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Introducing array comparative genomic hybridization into routine prenatal diagnosis practice: a prospective study on 1166 consecutive clinical cases. *F. Fiorentino[1], F. Caiazzo[1], S. Napoletano[1], L. Spizzichino[1], S. Bono[1], M. Sessa[1], A. Nuccitelli[1], A. Biricik[1], A. Gordon[2], G. Rizzo[1], M. Baldi[1].* 1) "GENOMA" - Molecular Genetics Laboratory, Rome, Italy; 2) Bluegnome Ltd, Cambridge CB22 5LD, UK.

Objective: Experience with array-based comparative genomic hybridization (aCGH) use for clinical prenatal diagnosis is still relatively limited. Prospective trials on a large sample size are necessary before aCGH can be recommended for routine clinical use in prenatal diagnosis. To assess the feasibility of offering aCGH for prenatal diagnosis as a first-line test, a prospective study was performed on a cohort of 1166 consecutive prenatal samples, comparing the results achieved from aCGH with those obtained from a conventional karyotype. Methods: Women undergoing amniocentesis or chorionic villus sampling (CVS) for standard karyotype, between 1 October 2010 and 31 May 2011, were offered aCGH analysis. A total of 1166 prenatal samples were processed in parallel using both aCGH, performed on DNA isolated from amniotic fluid (88.7%) or CVS (9.5%) and cultured amniocytes (1.8%), and G-banding for standard karyotyping. Results: Clinically significant chromosome abnormalities were identified in 37(3.2%) samples, 27(73.0%) of which were also detected by conventional karyotyping. In 10(27.0%) samples, aCGH identified pathogenic copy number variations (CNVs) that would not have been found if only a standard karyotype had been performed. Six were de novo, not recorded as benign CNVs, 4 resulted inherited. Eight of the above CNVs were concerning well-established syndromes. Benign CNVs were identified in 152(13.0%) samples. Following parental studies, no findings of unclear significance remained. aCGH was also able to detect chromosomal mosaicism as lower as 10% level. There was a complete concordance between the conventional karyotyping and aCGH results, except for 2 cases, that were correctly diagnosed by aCGH. Conclusions: This study demonstrates that aCGH represents an improved diagnostic tool for prenatal detection of chromosomal abnormalities, allowing identification of submicroscopic clinically significant imbalances that are not detectable by conventional karyotyping. The results of the study indicated that the aCGH approach was robust, with no false positive or false negative findings, suggesting that the technique has the potential to replace the traditional cytogenetic analysis without missing significant results. Our findings provide a further evidence on the feasibility of introducing aCGH into routine prenatal diagnosis practice as first-line diagnostic test to detect chromosomal abnormalities in prenatal samples.

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Validation of single cell Whole Genome Amplification (WGA) for Preimplantation Genetic Haplotyping (PGH) and its application using DNA microarrays. *G. Altarescu, H. El Harar, S. Zeligson, S. Perlberg, R. Beeri, D. Zeevi, T. Eldar-Geva, I. Varshaver, E. Margalioth, E. Levy-Lahad, P. Renbaum.* Zohar PGD Lab & IVF Unit, Shaare Zedek Medical Ctr, Jerusalem, Israel.

