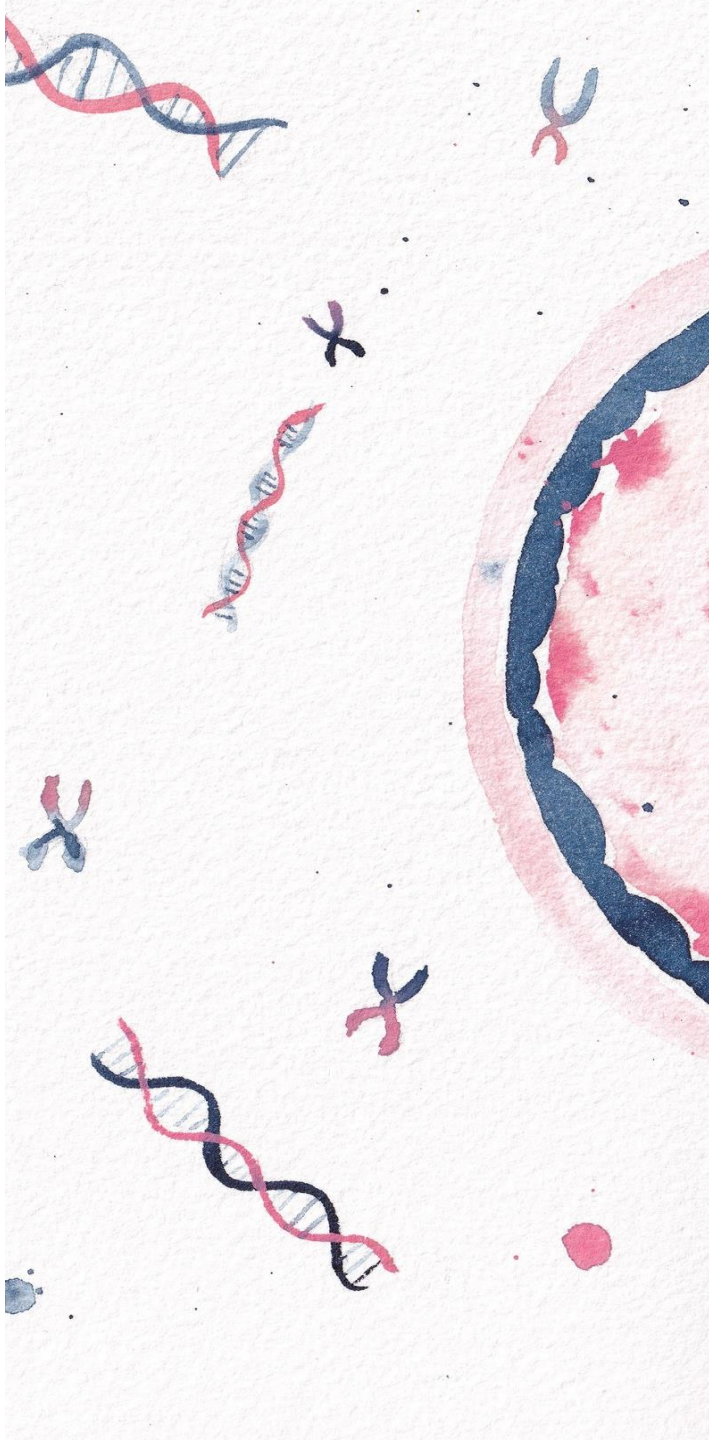
