Table 2 Mouse embryo development after co-cultured with human endometrial cells.

| # of days after feeder cell thaw | D3 (8 cells and above) | D4 ( morula and above) | D5 ( full/hatching blast) |
|---|---|---|---|
| 1 | 27/28 ( 96%) | 28/28 ( 100%) | 25/28 ( 89%) |
| 2 | 31/33 (94%) | 32/33 (97%) | 30/33 ( 91%) |
| 3 | 31/31 (100%) | 29/31 (94%) | 18/31 ( 58%)* |
| 4 | 5/30 ( 17%)* | 19/30 (63%)* | 0/ 30 ( 0%)* |
| 5 | 6/30 (20%)* | 1/30 ( 3%)* | 0/ 30 ( 0%)* |
| Control | 28/32 (88%) | 31/32 (97%) | 24/32 (75%) |

* $\chi 2$ test after adjusting for multiple comparisons $p<0.05$

CONCLUSION: Our study indicates that prolonged preparation of feeder cells may cause a detrimental effect on embryo growth. The acceptable time period of the feeder cell preparation is a maximum of 3 days if using granulosa cells and 2 days if using endometrial cells for co-culture. Therefore, to achieve the optimal development of embryos co-cultured with granulosa or endometrial cells, it is suggested to thaw the feeder cells at the earliest, the day before or, more preferably, the day of, oocyte retrieval.

*Supported by:* None.

**P-270**

**DETERMINING THE PREDICTIVE VALUE OF DAY 3 EMBRYONIC MORPHOLOGY AND CELL STAGE ON ULTIMATE EMBRYO QUALITY.** K. M. Silverberg, H. Werland, T. Turner, R. Fields, C. Crowl, T. C. Vaughn. Texas Fertility Center, Austin, TX; Austin IVF, Austin, TX.

OBJECTIVE: Although blastocyst transfer has been demonstrated to offer higher implantation rates than Day 3 embryo transfer (ET), few programs offer blastocyst transfer exclusively. Due to significant in vitro embryonic attrition between Days 3 and 5, optimal embryo selection is of paramount importance for patients undergoing Day 3 ET. Few data exist, however, regarding the predictive value of Day 3 embryo morphology and cell stage on ultimate (Day 5 or 6) embryo quality. This study was designed to assess the predictive value - if any - of both Day 3 embryo morphology and cell stage on ultimate in vitro embryo quality.

DESIGN: Prospective analysis of all patients undergoing IVF with a Day 5 embryo transfer in 2005 in a large private infertility practice.

MATERIALS AND METHODS: Oocytes were inseminated in Sage fertilization medium with 0.5% HSA and cultured to Day 3 in Sage cleavage media with 10% SPS. Embryonic appearance and cell stage on the morning of Day 3 were noted and embryos were then transferred into Sage blastocyst media with 10% SPS. The morphologically best blastocysts were transferred on Day 5, while other good quality blastocysts were cryopreserved on either Day 5 or 6. For purposes of data analysis, embryos that were transferred or cryopreserved were considered good quality (Group 1), whereas embryos that stalled or failed to become good quality blastocysts were considered poor quality (Group 2). Statistical analysis was performed using t-test and linear regression analysis.

RESULTS: 103 patients underwent IVF with Day 5 ET in our program in 2005. All 1448 embryos resulting from these cycles were included in this study. 202 blastocysts were transferred (14%), 265 were cryopreserved (18.3%), 439 stalled (30.3%), and 544 made poor quality blastocysts (37.6%). 81% of these patients achieved pregnancy, with 71% ongoing or delivered as of this time. Embryos were assessed using a combination of morphologic grade (G) (1=best, 4=worst) and cell stage (C). Statistical analysis identified 3 subgroups based on Day 3 embryonic appearance: Favorable criteria produced significantly more Group 1 embryos (>48%) than did unfavorable criteria (< 32%, p< 0.05) Favorable Criteria: >10C, 10C-G2, 9C-G1, 9C-G2, 8C-G1, 8C-G2, 7C-G1 Intermediate Criteria: Morula, 10C-G1, 7C-G2 Unfavorable Criteria: 10C-G2.5/3/4, 9C-G2.5/3/4, 8C-G2.5/3/4, 7C-G2.5/3/4, less than or equal to 6C-all grades

CONCLUSION: Day 3 embryonic morphology and cell stage afford significant information regarding ultimate in vitro embryonic quality. Regardless of morphologic grade, it appears as though embryos must achieve at least the 7 cell stage in order to have a better prognosis. Once this cell stage has been achieved, morphologic grade appears to be prognostic, as higher degrees of fragmentation and cellular irregularity portend a poorer prognosis. The use of these criteria may enable embryologists to better select the appropriate embryos for Day 3 ET.

