Q3 and Q4, for embryo development in Q2 and Q3 ($112 \leq$ apoB < 330 ng/ml) and for embryo quality in Q2 ($112 \leq$ apoB < 230 ng/ml).
Limitations, reason for caution: In our study, a small number of embryos were transferred and a larger study will be required to determine if FF apoB levels are associated with embryo implantation and pregnancy rates.
Wider implications of the findings: ApoB could be used as a new predictive biomarker of ART success parameters such as oocyte quality, normal fertilization and embryo quality. After oocyte retrieval, FF apoB levels could be analyzed to predict the developmental competence of each oocyte and subsequent embryo development in IVF patients. FF apoB assays are easily practicable in biochemistry laboratories and could be used in IVF labs in order to select the highest quality embryo to be transferred.
Study funding/competing interest(s): This study was supported by a grant from the Centre Hospitalier Universitaire of Dijon and by ASGOD. There is .
Trial registration number: NC

O-060 Leptin stimulates activation of STAT3 in human blastocysts

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Study question: Does exogenous human recombinant leptin protein promote activation of signal transducer and activator of transcription 3 (STAT3) in human blastocysts?
Summary answer: Activation of STAT3 is promoted by leptin in both trophectoderm and inner cell mass cells in human blastocysts.
What is known already: Activated STAT3 (p-STAT3) is present in mouse blastocysts and endometrial tissues around the implantation site and during the implantation window. The regulatory protein leptin activates STAT3, during which cytoplasmic STAT3 is phosphorylated and translocates to the nucleus to activate target genes. STAT3 has an important role in human stromal cell decidualisation, and trophoblast migration, however, no data are available about STAT3 in human blastocysts. Leptin is lower in the endometrium of implantation failure patients.
Study design, size, duration Laboratory study in medical school setting. Research on human embryos carried out with patient consent and ethical approval and under Human Fertilisation and Embryology Authority licence as part of PhD research project. Frozen-thawed embryos were cultured to day 6 in clinical conditions.
Participants/materials, setting, methods: Blastocysts were incubated with/without 20ng/ml human recombinant leptin for 10, 20, 30 minutes, or 24 hours, then labelled with FITC-conjugated anti-pSTAT3 antibody for confocal imaging. Quantitative analysis of fluorescence intensity was standardised for comparison of relative cytoplasmic and nuclear p-STAT3 in trophectoderm (TE) and inner cell mass (ICM).
Main results and the role of chance: In blastocysts not exposed to leptin, ICM cytoplasm and nuclei and TE cytoplasm and nuclei contained similar fluorescence intensities associated with p-STAT3. In embryos exposed to leptin, significantly higher p-STAT3 was detected, with ICM cytoplasm and nuclei generally higher than TE cytoplasm and nuclei respectively, Relative fluorescence intensity was highest in blastocysts exposed for 10 minutes. These results show that leptin increases activation of STAT3 in both ICM and TE.
Limitations, reason for caution: Human embryos are highly variable. Further quantitation of p-STAT3 using RT-qPCR is advisable; however, this would provide data on a per embryo basis, not individual cells and cell lineages, as has been undertaken using image analysis.
Wider implications of the findings: Since we show that leptin has a direct effect upon human embryos around the time of implantation, then maternal leptin has potential to affect embryonic development. Leptin is present at lower levels in the endometrium of women with implantation failure, which might indicate that embryonic STAT3 activation is suboptimal in this situation and could possibly be enhanced by leptin supplementation in vitro. Further study is essential to understand the STAT3 pathway at implantation.
Study funding/competing interest(s): Funding was provided by a PhD Fellowship from University Hospitals Coventry and Warwickshire NHS Trust.
Trial registration number: N/A

SELECTED ORAL COMMUNICATION SESSION

Session 16: Innovations in reproductive genetics

Monday 8 July 2013 **15:15 - 16:30**

O-061 Breakthrough/innovative approach: first genetic matching assay in an oocyte donation program using preconceptional screening arrays (Recombineô)

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Study question: Preconceptional-screening (PCS) is the diagnosis of genetic risks of couples. Laws for anonymous donation programs prevent recipients from choosing their donors and render IVF clinics to subjectively do it for them. Concomitant with the preventive PCS for the donor selection, we attempted a novel approach: an objective genetic matching.
Summary answer: We developed an algorithm that allows us to select objectively the most appropriate donor calculating a ratio for random genetic similarities based on 500 singular SNPs, thus significantly increasing the genetic correspondence between donor and recipient.

