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Human Oocyte Cryopreservation
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Introduction

Human oocyte cryopreservation, or "egg freezing," has long been an elusive goal of cryobiologists. Although sperm and embryos (fertilized eggs) have been successfully frozen and subsequently thawed to create healthy children for decades, egg freezing has only recently become a successful reality. The cryopreservation of human oocytes is highly beneficial for several reasons, most importantly to preserve a woman's fertility. Oocyte storage allows: (i) women at risk of becoming sterile due to cancer to preserve their oocytes prior to radio- or chemotherapy or ovariectomy; (ii) the salvage of an in vitro fertilization (IVF) cycle when no sperm is available; (iii) the alleviation of religious and ethical concerns of embryo storage; (iv) the elimination of donor-recipient synchronization problems; (v) a "quarantine period" on donated oocytes similar to that of donated semen; and (vi) women to delay reproduction until later in life, providing them with more reproductive choices.

Background Information

The theoretical basis for cryopreservation of cells was first proposed in 1965 (Mazur, 1965). These theories were not tested, however, until 1972 resulting in the first births of mice from cryopreserved embryos (Whittingham et al., 1972). In 1976, the first successful birth of mice from frozen-thawed oocytes was reported (Parkening et al., 1976). More recently, a review of the literature reported embryos from at least 22 other mammalian species have now been successfully cryopreserved (Rall, 2001; Leibo et al., 2002). In the early eighties, the first human pregnancy (Trounson et al., 1983) and births (Zeilmaker et al., 1984) were reported from cryopreserved human embryos. The freezing and storage of human embryos is now standard practice in clinical in vitro fertilization (IVF) laboratories around the world (Shaw et al., 1993). According to the CDC website, 5,047 children were born in the year 2001 alone from the transfer of frozen-thawed human embryos (Wright et al., 2004).

Oocyte Cryopreservation

The first report of a pregnancy from a frozen-thawed human oocyte was in 1986 (Chen, 1986). Until recently, progress with frozen oocytes was slow with few births reported (Al-Hasani et al., 1987; Van Uem et al., 1987; Chen, 1988). This was due mainly to two factors: concerns of a higher incidence of chromosomal abnormalities in the

cryopreserved oocyte as compared to fresh oocytes (Johnson et al., 1987; Pickering et al., 1987; Sathananthan et al., 1988), and poor fertilization due to "zona hardening" (Vincent et al., 1990; Wood et al., 1992; George et al., 1993).
Following the development of intracytoplasmic sperm injection (ICSI) to assist fertilization following cryopreservation of oocytes in the 1990's, significant progress has been reported in cryopreservation of oocytes (Gook et al., 1995; Kazem et al.,1995). Subsequent research demonstrated cryopreservation of oocytes was not as detrimental as previously believed (Gook et al., 1993,1994). By 2000, there were over 20 normal children born from frozen-thawed oocytes (Tucker et al., 1996, 1998; Porcu et al., 1997, 1998, 1999a,b; Antinori et al., 1998; Borini et al., 1998; Nawroth et al., 1998; Polk de Fried et al., 1998; Vidali et al., 1998;Yang et al., 1998,1999;Young et al., 1998; Porcu, 1999). Currently, there are approximately 100 children reported following oocyte freezing (Leibo, 2003; Stachecki et al., 2004).

Current Methodologies

Slow Freezing

Slow freezing is the most reported method for cryopreservation of oocytes, resulting in the vast majority of babies born worldwide. Slow freezing methods, with only slight modifications, are based on the procedure first described by Willadsen (1977) for the freezing of ovine and bovine embryos. The equilibration time and amount of permeating cryoprotective additive (CPA) as well as the concentration of sucrose are among most common changes between various protocols (Fabbri et al., 2001). Recent alterations include the use of low or sodium free freezing solutions (Azambuja et al., 2002; Quintans et al., 2002; Boldt et al., 2003). However, the basic principles, remains the same. Oocytes are suspended in a solution containing CPAs, cooled and then seeded to induce ice formation promoting dehydration of the oocyte as the solution is cooled slowly at 0.3 o C/min to -30 o C or below. Finally, the oocyte is plunged into liquid nitrogen solution for storage.

