Table 2.

Month	AMA (%)	Positive bhCG per ET (%)	CPR per ET (%)	IR (%)
Jan	94.73	20	10	6.7
Feb	77.27	30	20	13.3
Mar	88.88	40	33.3	22.7
Apr	83.3	33.3	33.3	25
May	76.43	33.3	17	13
Jun	72.72	50	44	30.4
Jul	69.04	61.9	57	46.7
Aug	95.23	35.7	29	23.5
Sep	70	60	60	50
Oct	84.09	31.3	25	15
Nov	86.48	29.4	29.4	20
Dec	72.72	71.4	57	40

Conclusions: One of the main concerns in an IVF unit is the stability and reliability of its results. It reflects the capability and performance of a team, particularly in a PGS laboratory. Advanced maternal age and ovarian ageing are key elements in the ART results. In this study we have demonstrated how there is a negative correlation between percentage of patients >38 years and the IR, pregnancy and CPR per ET in a PGS program through a year and month-by-month. These findings and how rates differ may be useful when analyzing your own unit results in short periods of time and additionally when comparing results among different units.

Keywords: preimplantation genetic diagnosis, advanced maternal age

P48 Application of aCGH for pre-implantation genetic diagnosis of chromosome rearrangements and additional unrelated chromosome aneuploidy

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Introduction: Fluorescent in situ hybridisation has historically been the method of choice for detecting aneuploid embryos when performing PGD for chromosome rearrangements. However, this only analysed the chromosomes involved in the rearrangement, and would not have detected additional unrelated aneuploidy. Our PGD program has always reported a lower pregnancy rates in couples seeking PGD for chromosome rearrangements than PGD for single gene disorders, with the suggestion this may have been due to the limited accuracy of FISH or an interchromosomal effect, meaning these patients may be at a higher risk of aneuploidy involving chromosomes beyond those involved in the rearrangement.

Materials and Method: Embryo biopsy was performed 3 days post oocyte retrieval. Blastomeres were tubed in 2 μl PBS and underwent whole genome amplification (GGAmp). The DNA quantity was assessed using gel electrophoresis and samples showing a high signal smear were labelled using the Bluegnome fluorescent labelling system and co-hybridised with labelled male control DNA onto Bluegnome v2.0 bacterial artificial chromosome microarrays. Results were analysed and reported using the Cytochip algorithm fixed setting in Bluefuse software.

Results: The data presented shows a higher clinical pregnancy rate in the patient group where chromosome analysis was performed by aCGH as opposed to FISH (63% v 38% per embryo transfer). The percentage of unbalanced embryos in the FISH cohort is 77% versus 42% in the aCGH cohort. In the aCGH cohort an additional 44% of embryos were balanced for the chromosome rearrangement but aneuploid, leading to a similar percentage of embryos suitable for patient use.

Conclusion: This work shows that aCGH can be successfully applied to pre-implantation diagnosis of chromosome rearrangements. It offers a quicker pathway to treatment for patients as no work up is required. The size of the chromosome segments involved, the position of breakpoints and array coverage in these regions allows a probability of detection to be calculated. These factors and the accuracy of the karyotype, method of biopsy and quality of amplification will affect accuracy.

This initial data indicates that analysis of a single blastomere by aCGH is a better indicator for embryo selection than FISH analysis of the chromosome rearrangement. The percentage of unbalanced embryos in both cohorts potentially indicates a high false positive rate with FISH analysis.

Additionally, the absence of any clinical miscarriages following the 28 cycles where aCGH was employed is very encouraging for clinics and patients.

P49 Detection of chromosome copy number mosaicism in mixed cells by array comparative genome hybridization

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Introduction: A central issue of current debate on the efficacy of preimplantation genetic screening (PGS) is that a single cell biopsied from D3 embryos may not truly represent the embryo due to mosaicism, resulting in false positive or negative diagnosis. Technological advances have allowed for more in depth chromosomal analysis – shifting from standard 9–12 chromosome FISH protocols to 23 pairs of chromosomes. Several studies have reported that PGS at the blastocyst stage may improve fertility treatment because studies by CGH and more recently microarray suggest that mosaicism is less common at the blastocyst stage. To test the sensitivity of a commonly used aCGH platform at detecting mosaicism in a multiple cell sample, a gradient of known normal and trisomic cell lines was mixed and aCGH results were analyzed.

Materials and Methods: Two cell lines, 46,XX and 47,XX,+21, were obtained from ATCC and Coriell respectively. To mimic a trophectoderm biopsy sample, where some 5 plus or minus cells are usually biopsied from blastocysts, a total of 5 cells were counted and loaded into PCR tubes; each with a mixed ratio of normal and trisomic cells. Group 1 (G1) consisted of 5 normal cells and 0 trisomic; group 2 (G2) consisted of 20% trisomic cells (1 trisomy, 4 normal); group 3 (G3) of 40% trisomic cells; group 4 of 60% trisomic cells; group 5 (G5) of 80% trisomic cells; and group 6 of 100% trisomic cells. A commercially available aCGHkit (24Sure V3, BlueGnome, UK) was used for 24 chromosome screening. Array CGH was performed according to the manufacturer's instructions. BlueFuseMulti software version 2.4 was used for analysis.

Results: All mixed specimens were successfully amplified and aCGH results were obtained from all 6 groups. Sample G1 was accurately reported as normal female, and sample G6 was accurately reported as abnormal female, with an extra copy of chromosome 21. Though the software did not report samples G3–G5 as abnormal, a clear increase of chromosome 21 is visible. The average log2 ratio of chromosome 21 when compared to the remaining euploid chromosomes of each sample is clearly elevated; a stepwise increase associated to the percent of trisomic cells in the original sample is presented in the table.

