Conclusions: HB-EGF partially restored the rate of *in vitro* blastocyst differentiation to nearly that achieved within the uterine environment. The acceleration of blastocyst differentiation by HB-EGF may be associated with its ability to increase the intracellular concentration of free Ca^{2+} and activate calmodulin. This signaling pathway appeared to be developmentally regulated during the first 14 h of blastocyst culture.

1) Das et al. HB-EGF gene is induced in the mouse uterus temporally by the blastocyst solely at the site of its apposition. Development 1994;120:1071
2) Stachecki et al. Mouse blastocyst outgrowth and implantation rates following exposure to ethanol or A23187 during culture in vitro. J Reprod Fert 1994;101:611

This work was supported by grant P50AA07606 from the National Institutes of Health

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Expression of the $\alpha_{IIb}\beta_3$ Integrin Receptor for Fibronectin Prior to Blastocyst Implantation. U. K. Rout, D. R. Armant. C. S. Mott Center for Human Growth and Development, Dept. of OB/GYN, Wayne State University, Detroit, MI.

Objectives: Integrin-mediated fibronectin (FN) binding activity is both temporally and spatially regulated during mouse blastocyst differentiation in accordance with the adhesive behavior of the implanting embryo.[1] Subunits of several FN-binding integrins, including α_5, α_v, β_1 and β_3, are expressed throughout preimplantation development,[2] however, no information is available regarding $\alpha_4\beta_1$, $\alpha_4\beta_7$ or $\alpha_{IIb}\beta_3$, which also recognize FN. Therefore, the levels of α_4 and α_{IIb} mRNAs were estimated in mouse embryos prior to implantation.

Design: Nucleotide sequences of α_4 and α_{IIb} cDNAs were obtained for the design of oligonucleotide primers to be used for semiquantitative analysis of the respective mRNAs in preimplantation embryos employing reverse transcription and the polymerase chain reaction (RT-PCR).

Materials and Methods: Partial sequence of mouse α_{IIb} was obtained using cDNA prepared from mouse B16 melanoma cell RNA and nested PCR with published oligonucleotide primers for the rat α_{IIb} sequence. A 711 bp product was cloned into a pCR3 vector and sequenced to confirm identity. Specific primers for mouse α_{IIb} were then designed using the OLIGO v 5.0 program. Primers for RT-PCR amplification of α_4 mRNA were designed similarly using the published sequence of mouse α_4 cDNA. Mouse embryos were collected for RNA isolation during the first 4 days of gestation and after 24 or 48 h of blastocyst culture in Ham's F10 medium. RNA equivalent to 200 embryos was reverse transcribed using oligo $(dT)_{12-18}$. PCR was carried out for 30 cycles using either 10 or 20 embryo equivalents of cDNA.

Results: Sequencing of the 711 bp mouse α_{IIb} amplicon revealed 79% identity to the human sequence. The α_4 mRNA was not detected at any stage of preimplantation development, although an amplicon of the correct size and sequence was produced from B16 cell RNA. The α_{IIb} mRNA was present from the unfertilized oocyte stage onward, reaching peak levels at the morula stage. Product band intensities were approximately linear with the amount of embryonic cDNA added during PCR under the conditions of assay.

Conclusions: Mouse preimplantation embryos do not express the α_4 integrins, which supports a previous finding that antibodies against α_4 do not label trophoblast cells adhering to FN.[3] The expression of α_{IIb} mRNA is consistent with its detection in trophoblast focal contact sites by immunohistochemical staining.[3] Thus, like several other FN-binding integrins, $\alpha_{IIb}\beta_3$ is transcribed throughout preimplantation development, supporting the view that blastocyst adhesion to the extracellular matrix during implantation is regulated by post-transcriptional mechanisms.

