Aneuploidy rates and morphokinetic parameters in sibling embryos cultured in distinct culture media

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Principal Investigator: Mitchell Rosen, MD

Background:
Embryonic aneuploidy is the underlying etiology for the majority of failed implantation and miscarriage. Preimplantation genetic screening (PGS) with transfer of a euploid embryo has been advocated as a strategy for increasing live birth rates with a single embryo transfer. Culturing embryos to the blastocyst stage for trophectoderm biopsy is a requirement for PGS. Several commercially-available single-step embryonic culture media with varying composition have been established for use in the IVF laboratory. Early reports have suggested differences in clinical outcomes, such as aneuploidy and miscarriage rates, with distinct culture media currently in standard use.[1,2] However, there have been no clinical trials demonstrating the superiority of any one commercially-available culture media formulation. As a result, clinics use media with varying composition based upon familiarity and cost.

Specific Aims:

1. To determine if aneuploidy rates differ between sibling embryos cultured in distinct media.
2. To determine if embryo quality differs between sibling embryos cultured in distinct media.
3. To determine if morphokinetic parameters, as assessed by time lapse imaging, differ between sibling embryos cultured in distinct media.
4. To determine if and how morphokinetic parameters and aneuploidy rates differ between embryos with zona breach performed on day three of embryo development versus at the blastocyst stage.
5. To assess the accuracy of non-invasive genetic testing of embryos from spent culture media.

Design:
Prospective sibling embryo study in an academic IVF laboratory comparing clinical parameters for embryos cultured in two different single-step culture media with time lapse imaging.

Study Population:
Couples planning in vitro fertilization (IVF) with intent for blastocyst culture for preimplantation genetic screening (PGS) will be offered enrollment prior to initiation of their treatment cycle. Intend to enroll 124 subjects (see sample size calculation below).

Inclusion Criteria:
All subjects planning blastocyst culture for preimplantation genetic screening (PGS) will be offered enrollment.
AFC $\geq$8
Male and female partner $\geq$ 18 years of age

Exclusion Criteria:
Female partner age >42 years of age
Cleavage stage biopsy

Outcome Measures:
Aneuploidy rate for embryos cultured in one of two distinct media from the same patient (Primary outcome)
Embryo quality at cleavage and blastocyst stages
Morphokinetic parameters from time lapse imaging

Study Implementation:

Couples planning in vitro fertilization (IVF) with intent for blastocyst culture for preimplantation genetic screening (PGS) will be offered enrollment prior to initiation of their treatment cycle. Ovarian stimulation will be performed with standard treatment chosen by the couple's primary physician. The day of ovulation trigger will be determined by the primary physician. Oocyte retrieval will be performed according to clinic standard 36 hours following ovulation trigger. All oocytes aspirated from the left ovary will be allocated to Global Media while all oocytes from the right ovary will be allocated to SAGE media (see supplement with media systems). Follicular fluid will be collected from a large and a medium-sized follicle from each ovary in order to perform a direct correlation of follicular size to oocyte recovery, maturation, and embryo development. The collected follicular fluid will also be assayed for estradiol, follicle stimulating hormone, leutinizing hormone, and progesterone.

On the day of oocyte retrieval, the male partner will produce a semen sample per usual clinic protocol. Following 2-3 hours of incubation, hyaluronidase will be used to denude cumulus cells surrounding oocytes per usual clinic protocol. Fertilization will be performed by intracytoplasmic sperm injection (ICSI) of all mature oocytes by a trained embryologist. After 16-18 hours, fertilization will be evaluated by the existence of two pro-nuclei. Fertilized embryos from each patient's cohort will remain split for culturing in SAGE or Global media systems as previously designated. All embryos will be cultured in the EmbryoScope+ time lapse incubator. Once placed in the EmbryoScope, embryos will be cultured at 37 degrees C with 6% CO_2 and 5.5% O_2 or up to 6 days without media exchange.

The EmbryoScope+ incubation chamber contains a built-in microscope and camera allowing for continuous monitoring of embryonic development. Image acquisition software will be used to obtain high-contrast images every 10 minutes from several focal planes to create time-lapse videos. Conventional embryonic assessment will be made by observations at pre-specified time points. Cleavage-stage embryos will be assessed for cell number, symmetry, percentage fragmentation, evidence of multinucleation, and progression of compaction. Blastocysts will be evaluated to assess for blastocele volume and expansion, inner cell mass development, and trophectoderm organization. Additional morphokinetic parameters will be assessed with time lapse videos including time to pronuclear fading or syngamy (tPNf), time to 2-8 cells, time to morula, start of blastulation, blastocyst, and expanded blastocyst. Cleavage anomalies will be recorded. Zona breach will be performed with the use of a laser per usual clinical protocol on day 3 of embryo development or at the blastocyst stage. All spent media will be collected and sent for quantification of embryonic DNA content.

