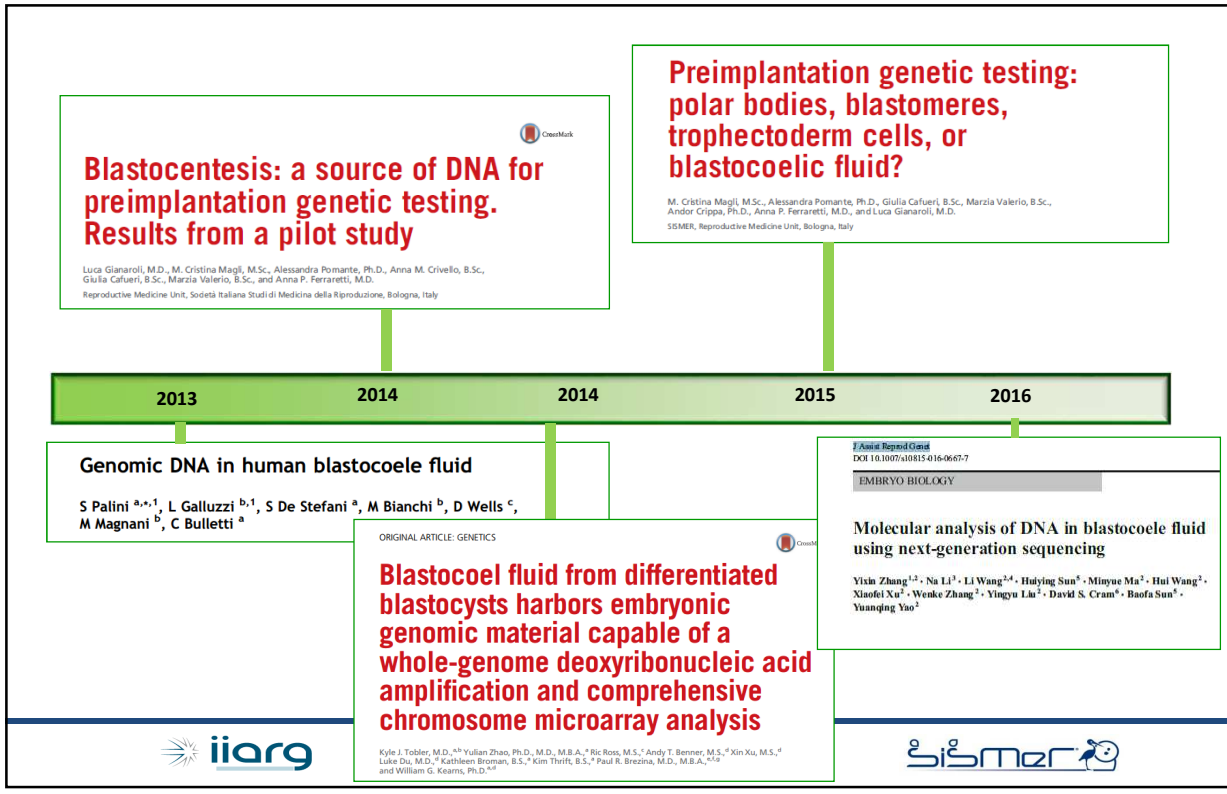
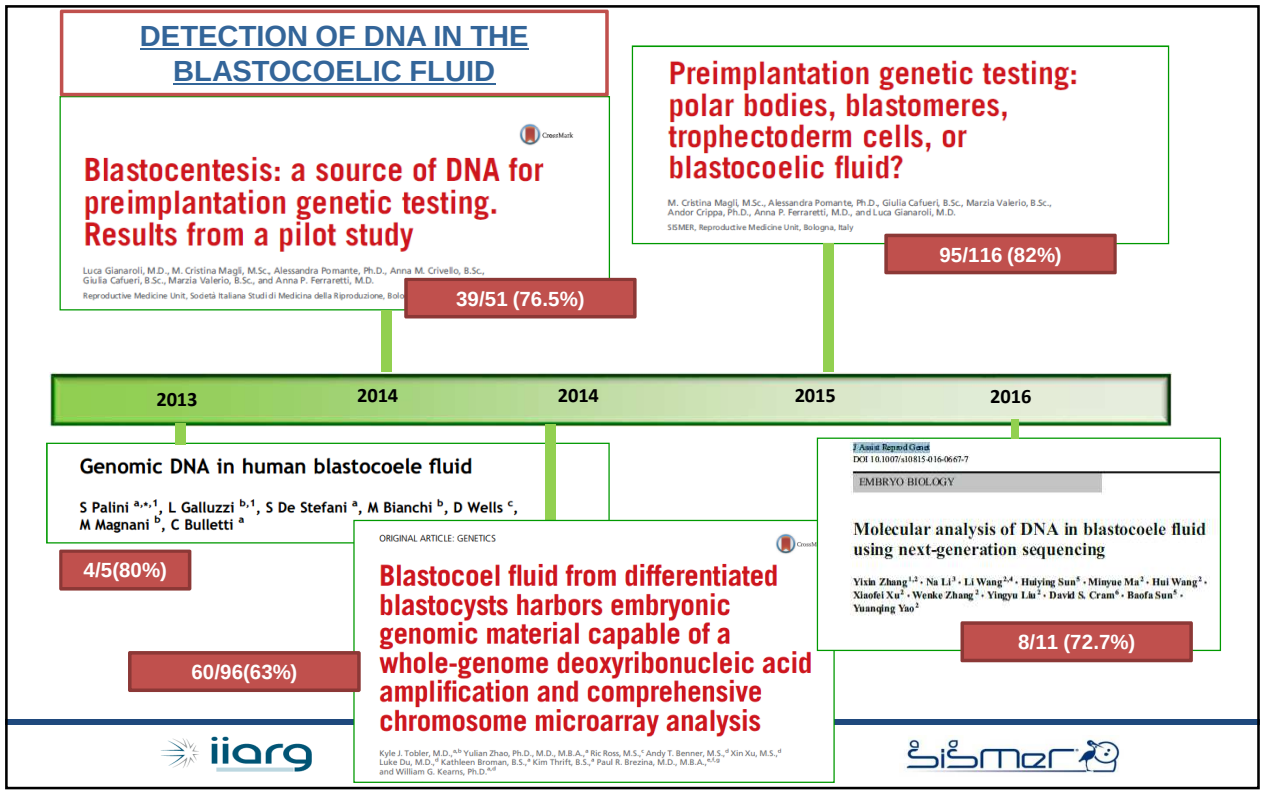
Blastocentesis: innovation in embryo biopsy

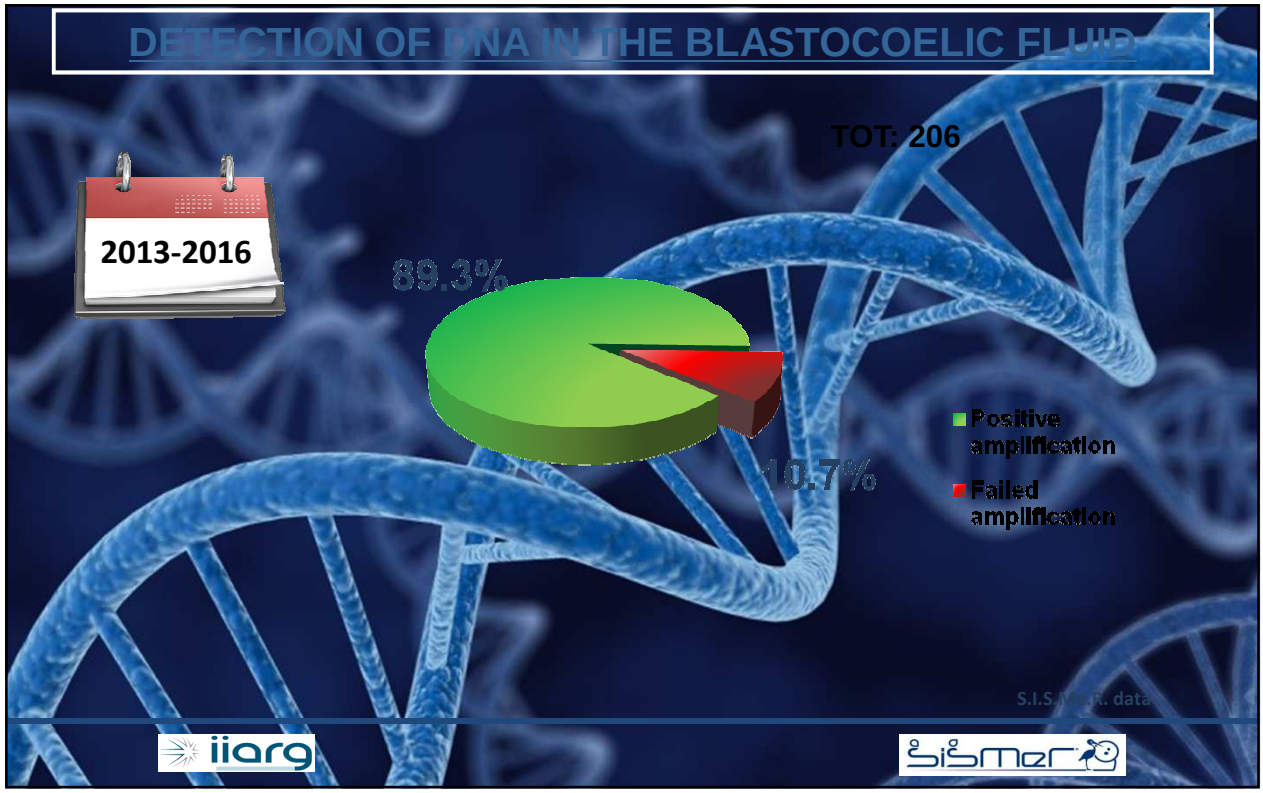
L. Gianaroli, MC Magli, A. Pomante, AP Ferraretti

