

## OA-2

### Characterizing the microbiome at embryo transfer: next generation sequencing of the 16S ribosomal gene

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**Objective:** The vaginal and placental microbiomes are partially characterized and impact obstetric outcomes. The uterine microbiome is largely uncharacterized given the limitations of cultivation-dependent analysis. Culture-free approaches to bacterial identification focus on sequencing of 16S ribosomal gene and allows for increased dynamic range in assessment. Here, we assess two Next Generation Sequencing (NGS) platforms for 16S sequencing, determine their advantages and disadvantages for 16S metagenomics, and study the endometrial microbial environment at the time of embryo transfer (ET).

**Design:** Method validation and cohort study.

**Materials and Methods:** The method validation of NGS 16S ribosomal gene sequencing was performed on known samples (*Escherichia coli*, *Staphylococcus epidermidis*, *Cyanobacterium Synechococcus*). The Ion 16S metagenomics workflow analyzed seven of nine hypervariable regions (V2,3,4,6,7,8,9) of 16S rRNA gene versus targeted sequences used in Illumina 16S workflow (V3-V4 and V4 only). Customized bioinformatics data analysis was used to improve the taxonomic assignments. Illumina V4 metagenomics workflow was further validated on Microbial Mock Community of 20 bacterial strains. The endometrial microbiome at the time of ET was characterized by analyzing transfer catheter tips with Illumina V4 metagenomics for 70 patients.

**Results:** The built-in analysis from both Ion PGM and Illumina MiSeq metagenomics failed to generate correct taxonomic classification for genus or species for known single- or poly-microbial samples. The customized bioinformatics improved alignment, however for Ion 16S metagenomics workflow, the majority reads were family level. For both amplicons V3-V4 and V4, Illumina metagenomics workflow identified the genus or species level in the single- and poly-microbial samples. V4 had higher Operational Taxonomic Unit (OTU) counts than V3-V4. Subsequently, Illumina V4 metagenomics workflow with customized bioinformatics detected the genus or species level for all the 20 bacterial strains in the Microbial Mock Community. Finally, the endometrial microbiome of the 70 patients was assessed with Illumina V4 metagenomics and customized bioinformatics. *Lactobacillus* was detected in all the 70 samples along with other bacteria native to the reproductive tract (*Corynebacterium*, *Bifidobacterium*, *Staphylococcus*, *Streptococcus*).

**Conclusions:** The Illumina V4 metagenomics workflow with customized bioinformatics provided a rapid and sensitive method for the identification of bacterial genus or species in single- or poly-microbial samples. Despite the limited starting material when analyzing clinical ET specimens, the Illumina V4 metagenomics approach provided adequate taxonomic identification. This sets the stage for a larger trial analyzing the relationship between endometrium microbial structure and implantation after ET.

**Disclosures:** Nothing to disclose.

**Funding:** None.

## OA-3

### Detection of a male translocation following an ovum donor preimplantation genetic screening cycle

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**Objective:** It is not standard of care to screen IVF patients for translocations due to their low existence in the general population at ~0.2%. However, the percentage of carriers of balanced translocations may be much higher in the infertile population and they are most likely unaware of their condition. We describe the clinical and laboratory outcomes of an advanced maternal age (AMA) couple seeking infertility care, and the inadvertent discovery of a male partner translocation.

**Design:** We describe a single case study of a rare preimplantation genetic screening (PGS) result, which could have been avoided with an update to genetic pre-screening for any patients initiating expensive IVF cycles.

**Materials and Methods:** A couple's case files were reviewed and are described to demonstrate the discovery of a male translocation carrier following a donor oocyte IVF/PGS cycle.

