Cryopreservation of human embryos and its contribution to in vitro fertilization success rates

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Cryopreservation of human embryos is now a routine procedure in assisted reproductive technologies laboratories. There is no consensus on the superiority of any protocol, and substantial differences exist among centers in day of embryo cryopreservation, freezing method, selection criteria for which embryos to freeze, method of embryo thawing, and endometrial preparation for transfer of frozen-thawed embryos. In the past decade, the number of frozen-thawed embryo transfer cycles per started in vitro fertilization (IVF) cycle increased steadily, and at the same time the percentage of frozen-thawed embryo transfers that resulted in live births increased. Currently, cryopreservation of human embryos is more important than ever for the cumulative pregnancy rate after IVF. Interestingly, success rates after frozen-thawed embryo transfer are now nearing the success rates of fresh embryo transfer. This supports the hypothesis of so called freeze-all strategies in IVF, in which all embryos are frozen and no fresh transfer is conducted, to optimize success rates. High-quality randomized controlled trials should be pursued to find out which cryopreservation protocol is best and whether the time has come to completely abandon fresh transfers. (Fertil Steril® 2014;102:19–26. ©2014 by American Society for Reproductive Medicine.)

Key Words: IVF, embryo cryopreservation, endometrial receptivity, slow freezing, vitrification, embryo transfer

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The first pregnancy resulting from transfer of a thawed cryopreserved human embryo was reported in 1983 in Australia (1), and the first live birth following embryo cryopreservation was reported in 1984 in The Netherlands (2). Subsequent to the first successful in vitro fertilization (IVF) treatment in 1978, the need for an effective cryopreservation program arose from rapid development and improvements of assisted reproductive technology (ART) protocols. Initially, all available embryos were transferred in IVF treatments owing to its low success rate, but improvements of clinical and laboratory aspects of IVF led not only to increased pregnancy rates but also to increased risk of multiple pregnancies. To prevent multiple pregnancies, fewer embryos were transferred and the supernumerary embryos cryopreserved for potential future use (3, 4). At that time, selection of embryos became important because the best available embryos had to be transferred fresh owing to the initially low success rates of embryo cryopreservation (5). Since those early days, cryopreservation of supernumerary embryos has become an integral part of IVF treatment. In addition, embryo cryopreservation is applied for women at risk for ovarian hyperstimulation syndrome (6), in embryo donation programs (7, 8), and for fertility preservation in women awaiting cytotoxic treatment (9).

Multiple variables, such as the selection criteria for embryos to be cryopreserved (10), the method of freezing and thawing (11), the synchronization between embryo and endometrial development (12), hormone supplementation during the frozen-thawed embryo transfer cycle (13), and patient characteristics, such as age of the woman (14, 15), determine the efficacy of embryo cryopreservation programs.

In this review, we will discuss the current state of affairs of embryo cryopreservation in IVF treatments and provide supportive evidence for the freeze-all strategy.

EMBRYO CRYOPRESERVATION

The principle of cryopreservation relies on preserving cellular viability in an

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arrested state by raising the intra- and extracellular viscosity to a level in which all molecular diffusion and chemical processes are halted. Freezing results in ice nucleation (change of state from liquid to solid around a certain focal point) and subsequent growth of ice crystals that turn water into ice, while all salts become confined to the remaining unfrozen fraction (16). These two phenomena, i.e., ice crystal formation (16) and increased salt concentrations (17), are the main causes of cell damage and possible cell death associated with cryopreservation. To prevent cell damage, cryopreservation protocols aim to dehydrate the intracellular space and as a result minimize intracellular ice formation while keeping the intracellular salt concentrations low (18, 19). Dehydration can be facilitated by adding cryoprotectant agents (CPAs) to the freezing medium. CPAs serve as antifreeze agents by disrupting hydrogen bonds in water. They come in two shapes, namely: 1) permeating CPAs that can enter the cell and directly displace water out of the cell, e.g., dimethyl sulphoxide (DMSO), ethylene glycol (EG), and propanediol (PROH); and 2) nonpermeating CPAs that remain outside the cell and draw water out of the cell by osmosis, e.g., sucrose. An additional effect of permeable CPAs is that they compensates for the increased intracellular salts that could be lethal at high concentrations. The addition of high concentrations of CPAs also lowers the freezing temperature of a solution and thereby reduces intracellular ice formation.