S.I.S.Me.R. Reproductive Medicine Unit, Bologna, Italy

Bologna, 8-11 May 2016

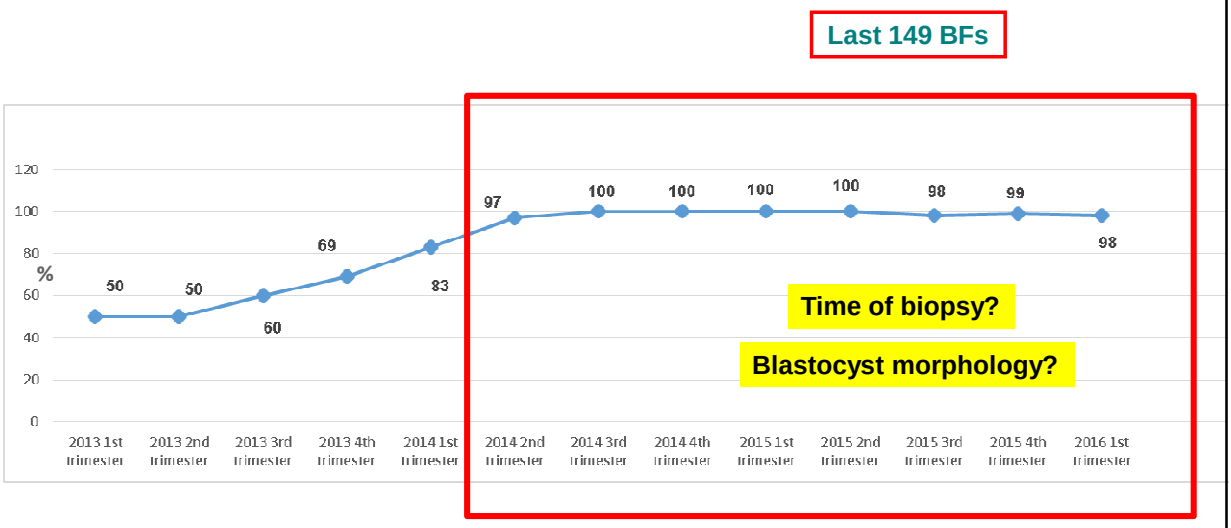



DETECTION OF DNA IN THE BLASTOCOELIC FLUID
Blastocentesis: a source of DNA for preimplantation genetic testing. Results from a pilot study
Luca Gianaroli, M.D., M. Cristina Magli, M.Sc., Alessandra Pomante, Ph.D., Anna M. Crivello, B.Sc., Giulia Cafueri, B.Sc., Marzia Valerio, B.Sc., and Anna P. Ferraretti, M.D.
Reproductive Medicine Unit, Società Italiana Studi di Medicina della Riproduzione, Bolo
39/51 (76.5%)
Preimplantation genetic testing: polar bodies, blastomeres, trophectoderm cells, or blastocoelic fluid?
M. Cristina Magli, M.Sc., Alessandra Pomante, Ph.D., Giulia Cafueri, B.Sc., Marzia Valerio, B.Sc., Andor Crippa, Ph.D., Anna P. Ferraretti, M.D., and Luca Gianaroli, M.D.
SISMER, Reproductive Medicine Unit, Bologna, Italy
95/116 (82%)
2013
2014
2014
2015
2016
Genomic DNA in human blastocoele fluid
S Palini a,*,1, L Galluzzi b,1, S De Stefani a, M Bianchi b, D Wells c, M Magnani b, C Bulletti a
4/5(80%)
ORIGINAL ARTICLE: GENETICS
Blastocoel fluid from differentiated blastocysts harbors embryonic genomic material capable of a whole-genome deoxyribonucleic acid amplification and comprehensive chromosome microarray analysis
Kyle J. Tobler, M.D.,a,b Yulian Zhao, Ph.D., M.D., M.B.A.,a Ric Ross, M.S.,c Andy T. Benner, M.S.,d Xin Xu, M.S.,d Luke Du, M.D.,d Kathleen Broman, B.S.,a Kim Thrift, B.S.,a Paul R. Brezina, M.D., M.B.A.,a,e,g and William G. Kearns, Ph.D.a,d
60/96(63%)
J Assist Reprod Genet
DOI 10.1007/s10815-016-0667-7
EMBRYO BIOLOGY
Molecular analysis of DNA in blastocoele fluid using next-generation sequencing
Yixin Zhang[1,2] · Na Li[3] · Li Wang[2,4] · Huiying Sun[4] · Minyue Ma[2] · Hui Wang[2] · Xiaofei Xu[2] · Wenke Zhang[2] · Yingyu Liu[2] · David S. Cram[4] · Baofa Sun[4] · Yuanqing Yao[2]
8/11 (72.7%)
iiarg
SISMER

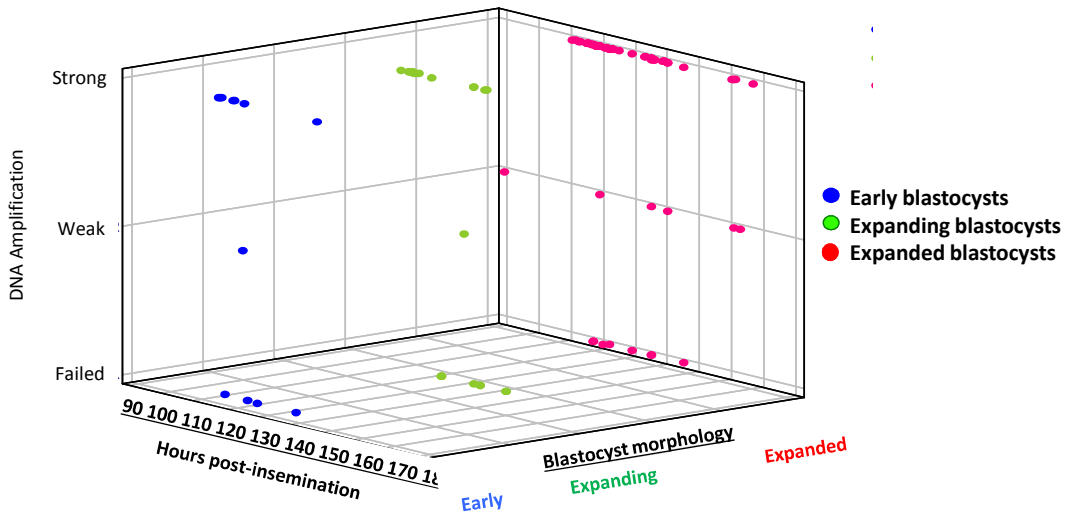

DETECTION OF DNA IN THE BLASTOCOELIC FLUID
TOT: 206
2013-2016
89.3%
10.7%
Positive amplification
Failed amplification
S.I.S.Me.R. data
iiarg
SISMER

DETECTION OF DNA IN THE BLASTOCOELIC FLUID

Our experience throughout time on 206 BFs



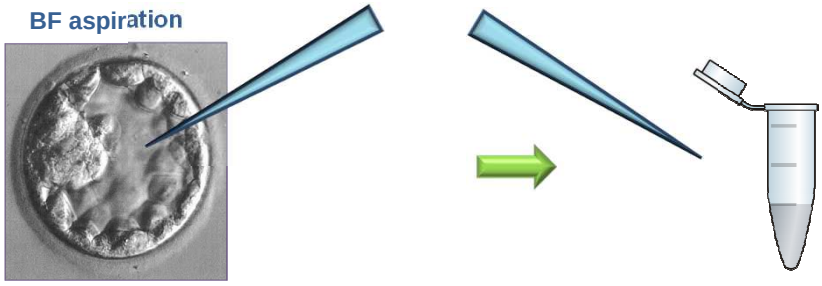
DETECTION OF DNA IN THE BLASTOCOELIC FLUID



