DESIGN: Research Study.

MATERIALS AND METHODS: Human zygotes with 3 pronuclei (PN) were cryopreserved with consent, thawed, and placed into control (CON; n=23) or reduced nutrient (RN; n=25) sequential culture medium, both supplemented with HSA. Embryos were cultured individually in an EmbryoScope and were assessed for blastocyst development on D5 and D6. On D6, blastocysts were placed into fibronectin coated dishes for outgrowth culture in IVC1 (Cell Guidance Systems) medium, along with thawed D6 human blastocyst controls donated to research after culture under standard clinical conditions (Sage CM/BM with SPS; CBL). After 48h in outgrowth, attachment was assessed and media was replaced with IVC2. Media was replaced daily until 96h of outgrowth (D10), at which point embryos were fixed and imaged to measure outgrowth area. Embryos were then stained with F-actin, DAPI, and POUF51, and imaged using confocal microscopy to determine outgrowth volume, total cell number, and epiblast cell number, respectively.

RESULTS: Blastocyst development was not different between 3PN embryos cultured in CON and RN medium on D5 (30.4% and 24.0%) or D6 (34.7% and 28.0%). All embryos placed into outgrowth were attached by 48h (CON n=11; RN n=7; CBL n=7). There was no difference in the area of outgrowth between treatments, either at 48h ($0.05\pm0.02mm^2$, CON; $0.09\pm0.04mm^2$, RN; $0.06\pm0.02mm^2$, CBL) or 96h ($0.12\pm0.06mm^2$, CON; $0.23\pm0.10mm^2$, RN; $0.20\pm0.10mm^2$, CBL). Of embryos placed into outgrowth, 73% CON, 60% RN, and 100% CBL had a 3D volume that was assessed using confocal microscopy. Of these embryos, 50% of CON, 67% of RN, and 40% of CBL embryos contained a visible epiblast; these embryos had similar average numbers of epiblast cells (59, CON; 41, RN; 67, CBL).

CONCLUSIONS: This data demonstrates that an environment of reduced nutrient concentration successfully supports the development of human zygotes to the blastocyst stage, with equal developmental potential to both those cultured in control medium and those cultured in standard clinical conditions. In addition, 3PN zygotes developed to the blastocyst stage and successfully organized peri-implantation embryonic development equivalent to normally fertilized embryos. This innovative approach to safely investigating novel culture conditions for human embryos could significantly enhance research in the development of more effective embryo culture media for human ART.

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**BLASTOCYST DEVELOPMENT AND EUPLOIDY RATE IN SINGLE MEDIUM WITH CONTINUOUS OR RENEWAL ON DAY 3 USING SIBLING EMBRYOS.** H. Ryu, J. Galiguis, A. Pham, A. Le. HRC Encino, Encino, CA.

OBJECTIVE: To investigate which culture system is suitable for our clinical IVF by comparing blastocyst development and euploidy rate.

DESIGN: Prospective cohort study on sibling embryos.

MATERIALS AND METHODS: A total of 698 embryos from 37 patients (ages 24-41) from November 2017 through March 2018 were included in the analysis. Study inclusion required cohorts of at least 12 fertilized day 1 sibling embryos to be randomly divided into two separate groups. Up to 5 embryos were cultured in 50 $\mu$l droplets of culture media (Global, Life Global Group) under oil. All sibling embryos were treated under identical conditions except for the renewal of medium on day 3. Culture occurred in Labotec C-top incubators using certified premixed gas with 7% $CO_2$ and 5% $O_2$. Overall blastocyst conversion rates, biopsy rates (defined as the blastocyst conversion rate for fair or better quality embryos) and euploidy rates (PGS tested by Next Generation Sequencing (NGS)) on day 5 and day 6 were compared (Chi square, P=0.05).

RESULTS: The overall blastocyst and biopsy rates for the continuous group (71% and 80%, respectively) were no statistically different from those of the renewal group (71% and 88%, respectively) (p>0.05). Similarly, early blastocyst development (d5) and late blastocyst development (d6) showed no significant difference between the two groups (p>0.05). A total of 109 of 201 embryos (54%) in control group and a total 116 of 217 embryos (53%) were euploid (p>0.05).

CONCLUSIONS: The culture system, whether continuous or renewal, did not affect our blastocyst development or euploidy rate. However, the renewal culture media demonstrated slightly higher D5 blastocyst conversion even though there are no significant differences.

