Comparison of single and sequential culture systems on embryo development and aneuploidy rates using sibling oocytes

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DESIGN: Quality management review of room RH, embryo development and pregnancy outcomes.

MATERIALS AND METHODS: A quality management review of 2011-2012 data from a Southwestern U.S. ART program comparing laboratory RH to embryo development and pregnancy outcomes. Data were divided between periods of high and low room RH and analyzed using t-test comparisons.

RESULTS: A total of 79 cycles were reviewed. Preliminary observations demonstrated that the majority of the chemical pregnancies (69.4%), occurred during cycles conducted during periods of relatively high laboratory RH. However, once established, equal percentages of pregnancies progressed to heart beat (63.6% vs. 69.9%; low RH: high RH respectively). Comparisons of embryo development and embryo quality are ongoing.

CONCLUSION: Results suggest that laboratory RH might impact the establishment of pregnancy in ART patients, but has little effect once pregnancy is established. Low lab RH might result in shifts in media osmolarity or shifts in gas control and pH adversely affecting embryo development. Ongoing studies are examining this relationship.

P-346 Tuesday, October 15, 2013

DO *IN VITRO* BLASTOCYST CULTURES CONSUME MORE EMBRYOS THAN *IN VIVO*? C. Fang, R. Huang, L. Li. Reproductive Medicine Research Center, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China.

OBJECTIVE: The aim of the current study was to determine if blastocyst transfer consumes more cleavage-stage embryos than day 3 embryo transfer.

DESIGN: A retrospective study.

MATERIALS AND METHODS: A retrospective analysis of 351 cases in the first cycle of IVF- ET from June 2010 to May 2012 was conducted.The selection criteria were women < 40 years of age with > 4 good quality embryos on day 3 after oocyte retrieval. In the D3 group, ET and extended culture of the surplus embryos were performed on day 3. In the D5 group, blastocyst transfer was performed on day 5 after oocyte retrieval. The number of cleavage-stage embryos required per gestational sac was compared between the two groups.

RESULTS: During the IVF cycles, the number of cleavage-stage embryos required per gestational sac was 2.76 in the D3 group compared to 4.39 in the D5 ET group ($P<0.05$). During the ICSI cycles, the number of cleavage-stage embryos required per gestational sac was 3.23 in the D3 group compared to 5.82 in the D5 ET group ($P<0.05$). The same results were obtained for patients with intrauterine pregnancies.

CONCLUSION: The day 5 blastocyst transfer consumed more cleavage-stage embryos than the day 3 transfer.

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P-347 Tuesday, October 15, 2013

DOSE-DEPENDENT EMBRYOTROPHIC EFFECT OF RECOMBINANT GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR AND BRAIN-DERIVED NEUROTROPHIC FACTOR IN CULTURE MEDIUM FOR MOUSE PREIMPLANTATION EMBRYO. J. H. Kim,[a] S. Kim,[b] B. Jee,[a] S. Kim.[c] [a]Department Obstetrics and Geynecology, Seoul National University Bundang Hospital, Seongnam, Kyonggi, Republic of Korea; [b]Maria Plus Fertility Hospital, Seoul, Republic of Korea; [c]Seoul National University Hospital, Seoul, Republic of Korea.

OBJECTIVE: To evaluate the dose-dependent effect of different concentrations of recombinant mouse granulocyte-macrophage colony-stimulating factor (rmGM-CSF) or brain-derived neurotrophic factor (BDNF) in culture medium on the development of in-vitro fertilized mouse embryos.

DESIGN: Experimental study.

MATERIALS AND METHODS: Mature oocytes were retrieved from superovulated female BDF-1 mice and inseminated by sperms from male BDF-1 mice. On day 1, two-cell stage embryos were divided and cultured until day 5 in embryo maintenance medium supplemented with 0, 1, 2, 5, or 10 ng/mL of rmGM-CSF or supplemented with 0, 5, 10, 20 ng/mL of BDNF. Blastocyst formation rate and their cell number were assessed.

RESULTS: The blastocyst formation rate and total cell count in blastocyst was similar in all rmGM-CSF treatment groups when compared with control. However, in the group supplemented by 10 ng/mL of BDNF, the blastocyst formation rate (63.9%) and total cell count (45.8 ± 11.5) was significantly higher when compared with control (52.3%, 38.0 ± 6.8, $p<0.05$ for each).

