

Le PGS est il efficace en PMA: Revue de la Littérature

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XXIV^e COLLOQUE **ACLF** &
Association des Cytogénétiens de Langue Française


XXVIII^e COLLOQUE **ATC**
Association des Technicien(ne)s en Cytogénétique

LE GRAND LARGE - PALAIS DES CONGRÈS DE SAINT-MALO



PGS ?

- ❑ **PGS: le screening génétique préimplantatoire**
- ❑ **Le PGS est une technique de sélection de l'embryon.**
- ❑ **Elle consiste à n'implanter par fécondation *in vitro* (FIV) que les embryons ne présentant aucune anomalie chromosomique.**
- ❑ **Le PGS devrait donc augmenter les chances de mener une grossesse à terme.**

- ❑ **Pratique interdite en France**

Article

Preimplantation genetic screening: results of a worldwide web-based survey



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- Etude Prospective
- **386** centres de FIV
- **70** pays
- **342 600** cycles



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IVF Blog

→ Dehydroepiandrosterone (DHEA) supplementation in women with low functional ovarian reserve

→ Open versus closed systems for vitrification of human oocytes and blastocysts

→ Universal warming protocol: comments on EM

Contenu de l'enquête

Table 1 – Geographic distribution of IVF units participating in the survey.

Continent	Total				Centers Performing PGS				Centers NOT Performing PGS			
	Annual IVF Cycles	%	Number of units	%	Annual IVF cycles	%	Number of units	%	Annual IVF cycles	%	Number of units	%
USA and Canada	65,800	19.2	97	25.1	63,000	23.9	93	34.3	2800	3.5	4	3.5
South America	18,500	5.4	34	8.8	15,900	6.0	26	9.6	2600	3.3	8	7
Australia and New Zealand	22,300	6.5	21	5.4	20,800	7.9	16	5.9	1500	1.9	5	4.3
Asia	83,900	24.5	78	20.2	62,200	23.6	56	20.7	21,700	27.3	22	19.1
Europe	137,900	40.3	137	35.5	91,600	34.8	70	25.8	46,300	58.2	67	58.3
Africa	14,200	4.1	19	4.9	9600	3.6	10	3.7	4600	5.8	9	7.8
	342,600	100	386	100	263,100	100	271	100	79,500	100	115	100

INDICATIONS

- ✓ Age maternelle avancée (27%)
- ✓ Défaut d'implantation récurrent (32%)
- ✓ Fausses couches à répétition (31%)
- ✓ (10%) offert à toutes les patientes.



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IVF Blog

- ➔ Dehydroepiandrosterone (DHEA) supplementation in women with low functional ovarian reserve
- ➔ Open versus closed systems for vitrification of human oocytes and blastocysts
- ➔ Universal warming protocols: comments on...

PGS: RANDOMIZED CLINICAL TRIALS

Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study

Zhihong Yang¹, Jiaen Liu², Gary S Collins³, Shala A Salem¹, Xiaohong Liu², Sarah S Lyle¹, Alison C Peck¹, E Scott Sills^{1*} and Rifaat D Salem¹

Molec Cytogenet 2012

In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial

Eric J. Forman, M.D.,^{a,b} Kathleen H. Hong, M.D.,^{a,b} Kathleen M. Ferry, B.Sc.,^a Xin Tao, M.Sc.,^a Deanne Taylor, Ph.D.,^a Brynn Levy, Ph.D.,^{a,c} Nathan R. Treff, Ph.D.,^{a,b} and Richard T. Scott Jr., M.D.^{a,b}

Fertil Steril 2013

Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial

Richard T. Scott Jr., M.D.,^{a,b} Kathleen M. Upham, B.S.,^a Eric J. Forman, M.D.,^b Kathleen H. Hong, M.D.,^b Katherine L. Scott, M.S.,^{a,c} Deanne Taylor, Ph.D.,^{a,b} Xin Tao, M.S.,^a and Nathan R. Treff, Ph.D.^{a,b}

Fertil Steril 2013



Le PGS augmente les chances des taux d'implantation, de grossesses et de naissance vivantes

Characteristics of included randomized and observational studies.

Study	Design	Indication	Embryo biopsy	Genetic platform	Main outcomes
Randomized studies					
Yang et al., 2012 (24)	Pilot RCT	Good-prognosis patients, 1st IVF cycle	Blastocyst	aCGH	Clinical PR, ongoing PR (>20 wk) Ongoing PR (>24 wk), multiple PR Sustained IR, delivery rate
Forman et al. 2013 (22)	Noninferiority trial (RCT)	Normal ovarian reserve, ≤1 previous IVF failure	Blastocyst	qPCR	
Scott et al., 2013 (75)	RCT	Normal ovarian reserve, ≤1 previous IVF failure	Blastocyst	qPCR	
Observational studies					
Sher et al., 2009 (58)	PCS	AMA + RIF + RPL	Cleavage	mCGH	IR, Live birth rate
Schoolcraft et al., 2010 (55)	PCS	Previous IVF failure	Blastocyst	aCGH	IR
Fishel et al., 2011 (79)	PCS	RIF	Polar Body	aCGH	IR
Forman et al., 2012 (23)	RCS	1st SET cycle	Blastocyst	qPCR	Ongoing PR (>12 wk)
Keltz et al., 2013 (76)	RCC	AMA + RIF + RPL	Cleavage	aCGH	IR
Greco et al., 2014 (43)	PCS	RIF	Blastocyst	aCGH	IR
Lee et al., 2015 (77)	RCS	AMA	Blastocyst	aCGH	IR, Live birth rate
Feichtinger et al., 2015 (78)	RCS	RIF + AMA	Polar Body	aCGH	Live birth rate

Note: aCGH = array comparative genomic hybridization; AMA = advanced maternal age; IR = implantation rate; mCGH = metaphase comparative genomic hybridization; qPCR = quantitative polymerase chain reaction; PCS = prospective cohort study; PR = pregnancy rate; RCC = retrospective case-control; RCS = retrospective cohort study; RCT = randomized controlled trial; RIF = repeated implantation failure; RPL = recurrent pregnancy loss; SET = single-embryo transfer.

Reserve ovarienne normale → Le PGS augmente les chances du taux d'implantation et de grossesse.