Table 1Blastocyst Development and Euploidy Rate Between Two Groups

| Group | Embryos, n | Blastocyst development (%) | D5 Biopsied (%) | D6 Biopsied(%) | Total # of biopsied (%) | Euploid (%) |
|---|---|---|---|---|---|---|
| Continuous | 352 | 251(71) | 109(43) | 92(37) | 201(80) | 109(54) |
| Renewal | 346 | 247(71) | 131(53) | 86(35) | 217(88) | 116(53) |

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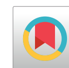

**STRESS RELIEF: CAN CONTINUOUS CULTURE IN A LOW LACTATE CULTURE MEDIA REDUCE NUMERICAL CHROMOSOMAL ABNORMALITIES AND THEREFORE IMPROVE EUPLOIDY RATES?.** M. VerMilyea,[a,b,c] C. Rios,[d] A. M. Abu Maizar,[c] R. M. James,[e] A. Picou,[a] H. J. Werland,[a] R. P. Marrs,[c] K. Silverberg.[b,a] [a]Ovation Fertility, Austin, TX; [b]Texas Fertility Center, Austin, TX; [c]California Fertility Partners, Los Angeles, CA; [d]Ovation Fertility Genetics, Henderson, NV; [e]Texas A&M College of Medicine, Sugar Land, TX.

OBJECTIVE: To compare PGT-A results from embryos cultured in a new formulation of culture media against those embryos cultured in a sequential or single-step culture system.

DESIGN: Retrospective analysis in private reproductive technology programs.

MATERIALS AND METHODS: From 2016 - 2018, embryos in two separate IVF laboratories (Lab A & B) were either cultured to the blastocyst stage in a sequential culture system (G-Series™, Vitrolife), Continuous Single Culture® (CSCM) or Continuous Single Culture®- NX (CSCM-NX) (Irvine Scientific®). CSCM-NX contains a lower concentration of lactate compared to traditional culture media. All embryos were cultured in 6% $CO_2$ in reduced oxygen. A sequential media change was performed with embryos cultured in G-Series™ on Day 3 (from G1™ to G2™) whereas embryos cultured in both CSCM and CSCM-NX were cultured undisturbed for 7 days. Trophectoderm biopsy was performed on either Day 5, 6, or 7 of blastocyst development and embryo ploidy status was determined by PGT-A through NextGen Sequencing (Ovation Genetics). Embryos reported as normal had no detectable copy number aberration, at a threshold of $\leq$30%.

RESULTS: A total of 6,655 embryos cultured in either sequential, CSCM and CSCM-NX culture media were biopsied for PGT-A. 53.9% of embryos cultured in CSCM-NX were identified as euploid compared to 44.3% of embryos cultured in the sequential system. This difference was highly significant ($P<0.001$). Embryos cultured in CSCM or CSCM-NX low lactate medium and subsequently biopsied, 46.6% were euploid compared to 57.1% respectively. This difference was also highly significant ($P<0.001$).

CONCLUSIONS: An excess of lactate present in culture medium may be a contributing factor to unwarranted stress on an embryo. This strain on metabolic efficiency can subsequently alter embryo development and cellular integrity which may increase the occurrence of mitotic aneuploidy by affecting spindle assembly and chromosome segregation in dividing cells. For the first time, we report a highly significant difference in euploidy rates from embryos cultured in a low lactate, single-step culture media. Our results show that by simply changing our selection of culture media we experienced a 10% increase in euploid embryos. Stimulation protocols, the PGT-A testing platform and overall embryo culture protocols remained consistent. Further assessment of subsequent pregnancy outcomes is currently ongoing.

% PGT-A Euploid Blastocysts

| LAB A | Normal | % Normal | Abnormal | Total |
|---|---|---|---|---|
| Sequential | 697 | 44.3% | 876 | 1573 |
| CSCM-NX | 558 | 53.9% | 478 | 1036 |
| **LAB B** | | | | |
| CSCM | 1594 | 46.6% | 1823 | 3417 |
| CSCM-NX | 359 | 57.1% | 270 | 629 |

*References:* Lee, A., & Kiessling, A. A. (2017). Early human embryos are naturally aneuploid—can that be corrected?. *Journal of assisted reproduction and genetics*, *34*(1), 15-21.

Mantikou, E., Wong, K. M., Repping, S., & Mastenbroek, S. (2012). Molecular origin of mitotic aneuploidies in preimplantation embryos. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1822*(12), 1921-1930.