Expanded Day 5 blastocysts have the highest chances of having DNA in the BF

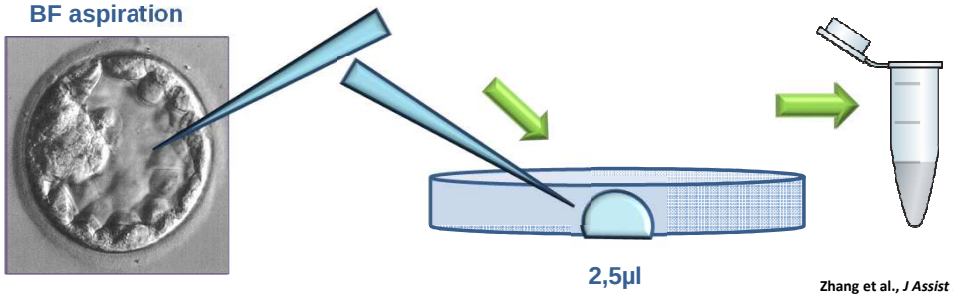
COLLECTION PROCEDURE

1. BF aspiration



Gianaroli et al., *Fert Ster*, 2014

2. BF aspiration



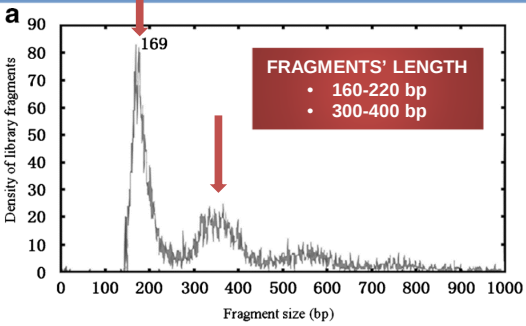
2,5µl

Zhang et al., *J Assist Reprod Genet*, 2016

THE BLASTOCOELIC FLUID

Where does this DNA come from?

Direct sequencing of 3 pooled blastocoelic fluids

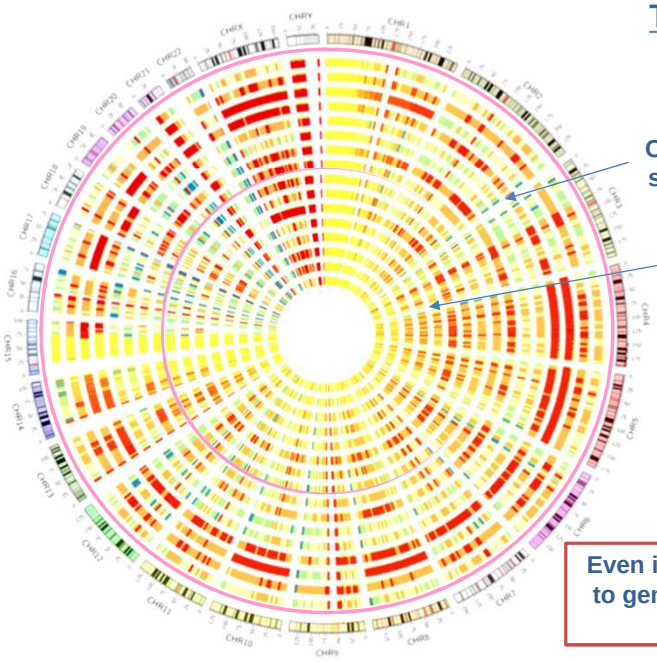


FRAGMENTS' LENGTH
- 160-220 bp
- 300-400 bp

DNA FRAGMENTS' LENGTH SIMILAR TO CIRCULATING PLASMA DNA

TYPICAL LENGHT OF APOPTOTIC FRAGMENTS

Zhang et al., *J Assist Reprod Genet*, 2016



THE BLASTOCOELIC FLUID WGA COVERAGE

(SurePlex DNA Amplification System)

Chromosome density plots of mapped sequencing reads from 8 blastomeres

Chromosome density plots of mapped sequencing reads from 8 corresponding Blastocoel fluids

Density pattern similar within each group and between the two groups

Even if BF DNA is highly fragmented, it is possible to generate a WGA product similar to that of cells with an intact genome

Zhang et al., *J Assist Reprod Genet*, 2016

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CONCORDANCE STUDY

105 BFs

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CONCORDANCE STUDY

Indicators

1.Ploidy condition

Correspondence in terms of euploidy/aneuploidy
(Transferrable/Not Transferrable)

FULL	PARTIAL	NULL

2.Chromosome concordance

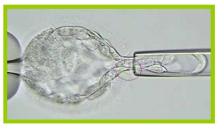
Percentage of correspondence of all studied chromosomes between the different stages of the analyzed embryos.

CONCORDANCE STUDY

CONCORDANCE PER SAMPLE

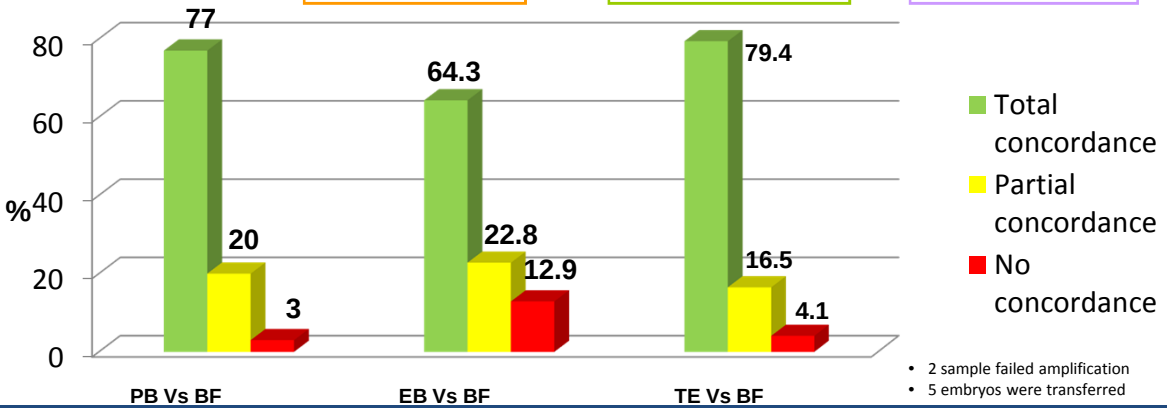
Polar bodies(n=35)

Blastomeres (n=70)



Trophectoderm (n=98*)

Blastocoelic fluid (n=105)



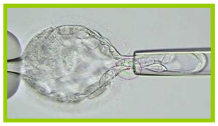
- 2 sample failed amplification
- 5 embryos were transferred

CONCORDANCE STUDY

PLOIDY CONCORDANCE

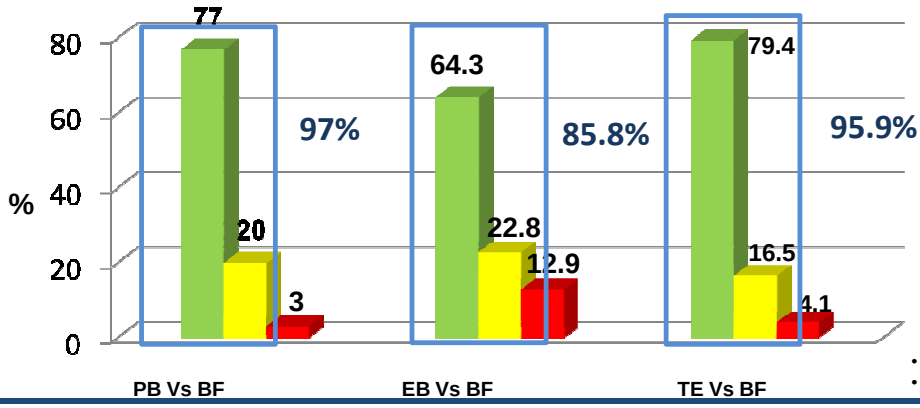
Polar bodies(n=35)

Blastomeres (n=70)



Trophectoderm (n=98*)

Blastocoelic fluid (n=105)

