Wider implications of the findings: RIF is a big challenge since its multiple causes and because no diagnostic tests are available to identify the main factor. Therapies are empirical and produce controversial results. Our preliminary data suggest that the proportion of aneuploidies by PGS can have a diagnostic role in identifying the RIF etiology.
Trial registration number: Not applicable.

P-632   A novel preimplantation genetic diagnosis strategy for Duchenne muscular dystrophy based on targeted next-generation sequencing and linkage analysis

J. Du[1], X. Hu[1], T. Feng[2], L. Xing[2], J. Dai[1], W. He[1], X. Liu[2], K. Luo[1], F. Gong[1], W. LI[1], G. Lu[1], G. Lin[1]
[1]Reproductive & Genetic Hospital of CITIC-Xiangya, Genetic Centre, Changsha, Hunan, China
[2]Peking Jabrehoo Med Tech. Ltd., Peking, China

Study question: To establish a rapid and broadly applicable strategy of PGD for DMD carriers based on target sequencing and linkage analysis.
Summary answer: Targeted next-generation sequencing (NGS) combined with SNP linkage analysis is a efficient strategy than previous methods and being applicable to 99% of DMD carriers.
What is known already: DMD is the most common fatal genetic disorder diagnosed in childhood, with an incidence of 1/3500 in live male births. The gene is about 2.3 Mb and has 79 exons. Multiple mutations were found, including large deletions (60%), large duplications (10%) and point mutations (30%). The traditional PGD approaches take up to three months to complete the pre-experiment. Although karyomapping is a faster PGD technology and suit for a wide range of conditions, it is not suit for DMD due to inadequate SNPs. Now we are still lack of a high efficiency and easy to clinical extension approach for DMD-PGD.
Study design, size, duration: Targeted NGS workflow based on an AmpliSeq panel was designed for sequencing 480 SNPs and coding region of DMD gene on Ion PGM™ Sequencer. Total 99 female carriers with DMD heterogeneous mutation were recruited for testing the application range. Of them 3 patients were carried out 3 PGD cycles to verify the efficacy of the novel DMD-PGD scheme. The work was carried out from January 2014 to December 2015.
Participants/materials, setting, methods: The study was set at the Reproductive and Genetic Hospital of CITIC-Xiangya, China. The designed AmpliSeq panel includes the coding region of DMD gene and 480 SNPs selected from 1 Mb upstream to 1 Mb downstream of the gene. Ninety-nine females carrying heterogeneous DMD gene mutation, including 51 large deletions, 15 large duplications and 32 point mutations, were recruited to determine the efficacy of the panel. Among them 3 carriers underwent PGD and 14 embryos were tested.
Main results and the role of chance: Among the 99 female DMD carriers, 98 had at least 3 informative SNPs in the upstream and downstream of the mutation loci of the gene, respectively. The successful rate of haplotype construction was 99% (98/99). The point mutations were detected in all 32 carriers and the efficacy rate was 100%. Of the 14 embryos from 3 PGD cycles for 3 families, 1 was normal male embryo, 4 were normal female embryos, 5 were female carrier embryos and 4 were in affected embryos. Among them, 2 families were transferred 2 normal female embryos and 1 female carrier embryos, respectively. The PGD results were confirmed by the following prenatal diagnosis.
Limitations, reasons for caution: This strategy can not directly detect the large deletions and duplications of DMD gene. In addition, 1 case (1%) had less than 3 informative SNPs within 1 Mb upstream and 1 Mb downstream of the mutation loci of the gene, which might decrease the accuracy of the results.
Wider implications of the findings: We establish a new DMD-PGD strategy based on targeted NGS and linkage analysis. Ninety–nine percent of female carriers were successfully constructed haplotype. The new scheme is rapid, accurate, low cost and easy to clinic extension.
Trial registration number: Not applicable.