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**INFLUENCE OF COMMERCIAL EMBRYO CULTURE MEDIA ON IN VITRO EMBRYO DEVELOPMENT AND PREGNANCY OUTCOME AFTER IVF: A SINGLE-CENTER RCT.** M. Murakami, S. Mizumoto, K. Tanaka, H. Otsubo, Y. Nagao, T. Kuramoto. Kuramoto Women's Clinic, Fukuoka, Japan.

OBJECTIVE: Numerous commercial embryo culture media are now available for IVF, raising the question of whether any medium is superior to others. Notably, the ability of a medium to yield a high embryo development percentage *in vitro* does not necessarily mean that the embryos are viable. For example, previously it was common to add blood serum to animal embryo culture media to stimulate blastocyst formation, but this impaired embryonic, fetal, and offspring health. Given the importance of culture media in treatment outcome, well-designed RCTs are needed, but the existing data are insufficient to select the best medium. In this study, we report updated data of an RCT conducted to compare the clinical outcome between three embryo culture media systems widely used in IVF.

DESIGN: Single-center RCT.

MATERIALS AND METHODS: This study included 795 healthy patients undergoing their first IVF treatment cycle at our clinic between February 2016 and August 2017. They were randomized by computer-generated tables into three groups and underwent our standard oocyte retrieval and IVF/ICSI procedures. Embryos were cultured in G1/G2 Plus (Vitrolife) (A), Global Total (LifeGlobal) (B), or Sequential Cleav/Blast (Origio) (C) media. Thirty-seven patients with no 2PN oocytes 18 h after insemination were excluded from the study. During embryo culture (1-5/50 $\mu$L), for cycles where the patients had only one good-quality (GQ) embryo by D3, the embryos were vitrified on D2/3. When the patients had $\geq$2 GQ embryos by D3, $\leq$2 GQ embryos were vitrified on D2/3, culture period of the remaining embryos was extended, and all GQ blastocysts were vitrified on D5/6. Data for vitrified ET performed until the end of March 2018 were analyzed.

RESULTS: Patient age (y) and vitrified D2/3 embryo percentages/cultured 2PN oocytes were similar for Groups A (36.4 $\pm$ 0.3 and 339/1646 (20.6%), respectively, n = 251), B (36.2 $\pm$ 0.3 and 352/1749 (20.1%), respectively, n = 256), and C (36.5 $\pm$ 0.3 and 339/1681 (20.2%), respectively, n = 251). Vitrified D5/6 blastocyst percentages/2PN oocytes were 26.1% (A), 36.9% (B), and 30.6% (C) (A vs. B, $P$ < 0.0001; A vs. C, $P$ = 0.0039; B vs. C, $P$ < 0.0001). Groups A, B, and C underwent 316 (D2/3 ET, 133; D5/6 ET, 183), 346 (D2/3 ET, 107; D5/6 ET, 239), and 318 (D2/3 ET, 107; D5/6 ET, 211) vitrified ET cycles, respectively (ET cancellation: A, 0.6%; B, 0.6%; C, 0.3%). The mean number of embryos transferred, implantation rates, clinical PRs/ET, and ongoing/delivered PRs/ET did not differ for Groups A (1.12 $\pm$ 0.02, 40.5%, 43.6%, and 32.8%, respectively), B (1.07 $\pm$ 0.02, 42.9%, 44.8%, and 32.0%, respectively), and C (1.11 $\pm$ 0.02, 37.7%, 40.1%, and 28.1%, respectively).

CONCLUSIONS: Overall PR of a culture system yielding fewer blastocysts was comparable to or slightly better than those of other systems. Differentiation of the ability of culture media to support preimplantation development with its ability to yield viable embryos would be important. Follow-up on perinatal and long-term health of children born after embryo culture with more participants is required.

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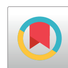

**KEY PERFORMANCE INDICATORS (KPIS) BASED ON POOR PROGNOSIS PATIENTS ARE MORE SENSITIVE TO THE EVALUATING EFFECTIVENESS OF DIFFERENT EMBRYO CULTURE INCUBATORS.** Y. Du, Y. Geng, Y. Zhang. Department of Reproductive Medicine, Tianjin Central Hospital of Gynecology Obstetrics, Tianjin, China.

OBJECTIVE: To study whether it is better to follow the poor-prognosis patients compared with the gold standard patients in IVF before further integrating a change into routine human IVF practice.

DESIGN: A prospective cohort study.

