FROZEN Sperm and FROZEN Eggs:
LATEST DEVELOPMENTS

by Haimant Bissessar

In 1776, the Italian priest and physiologist Lazzaro Spallanzani was the first to observe that human sperm cooled by snow became motionless.[1] However, the actual freezing of sperm did not occur until the mid-1800s. A major breakthrough came in 1949 when Christopher Polge, a graduate student, serendipitously discovered that if he added as little as 10% glycerol to a suspension of rooster sperm and then froze them to -80°C, almost all of the sperm exhibited normal motility when thawed.[2] The freezing process was further refined in 1953 by American scientists Drs. J. Sherman and R. Bunge, who froze human sperm with 10% glycerol on dry ice, and reported a 67% survival rate. They subsequently reported three pregnancies with artificial insemination using frozen-thawed sperm.[3]

The cryopreservation of sperm skyrocketed in the 1980s with the onset of the human immunodeficiency virus (HIV) epidemic,[4] and the devastating consequences of the sexually-transmitted acquired immune deficiency syndrome (AIDS). Cryopreservation is a specific term that refers to the freezing, storage and thawing processes.[5] It is no longer acceptable to use fresh semen for assisted conception due to the potential risk of transmitting any infectious agents (HIV, hepatitis virus), which may be present in the semen, to the recipient. Donated semen for use in assisted conception is required by law to be frozen and quarantined for at least 180 days, and the donor testing negative for infectious diseases before the semen can be released for use by the recipient.[6]

Today, cryopreservation of human sperm is routine. While sperm from donors make up most of the frozen sperm, sperm from men facing voluntary sterilization, exposure to reproductive hazards, or undergoing cancer treatments that could reduce fertility potential, are also frozen and stored for future use.

In contrast, oocyte (egg) cryopreservation technology has lagged behind that of sperm cryopreservation.[5] The human egg is the largest cell in the body and is composed mostly of water.[5] When the slow-cooling sperm cryopreservation technique was used to freeze eggs, very few eggs survived. The water turned to ice upon freezing, and this intracellular ice formation disrupted the eggs' metaphase spindles and membrane structures.[5,7] To reduce the formation of ice crystals, cryoprotectants ("antifreezing solutions") were used to replace the water in the egg, but it was impossible to remove all of the water. Thus, post-thaw egg survival and fertilization rates were low. However, despite these poor early results, the

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first successful pregnancy from human egg freezing was reported in the medical journal *Lancet* in 1986,[8] and the first pregnancy using microinjection of sperm into a frozen and thawed egg was reported in Italy in 1997.[9]

More encouraging results using slow freezing of eggs were published in 2003 by Dr. Jeffrey Boldt, one of the early pioneers in egg freezing in the US. He reported a thaw survival rate of 74%, and fertilization rate of 59%. Overall, four of his 11 infertile patients delivered five babies, for a pregnancy rate of 36%.[10]

Other scientists looking at ways to overcome the formation of intracellular ice and its damaging effect on egg survival explored a freezing technique first investigated and described in the 1930s by Basile Luyet, the founder of cryobiology.[11] This technique, vitrification, or ultra-rapid freezing, is a process that produces a glass-like solidification of living cells that completely avoids ice crystal formation during cooling.[11] This is accomplished by exposing the eggs to high concentrations of cryoprotectants, and high cooling rates. Vitrification is performed by immersing the device containing the egg directly into liquid nitrogen at minus 196°C, a procedure that leads to cooling rates of 15,000 to 30,000°C per minute.[12] The first successful cryopreservation by vitrification was reported in 1985 by Rall and Fahy, who successfully cryopreserved mouse embryos.[13]

The first baby born from a vitrified egg was in Italy on June 20, 1999. The baby girl was delivered by Caesarian section at 37 weeks gestation, to a 47-year-old mother who received the donated egg.[14] In South Korea, three women became pregnant with vitrified eggs which were stored between one and nine months before thawing. Two of the women delivered healthy male babies in August and December of 1999, and the third pregnancy was ongoing at the time the study was reported.[15] Subsequently, other scientists have reported comparably high implantation and pregnancy rates in both fresh and in vitrified/warmed eggs.[16,17]

