Vitrification of Mouse Metaphase II Oocytes: Prelude to Successful Preservation of Reproductive Capacity in Female Cancer Patients

Cristine A. S. Silva[1], Galina Gavrilina[2], Thomas L. Saunders[2], Keiko Hata[6], Masashige Kuwayama[6], Rebecca Gilbert[7], Gary D. Smith[1,3,4,5]
Departments of OB/Gyn[1], Internal Medicine[2], Urology[3], Physiology[4], Reproductive Sciences Program[5]; University of Michigan, Ann Arbor, MI; Kato Ladies' Clinic[6], Tokyo, Japan; Irvine Scientific[7], Santa Ana, CA, USA

University of Michigan Medical School

ABSTRACT

While births have been reported following human oocyte vitrification, experimental evidence indicating realistic expectations of this technology is lacking. Objectives of this study were to evaluate mouse metaphase II (MII) oocyte vitrification in a new secure closed-pulled straw system. Oocytes were vitrified in closed-pulled straws, warmed, and assessed for recovery, survival, ability to fertilize by conventional *in vitro* fertilization (IVF) or Piezo intracytoplasmic sperm injection (ICSI), and embryo development. In addition, two-cell embryos were transferred to recipients to assess live-birth rates. Statistics were performed with Chi-square analyses and ANOVA, P<0.05 was considered significantly different. Vitrification and warming of 2 (n=40) or 10 (n=1034) oocytes / straw resulted in 100 and 95% recovery, and 95 and 98% survival, respectively. Conventional IVF was unsuccessful with denuded vitrified oocytes (n=115; 18%) compared to denuded non-vitrified oocytes (n=233; 52%), and likely represented parthenogenic activation (vitrified oocytes without insemination; n=88; 15%). Poor fertilization of vitrified / warmed oocytes by conventional *in vitro* insemination was due to zona pellucida (ZP) modifications ("hardening") as indicated by significantly greater control-normalized time for ZP dissolution (57.8 ± 7.6 sec; mean ± SE) compared to no treatment (control; 0 sec) and solution exposure only (14.3 ± 7.2 sec). Transmission electron microscopy revealed premature release of cortical granules in vitrified / warmed oocytes. Fertilization following Piezo ICSI of vitrified oocytes (n=74; 80%) was not significantly different than non-vitrified oocytes (n=89; 90%). Resulting 24hr cleavage and 96hr blastocyst development rates were significantly reduced following oocyte vitrification (73% and 48%, respectively) compared to non-vitrified oocytes (89 and 66%, respectively). Embryos transferred after oocyte vitrification / warming / ICSI (n=124) or non-vitrified / ICSI (n=89) resulted in similar live-birth rates (31% and 36%; respectively). Thus, vitrified mouse MII oocytes in closed-pulled straws have excellent survival rates, require ICSI for fertilization, and can be used efficiently to generate embryos and offspring.

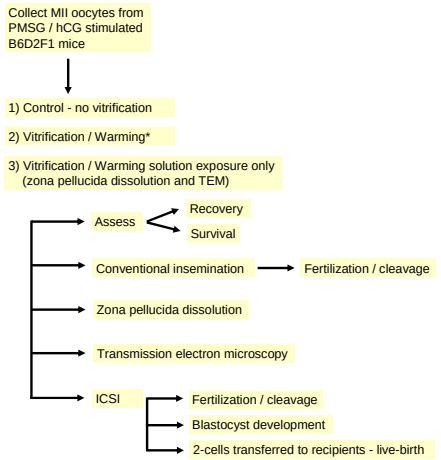
INTRODUCTION

Ability to cryopreserve human oocytes holds great promise for preserving fertility in cancer patients that must undergo radiation- or chemotherapy. These therapies are know to be gonadotoxic. Success in cryopreservation of oocytes is directly related to their large size and formation of cell-damaging intracellular ice crystals. Vitrification is a technology that results in transition from liquid to solid that contains the normal molecular and ionic distributions of the liquid state, but is considered to be an extremely viscous, supercooled liquid. If vitrification, which requires an extremely rapid rate of heat transfer, is performed correctly extracellular and intracellular ice crystals will not form. Success of vitrification depends upon the container and the solutions, which directly influence the rate of heat transfer. While feasibility of human oocyte vitrification has been reported (2 live-births; Katayama et al., 2003), efficiency and safety remain to be determined. Thus we initiated studies in the mouse model-system to delineate efficiency and safety of oocyte vitrification with novel secure closed-pulled straws and cryosolutions.