P-633   Next generation sequencing (NGS) metrics following DOP-PCR whole genome amplification (WGA) of single and multi-cell samples for PGS

M.J. Jasper[1], T. Hardy[3], M. Pinese[2], M. McCabe[2], W. Ledger[3], M. Fraser[1]
[1]Reproductive Health Science Ltd, Thebarton, SA, Australia
[2]Kinghorn Centre for Clinical Genomics, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia
[3]School of Women's & Children's Health, University of New South Wales, University of New South Wales, Randwick, NSW, Australia

Study question: Evaluate single cell and limited DNA template sample NGS metrics following WGA and sequencing on alternate capacity sequencers.
Summary answer: The amount of sequencing data available for interrogation is highly dependent on the number of samples multiplexed in a library, read length and platform configuration.
What is known already: When limited source material for genome wide evaluation is available, WGA is often used to generate sufficient DNA for downstream analysis. Limited WGA technologies are commercially available, and WGA using the EmbryoCellect™ kit (Reproductive Health Science Ltd) protocol utilizes a proprietary degenerate oligonucleotide primed PCR (RHS-DOP-PCR). When used in conjunction with the kit array, screens low template samples for aneuploid status. Aneuploidy screening using NGS technology is becoming increasingly popular in the IVF setting. The total aligned genomic and mitochondrial DNA sequence data generated from an NGS run is potentially sufficient for subsequent sample screening for other genetic anomalies.
Study design, size, duration: The aim of this study was to determine NGS run and aligned read data metrics from a range of NGS platforms as models for PGS utilizing the DOP-PCR based WGA of single cell and multi-cell aliquots as described (EmbryoCellect™ kit). Additionally, fluorescently labelled test and reference WGA DNA was hybridized to the EmbryoCellect™ kit microarray, consisting of repeat-depleted chromosome-specific probes. The dye ratio was determined and compared to the known karyotype of the sample.
Participants/materials, setting, methods: Single cell and 5-cell aliquots sorted from euploid and aneuploid cell lines (Coriell Institute) were subjected to WGA using EmbryoCellect™ kit protocol. Nextera libraries were prepared from WGA and unamplified gDNA samples and subsequently sequenced (paired-end) on either a MiSeq ($n = 48$; read length 300 bp), NextSeq ($n = 23$; 150 bp) or X-10 (150 bp) platform according to standard protocol (Illumina). The sequencing data was bioinformatically aligned to hg19. Sequencing run and aligned data metrics were tabulated for comparison.
Main results and the role of chance: On completion of the sequencing runs, approximately 4.5 fold more reads were generated using the NextSeq compared to MiSeq platform (mean; 1.3 million reads versus 5.8 million reads per sample were mapped to hg19). The sequencing of single cells and 5-cell aliquots on the X-10 yielded 110,000–120,000 Mb in comparison to 126 000 Mb from sequencing of the unamplified gDNA sample or approximately 400 million reads per sample. The average single cell Q30 scores were >89% for the Miseq run and >82% for the NextSeq NGS run. The average X-10 Q30 scores for single cells, 5-cell aliquots and gDNA were 83%, 81% and 87% respectively.
Limitations, reasons for caution: This data has been generated from limited NGS runs, and therefore increasing the number of sequencing runs is necessary to increase the number of samples per sequencing parameter/condition.
Wider implications of the findings: While specific gene sequence and mitochondrial DNA is present in single cell NGS data, breadth and depth of coverage to acquire a level of resolution suitable for PGD will be a key indicator for PGS + PGD success.
Trial registration number: –.

P-634   Development of a universal method for the preimplantation diagnosis of β-thalassemia and sickle-cell anaemia using a novel next-generation sequencing approach: a new paradigm for PGD

N. Kubikova[1,2], J. Sarasa[1,3], D. Wells[1,2]
[1]Reprogenetics UK, Institute of Reproductive Sciences, Oxford, UK
[2]University of Oxford, The Nuffield Department of Obstetrics & Gynaecology, Oxford, UK
[3]IVF Spain, Alicante, Spain

Study question: To develop a novel, low-cost PGD protocol applicable to all couples undergoing PGD for β-thalassemia and sickle-cell anaemia.
Summary answer: The study successfully harnessed the power of next-generation sequencing (NGS) technology, delivering a PGD protocol for diagnosis of any *HBB* gene mutations in preimplantation embryos.
What is known already: Worldwide, the most common inherited disorders are those caused by mutation of the beta-globin gene (*HBB*), responsible for beta-thalassemia and sickle cell anaemia. There are various challenges in providing

PGD for these conditions. Traditional PGD protocols involve the time-consuming process of designing customized tests for each mutation. This is problematic in the case of *HBB* as there is a wide diversity of mutations. Not only does the need to develop customized tests substantially delay treatment, but the work entailed greatly increases the cost of PGD. This is problematic considering the high prevalence of *HBB* mutations in many resource poor countries.

