrane damage that accompanies temperature-induced phase changes. The biochemically and physically diverse compartments of the sperm cell (acrosome, nucleus, mitochondrial-flagellar network) complicate the process, since they may respond quite differently to freezing and thawing. The sperm also are subject to damaging oxygen radical exposure during their transit through wide temperature changes. Attempts to maximize post-thaw survival have led to the development of sperm cell diluents (semen extenders), cryoprotectants, and various rates of temperature change to control alterations in extracellular and intracellular solvents and solutes.

In a typical slow-freeze cryopreservation protocol, the semen is mixed with a buffered diluent or extender that contains lipids (often egg yolk), a penetrating cryoprotectant such as glycerol and an energy source such as fructose or glucose. After dilution, the sperm initially undergo rapid shrinkage as intracellular water leaves the cell, and then slowly return to their original volume as the glycerol enters. Rapid cooling is initiated at a rate of about - 20°C per minute. Extracellular formation of ice crystals begins and, as water freezes, the solutes present in the liquid phase surrounding the sperm rapidly become concentrated. Glycerol lowers the intracellular water freezing point, thus the cells remain unfrozen and become supercooled well below their actual freezing point. In response to high extracellular solute concentration and the osmotic tendency of supercooled intracellular water to leave the cells, sperm undergo a second volume adjustment as water moves outward, and the cells become dehydrated. When extracellular water freezes and therefore solidifies, an exothermic reaction known as the “heat of fusion'' occurs, which can cause serious disruption of the cells, unless deliberately reduced by

controlled cooling of the environment. Upon reaching the temperature of liquid nitrogen, -196oC, the sperm are placed in liquid nitrogen or vapor phase storage, where they are presumed to reside in a quiescent state of minimal molecular motion indefinitely.

During thawing, the sperm are subjected to similar rapid and dramatic changes in cell volume and membrane permeability. When the extracellular ice melts and becomes liquid, solute concentrations are rapidly diluted and water rushes into the sperm. As the temperature rises, and as glycerol leaves the cells, the sperm cell volume continues to expand. In order for sperm function to be restored, the surface area and volume must return to normal, the membrane proteins and lipids must redistribute to restore molecular structure and mobility and bioenergetic demands must be met.

For maximum functional recovery to take place, both the freezing and the thawing protocols must be optimized, a very difficult task given the paucity of data available about these processes. Recent research has focused on mechanisms of freeze-thaw damage and alternate freezing methods such as vitrification, ultra-rapid freezing without a cryoprotectant.

Human clinical applications of sperm banking

Clinically, sperm banking encompasses donor sperm or client depositor (autologous) sperm cryopreservation. In either case, sperm are obtained most commonly by masturbation, but alternatively can be retrieved by electro-ejaculation or through surgical procedures such as epididymal aspiration, testicular aspiration or extraction. In the USA, the Food and Drug Administration (FDA) regulates human sperm banking under Human Cells, Tissues, and Cellular and Tissue Based-Products (HCT/Ps) Regulations. Under the authority of Rule 1271, sperm banks must register with the FDA, adhere to guidelines for donor screening and follow standards for processing, storage and distribution. The American Association of Tissue Banks (AATB) has standards for both donor and client depositor sperm banking, and accredits banks by inspection. Several states also require separate licensure with inspection. Many other countries also have regulations about sperm banking.

The most common use of donor sperm is treatment of infertility caused by absent or defective sperm, and for individuals or couples without a sperm producing partner, especially single women and women only couples. In a 1987 survey, the United States Office of

Technology Assessment estimated that 30,000 births resulted from artificial insemination with donor sperm, with approximately 11,000 physicians providing the treatment to about 86,000 women. A more recent study estimates that roughly half a million women were estimated to have used donor or mixed (husband and donor) sperm. However the U.S. does not maintain records on the usage of donor sperm, thus these numbers are extrapolated through surveys.

The practice of "quarantining" cryopreserved sperm for donor insemination arose in the mid-1980s after the emergence of human immunodeficiency virus (HIV). The incubation period before the disease could be detected made screening at the time of collection unreliable. The recognition that this problem also exists for most other semen-borne infectious diseases led to the use of a cryopreservation quarantine to improve safety. This practice permits repeated examination of the donor for disease exposure over weeks, months or years before the sperm are used. After a minimum of 180 days quarantine, the sperm can be used as long as the donor retesting is negative for infectious disease. As nucleic acid testing for diseases improves and replaces traditional serologic testing, reduction of the quarantine period may be possible.

