

## Abstracts for 2017 Foundation for Reproductive Medicine Translational Reproductive Biology and Clinical Reproductive Endocrinology Conference

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### Young Investigator Award winner

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#### Does embryo mosaicism affect clinic results in assisted reproduction cycles?

Andrea Bernabeu<sup>1</sup>, Jose Antonio Ortiz<sup>3</sup>, Belen Lledo<sup>3</sup>, Ruth Morales<sup>1,2</sup>, Elena Fernandez<sup>2</sup>, Jorge Ten<sup>1</sup>, Rafael Bernabeu<sup>1</sup>  
<sup>1</sup>Reproductive Assisted Treatment Department, Bernabeu Institute, Alicante, Spain  
<sup>2</sup>Reproductive Assisted Treatment Department, Bernabeu Institute, Madrid, Spain  
<sup>3</sup>Bernabeu Institute, Biotech Institute, Alicante, Spain

**Objective** To evaluate if embryo mosaicism is linked to poorer clinic pregnancy rates.

**Design** Retrospective case-control study between January 2013 and January 2017.

**Materials and Methods** Complete Chromosomal Screening (CCS) was performed on embryos of couples attending our fertility clinic between January 2013 and January 2017. In the first phase, array-CGH (aCGH) analyses were performed using the Agilent SurePrint platform G3 8 × 60 K CGH. Cells from the trophectoderm of the embryos were biopsied at the blastocyst stage (D + 5 or D + 6). We analyzed 1923 blastocysts from 704 cycles of IVF. An embryo was considered mosaic when the percentage of mosaicism was >25% and <80%. Embryos >80% were classified as aneuploid. Subsequently, embryos, in single embryo transfers previously diagnosed by array-CGH as euploid, were reanalyzed by NGS ( $n = 102$ ). The chromosomal analysis was performed using the Veri-Seq Illumina kit and bioinformatic analysis was performed with the BlueFuse Multi software program (Illumina). Here an

embryo was considered mosaic when the percentage of aneuploid cells was 20%–80%. Differences were assessed using the Chi-square test and binary logistic regression (SPSSv20.0).

**Results** Clinical results were compared between cycles where euploid embryos with embryonic mosaicism were transferred and cycles in which only euploid embryos were transferred, both diagnosed by array-CGH. Both groups were homogenous, with no differences in implantation rates (26.9% vs. 40.3,  $P = 0.224$ ), clinical miscarriages (7.1 vs. 18.1%,  $P = 0.354$ ), biochemical miscarriages (21.2% vs. 12.3%,  $P = 0.102$ ), clinical pregnancy rates (20.6% vs. 38.9%,  $P = 0.127$ ). In a second phase, embryos previously diagnosed as euploid by array-CGH were reanalyzed by NGS ( $n = 102$ ). In this group of 33 (32.4%) were found to be mosaic. Embryos from ongoing pregnancies demonstrated non-significantly lower mosaicism rates than failed cycles (23.1% vs. 38.1%,  $P = 0.115$ ). In patients with repeated implantation failure and repeated pregnancy loss, percentages of mosaicism were also only non-significantly higher than in control group (27.8% vs. 20.2%,  $P = 0.365$ ; 41.7% vs. 27.4%,  $P = 0.123$ ), and chemical and ongoing pregnancies also did not differ (16.7% vs. 23.1%,  $P = 0.743$ ).

**Conclusions** The management of mosaic embryos is still very controversial. The new techniques of CCS have given us better diagnostic power, allowing embryos previously classified as euploid with array-CGH to now with techniques such as NGS to be diagnosed as mosaic. After repeat analyses with NGS, this study found mosaicism in approximately one-third of previously by array-CGH reported euploid embryos. Mosaic embryos only demonstrated non-significant trends toward poorer IVF cycle outcomes, though lack of significance may reflect small cycle numbers. Though comparisons between mosaic and

euploid embryos did not demonstrate significant outcome difference, transfer of mosaic embryos should, especially in RPL and RIF patients, still only be done cautiously.

*Support* None.