Study design, size, duration: A large multiplex PCR protocol was designed, allowing simultaneous amplification of multiple overlapping DNA fragments encompassing the entire *HBB* gene sequence. Additionally, 22 linked polymorphisms (SNPs) flanking the *HBB* gene were amplified in additional PCR fragments. The resulting DNA was subjected to NGS to reveal the genotype/mutation status.

Participants/materials, setting, methods: The protocol was validated in samples from 4 families carrying different β-thalassemia mutations. The method was also applied to whole-genome amplified (WGA) DNA from 24 embryos derived from couples carrying various mutations in the *HBB* gene (thalassemia and sickle cell). In total, 12 mutations were assessed and the results obtained using the new methodology were compared to those obtained from conventional PGD methods for diagnosis of single gene disorders.

Main results and the role of chance: The new NGS-based protocol accurately detected the mutations in the DNA samples tested, confirming all patient genotypes. Seventeen single nucleotide polymorphisms (SNPs) in close proximity to the *HBB* locus (all within 1 Mb) were also successfully sequenced. Five further SNPs were identified within the *HBB* gene locus (i.e., intragenic) in the patient samples. Of the 22 SNPs analysed, each was informative for at least one family. On average, the families tested had 10 informative SNP loci. This allowed the inheritance of haplotypes associated with mutant genes to be tracked with high precision, providing a supplementary means of diagnosis, additional to direct mutation detection. The combination of direct mutation detection and analysis of multiple informative polymorphisms provides a redundant diagnostic, highly resistant to misdiagnoses due to problems such as allele dropout. Concerning the WGA DNA derived from embryo biopsy specimens, the protocol was able to correctly diagnose all 24 associated embryos. When compared to the results obtained from conventional PGD or karyomapping the new test displayed 100% concordance. Importantly, no patient-specific test design or optimization was needed. As far as we are aware, this is the first report of an NGS-based method for PGD of a monogenic disorder.

Limitations, reasons for caution: As part of the clinical validation of this newly-designed protocol for diagnosis of β-thalassemia and sickle-cell anaemia in preimplantation embryos, analysis on additional embryos with a wider variety of mutations should be undertaken. This will permit a more robust and comprehensive evaluation of the sensitivity and specificity of the method.

Wider implications of the findings: For disorders characterized by large numbers of different mutations, NGS-based PGD protocols, such as that described here, provide a simple generic approach, which is substantially less time-consuming and more cost-effective than conventional PGD methods. Lower costs should improve patient access to PGD, especially in less affluent parts of the world.

Trial registration number: N/A.

P-635 Clinical outcome of SET transfer of euploid embryos by Next Generation Sequencing with and without MitoGrade (mitochondrial DNA selection)

K. Ravichandran[1], C. Mccaffrey[2], J. Grifo[2], A. Morales[3], M. Perloe[4], S. Munne[1], D. Wells[5], E. Fragouli[5]
[1]*Reprogenetics, Research and Development, Livingston, NJ, USA*
[2]*NYU Langone Medical Center, NYU Fertility Center, New York, NJ, USA*
[3]*Fertility Specialists Medical Group, Medical Director, San Diego, CA, USA*
[4]*Georgia Reproductive Specialists, Medical Director, Atlanta, GA, USA*
[5]*Reprogenetics UK, Institute of Reproductive Sciences, Oxford, UK*

Study question: What is the overall value of mitochondrial DNA (mtDNA) assessment in a clinical setting, and how can it help improve IVF outcomes?

Summary answer: Retrospective analysis demonstrates that mtDNA quantification has a high negative predictive value and can be used as an independent biomarker in determining embryo viability.