1) Schultz JF, Armant DR. β1- and β3-class integrins mediate fibronectin binding activity at the surface of developing mouse peri-implantation blastocysts. J Biol Chem 1995;270:11522
2) Sutherland AE, Calarco PG, Damsky CH. Developmental regulation of integrin expression at the time of implantation in the mouse embryo. Development 1993;119:1175
3) Yelian FD, Yang Y, Hirata JD, Schultz JF, Armant DR. Molecular interactions between fibronectin and integrins during mouse blastocyst outgrowth. Mol Reprod Dev 1995;41:435

This work was supported by grant P50AA07606 from the National Institutes of Health

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Fibronectin (FN) Concentrations in Human Embryo Culture-Conditioned Media. Their Relationship With Embryo Morphology, Pregnancy Outcome and IVF or ICSI. [1]R. I. Barañao and [2]E. Polak de Fried. [1]Instituto de Biología y Medicina Experimental Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and [2]CER Instituto Medico, Department of Reproductive Medicine, Buenos Aires, Argentina.

Objectives: FN is an adherence protein from the extracellular matrix that could be involved in the implantation process and that is associated with the embryo differentiation in diverse species of vertebrates. The aim of this study was to evaluate the concentration of FN in 24-hour human embryo culture-conditioned media (HECCM) and the relationship between FN levels, embryo morphology and pregnancy outcome, as well as that the possibility of a difference between FN levels in embryos obtained by IVF and ICSI. The connection between IL-1β concentration in HECCM and FN levels was also evaluated in consideration of pregnancy outcome.

Design: We developed an enzyme-linked immunosorbent assay (ELISA) for FN in which the lowest concentration detectable was 5 ng/ml and the intra and inter assay variability were 5% and 6% respectively. We analyzed HECCM from individual embryos obtained by IVF or ICSI. Their morphology was classified according to the Lucinda Vek criteria.

Materials and Methods: We analyzed 38 samples of HECCM (medium which surrounds individual human preembryos of 2–4 cells, 24 hour post-fertilization) and 6 controls. HECCM consists of Human Tubal Fluid containing 10% (v/v) synthetic serum substitute. Controls were HTF that had not been exposed to embryos. FN was determined by an original development of ELISA, and IL-1β levels were quantified using a high sensitivity commercial kit.

Results: FN levels were directly related to the morphology of the embryos. Values obtained in HECCM from embryos with different qualities of morphology were: I=859 ± 325 ng/ml (n=10), II=649 ± 301 ng/ml (n=7), III=251 ± 136 ng/ml (n=5), IV=128 ± 52 ng/ml (n=5) and V=83 ng/ml (n=1). We observed that in 23 HECCM which corresponded to embryos that did not render viable pregnancies the concentration of FN was 206 ± 28 ng/ml, while in 15 HECCM from embryos which rendered viable pregnancies the concentration of FN was 1280 ± 284 ng/ml ($p<0.001$). The FN concentration in control samples was 90 ± 10 ng/ml ($p<0.001$ vs. HECCM). No significant differences were observed in the concentration of FN from embryos obtained by the IVF and ICSI fertilization methods. We also determined a direct relationship between IL-1β and FN concentration since in HECCM from embryos which rendered viable pregnancies the average concentration was 4.0 ± 1.0 pg/ml while in those that did not render pregnancies the average was 0.4 ± 0.3 pg/ml.

Conclusions: According to our results the highest FN levels were found in embryos with the best morphology. In addition there is a strict relationship between soluble FN levels in HECCM from 24-hour human embryos and pregnancy. There are no differences in FN production from embryos obtained by IVF and ICSI. Finally, FN production could be induced by an embryonic IL-1β.

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Apoptosis and Human Embryo Survival. [1]C. A. Brenner, [2]G. E. Exley, [1]M. Alikani, [1]J. Cohen, [2]A. S. McElhinny, [1]J. J. Stachecki, [1]R. T. Scott, [2]C. M. Warner. [1]Gamete and Embryo Research Laboratory, The Institute for Reproductive Medicine and Science of Saint Barnabas, West Orange, NJ; [2]Department of Biology, Northeastern University, Boston, MA.