This new approach allows a safer and comprehensive genetic matching for OD programs.
What is known already: SNP-array based PCS became recently available. It was originally designed for recessive genetic sceening of couples or IVF patients, and if carriers, transition them to PGD or PND. Nobody has used this technology in an OD program before, neither to avoid risky matchings nor to improve the matching of donor candidates to a recipient by the bias of highest genetic correspondence.
Study design, size, duration: Cross sectional study with following sample size calculation: variability was calculated (independent sample), for a statistical power of 90%, we estimated a sample size of 79 with an estimated difference 2,5%.

Total number of subjects: 400 donors and 80 recipient couples, in total 560 tests performed during 3 months.
Participants/materials, setting, methods: We used the Recombine™ array with 1500-SNPs covering 174 genetic diseases and with 500 random SNPs for genetic matching. The genetic correspondence analysis between phenotypically selected donors, recipient and genetically best-matching donor was performed using following formula:$Vr / Vf = (((v_{m?\ 400}\sum((v_{0\leq a\leq 500}\sum_{SNPa\ x=\ SNPa\ Rm/fm}SNPa)/500)/m))/((v_{0\leq a\leq 400}\sum((v_{0\leq a\leq 500}\sum_{SNPa\ x=\ SNPa\ Dm}SNPa)/500)/400))-1)x100$
Main results and the role of chance: From 400 donors, 2 had to be rejected for being carriers of mutations for dominant diseases, although with unknown penetration. The classical phenotype matching showed to be risky for recessive diseases in 5,7% of cases comparing donor candidates and male profiles, as the mean value of presence of recessive mutations per subject was 14% in both populations.

Afterwards we compared coincidental similarities between these outstanding donor groups to define the mean interindividual variability. Regarding the genetic correspondence between donor and recipient we could show a significant improvement with the genetic matching of 12,7% (ratio:Vr/Vf with $p < 0.05$), comparing (Vf) as the mean genetic variability with a simple phenotypic matching (20 donor candidates per couple) with (Vr) selecting from this group the best genetically matching candidate.
Limitations, reason for caution: Limitations: Recombine™ tests 174 diseases with 1500 SNPs and 500 random SNPs, covering the most prevalent disorders with high sensitivity&specifity, but does not cover all possible mutations associated to diseases.
Wider implications of the findings: This new approach allows first time a comprehensive genetic matching for an OD program. It is not only able to dismatch dominant disease carriers and risky donor/recipient combinations for recessive diseases. Moreover our algorythm using random SNPs makes definitely a **genetic matching possible**. It is objective and covers a high demand for a proven genetic correspondence between donor and recipient. We think this new tool of PCS may open a new era in OD.
Study funding/competing interest(s): No funding, no conflict or competing interests.
Trial registration number: AE-009

O-062 Clinical application of massively parallel sequencing on chromosomal abnormalities detection of human blastocysts