What is known already: Chromosomal abnormalities are a major cause of implantation failure. Recently our group has validated the relationship between mtDNA quantification and IVF outcomes in a non-selection study. In particular we have been able to establish a threshold above which the probability of implantation is greatly reduced. While further randomized testing is being conducted, we took this opportunity to retrospectively analyze the overall value of the established mtDNA threshold in previously completed clinical cases.

Study design, size, duration: Retrospective study in which mtDNA was assessed in a total of 572 euploid blastocysts obtained from 328 couples (average maternal age 34.95 ± 0.27 years) undergoing preimplantation genetic screening (PGS). Outcome data was collected from 6 different IVF centers for routine follow up. Implantation outcomes were then utilized to determine the validity of the established threshold.

Participants/materials, setting, methods: DNA from blastocyst biopsy samples was amplified (Sureplex, Illumina, USA) and then subjected to aneuploidy analysis using next generation sequencing (NGS, Veriseq protocol, Illumina, USA). Only those embryos classified as chromosomally normal had their mtDNA levels assessed using MitoGrade (Reprogenetics). mtDNA levels were then compared to the pregnancy outcomes to confirm implantation predictions. All embryos were single embryo transfers (SET).

Main results and the role of chance: Nearly 14% (80/572) of all blastocysts analyzed contained mtDNA levels above the established threshold and were predicted to have lower chances of implantation. To date, 246 euploid embryos were replaced in SET with a pregnancy rate of 62.3% (153/246). Retrospective assessment of mtDNA levels revealed 216 embryos to contain normal mtDNA levels. Therefore the pregnancy rate post mtDNA quantification was 71% (153/216) in the normal level mtDNA group. Furthermore of the 30 embryos with elevated mtDNA levels, only one led to a successful pregnancy. Therefore the negative predictive value of mtDNA quantification was 96.7% (29/30). This highly significant ($p < 0.001$) difference between implanting and non-implanting embryos validates the clinical applicability of mtDNA quantification.

More importantly, the mtDNA threshold retained its validity across six different IVF centers and was unaffected by maternal age.

Limitations, reasons for caution: The study was retrospective and the number of cycles was not enough to show a difference between no selection (62% pregnancy rate) and selection against MitoGrade elevated embryos (71% pregnancy rate). It was however large enough to show a significant difference in implantation rates between elevated and normal MitoGrade embryos.

Wider implications of the findings: This study demonstrates the validity of MitoGrade as an independent variable in predicting embryonic implantation potential of euploid embryos. Further research involving the biological significance of mtDNA levels and implantation rates would be invaluable.

Trial registration number: N/A.

P-636 XRCC1 polymorphism Arg399Gln is associated with male infertility

A. Garcia[1], M. de la Casa[2], J. Gosalvez[1], R. Roy[1]
[1]*University Autonoma of Madrid, Biology Faculty, Department of Biology, Genetics Area, Madrid, Spain*
[2]*Ginefiv Assisted Reproduction Center, Laboratory of Andrology, Madrid, Spain*

Study question: Is XRCC1 polymorphism Arg399Gln associated with male infertility?

Summary answer: The Gln variant of the XRCC1 polymorphism Arg399Gln is associated with a decrease in the risk of male infertility.

What is known already: XRCC1 is a critical enzyme in the BER DNA repair system. Its expression in testis in higher than in any other tissue; it appears to have an important role in repairing DNA damages during spermatogenesis. XRCC1 polymorphism Arg399Gln is located in the PARP (poly-ADP ribose polymerase) binding domain.

Study design, size, duration: Case-control study with 120 seminal samples from infertile patients and 79 from donors with probed fertility.

Participants/materials, setting, methods: All samples where genotyped using the PCR-RFLP system. The primers used in the PCR where F 5´-TTGT-GCTTTCTCTGTGTCCA-3´ and R 5-TCCTCCAGCCTTTTCTGATA-3´ and the selected restriction enzyme was the MspI.

Main results and the role of chance: The allele frequencies where 0.66 (Arg) and 0.34 (Gln) in the patients group and 0.56 (Arg) and 0.44 (Gln) in the controls group. The genotype frequencies were 0.488 (Arg/Arg), 0.344 (Arg/Gln) and 0.172 (Gln/Gln) in the patients group and 0.278 (Arg/Arg), 0.557 (Arg/