*Supported by:* None

**P-271**

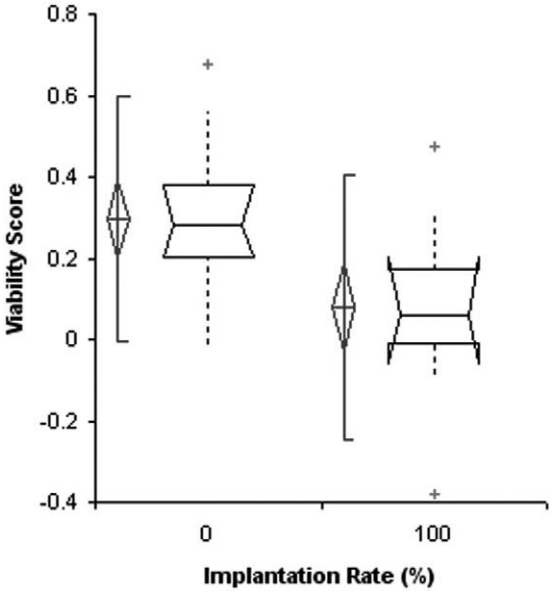
**A PROSPECTIVE BLINDED EVALUATION OF THE RELATIONSHIP BETWEEN METABOLOMIC PROFILING OF SPENT EMBRYO CULTURE MEDIA AND HUMAN EMBRYONIC REPRODUCTIVE POTENTIAL.** R. Scott, K. Miller, S. Picnic, S. Rosendahl, J. B. Massey, D. Burns. RMA of NJ, Morristown, NJ; McGill Univ., Montreal, PQ, Canada; Reproductive Biology Associates, Atlanta, GA.

OBJECTIVE: To determine if metabolomic profiling of embryonic development using biospectroscopy-based metabolomics was associated with implantation and delivery rates in IVF

DESIGN: Prospective blinded.

MATERIALS AND METHODS: *Population:* Patients undergoing IVF who gave informed consent to evaluate discarded media used during in vitro embryo culture and subsequent clinical outcome were studied. Specimens were collected on each embryo for all patients. A subset of patients who had either 0% or 100% ongoing implantation rates were selected for further study. *Specimen Collection:* The embryology laboratory routinely cultures embryos in individual droplets. Each is uniquely identified to allow tracking of individual embryonic development and specific collection of the spent media. In this study, spent media were collected on day 3 after 44 hours of embryo culture in cleavage stage media. All samples immediately frozen and stored at -80 C until analyzed. *Specimen Analysis:* Five to 10 $\mu$l of spent culture media from each individual embryo that was used in an embryo transfer was evaluated using both RAMAN and Near Infrared spectroscopy. Both of these tools are sensitive to changes in the relative hydroxyl modifications of various molecular constituents in the media. *Data Analysis:* Analysis of the spectral data was done by a single investigator who was blinded to all clinical data. The spectra obtained from each instrument were separately analyzed by a proprietary modification of a wavelength selective genetic algorithm (Molecular Biometrics, LLC, Chester, NJ) which had previously been developed following analysis of specimens with known implantation outcomes. A viability score was obtained for each sample based on its unique metabolomic spectral profiles. These scores were subsequently compared with the clinical outcome data using a t-test.

RESULTS: Thirty five embryos transferred to 14 patients were evaluated. Eight patients had a total of 18 embryos transferred and had an ongoing implantation rate of 100%. Six patients had 17 embryos transferred with no implantations. There were significant differences in the spectral scores between those embryos which implanted and those which did not (P=0.003). The scores were then regrouped by patient with each subject being assigned the mean of the scores of their individual embryos. There was a significant difference between those patients whose embryos were reproductively competent (100% sustained implantation rate) and those embryos that were not competent (P=0.01).




CONCLUSION: This prospective blinded analysis demonstrates a clear relationship between the reproductive potential of human embryos and their modification of the culture media in which they have been cultured. These