Study question: Determine whether a human endometrial receptivity biomarker (S100A10) plays a role in the decidualization and secretory transformation of endometrial cells in normal responder patients.
Summary answer: S100A10 protein silencing inhibited prolactin secretion in stromal endometrial cells, but significantly enhanced it in epithelial endometrial cells.
What is known already: The role(s) of human biomarkers of endometrial receptivity during the implantation window are rarely determined. Stromal cells decidualization and epithelial cells secretory transformation are critical for the establishment of uterine receptivity, and consequently, for successful implantation. Decidualization characterizes the differentiation of endometrial stromal fibroblasts into specialized secretory decidual cells that expressed specific markers such as prolactin. Decidualization is endowed with the secretory transformation of the uterine glands. As for decidualization, uterine gland maturation is mediated through cAMP allowing expression of implantation-related factors including the prolactin. The behaviour of epithelial cells during the decidualization of stromal cells has rarely been studied.
Study design, size, duration: Primary epithelial and stromal cells were purified from endometrial biopsies obtained during the implantation window of two fertile patients. Then, we targeted the extinction of the S100A10 using shRNA in each endometrial cell type. The obtained phenotype was analyzed in regards of the decidualization and the secretory transformation of stromal and epithelial cells respectively.
Participants/materials, setting, methods: Primary endometrial cell cultures of epithelial and stromal cells were performed. Then, to determine whether S100A10 had a role in the decidualization of endometrial stromal cells and the secretory transformation of epithelial cells, each cell type transduced with S100A10 shRNA or control shRNA were incubated or not with 8-Br-cAMP for 9 days. Spent culture medium was collected for prolactin quantification with the automated immunoassay system BRAHMS KRYPTOR.
Main results and the role of chance: The incubation with 8-Br-cAMP of the stromal and epithelial endometrial cells transduced with control shRNA, induced a typical decidual phenotype and prolactin secretion. In the stromal cells, prolactin secretion was increased by 16.3-fold in the culture medium of 8-Br-cAMP treated cells transduced with control shRNA compared with untreated cells (215 ± 33 vs. 13 ± 1 µUI/ml, $P < 0.0001$) and only by 8.3-fold in S100A10 silenced cells (110 ± 10 µUI/ml, $P = 0.009$) compared to control shRNA. These data indicate that for the stromal endometrial cells there is an inhibitory effect of prolactin secretion in S100A10 silenced cells. In endometrial epithelial cells, 8-Br-cAMP treatment induced prolactin secretion in both cells transduced with control shRNA (62 ± 11 vs.13 ± 1 µUI/ml, $P < 0.0001$) compared to untreated cells and with the S100A10 shRNA (98 ± 10 µUI/ml, $P = 0.025$) compared to control shRNA, indicating that in epithelial endometrial cells, S100A10 silencing promotes prolactin secretion. Therefore, S100A10 silencing inhibited prolactin secretion in stromal endometrial cells, but significantly enhanced it in epithelial endometrial cells.
Limitations, reasons for caution: Culture of luminal and glandular epithelial cells, as well as co-culture system between epithelial and stromal cells, should be considered to better understand cross-talk between these cellular compartments.
Wider implications of the findings: This study should open new perspectives in the understanding of molecular mechanisms regulating human endometrial receptivity and reveals the key role of S100A10 as a player in endometrial receptivity acquisition.
Trial registration number: Not applicable.

SELECTED ORAL COMMUNICATIONS

SESSION 33: DEEP SEQUENCING THE EMBRYO.

Tuesday 05 July 2016 **Hall 3 AB** **10:00–11:30**

O-117 Quantification of low frequency variants of the mitochondrial DNA (mtDNA) in single cells by massive parallel sequencing (MPS)

