

The application of post-light semiconductor-based next-generation sequencing in clinical cases of preimplantation aneuploidy screening (PGS) with fresh embryo transfer.

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Introduction

Although next generation sequencing (NGS) techniques are today best method of choice they require more than 24 hours to perform, consequently blastocyst vitrification is needed. We report the post-light semiconductor-based next-generation sequencing (PLS-NGS) application in clinical cases of preimplantation genetic diagnosis processed with innovative protocol that is compatible with fresh embryo transfer.

Materials and methods

The study population consisted of patients treated by intracytoplasmic sperm injection (ICSI) between 08.2013 and 01.2014. 28 patients decided to undergo preimplantation genetic diagnosis of aneuploidy because of repeated implantation failures (≥ 2). A control group was created consisting of 106 patients, matched to the PGD patients in term of infertile etiology, number of failed cycles, age, anti-Mullerian hormone, antral follicles count and range of other prognostic markers. All women were treated on a long agonist protocol.

Aneuploidy screening was performed on single blastomere biopsied on day-3 of culture of cleavage stage embryos composed of 6-8 cells. The short duration of the procedure (12 hours) allowed fresh embryo transfer without need of blastocyst vitrification.

Results

We performed 28 cycles in the study group. The average number of retrieved oocytes was relatively low (8.4 oocytes per patient) but sufficient to find at least one chromosomally normal embryo in 27 cases (96.4%). The pregnancy rate (as ultrasound fetal heartbeat detection on 6 weeks and 1-3 days) per transfer was much higher in the group having NGS-based embryo selection compared to control group.

An increase was also found in implantation rate. In the current study, only one miscarriage occurred in the group that received screening using NGS, whereas in control group without embryo genetic testing three miscarriages occurred before 12 week of pregnancy and additional four after biochemical pregnancy was stated (Table 1).

Fig 1. PGS NGS application process.

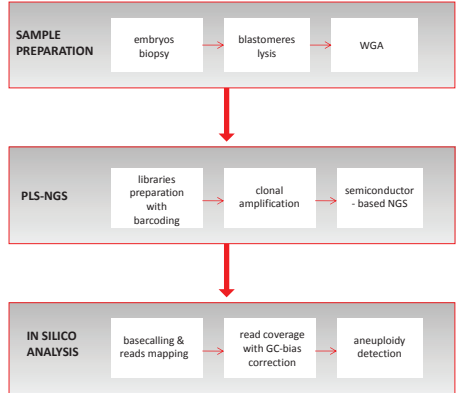


Table 1. IVF program characteristics of the investigated groups.

Variable	PGD NGS group	Control group - no PGD	p value
No. of cycles	28	106	
No. of transfers	27	106	
NGS results (% of biopsied embryos):			
Normal	48 (33.1)	-	
Abnormal	84 (57.9)	-	
No diagnosis	13 (8.9)	-	
Read number (SD) per embryo	61372.2 (9026.0)	-	
Average no. of embryos transferred	1.3	1.6	0.003
Pregnancy rate (per ET- %)	21 (77.8)	48 (45.3)	0.001
Implantation rate (%)	48.2	33.9	0.3
Multiple pregnancy rate of pregnancies (%)	1 (4.8)	16 (33.3)	0.001
Ectopic pregnancy (%)	1 (4.8)	0	0.13
OHSS ^b (%)	0	0	
Biochemical pregnancy	0	4 (8.3)	0.17
Spontaneous abortion below 12 weeks rate (%)	1 (4.8)	3 (6.3)	0.8

^a human menopausal gonadotropin; ^b ovarian hyperstimulation syndrome

Fig 2. PGS-NGS - result of healthy embryo 46, XY.

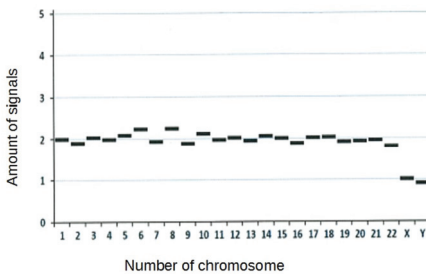
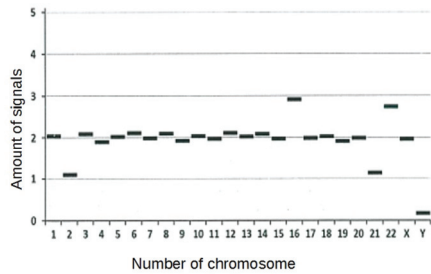


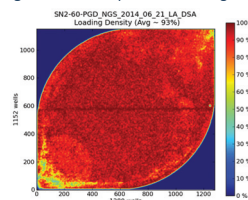
Fig 3. PGS-NGS - result of two monozygotic, two trisomic embryo 46XX -2, +16, -21, +22.



Conclusion

New technology of PLS-NGS possess strong research potential allowing for generation of large amount of data in scale of hours. Hereby, we report successful application in clinical preimplantation diagnosis. Data from this prospective trial indicated that single blastomere biopsy followed by fresh transfer of euploid embryos on day-5 was associated with excellent implantation and pregnancy rates and a low miscarriage rate, suggesting that NGS may be a useful tool for embryo selection.

Fig 4. PLS-NGS chip - beads loading density



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