Ultra Rapid Freezing (Vitrification)

Vitrification, or achieving a glass-like state, represents a potential alternative to slow freezing (first described in 1985 (Rall et al., 1985)). Since chilling injury to oocytes is time dependent, the rationale is to prevent ice formation and injury by freezing at a rate fast enough to solidify the intracellular water before it can crystallize (Martino et al., 1996). This is accomplished by exposing the cell to high concentrations of CPAs for a very short equilibration followed by very rapid cooling by plunging into liquid nitrogen. The high osmolarity of the vitrification solution rapidly dehydrates the cell and the submersion into liquid nitrogen quickly solidifies the cell before the remaining intracellular water has time to form damaging ice crystals. Two important concerns with this technique are the increased toxicity of high levels of CPAs at room temperature (Shaw et al., 1992) and the ability to freeze and thaw fast enough to avoid crystal formation and devitrification (Vajta et al., 1998). Several recent reports describe their ability to overcome these issues resulting in healthy children from vitrified oocytes (Kuleshova et al., 1999; Kuwayama et al., 2000; Yoon et al., 2000, 2003; Katayama et al., 2003).

Validation Studies

Over the past 9 years, the cryopreservation of more than 5000 human oocytes have been described in over 20 articles and abstracts resulting in over100 births (summarized in reviews: Leibo, 2004; Stachecki et al., 2004). However, these numbers are conservative and anecdotal evidence points to over 200 healthy live births to date. Extrapolating from reports, estimations of the average oocyte survival rates, fertilization rates, and clinical pregnancy rates are 54%, 61%, and 35% respectively (Kazem et al., 1995; Gook et al., 1995; Tucker et al., 1996, 1998; Porcu et al., 1997, 1999, 2000; Polak de Fried et al., 1998; Young et al., 1998; Kulehova et al., 1999; Hong et al., 1999; Yoon et al., 2000, 2003; Fabbri et al., 2001; Winslow et al., 2001; Yang et al., 2002; Boldt et al., 2003; Katayama et al., 2003; Fosas et al., 2003; Borini et al., 2004).

In May 2005, an abstract presented by the Huntington Reproductive Center at the annual Pacific Coast Reproductive Society demonstrated human oocyte survival and fertilization rates as high as 79% and 81% respectively. Also, in June 2005, Porcu presented his experience with over 500 cycles at the European Society for Human Reproduction and Embryology (ESHRE). His results supported pregnancy success rates with cryopreserved oocytes that were comparable to cryopreserved embryos.
Huntington Reproductive Center and Extend Fertility, Inc.

Extend Fertility is a company committed to furthering the advancement of oocyte cryopreservation research and providing services to women in need of preserving their fertility due to cancer, age, or other medical conditions.

Experience & Innovation

Extend Fertility has partnered with six premier medical centers across the U.S., including Stanford University's Reproductive Endocrinology and Infertility program (Northern CA), Huntington Reproductive Center (Southern CA), Reproductive Medicine Associates of New York (NY), IVF New Jersey (NJ), Reproductive Science Center (MA) and Texas Fertility Center (TX), to further research of oocyte cryopreservation through multi-center clinical trials.

Together, Huntington Reproductive Center and Extend Fertility provide state of the art technology for women interested in cryopreservation of oocytes. An IRB protocol has been established to continue researching new techniques for cryopreserving oocytes. Any woman under the age of 38 can be screened to determine whether she is a candidate to cryopreserve oocytes for future reproduction. Currently, a pharmaceutically sponsored multicenter study made possible through extend is available for women meeting inclusion and exclusion criteria (publication expected in 2006).

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