Dahdouh et al., Fertil Steril 2015

BIOPSIE EMBRYONNAIRE

Day 1

Day 2

Day 3

Day 4

Day 5

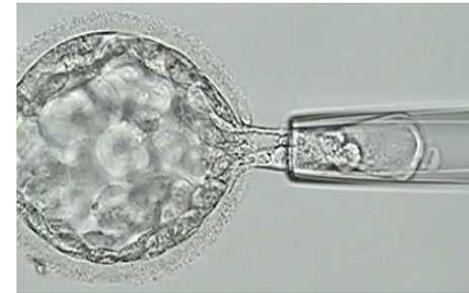
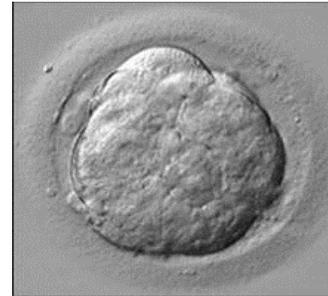
Day 6

**Polar Body
Biopsy**

**Cleavage stage
biopsy**

**Morula stage
biopsy**

Blastocyst biopsy
Trophectoderm **Blastocoelic fluid**



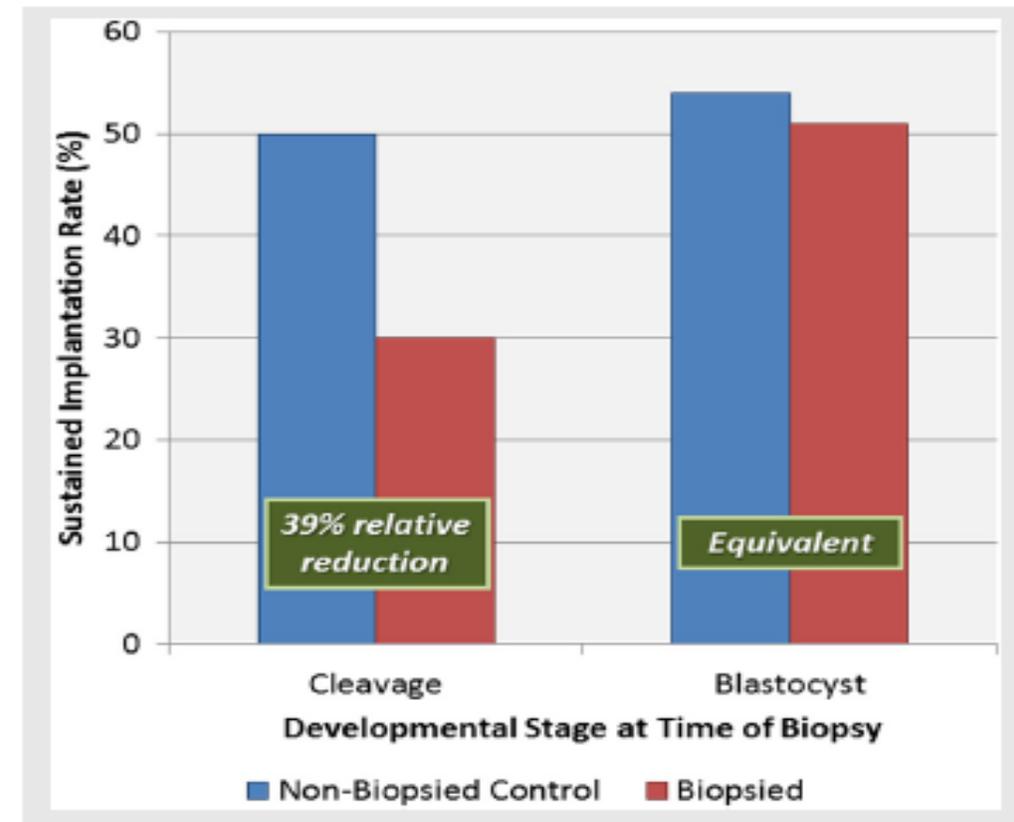
STADE DE BIOPSIE ET IMPACT SUR LA PROCEDURE??

[Fertil Steril](#). 2013 Sep;100(3):624-30. doi: 10.1016/j.fertnstert.2013.04.039. Epub 2013 Jun 15.

Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial.

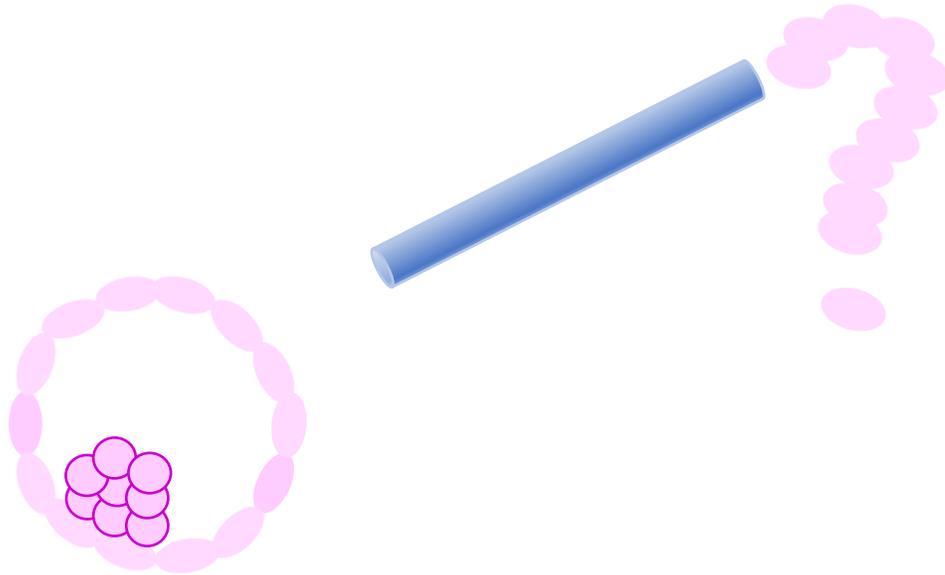
Scott RT Jr¹, Upham KM, Forman EJ, Zhao T, Treff NR.

- ❑ La Biopsie au stade j3: réduit de façon remarquable le taux d'implantation embryonnaire.
- ❑ Au stade J5: aucun impact sur le taux d'implantation.



Scott et al., 2013

Biopsie de Trophoectoderme



Nombre de cellules à biopsier?