The ability to store sperm from men with many different phenotypes and genotypes increases the selection that patients have in choosing a donor and reduces excessive use of any single donor within a limited geographic area with resulting risk of consanguinity in future generations. Population statistics can estimate this risk; generally, sperm from a single man should be limited to 10-15 pregnancies in a medium-sized city (500,000 to 1,000,000 inhabitants) in the United States. In other countries where ethnic diversity and ethnic intermarriage are not as common, the number could be smaller, but it depends on the live birthrate, number of inhabitants and ethnic composition.

Usually, sperm banks attempt to package donor sperm in plastic vials or straws containing at least 10 million motile sperm post-thaw; this has been accepted as the minimum adequate insemination dose. Frozen- thawed sperm have shorter longevity and less ability to penetrate cervical mucus than fresh sperm, making the route and timing of insemination critically important in achieving a successful pregnancy. Using urinary luteinizing hormone (LH) measurement to predict ovulation, and one or two intrauterine inseminations (IUI) within 20 to 40 hours after the LH surge, approximately 70% of patients who elect donor sperm insemination conceive, most within six insemination cycles.

Client depositor sperm banking, where a man preserves his own sperm for later use, is useful in the following situations:

1) Medical disorders that inherently, or through the treatment used to cure or stabilize the disease, can impair fertility by causing decreased sperm count and function, early fetal loss, genetic mutation, or impotence. Common examples include cancers, Hodgkin's and other lymphomas, leukemia and myelodysplastic disorders, nephrotic syndrome, diabetes and multiple sclerosis. The new medical practice of oncofertility promotes fertility preservation as an essential part of oncology treatment, and hopefully will increase the utilization of sperm banking.
2) Prior to elective sterilization such as vasectomy.
3) Anticipated exposure to hazardous environments. Occupational exposure to radiation, pesticides, and chemicals can affect sperm function or genetic integrity. Men engaging in military operations where risk of death or exposure to sperm toxicants exist are also candidates for sperm storage.
4) Fertility treatments that require semen collection at a specific time. For men who develop anxiety-related impotency or emission failure, sperm banking ensures that treatment cycles can proceed as planned. Patients whose occupation require unscheduled travel also use sperm banking to reduce the risk of cancelled treatment cycles.
5) Gender affirmation surgery and/or hormone replacement therapy (HRT).

The relatively few referrals for client depositor sperm banking may be linked to concerns about the quality and utility of sperm in men with systemic diseases. Although sperm count, motility and physiology may be impaired before treatment is initiated, the technological advances in assisted reproduction, such as direct single sperm injection into the ooplasm (intracytoplasmic sperm injection, ICSI), greatly improve chances of successful pregnancy with limited numbers of sperm. Having many sperm stored is definitely an advantage since it may reduce the need for in vitro fertilization or allow multiple cycles of fertility treatments. However, the desire to bank multiple ejaculates with adequate abstinence periods between collections, must be balanced against the urgency of treatment initiation. Given the increasing number of

sperm banks, reproductive centers and laboratories and overnight mail-in banking services, all men and boys who might have compromised fertility as a consequence of their disease or its treatment should be offered reproductive consultation and the opportunity to cryopreserve as many sperm samples as possible.

Sperm banking in animals

Sperm cryopreservation has important uses in the livestock industry, especially in the breeding of cattle, pigs, sheep and poultry, and in animal husbandry for domesticated animals such as horses, cats and dogs. Sperm from genetically desirable or "prized'' animals can be used to inseminate many females to increase the number of offspring with the desired characteristics. The ability to easily transport sperm has permitted the improvement of existing herds or the establishment of new herds in regions of the world needing development of native food sources. Sperm banking has also become an important way to perpetuate endangered or exotic species in the wild and in zoological parks and increase genetic diversity.

The ability to use sperm banking to preserve important research animal strains has been appreciated recently. Sperm cryopreservation can reduce the extraordinary cost of maintaining genetic lines that would otherwise be preserved by continual breeding of the animals. It also increases the accessibility of various strains to researchers since frozen sperm are easier to transport than live animals. Sperm banking reduces the risk of losing a valuable genetic line through catastrophic accident, impaired reproductive efficiency, genetic drift or disease. Because the millions of sperm normally present in a single ejaculate also represent millions of meiotic recombination events, cryopreserved sperm can be stored for future studies of gene recombination frequency and mapping of genetic loci when new DNA probes become available.

Summary and opportunities

Research efforts to improve sperm banking techniques and post-thaw survival have intensified in the past few decades and have been renewed by the emergence of oncofertility as a medical specialty. As protocols improve, the success of cryopreserved sperm applications will undoubtedly increase. Numerous sperm banking career opportunities exist for basic and applied research, as well as for

clinicians and entrepreneurs in both human and animal applications from the laboratory to the bedside or "barnside".

Suggested reading

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