Background: Preimplantation genetic diagnosis (PGD) for molecular disorders involves development of disease and family specific protocols that allow simultaneous amplification of the mutation with multiple polymorphic markers in single cells. The development of PGD tests for single-gene disorders is challenging and only a limited number of amplified markers can be analyzed. An alternative and universal approach, PGH relies on whole genome amplification from a single cell to give microgram quantities of DNA, which allows the testing of multiple loci using PCR based protocols. Aims: To validate a WGA protocol for single and multiple cell amplification, including assessment of amplification and allele drop-out (ADO) rates, using both standard PCR protocols and DNA microarray chips. Methods: Single cells from embryos that reached the 6-8 cell stage, along with 2-5 cells from blastocyst biopsies were used for PGH analysis using the Qiagen RepliG multiple strand displacement amplification kit. Multiple loci on the genome from our PGD database were tested. Marker informativity was assessed using family haplotypes for each biopsied embryo for the calculation of ADO rates. In addition, WGA samples from a single cell and two-cell biopsy were analyzed using an Affymetrix microarray SNP chip. Results: Seven samples of blastocyst biopsy and nine single cell blastomeres were amplified using WGA. Fifty three loci on four different chromosomes were analyzed from the WGA samples obtained from single blastomeres and blastocyst biopsied cells. Using standard PCR protocols the amplification rate for blastocysts was 99.5% and for singles blastomeres 87.5%. ADO rates ranged between 8% for blastocyst biopsied cells to 16.3% for blastomeres. The results were in concordance to the parental haplotypes built from genomic DNA. Analysis using DNA microarrays yielded 90-92% call rates with ADO rates between 26-29%. An unbalanced translocation, confirmed by FISH could also be detected. Conclusions: WGA appears to be an efficient and accurate method for obtaining significant amounts of DNA from single cells PGH and can also be used for microarray anlysis. PCR based ADO rates are similar to those obtained by conventional PGD methods. The WGA approach incorporates information from multiple polymorphic markers and thus identifies high-risk haplotypes, creating a universal generic test for any single-gene disorder and chromosomal imbalances.

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Are heterozygous carriers of cohesin mutations at an increased risk of aneuploidy? *B.M. Murdoch, N. Owen, S.I. Nagaoka, T.J. Hassold, P.A. Hunt.* Washington State University, Pullman, WA.

Cohesion proteins (cohesins) tether sister chromatids prior to cell division, and thereby play a critical role in chromosome segregation. Not surprisingly, mutations in genes encoding cohesin proteins have profound effects on genome stability: in somatic tissues, defects in cohesins have been linked to a number of malignancies and, in germ cells, to infertility or to an increase in aneuploid gametes. However, the germ cell studies have been based on studies of model organisms and, in particular, on mice homozygous for null mutations encoding the meiosis-specific cohesins REC8 or SMC1B. Since more subtle allelic variation - a likely scenario in the human population - has not been examined, we were interested in asking whether "milder" genotypes might also have deleterious effects on meiosis. Accordingly, we assessed the consequences of haploinsufficeny for either *Rec8* or *Smc1*B on meiotic chromosome dynamics in female mice. We generated surface spread preparations from fetal ovarian samples to examine the formation of the synaptonemal complex (SC), the incidence of synaptic errors and the level of meiotic recombination. Surprisingly, for each of these variables - and for both *Rec8* or *Smc1*B - we identified significant differences between heterozygous females and their wildtype sibling controls. For example, we found that haploinsufficency resulted in shorter and less compact SCs and an approximate two-fold increase in asynapsis and other synaptic defects. Further, in both *Rec8* and *Smc1*B heterozygotes we observed significant 5-10% decreases in the genome-wide number of MLH1 foci, a marker of meiotic crossovers. The increase in meiotic defects in prophase oocytes prompted us to examine conventional cytogenetic preparations in metaphase II (MII) eggs from adult females, asking whether chromosome abnormalities were increased in heterozygotes. Indeed, carriers of each mutation exhibited an approximate two-fold increase in chromosome errors, including both numerical and structural abnormalities. Thus, our results indicate that haploinsufficency for meiotic cohesins increases the likelihood of meiotic errors, suggesting that women who are asymptomatic carriers of a cohesin mutation may have an increased risk of a chromosomally abnormal pregnancy.