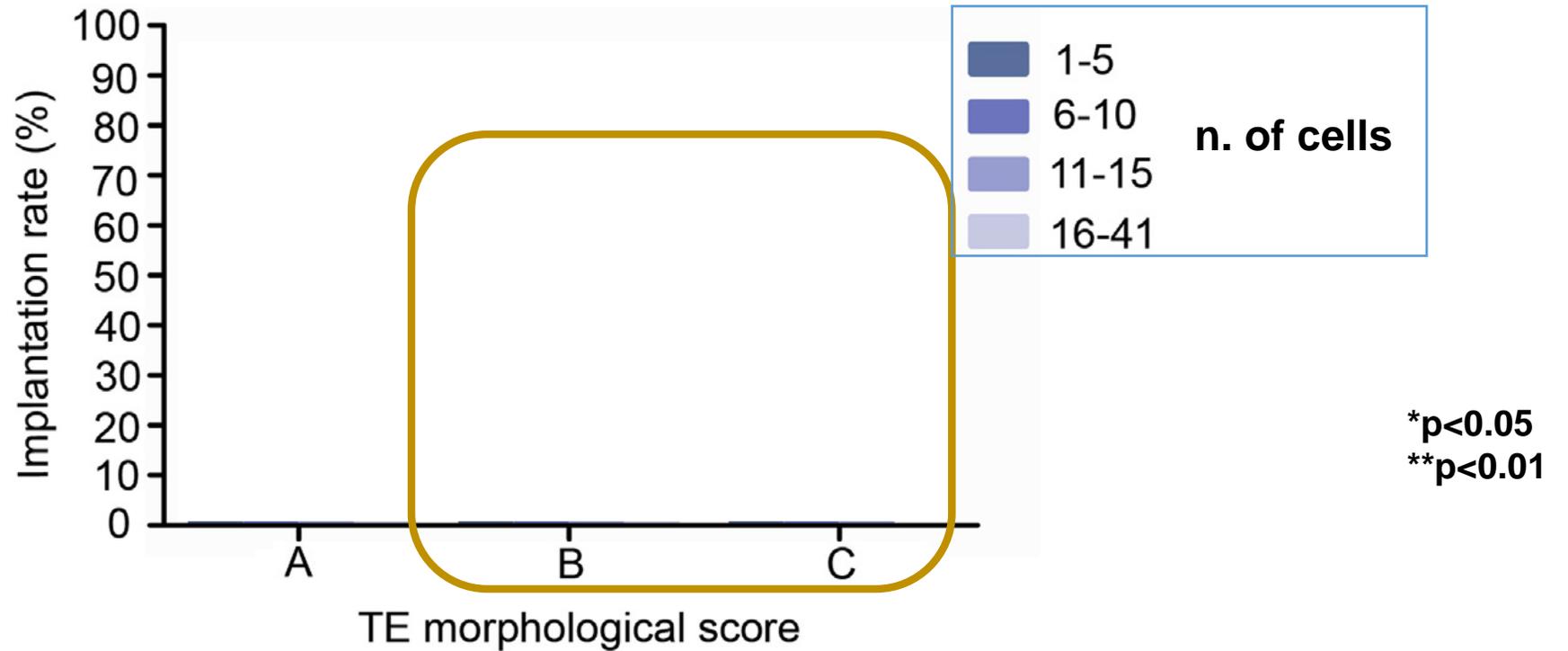


Number of biopsied trophectoderm cells is likely to affect the implantation potential of blastocysts with poor trophectoderm quality

Shuoping Zhang, M.Sc.,^{a,b} Keli Luo, M.D., Ph.D.,^{a,b,c} Dehua Cheng, M.Sc.,^{a,b} Yueqiu Tan, Ph.D.,^{a,b,c} Changfu Lu, Ph.D.,^{a,b,c} Hui He, M.Sc.,^{a,c} Yifan Gu, Ph.D.,^{a,b,c} Guangxiu Lu, M.D.,^{a,b,c,d} Fei Gong, M.D., Ph.D.,^{a,b,c} and Ge Lin, M.D., Ph.D.,^{a,b,c,d}

^a Institute of Reproduction and Stem Cell Engineering, Central South University, ^b Reproductive and Genetic Hospital of Citic-Xiangya, ^c Key Laboratory of Stem Cells and Reproductive Engineering, Ministry of Health, and ^d National Engineering and Research Center of Human Stem Cell, Changsha, People's Republic of China

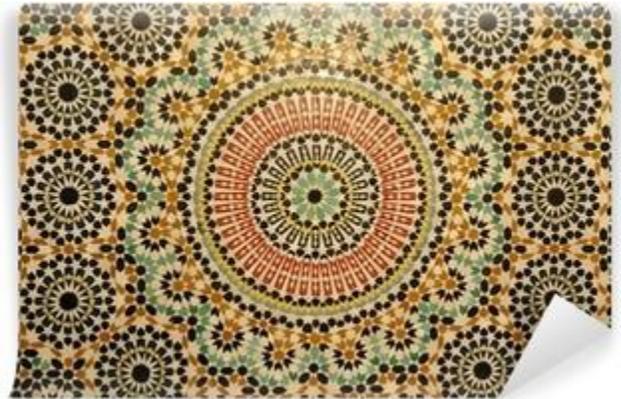
TROPHECTODERM BIOPSY



Dans les embryons à faible score, le nombre de cellules biopsiées impact négativement les taux d'implantation.

LE STATUT CHROMOSOMIQUE DE L'EMBRYON??

LE PGS ET MOSAICISME



Mosaicism type	Possible TE biopsy	Diagnoses accuracy
Total Mosaic 	 Euploid	Misdiagnosis
	 Mosaic	Accurate
	 Aneuploid	Misdiagnosis
ICM Mosaic 	 Euploid	Misdiagnosis (Mosaicism never detectable)
TE Mosaic 	 Euploid	Misdiagnosis
	 Mosaic	Accurate
	 Aneuploid	Misdiagnosis
ICM/TE Mosaic Type I 	 Euploid	Misdiagnosis (Mosaicism never detectable)
ICM/TE Mosaic Type II 	 Aneuploid	Misdiagnosis (Mosaicism never detectable)



Assessing the true incidence of mosaicism in preimplantation embryos

Maria Vera-Rodriguez, Ph.D. and Carmen Rubio, Ph.D.
Igenomix and Igenomix Foundation, Valencia, Spain

(Fertil Steril 2017;107: 1107–12. 2017 by American Society for Reproductive Medicine.)

MOSAICISME - ANEUPLOIDIE RESCUE

Incidence du mosaïcisme



de 15 to 90% au stade clivage
de 15 to 30% au stade blastocyste
de 1 to 2% en DPN



Un mécanisme de sélection contre le mosaïcisme / la correction de l'aneuploïdie aux stades ultérieurs du développement embryonnaire

[Hum Reprod Update](#). 2014 Jul-Aug;20(4):571-81. doi: 10.1093/humupd/dmu016. Epub 2014 Mar 25.

The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans.

[Taylor TH](#)¹, [Gitlin SA](#)², [Patrick JL](#)³, [Crain JL](#)³, [Wilson JM](#)³, [Griffin DK](#)⁴.