**Results:** The couple: 40-year old, G1, P0 (SAB) female with unremarkable hormonal/ultrasonic findings; and a low teratozoospermic, otherwise unremarkable male. The male had a normal genetic carrier screening panel. The couple was counseled about AMA and underwent an autologous IVF cycle with PGS. Two blastocysts developed, they were biopsied and the genetic results were: Monosomy 16/Trisomy 21 and Trisomy 3/Monosomy 16. The couple decided to proceed with donor oocytes for a subsequent cycle with PGS to transfer only a single normal embryo. In the donor cycle, using partner sperm, 20 blastocysts developed, they were biopsied and the PGS results showed 6 normal embryos (30%); 11 embryos (55%) with abnormalities always involving chromosomes 3 and/or 16 (monosomies, trisomies and complex abnormal). Very low numbers of normal embryos from an oocyte donor and repeated abnormalities in chromosomes 3 and 16 from both the autologous and donor cycles resulted in the male being karyotyped, discovering a balanced Reciprocal Translocation: 46 XY; t(3;16)(q22; q22).

**Conclusions:** Although standard pre-screening for an AMA couple seeking infertility treatment was followed, along with completing an autologous IVF cycle with PGS demonstrating findings that would be considered standard in an AMA couple, we discovered a male translocation following a donor oocyte/PGS cycle. The very low cost of a standard karyotype may be warranted prior to initiating donor oocyte cycles. It may even be preferable to change the standard of care to karyotype all couples prior to any infertility treatments to increase the likelihood success.

**Disclosures:** None.

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## OA-4

### Ploidy variation in trophoctoderm biopsies from day 5, 6, and 7 blastocysts

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**Objective:** To compare ploidy rates amongst day 5, 6, and 7 blastocysts.

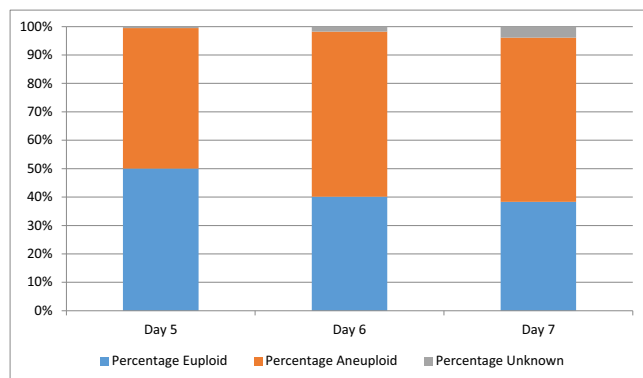
**Design:** Retrospective study in a private in vitro fertilization laboratory.

**Aim:** Some data suggests that Day 7 (D7) blastocysts can give rise to nearly 27% ongoing pregnancy success by frozen embryo transfer (Kovalevzky et al., 2013). We chose to compare the aneuploidy rate

observed amongst day 5, 6, and 7 blastocysts in order to determine the efficacy of extended D7 culture in our laboratory.

**Materials and Methods:** Over the span of 3 years, a total of 1915 blastocyst stage embryos (average maternal age of  $37 \pm 4.1$  years) underwent trophoctoderm biopsy for genetic testing of ploidy status. Pre-implantation genetic screening (PGS) results were retrospectively compared to day of trophoctoderm biopsy. Mature oocytes were fertilized using ICSI and embryos were group cultured in a continuous single culture medium (Irvine Scientific). On Day 3 of embryo development, all multi-cell embryos ( $\geq 6$  cells) were artificially hatched by laser ablation of the zona pellucida. Hatched embryos were then cultured to the blastocyst stage. Trophoctoderm biopsy was dependent on development of a well-defined inner cell mass and trophoctoderm. Hatching or completely hatched blastocysts were biopsied on either Day 5, 6 or 7 and subsequently vitrified for future use in a warmed embryo transfer cycle dependent on ploidy status. Biopsied samples were analyzed with comprehensive chromosomal screening involving the use of array comparative genomic hybridization (aCGH) or next generation sequencing (NGS) technology by a reference laboratory.

**Results:** 1915 embryos were biopsied yielding a total of 862 (45%) euploid embryos: 485(56%), 328(38%) and 49(5.7%) from D5, D6, and D7, respectively. The incidence of euploidy was 50%, 40%, and 38% among D5, D6, and D7. Chi-square analysis demonstrated that euploid status is dependent on day of biopsy/blastocyst development with chi square statistic calculated to be 17.5847 and a  $P$  value  $< 0.0001$  (Figure 1).



