for 1 min, 0.5M sucrose for 4 min, and MOPS-buffered media for 6 min. Survival, blastulation, and hatching rates were monitored daily. Chi-square test was used for statistical analyses, with post-hoc Tukey testing for multiple comparisons.

RESULTS: A total 302 embryos were vitrified and recovered. Results are summarized below.

Effects of Sucrose in ES on Mouse Embryo Development

	Control n(%) n=101	0.25M n(%) n=100	0.5M n(%) n=101	P-value
Survival	94 (93%)	99 (99%)	98 (97%)	0.09*
Blast stage	57 (56%)	69 (69%)	60 (59%)	0.161
Hatch stage	37 (36%)	46 (46%)	45 (45%)	0.350

*0.25M vs. control p=0.037

Post-thaw survival rates were similar among all three groups. However, pair-wise analysis revealed a significant increase in survival between the 0.25M group vs. control (p=0.037). There was also a trend towards increased blast formation in the 0.25 M group compared to control (p=0.06). While there were no differences in hatching rates between all three groups, there was a trend towards increased hatching in the 0.25M group compared to control (p=0.09). No differences in survival, blastulation, and hatching rates were noted between the 0.25M and 0.5M groups. All three outcomes were also similar between the 0.5M and control groups.

CONCLUSION: The addition of sucrose, a non-permeating osmolyte, to ES may enhance intracellular dehydration at an earlier stage. This may promote a higher efficiency of dehydration during rapid exposure to VS without the requirement of high amounts of permeating cryoprotectants. Addition of 0.25M sucrose to ES resulted in higher embryo survival and a trend towards increased blastulation. Further study is warranted to determine optimal concentrations of disaccharides in ES to optimize vitrification.

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NON-INVASIVE TEST FOR EMBRYO COMPETENT SELECTION BY QUANTIFICATION OF CELL-FREE NUCLEIC ACIDS IN EMBRYO CULTURE MICRO DROP. S. Assou,[a] A. Gala,[a] T. Al-Edani,[a] A. Ferrières,[a] A. R. Thierry,[b] S. Hamamah.[c] [a]Université Montpellier 1, Inserm U1040, IRMB, Montpellier, Hérault, France; [b]INSERM U896, Montpellier, Hérault, France; [c]ART-PGD Department, Université Montpellier 1, Inserm U1040, IRMB, Montpellier, Hérault, France.

OBJECTIVE: In order to increase the success rates of IVF cycles, improved methods for embryo selection to produce a baby are required. We determined the cell-free nucleic acids (cfNA) levels in human embryo spent culture media and we evaluated their possible use as biomarkers for embryo competent selection.

DESIGN: Human fertilized oocytes were individually cultured from zygote to blastocyst satge. A total of 60 spent culture media were collected on day 3 (6-8 cells) and day 5/6 blastocyst stage.

MATERIALS AND METHODS: MicroRNAs (miRNAs) such as MiR-21 and Let-7b were extracted from drops with the QIAamp kit and quantified by RT-qPCR using TaqMan technology. Cell-free DNA (cfDNA) was quantified using Bio-Rad Supermix SYBR Green. Statistical analyses defined relationship between nucleic acid content and embryo outcome.

RESULTS: We demonstrate that the embryo culture medium samples, during in vitro early embryo development, contained embryonic cfDNAs and miRNAs. The concentration values of cfDNA are lower in the culture medium in which emerge top quality embryo compared to no top ($p<0.05$). In the embryos that reached good blastocyst quality and leading to pregnancy, the variation in the cfDNA concentration between day 3 and day 5/6 is significantly decreased significantly and drastically of 88% (22.16 ng/ml and 2.75 ng/ml at day 3 and day 5/6 respectively). This variation is very low between day 3 and day 5/6 in the no good blastocyst quality (6.46 ng/ml and 3.78 ng/ml at day 3 and day 5/6 respectively, 41% decrease). Relate to the expression of the miRNAs which identified in spent media and was correlated with embryo outcome, MiR-21 and Let-7b were more highly concentrated in both day-3 and day-5 media samples when compared with day-0 samples (cycle threshold=33 and 34 versus 39.5, respectively).