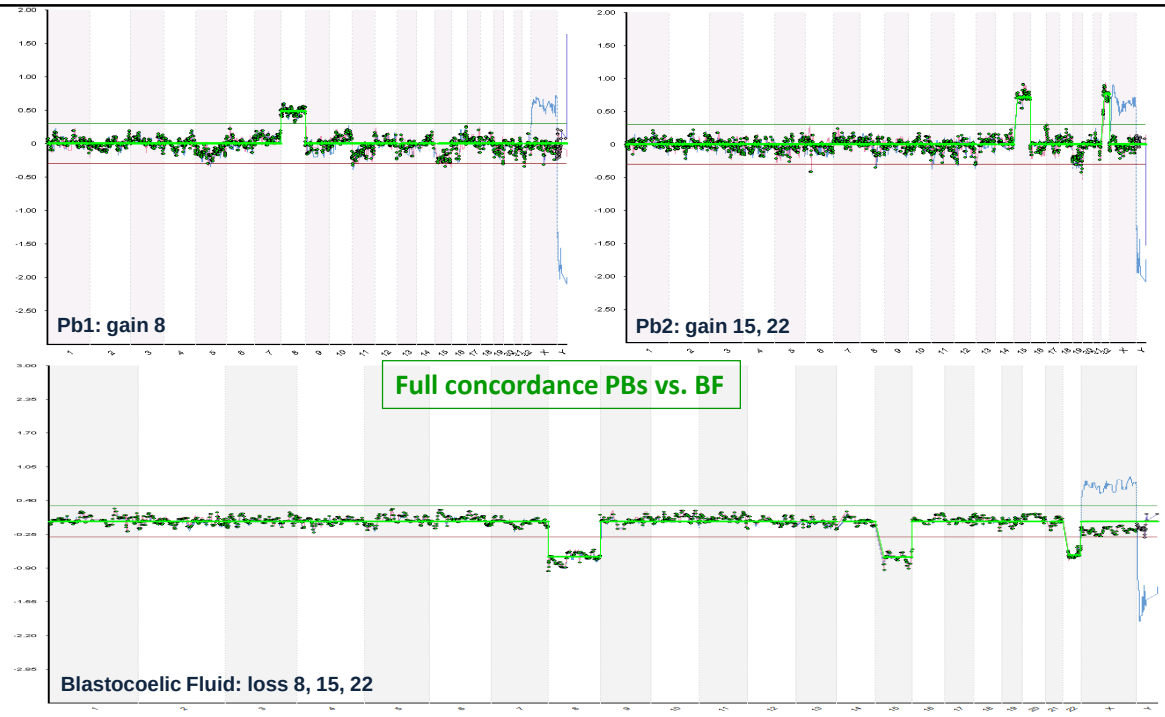


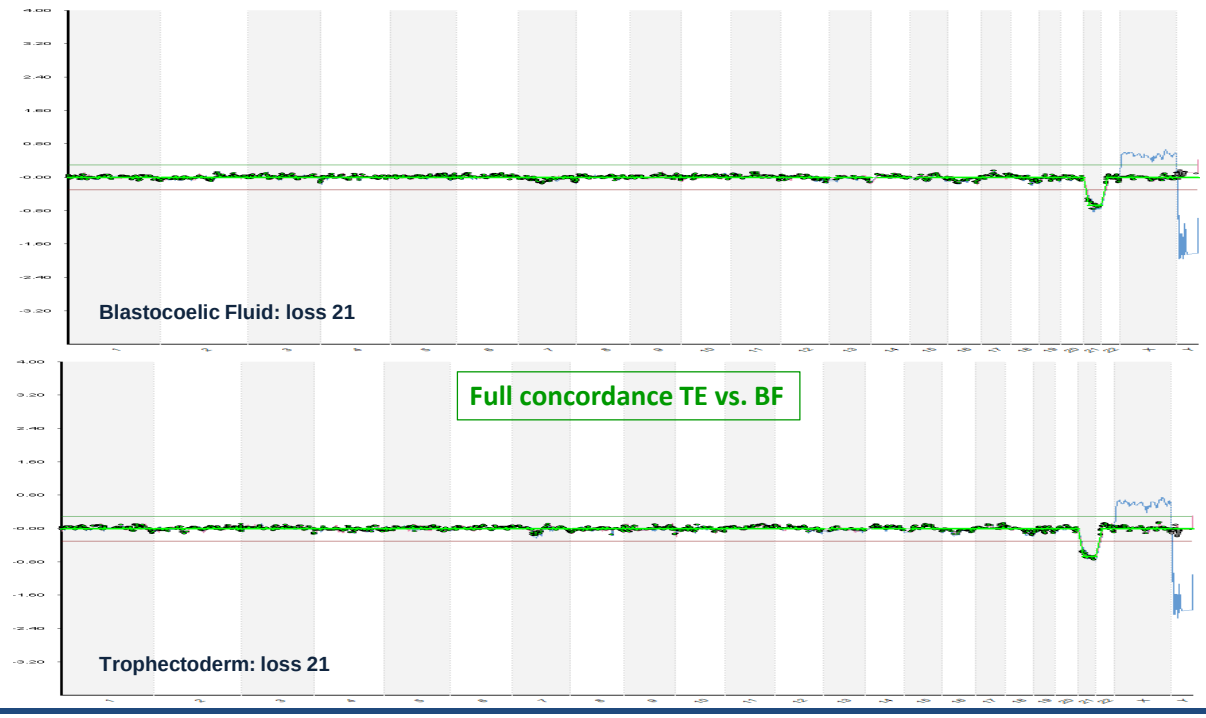
Total

CONCORDANCE OF PLOIDY CONDITION

No concordance

- 2 sample failed amplification
- 5 embryos were transferred

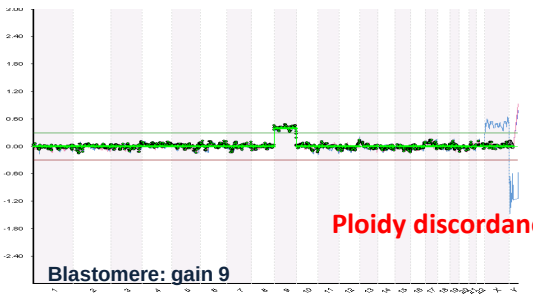




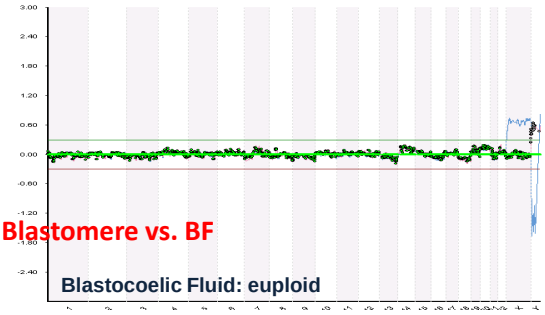
CONCORDANCE STUDY

9 DISCORDANT CASES BLASTOMERES VS BF:

ID sample	DAY 3		DAY 5/6		
	morphology	Chromosomal status	morphology	TE Chromosomal status	BF Chromosomal status
68	10c1	-2p	Bl1	NR	euploid
206	8c1	Xp22.3->Xq21.31	Bl 3,1,1	ET	euploid
192	8c1	+9	Bl 2,1,1	-9	euploid
225	10c2f1	+5,6	Bl 2,2,2	+10,-6	euploid
258	8c1	+9	Bl 3,0,3	euploid	euploid
240	8c1	+12,19	Bl 3,1,2	euploid	euploid
232	8c1	+15,17-1,14,X	Bl 3,1,1	euploid	euploid
263	8c1	-1,9	Bl 3,1,1	euploid	euploid
271	7c2	-Y	Bl 3,1,1	euploid	euploid

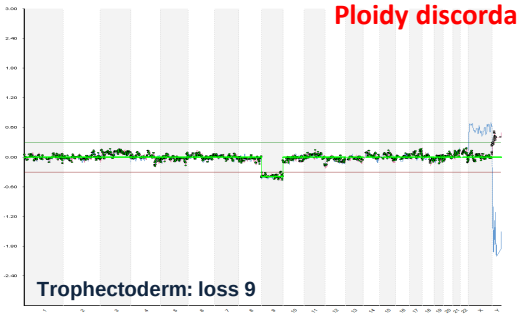


Blastomere: gain 9



Blastocoelic Fluid: euploid

Ploidy discordance Blastomere vs. BF



Trophectoderm: loss 9

Ploidy discordance TE vs. BF

Embryo: loss 9

Mosaicism?