For sample G2, though the expression ratio chart does not offer an alarming visual increase at chromosome 21, closer

examination does suggest an aberration. No other false positive or negative call was made or visible on any other chromosomes.

Group	% Trisomy 21	Average log2 ratio (SD)
G1	0	0.037 (0.038)
G2	20	0.123 (0.051)
G3	40	0.145 (0.049)
G4	60	0.185 (0.038)
G5	80	0.214 (0.041)
G6	100	0.257 (0.043)

Conclusion: Our study demonstrated that this aCGH platform with the latest version of software allowed for the detection of mosaicism in a known mix. However, determination of the degree of mosaicism may be difficult in multiple cell specimens, such as the TE biopsies, and caution must be taken when interpreting results with low confidence scores.
Keywords: aCGH, mosaicism

P50 Aneuploidy testing of polar bodies by array comparative genomic hybridization (array CGH)

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Introduction: Advanced maternal age (AMA) is positively correlated with an increased risk for aneuploidy in the oocyte. Therefore, aneuploidy testing of polar bodies 1 and 2 (PB1, PB2) was performed prior to transfer using array CGH, in order to improve fertility rates in these couples. The procedure of single cell analysis was evaluated in terms of performance robustness, run-to-run variability and assisted reproductive technique (ART) outcome.
Material and Methods: At the Center for Human Genetics and Laboratory Medicine[1], 46 polar bodies from 30 oocytes were analyzed for aneuploidies by whole genome amplification (WGA) followed by array CGH (24sureV2, Bluegnome, UK). The average maternal age of the patient cohort (5 patients, 6 cycles) was 42.8 years.
Results: On average, 4.8 oocytes were retrieved per cycle. WGA was successfully performed in 80% of all PB1 and 86% of all PB2. The hybridization-to-hybridization variability was very low depicted by a mean percentage of included BAC clones of 96.2% (required minimum >90%) and a mean signal to background ratio (SBR) for Cy3 and Cy5 above 3.8 (required minimum >3). The averaged local noise indicator DLR (derivative log ratio) equals 0.10 (required minimum <0.2). In summary, our results met consistently the manufacturer's recommended quality criteria. Euploidy was detected in 7 out of 24 PB1 (= 29%) and 3 out of 21 PB2 (= 14%). The overall euploidy rate was 22%. In 3 cases, we observed a correction of a PB1 aneuploidy in PB2. To date, no transfer resulted in an implantation of an embryo.
Conclusion: Array-CGH analysis of 46 polar bodies obtained from patients with AMA showed robust performance metrics in our center. The outcome of ART in this patient cohort however maybe compromised by a low number of retrieved oocytes per cycle combined with the increased aneuploidy detection rate of array CGH. If the cumulative implantation rate can be improved by applying array CGH remains to be elucidated.

P51 Multiple Factor PGD – 4 case reports involving testing for up to 4 indications in a single blastomere

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Introduction: PGD necessitates the use of IVF to facilitate embryo testing. There can be a general presumption that, since patients are accessing IVF due to a genetic rather than fertility issue, no fertility issues exist. However, since many patients are not actively trying to conceive, this may not be the case.
We present 4 cases where the standard approach of solely testing for the single gene disorder in question was insufficient due to the patients presenting with further complex factors including recurrent implantation failure, miscarriage, maternal age and parental translocations.
Materials and Methods: DNA samples were taken from the couples undergoing treatment and key family members Mutant and normal haplotypes were assessed with a combination of DNA sequencing and multiple-allele genotyping for each at-risk gene, as well as 24-chromosome CGH.
Patients commenced downregulation with a GnRH agonist by injection or nasal spray on day 21 of their cycle, then started stimulation, taking daily gonadotrophin injections. hCG was taken to trigger oocyte maturation and ovulation when at least 3 follicles were identified measuring 17 mm or above on ultrasound. Transvaginal oocyte retrieval was performed under sedation 36 h after administration of hCG. Metaphase II oocytes underwent ICSI and were checked for fertilisation 16–18 h post insemination.
Embryo biopsy was performed three days post oocyte retrieval. The embryo was placed in calcium magnesium free medium to reduce cell adhesion. A hole was made in the zona with a laser and a blastomere aspirated. Blastomeres were tubed in 2 μl PBS and underwent whole genome amplification (GGAmp). The DNA quantity was assessed using gel electrophoresis and samples showing a high signal smear were labelled using the BlueGnome Fluorescent labelling system and co-hybridised with labelled control male DNA onto Bluegnome Cytochip v2.0 bacterial artificial chromosome microarrays. Results were analysed and reported using the CytoChip algorithm fixed setting in BlueFuse software. In tandem, some of the WGA product was used to perform nested PCR with multiple markers for single gene disorders and HLA tissue typing.
Results: Results were returned allowing reporting of results and embryo transfer on Day 5 post oocyte retrieval. One patient progressed to embryo transfer, but unfortunately did not achieve a pregnancy.
Conclusions: While PGD for single gene disorders and HLA tissue typing is performed using molecular genetic methods, the previous use of cytogenetic methods, such as FISH, for the examination of chromosomes, would have necessitated the biopsy of multiple cells to gain information at the level of both gene and chromosome. aCGH is a molecular method, and so by first employing WGA, it is then possible to generate highly accurate chromosome information from a single cell in addition to single gene information. These cases have shown that we have employed a robust WGA protocol that produces amplified product that can be utilised by two different molecular techniques. To our knowledge this is the most information generated from a single blastomere in a fresh IVF treatment cycle in a clinical setting.