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commercial standard vitrification media; Tvitriv-4, produced in small scale for research by INVITRA, based on the standard composition with four modifications including carbohydrate trehalose instead sucrose, reduced non-permeant cryoprotectant concentration, and addition of two aminoacids; and Tvitriv-4 supplemented with L-carnitine (LC) and oleic and linoleic fatty acids (FA).

DESIGN: Experimental study.

MATERIALS AND METHODS: 23 C57BL/6J females were superovulated with 5UI eCG followed by 5UI hCG and oocytes (n=562; 4 replicates) were randomly divided in 4 groups: fresh control group (FC), vitrified using Irvine (IRV), Tvitriv-4 (T4), or supplemented Tvitriv-4 (T4-LC/FA) media. Fresh or vitrified-thawed oocytes were inseminated with 1x10^6 sperm/ml and cultivated for 96 or 120 hours in KSOm (Cosmo bio Co., LTD) incubated at 37°C with 5% CO2. Blastocysts of each group were individually fixed in methanol/water. Lipids were extracted using One Step Methanol protocol (9 blastocysts/group) and flow injected into the triple quadrupole spectrometer equipped with an electrospray ion source. Lipids were analyzed using the multiple reaction monitoring profiling (MRM-profiling) method and values of relative intensities of ions detected in each group were compared using univariate (one-way ANOVA, volcano plot) and multivariate analysis (PLS-DA).

RESULTS: One-way ANOVA (p-value ≤0.05) showed that 90 out of the 125 lipids were differently expressed among the four groups, while a comparison between the vitrified groups showed no difference. Two by two comparisons between the control and vitrified groups using volcano plot (p-value ≤0.05) showed 18 features of importance (VIP scores) higher than 1.3 detected among the FC vs. IRV, FC vs. T4, and FC vs. T4-LC/FA, respectively; all of them more abundant in the FC group. Partial least square discriminant analysis (PLS-DA) variables of importance (VIP scores) higher than 1.3 followed the same pattern and identified the phosphatidylinositol containing 36 carbon atoms (PI(36:1)) and phosphatidyl-cholines PC(38:4), PC(36:5), PC(34:1), and PC(30:0) among the top features; the exception being the free stearic acid (C18:0), which was the top feature in the FC x T4-LC/FA comparison, being more abundant in the latter. CONCLUSIONS: Vitrification changed the lipid profile of mice blastocysts causing an overall reduction on lipid abundances that affected PC lipids the most. This effect was more apparent in IRV, followed by T4, and T4-LC/FA suggesting that supplementation of media with L-carnitine and unsaturated fatty acids may have protective effects on lipid content of blastocysts from vitrified oocytes, whose impacts needs further investigation.

CNPq (process n. 305173/2019-7), Trial registration number: CEUA-FMRP/USP-107/2017.

SUPPORT: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – process n. A 88887.371487/2019-00; Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas da FMRPR – USP (FAEPA); Invitira Assisted Reproductive Technologies LTD.

P-188 4:30 PM Saturday, October 17, 2020

OPTIMAL STORAGE TEMPERATURE FROM THAWING OF CRYOPRESERVED OVARIAN TISSUE TO TRANSPLANTATION: XENOTRANSPLANTATION INTO NUDE MICE. Nobuya Aono, Ph.D.,1 Ena Nakatsukasa, Ph.D.,2 Noriyuki Okuyama, M.S.C.,2 Yusuke Nakamura, BS,1 Sena Shibasaki, BS,2 Mio Mori, BS,2 Tonomoki Hashimoto, M.D., Ph.D.,1 Toshikuni Sasaoka, MD, Ph.D.,1 Koichi Kyono, M.D., Ph.D.1
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OBJECTIVE: Human ovarian tissue cryopreservation, an essential method of fertility preservation, has led to the birth of more than 180 healthy babies around the world. Although there are many reports on cryopreservation and transplantation of ovarian tissues, the optimum storage conditions after thawing are still unclear. In this study, we performed xenotransplantation of ovarian tissue into nude mice to determine what kind of storage conditions are appropriate.

DESIGN: Prospective controlled animal study.