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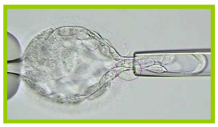
CONCORDANCE STUDY

CONCORDANCE PER CHROMOSOME

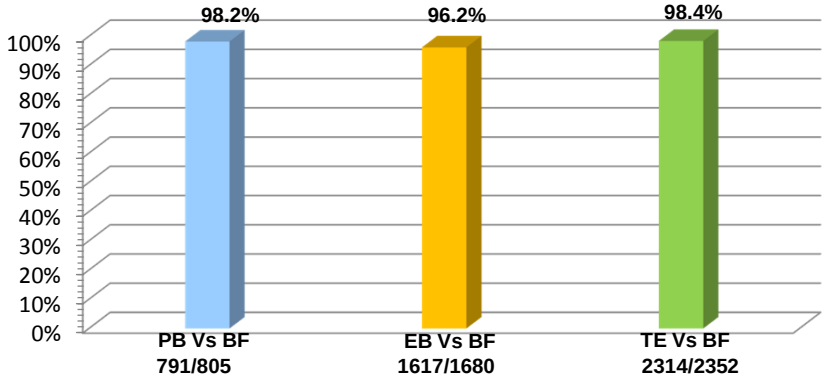
Polar bodies(n=35)

Blastomeres (n=70)



Trophectoderm (n=98*)

Blastocoelic fluid (n=105)



- 2 sample failed amplification
- 5 embryos were transferred

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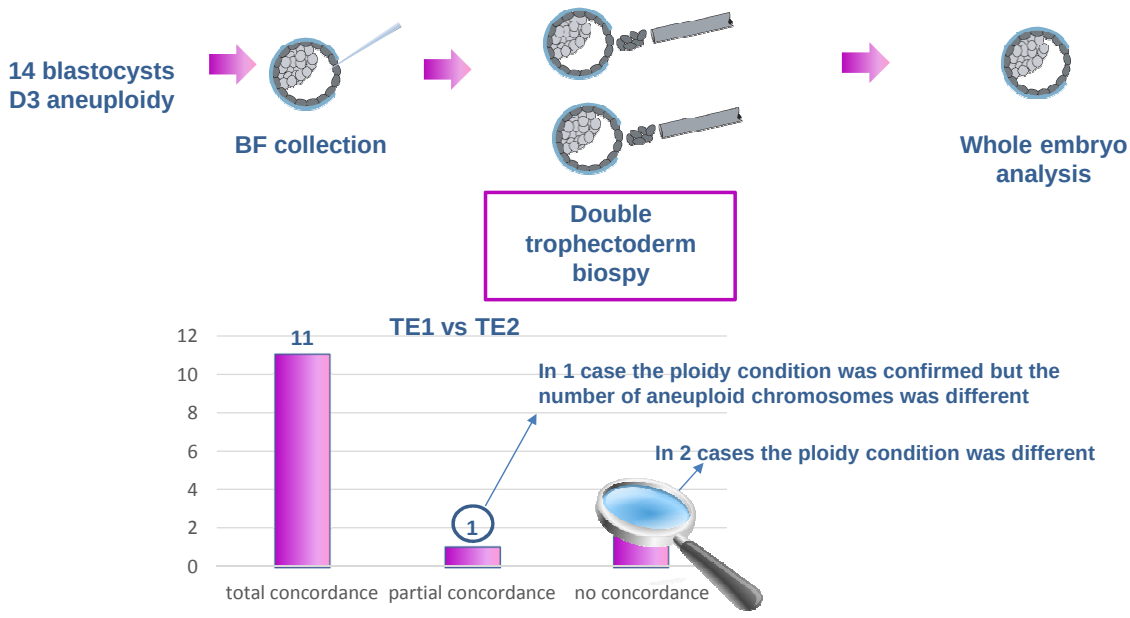
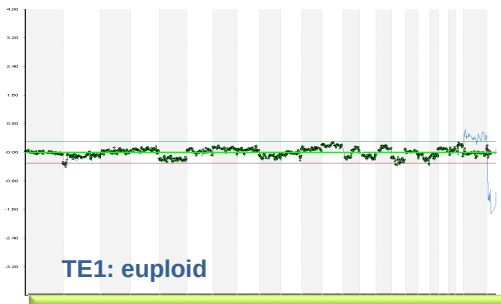
DOUBLE TROPHECTODERM BIOPSIES

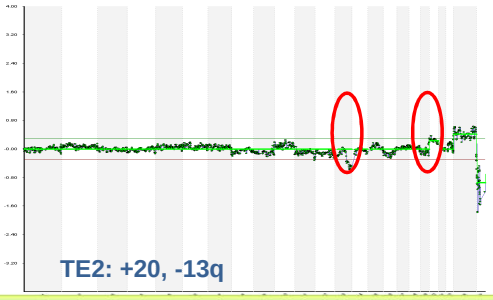
DOUBLE TE BIOPSY



DOUBLE TE BIOPSY

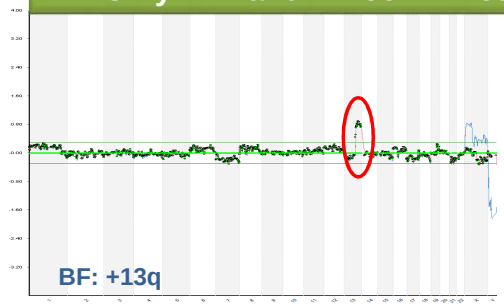


TE1: euploid

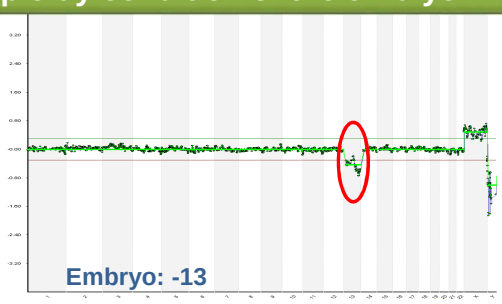


TE2: +20, -13q

Only TE2 and BF confirmed the ploidy condition of the embryo

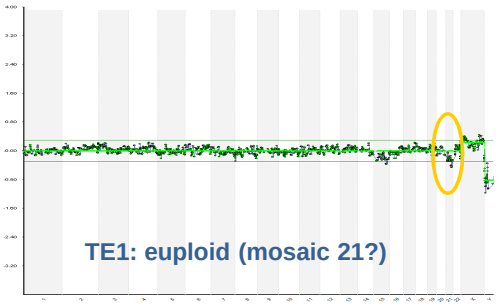


BF: +13q

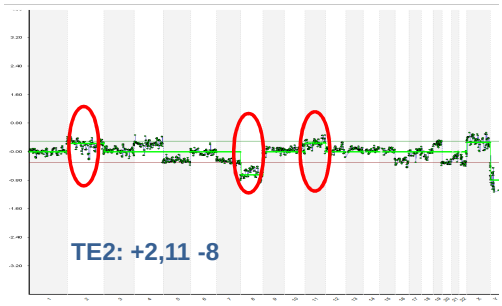


Embryo: -13

DOUBLE TE BIOPSY

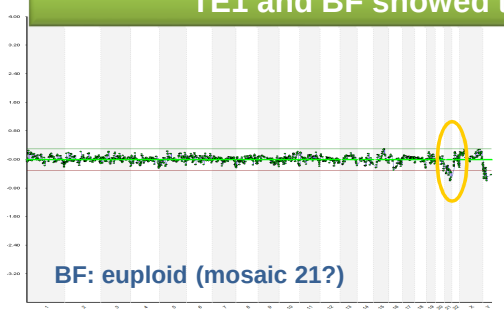


TE1: euploid (mosaic 21?)

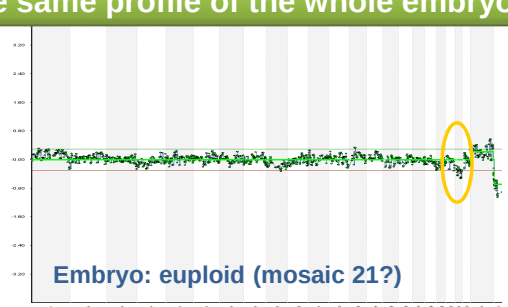


TE2: +2,11 -8

TE1 and BF showed the same profile of the whole embryo



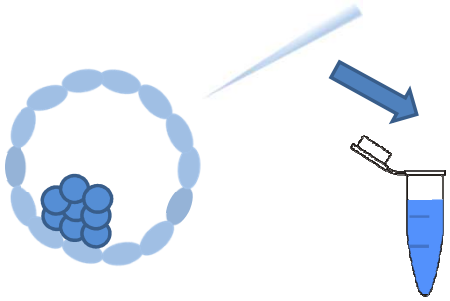
BF: euploid (mosaic 21?)



Embryo: euploid (mosaic 21?)