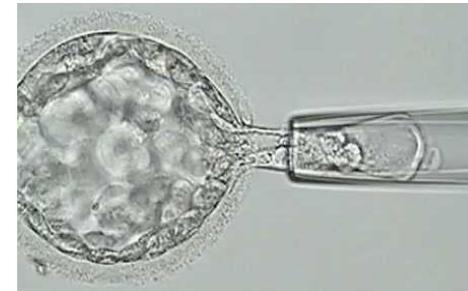
Preimplantation aneuploid embryos undergo self-correction in correlation with their developmental potential.

Barbash-Hazan S¹, Frumkin T, Malcov M, Yaron Y, Cohen T, Azem E, Amit A, Ben-Yosef D.



83 embryos aneuploïdes à J3

Ré analyser à j5



27 embryos euploïdes à j5

Au stade blastocyste **32,6%** de self correction partielle ou totale

ANEUPLOIDIE RESCUE

Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts

N Engl J Med 2015; 373:2089-2090 | November 19, 2015 | DOI: 10.1056/NEJMc1500421

Greco E, et al.

Table 1. Clinical Outcomes of Single Mosaic Blastocysts Transferred.*

Patient No.	Chromosomal Constitution	Mosaicism† percent	Karyotype‡	Clinical Outcome
1	arr(4)x1,(10)x1	40	46,XX	Baby healthy at birth
2	arr(6)x1,(15)x1	50	46,XX	Baby healthy at birth
3	arr(2)x1	40	46,XX	Baby healthy at birth
4	arr(2)x1	35	46,XY	Baby healthy at birth
5	arr(5)x1	50	46,XX	Baby healthy at birth
6	arr(5)x1,(7)x1	40	46,XX	Baby healthy at birth
7	arr(11)x1,(20)x3,(21)x3	30	NA	No pregnancy
8	arr(1)x1,(6)x3,(10)x3,(12)x3,(13)x3,(14)x3,(21)x3	50	NA	No pregnancy
9	arr(3)x1,(10)x3,(21)x3	35	NA	No pregnancy
10	arr(1)x3	50	NA	Biochemical pregnancy§
11	arr 9p21.2q34.3(26,609,645-140,499,771)x3	45	NA	Biochemical pregnancy§
12	arr(15)x3	30	NA	No pregnancy
13	arr(18)x1	50	NA	No pregnancy
14	arr(18)x1	50	NA	No pregnancy
15	arr(18)x1	40	NA	No pregnancy
16	arr(4)x1	50	NA	No pregnancy
17	arr(5)x3	40	NA	No pregnancy
18	arr 10q21.3q26.3(67,216,644-134,326,648)x3	50	NA	No pregnancy

* NA denotes not available.

† The approximate percentage of aneuploid cells in the transferred blastocyst is listed (see the Supplementary Appendix).

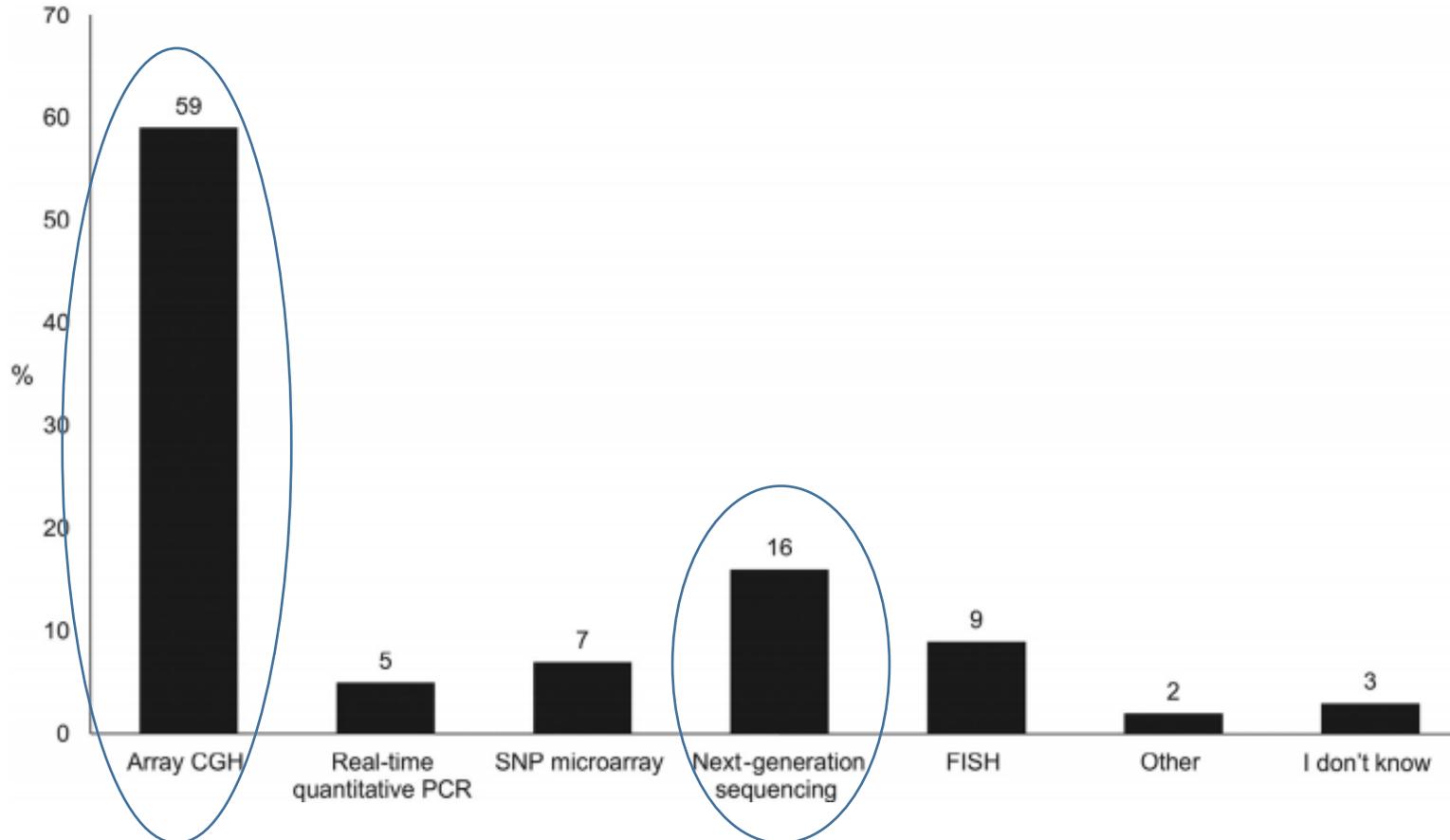
‡ The karyotype was determined by means of chorionic-villus sampling.

