

Cryopreservation did not interfere with cancer treatment. Patients felt well informed, well treated and were glad they had the procedure done, regardless of ovarian function at follow up.

With the current settings, the balance of the risk of ovarian failure on one side, and the potential surgical complications and possible delay of cancer treatment on the other, seems agreeable and provides the opportunity of resumption of ovarian function following an autotransplantation.

P-363 Poster Analysis of post-warming degeneration & apoptosis following porcine ovarian tissue vitrification using the ohio-cryo device

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Introduction: Ovarian tissue cryobanking for cancer patients is now offered in many reproductive centers. Although different methods of cryopreserving this tissue exists, vitrification seems to be the most efficient. The Ohio-Cryo is a novel method that allows the vitrification of ovarian tissue in a closed system device. The device allows rapid handling of tissue, rapid heat transfer & the vitrification in a minimal volume of ovarian fragments processed from one hemiovary in one vial. Our objective is to evaluate the use of this device for vitrification of ovarian tissue.

Materials and methods: Seven porcine ovaries from 4 different pigs were collected immediately post-mortem. After removal of the medulla, the ovarian cortex was carefully sliced into 0.5 mm thickness slices, then chopped at 0.5 mm thickness using Mcwillian tissue chopper to make tiny ovarian cubes of 0.1253 mm. From each ovary, a sample was fixed as fresh control. Another sample was incubated in Leibovitz media in a 4°C refrigerator for 5 days to spontaneously degenerate, then fixed. Another sample was directly plunged into liquid nitrogen to induce cryo-damage. The remaining sample was vitrified using the Ohio-Cryo. After warming, tissues were incubated for 2 hours in culture media at 37°C then fixed. All samples were paraffin embedded & cut at 5µm thickness. For each sample, one slide was stained for H&E for histological assessment of degenerative changes. Degenerative changes were judged according to Wood's criteria (Wood et al 1998) with slight modification. The scores were numerically displayed as 0 for no degeneration, 1 for slight degenerative changes & 2 for severe degenerative changes. An adjacent section was assessed for apoptosis using TUNEL. This was calorimetrically assessed as (0 no TUNEL signal), (1 for mild, 2 for moderate & 3 for intense (Mahmoud et al. 2006).

Results: The histologic degeneration scores for primordial follicles was 0.15 ± 0.16 for fresh samples, 0.17 ± 0.23 for vitrified samples, 0.56 ± 0.55 for the cryo-induced damage samples & 1.75 ± 0.08 for the degenerating samples. The degenerative scores for preantral follicles were 0.47 ± 0.4 for fresh samples, 0.36 ± 0.27 for vitrified samples, 1.00 ± 0.34 for the cryo-induced damage samples & 1.52 ± 0.23 for the degenerating samples. As for the TUNEL, the apoptotic index was 0.23 ± 0.08 for the fresh samples, 0.32 ± 0.05 for the vitrified samples, 1.01 ± 0.34 for the cryo-induced damage samples & 1.34 ± 0.59 for the degenerating samples.

There was no significant difference between vitrification & fresh tissue, with a p value of 0.14 for TUNEL, 0.82 & 0.64 for the degenerative scores of primordial & preantral follicles respectively. These indices were all significantly higher than fresh & vitrified tissues when cryo-damage or spontaneous degeneration were applied. Compared to fresh tissue, TUNEL showed a significant increase in the apoptotic index with a P-value of 0.006 for cryo-damaged samples & 0.036 for degenerating samples. Histologic degeneration assessment score showed a similar increase for primordial follicles with a P value of 0.22 (NS) & <0.001 for primordial cryo-damaged & degenerating respectively. Preantral follicles showed an increase that was significant for cryo-damage ($P = 0.043$) & apoptosis induced ($P = 0.015$).

Conclusions: The vitrification of ovarian tissue in the Ohio-Cryo did not induce degenerative changes nor increase the apoptotic index in the ovarian tissue as evidenced morphologically by H&E or using TUNEL assay. With the swiftness of its handling the ovarian tissue for vitrification in a closed system, it may provide a suitable solution for a reliable ovarian tissue vitrification.