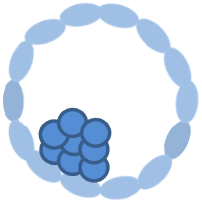
DOUBLE BF BIOPSY

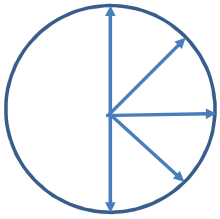
DOUBLE BF COLLECTION



1ST BF collection

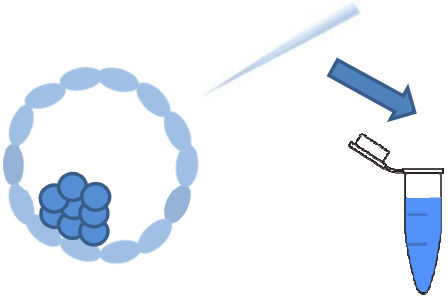
DOUBLE BF COLLECTION





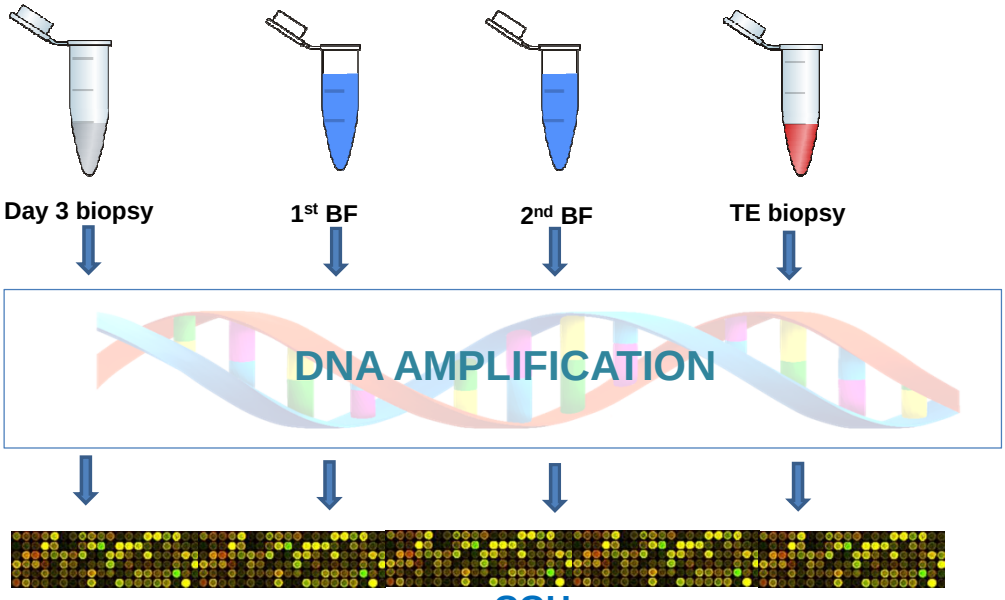
6 hours later

DOUBLE BF COLLECTION



2nd BF collection

DOUBLE BF COLLECTION



DOUBLE BF COLLECTION

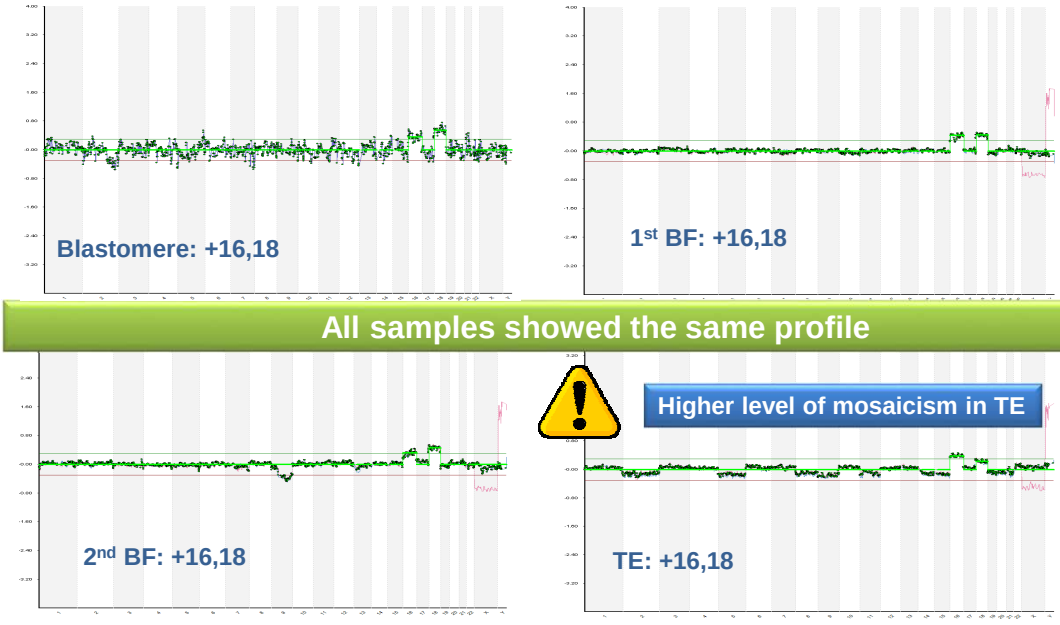
RESULTS:

All the analyzed samples gave the same result for both BF biopsies

ID SAMPLE	DAY 3 BIOPSY	1st BF	2nd BF	TE biopsy
2	-Y	euploid	euploid	euploid
3	+19,22	+19,22	+19,22	+19,22
8	+16,18	+16,18	+16,18	+16,18

DOUBLE BF COLLECTION

RESULTS



BLASTOCOELIC FLUID: WHAT ABOUT PGD?

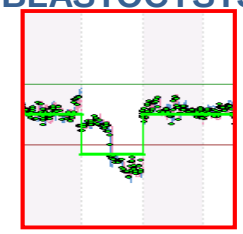
- Translocations
- Monogenic diseases

PGD FOR TRANSLOCATIONS

105 BLASTOCYSTS
(2013-2015)

17

BLASTOCYSTS



6 COUPLES
TRANSLOCATION CARRIERS

6 COUPLES
NORMAL KARYOTYPE

12 COUPLES

INCIDENCE OF *DE NOVO* SEGMENTAL ABNORMALITIES: 8/77 (10.4%)

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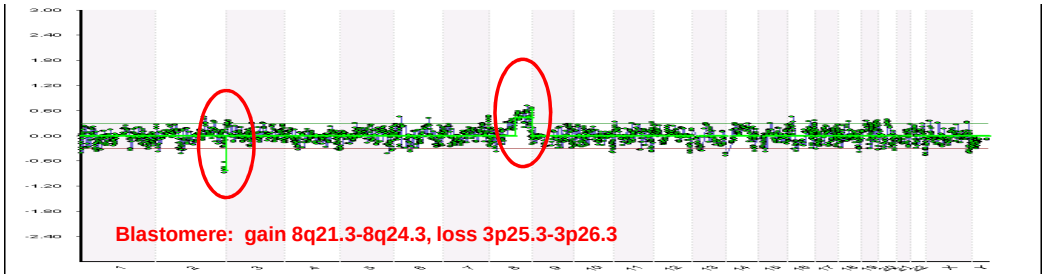
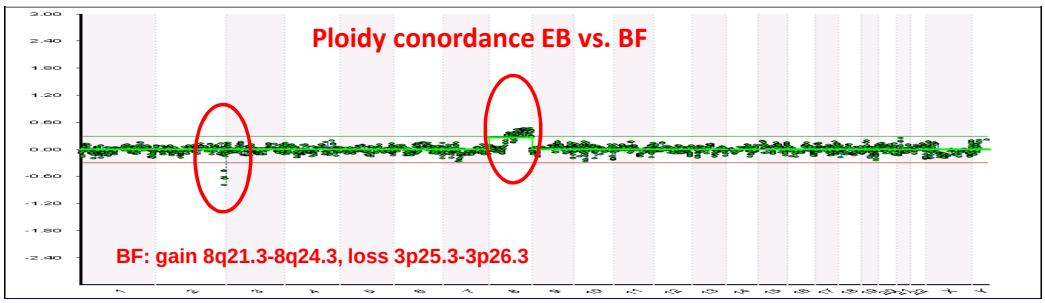
Segmental abnormality in translocation carriers
46 XX,t(3;8)(p25;q12)