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Study question: To comprehensively evaluate the performance of massively parallel sequencing(MPS)-based approach for chromosomal abonormalities detection in clinical setting.
Summary answer: MPS-based chromosomal abnormalities detection method may be readily used for preimplantation genetic diagnosis and screening in clinical setting.
What is known already: MPS is well known for its high throughput sequencing capacity and its low cost,which is a powerful platform for both research and clinical study. Many clinical tests based on MPS have been reported, such as monogenic desease diagnosis, non-invasive prenatal genetic test, etc. Recently, MPS-based chromosomal abonormalities detection has been reported.
Study design, size, duration: we applied MPS-based approach to detect chromosomal abnormalities for human blastocysts from 41 randomly selected couples and report its performance. All couples participated in this trial signed a consent form
Participants/materials, setting, methods: A total of 150 trophectoderm (TE) samples were collected by day 5 or 6 blastocyst biopsy in the CITIC Xiangya Reproductive & Genetic Hospital. Whole Genome Amplification (WGA) products were obtained using the WGA4 GenomePlex Single Cell Whole Genome Amplification Kit. After library preparation and low coverage sequencing,an average of 8.2 million reads were generated for each blastocyst, covering 5.5% $\pm$ 1.2% of the whole human genome.
Main results and the role of chance: We identified 71 (47.3%) uniformly euploidy and 79 (52.3%) chromosomal abnormality blastocysts using our own bioinformatic method. Among the abnormalities, 26 blastocysts were uniformly aneuploidy and 53 were with unbalanced chromosomal rearrangements. According to the results there were 33 (80.5%) couples have euploid embryos with qualified morphology for transfer. 58.5% of ongoing pregnancy rate was observed in 24 women in first trimester, and seven in 41 women (17.1%) had live birth.
Limitations, reason for caution: The whole process of the test takes more than 10 days, embryos vitrification are needed
Wider implications of the findings: Our study for the first time reported the performance of MPS-based approach in clinical setting, which empowers its utilization of chromosomal abnormalities detection in preimplantation genetic diagnosis and screening (PGD/PGS).
Study funding/competing interest(s): This project is supported by Key Laboratory Project in Guang Dong Provicine, (2011A060906007), Key laboratory Project in Shenzhen (Shenzhen Municipal Commission of development and Reform (2011) No.861). No competing interest.
Trial registration number: None

O-063 A novel embryo screening technique provides new insights into embryo biology and yields the first pregnancies following genome sequencing

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Study question: Can powerful next generation sequencing (NGS) techniques be adapted for the analysis of single cells, allowing unprecedented amounts of genetic information to be obtained from human embryos for diagnostic and research purposes?
Summary answer: A new NGS method was successfully developed, capable of simultaneously detecting monogenic disorders, diagnosing aneuploidy and quantifying mitochondrial DNA (mtDNA) mutations. The method was applied clinically, allowing identification and transfer of euploid embryos, resulting in healthy pregnancies. Additionally, a potentially important association between mtDNA content and blastocyst aneuploidy was discovered.
What is known already: Next generation sequencing, a class of methods involving the production of vast quantities of DNA sequence data, is revolutionizing genetic diagnostics. However, these techniques have not been applied to research involving human embryos, or to their clinical diagnosis, due to significant technical obstacles associated with analysis of single cells. Theoretically, NGS methods could allow simultaneous analysis of gene mutations and aneuploidy and could lead to reduced costs for patients requesting genetic diagnosis of their embryos.
Study design, size, duration: The NGS method was validated using single cells from cell-lines with known genetic defects (aneuploidies, cystic fibrosis mutations, or mitochondrial DNA defects) (n = 30). Additionally, 45 embryos, previously shown to be abnormal using aCGH, were reanalysed in a blinded fashion. Subsequent clinical application involved testing of seven blastocysts from two patients.
Participants/materials, setting, methods: The method involved multiple displacement amplification followed by NGS using the Ion Torrent platform. Data was analysed with tools developed in our laboratory. The entire process could be completed within 16 hours, allowing fresh embryo transfer. The two patients were 35 and 39 years old, with a history of miscarriage.
Main results and the role of chance: The NGS technique was robust, with 82/82 samples yielding results. Aneuploidy diagnoses were concordant with those obtained using established cytogenetic techniques in all cases (100%). Detection of DNA sequence mutations was confirmed in 10/10 cells carrying a cystic fibrosis mutation. Application of NGS to cells with a heteroplasmic mtDNA mutation, succeeded in identifying the mutation and quantifying the proportion of affected mtDNA molecules. Clinical application of NGS revealed 3/5 euploid blastocysts from the first couple and 2/2 from the second. Single embryo transfers, based upon these results, led to healthy pregnancies in both cases. Simultaneous chromosome screening and mtDNA quantification, revealed an association between blastocyst aneuploidy and mtDNA content ($P < 0.05$), an interesting biological finding with potential implications for the origin and fate of aneuploid cells.
Limitations, reason for caution: The NGS method developed provides an unprecedented insight into embryo genetics and has the potential to dramatically reduce the costs of preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS). However, before recommending widespread application, a randomized clinical trial, confirming efficacy, is advisable. Such a trial is now underway.
Wider implications of the findings: The NGS technique developed allows simultaneous testing for aneuploidy, gene mutations and mtDNA with exceptional accuracy. This strategy may revolutionize research and diagnosis in fields where the amount of tissue available is extremely limited (e.g. PGD). The cost of NGS was significantly lower than existing methods, suggesting that this approach may ultimately bring genetic analysis within the reach of a much larger number of patients. The feasibility of applying NGS in clinical cycles was confirmed.
Study funding/competing interest(s): Institutional funding was used for this investigation . None of the authors have competing interests.
Trial registration number: Not applicable.

