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Resiliency of Human Blastocysts to Cryotoxicity and Repeated Osmotic Stress Using a Re-Vitrification Model

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Objective: Vitrification (VTF) has proven to be a highly efficient mode of embryo cryopreservation. Survivability is dependent on 1) exposure to concentrated cryoprotective solutions that form a metastable glasseous solid state upon rapid cooling, and 2) high warming rates followed by cellular elution to an isotonic, equilibrated condition. We have previously shown that human embryos are highly adaptive to osmotic shifts during elution and that the re-VTF (rVTF) of blastocysts can serve as an effective research model. The aim of this study was to assess the cryotolerance of human blastocysts to repeated rVTF and determine the degree to which cytotoxicity of VTF solutions or osmotic membrane stress represents potential sources of cryoinjury.

Design: Using two 2x3 factorial arrangement of treatments, 160 research consented, discard blastocysts were randomly assigned to: Expt.1) either repeated rVTF (1, 3 or 5 times) with or without elution/equilibration; or Expt.2) extended rVTF exposure (1, 3 or 5 min) to either a DMSO-free, glycerol-based VTF solution (Innovative Cryo Enterprises) or a commercial EG/DMSO VTF solution (LifeGlobal). Differences in %survival and %24h development were statistically compared by chi-square analysis.

Materials and Methods: Standard microSecure vitrification (mS-VTF) and rapid warming of flexipettes (Cook, 300 μmID) was performed. In Expt.1, half the embryos were eluted in decending sucrose solutions (1.0M to 0.125M; 3 min/step) and equilibrated for 30 min before rVTF (with a controlled 1 min exposure to V3 solutions), while the other flexipettes (containing embryos) were directly dried with sterile gauze, reinserted into new straws and vitrified 1 min post-warming (i.e., non-elution). In Expt.2, mS-vitrified, warmed, eluted and rediluted blastocysts were exposed to final VTF solutions for 1-5 min before elution, equilibration and 24h embryo culture.

Results: Human blastocysts proved to be highly cryotolerate to extended VTF solution exposure (with or without VTF), dilutions/elutions and rVTF, with no differences in post-warming or developmental survival being observed in Expt.1 or 2 treatment groups. Overall, 97.5% survival of intact blastocysts and 94.8% sustained development was observed.

Discussion: Human blastocysts are highly resilient to extended exposures (≤6 min total) to VTF solutions, independent of the permeating cryoprotectants used. Routine production of healthy live births occurs following a single rVTF, but it is not known what genetic alterations could be associated with >5 min combined exposures to VTF solutions, if any. The safety and efficacy of VTF was clearly shown to be highly tolerable by the functional cell membrane integrity sustained after repeated metastable rVTF.

Disclosures: This study was conducted, in part, as a summer student training program research project (V. Smetona).

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The GENETWORx Personal Health Panel: A Pharmacotherapy Guided Approach to Genital Pathogen Diagnosis and Treatment

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Objective: To evaluate a urogenital pathogens molecular diagnostic panel that includes therapeutic recommendations for its utility in detecting the presence or absence of genital pathogens and to determine how rate of co-infection of pathogens found in different groups of pathogens affects the recommended treatment for infection.

Methods: A total of 659 deidentified patient results were evaluated. The GENETWORx Personal Health Panel is comprised of a comprehensive panel of 23 urogenital pathogens. The panel is composed of three leukorrhea (Leuko) strains, six bacterial vaginitis (BV) strains, six candida vaginitis (CV) strains, four ureoplasma/ mycoplasma strains (Urogen), Herpes simplex viruses (HSV) 1 and 2, and group B streptococcus (GBS) as well as 24 high-risk and 2 low risk human papilloma virus (HPV) strains. All tests were performed from a single swab collection shipped in universal transport media. Pathogen detection was performed using multiplex PCR followed by fluorescent primer extension and microarray hybridization. Therapeutic recommendations for treatment of positive patients were provided by pharmacists based on CDC and medical treatment guidelines. Rates of infection of each pathogen were determined as well as rates of co-infection and incidence of less common HPV strains.

Results: The frequency of detection for each pathogen fell within expected limits with regard to population and ethnicity. 68% (445/659) of patient reports evaluated were positive for at least one pathogen. Of those 445 positive patients, 47% (208/445) of patients were positive for more than one pathogen requiring differential treatment. The most common pathogens observed during co-infections was the bacterial vaginosis strains *Gardnerella vaginalis* (GV) and *Prevotella bivia* (PB); 63% of individuals infected with *Candida* species were also infected with GV and or PB, and 50% of individuals infected with *Trichomonas vaginalis* were co-infected with GV. Additional co-infection rates will be discussed. It is also of interest to note that of the 26 high risk-HPV positive patients, 65% (17/26) were not among the four more common high-risk HPV types screened (HPV16, 18, 31 and 45).

Conclusions: The use of a panel of urogenital health tests provides a more comprehensive view of the presence of the vaginal flora as compared to timely and individualized culture methods and provides more personalized treatment for individuals infected by one or multiple pathogens. This approach is of special interest in HPV detection which may detect high-risk strains absent in smaller screening panels.

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Use of Comprehensive Chromosome Screening as Part of Routine Infertility Care is Associated with Decreased Transfer Order and Increased Elective Single Embryo Transfer

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Objective: Multiple gestations due to multiple embryo transfer are the leading cause of maternal and neonatal morbidity related to infertility treatment. Many technologies have been employed to apply selective pressure to an embryo cohort with the hopes of selecting fewer embryos with greater reproductive potential and thus lowering transfer order. We sought to evaluate the impact of offering routine comprehensive chromosome screening (CCS) in the general infertility population on the number of embryos selected for transfer.

Design: Retrospective cohort study at a single, academic center.

Materials and Methods: The electronic medical record was utilized to collect data on all first time IVF cycles from 1/2012 to 12/2014 along with the first fresh or frozen embryo transfer cycle following oocyte retrieval. During this period of time CCS was routinely offered in this clinic to all patients as a way to increase pregnancy rates and decrease miscarriage rates. Data collected included use of CCS or not, number of aneuploid/euploid embryos if CCS was performed, number of embryos transferred, and number of embryos cryopreserved which was used to determine the rate of elective single embryo transfer in cases where there were embryos which were cryopreserved in excess of those transferred. Patients with known single gene cases or translocations were excluded as were study protocol cycles.

Results: During the study timeframe there were 3312 unique patients identified. Of these 1562 (47%) underwent preimplantation genetic screening with CCS and 1750 (52.8%) did not. Of those undergoing PGD, the average number of embryos biopsied was 5.4 (1-34), number euploid was 3.7 (0-27), number aneuploid was 1.7 (0-13), and 99 (6.3%) had no euploid for embryo transfer. Of the 3213 remaining, when CCS was employed the SET rate was 71.9% and DET rate was 28.1% compared to an SET rate of 43.0% and DET rate of 57.0% when patients did not elect CCS. There were no transfers which had more than 2 embryos put back. The mean number of embryos transferred was 1.3 with CCS and 1.6 without CCS ($p<0.0001$). The rate of elective single embryo transfer (eSET) was 19.1% in the CCS group and 15.7% in the group without CCS ($p=0.0095$).

Conclusions: When patients and physicians incorporate CCS into routine IVF care both the average numbers of embryos and the rates of eSET are increased. Given the significant maternal and neonatal morbidity associated with multi-fetal births, any intervention which decreases transfer order represents a significant advancement in infertility care.

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