CONCLUSION: Supplementation of 10 ng/mL of BDNF enhanced the developmental potential of mouse preimplantation embryo, but supplementation of rmGM-CSF did not. The embryotrophic effects of these growth factors were not dose-dependent.

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P-348 Tuesday, October 15, 2013

COMPARISON OF SINGLE AND SEQUENTIAL CULTURE SYSTEMS ON EMBRYO DEVELOPMENT AND ANEUPLOIDY RATES USING SIBLING OOCYTES. L. Nanassy,[a] B. Dudas,[a] Z. Kosa,[a] S. Savay,[a] D. Debreceni,[b] A. Vereczkey.[a] [a]Versys Clinics Human Reproduction Institute, Budapest, Pest, Hungary; [b]Reprogenex Genetic Diagnostic Laboratory, Budapest, Pest, Hungary.

OBJECTIVE: The objective of this study was to evaluate the effect of different culture systems on the development of human preimplantation embryos.

DESIGN: Interim analysis of a prospective study with no true randomization to support decision making regarding which culture systems to use in IVF setting.

MATERIALS AND METHODS: A total of 137 zygotes from 25 women with at least two embryos fertilized by IVF or ICSI were included in the study. Before fertilization check, zygotes from each patient were allocated to either to sequential (n=71) (Vitrolife G5, Vitrolife) or single (n=66) (Global™, LifeGlobal) culture media. Global™ medium was replenished at day 3. Embryo biopsy was carried out for 17 patients at day 3, and the number of chromosomes was determined by CGH microarray (24Sure, Bluenome). Embryo scores (ES) at day 3 and day 5, as well as blastulation and aneuploidy rates were recorded. Data comparison was carried out using paired t-test and Chi-square analysis.

RESULTS: There was a significant difference in ES on day 3 (4.01 ± 0.065 versus 5.30 ± 0.086, $p < 0.01$) but not at day 5 (3.24 ± 0.120 versus 4.10 ± 0.118, $p > 0.05$) between the Vitrolife and Global culture systems respectively. The blastulation rate was likewise not different between groups (37.83 ± 1.754 versus 51.59 ± 1.727, $p < 0.05$). A total of 68 embryos were evaluated for chromosome content. No difference was observed in the frequency of euploid embryos (33.33% (11/33) versus 40% (14/35), $p = 0.57$) culturing in Vitrolife and Global™ media, respectively.

CONCLUSION: In our setting, higher ES was seen at day 3 using single culture system, but no difference was observed beyond this point in embryo quality and development. Also, the frequency of euploid embryos between culture systems did not differ. At this early point in our comparison, no detrimental effect could be observed using a single culture system. Continuation of the study to include a larger number of embryos along with the evaluation of pregnancy and implantation rates in an extended study is warranted.

P-349 Tuesday, October 15, 2013

CAN EMBRYO QUALITY BE IMPROVED BY IN VITRO EXPOSURE TO LOW OXYGEN CONCENTRATION OR BY USING A MINI-INCUBATOR? TWO RANDOMIZED CONTROLLED TRIALS. G. Paternot, S. Debrock, T. M. D'Hooghe, C. Spiessens. UZ Gasthuisberg, Leuven, Vlaams-Brabant, Belgium.

OBJECTIVE: The aim of this study was to evaluate the difference between embryos placed in an undisturbed environment (mini-incubator) (RCT1), or in a lower O2 tension (5%) (RCT2), compared to a standard incubator or in 20% oxygen.

DESIGN: Two randomized controlled trials.

MATERIALS AND METHODS: In both studies, patients younger than 36 years who entered their first or second IVF/ICSI cycle gave informed consent to participate in RCT 1 or RCT 2. Embryos were cultured in a mini- incubator or standard incubator (RCT 1) or in 5% or 20% oxygen (RCT 2). In each study, 395 embryos were needed in each group to detect a significant difference in embryo quality of 10% (reference value 35%) at a statistical power of 0,80.

RESULTS: The results of the first randomized study, evaluating an extended set of individual characteristics on day 1, day 2 and day 3 of embryo development, showed no overall impact of culturing the embryos in a mini-incubator. There was only a significant higher number of embryos with local fragments when cultured in the standard incubator on day 3 (48% versus 39%; p=0.027). The results of the second randomized study failed to show