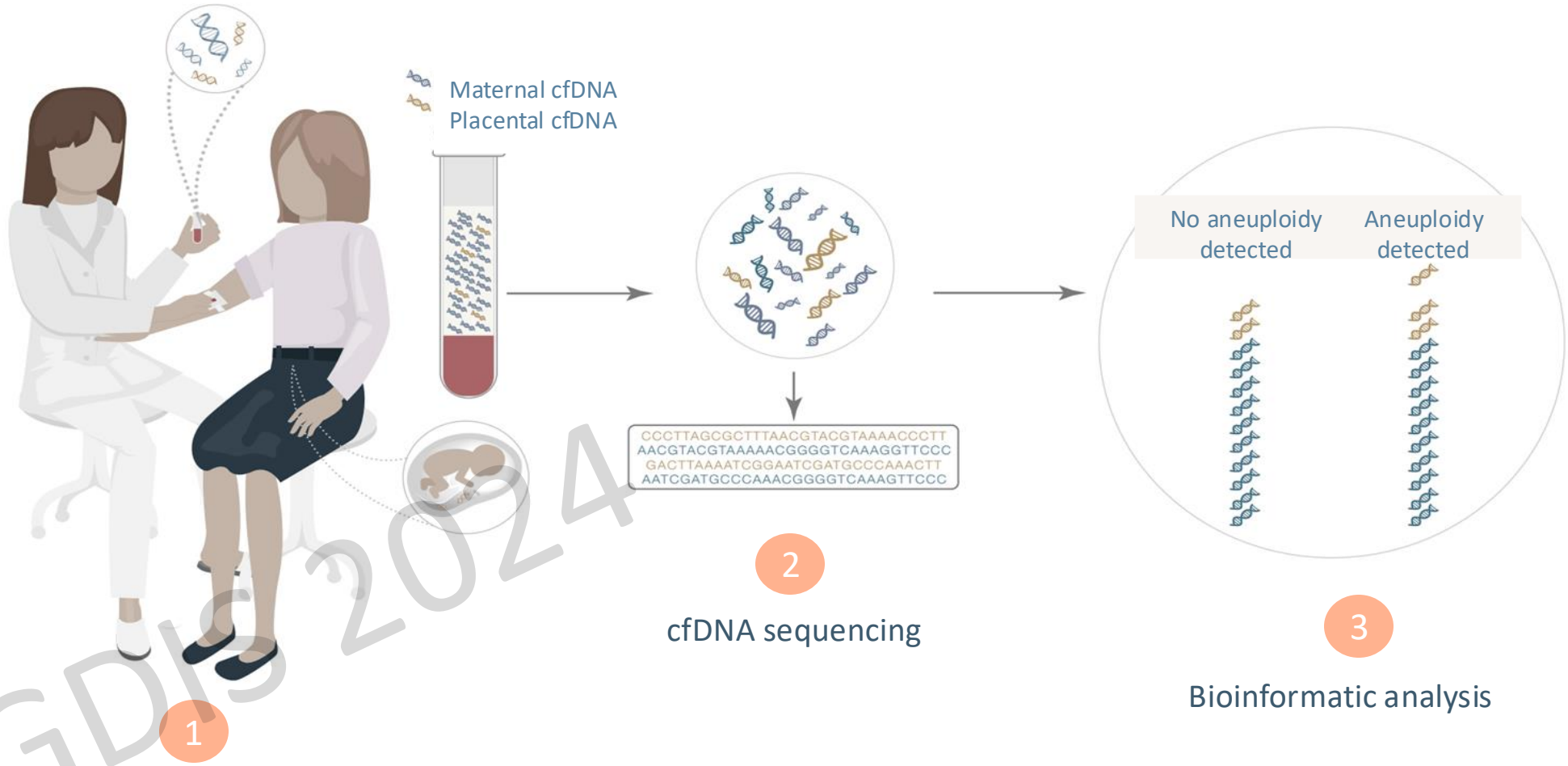
Should we down the pathway of niPGT-A?