### Young Investigator Award winner

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#### Immunotoxin to cell surface associated OOCYTE-SAS1B protein kills human uterine cancer cells

Eusebio Pires<sup>1</sup>, John Herr<sup>2</sup>

<sup>1</sup>Ob/Gyn, University of Virginia, Charlottesville, United States of America

<sup>2</sup>Cell Biology, University of Virginia, Charlottesville, United States of America

*Objective* SAS1B (Ovastacin/ASTL) is an oocyte specific matrix metalloprotease. The goal was to examine the transcripts and proteins in uterine tumors and test SAS1B antibodies, including a prototype immunotoxin (Saporin), for their effect in vitro on growth and viability.

*Design* Study examines: 1) SAS1B in normal oocyte biology and fertilization 2) internalization of cell-surface SAS1B 3) antibody-dependent cell cytotoxicity and 4) immunotoxin effects on tumor growth and viability.

*Materials and Methods* Unique gene-specific primers were designed. An MMT-derived cell line, SNU539, was used for cell-based experiments. A rabbit SAS1B-polyclonal antibody to cell cytotoxicity studies.

*Results* SAS1B was found at message or protein levels in 77% of uterine tumors, with the incidence higher in MMT tumors (87%,  $N = 16$ ) than endometrioid carcinomas (74%,  $N = 59$ ). SAS1B showed cell surface localization in live cells by immunofluorescence. Incubating SNU539 cells with SAS1B antibodies caused a transformation from a regular polygonal to rounded cells with redistributed actin cytoskeletons and shrinkage in diameter. When SNU539 cells were exposed to antibody under cold conditions followed by warming, antibody-SAS1B complexes were internalized into the cell cytoplasm where they co-localized with endocytic pathway markers EEA1/LAMP1. In a real-time cell-based assay using the XCELLigence system, tumor cells were killed in the presence of SAS1B-antibody and active complement. Inhibition of tumor cell growth and division followed by cell death occurred in culture over 72 h using a complex of SAS1B primary antibody and secondary immunotoxin conjugate.

*Conclusions* SAS1B offers a candidate cell-surface target for development of a therapeutic tumor-selective antibody that is directed only to the tumor and an expendable

population of mature oocytes thereby potentially reducing off target effects.

*Support* Funding was received from the Center for Innovative Technology (CIT): “mAbs to target surface metalloprotease” ER14S-003-LS awarded to Eusebio Pires and John Herr.

### Young Investigator Award runner-up

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#### The MinION Access Project: A first attempt at pre-implantation genetic screening with nanopore sequence sensing

Ryan Miller<sup>1,2</sup>, Chin-Fu Chen<sup>3</sup>, Jane DeLuca<sup>1</sup>, Henry Malter<sup>2</sup>

<sup>1</sup>Healthcare Genetics, Clemson University, Clemson, United States of America

<sup>2</sup>Fertility Center of the Carolinas, Greenville Health System, Greenville, United States of America

<sup>3</sup>Center for Bioinformatic and Epidemiological Studies, Greenwood Genetic Center, Greenwood, United States of America

*Objective* Morphological assessment of viable embryos for selection prior to transfer remains unreliable and subjective. Improved selection methods such as pre-implantation diagnostics are likely to increase the current live birth rates in assisted reproductive technology cycles by preventing the transfer of aneuploid embryos. Here, we present data collected as part of the MinION Access Project (MAP) – an early access program designed to acquaint researchers with this new technology.

*Design* A blastocyst stage embryo, previously diagnosed as karyotypically abnormal by array comparative genomic hybridization (aCGH) was thawed and briefly cultured to allow for two additional trophectoderm biopsies before being discarded per laboratory protocol. The biopsy samples were analyzed using an Oxford Nanopore MinION to compare a newly generated karyotype with the aCGH results.

*Materials and Methods* Genomic material in the biopsy samples was isolated and amplified utilizing a SurePlex Amplification kit. The entire 25ul reaction was utilized for downstream MinION library preparation. Four sequencing runs of library 1 and one sequencing run of library 2 were performed with a flowcell wash in between samples. Base called FAST5 files were aligned to the human genome with the LAST alignment tool. Embryo karyotype was obtained utilizing normalized read counts for each chromosome.

*Results* The original aCGH diagnosis of monosomy 16 was not reflected in this data. Chromosomes 1 and 2 had read counts of approximately 8–10 fold higher than all other