§ Biochemical pregnancy was defined by the presence of a low peak in levels of the beta subunit of human chorionic gonadotropin (β -hCG) (<100 mIU per milliliter), a rapid decrease in the urinary or serum β -hCG concentration, and no substantial delay in the onset of the next menstrual period, but with no detection of an identifiable pregnancy by means of ultrasonographic examination.



Nouvelles Techniques de Diagnostic

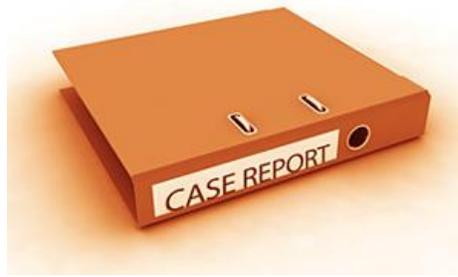
TECHNIQUES DE GENETIQUE ET PGS



PGS 2.0

Figure 3 – Which method of genetic testing is predominantly used in your clinic for determination of embryo ploidy status? Results are expressed in percentage, which represents the proportion of replies from the clinics relative to the number of cycles performed in each clinic. CGH, comparative genomic hybridization; FISH, fluorescence in-situ hybridization; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism. Figure adapted the [IVF-Worldwide \(2017\)](#).





J Assist Reprod Genet
DOI 10.1007/s10815-017-0954-y



GENETICS

Complex chromosomal rearrangement—a lesson learned from PGS

Tsvia Frumkin¹ • Sagit Peleg¹ • Veronica Gold¹ • Adi Reches^{1,2} • Shiri Asaf¹ •
Foad Azem¹ • Dalit Ben-Yosef^{1,3,4}  • Mira Malcov¹

[J Assist Reprod Genet](#). 2017 Aug; 34(8): 1095–1100

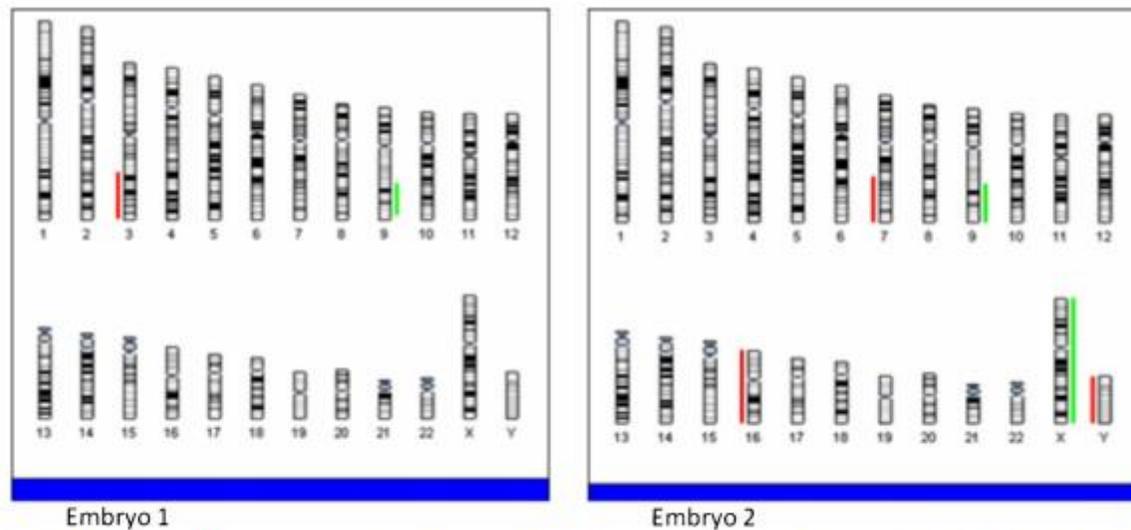


Fig. 1 Representative figure of CMA results of abnormal embryos that were diagnosed by PGS. The *green lines* demonstrate a gain of chromosomal regions and *red lines* demonstrate a loss of chromosomal regions

Table 1 Results of all embryos analyzed in the first CMA cycle

Embryo ID	Result
1	Unbalanced (-3s, +9s)
2	Unbalanced (-7s, -16, +9s)
3	Unbalanced (-9s, +7s, +22)
8	Abnormal (-8)
12	Unbalanced (-9s, +3s)
14	Unbalanced (-7s, +3s, +22)
16	Unbalanced (-3 s, +7s)

s structural aberration

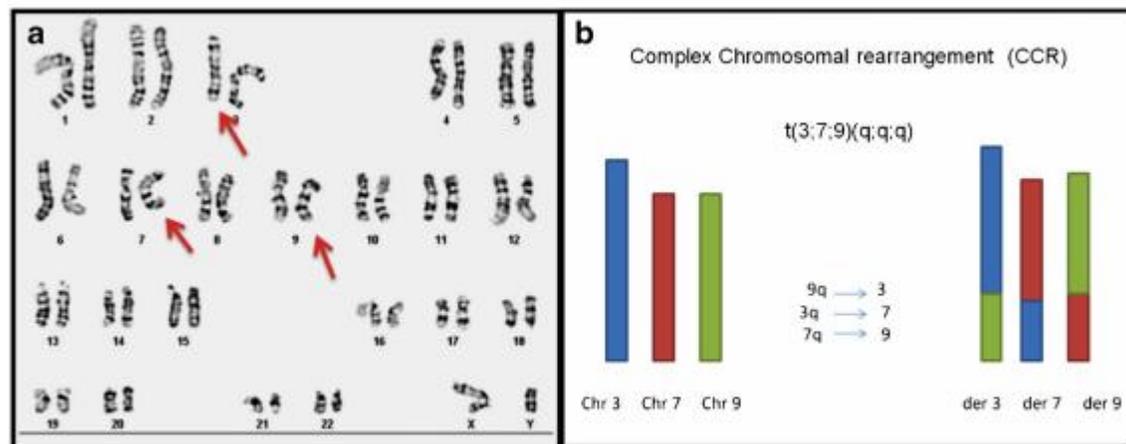
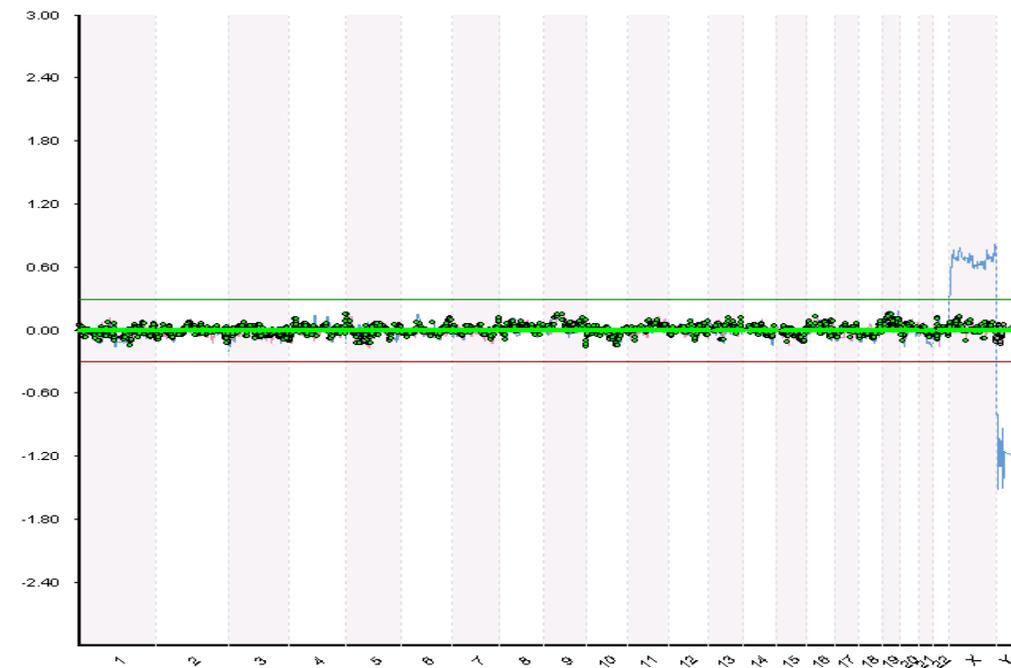