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Study question: Is it possible to detect and quantify low frequency heteroplasmic variants, large deletions and single nucleotide variants (SNV) in the mtDNA of single cells?
Summary answer: Our method detects heteroplasmic SNVs and large deletions in single cells; data on single oocytes provides interesting insight on SNV segregation during oogenesis
What is known already: The advent of MPS has opened new possibilities for the analysis of low frequency heteroplasmies in the mtDNA, but no methods are currently available to accurately detect and quantify low frequency SNVs and large deletions. Mutations in the mtDNA have been previously reported in ART-derived oocytes and embryos, as well as methods for the analysis of individual mutations in preimplantation genetic diagnosis. Conversely, up to now, it has not been possible to identify all mutations in one single cell simultaneously, nor to establish their mutation load
Study design, size, duration: We developed a method to analyze large deletions and SNVs in DNA samples and scaled it down to the level of single cells. We sequenced six single fibroblasts from a control individual, two muscle fibers from a patient with mtDNA deletions, and 11 oocytes from three different patients treated at our IVF center (5 GV, 3 MI, 3 MII)
Participants/materials, setting, methods: Single cells were lysed in alkaline lysis buffer. MtDNA was enriched by long-range PCR. Sequencing was done at 6,000×. SNVs called with CLCBio Genomic Workbench and large deletions were identified using BLAST. Mutation load was established using the sequencing depth. The lower detection limits are linked to the number of PCR cycles needed, down to loads for deletions and SNVs of 0.5 and 1.5% at 35 cycles, and 0.5 and 2% at 45 cycles, respectively
Main results and the role of chance: The single fibroblasts revealed somatic mosaicism, with two variants appearing in 1/6 of the cells at high loads, and not present in the other cells. The muscle fibers showed a low number of large deletions at a high frequency and most of them were also identified in the blood sample of the same patient. In the oocytes, one large deletion at 0.51% was found in the cohort analyzed. We detected SNVs in 9/11 oocytes, at frequencies ranging from 1.5 to 20%. The same SNVs were found recurrently in the oocytes from the same donor but often at different frequencies. In one patient we observed a variant in 3/4 oocytes at loads of 5, 15 and 20%. In the same patient, another variant was detected in 2/4 oocytes at frequencies of 5 and 15%. In 2/4 oocytes from a different patient, a third SNV was detected at frequencies of 2.5 and 3%. Half of the detected variants mapped to the hypervariable region (12/24) and only one variant at load of 4% was predicted to be pathogenic. There was no correlation between the oocyte maturation stage and the presence of variants
Limitations, reasons for caution: The results we obtained with the oocytes must be matched with the variants in somatic tissues of the same patient to have a full validation of the variants called and to follow the variants' segregation
Wider implications of the findings: Our method for low frequency detection can be used to study mtDNA variants in single cells with high accuracy. This tool can be applied also to the diagnostic of mitochondrial diseases involving large deletions or SNVs. Finally, our data provides interesting preliminary information about heteroplasmy shifts during gametogenesis.
Trial registration number: N.A.

O-118 Design, validation and clinical application of a novel next-generation sequencing protocol for detection of mitochondrial disease and simultaneous aneuploidy screening in preimplantation embryos

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Study question: Can a next-generation sequencing (NGS) protocol be clinically applied to perform preimplantation genetic diagnosis (PGD) of mitochondrial DNA (mtDNA) disease and aneuploidy in preimplantation embryos?
Summary answer: The NGS technique enables accurate PGD of mtDNA disease in cleavage- and blastocyst-stage biopsies. Additionally, comprehensive chromosome screening (CCS) can be incorporated in the protocol.
What is known already: Maternally inherited mutations can be present in all (homoplasmy) or a fraction (heteroplasmy) of mtDNA copies. Although most female carriers are phenotypically unaffected, due to low mutation load, high levels can be present in oocytes and passed to the offspring resulting in mtDNA disease. To prevent transmission, PGD can be performed to select embryos free of the mutation or unlikely to be affected by disease. Current PGD techniques of mtDNA disease do not allow incorporation of CCS. Since aneuploidy is extremely common in preimplantation embryos, we aimed to develop a test that enables accurate mtDNA mutation detection and aneuploidy screening.
Study design, size, duration: To combine mtDNA mutation and aneuploidy screening, the technique was developed and optimised on whole genome amplified (WGA) DNA. This was then subjected to targeted sequencing of the mtDNA for PGD and to microarray comparative genomic hybridisation (aCGH) or NGS for the purpose of CCS. Prior to clinical application, the protocol was extensively validated on genomic DNA and isolated buccal cells and/or lymphocytes, derived from four mtDNA mutation carriers presenting varying mutation levels (50–100%).
Participants/materials, setting, methods: The method was applied to developmentally competent and arrested embryos and unfertilised oocytes from three patients, each having a child affected by Leigh Syndrome (Patient 1 and 2: m.8993T > G; Patient 3: m.10191T > C). The mutation was not detectable in somatic cells of Patients 1 and 3, however children of both presented with heteroplasmy (95 and 78% mutant mtDNA, respectively). Somatic heteroplasmy of 56% was detected in Patient 2 who had a child homoplasmic for the mutation.
Main results and the role of chance: The NGS method provided qualitative (DNA sequence) information and also quantitative data (proportion of mtDNA molecules with the mutation). Initial validation using genomic DNA and cells of mutation carriers yielded 100% diagnostic accuracy. Five PGD cycles were performed with 50 specimens analysed: 19 blastocyst- and cleavage-stage embryos, three unfertilised oocytes, eight arrested embryos. Additionally, three embryos were divided into 20 single sister blastomeres. Patient 1 generated 14 blastocysts. None carried detectable mutation levels, but four were aneuploid. No mutations were detected in one unfertilised oocyte, one arrested embryo and 16 sister blastomeres from two embryos. The patient underwent a single embryo transfer and is pregnant (delivery in May). Patient 2 generated one blastocyst after two cycles, which was unaffected by the mutation, but aneuploid. One unfertilised oocyte and 2 arrested embryos carried 91, 89 and 0% of mutated mtDNA copies, respectively. Patient 3 produced four embryos from two cycles, which were assessed at the cleavage-stage. None carried the mutation, but two were aneuploid. No mutation was detected in one unfertilised oocyte, four arrested embryos and four sister blastomeres of one embryo. Transfer of a single embryo yielded no pregnancy and the patient is awaiting transfer of the second euploid embryo.
Limitations, reasons for caution: The accuracy of PGD for mtDNA mutation analysis may be reduced due to possible biological variations in mutation levels between the biopsy specimen and the remaining embryo. Hence verification by prenatal testing should be performed to confirm that the fetus is not affected by mtDNA disease.
Wider implications of the findings: This is the first study utilising NGS for the detection and quantification of mtDNA mutations in preimplantation embryos. The protocol was to be demonstrated highly accurate and can be combined with chromosome screening. The study provided evidence of low recurrence risk of mtDNA disease in patients with undetectable somatic mutations.
Trial registration number: NA.

