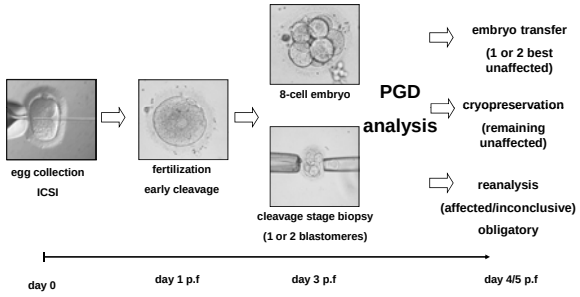
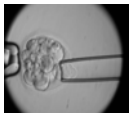
Quality control
by means of spare embryo re-analysis

Dreesen et al., 2008
Validation of Preimplantation Genetic Diagnosis by PCR analysis:
genotype comparison of the blastomere and corresponding embryo, implications for clinical practice.
Mol Hum Reprod. Sep [Epub]

PGD - PCR routine



PGD – PCR protocol



embryo biopsy
one/two blastomeres

⇩

alkaline lyses step

⇩

PCR with fluorescently labelled primer (40-50 cycles)

⇩

mutation detection / genotyping

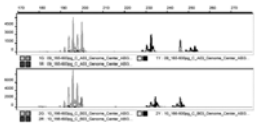
⇩

analyses on ABI 3100/3730 capillary automatic
sequencer with laser detection

PGD-PCR protocols

Monogenic disorders

Spinal Muscular Atrophy
Cystic fibrosis
Myotonic Dystrophy
Fragile-X syndrome
Huntingtons disease
Spinocerebellar Ataxia 3
Marfan syndrome
AR Polycystic kidney disease
Adrenogenital syndrome
Phenylketonuria
Incontinentia pigmenti
Pelizaeus Merzbacher



Fanconi Anemia
Cogenital Deafness
Familial Adenomatous polyposis coli
Tyrosine hydroxylase defeciency
enzyme complex I defficiency
Ehlers-Danlos syndrome
Hypochondroplasia
Achondroplasia
Metachromatic Leukodystrophy
Citrullinaemia
Tuberous Sclerosis type 1
Tuberous Sclerosis type 2
Krabbe disease
CADASIL
Familial Atypical Mole-Malignant Melanoma Syndrome
Holoprosencephaly
Nail Patella syndrome
Mucolipidosis type 2

Mitochondrial disorders

NARP/Leigh syndrome (T8993C/G mutation)
Leigh syndrome (T9176C mutation)
MELAS (A3243G mutation)

CUMULATIVE PGD-PCR DATA until 2007

No. of patients	No. of cycles	No. cancelled	No. of OR	No. of embryos diagnosed	No. of cycles to ET	No. of HCG positive	No. of FHB positive	Clinical pregnancy rate		No. children Born
								FHB/OR	FHB/ ET	
115	257	52	205	1241	182	52	45	22,0%	24,7%	53

Frozen cycles not included

ESHRE PGD consortium data collection I-VII 20% FHB/OR , 26% FHB/ET

How valid is PGD-PCR diagnosis ?

Validation of PGD may be performed by:

- follow up of children born after PGD
- reanalysis of spare embryos after PGD

aims of the study

- validate PGD-PCR analysis through a diagnostic-test analysis
- to determine the diagnostic value

validation PGD-PCR analysis

422/1241 embryos with initial genotype based on biopsied blastomere were reanalyzed

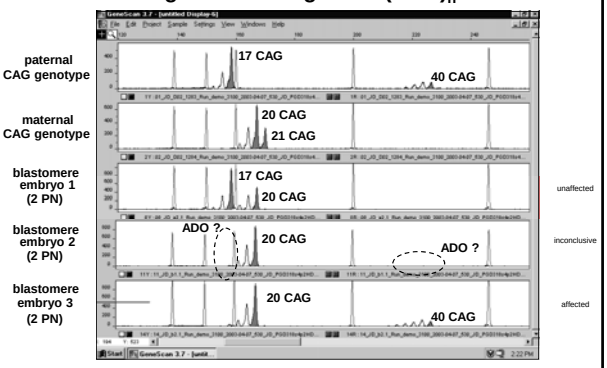
- removal of the zona
- several washing steps
- embryo reanalysis by single cell PCR, using same initial strategy

initial biopsied blastomere genotype was compared to reanalyzed embryo genotype

distribution of blastomere/embryo comparison results over the different genotype outcome groups

different genotype outcome groups	**no. blastomeres / embryos compared**
	n
blastomere genotype confirmed by embryo genotype (allele match)	367
ADO in blastomere genotype confirmed by embryo genotype	32
blastomere genotype not confirmed by embryo genotype due to contamination	4
blastomere genotype not confirmed by embryo genotype	19
total	422