Table 2 Results of all embryos analyzed in the second CMA cycle

Embryo ID	Result
1	Unbalanced (-9s, +7s)
3	Unbalanced (+3s, +7s)
4	Unbalanced (-7s, +3s)
6	Unbalanced (+3s, +7s)
9	Balanced
16	Unbalanced (-3s, +7s)
17	Balanced

s structural aberration

Fig. 3 a Re-karyotyping for the male partner demonstrates CCR 46,XY,t(3;7;9)(q;q;q). The *red arrows* point at derivatives 3, 7, and 9. b A schematic presentation of the balanced CCR of the male partner. *Chr* chromosome, *der* derivative



PGS + ACPA = méthode de choix pour la détection de RCC.

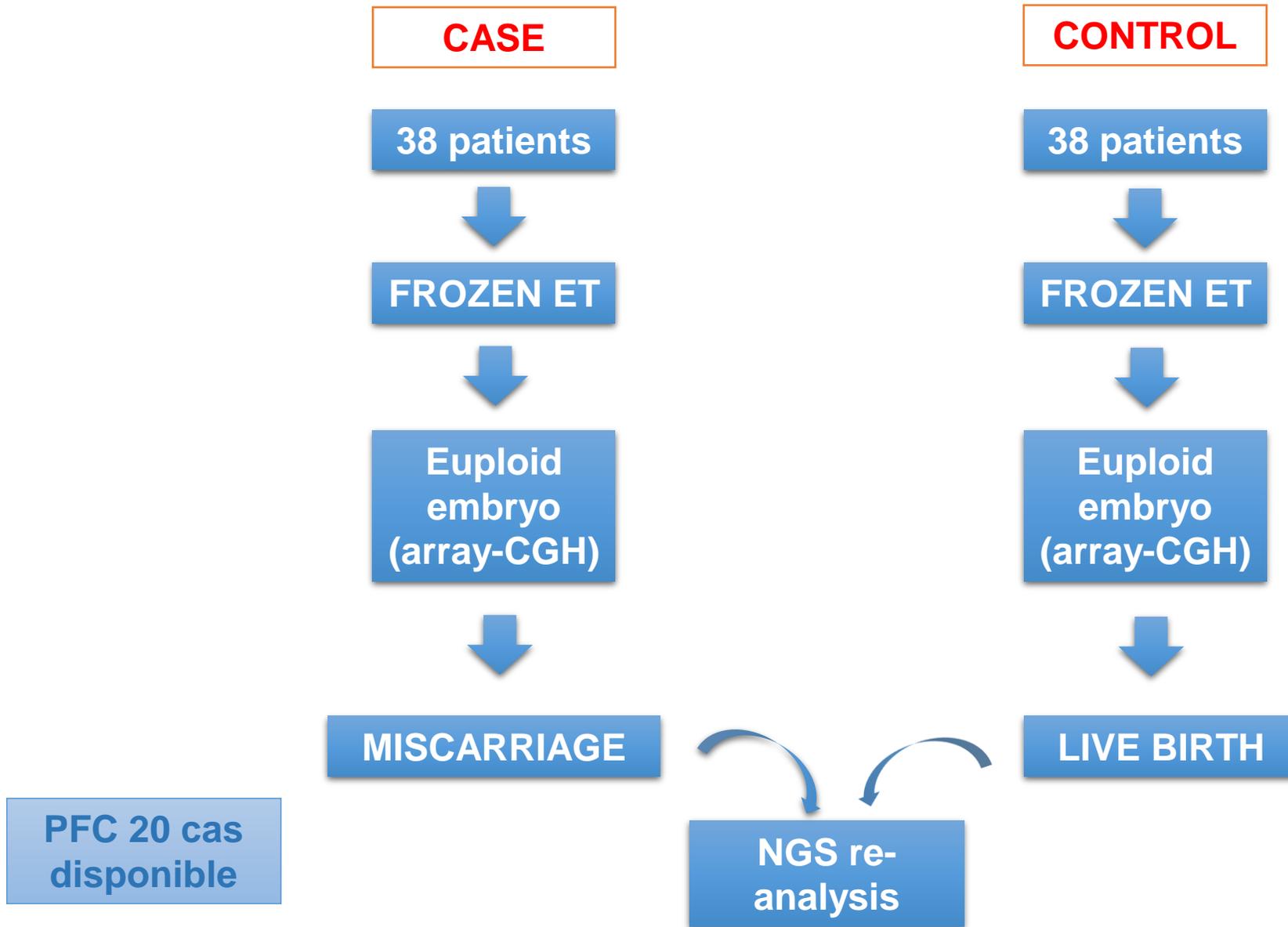
Why do euploid embryos miscarry? A case-control study comparing the rate of aneuploidy within presumed euploid embryos that resulted in miscarriage or live birth using next-generation sequencing.

Maxwell SM¹, Colls P², Hodes-Wertz B³, McCulloh DH³, McCaffrey C³, Wells D⁴, Munné S², Grifo JA³.

⊕ Author information

Abstract

OBJECTIVE: To determine whether undetected aneuploidy contributes to pregnancy loss after transfer of euploid embryos that have undergone array comparative genomic hybridization (aCGH).



RESULTS

TABLE 2

Results of reanalysis of embryo biopsy specimens with next-generation sequencing.

