DESIGN: Research Study.

MATERIALS AND METHODS: Human zygotes with 3 pronuclei (PN) were cryopreserved with consent, thawed, and placed into culture (CON; n = 25) 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/BN 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 POUFS1, 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±0.02mm²; CON: 0.09±0.04mm²; RN: 0.06±0.02mm²; CBL: 0.06±0.02mm²) or 96h (0.12±0.06mm²; CON: 0.23±0.10mm²; RN: 0.20±0.10mm²; 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 μl 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% CO2 and 5% O2. 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 significantly 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 of 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 1: Blastocyst Development and Euploidy Rate Between Two Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Embryos, n</th>
<th>Blastocyst development (%)</th>
<th>D5 Biopsied (%)</th>
<th>D6 Biopsied (%)</th>
<th>Total # of biopsied (%)</th>
<th>Euploid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>352</td>
<td>251(71)</td>
<td>109(43)</td>
<td>92(37)</td>
<td>201(80)</td>
<td>109(54)</td>
</tr>
<tr>
<td>Renewal</td>
<td>346</td>
<td>247(71)</td>
<td>131(53)</td>
<td>86(35)</td>
<td>217(88)</td>
<td>116(53)</td>
</tr>
</tbody>
</table>

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OBJECTIVE: Numerous commercial embryo culture media are now available for IVF, raising the question of whether any medium is superior to the gold standard patients in IVF before further integrating a change into routine human IVF practice.

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 procedure. Embryos were vitrified on D2/3. When the patients had ≥2 GQ embryos by D3, the embryos were vitrified on D2/3. When the patients had ≥2 GQ embryos by D3, the embryos were vitrified on D2/3. When the patients had ≥2 GQ embryos by D3, the embryos were vitrified on D2/3. 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 ± 0.3 and 339/1646 (20.6%), respectively, n = 251), B (36.2 ± 0.3 and 352/1749 (20.1%), respectively, n = 256), and C (36.3 ± 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), 356 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 ± 0.02, 40.5%, 43.6%, and 32.8%, respectively), B (1.07 ± 0.02, 42.9%, 44.8%, and 32.0%, respectively), and C (1.11 ± 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. Difference in embryo 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.