Objectives: Cellular fragmentation in human embryos is a poorly understood phenomenon. It has recently been hypothesized that fragmentation in these embryos is a consequence of programmed cell death (PCD) or apoptosis and that highly fragmented embryos have a poor prognosis for survival. The main objective of this work is to identify genes whose activation or suppression predict human embryo survival. In this study we examined the transcription of the apoptotic genes *bax* and *bcl-2*.

Design: Using single-oocyte and single-embryo RT-PCR we characterized the mRNA expression of *bax,* a regulatory gene that promotes cell death and *bcl-2,* a regulatory gene which functions to enhance cell survival in various cell types.

Materials and Methods: RNA was isolated from spare human oocytes (N= 10) and embryos (N=50) at various stages of development. The RNA was converted to single-stranded cDNA in the presence of random primers with reverse transcriptase. Nested primer PCR was performed with *bax, bcl-2,* and GAPDH primers.

Results: The results show that *bax* mRNA is expressed at all stages of preimplantation human embryonic development implying that the transcripts are both maternal and embryonic. There are varying levels of *bax* mRNA expressed in different stages of oocyte maturation (GV, MI, and MII). The *bax* transcript was also present in 2-cell, 8-cell, 16-cell, compacted morula, and blastocyst stages.

The *bax* mRNA was present in $\frac{19}{21}$ embryos. The abundance of the mRNA varied among day 4 embryos. Similarly, *bcl-2* transcript was also expressed in 2-cell, 8-cell, 16-cell, and compacted morula stages but with much greater variability in message abundance. The *bcl-2* mRNA was present in $\frac{13}{20}$ embryos, but only abundant in $\frac{4}{20}$. As a result, we began to look at the *bax/bcl-2* ratios to predict embryo survival. In 9 embryos from day 4 of development we found the following qualitative mRNA levels: $\frac{6}{9}$ embryos had *bax* > *bcl-2,* $\frac{2}{9}$ embryos had *bax* = *bcl-2* and $\frac{1}{9}$ had *bax* < *bcl-2.* The highly fragmented embryos had *bax* mRNA > *bcl-2* while the normal embryos had *bcl-2* mRNA either equal to or greater than *bax* mRNA.

Conclusions: Since *bcl-2* promotes cell survival its overexpression may be important for human embryo survival during culture in vitro. Our results show that $\frac{4}{19}$ spare human embryos had abundant expression of *bcl-2,* and that these embryos were less than 15% fragmented. We also found that the ratio of *bax/bcl-2* may be correlated to the degree of fragmentation in the human embryo. Moreover, the *bax/bcl-2* mRNA ratio may be useful to predict human embryo survival. More experiments are in progress to determine if this result is sustained and whether the ratio can be applied as a diagnostic tool.

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Increased Incidence of Cell Death in Rat Blastocysts Exposed to High Glucose and TNF-α In Vitro or to Maternal Diabetes In Vivo. S. Pampfer, I. Vanderheyden, F. Cecchi, R. De Hertogh. Physiology of Human Reproduction Research Unit, UCL Medical School, 1200 Brussels, Belgium.

Objective: Exposure of rat blastocysts to high concentrations of glucose or TNF-α in vitro results in a marked decrease in the expansion of their inner cell mass (ICM), the cell lineage expected to give rise to all the fetal tissues following implantation. This effect is also observed in blastocysts recovered from diabetic rats. In this study, we investigate whether the impact of glucose, TNF-α or maternal diabetes is mediated via an increase in the incidence of either necrosis or apoptosis.

Design: Different techniques were used to visualize the occurence of cell death in rat blastocysts.

Materials and Methods: Rat blastocysts were treated for 24 hours with high glucose or with rat recombinant TNF-α and processed through a differential staining procedure that allows for the distinction between ICM and trophectoderm cells. The proportion of fragmented nuclei was