Huntington PGD single cell $(CAG)_n$



distribution of blastomere/embryo comparison results
over the different genotype outcome groups

different genotype outcome groups	**no. blastomeres / embryos compared**
	n
blastomere genotype confirmed by embryo genotype (allele match)	367
ADO in blastomere genotype confirmed by embryo genotype	32
blastomere genotype not confirmed by embryo genotype due to contamination	4
blastomere genotype not confirmed by embryo genotype (mainly additional maternal allele)	19
total	422

diagnostic test analysis

genotype outcome was converted to diagnostic outcome

- embryos diagnosed by PGD as unaffected; test negative (T-)
- embryos diagnosed by PGD as affected or aberrant; test positive (T+)
- embryos reanalyzed after PGD as unaffected; disease negative (D-)
- embryos reanalyzed after PGD as affected or aberrant; disease positive (D+)

compare diagnostic outcome of blastomere to reanalyzed embryo

- reanalyzed embryo was used as golden standard

diagnostic-test analysis performed on diagnostic outcome

Conversion of genotype outcome to diagnostic outcome for the different blastomere/embryo genotypes outcome groups

different genotype outcome groups	blastomeres / embryos compared	different diagnostic outcome groups			
	n	T-D-	T-D+	T+D+	T+D-
blastomere genotype confirmed by embryo genotype	367	167	0	200	0
ADO in blastomere genotype confirmed by embryo genotype	32	5	0	8	19
blastomere genotype not confirmed by embryo genotype due to contamination blastomere	4	0	0	3	1
blastomere genotype not confirmed by embryo genotype	19	2	7	7	3
total	**422**	**174**	**7**	**218**	**23**

false positives

Conversion of genotype outcome to diagnostic outcome for the different blastomere/embryo genotypes outcome groups

different genotype outcome groups	blastomeres / embryos compared	different diagnostic outcome groups			
	n	T-D-	T-D+	T+D+	T+D-
blastomere genotype confirmed by embryo genotype	367	167	0	200	0
ADO in blastomere genotype confirmed by embryo genotype	32	5	0	8	19
blastomere genotype not confirmed by embryo genotype due to contamination blastomere	4	0	0	3	1
blastomere genotype not confirmed by embryo genotype	19	2	7	7	3
total	**422**	**174**	**7**	**218**	**23**

false negatives

Conversion of genotype outcome to diagnostic outcome for the different blastomere/embryo genotypes outcome groups

different genotype outcome groups	blastomeres / embryos compared	different diagnostic outcome groups			
	n	T-D-	T-D+	T+D+	T+D-
blastomere genotype confirmed by embryo genotype	367	167	0	200	0
ADO in blastomere genotype confirmed by embryo genotype	32	5	0	8	19
blastomere genotype not confirmed by embryo genotype due to contamination blastomere	4	0	0	3	1
blastomere genotype not confirmed by embryo genotype	19	2	7	7	3
total	**422**	**174**	**7**	**218**	**23**

false negatives/potential misdiagnosis

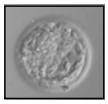
Embryo Quality

- embryo morphology

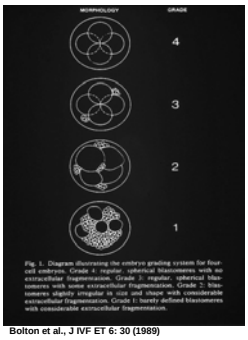
morphology grading systems used for

cleavage stage

morulae



blastocysts

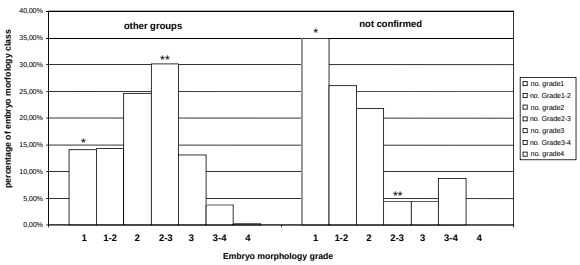


Bolton et al., J IVF ET 6: 30 (1989)