OBJECTIVES

- Evaluate feasibility of mouse metaphase II (MII) oocyte vitrification by analyzing:
  - * Recovery and survival
  - * Ability to fertilize by conventional IVF
  - * Ability to fertilize by intracytoplasmic sperm injection (ICSI)
  - * Blastocyst development
  - * Ability to establish live-births

MATERIALS & METHODS



*Secure close-pulled straws and solutions (Gilbert et al., 2002)

RESULTS

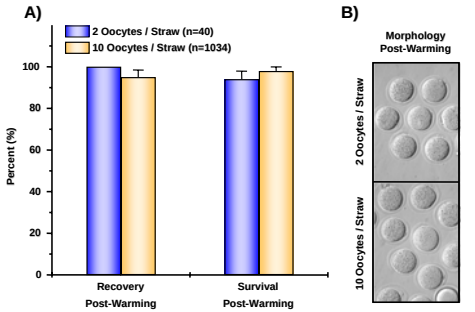


Figure 1. A) Percent recovery and survival of MII mouse oocytes vitrified and warmed as either 2 or 10 oocytes per straw. **B)** Representative micrographs of vitrified / warmed oocytes

RESULTS

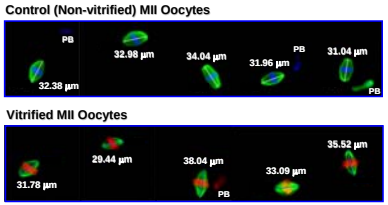


Figure 2. Representative micrographs of spindles (green) and chromatin (blue and red) in non-vitrified and vitrified MII oocytes. Spindle length measurements, from pole to pole are presented in microns. When visible within the focal plan polar bodies (PB) are indicated.

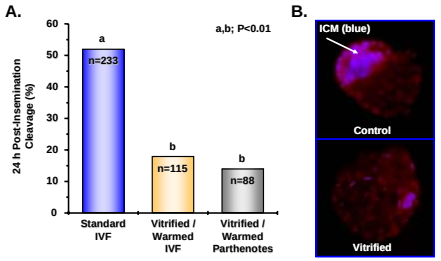


Figure 3. A) Twenty-four hour cleavage following insemination of control (blue), vitrified (gold), and vitrified non-inseminated (parthenotes; gray) oocytes. Statistical analysis by X^2. **B)** Representative micrographs of blastocysts from non-vitrified / inseminated (top) and vitrified / inseminated (bottom) oocytes.

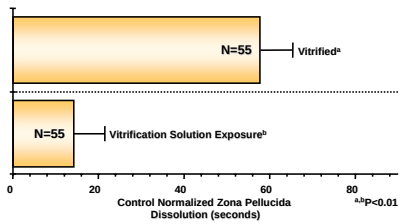


Figure 4. Zona pellucida dissolution following isolation (control), exposure to vitrification and warming solutions only, and vitrified / warmed. Data are expressed as dissolution times normalized for controls (sec=0). Solution exposure was not significantly different from control. Statistical analysis by ANOVA.

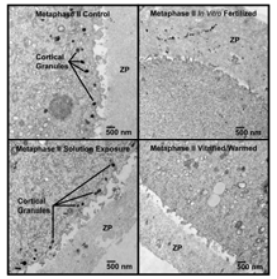


Figure 5. Representative electron micrographs of metaphase II oocytes freshly isolated (control), fertilized, exposed to vitrification and warming solution, and following vitrification and warming. Note presence of cortical granules in control and exposed oocytes, and lack of cortical granules in fertilized and vitrified oocytes. ZP = zona pellucida. 10500X

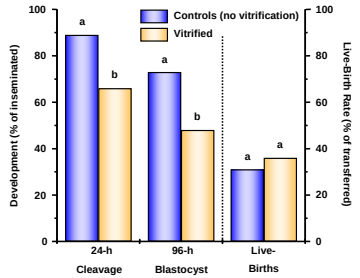


Figure 6. Embryo development and ability to establish a live-birth after oocyte vitrification / warmed / ICSI. Columns within a heading with different letters are significantly different (P<0.05) by X^2 analysis.

CONCLUSIONS

The use of closed-pulled straws, and a two-step method of oocyte vitrification, provides a means of cryopreserving oocytes with excellent post-thaw viability and morphology. Conventional IVF is unsuccessful due to zona pellucida modifications necessitating ICSI for fertilization. Vitrified and fertilized oocytes can be used efficiently to generate embryos and offspring. Studies are on-going assessing developmental normality of offspring born following oocyte vitrification.