Is vitrification of oocytes useful for fertility preservation for age-related fertility decline and in cancer patients?

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The aim of this review is to provide current knowledge on oocyte cryopreservation, with special emphasis on vitrification as a means to preserve fertility in different indications. Major advancements achieved in the past few years in the cryolaboratory have facilitated major changes in our practice. Areas such as fertility preservation for social or oncologic reasons, the possibility to create oocyte banks for egg donation programs, the opportunity to avoid ovarian hyperstimulation syndrome, or to accumulate oocytes in low-yield patients, or even to offer treatment segmentation by stimulating the ovaries, vitrifying, and then transferring in a natural cycle are some of the options that are now available with the development of cryopreservation. We present general experience from our group and others on fertility preservation for age-related fertility decline as well as in oncologic patients, confirming that oocyte vitrification is a standardized, simple, reproducible, and efficient option. (Fertil Steril® 2013;■:■–■. ©2013 by American Society for Reproductive Medicine.)

Key Words: Fertility preservation, oocyte vitrification, cancer patients, social freezers, fertility decline

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Fertility preservation (FP) is an emerging, rapidly evolving branch of reproductive medicine comprising the preservation of gametes (sperm, oocytes) and reproductive tissue (ovarian, testicular), giving individuals at risk of losing their reproductive ability the chance to conceive and have their own genetic offspring. Cancer patients who are to undergo surgery or start chemotherapy or radiotherapy, women with other medical conditions leading to premature menopause, and healthy women wishing to postpone childbearing are the main beneficiaries of this strategy. Options for women to safeguard their fertility include the cryopreservation of ovarian tissue or oocytes.

The introduction of vitrification into assisted reproduction (AR) has established efficient female gamete cryopreservation, which provides outcomes similar to those achieved with fresh oocytes and opens up a wide range of applications, including for FP candidates.

The present review addresses the clinical use of oocyte vitrification in the FP context for nononcologic and oncologic patients.

BACKGROUND

History

Since its inception, assisted reproduction has accomplished important advances, true milestones to help increase many couples' means to conceive. Cryopreservation is one of these means and preserves biologic materials at cryogenic temperatures to completely stop biologic reactions. From 1938 to 1945, scientists observed that sperm survived freezing and storage at temperatures as low as −160°C. The first major breakthrough came in 1949 when Polge developed a method using glycerol to protect semen. Embryo cryopreservation has been widely and successfully applied since the very beginning of AR, and the first pregnancy after cryotransfer was published in 1983 (1). In 1985, Lasalle introduced the use of propanediol (2) into a protocol that is still being used with minimal modifications.

Conversely, following the first report of a successful pregnancy using a frozen thawed oocyte in 1986 (3), most efforts have been made to develop an ideal oocyte cryopreservation

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method. Several reasons explain the low success rates traditionally observed, including oocyte size and shape. A large water content probably leads to intracellular ice formation, chilling injury, and osmotic damage, which are the main causes of high oocyte sensitivity to cryopreservation. These effects can be controlled depending on the cryopreservation method applied. There are two main cryobiology strategies: slow freezing and vitrification. During the former, cells are gradually dehydrated in the presence of cryoprotectants (CPAs) and the temperature is lowered at a very slow cooling rate (−0.3°C) (4). Cells are exposed to low temperatures for a long period, which can lead to chilling injury, defined as irreversible damage after exposing cells to low temperatures (+15°C to −5°C) before the nucleation of ice (5, 6). This detrimental event affects mainly the cytoskeleton (7) and cell membranes (8). Ice crystal formation within the cytoplasm must be avoided at all costs to guarantee the survival and integrity of cells when later thawed. The outcome of IVF cycles conducted with slow-frozen/thawed oocytes is limited and has never equalled that achieved with fresh oocytes. Therefore, there is a pressing need to cryopreserve oocytes more effectively. The protocol must reduce the damage to cells caused by ice crystal formation and chilling injury during the freezing process.