**Figure 1** Ploidy status based on day of biopsy.

**Conclusion:** Embryos biopsied on day 5 and 6 have a significantly higher probability having normal chromosome complements compared to day 7 embryos as a whole. These results contradict previous reports of D5 and D7 blastocysts contributing equal numbers of euploid embryos (Vaccari et al., 2014). Our findings suggest that perhaps a delay in blastulation (until D7) may be associated with a greater likelihood of aneuploidy. Despite this, culturing embryos to D7 appears to be beneficial as a substantial number of blastocysts biopsied on D7 were euploid. Further investigation of ploidy status from D5, D6, and D7 embryos stratified by various age groups, may provide further information regarding the efficacy of D7 blastocyst culture and screening.

**Disclosures:** None.

**Funding:** None.

## References

Kovalevzky, G., Carney, S.M., Morrison, L.S., Boylan, C.F., Neithardt, A.B., Feinberg, R.F., 2013. Should embryos developing to blastocysts on day 7 be cryopreserved and transferred: an analysis of

pregnancy and implantation rates. *Fertil. Steril.* 100, 1008-1012.

Vaccari, S., Conaghan, J., Chenette, P., 2014. Extended culture to day7 increases euploid embryo yield per cycle. *Fertil. Steril.* 102, 0-391.

## OA-5

**Growing zona pellucida-free (ZPF) oocytes from ICSI to biopsy: it works**

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**Objective:** To observe and document the growth of an intact, mature, accidentally zona pellucida-free oocyte (ZPF) that underwent ICSI and culture to blastocyst-stage with subsequent trophoctoderm biopsy on day six.

**Design:** Retrospective case study where a ZFP oocyte observed after oocyte processing for ICSI, and subsequently grown to day 6 for trophoctoderm biopsy.

**Materials and Methods:** Anonymous donor oocytes were retrieved and processed according to standard laboratory protocols. After processing, 1 of the 9 oocytes was intact but outside of its zona pellucida. All oocytes were mature and underwent ICSI, including the zona-free oocyte. Oocytes were incubated using the standard continuous culture protocol, under oil with 5% O<sub>2</sub>, 8%CO<sub>2</sub> and balance nitrogen. The ZPF oocyte was cultured individually. Injected ova were evaluated on day1 (day 0 = day of retrieval) and on day 3 to check fertilization and development respectively. The embryos were checked again on day 5; there were 5 zona-intact blastocysts for biopsy. On day 6, there was 1 zona-intact blastocyst, and the blastocyst from the ZPF oocyte available for biopsy. Immediately post-biopsy, embryos were vitrified and the trophoctoderm samples were sent for genetic testing.

**Results:** Using routine laboratory protocols the ZPF embryo underwent appropriate development, as visualized by formation of a structured inner cell mass and trophoctoderm. No special techniques were utilized, e.g. placing cells into the empty zona, or culturing with increased protein concentration. Genetic testing showed normal embryo. 4 of the 7 biopsied blastocysts were euploid, including the ZPF embryo.

**Conclusion:** There have been several case reports describing the successful development of ZPF embryos, and subsequent pregnancy after transfer, however this report further demonstrates that ZPF embryos can not only display expected developmental patterns, but that these embryos can undergo biopsy and have a normal chromosome complement. Embryologists should consider mature ZPF oocytes as available for ICSI, though careful attention to the ICSI process is required.

**Disclosures:** Authors have nothing to disclose.

**Funding:** No funding was provided nor needed.

## OA-6

**Human blastocyst toxicity potential of different vitrification solutions: experiment II**

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**Objectives:** We exhibited that human blastocysts are highly resilient to repeated metastable re-vitrification (rVTF), with or without elution, and to 2-6 min exposures to non-DMSO containing VTF solutions (Experiment I). The aim of Experiment II was to comparatively assess the potential toxicity of different VTF solutions to extended exposures.

**Design:** 360 research consented, discard blastocysts were randomly assigned to a  $6 \times 3$  factorial design: comparing different ex-