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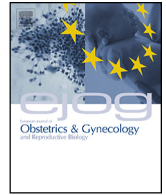

Full length article

Clinical outcomes of a vitrified donor oocyte programme: A single UK centre experience

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ABSTRACT

Objective: To assess the survival rate of vitrified oocytes used in an egg recipient programme and compare the clinical outcomes of pregnancy and live-birth rates per warmed oocyte with fresh autologous oocytes. The differences in the obstetrical outcomes between the two groups were also studied.
Design: A prospective case control study from a single in-vitro fertilisaton (IVF) Centre in UK
Setting: Centre of Reproductive and Genetic Health (CRGH), London
Population: Vitrified oocytes from egg donors and autologous fresh oocytes from patients attending for an IVF cycle
Methods: The study group consisted of 1490 vitrified oocytes, which were obtained from 145 egg donors who underwent a stimulation cycle at CRGH Centre. The control group included 145 age-matched women who underwent intra cytoplasmic sperm injection (ICSI) treatment with their own oocytes (n = 1528). The clinical outcomes clinical pregnancy rates (CPR) and live-birth rates (LBR) and obstetrical outcomes (gestational age and weight at delivery) were compared between the two groups. Statistical analysis of the summary data and logistic regression analysis was performed using statistical packages (SPSS Version 23 and Stata 2015). The percentages of all parameters in the cases and control groups were compared by Fisher's exact test. A statistical significance level of 5% was adopted throughout the study.
Main outcome measures: Survival rate per thawed oocyte, clinical pregnancy rate and live-birth rate per embryo transfer was compared to the autologous oocyte group
Results: The survival rate of vitrified oocytes was 73.6% (95% CI: 71.3–75.8%). The clinical pregnancy rate (per embryo transfer) using vitrified oocytes was found to be 51.8% compared to 59.3% in the control group. The live birth rate per embryo transfer in the vitrified oocyte group was 46% (95% CI 37.4–54.7%) compared to 57.1% (95% CI 48.5–68.5%) in the control group. The live-birth rate per thawed oocyte was found to be 4.2%. The gestational ages of the fetus at delivery in both the groups were comparable 39.0 (95% CI 32.7–41.9%) and 39.1 (95% CI 25.6–42.0) (p = 0.38). There was no statistically significant difference in the birth weight between the study and the control group 3100 g (750–4337) and 3232 g (1616–4500) respectively (p = 0.28).
Conclusions: This is the first study reporting on the efficacy of a vitrified donor oocyte programme from within the UK. There were no significant differences in the obstetrical outcomes between vitrified donor oocytes and autologous oocytes. The above data will be encouraging for women who are undertaking egg freezing for medical and or social reasons.

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Introduction

Oocyte freezing is no longer considered an experimental method by the American Society for Reproductive Medicine (ASRM) or American College of Obstetricians and Gynaecologists (ACOG 2014). Oocyte freezing techniques have improved dramatically over the last few years. 'Slow freezing' is no longer popular due to its low oocyte survival rate after thawing. Vitrification is a relatively new freezing technique that is based upon the principle that short exposure to high concentration of cryoprotectants and concurrent rapid cooling of metabolically active cells prevents the formation of intracellular ice. Vitrification is now an established method of gamete preservation compared to the traditional

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method of slow freezing [1]. During vitrification, the toxicity of electrolytes during the transition from liquid to solid phase is limited and thereby prevents a sudden drop in extracellular osmotic pressure during rewarming, which is thought to preserve the structure of the oocytes. A systematic review and meta-analysis of five trials (4282 vitrified oocytes, 3524 fresh oocytes, 361 slow-frozen oocytes), demonstrated that vitrification resulted in a higher oocyte survival rate, a higher fertilisation rate, and a higher rate of top-quality embryos compared with slow freezing [2]. A meta-analysis of 10 studies on the outcomes of vitrified versus slow frozen oocytes (2265 cycles from 1805 patients), it was noted that vitrification was associated with a significantly higher rate of oocyte survival (85 vs. 65%), fertilisation (79 vs. 74%), and implantation than slow-freezing [3]. A study on the assessment of clinical outcomes comparing the usage of fresh donor oocytes to vitrified donor oocytes; found the clinical pregnancy rates to be similar [4]. Concerning safety, studies have demonstrated that there was no difference in birth weight [5] and congenital malformations [6,7] in infants born following oocyte vitrification compared to those born from natural conception or through conventional in vitro fertilisation (IVF).

There is a significant decrease in the women's fertility with advancing age [8,9] due to a reduction in the ovarian follicular reserve. There is also a greater prevalence of chromosomal alterations in the oocyte, which leads to a significant reduction in pregnancy rates [10,11], and an increase in miscarriage rates [12,13]. In addition, there are other conditions affecting fertility potential including premature ovarian failure, unexplained recurrent implantation failure and inherited conditions that may result in women opting for oocyte donation [14].

Oocyte donation is a successful and well-established treatment for age-related female infertility, where the oocyte and subsequent embryo qualities are optimized by donated oocytes from young women [15], resulting in high pregnancy rates and good obstetric outcomes observed in recipients [14,16].