Conversion of genotype outcome to diagnostic outcome for the different blastomere/embryo genotypes outcome groups

different genotype outcome groups	blastomeres / embryos compared	different diagnostic outcome groups			
	n	T-D-	T-D+	T+D+	T+D-
blastomere genotype confirmed by embryo genotype	367	167	0	200	0
ADO in blastomere genotype confirmed by embryo genotype	32	5	0	8	19
blastomere genotype not confirmed by embryo genotype due to contamination blastomere	4	0	0	3	1
blastomere genotype not confirmed by embryo genotype	19	2	7	7	3
total	**422**	**174**	**7**	**218**	**23**

distribution of embryo morphology on day 4 p.f in different genotype outcome groups



* And ** significantly different; p<0,01

re-validation of PGD-PCR analysis

Validation of the PGD-PCR analysis

- total group reanalyzed embryos
- embryo group grade 1 day 4 p.f
- grade 1 day 4 p.f embryo group only

Validation of PGD-PCR analysis by calculating sensitivity, specificity, accuracy and Likelihood Ratio

Establish the diagnostic value by calculating Positive- and Negative Predictive Value

diagnostic-test analysis total group

		affected/aberrant embryos at reanalysis (D+)	unaffected embryos at reanalysis (D-)	total
affected/aberrant embryos at PGD	(T+)	218	23	**241**
unaffected embryos at PGD	(T-)	7	174	**181**
total		**225**	**197**	**422**

Sensitivity; the proportion of affected/aberrant embryos (D+) correctly diagnosed by PGD (T+), 218/225)

Specificity; the proportion of unaffected embryos (D-) correctly diagnosed by PGD (T-), (174/197)

PPV; the proportion of PGD analysis predicted correctly as affected/aberrant (218/241).

NPV; the proportion of PGD analysis predicted correctly as unaffected (174/181).

diagnostic-test analysis total group

		affected/aberrant embryos at reanalysis (D+)	unaffected embryos at reanalysis (D-)	total
affected/aberrant embryos at PGD	(T+)	218	23	**241**
unaffected embryos at PGD	(T-)	7	174	**181**
total		**225**	**197**	**422**

Sensitivity; the proportion of affected/aberrant embryos correctly diagnosed by PGD (218/225).

Specificity; the proportion of unaffected embryos correctly diagnosed by PGD (174/197).

PPV; the proportion of PGD analyses correctly predicting embryos to be affected/aberrant (218/241)

NPV; the proportion of PGD analyses correctly predicting embryos to be unaffected (174/181)

total group

Validity of the PGD-PCR analysis	Total group
Sensitivity	96,9 % (218/225)
False Negative	3,1 % (7/225)
Specificity (low due to ADO)	88,3 % (174/197)
False Positive	11,7 % (23/197)

Diagnostic value of the PGD-PCR analysis	Total group
Negative predictive value	96,1 % (174/181)
Positive predictive value	90,5 % (218/241)

Validity of the PGD-PCR analysis	**Grade 1 (day 4 p.f)**	**Grade 1 (day 4 p.f) excluded**
Sensitivity	82,9 % (29/35)*	99,5 % (189/190)*
False Negative	17,1 % (6/35)*	0,5 % (1/190)*
Specificity	93,1 % (27/29)	87,5 % (147/168)
False Positive	6,9 % (2/29)	12,5 % (21/168)

*significant; p<0,001

Diagnostic value of the PGD-PCR analysis	Grade 1 (day 4 PF)	Grade 1 (day 4 PF) excluded
Negative predictive value	81,8 % (27/33)*	99,3 % (147/148)*
Positive predictive value	93,6 % (29/31)	90,0 % (189/210)

*significant; p<0,001

summary validation (1)

ADO is the major cause of false positives, no false negatives were obtained due to the fact that ADO is taking into account in developing PGD-PCR protocols

Grade 1 embryos on day 4 p.f are overrepresented in the group in which the blastomere genotype is not confirmed by the embryo genotype.

If grade1 embryos are excluded from transfer in a PGD-PCR procedure, sensitivity increases from 96.6% up to 99.5%

summary validation (2)

We would unnecessarily discard 27(unaffected)/422 embryos by excluding grade 1 embryos from transfer

We would prevent transfer of 6 affected/aberrant embryos and increase the negative predictive value from 96.1% to 99.3%.

Conclusions

The PGD-PCR method is validated as a diagnostically valid method with a good diagnostic value for selecting unaffected embryos for transfer

By not accepting grade1 embryos on day 4 p.f for transfer,
the negative predictive value will increase from 96.1 to 99.3%
at the cost of unnecessarily excluding 6.4% unaffected grade 1 embryos

Implication

In PGD-PCR,
we do no longer consider embryos grade 1 on day 4 p.f. transferable

THANK YOU

Distribution of embryo morfology on day 3 post OR in different genotype outcome groups.