the MII stage. After all MII oocytes were injected with sperm, the maturity of the remainder of the ooctyes was re-evaluated. Any oocytes that developed a polar body since the last observation (Late MII) were also injected with sperm. If <5 oocytes were injected, the observation for appearance of Late MII oocytes was continued until 5-6 hours post-retrieval. Embryos were cultured individually in Sage media in 5% O_2. Fertilization rate, day 3 embryo quality and blastocyst quality (Day 5 transfers only) for MII and Late MII oocytes were compared.

Determination of transfer day:
Transfer was performed on day 3 if <35 years and fewer than 4 Top quality embryos; 35-40 years with fewer than 6 Top quality embryos; or patients >40 years regardless of embryo quality. All other patients were transferred on day 5.

Definitions of embryo quality:
Good Quality Day 3 Embryo: An embryo with the following characteristics at the indicated time points post-insemination: 25 hours: Between the 2 pronuclear stage and 2 cell stage, even cells, no multinucleate blastomeres; 42-44 hours: 2-5 cells, even cell divisions, no multinucleate blastomeres; 66-68 hours: 7-8 cells with <20% fragmentation or 6-9 cells with <15% fragmentation, ≥2 more cells than on day 2, even cell divisions, no multinucleate blastomeres.

Good Quality Blastocyst: A blastocyst with the following characteristics on either day 5 or day 6: Expanded blastocoel cavity, organized and compacted inner cell mass, medium to large number of cohesive cells in the trophectoderm.

Statistical analysis: Data were analyzed using Pearson Chi Square.

Results:

Oocyte Type	% Fertilization	% Good Quality Day 3 Embryos	% Good Quality Blastocysts
MII	74.6% (1207/1617)	57.3% (693/1209)	15.6% (80/512)
Late MII	53.4% (127/238)	52.0% (66/127)	13.5% (5/27)
P	0.000	0.247	0.064

Conclusion:
Late MII oocytes fertilize at a fairly good rate, even though it is significantly lower than that of MII oocytes. The Late MII oocytes that fertilize develop into good quality embryos/blastocysts at a rate equivalent to that of MII oocytes. Insemination of Late MII oocytes is therefore of benefit to patients.

MicroSecure Vitrification (µS-VTF): Evaluation of Thaw Intervals for Human Blastocysts

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Objective: MicroSecure vitrified (µS-VTF) blastocysts (BLs) were thawed to determine whether the ambient temperature thawing interval could be reduced safely without compromising viability and allow further study on VTF-oocyte thawing intervals. Cryophysical models suggest a delicate balance between cellular rehydration/equilibration and membrane plasticity. BLs vitrified in non-DMSO solutions were thawed and evaluated in three test time groups for survival and viability of shorter sucrose dilution intervals. We aimed to determine that a 5 min dilution at each of 5 dilution steps, as indicated by the manufacturer, is excessive. We proposed that the dilution intervals could be reduced to 3 min without adversely altering cellular viability.

Methods: In Phase I, 55 discard µS-VTF BLs were randomly separated into three treatment group dilution intervals (n=14/trt): 1min, 3min, and 5min (positive control). All embryos used were research consented. BLs were diluted stepwise from 1.0M sucrose (T1) to isotonic LG-H (HEPES buffered T5) in five intervals. Note, in the 1min treatment group BLs were exposed to T1 solution for 3 min before applying the 1min intervals (T2-T5). In addition, a negative control group (n=13) was added to evaluate BLs in T1 solution for 3 min before direct placement into LG-H. After dilution, all VTF BLs were incubated with 5% CO_2 at 37°C and cultured using LG medium + 7.5%SS in microdrops under oil for 24 hrs. The BLs were assessed for continued development (BL expansion/hatching=survival). In the 1st quarter of 2012, Phase II of this study was implemented to evaluate the effectiveness of 3 min µS-VTF BL dilution intervals on FET cycle outcomes (n=44, all age groups). We contrasted these preliminary outcomes to our µS-VTF FET cycles in 2010-2011 (n=206).