NGS results	Spontaneous abortion cases (n = 38)	Live birth controls (n = 38)	P value ^a
Euploid embryos, n (%)	24 (63.2)	32 (84.2)	.035 ^b
Mosaic embryos, n (%)	12 (31.6)	6 (15.8)	.092 ^c
Triploid embryos, n (%)	2 (5.2)	0	.25

Note: NGS = next-generation sequencing.

^a Mid-P Exact test.

^b Odds ratio with 95% confidence interval: 3.67 (1.085–16.37).

^c Odds ratio with 95% confidence interval: 3 (0.848–13.74).

Maxwell. NGS reanalysis of aCGH euploid embryos. *Fertil Steril* 2016.

Triploidie non détecté par ACPA

L'ACPA est incapable de détecter certain cas de mosaïcisme et de polyploïdie

RESULTATS



TABLE 3

Euploid embryos by aCGH resulting in spontaneous abortion with results of POC and reanalysis with NGS.

Euploid embryo no.	Cytogenetic method	Cytogenetic result	NGS result
1	SNP	46 XY	46 XY
2	SNP	47 XX+7	46 XX
3	SNP	46 XX	46 XX
4	Cell culture, G-banding	46 XX	46 XX
5	SNP	46 XX	46 XX
6	Cell culture, G-banding	46 XY →	Mosaic partial trisomy 11pter-p15.1
7	Cell culture, G-banding	46 XX →	Mosaic monosomy 1, mosaic monosomy 17
8	Cell culture, G-banding plus FISH	Mosaic trisomy 21	Mosaic trisomy 21
9	SNP	46 XY	46 XY
10	Cell culture, G-banding	46 XY	46 XY
11	Cell culture, G-banding	46 XX	46 XX
12	SNP	Mosaic trisomy 13	46 XX
13	SNP	46 XX	46 XX
14	Cell culture, G-banding	46 XY	46 XY
15	Cell culture, G-banding	46 XY →	Mosaic partial monosomy 11pter-p11.12, Mosaic partial monosomy 22q12.1qter
16	SNP	46 XX	46 XX
17	Cell culture, G-banding	46 XY	46 XY
18	SNP	Mosaic trisomy 11	Mosaic trisomy 11
19	SNP	46 XY	46 XY
20	Cell culture, G-banding	46 XY →	Mosaic partial monosomy 1, Mosaic monosomy 8

Note: aCGH = array comparative genomic hybridization; FISH = fluorescent in situ hybridization; NGS = next-generation sequencing; POC = products of conception; SNP = single nucleotide polymorphism.

Maxwell. NGS reanalysis of aCGH euploid embryos. *Fertil Steril* 2016.

Mosaïcisme détecté par NGS non présent dans le PFC

BIOPSIE EMBRYONNAIRE

Day 1

Day 2

Day 3

Day 4

Day 5

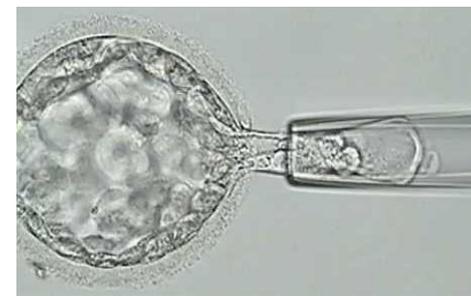
Day 6

**Polar Body
Biopsy**

**Cleavage stage
biopsy**

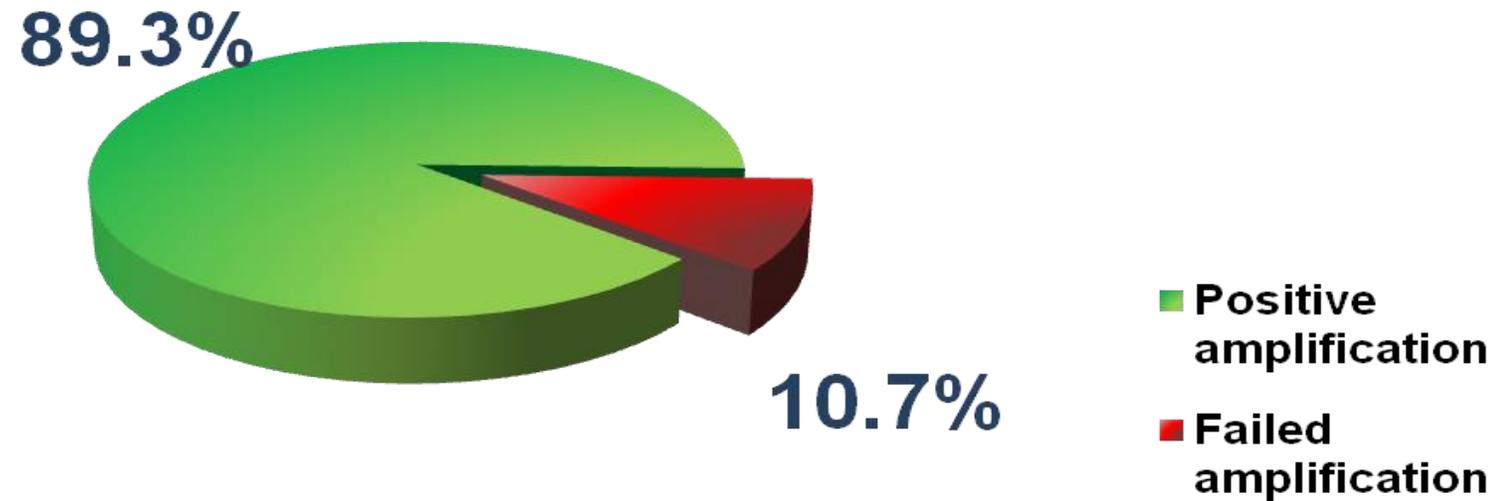
**Morula stage
biopsy**

Blastocyst biopsy
Trophectoderm **Blastocoelic fluid**



DETECTION de L'ADN DANS LE LIQUIDE BLASTOCOELIQUE

SAMPLES: 206

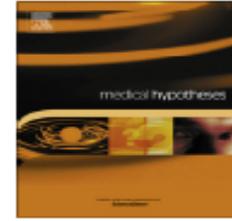




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Non-invasive pre-implantation genetic diagnosis of X-linked disorders