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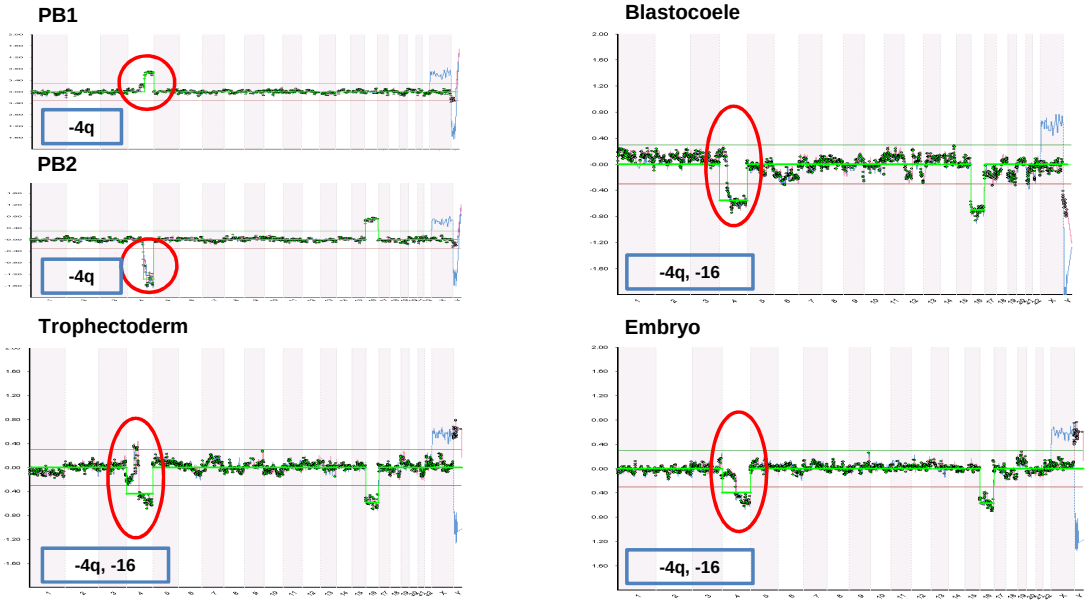
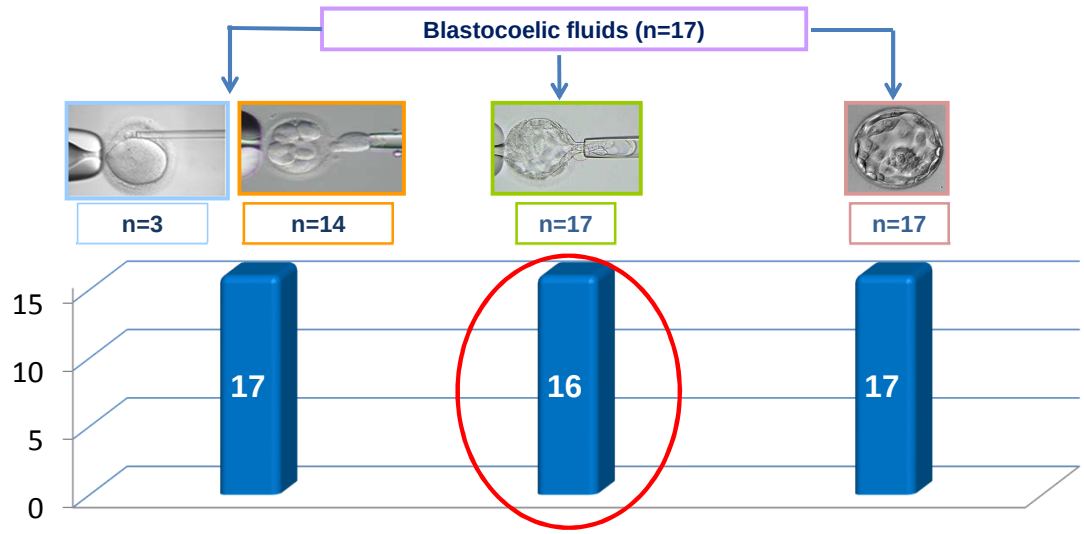
De novo segmental abnormality



DETECTION OF SEGMENTAL ABNORMALITY

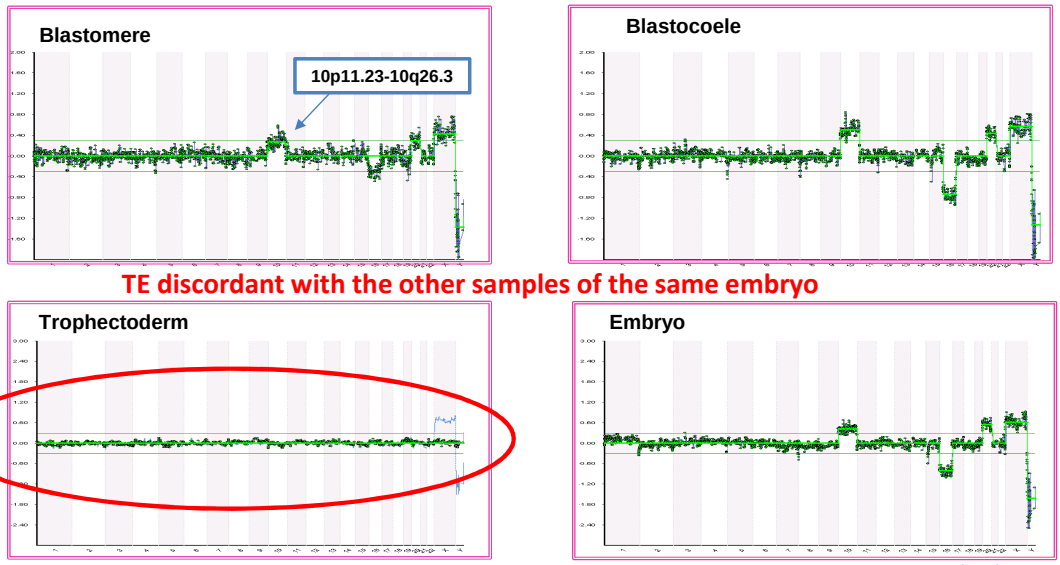
In all analyzed cases the segmental abnormality was detected



DETECTION OF SEGMENTAL ABNORMALITY

DISCORDANT CASE

Patient: 46XX,t(5;10)(q13;p12).



Magli et al, Fert Ster 2016

BLASTOCOELIC FLUID: WHAT ABOUT PGD?

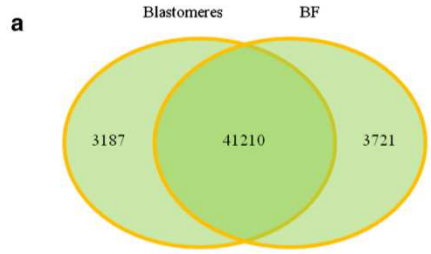
Translocation

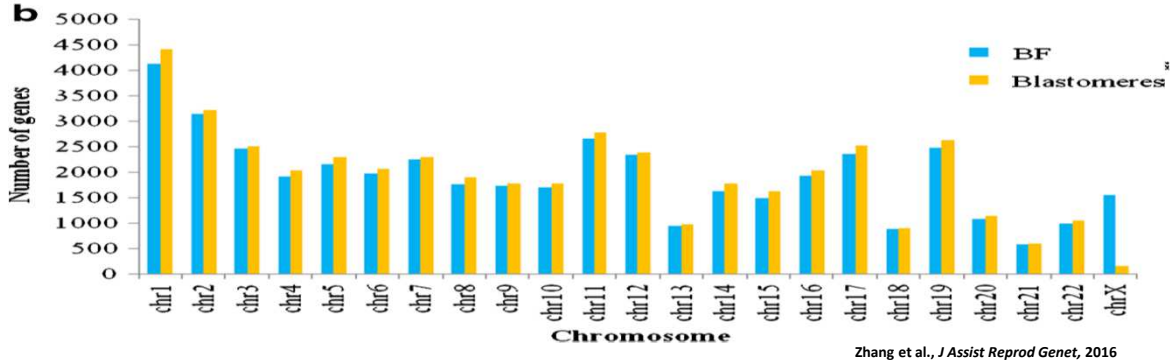
Monogenic diseases

PGD FOR MONOGENIC DISEASES

41,210 genes co-detected in both the BF and blastomere samples suggesting similar gene coverage after WGA

BF WGA product contained sequences of the majority of known genes and, specifically, disease genes, which are commonly the subject of different single gene PGD cases.





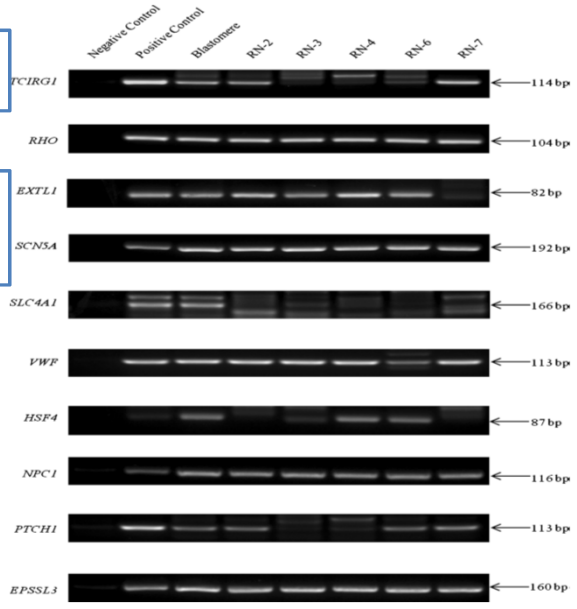
Zhang et al., *J Assist Reprod Genet*, 2016

