Aneuploidy screening of human blastocysts from PGS patients using next generation sequencing: a pilot study

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BACKGROUND

Recent advances in next generation sequencing (NGS) technology have provided new tools for detecting DNA mutations and/or chromosome aberrations for research and diagnosis purposes. The parallel nature of NGS data offers a unique platform to evaluate multiple genomic loci and multiple samples on one chip. Embryonic DNA from different patients could be evaluated on the same sequencing chip with the use of standard DNA barcoding methods (Knapp et al. 2012). One of the NGS platforms has been recently evaluated using embryonic biopsy samples derived from PGS patients (Fiorentino et al, 2014). However, there is still very limited information about the clinical applications of NGS in IVF and / or PGS treatment.

References

1. Knapp M, Stiller M, Meyer M. Generating libraries for multiplex high-throughout sequencing. Methods Mol Biol. 2012, 840:155-170.
2. Fiorentino F, Biriclik A, Bono S, Spizzichino L, Cotroneo E, Cottone G, Kokocinski F, Claude M. Development and validation of a next-generation sequencing-based protocol for 24-chromosome aneuploidy screening of embryos. Fert Steril. 2014, 101:1375-1382.

OBJECTIVE

Our current study aimed at evaluating the efficiency of aneuploidy screening using NGS for patients with history of recurrent pregnancy loss and previous aneuploid conception in terms of clinical pregnancy and implantation outcomes.

DESIGN

Step one:

Blinded DNA from blastocysts derived from patients undergoing preimplantation genetic screening (PGS) with NGS and array CGH (BlueGnome/Illumina, Cambridge, UK). 3 to 5 trophectoderm cells were biopsied from each blastocyst. All embryos were vitrified after trophectoderm biopsy. Results obtained from the same embryos were compared and the two methods were evaluated for consistency.

Step two:

Based on the results of NGS analysis, one to two euploid blastocysts with the best morphological grade available were thawed and transferred to individual patients. Clinical pregnancy rate and implantation rate were recorded. Clinical pregnancy was defined as an intrauterine gestational sac containing one embryo which demonstrated cardiac action with rate >110/min. Implantation rate was calculated as percentage of the numbers of heartbeats over total numbers of embryos transferred.

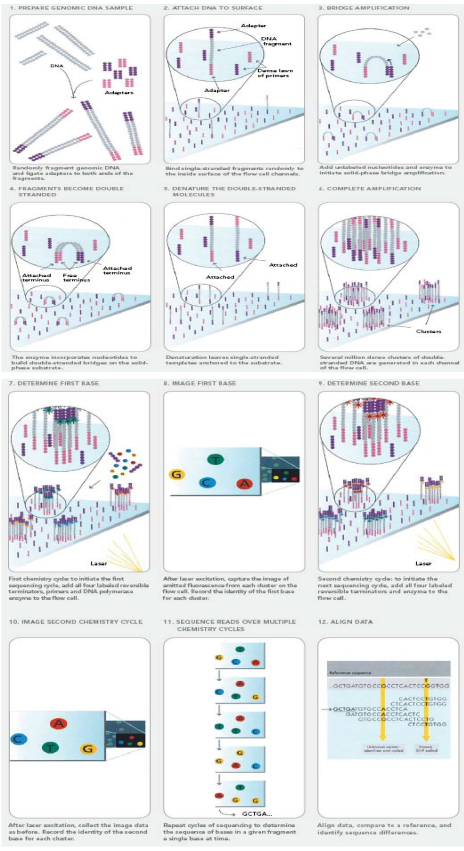
MATERIALS & METHODS

Patient groups: A total of fifteen PGS patients at mean age 38.1 ± 2.3 years in our IVF clinics were recruited in this pilot study after signing a informed consent with the clinical indications of 1) Unexplained recurrent pregnancy loss (RPL): patients (n=9) with two or more unexplained miscarriages and 2) Previous aneuploid conceptions (PAC): Patients (n=6) with previous aneuploid conceptions (PAC) (e.g. Down Syndrome).

Biopsy and whole genomic amplification (WGA): A total of 56 blastocysts were biopsied and vitrified on day 5. 3 to 5 trophectoderm (TE) cells were removed from each embryos and loaded into a PCR tube containing 2.5 ul 1x PBS. Whole genome amplification was performed using the SurePlex kit (BlueGnome/ Illumina, UK).

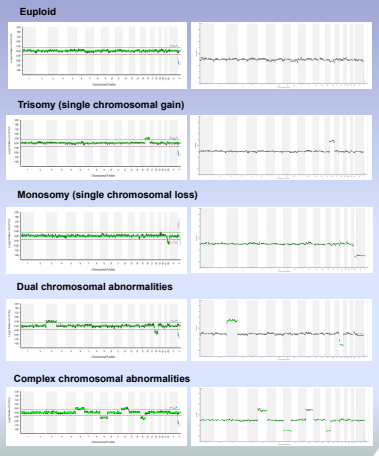
Array CGH: Sample and/or control DNA were labeled with Cy3 and/or Cy5 fluorophores. Labeled DNA was re-suspended in the dexsulphate hybridization buffer and hybridized under cover slides. After a series of washing, the hybridized 24sure slides (BlueGnome/ Illumina, UK) were scanned at 10 um using a laser scanner (Agilent, USA). The data was analyzed using the BlueFuse software (BlueGnome/Illumina, UK) for whole chromosome loss or gain.

NGS procedures: sequencing by synthesis



RESULTS

Comparison of PGS graphs: aCGH (left) vs. NGS (right)

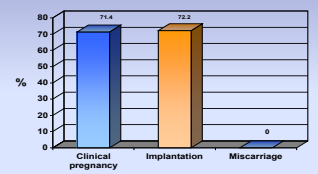


Comparison of PGS results: aCGH vs. NGS (n=56)

Ploidy	aCGH	NGS
Euploid	39.3%	39.3%
Trisomy	10.7%	10.7%
Monosomy	12.5%	12.5%
Dual abnormalities	16.1%	16.1%
Complex abnormalities	21.4%	21.4%

No differences in each category between aCGH and NGS

Clinical pregnancy and implantation rates in the cohort patients (n=15)



* Clinical pregnancy is defined as gestational sacs with fetal heartbeats.
** Implantation rate is calculated as number fetal heartbeats over total numbers of embryos transferred.

CONCLUSION

Our data demonstrate that NGS analysis detects all types of aneuploidies of human blastocysts accurately as compared with array CGH and provides PGS results with a high level of consistency with the well established methodology. Moreover, NGS screening identifies euploid blastocysts for transfer and may improve pregnancy and implantation rates for PGS patients. Further randomized clinical trials with a larger sample size are planned to verify these preliminary findings.