POSTER SESSION

ART, LABORATORY: CRYOPRESERVATION OF EMBRYOS

P-364 Poster The effect of pre-freeze embryo culture media protein supplementation on post-thaw live birth outcomes

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Introduction: Due to the danger of disease transmission and the increasing quest for standardized culture conditions in human IVF, there are significant incentives to consider recombinant sources of protein for supplementation or to replace biological proteins in culture media with other non-protein macromolecules. However, experience suggests that a certain quality and complexity of proteins in animal and human embryo culture media are essential for optimum results (Esfandiari et al., 2005). Furthermore, there are strong indications in animals that the culture conditions of IVF embryos prior to cryopreservation may affect post-thaw clinical outcomes. Therefore, the objective of this study was to evaluate the post-thaw live-birth outcomes for blastocysts frozen after being cultured in media supplemented with either a simple or more complex protein source and to apply the best outcome to a commercial blastocyst-transfer program.

Materials and methods: In a randomized prospective study, 528 patients were randomly assigned to a control group with embryos cultured in media with 0.3% human serum albumin (HSA) or a treatment group with all embryos cultured in media with 0.3% HSA and 10% Serum Supplement Substitute (SSS) (Irvine Scientific, Santa Ana, California). Supernumary blastocysts of 109 of these patients were cryopreserved for later use. Since the completion of this study, 28 patients from the control group and 17 patients from the treatment group used some or all of their frozen blastocysts. Endpoints evaluated include post-thaw survival rate, implantation rate, live-birth implantation rate (number of embryos transferred that resulted in a live baby) and live birth rate. Results were evaluated for significance using a chi-square analysis with Fisher exact probability.

Results: Survival rates for the frozen-thawed blastocysts were almost identical with 81% and 80% for the control and treatment groups, respectively. The implantation rate for embryos originally cultured in HSA only was 41% and for embryos cultured in 10% SSS was 43%. The live-birth implantation rate (11/34–32.4%) and live birth rate (8/17–47.1%) for the SSS-cultured group appeared superior to those of the HSA-cultured group (10/44–22.7% and 8/28–28.6%, respectively). These results did not achieve statistical significance. However, based on these indications, all embryos in the commercial program were now cultured in SSS-supplemented media. After supplementing the culture media with SSS, implantation rates improved from 32% ($n = 354$ patients) to 47% ($n = 673$ patients) and live birth rates from 38% to 55% which was highly significant ($P < 0.0001$).

Discussion: Morphological blastocyst criteria, post-thaw survival and even implantations failed to benefit from culture media supplementation in this study with more complex protein supplementation. Interestingly, it appears that more implantations were lost when supplementing with HSA alone, resulting in fewer live births and babies per embryo transferred. It may be that the inner cell masses were preferentially compromised with sub-optimum culture conditions which were not discernable before transfer. Even though 528 patients were initially randomized in this study, only 45 patients could be followed up to include live births, thereby compromising statistical resolution. However, when applying SSS supplementation as suggested by the results of this study to a larger population (more than a 1000 patients), live births improved dramatically due to improved implantations accompanied by a decrease in miscarriages. The results from this study as well as the follow-up data after clinical application strongly suggest that one should be careful to simplify the embryo culture environment too much due to regulatory pressures or due to a natural quest for simplification and standardization.

P-365 Poster Comparison of liquid nitrogen and liquid nitrogen vapour phase for storage of vitrified human blastocysts

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Introduction: Storage of embryos in liquid nitrogen vapour (LNV) following cryopreservation has been proposed as a “safer” alternative to storage in liquid nitrogen, potentially decreasing the risk of cross contamination. In addition, the use of LNV tanks for embryo storage may be economically desirable since they use significantly less nitrogen than traditional automated liquid nitrogen storage vessels and have an increased storage capacity. Despite this many ART laboratories are understandably hesitant to move towards LNV storage before there is clear data supporting the efficacy of this method. This study compares blastocyst survival following warming of vitrified embryos as well as pregnancy outcomes for embryos stored in either system.

