Successful Pregnancies after Application of Array-CGH in PGD-Aneuploidy Screening

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Applications of preimplantation genetic screening (PGS)

PGS has been suggested and used to improve pregnancy rates for the following indications: Advanced maternal age Repeated IVF failure Repeated miscarriage Testicular sperm extraction (Kuliev and Verlinsky., 2008; Donoso *et al.*, 2006; Gianaroli *et al.*, 2005, Caglar *et al.*, 2005).

Hellani et al; ESHRE 2009

PGS and chromosomal abnormalities

Although, improvements in IVF outcome after PGS have been observed in multiple case-controlled studies (Munne *et al.*, 2007a; Munne *at al.* 2007b) its effectiveness in randomized controlled studies is still limited. One of the main reasons for this limitation is in the FISH technique.

Background

Aneuploidy is the main cause of recurrent IVF failure (RIF)

Majority of the applications has been done using

FISH Technique

Number of chromosomes assessed varies from 5 to 12

Hellani et al; ESHRE 2009

Rational

Screening the entire set of single cells chromosomes in patients suffering from RIF using agilent A-CGH platform

Assess the outcomes in term of pregnancy rate

Validation of A-CGH technique

	Chr.13		Chr.16		Chr.18		Chr.21		Chr.22		Chr.X/Y
	Trizomy	Monozomy									
of embryos iagnosed by ISH	3	1	2	1	2	1	3	2	2	1	XXY 2
of embryos iagnosed by CGH	2*	1	2	1	2	1	1*	2	1*	1	XXY 1*

- * Discrepancy in the diagnosis observed between the two methods.

- Chr.: Chromosome

-In FISH, one blastomere was used whereas in aCGH two blastomeres were used due to protocol requirements for each method (see materials and methods section).

- Each embryo examined by aCGH had only one chromosome abnormality as detected by FISH.

-Possible reason for discrepancies between the FISH and aCGH is embryo-mosaicism due

to the number of blastomeres diagnosed

Recurrent IVF failure patients selection criteria

A minimum of 7 IVF failures was the main criteria set for patient selection

Hellani et al; ESHRE 2009

PGS steps

- Embryos are biopsied on day 3 where two cells are
- systematically taken
- Multiple Displacement Amplification (MDA) was performed
- on the 2 cells of each embryo, labeled and hybridized agains
- a normal control DNA
- After 20 hours of hybridization slides are washed and scanned using the agilent platform.

atient age	# of recurrent IVF failure	# of embryos assessed by Acgh	# of normal embryos detected	# of abnormal embryos detected and type of abnormalities	Pregnancy (heartbeat)	Pregnancy duration
8	10	6	3	E1: Trizomy 18 E2*: Trizomies: 13, 16, 22 E3: Trizomy 20	Yes	Third- trimester
1	11	4	2	E1*: Trizomies: 13, 21; Monozomies: 22, 14 E2: 8q deletion	Yes	Third- trimester
4	9	3	None	E1: Trizomy 15 E2: Monozomy 10 E3: Trizomy 14	NA	Third- trimester
2	10	4	None	E1: Monozomy 17 E2: Trizomy 15 E3: Trizomy 2 E4: Monozomy 10	NA	Third- trimester
4	7	6	4	E1: Monzomy 1 E2: Trizomy 21 E3: Trizomy 15 E4: XXY	Yes	Third- trimester
.8	9	6	2	E1: Trizomy 4 E2: Trizomy 16 E3: Monozyomy 12 E4: X0	Yes	Third- trimester
31	7	6	3	E1: Trizomy 16 E2: Trizomy 21 E3: Monozomy 18	No	Third- trimester
32	12	6	2	E1: Duplication 1p E2: Trizomy 13 E3: Monozomy 5 Heliani et al; ESHRE 2009 E4: Trizomy 12	Yes	Third- trimester

Result summary

Number of	Transfe	er	Pregnancy
patients	perfor	med	
24	19/24	(79%)	13/19 (68%)

The first clinical application of A-CGH in PGS

The technique is reliable and paves the way for more applications

The discrepancy observed between A-CGH and the FISH is most probably due to the mosaicism mechanism known in the embryos

Many abnormalities detected by aCGH were missed using the FISH technique.

Abnormalities in chromosomes 1, 2, 4, 5, 8, 10, 12, 14, 15, 17 and 20 in many embryos tested. Such abnormalities could not be detected using the seven probes FISH panel used in our routine PGS.

The percentage of embryos with those abnormal chromosomes was 60%, when compared to the total number of abnormal embryos.

Therefore, FISH panel for chromosomes 13, 16, 18, 21, 22 and sex chromosomes, could only detect 40% of the chromosomal abnormalities

The confirmation of aCGH result on the nontransferred embryos by FISH showed the presence of embryos with mosaicism involving more than one chromosome (18%)

The 18% figure should be added to the percentage of biased factor due to missed mosaicism by aCGH (embryo diagnosed as normal and transferred).

Two cells are systematically diagnosed for each embryo; consequently, mosaicism could not be easily detected which may explain the high percentage of normal embryos (40%) compared with the 25% previously reported (Voullaire *et al.*, 2000).

During the aCGH validation and confirmation processes, 25% and 18% of the embryos were mosaics.

Challenges and possible improvements duration of diagnosis



Hellani et al; ESHRE 2009

Future directions

A-CGH and Recurrent miscarriages Study design: 3 or more miscarriages during the first trimester Ten recurrent miscarriages patients She produced 10 embryos after PGD, 9 embryos were found abnormal Abnormalities observed in the following chromosomes: 19, 14, 20, 21, 11, 2, 22 Patient did not get pregnant with single embryo transferred.



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Agilent slides used

8x15 k format: Customized array for our laboratory 4x4 k format: Generic and commercially available

In conclusion, aCGH can be used routinely in PGD with a potential of high successful pregnancy rate and had superior efficiency over the seven probes FISH panel. Agilent technology made it more convenient with the possibility of screening more than one embryo using one slide. More cases should be performed in order to define more Accurately the importance of A-CGH in IVF recurrent Failures And recurrent miscarriages

Hellani et al; ESHRE 2009