O-064 Targeted sequencing of SNPs results in highly accurate non-invasive detection of fetal aneuploidy of chromosomes 13, 18, 21, X, and Y: a validation study

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Study question: To non-invasively detect fetal aneuploidy at chromosomes 13, 18, 21, X, and Y via analysis of fetal cell-free DNA (cfDNA) from maternal blood. The method targets ~19,500 single-nucleotide polymorphisms (SNPs) in a single multiplex PCR reaction and uses the Next-generation Aneuploidy Test Using SNPs (NATUS) algorithm to analyze data.
Summary answer: This is the first large-scale study that includes blinded sample analysis using the NATUS algorithm, showing detection of chromosomally

abnormal fetuses from a maternal blood sample with high accuracy at chromosomes 13, 18, 21, X, and Y. This method calculates a personalized accuracy for each chromosome within each sample.
What is known already: Current non-invasive methods all use a quantitative 'counting' approach that requires a reference chromosome and falters at low fetal fractions. There are no available methods that leverage the increased information offered by SNPs and parental genotypic information, which allows identification of fetal ploidy state, parental origin, and will allow detection of uniparental disomy, triploidy, microdeletions/microduplications, and haplotype reconstruction.
Study design, size, duration: Analysis was performed on 764 maternal plasma samples (673 euploid; 52 trisomy 21; 17 trisomy 18; 8 trisomy 13; and 14 Monosomy X samples) collected from patients at greater than 9 weeks of gestation under an institutional review board (IRB)-approved protocol.
Participants/materials, setting, methods: Isolated cfDNA was amplified using multiplex PCR targeting ~19,500 SNPs covering chromosomes 13, 18, 21, X, and Y. Sequencing data was analyzed using NATUS technology employing Bayesian statistics to analyze multiple copy number hypotheses and determine the Maximum Likelihood hypothesis, calculating sample- and chromosome-specific accuracy without requiring reference chromosome.
Main results and the role of chance: Samples were required to pass stringent quality control thresholds. Samples that failed to meet this threshold (5.8%) were typically of poor DNA quality and/or low fetal cfDNA fraction. In those samples that passed, the NATUS algorithm detected trisomy 13 (n = 7; sensitivity: 100% [CI: 59.0-100%]), trisomy 18 (n = 15; sensitivity: 100% [CI: 78.2-100%]), trisomy 21 (n = 47; sensitivity: 100% [CI: 92.5-100%]), 45,X (n = 12; sensitivity: 92.3% [CI: 64.0-99.8%]), with average calculated accuracies for all calls of >99%. The NATUS algorithm accurately identified fetal chromosome copy number as low as 3.9% fetal fraction and as early as 9 weeks gestation.
Limitations, reason for caution: Because the method incorporates maternal genotypic information, samples from pregnant women who used egg donors, surrogates, or have undergone bone marrow transplants are not accepted at this stage. Additionally, the method only detects those chromosomes that are specifically targeted. Current cell-free DNA-based NIPT methods are not considered diagnostic.
Wider implications of the findings: SNP- and NATUS-based analysis of cfDNA isolated from maternal plasma offers a clinically viable, highly accurate approach to identifying fetal aneuploidies. The method has the potential to expand to detect microdeletions and microduplications, as well as single-gene anomalies.
Study funding/competing interest(s): Partially funded by a grant from the National Institute of Health (4R44HD062114-02). Industry employment noted under affiliated institutions.
Trial registration number: NCT01545674

O-065 Development and validation of rapid and cost-effective blastocyst comprehensive chromosome analysis methods utilising quantitative real-time PCR and microbead-based technologies