Materials and methods: 223 frozen embryo transfer cycles were included in this retrospective study. Patients had varying ovarian stimulation protocols and embryos were vitrified on either day 5 or day 6 following fertilisation of oocytes. Embryos were vitrified in a final solution containing 16% DMSO/16% ethylene glycol/0.68M trehalose using the fibreplug technique (CVM Cryologic, Australia) and stored in either a liquid nitrogen or LNV tank (Chart, Australia). Following warming, all embryos were assessed for percentage cell survival (defined as % cells surviving in each embryo). Pregnancy outcomes following embryo transfer were also assessed.

Results: A total of 330 embryos were warmed, 113 of which were stored in liquid nitrogen and 217 of which were stored in LNV. When embryos were stored in liquid nitrogen, 62% ($n = 70$) gave cell survival rates of $\geq 90\%$ following warming, compared to 65% ($n = 142$) for embryos stored in LNV. A cell survival rate of $\geq 50\%$ was observed in 93% ($n = 105$) of embryos stored in liquid nitrogen compared to 91% ($n = 198$) for vapour storage. None of these values were statistically different. For patients who had embryos stored in liquid nitrogen (average age at freezing 35.7), a positive pregnancy was achieved in 39% ($n = 33$) of cycles. Where patients had embryos stored in LNV (average age at freezing 34.6), a positive pregnancy was achieved in 35% ($n = 46$) of cycles. These results were not statistically different. The mean number of embryos transferred per cycle was 1.2 for both groups. Based on the scan results available to date for the conversion from positive pregnancy to fetal heart pregnancy (FHP), the projected FHP rate for all ages combined is the same in both groups (25%).

Conclusions: This study found no difference in either embryo survival or clinical pregnancy rates following transfer of vitrified blastocysts stored in liquid nitrogen or LNV. While the numbers included in this study are low, it does provide preliminary evidence that, along with the theoretically safer storage and benefits to laboratory economics, storage of human embryos in LNV is a viable alternative to storage in liquid nitrogen. The outcomes following LNV storage will continue to be monitored in order to improve the statistical comparison between the two storage methods.

P-366 Poster Vitrification of human blastocysts: clinical outcome in 2111 cycles

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Introduction: The need to cryopreserve human blastocysts is increasing. The first pregnancy of vitrification of human blastocysts was reported in 2000 by Yokota. Since then several techniques using different cryoprotectants and various embryo carriers were developed. However, the survival and pregnancy rates after human blastocyst vitrification are still inconsistent. The purpose of this study was to evaluate the survival and subsequent pregnancy rates of human blastocysts vitrified after artificial shrinkage (AS).

Materials and methods: Retrospective study was performed for 2111 cycles participated in our thawing-ET program from November 1, 2002 to June 31, 2007. All the blastocysts which derived from conventional IVF or ICSI were cocultured with cumulus cells in a 10 ul YS medium containing 20% hFF. In the course of culture, maximum 3 of good quality embryos were transferred into the uterus on day 3 or 5. The surplus embryos developed to the expanded blastocyst stage were vitrified on day 5 or 6 using EM-grid following AS (Son

et al., 2003). The solution for vitrification was composed of DPBS containing 20% (v/v) hFF, 40% (v/v) ethylene glycol, 18% (w/v) Ficoll, and 0.3 M sucrose. Thawing of vitrified blastocysts was carried out by 2-steps on day 3 after ovulation (Lee *et al.*, 2006). Thawed blastocysts were overnight cultured and then transferred on the next day. The viability was assessed through the survival, hatching embryo, and implantation rates as possible.

Results: The total of 5810 vitrified blastocyst was thawed for 2111 cycles. The survival rate of thawed blastocysts was 90.7% (5269/5810) and the hatching or more rate was 85.0% at the time of transfer (4484/5269). The total of 5216 blastocyst was transferred into 2076 cycles. The mean number of blastocysts transferred per cycle was 2.5 ± 0.8 . The implantation rate was 23.4% (1218/5216) and the pregnancy rate was 45.5% (945/2076). In this study, embryo transfer was not conducted for 35 cycles with no blastocysts survived.