Vitrification

Vitrification is highly effective in avoiding crystallization (9). The first successful vitrification of mammalian (spermatozoa) cells was described more than 60 years ago (10) and was applied to mouse oocytes almost four decades later (11). Initial protocols subjected oocytes to high CPA concentrations for long periods, up to 50 minutes, involved high cytotoxicity, and caused remarkable osmotic stress. Subsequent studies substantially improved these initial experiments. One of the most notable changes was using CPA mixtures to overcome osmotic stress. The ethylene glycol–dimethyl sulfoxide 1:1 combination proved to be highly effective (12). Moreover, the probability of achieving vitrification is related directly to the cooling rate and solution viscosity and inversely to sample volume (13). The simplest way of balancing this equation is by reducing the vitrification solution volume when loading samples, followed by direct immersion in liquid nitrogen, which considerably increases the cooling rate. This strategy, in turn, lowers CPA requirements (14). Accordingly, several vitrification systems and protocols using different devices were introduced (15–22). Most of these devices are known as “open systems” because samples come into direct contact with liquid nitrogen during vitrification. Devices hermetically sealed before vitrification are known as “closed systems,” which prevent samples from coming into direct contact with liquid nitrogen during vitrification. Although it is true that high cooling rates are required to achieve vitrification, the warming rate is perhaps the best determinant factor for survival, as demonstrated recently (23), leading to the inference that possibly the extremely high cooling rates achieved with open systems are not absolutely necessary. It is more likely that the high viability associated with open systems is due to the extremely high warming rate achieved with these devices (e.g., ~40,000°C/min with the Cryotop system [21]). Conversely in closed systems, samples must pass an intermediate stage while they are released from the sealed device. We speculate that this intermediate phase conditions the warming rate and increases the likelihood of ice formation during the process. It probably explains the lower outcomes achieved after oocyte vitrification with the use of closed systems (24, 25). For embryos, this situation differs somewhat, because the results attained with blastocysts or cleavage-stage embryos with the use of closed systems can be most satisfactory (22, 26).

Undoubtedly, the great detractor of open systems is the risk of cross-contamination. It is essential to indicate, therefore, that to date there has never been a single cross-contamination case in AR involving a cryotransfer, even with open vitrification systems (27). Nonetheless, certain measures can be taken to avoid this hypothetical risk while applying open vitrification (28, 29).

Safety of Vitrification in Relation to Its Effects on Meiotic Spindle: The Chance of Increased Aneuploidy Incidence

The extent of clinical oocyte vitrification application can not be reviewed without mentioning the effect on the meiotic spindle (MS), a particularly sensitive structure responsible for chromosome segregation, given the possibility of generating aneuploid embryos. Years ago, it was suggested that the impaired potential of slowly frozen oocytes is related to the MS’s high sensitivity to cryopreservation, which may increase the aneuploidy rates in resulting embryos (30, 31). Spindle apparatus disruption caused by low temperature and or cryopreservation procedures is well documented in mice (32–35), cows (36, 37), and humans (38–40). This highly dynamic structure has also been demonstrated to be possibly repolymerized, with a normal appearance in >80% of cases when physiologic conditions return (31, 33–35, 41–45). There is considerable evidence that MS restoration in humans occurs without alterations, with the absence of scatter chromosomes (30, 46). Preliminary studies attributed a stabilizing effect of CPAs on tubulin fibers (31, 47–49), as confirmed by noninvasive studies conducted in living metaphase (metaphase II [MII]) human oocytes, showing complete MS repolymerization in a post-thawing incubation time-dependent manner (50, 51). It was also suggested that suboptimal protocols (44) and suboptimal material, such as aged or spare oocytes (52), compromise their viability and spindles’ restoration ability.

Several studies using freshly collected oocytes assessed spindle restoration among slow-freezing and vitrification protocols. Most revealed repolymerization, regardless of the cryopreservation method applied (52), although they might depend on temperature (53) and postincubation times (50, 51). Indeed short incubation may be responsible for the poor restoration observed in another study (54).

A more recent study confirming MS restoration after crypreservation showed the IVF outcome of 26 cycles conducted with vitrified or slow frozen oocytes (55). Ninety