Embryonic biopsy for preimplantation genetic screening will be performed at blastocyst stage. As per usual clinical protocol, on the day of biopsy, 5-10 trophectoderm cells will be gently aspirated. All embryos making it to the blastocyst stage will be eligible for biopsy, including low quality blastocysts. Arrested embryos may be biopsied to determine a cause for arrest.

Preimplantation genetic screening will be performed per usual clinical protocol by PacGenomics. All embryos will be vitrified following biopsy according to standard clinic protocol awaiting PGS results. Embryos subsequently determined to be euploid will be transferred as a controlled or natural cycle frozen embryo transfer according to standard clinical protocols. Number of embryos to be transferred will be determined by the patient's primary physician.

Additional data will be collected on clinical pregnancy rate (ultrasound demonstrating gestational sac with yolk sac per transfer) and live birth rate. These data are routinely collected and reported to the Society for Assisted Reproductive Technology Clinical Outcomes Reporting System (SART CORS) according to the Fertility Clinic Success Rate and Certification Act of 1992.

Statistical Analysis:
The primary outcome is rate of aneuploidy for embryos cultured in one of two distinct media from the same patient. We will perform a paired t-test to compare number of euploid blastocysts in embryos split for culture in two different media from the same patient. Our secondary outcome is embryo quality for split embryos. Embryo quality will be graded at the cleavage and blastocyst stages. High quality cleavage embryos will have at least 6 blastomeres or greater and scores of "1"or "2" for symmetry and fragmentation. Expanded blastocysts will be considered high quality where inner cell mass and trophectoderm score are "A" or "B." We will perform paired t-tests to compare number of high quality cleavage and blastocyst stage embryos from the cohort of sibling embryos cultured in different media.

Sample Size Calculation:
The primary outcome is aneuploid fraction for embryos cultured in two distinct media from the same patient. To detect same difference in euploid fraction as Hickman et al (29% vs 16%) we would need 176 blastocysts per arm. This calculation does not account for clustering of embryos from the same patient; however, we will obtain a substantially higher number of blastocysts per arm as we are planning to power for our secondary outcome (see below).

Our secondary outcome relates to differences in embryo quality among split embryos cultured in distinct media. Previous review of internal data demonstrates that interventions targeting couples with a history of poor embryo quality have been able to demonstrate a four-fold increase in high quality day 3 fraction. We need 62 subjects (31/arm) to detect four-fold increase in high quality day 3 fraction within this population with history of poor embryo quality (< 40% good quality cleavage stage fraction). We estimate that 25% of clinic patients have poor quality cleavage stage fraction. Therefore, would need 4 x 62= 248 patients. However, since we are splitting embryos from the same patient, each cohort of embryos serves as its own control. Therefore, we need to enroll **124 patients** to power for our secondary outcome of clinically meaningful differences in embryo quality.

References:

[1]Campbell A, Smith R, Montgomery S et al. A prospective multi-center comparison of two different single step culture media using sibling embryos. In: Human Reproduction Vol 31, Suppl 1; 2016 July 3-6; Helsinki, Finland: ESHRE 2017. Abstract 265.

[2]Hickman C, Wells D, Gwinnett D et al. Euploid rate sensitivity to laboratory culture environment: a blind, prospective, randomized, sibling study. In: Human Reproduction Vol 31, Suppl 1; 2016 July 3-6; Helsinki, Finland: ESHRE 2017. Abstract 203.

[3]Goodman LR, Goldberg J, Falcone T, Austin C, Desai N. Does the addition of time-lapse morphokinetics in the selection of embryos for transfer improve pregnancy rates? A randomized controlled trial. Vol 105 (2); 2016: 275-85.

[4]Minasi MG, Colasante A, Riccio T, Ruberti A, Casciani V, Scarselli F et al. Correlation between aneuploidy standard morphology evaluation and morphokinetic development in 1730 biopsied blastocysts: a consecutive case series study. Hum Reprod 31(10); 2016:2245-54.

[5]Morbeck DE, Baumann NA, Oglesbee D. Composition of single-step media used for human embryo culture. Fertil Steril 107(4);2017: 1055-60.