Conclusions: When thawed, the blastocysts vitrified in solution containing 40% (v/v) ethylene glycol, 18% (w/v) Ficoll, and 0.3 M sucrose using EM-grid after AS were high in their survival. Their pregnancy rate was also a tendency to be similar to that obtained for fresh COH-ET cycles, generally. These data indicate that the vitrification technique above should be very effective for an increase of the cumulative pregnancy rate in ART program.

P-367 Poster Vitrification of human blastocysts with either DMSO or PrOH as a cryoprotectant

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Vitrification of human blastocysts with either DMSO or PrOH as a cryoprotectant

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Background: Several studies have shown that transfer of blastocysts cryopreserved by use of vitrification results in increased pregnancy rates compared to cryopreservation with slow freezing methods. The cryoprotectant most commonly used for vitrification is dimethylsulphoxide (DMSO). DMSO is however considered potentially toxic to handle and it could be of advantage to replace DMSO with less toxic chemicals such as 1,2 propanediol (PrOH). In addition, it has been shown that slow freezing of human cleavage stage embryos can affect the aneuploidy rate, while there are as yet no studies concerning the aneuploidy rate of embryos cryopreserved using vitrification.

The aim of this study was to perform a randomised study where survival rate, reexpansion rate and chromosomal status of blastocysts vitrified with DMSO are compared to blastocysts vitrified with PrOH, and to a control group.

Materials and methods: Embryos not fulfilling the criteria for good quality embryos (GQE) on day two after oocyte pick-up and donated by patients going through IVF treatment were cultured to blastocysts. Blastocysts scored as expanded (day 5 or 6) or hatched (day 6 or 7) were randomized into one of the three groups. The study includes a total of 100 blastocysts, where 33 were vitrified with cryopreservation media including DMSO, 34 vitrified with cryopreservation media including PrOH and 33 were fixated as controls, without cryopreservation. All media were manufactured by Vitrolife AB, Kungsbäcka, Sweden. To optimize the vitrification process and enhance the penetration of the cryoprotectant, the liquid-filled blastocoel was collapsed by use of a laser. The initial survival rate was estimated directly after thawing. The reexpansion rate was determined 2, 6 and 24 hours later. At 24 hours reexpanded or hatched blastocysts were fixated for fluorescence in situ hybridisation (FISH) analysis for chromosomes 13, 18, 21, X and Y.

Results: Directly after thawing the group vitrified with DMSO showed a significantly lower initial survival rate than the group vitrified with PrOH ($P = 0.001$). However, there were no statistical differences in the reexpansion/hatching rates after 2 hours (53% and 60%), 6 hours (53% and 60%) and 24 hours (40% and 39%) for DMSO and PrOH respectively. The mean number of cells after thawing and fixation was similar for the DMSO group (mean = 146) and the PrOH group (mean = 148). The FISH results showed a higher mean number of chromosomally normal cells for the DMSO group (67%, $n = 6$) compared to the PrOH group (49%, $n = 17$) and the control group (41%, $n = 28$). Reasons for this might be the low number of fixated blastocysts in the DMSO group, and/or that, despite randomization, the DMSO group included a higher percentage of top and good quality blastocysts. The control group had the lowest number of top quality blastocysts and also the lowest rate of normal cells.

Conclusions: Vitrification of blastocysts does not seem to be detrimental compared to non-vitrified controls regarding development and chromosomal status. In addition, results after vitrification using PrOH are found to be similar or better compared to DMSO.