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Study question: Can accurate, rapid, low-cost methods, allowing 24-chromosome aneuploidy screening of blastocysts be developed using quantitative real-time PCR (real-time qPCR) and BACS-on-Beads (BoBs) technologies?
Summary answer: Real-time qPCR and BoBs approaches enable accurate detection of chromosome losses and/or gains in blastocyst biopsies with significant cost advantages over the most widely used preimplantation genetic screening (PGS) methods. Additionally, real-time qPCR allows the detection of aneuploidy within four hours following embryo biopsy.
What is known already: PGS has been proposed as a strategy for detecting chromosomally abnormal embryos. However, current techniques require approximately 24 hours. Given that some blastocysts are not ready for biopsy until late on day-5 or even day-6, a 24-hour chromosome screening test may often be incompatible with fresh embryo transfer. Furthermore, existing chromosome screening methods are relatively expensive, significantly increasing the costs of IVF treatment. Rapid, low-cost methods for 24-chromosome analysis of blastocysts are urgently required.
Study design, size, duration: Trophectoderm samples were taken from 17 surplus embryos, donated by eight patients undergoing IVF treatment. The same embryos were analysed using real-time qPCR as well as BoBs technology. Additionally, microarray comparative genomic hybridisation (aCGH) was performed on all embryos to ensure consistency with a well-established, highly validated aneuploidy screening method.
Participants/materials, setting, methods: Both, real-time qPCR and the BoBs technology are based on the analysis of distinct genomic regions spread across all 24 chromosomes (96 and 90 regions, respectively). However, real-time qPCR utilises a targeted amplification of each genomic sequence, whereas BoBs technology is based on whole genome amplification (WGA) followed by CGH.
Main results and the role of chance: Real-time qPCR, analysis using BoBs as well as aCGH was carried out on distinct trophectoderm biopsies derived from the same blastocyst stage embryos. Results of real-time qPCR and BoBs technology were analysed blindly and compared to array CGH results. Both methodologies allowed the accurate detection of whole chromosome losses/gains as well as sex determination of all 17 embryos. Similar to microarray-based techniques, analysis using BoBs technology requires approximately 24 hours. However, with lower reagent and hardware costs, it is less expensive compared to traditional aCGH strategies. Real-time qPCR enables aneuploidy detection within 4 hours upon embryo biopsy. Furthermore, the real-time qPCR protocol is less labour-intensive and has significant cost advantages over current PGS techniques.
Limitations, reason for caution: As with aCGH, the detection of haploidy/polyploidy using these methods is not straightforward. Microarray-based techniques involve analysis of hundreds of loci on each chromosome, but real-time qPCR and BoBs technology are based on analysis of ~4 regions per chromosome. Therefore, imbalances related to structural rearrangements may be less reliably detected.
Wider implications of the findings: There is growing evidence that embryo selection, based upon comprehensive chromosome screening of blastocysts, improves IVF outcomes. However, testing remains expensive, restricting access to this promising strategy. This study demonstrated that accurate aneuploidy screening can be achieved at substantially lower cost than existing methods, potentially improving patient access. Additionally, real-time qPCR was compatible with same-day biopsy and transfer, avoiding the need for cryopreservation, even for embryos not reaching the blastocyst stage until day-6 (morning).
Study funding/competing interest(s): Institutional funding was used for the pilot study. None of the authors have any competing interest.
Trial registration number: N/A

SELECTED ORAL COMMUNICATION SESSION

Session 17: Promises of AMH

Monday 8 July 2013 **15:15 - 16:30**

O-066 Is ovarian reserve a good marker of spontaneous conception in infertile women? A retrospective study of natural conception in 1551 women trying to conceive

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Study question: Ovarian reserve, as measured by FSH and/or AMH, is a common marker used to estimate the likely success of IVF treatment. Given a dataset of 1551 infertile women trying to conceive naturally, we ask if AMH, FSH predict spontaneous pregnancy and live birth beyond age and time trying to conceive.
Summary answer: From the data available (FSH n = 389, AMH n = 174), it appears that ovarian reserve is poor marker of spontaneous pregnancy and live birth rate for infertile women. This should be taken into account when counselling women of their chances of success with and without fertility treatment.
What is known already: Ovarian reserve markers are key indicators of the likely success of an IVF/ICSI cycle, in particular regarding the effectiveness of ovarian stimulation. There is very little data regarding the effect of ovarian reserve markers on the likely spontaneous pregnancy rate and live birth of infertile women,