MATERIALS AND METHODS: We used ovaries derived from four SD rats at 10 weeks of age. Each ovary was cut in half, then 16 slices were cryopreserved by the slow freezing method (using 1.5M DMSO as cryoprotectant). Then, the tissues were thawed and stored at 4°C, RT (24°C), or 37°C for 2.5 hours in DPBS buffer. They were grafted under the kidney capsule of an ovarectomized nude mice (8-20 weeks old). Five IU PMSG and 5 IU hCG were given once into mice at about 4 weeks after transplantation. Engraftment and follicle development in each group was compared with that in the control group (immediately grafted after being thawed).

RESULTS: The engraftment rates of frozen-thawed ovarian tissues were assessed in the control group (100%), 4°C group (87.5%), RT group (84.6%), and 37°C group (50.0%). The engraftment rate was significantly decreased when the tissues were stored at 37°C compared with those of the other groups (P < 0.05). The rates of engrafted ovarian tissues with macroscopically confirmed follicles were assessed in the control group (61.5%), 4°C group (71.4%), RT group (45.5%), and 37°C group (33.3%). Those rates in the RT and 37°C groups decreased compared with the control and 4°C groups although there was no significant difference. The follicles were aspirated and oocytes were collected in the control group (9), 4°C group (12), RT group (3), and 37°C group (1).

CONCLUSIONS: Storing the frozen-thawed tissues at 4°C led to a successful engraftment and follicle development. Although the tissues were well engrafted, there was little follicular growth in the RT group compared with the 4°C group. The storage at 37°C resulted in poor engraftment and follicle development. This study showed that the storage temperature of frozen-thawed ovarian tissues affects the engraftment and/or follicle development. Frozen-thawed ovarian tissues should be transplanted immediately after being thawed. In case they need to be kept a while before transplantation, storage at lower temperatures is recommended.
Although we agree that the relative risks of embryo disease transmission is no longer a clinical reality for cryopreserved blastocysts. blastocyst implantation efficiency (46% vs 69% implantation using 1.91 vs 1.07 (1066 of 1126 BL) to 99.4% (3352 of 3373 BL) with increased (p < 0.05) our survival rates from 95%
realized by recent tank failure experiments and known catastrophic events. Finally, it is worth noting that embryos vitrified in an insulated straw environment are more resistant to detrimental additive temperature fluxes that can occur under sub-optimal cryostorage handling procedures. So, we ask, is it time to reconsider the status quo of embryo good tissue practices when viral pandemics are a reality?

DESIGN: Retrospective analysis of clinical practices that may alter the risk of embryo disease transmission in cryostorage. We looked at the impact of cryopreservation guidelines relative to disease transmission potential.

MATERIALS AND METHODS: Human blastocysts were vitrified in a closed, aseptic device system and rapidly-warmed and sucrose diluted using non-DMSO solutions. Specifically, we will investigate the effectiveness of a validated cryopreservation guidelines that cross-contamination of infectious agents is a negligible risk. Fertil Steril. 2010;94:1181–1188.

OBJECTIVE: The current global pandemic has triggered concerns regarding the potential infectivity of the SARS-CoV-2 virus to blastomeres known to possess ACE-2 receptors. In 2010, Pomeroy and coauthors reviewed the negligible risks associated with the potential cross contamination of human reproductive tissues, gametes and embryos in cryostorage. The purpose of this investigation is to explore changes in ART lab practices over the last decade that could warrant a reassessment of the latter AAB/CRB embryo vitrification (VTF) and cryostorage guidelines relative to disease transmission potential.

RESULTS: The routine application of ZP-exposed trophectoderm and blastocyst biopsying improved (p < 0.05) in ART lab practices over the last decade that could warrant a reassessment of the latter AAB/CRB embryo vitrification (VTF) and cryostorage guidelines relative to disease transmission potential.

CONCLUSIONS: The protective barrier of an intact ZP to potential pathogen exposure is no longer a clinical reality for cryopreserved blastocysts. Although we agree that the relative risks of embryo disease transmission in cryostorage remain negligible, why take any risks when highly effective closed VTF systems (ICE straw; HSV, μS-VF, VitrSafe) have been established over the last decade? Alternatively, we question whether the use of LN2-vapor storage tanks for open-VTF systems alleviates potential airborne viral cross-contamination, while they most certainly create a greater risk for potential embryo wastage as discussed by Pomeroy et al. (2010) and overtly

No statistically significant differences was observed between the two groups in biological and clinical outcomes.