MATERIALS AND METHODS: This study included 1260 infertile patients from April 2017 to March 2018. Patients were categorized as poor prognosis patients and gold standard patients group based on age and functional ovarian reserve parameters (FSH and/or AMH). We performed a two-part prospectively study in IVF. Part A involved 202 infertile poor prognosis patients; Part B involved 1058 infertile gold standard patients. After oocyte collection, randomization was carried out and all of the patients' oocytes were allocated to culture in either a conventional incubator (control group) or a Flat Bed incubator (FBC) until embryo transfer on Day 3. The primary KPIs of this study was the implantation rate (IR) per embryo transferred. We then compared pregnancy-, ongoing pregnancy- and good quality embryos rate on day-3 after fertilization. Statistical analysis was performed using $\chi^2$ test for categorical data and Student's t-test for continuous data with $p$<0.05 considered statistically significant.

RESULTS: Part A revealed in poor prognosis patients no significant difference in the good quality embryos rate between groups: the control group and FBC group. The IR per embryo cultured in Flat Bed incubator (28.0% (23/82)) was significantly higher after transfer than the control group (16.4% (40/244), $P$=0.031; $\chi^2$=4.626). Similarly, a significantly higher rate in pregnancy-, ongoing pregnancy was found in the FBC group compared with the control group (46.2 and 29.7%, $p$=0.043, $\chi^2$=4.088; 42.3 and 24.8%, $p$=0.016, $\chi^2$=5.033). In Part B (the gold standard patients' group), the data displayed that no significant differences were found in the rate of GQEs on day 3 embryos, or implantation-, pregnancy-, and ongoing pregnancy rate between two embryo culture incubators groups.

CONCLUSIONS: The poor-prognosis patients may be a more sensitive group on evaluating the effectiveness of implementing a change in IVF laboratory process.

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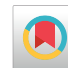

**HUMID VS. DRY EMBRYO CULTURE CONDITIONS ON EMBRYO DEVELOPMENT: A CONTINUOUS EMBRYO MONITORING ASSESSMENT.** R. Del Gallego,[a] C. Albert,[a] J. Marcos,[b] Z. Larreategui,[c] L. Alegre,[a] M. Meseguer.[a] [a]IVIRMA Valencia, Valencia, Spain; [b]IVIRMA Murcia, Murcia, Spain; [c]IVIRMA Bilbao, Leioa, Spain.

OBJECTIVE: Advances in embryo culture strategies seeking to mimic in vivo conditions raise questions regarding the adequacy of the current use of dry incubators combined with oil covered media systems. The aim of the present study is to determine the effects of atmospheric humidity on embryo development by performing a continuous embryo monitoring assessment and an oxidative stress profiling.

DESIGN: Prospective randomized controlled trial including a total of 1,734 embryos from 176 patients.

MATERIALS AND METHODS: Embryos were cultured in the time-lapse incubator Geri® (Genea Biomedx, Australia) with 6 patient-individual chambers. 3 chambers worked under humid conditions (**HC**) and 3 under dry conditions (**DC**), 83 and 93 patients respectively. Geri dishes with 80 $\mu$L of culture media were covered with an oil overlay, and cultured for 5-6 days. The effects of humidity were assessed retrospectively with regard to blastocyst, pregnancy and miscarriage rates. Its influence over morphokinetic parameters was evaluated by using the time-lapse system. Additionally, a preliminary study regarding the oxidative status of the spent embryo culture media was conducted using the TCL (Thermochemiluminescence) Analyzer™.

RESULTS: Odds ratios were adjusted for patient's age, body mass index, fresh sperm concentration, number metaphase II oocytes, fertilization rate and number of transferred and vitrified embryos. A significantly higher blastocyst rate (p< .05) was found in embryos cultured under HC (**HC** = 74.5% vs. **DC** = 69.2%). Although no statistical differences were found in pregnancy and miscarriage rates, due to the reduced sample sizes, a clear trend was observed towards a higher number of pregnancies (**HC** = 83.3% vs. **DC** = 66.7%) and less miscarriages (**HC** = 16% vs. **DC** = 26.5%) in the HC group. Regarding the morphokinetic parameters, embryos cultured under HC reached the 5-cell stage significantly earlier (p< .05) than DC incubated embryos (**HC** = 46.3h vs. **DC** = 49.7h). Finally, the preliminary assessment of the culture media's oxidative status showed higher oxidative stress levels in media cultured under DC.

CONCLUSIONS: Our results suggest a clear improvement on embryo development and subsequent IVF outcome when embryos are cultured in a humidified atmosphere, replicating an *in vivo* state. Additionally, oxidative status profiling proved a potential impact of humidity on culture media