CONCLUSION: Under in vitro IVF/ICSI conditions, changes in the nucleic acids levels in the embryo culture medium on day 3 and day 5/6 predict the embryo quality and may be used as a new potential biomarker for selecting top quality embryos. Our data strongly open the possibility to develop a new quick and low-cost test for the selection of the embryos viable with the highest implantation potential.

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DO EMBRYO DESCRIPTORS ON CULTURE DAYS 2 AND 3 AID IN SELECTION OF BLASTOCYSTS WITH IMPLANTATION POTENTIAL? D. H. McCulloh, K. Goldman, B. Hodes-Wertz, C. McCaffrey, J. A. Grifo. NYU Fertility Center, NYU Langone Medical Center, New York, NY.

OBJECTIVE: To determine if embryo descriptors on days 2 and 3 provide selective value over and above the quality descriptors on day 5.

DESIGN: Retrospective Analysis of Embryo Scores and Implantation.

MATERIALS AND METHODS: Data from our electronic medical record system were accumulated for patients who became pregnant between 1/1/2003 and 12/31/2012 but for whom all or only some of the transferred embryos implanted. This method was used previously to assess embryo characteristics associated with implantability, greatly reducing the impact of uterine factors on outcome. Embryos were selected for transfer by their characteristics (Stage, ICM and TE scores) on Day 5. Multiple logistic regression was used to determine if the number of cells, the fragmentation, or the embryo grade on days 2 and/or 3 provided any further predictability of implantation. Significant parameters were chosen using the Akaike Information Criterion (AIC) and the ability to predict outcome was assessed using Receiver Operator Characteristic (ROC) curves, and in particular the Area Under the ROC Curve.

RESULTS: 3971 embryos transferred to 2111 patients who experienced a clinical pregnancy were analyzed. ROC curves were constructed using the best fit MLR equations using Day 5 Blastocyst Scores alone, Day 5 Blastocyst scores plus day 3, plus day 2 and plus days 2 and 3 descriptors. The area under the ROC curve assessing the ability of Blastocyst scores on day 5 to predict implantation was 0.57. The AUC was not improved by adding day 2, day 3 or both day 2's and day 3's descriptors to the MLR best fit equation.

CONCLUSION: We conclude that consideration of embryo descriptors on days 2 and 3 for embryos without preimplantation genetic screening, provides no additional selective value over the blastocyst descriptors on day 5 alone. Therefore, neither the number of cells on day 2 or 3, the fragmentation nor the grades provide additional information about the embryos implantation potential that is not already present in the description of the blastocyst on day 5. Since we only considered blastocysts on day 5, we cannot determine if day 2 and day 3 descriptors will predict which embryos will become blastocysts. Our results indicate to us that descriptions of blastocysts on day 5 provide discrimination of morphological features associated with implantabilty that are superior to any features that we observed on days 2 and 3.

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DO MICROMANIPULATION TECHNIQUES AND CRYOPRESERVATION ALTER PRE-IMPLANTATION DEVELOPMENT AND GENE EXPRESSION IN CULTURED EMBRYOS? M. Paczkowski, M. Reyes, D. Jones, T. J. Kuehl. Baylor Scott & White Healthcare, Temple, TX.

OBJECTIVE: To examine the effects of micromanipulation and cryopreservation of mouse blastocysts cultured in vitro using morphologic and gene expression endpoints.

DESIGN: Research study.

MATERIALS AND METHODS: Two-cell mouse embryos were cultured for 42 hrs until the blastocyst stage and divided into 3 treatment groups: control, laser assisted hatching (LAH), and biopsy. A subset of the LAH (LAH-cryo) and biopsied (biopsy-cryo) embryos was cryopreserved via slow cooling and subsequently thawed for analysis. After manipulation, or thaw, embryos were cultured for an additional 24, 28 and 40 hrs and blastocyst development was recorded. Fully hatched blastocysts were collected at