P-368 Poster Vitrified egg donation program with blastocyst transfer. A successful tool to improve results. one year follow up study in Mexico cityS. Cubillos-Garcia¹, S. Sanchez¹, G. Charria², E. Obregon², H. Aparicio², S. Cuneo²¹*Concibe Reproduccion Asistida, Assisted Reproduction Laboratory, Mexico D.F., Mexico,* ²*Concibe Reproduccion Asistida, Reproductive Medicine, Mexico D.F., Mexico*

Introduction: Recent advances in freezing technology and the modifications of conventional protocols are still improving day by day so as to arise the method's efficiency. Oocyte banking is the best option not only to preserve female's fertility but also to diminish the cost and time for eggs donation. With this procedure we can assure to have better opportunities to fulfill and have high percentage of what people have desire to get as immediate results, and to avoid any regret for the overtime egg donation.

Up to now, few clinical data are available about large series of vitrified egg donation and there are a lot of doubts in relation with affect spindle morphology, aberrant chromatin alignment or premature cortical granule release that can be affect embryo development and pregnancy, implantation and/or miscarriage rates.

We use culture to the blastocyst stage thereby allowing self selection from those embryos capable of blastulation and self exclusion from those which have a lack of embryonic development or arrestment. Our objective is to show the vitrified egg donation ability and the sequential culture in our own results.

Materials and methods: This is a retrospective study, that included sixteen patient's cycles receptors, done from May 2007 to December 2008.

30 oocyte donors were frozen by vitrification since October 2006 to the present day.

donators were stimulated with conventional down regulation protocols

For this study a requirement was that all of the above had proved fertilized *in vivo* or *in vitro*.

Oocytes were retrieved by ultrasound-guided transvaginal aspiration and vitrified with the Cryotop method, with 7.5% ethylene glycol, 7.5% propanediol, and 0.5 mol/L sucrose. Viability was assessed 1 hours after thawing. 154 oocytes were thawed, and a mean of 7 oocytes MII were assigned for each recipient. The survived oocytes were inseminated by intracytoplasmic sperm injection. the fertilization was evaluated after 24 hours. The zygotes were further cultured *in vitro* for up to 120 hours until time of embryo transfer, day 5 or 6 according to embryo development.

Results: The survival rate observed was 96.7%. Fertilization rates 85.71% day 2 cleavage (93.9%), day 3 cleavage (77.41%), and blastocyst formation (41.6%). A total of 29 embryos were transferred on the 5th or 6th day. The medium of embryos transferred were two.

The refreezing rate by vitrification was (27.5%) blastocyst, that are still frozen. Pregnancy, implantation and miscarriage rates, were 66.67%, 31.06%, and 22.22%, respectively.

Our pregnancy, implantation and miscarriage rates in fresh egg donation are (70.55%), (36.11%) and (13.04%) respectively.

Conclusions: Excellent clinical outcome indicates the possible use of the egg donation program technology and establishes oocyte banking. The costs and time are meant to benefit the patients in terms of oocytes disposal.

Sequential culture is a strategy to consider to selected embryos with a high potential implantation to transfer. As a result of our pregnancy, implantation and miscarriage rates in fresh egg donation are not that different from the vitrified eggs donation. More studies should be done to confirm these results.

POSTER SESSION**ART, LABORATORY: CRYOPRESERVATION OF GAMETES****P-369 Poster** Clinical evaluation of blastocyst transfer in oocyte cryopreservation cyclesC.C. Chang¹, D.P. Bernal¹, H.I. Kort¹, C.W. Elsner¹, D. Mitchell-Leaf¹, D.B. Shapiro¹, A.A. Toledo¹, Z.P. Nagy¹¹*Reproductive Biology Associates, IVF center, Atlanta GA, U.S.A.*

Introduction: Cryopreservation of human oocytes has been of interest in assisted reproduction for many years. Although, oocyte cryopreservation is a

sophisticated technology in the field of human Assisted Reproductive Technology, it still requires clinical enhancements to improve the overall efficiency (implantation and pregnancy rates). Blastocyst transfer has proven effective for the selection of embryos resulting in the highest implantation and pregnancy rates in fresh IVF cycles recently. However, there is virtually no data to evaluate the clinical results of the blastocyst transfer for oocyte cryopreservation cycles. Therefore, our objective was to investigate the feasibility of blastocyst transfer specifically for oocyte cryopreservation cycles.