PGD FOR MONOGENIC DESEASES

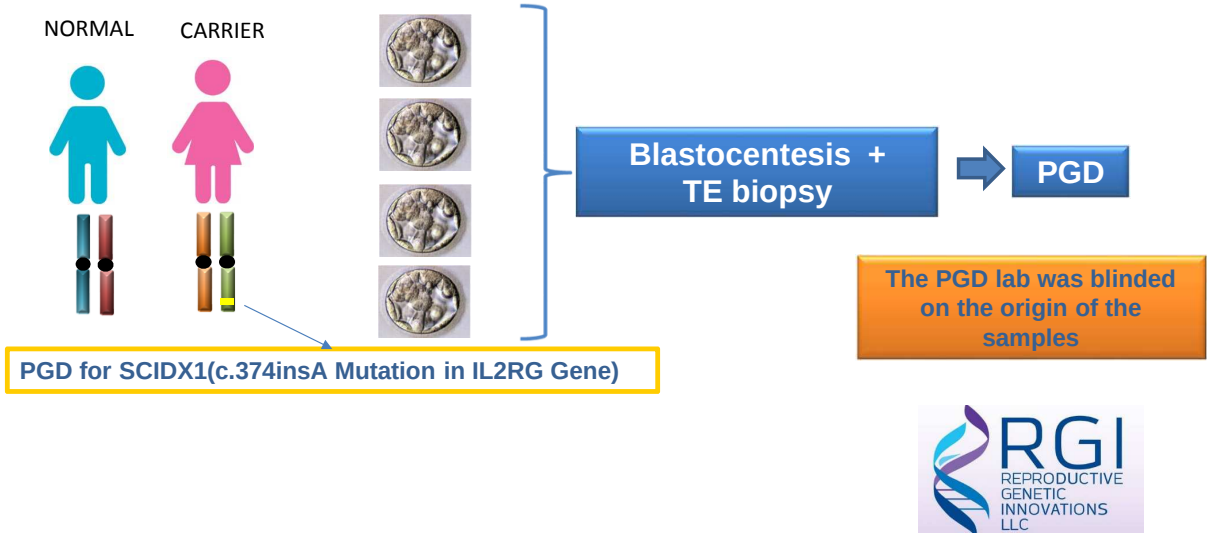
PCR validation of randomly selected genes in WGA products from 5 BF samples

The majority of samples showed a PCR fragment of the expected size for the ten tested genes

42/50 POSITIVE RESULT FOR TESTED GENES (84%)



PGD FOR MONOGENIC DISEASES
CASE REPORT



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PGD FOR MONOGENIC DISEASES
CASE REPORT

In all cases BF gave the same result of trophectoderm biopsy

		DXS 1216	DXS 8031	IL2RG Mutation	DXS 559	DXS 8079	DXS 8066	DXS 8060	DXYS 1223	DXYS 1071	Genotype
	BF	121	157	N	137/133	99	121/123	130/132	134/144	122/120	Normal Female
	TE	121	157	N	137/133	99	121/123	130/132	134/144	122/120	Normal Female
	BF	123	148	c.374insA	140	95	119	128	124/144	118/120	Affected Male
	TE	123	148	c.374insA	140	95	119	128	124/144	118/120	Affected Male
	BF	FA	FA	c.374insA	FA	99	121/123	130/132	FA	FA	No result
	TE	FA	FA	FA	FA	FA	FA	FA	FA	FA	No result
	BF	121/123	157/148	N/c.374insA	133/140	99/95	121/119	130/128	133/144	122/120	Carrier Female
	TE	121/123	ADO/148	N/c.374insA	133/140	99/95	121/119	130/128	133/144	122/120	Carrier Female
MALE		121	157	N	137	99	121	130	133/124	122/118	
FEMALE		121/123	157/148	N/c.374insA	133/140	99/95	123/119	132/128	133/144	118/120	

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IS BLASTOCENTESIS A SAFE PROCEDURE?

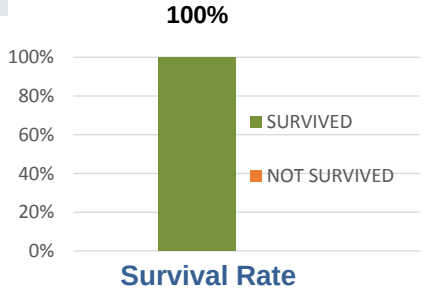
COLLAPSING OF BLASTOCYSTS BEFORE VITRIFICATION

37 couples

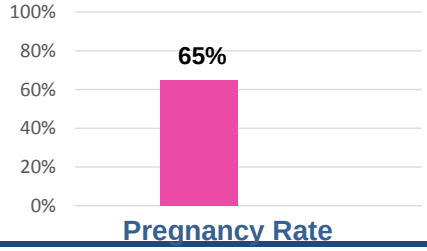
Blastocentesis *per se* does not affect embryo viability

VITRIFICATION

THAWING → EMBRYO TRANSFER → 24 PREGNANCIES (19 ongoing)



Survival Rate



Pregnancy Rate

J Assist Reprod Genet
DOI 10.1007/s10815-016-0655-y

EMBRYO BIOLOGY

Artificial shrinkage of blastocysts prior to vitrification improves pregnancy outcome: analysis of 1028 consecutive warming cycles

Paolo Emanuele Levi-Setti[1] · Francesca Menduni[1] · Antonella Smeraldi[1] · Pasquale Patrizio[2] · Emanuela Morenghi[3] · Elena Albani[1]

RESULTS

	No Blastocentesis	Blastocentesis	Total	*P*
Cycles (*n*°)	448	580	1028	
Mean age[a]	36.3 ± 3.9	36.4 ± 3.7	36.3 ± 3.8	0.909
Frozen Embryo Transfers (n°)	437	570	1007	
FET canceled (*n*°)	11	10	21	
Thawed blastocysts (*n*°)	625	820	1445	
Survived blastocysts (*n*°)	604	804	1408	
Blastocyst survival rate (%)	96.6	97.8	97.3	0.192
Transferred blastocysts (*n*°)	604	804	1408	
Mean transferred blastocyst[a]	1.38 ± 0.50	1.41 ± 0.49	1.40 ± 0.49	0.318
Implants (*n*°)	140	240	379	
Implantation rate (%)	23.2	29.9	27.0	0.005
Pregnancies (n°)	122	207	329	
Pregnancy rate (%)	27.9	36.3	32.7	0.005
Delivery (*n*°)	79	152	231	
Delivery rate (%)	18.1	26.7	22.9	0.001

[a] Mean ± SD

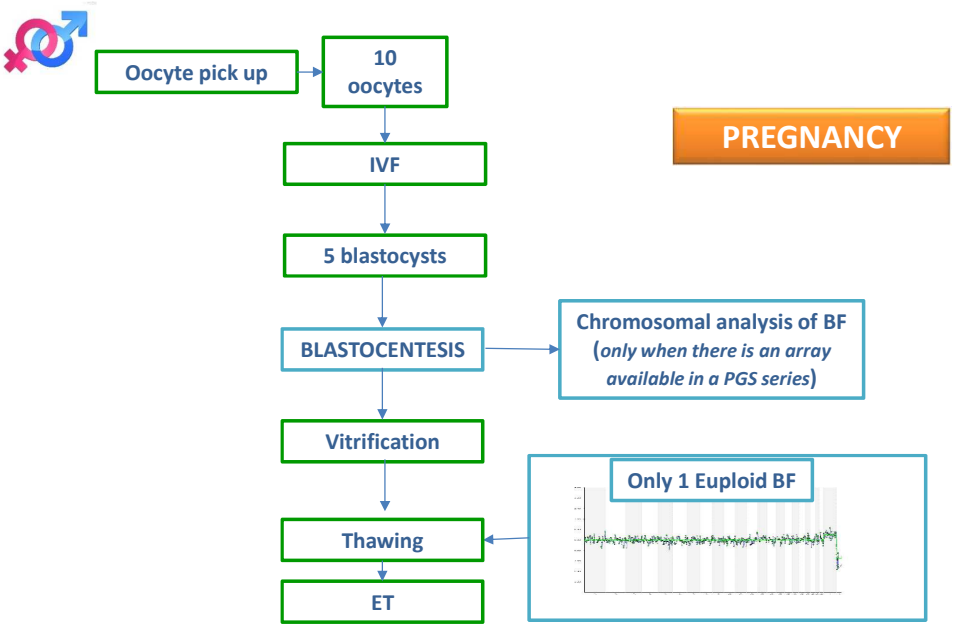
Improvement in frozen embryo transfer outcome (better implantation, pregnancy, and delivery rates) when expanded blastocysts undergo artificial shrinkage prior to vitrification.

No difference in the mean age, survival rate and mean transferred blastocysts

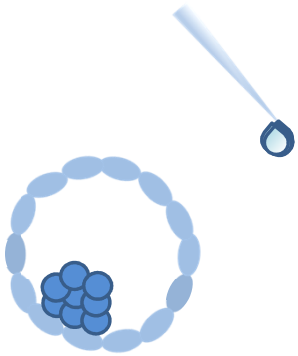
Significantly higher implantation rate, pregnancy rate and delivery rate in Artificial Shrinkage group

CAN BLASTOCOELIC FLUID BE USED FOR *GRADING* OF EMBRYOS?

CASE REPORT



CONCLUSIONS



DNA can be detected in the majority of Blastocoelic fluids (best performance on Day-5 expanded blastocysts)

The DNA in the Blastocoelic Fluid is highly representative of the blastocyst chromosomal status (95.5% of cases)

Blastocentesis is less invasive for the embryo

Blastocentesis is more convenient for the lab

Blastocentesis could be ethically more acceptable

Segmental abnormalities can be detected in Blastocoelic fluid → possible use of bastocentesis for PGD for translocations.

PGD for monogenic deseases

BF analysis can be used for grading of embryos

The Blastocoelic Fluid can be an alternative source of DNA for preimplantation genetic testing