Each cell type has a specific optimal cooling rate determined by its volume-to-surface area ratio and its membrane permeability for water and cryoprotectants. If the cooling rate (decrease in temperature over time) is low, cells have sufficient time to lose water and achieve maximal dehydration, resulting in minimal ice crystals formation. If the cooling rate is high, the time for movement for water out of the cells is limited and ice crystals can be formed.

All cryopreservation methods can basically be divided into slow freeze and vitrification methods. Although slow freezing is likely still the predominant mode of embryo cryopreservation, there has been a major switch to vitrification in recent years. In 1984 the first human live birth occurred from a slow-frozen and subsequently thawed embryo, and the first successful pregnancies and deliveries after vitrification and warming of human cleavage-stage embryos were reported in 1990 (20). Since then, a significant improvement in post-thaw survival rates of human embryos has been reported for both freezing techniques (11).

Slow freezing and vitrification use similar chemicals but differ greatly in the concentration of those chemicals as well as in cooling and warming rates (Table 1). Slow freezing uses relatively low concentrations of CPAs, low cooling rates, and fast warming rates, whereas vitrification uses high CPA concentrations and ultrafast cooling and warming rates. Under slow cooling conditions, dehydration without excessive shrinkage is achieved by exposure to permeating cryoprotectants and exposure time to extracellular hyperosmotic conditions is limited. The principle of vitrification is to reach a glass-like state of the cell without formation of harmful ice crystals (18, 21). Under vitrification conditions, extreme dehydration is achieved by a very short exposure to high concentrations of permeating and nonpermeating cryoprotectants in the context of a high cooling rate. To achieve ultrafast cooling rates, open embryo carriers (e.g., electron microscopy grid, open pulled straw, Cryoloop, McGill Cryoleaf, Hemi-Straw, Cryotop, Cryolock) were designed to allow direct contact of the medium containing the embryo with liquid nitrogen (22, 23).

EMBRYO CRYOPRESERVATION AND CELLULAR CHANGES

Although implantation potential and resulting pregnancy rates are indicators for efficacy, little is known about the effect of cryopreservation on the physiology and cellular changes of the human embryo. Only a few studies have reported on molecular differences between frozen-thawed embryos and freshly cultured human embryos. For example, increased spindle abnormalities have been demonstrated in (day 5) vitrified blastocysts compared with fresh blastocysts (24). Although vitrified blastocysts contained an increased level of abnormally shaped spindles, they showed a high survival rate, suggesting that an increased occurrence of spindle abnormalities in post-thaw blastocysts does not per se impair

TABLE 1

Differences and similarities between slow cooling and vitrification protocols.

Factor	Slow cooling	Vitrification
Cryoprotectant	DMSO/ethylene glycol/propanediol	DMSO/ethylene glycol
Concentration of cryoprotectant (initial)	Low (1.5 mol/L)	High (2–5 mol/L)
Incubation time	Long (~3–5 h)	Short (a few minutes)
Cooling rate	Slow (−0.3 to −1°C/min)	Fast (−20°C/min)
Osmotic stress	Yes	Limited
Toxic stress	Yes	Yes
Chilling injury	Yes	Limited
Mechanical stress (ice crystal formation)	Yes	No
Programmable freezing equipment	Yes	No
Carrier	Ampule/straw (“closed”)	EM grid/Cryoloop/Cryotip/Cryotop (semiclosed)/ high-security straw (“closed”)
Direct contact with LN_2	No	Yes/no (in case of high-security straw)

Note: DMSO = dimethylsulfoxide; EM = electron microscopy; LN_2 = liquid nitrogen.

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