While many studies have shown that vitrified eggs have a higher survival rate than slow-cooled eggs, fertilization rate and embryonic development in vitro[18,19,20] in at least one recent study (2012) has shown that with a modified slow-cooling protocol, the results obtained with slow freezing are comparable to those reported in the literature using vitrification.[21]

Regardless of which freezing method is used, oocyte (egg) cryopreservation offers many advantages and options to women. Women may wish to cryopreserve their eggs for future use in treatment for a number of reasons, including if they:

- are about to lose ovarian function from surgery, chemotherapy or radiotherapy
- face early menopause
- wish to postpone childbearing age
- face certain ethical issues relating to the storage and potential disposition of embryos created through IVF treatments

The establishment of donor oocyte cryobanks, or egg banks, allows frozen donated eggs to be quarantined until the appropriate infectious diseases-screening of the donor is completed, as is currently done with frozen donor sperm.[22] Egg banks can also aid the more efficient sharing of donor eggs between multiple recipients, improving the cost effectiveness through better use of resources.[22]

US egg banks follow the Food and Drug Administration (FDA) regulations for infectious screening of oocyte donors (Code of Federal Regulations Title 21, Part 1271),[23] or the American Society of Reproductive Medicine (ASRM) guidelines on oocyte donation.[24] However, since 2008 the ASRM has classified the technology of egg freezing as experimental and investigational.[25] The ASRM recognized that there is potential for clinical application of cryopreserved oocytes, but concluded that it will need to be studied in an adequate number of patients for a sufficient

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length of time to determine whether the development of children is comparable to that of children conceived from established assisted-reproduction techniques.[25] Some scientists are questioning whether the time has come to remove the experimental label from egg cryopreservation.[26,27] To support their argument, they reference studies[28,29] from multiple centres where over 1000 babies were born from frozen oocytes, with no apparent increase in the rate of congenital anomalies when compared to statistics on natural conceptions as reported by the US Centers for Disease Control (CDC).[26]

Fertility clinics in Canada have been slow to offer their patients the option of using frozen donated eggs. One reason for this is the limited availability of donated eggs as a result of the prohibition on payment to egg donors in Canada.[30] The other reason is that although regulations in Canada governing the screening and infectious disease-testing of egg donors and cryopreservation of eggs have been addressed in the standards for tissues to be used in assisted reproduction, developed in June 2010 by the Canadian Standards Association (CSA) in collaboration with Health Canada,[31] these standards are still in draft form and have yet to be approved and implemented.

Canadian women needing frozen donated eggs can now contact a Canadian fertility clinic offering imported eggs. As more and more Canadian clinics are offering this, patients should make inquiries at their clinic about this option. With over a hundred egg banks in the US offering frozen eggs from thousands of women, the likelihood of finding a suitable match is high.

However, this is a very expensive option as the frozen donor eggs are usually sold in batches of six, which can cost as much as US$15,000 to 20,000, not including shipping and administrative costs. Caveat emptor, or "let the buyer beware," is appropriate in the absence of government regulations and oversight, so women should thoroughly investigate the egg bank they plan to use.

Cryopreservation of sperm, eggs and embryos has had a significant impact on the way reproductive endocrinologists, urologists and oncologists practice.[32] Sperm cryopreservation is a well-established practice that has afforded men the option to store their sperm for future use, and women access to donated sperm. Egg cryopreservation, despite being labeled experimental, is of great potential to women whose fertility is compromised due to disease or age, and for women seeking donated eggs. ■

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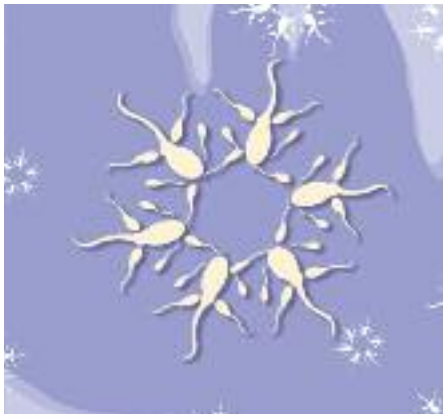

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