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Human Embryos with Aneuploid Cells Documented at the Cleavage Stage Undergo Genetic Correction During Differentiation to the Blastocyst Stage. *P.R. Brezina[1], A. Benner[2], R. Ross[3], A. Barker[4], K. Richter[5], G.R. Cutting[6], W.G. Kearns[7].* 1) GYN/OB; Johns Hopkins Medical Institutions, Baltimore, MD. U.S.A; 2) Genetics, The Center for Pre-Implantation Genetics, LabCorp: Rockville, MD, U.S.A; 3) IVF Lab, La Jolla IVF; La Jolla, CA, U.S.A; 4) IFV Lab, Arizona Center for Fertility Studies, Phoenix, AZ, U.S.A; 5) Research, Shady Grove Fertility Reproductive Science Center, Rockville, MD. U.S.A; 6) The McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Medical Institutions, Baltimore, MD, U.S.A; 7) Genetics and GYN/OB, The Center for Preimplantation Genetics Labcorp and Johns Hopkins Medical Institutions, Rockville and Baltimore, MD.

Background: We determined if day 3 aneuploid embryos can undergo genetic normalization during differentiation to the blastocyst stage. **Materials and Methods:** Patients underwent in vitro fertilization (IVF) and preimplantation genetic screening (PGS) due to repeat pregnancy loss (RPL). Single blastomeres taken from Day-3 embryos underwent two rounds of DNA amplification followed by a 23-chromosome SNP microarray analysis using HumanCytoSNP-12 DNA beadchips and GenomeStudio and KaryoStudio software. Following Day-3 biopsy, all embryos remained in a standard commercially available media until Day-5 post oocyte fertilization. Day-5 blastocysts with euploid Day-3 results either underwent uterine transfer or were cryopreserved. Day-5 blastocysts grown from embryos with aneuploid Day-3 results underwent surgery to separate the inner cell mass (ICM) from the trophectoderm (TE). An average of 100 TE cells and a range of 40 ICM cells to the entire ICM cell population were obtained from each embryo and immunocytochemistry was performed using anti-oct3/4 to confirm the ICM and anti-cdx2 to identify the TE cells. DNA amplification and microarray analyses were then performed as described above. Binomial confidence intervals for proportions were calculated. **Results:** 12 patients were enrolled. 126 embryos were subjected to Day-3 biopsy. Of cleavage stage embryos with euploid karyotypes, 43 (69.4%) developed to the blastocyst stage. In contrast, only 25 (39.1%) of the embryos with an aneuploid karyotype at the cleavage stage progressed to the blastocyst stage. A euploid karyotype obtained at the cleavage stage was predictive of progression to the blastocyst stage with a positive predictive value of 69.4% [95% CI: 57-79%] and a negative predictive value of 60.9% [95% CI: 49-72%]. Evaluation of the 25 blastocysts that developed from aneuploid day 3 embryos revealed that 68% (17/25) [95% CI: 48-83%] possessed a euploid ICM and 76% (19/25) [95% CI: 56-89%] possessed a euploid TE with 64% (16/25) [95% CI: 44-80%] having both a euploid ICM and TE. No mosaicism, detected at a level of 5%, was observed. **Conclusions:** The genetic normalization observed in this study has significant implications in numerous scientific fields. An understanding of such in vitro reparative mechanisms could further gene repair and stem cell transplant therapy and could impact the management of patients undergoing infertility care by reassessing the disposition of abnormal Day-3 aneuploid embryos.

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Mosaicism do not affect accuracy of 24 chromosomes preimplantation genetic screening on cleavage stage embryos. *A. Biricik[1], F. Fiorentino[1], G. Kokkali[2], L. Rienzi[3], L. Spizzichino[1], S. Bono[1], A. Gordon[4], F.M. Ubaldi[3], K. Pantos[2].* 1) "GENOMA" - Molecular Genetics Laboratory, Rome, Italy; 2) Centre for Human Reproduction, Genesis Athens Hospital, Athens, Greece; 3) G.EN.E.R.A Centre for Reproductive Medicine, Clinica Valle Giulia, Rome, Italy; 4) Bluegnome Ltd, Cambridge CB22 5LD, UK.