The clinical outcome of oocyte donation requires a receptive endometrium, usually prepared by exogenous hormone replacement [17,18], and well-synchronized replacement of good-quality embryos. For the synchronization, several strategies have been employed with varying levels of success, but certainly an important advance is the availability of oocyte banks. The drawbacks of oocyte donation, such as long waiting-times subject to the availability of a suitable donor, are responsible for the poor efficiency of oocyte donation programs, causing great stress and discomfort in patients. Furthermore, current regulations demand that donors are tested to avoid the transmission of infectious diseases and a quarantine period is recommended (UK guidelines) [19]. All these limitations could be solved with the establishment of efficient banks of donated cryopreserved oocytes [20]. In United Kingdom, CRGH (Centre for Reproductive and Genetic Health) has established a large oocyte bank. We hereby present our centre's data on survival rates and the clinical outcomes of vitrified donor oocytes whilst comparing it to outcomes from use of fresh autologous oocytes.

Materials & methods

A prospective case control study was undertaken from January 2010 to December 2015 at the CRGH clinic. 145 egg donors went through a stimulation cycle resulting in 1490 vitrified oocytes. Subsequently the donated oocytes were thawed for ICSI in the recipient treatment programme. The control group consisted of 145 age-matched patients, who underwent ICSI with fresh oocytes and were undergoing IVF/ICSI treatment for male factor infertility. The clinical and obstetrical outcomes were compared in both the egg provider groups. Summary data was obtained and logistic regression analysis performed using SPSS Version 23 and Stata 2015. Case and control percentages were compared by Fisher's exact test. A significance level of 5% was adopted throughout.

Vitrification

A total of 1490 MII oocytes were vitrified correlating to the parameters of our study group. A total of 1490 MII oocytes were thawed correlating to the parameters of our control group. All oocytes were mechanically denuded at 39 h post oocyte maturation trigger in Cumulase (Origio, Denmark) for a maximum of 30 s and then in HEPES (SAGE, CT, USA) buffer for a maximum of 30 s. Maturity was assessed via a Nikon stereomicroscope. All oocytes identified as metaphase II were immediately vitrified at room temperature (25 °C) using the Kitazato Vitrification Media Kit (Kitazato, Shizuoka, Japan). The composition of the media in the kit was undisclosed by the manufacturer. The oocytes were placed in Basic Solution (BS) as a holding solution, before adding 20 μl of Equilibrium Solution (ES) for 3 min, followed by the addition of a further 20 μl of ES for a further 3 min and a final addition of 240 μl of ES. The oocytes were left to equilibrate for a further 9 min after which they were washed through 2×300 μl of Vitrification Solution and loaded onto Cryolocks (Biotech, GA USA) in ≤ 1 μl of media. Oocytes were stored in liquid nitrogen.

Oocyte warming

Oocytes were warmed using Kitazato Warming Media Kits (Kitazato, Shizuoka, Japan). The initial Thawing Solution (TS) was warmed to 37.5 °C. All other components were used at room temperature (25 °C). The composition of the media in the kit is undisclosed by the manufacturer. The Cryolock was rapidly taken out of the liquid nitrogen and submerged into 300 μl TS solution for 1 min. Oocytes are moved into 300 μl Diluent Solution (DS), aspirating approximately 2 mm of TS below, in order to allow gradual displacement of medium. Oocytes remain in DS for 3 min and are then moved into 300 μl TS1 for 5 min before being released at the top of 300 μl of TS2 and being allowed to sink to the bottom of the well. This release/sink cycle was repeated for the duration of 1 min. The oocytes were then washed through 3×0.5 ml wells of Fertilisation Medium (Sage, Cooper Surgical, USA) and placed into a fourth well of 0.5 ml FM and were incubated at 37.5 °C/6% CO_2.

Hormonal preparation for preparation of endometrium in oocyte recipients: Egg recipients with ovarian function were given down regulation in the luteal phase of the preceding cycle with the buserelin nasal spray 200 micrograms four times a day (Suprefact nasal spray). After menstruation, all subjects received oral estradiol valerate (EV; Progynova, 6 mg/d; Schering). Approximately 10–15 days after initiating EV, serum E2 levels and endometrial thickness were measured. Administration of micronized progesterone (Crinone 8%; vaginally; Merck Serono LTD) was initiated the day of the oocyte thawing. The survived oocytes were injected with the partner's sperm from a fresh ejaculate. The Fertilization rate and the number of embryos that survived to day 3 of development were noted. Dependent on the number and the morphology of day 3 embryos, a day 3 embryo transfer was performed. Otherwise, an extended culture to the blastocyst stage was performed and patients underwent a transfer on day 5. If pregnancy was achieved, administration of EV and P was maintained until gestation week 12.

Statistical methods

Numerical data were summarized either as means with 95% confidence intervals (CI) if the data were approximately normally distributed or as medians with minimum and maximum values otherwise. Categorical data were summarized as percentages with

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