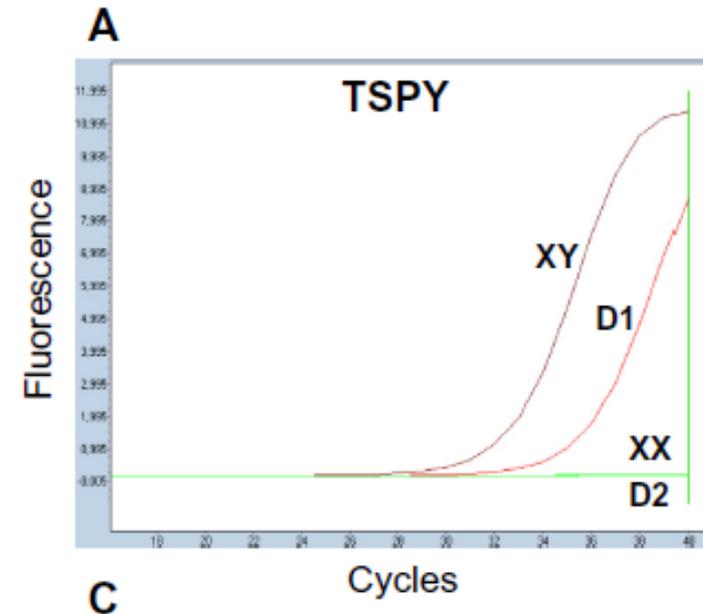
Said Assou ^{a,b}, Ounissa Aït-Ahmed ^{a,b}, Safia El Messaoudi ^c, Alain R. Thierry ^c, Samir Hamamah ^{a,b,d,*}

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Pushing the limits of detection: investigation of cell-free DNA for aneuploidy screening in embryos

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Concordance rates between cell-free DNA (cfDNA), trophoctoderm biopsy, and whole embryos, n (%).

Pair	Concordance for ploidy				Concordance for sex			
	Total	AH	No AH	P value ^c	Total	AH	No AH	P value ^c
Day 3 cfDNA vs. whole embryo ^a (n = 16)	9/16 (56.3%)	4/8 (50.0%)	5/8 (62.5%)	.61	13/16 (81.3%)	5/8 (62.5%)	8/8 (100%)	.06
Day 5 cfDNA vs. whole embryo ^a (n = 33)	15/33 (45.5%)	5/16 (31.3%)	10/17 (58.8%)	.11	26/33 (78.7%)	12/16 (75.0%)	14/17 (82.4%)	.61
Day 5 cfDNA vs. trophoctoderm biopsy ^b (n = 40)	26/40 (65.0%)	16/28 (57.1%)	10/12 (83.3%)	.16	28/40 (70.0%)	17/28 (60.7%)	11/12 (91.7%)	.07
Day 5 trophoctoderm biopsy vs. whole embryo ^a (n = 27)	25/27 (92.6%)	12/14 (85.7%)	13/13 (100%)	.22	26/27 (96.3%)	14/14 (100%)	12/13 (92.3%)	.48

Note: AH = assisted hatching.

^a Includes research embryos only.

^b Includes both research embryos and clinical samples.

^c Chi-square analysis or Fisher exact test used to compare AH vs. no AH groups; P < .05 was considered to be significant.

Ho. Cell-free DNA for aneuploidy screening. *Fertil Steril* 2018.

CfDNA du milieu de culture embryonnaire n'est pas actuellement optimisé pour dépistage des aneuploïdies mais il reste un outil prometteur pour le

PGDIS Newsletter, July 19, 2016

PGDIS POSITION STATEMENT ON CHROMOSOME MOSAICISM AND PREIMPLANTATION ANEUPLOIDY TESTING AT THE BLASTOCYST STAGE

- ❑ ***Pour déclarer un mosaicism embryonnaire, le seuil suggéré doit être > 20%, donc les niveaux inférieurs doivent être traités comme normaux (euploïdes), > 80% anormaux (aneuploïdes) et les autres entre 20-80% mosaïque (mosaïques euploïdes-aneuploïdes).***



Les guidelines suggérées pour prioriser les embryons mosaïques à transférer

Les embryons présentant une mosaïque **euploïdie / monosomie** sont préférables à **l'euploïdie / trisomie**, étant donné que les embryons monosomiques (sauf 45, X) ne sont pas viables.

Si une décision est prise de transférer des embryons trisomiques mosaïque pour un seul chromosome, on peut prioriser la sélection en fonction du chromosome impliqué:

- ❑ **Transfert préférable** pour des embryons trisomiques en mosaïque concernant **les chromosomes 1, 3, 4, 5, 6, 8, 9, 10, 11, 12, 17, 19, 20, 22, X, Y.**
- ❑ **Transfert moins prioritaire:**
 - embryons mosaïques pour les trisomies associés à l'UPD (14,15).
 - embryons mosaïques pour les trisomies associées à un retard de croissance intra-utérin (**chromosomes 2, 7, 16**).
 - embryons pour les trisomies capables de donner des naissances vivantes !!!!! (**chromosomes 13, 18, 21**).

Systematic review of worldwide trends in assisted reproductive technology 2004–2013

[Vitaly A. Kushnir](#)^{1,2}, [David H. Barad](#)^{1,3}, [David F. Albertini](#)^{1,4,6}, [Sarah K. Darmon](#)¹ and [Norbert Gleicher](#)^{1,3,5,6}



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[Reprod Biomed Online](#). 2018 Mar;36(3):288-289. doi: [10.1016/j.rbmo.2017.12.012](https://doi.org/10.1016/j.rbmo.2017.12.012). Epub 2017 Dec 29.

Time-lapse systems for ART.

[Armstrong S](#)¹, [Bhide P](#)², [Jordan V](#)², [Pacey A](#)², [Farquhar C](#)².



Letter

How PGS/PGT-A laboratories succeeded in losing all credibility



Norbert Gleicher MD et al (2018)

« (...)resulted in couples paying more money for a less effective treatment.»



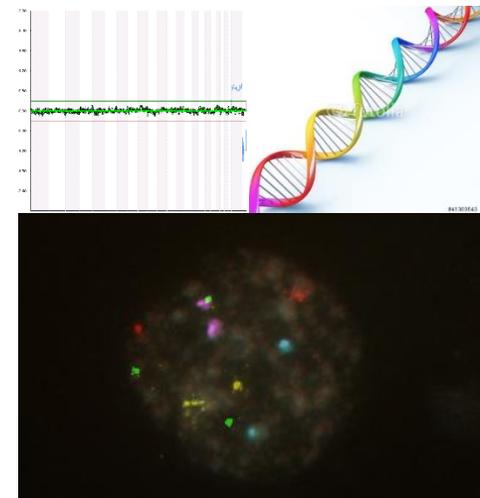
« L'important
n'est pas de
convaincre, mais
de donner à
réfléchir. »

Bernard Werber



THE

TAKE-HOME MESSAGE



« Les enfants sont des soucis certains et des réconforts incertains. » J. Clarke



Essaouira



Saint Malo