O-119 Mitochondrial quantification during human embryogenesis *via* next-generation sequencing: relation to nuclear ploidy status

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Study question: What is the association between embryo mitochondrial load and nuclear ploidy status during early human embryo development?
Summary answer: Mitochondrial quantity appears to vary throughout the sequence of human embryo development and does correlate with nuclear ploidy status.
What is known already: Mitochondria are recognized as the critical source of cellular ATP although they are also involved in redox & calcium homeostasis, provide intermediary metabolites, and store proapoptotic factors. They are maternally transmitted, deriving from a restricted founder population amplified during oogenesis. While mitochondrial segregation to blastomeres during early embryogenesis is strictly regulated, the distribution of mitochondria during this critical phase remains poorly understood.
Study design, size, duration: This retrospective, multi-cohort pilot study was undertaken to quantify mitochondrial load during early embryogenesis and to assess the relation of mtDNA to nuclear ploidy status. Data from embryos (n = 325) were reviewed for patients undergoing IVF at a single institution during the 9 month interval ending December 2015.
Participants/materials, setting, methods: Embryo records were evaluated from patients (n = 69) who completed IVF treatment with preimplantation screening at an urban referral fertility unit in southern California. High throughput sequencing of mitochondrial and nuclear DNA was achieved simultaneously after whole genome amplification using an ion semiconductor platform (ThermoFisher Scientific, Inc). Nuclear and mitochondrial DNA ratios were then tabulated according to time of single biopsy: day 3 (n = 90), day 4 (n = 145), or day 5 (n = 90).
Main results and the role of chance: Successively declining mitochondria quantities were observed from the blastomere stage (d3) compared to morula (day-4) and blastocyst stage (day-5), validating a prior observation that mitochondria are not newly made during the first 5 days of human embryo development. Among euploid embryos, we noted a relatively high mitochondria load at d3 but successively fewer mitochondria at d4 and d5 vs. aneuploid embryos.
Limitations, reasons for caution: Absolute quantity of mitochondria may not always correlate to mtDNA copy number estimates, so additional study is planned to measure discrete mitochondrial parameters at our center in the future. These data also cautiously align with previous work in that fewer mitochondria were present in embryos obtained from older IVF patients.
Wider implications of the findings: We hypothesize that the observed decline in mitochondrial load at d4 and d5 among euploid embryos results from an enhanced metabolic milieu, where less ATP is needed to execute vital cellular processes. Additionally, impaired cell fission may allow aggregation of mtDNA that would otherwise be dispersed in "healthy" embryo cells.
Trial registration number: N/A.

O-120 The impact of simultaneous mitochondrial DNA (mtDNA) content assessment in comprehensive chromosomal screening (CCS): a prospective pilot study

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Study question: Does simultaneous mtDNA copy number assessment bring additional benefit on cycle outcome in frozen embryo transfer cycles where euploid blastocysts are replaced after CCS?
Summary answer: Our study shows a high correlation between mitochondrial DNA copy number assessment and implantation potential of euploid human embryos in CCS cases.