Introduction: Different randomized clinical trials (RCTs) have shown that preimplantation genetic screening (PGS), as it was practiced, has not provided the expected benefits. The possible explanation for this poor clinical performance has been mainly attributed to the embryonic chromosomal mosaicism, that is present on day-3 of development, which may decrease the chances of a live birth by prematurely labeling an embryo as abnormal. In this study we aimed to evaluate the accuracy of the 24-chromosomes PGS performed on cleavage stage embryos, in order to ascertain if the tested blastomeres were representative for the whole embryo. Methods: embryos biopsy was carried out at day-3. Single cell DNA was amplified by whole genome amplification (WGA) and processed by Array-CGH according to the 24sure protocol, BlueGnome. Euploid embryos were then selected for transfer on day-5 or day-6 of the same cycle. In order to verify the results obtained following day-3 PGS, chromosomally abnormal embryos that developed to blastocyst stage were re-biopsied on day-6 and reanalyzed. Results: 111 PGS cycles were carried out for 104 couples. The mean maternal age was 39.0±3.7 years. A total of 838 embryos were biopsied on day 3. Overall, 768 (91.6%) embryos were successfully diagnosed, 553 (72.0%) of which resulted aneuploid. Embryos suitable for transfer where identified in 75 cycles (67.6%). Following transfer of 124 embryos, 50 women (mean maternal age 38.1±3.2 years) had a clinical pregnancy (66.7% pregnancy rate/ET). A total of 63 embryos implanted (50.8% implantation rate/ET), for 60 of which heart beat was also detected. After the clinical cases, 218 non-transferred embryos from 64 PGS cycles were successfully reanalyzed. Aneuploidy mosaicism was detected in 100/218 (45.9%) embryos. Despite high levels of mosaicism found, all day-3 aneuploid embryos followed-up were again diagnosed as abnormal after re-analysis on trofectoderm cells, confirming at the end the previous results regardless of the actual abnormal genotype. Conclusions: Post-zygotic errors leading to mosaicism were common. However, mosaic embryos were confirmed as chromosomally abnormal after re-analysis at blastocyst stage. Although a larger follow-up study are required in order to confirm the above findings, array-CGH analysis on single blastomeres has demonstrated an accurate aneuploidy detection tool and may assist in identifying abnormal embryos at cleavage stage.

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The transcriptome of a human polar body accurately reflects its sibling oocyte. *A. Reich[1], P. Klatsky[2], S. Carson[2], G. Wessel[1].* 1) Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI 02912, USA; 2) Division of Reproductive Endocrinology and Infertility, Women and Infants Hospital, Alpert School of Medicine, Brown University, Providence, RI 02905, USA.

Improved methods are needed to reliably and accurately evaluate the developmental potential of human embryos created through in vitro fertilization (IVF). Currently embryos are prioritized by morphology, an imperfect criterion, and this inaccuracy necessitates the creation and transfer of multiple embryos to produce a successful live birth. The first polar body (PB) is extruded from the oocyte before fertilization and can be biopsied without damaging the oocyte and because much of early development is driven by maternally deposited mRNA in the oocyte, we tested the hypothesis that the PB transcriptome is representative of that of the oocyte. Polar body biopsy was performed on metaphase II (MII) oocytes followed by single-cell transcriptome analysis of the oocyte and its sibling PB. Over 12,700 unique mRNAs and miRNAs from the oocyte samples were compared to the 5,431 mRNAs recovered from the sibling PBs (5,256 shared mRNAs or 97%, including miRNAs). Analysis of the mRNA expression levels of transcripts expressed in oocytes and PBs reveals that no genes are significantly differentially expressed between the two populations, indicating that the human PB transcriptome reflects that of the oocyte in both the expressed genes as well as the abundance levels of those gene products. This analysis was able to detect up to a four orders of magnitude difference in transcript abundance between individually sequenced oocytes; the same order of magnitude difference was also detected in the individually sequenced sibling PBs. These results suggest that this single cell analysis is accurate and sensitive over the wide dynamic range of mRNA transcript abundance that is generally found within transcriptomes. Furthermore we identified a conservative list of 215 genes that could be used as potential candidates for future clinical applications. Refinement of this approach could lead to the first molecular diagnostic of gene expression in MII oocytes, possibly allowing for both oocyte ranking and embryo preferences in IVF.