Materials and methods: Oocytes were donated for the study by 10 individuals less than 35 years of age. Recovered oocytes from donors were denuded using a combination of enzymatic (Cumulase) and mechanical cleansing. Oocytes that were at the metaphase-II stage at the time of denudation were kept in culture for 2 hours before freezing them. Cryopreservation on all oocytes was performed by the vitrification method. The oocytes were first equilibrated in 7.5% ethylene glycol and 7.5% DMSO for 5 min, and then they were transferred directly into the vitrification solution containing 15% ethylene glycol and 15% DMSO for 45–60 seconds before freezing. Oocytes were placed onto the plastic film of cryotop and immediately plunged into liquid nitrogen. Oocyte thawing was performed by serial dilutions in three steps using 1.0M, 0.5M, and 0M sucrose solutions. Oocytes that survived were inseminated with the sperm of recipient husbands by ICSI 2–3 hours after thawing. On day 5, blastocyst formation was assessed and graded for embryo replacement.

Results: One hundred and fifty-five oocytes were vitrified and thawed (average 7.75 oocytes/recipient) and 135 survived (87.1%). Fertilization (2PN) rate was 86.6% (117/135). The embryo cleavage was assessed on day 3 (114/117; 97.4%). Eighty-five of 117 embryos (72.6%) developed to blastocyst stage until day 6. Embryo transfer was performed on day 5; 52 blastocysts were transferred to 20 recipients; 18 out of 20 recipients had positive b-hCG and a total of 29 FCAs were detected in 17 recipients (implantation: 29/52, 55.7%). Nine out of the 17 recipients have delivered 17 healthy babies, and the other 8 pregnancies are still ongoing.

Conclusions: In the present study, we showed that blastocyst transfer was successfully integrated into the oocyte cryopreservation program that achieved high implantation and pregnancy rates. The efficiency of blastocyst transfer of cryopreserved oocytes can be comparable to fresh oocytes with regard to clinical outcomes; hence, the idea of blastocyst transfer seems to render an efficient criterion for selecting embryo with high developmental competency and may be used routinely in the human oocyte cryopreservation protocol.

P-370 Poster Fertility preservation in breast cancer: oocyte freezing after IVM & ovarian cortex cryopreservation versus oocyte freezing after modified stimulationS. von Otte¹, M. Thill¹, B. Schöpfer¹, A. Schultze-Mosgau¹, S. Al-Hasani¹, K. Diedrich¹, G. Griesinger¹¹*University of Schleswig-Holstein Campus Luebeck, Department of Obstetrics and Gynecology, Luebeck, Germany*

Introduction: Breast cancer (BC) is the leading cancer in women of reproductive age. Improved treatment strategies in breast cancer patients lead to an increased long-term survival. In general BC is treated with surgery followed 4 to 6 weeks later by a combination chemotherapy containing the alkylating agent cyclophosphamide (CP) causing a significant loss in ovarian follicle reserve and premature ovarian failure. With increasing survival rates a growing number of breast cancer patients are seeking fertility preservation (FP) strategies.

Methods: In 9 premenopausal breast cancer patients (mean age 31.3 ± 3.2; 5 estrogen receptor positive, ER+) two different FP strategies prior to chemotherapy where arbitrarily chosen: in group 1 (n = 5) all visible antral follicles were aspirated regardless of the menstrual cycle 36 hours after 5000 IU hCG. All aspirated immature oocytes were matured in-vitro (IVM). In the same anesthesia one third of the ovarian surface was laparoscopically removed and cryopreserved. In group 2, ovarian stimulation was performed using a modified antagonist regimen employing rFSH, tamoxifen, and letrozole. For rapid reduction of estradiol (E2) levels and prevention of OHSS, a GnRH-agonist instead of hCG was used for triggering final oocyte maturation. Depending on the availability of a male partner, mature oocytes were vitrified in both groups either fertilized (ICSI technique) or unfertilized.

Results: In group 1, a total of 50 GV-stage oocytes was aspirated without any delay of cancer treatment and without alteration of serum E2 levels (mean