Results: No differences in post-dilution survival of VTF BLs were observed in all treatment groups. Furthermore, survival in our negative control group was not different.

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Dilution Interval	5min	3min	1min	Negative Control
# Thawed	14	14	14	13
# Survived (%)	13 (93%)	13 (93%)	13 (93%)	12 (92%)

In the clinical application of the 3 min dilution treatment, 80 of 81 BLs survived (98.8%), and 32 of 44 (72.7%) developed a clinical pregnancy. The outcomes compare favorably to 2010-11 outcomes for BL survival (398/420, 95%) and clinical pregnancy rates (n=134, 65%).

Conclusion: VTF BLs were more resilient to osmotic changes than theoretical modeling would predict. 1M sucrose effectively and safely dehydrates BLs, eliminating the potentially toxic intracellular cryoprotectants, with normal membrane fluidity post-VTF. Most notable, the negative control demonstrates that an initial 3 min 1 M sucrose exposure resulted with comparable survival. Based on our results, the 3 min/ 5 dilution step protocol has been successfully implemented into our clinical practice with continued consistency in survival and pregnancy. Additional viability studies are needed before more extreme thawing practices (e.g., 1 min dilutions or single step only thaw) are adopted for clinical application. Considering the membrane resiliency of VTF BLs during rehydration without lysis, we are hopeful that these findings can be applied to human oocytes. Ongoing investigations are aimed at optimizing our aseptic vitrification system for oocytes using the non-commercial, FDA compliant µS-VTF system. Overall, microSecure vitrification (Schiewe, JCE, 2010) offers unparalleled facets of quality control (e.g., labeling, security, technical ease, repeatability, and storage) which should be seriously considered in the clinical application of vitrification.

Disclosure: The authors have no commercial interests in the µS-VTF product.

Potential For Empowering And Broadening The Application Of SART Embryo Grading System

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Objective: Morphological assessment has been the method of embryo selection since the application of IVF for infertility treatment. The necessity of developing a unifying embryo grading system acceptable to all has since been felt. Recently SART took such an initiative so that embryo grading is done using a uniform grading system in reporting embryo data to SART registry. SART elected three growth phases, cleavage, morula and blastocyst, to be evaluated by such grading. The objective of the present study was to see if the SART grading can be strengthening further, and be applied to other growth phases of the embryo.

Study Design: Possibility of integration of more morphological determinants into the SART grading system, and its application to all growth phases are explored.

Materials and Methods: The grading system developed by SART task force and the preliminary outcome of the collection of the information based on such grading were assessed. Proficiency test (PT) data on SART grading system were analyzed. Published literatures on different embryo grading models were reviewed. Morphological determinants used in previous grading systems but not in SART system were attempted to incorporate in the SART three point (good, fair, poor) grading system, and the possibility of its uses to all growth phases is assessed.

Results: The SART three point grading (good, fair, poor) utilized an independent set of three parameters for cleavage and blastocyst, and two parameters for morula. Grading morula is an unpopular event. Proficiency testing did not include day 4 morula; instead it targeted day 1 zygote, day 3 cleavage and day 5 blastocyst. The post insemination timing of grading embryos was 17-18 hrs, 42-44 hrs, 64-68 hrs, 88-90 hrs, 115-117 hrs and 140-142 hrs for day 1, day 2, day 3, day 4, day 5 and day 6 of growth, respectively. A set of four morphological parameters in each growth phase have been proposed in which all relevant morphological determinants that were utilized so far in the previously reported embryo grading models can be accommodated. Further, these growth phase specific morphological parameters can be integrated into the SART three point grading system for evaluating any developmental stage starting from day 0 to day 6 of in vitro development.

Conclusion: The SART embryo grading can be made powerful by incorporating more relevant morphological determinants into it without compromising its simplicity and uniqueness. Secondly, the SART system is applicable in any preferable day of embryo assessment.

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