Carmen Rubio, PhD
R&D VP
IGENOMIX (Vitrolife Group), Valencia, Spain

Igenomix®
PART OF VITROLIFE GROUP™



Non-invasive approaches in prenatal diagnosis and oncology

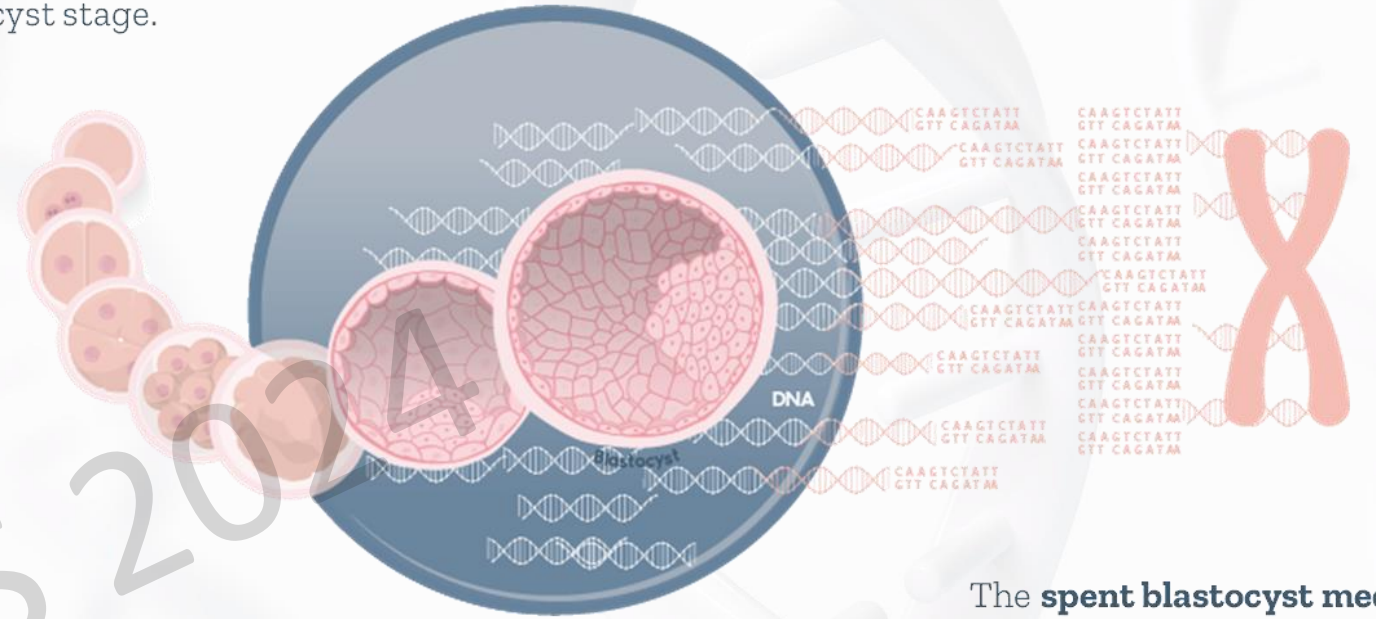


Bianchi DW, Parker RL, Wentworth J, Madankumar R, Saffer C, Das AF, Craig JA, Chudova DI, Devers PL, Jones KW, Oliver K, Rava RP, Sehnert AJ; CARE Study Group. DNA sequencing versus standard prenatal aneuploidy screening. N Engl J Med. 2014 Feb 27;370(9):799-808.

Ellen Heitzer, Samantha Perakis, Jochen B. Geigl, Michael R. Speicher. The potential of liquid biopsies for the early detection of cancer NPJ Precis Oncol. 2017; 1: 36.

Embryo cell-free DNA is released during embryo development

During in vitro development, mostly from day 4 to day 6, embryo **cell-free DNA (cfDNA)** is released to the culture medium, with higher concentrations as the number of cells increases at blastocyst stage.



The **spent blastocyst medium (SBM)** containing the embryo cell-free DNA can be analysed by next generation sequencing, representing a non-invasive approach to estimate the chromosome copy number of the blastocyst without the need of a trophectoderm biopsy.

Concordance of cfDNA vs TE biopsies & whole blastocysts

Authors	No. of SBM	Informative media	Ploidy Concordance With TE or WB	False positives	False negatives	Embryo manipulation	Time in culture	WGA method	NGS platform
Xu et al., 2016	42	100%	85.7% (WB)	9.5%	4.8%	D3 Vitrification	D3-D5	MALBAC (Yikon)	NGS (Illumina)
Vera-Rodríguez et al., 2018	56	91.1%	33.3% (TE)	-	66.7%	D3 AH	D3-D5	Sureplex + ReproSeq (Thermo)	NGS (Thermo)
Ho et al., 2018	41	97.6%	[illegible] (TE)	-	-	D3 AH vs no AH	D1 to D5	Picoplex (Rubicon)	NGS (Thermo)
Huang et al., 2019	52	92		2.2% (TE) 6.3% (WB)	8.7% (TE) -	D3 AH + TE biopsy + vitrification D5/6	24h culture after thawing	MALBAC (Yikon)	NGS (Illumina)
Yeung et al., 2019	168	[illegible]		12.9% D5: 12% D6: 13.6%	13.8% D5: 12% D6: 15.2%	D3 AH	D3-D5 D3-D6	Sureplex (Illumina)	NGS (Illumina)
Rubio et al., 2019	115			13.9% D5: 29.6% D6/7: 8.6%	2.8% D5: 3.7% D6/7: 2.5%	NO		Reproseq (Thermo)	NGS (Thermo)
Rubio et al., 2020[4]	1301			12.4% (TE) 6.2% (ICM)	8.3% (TE) 9.4% (ICM)			Reproseq (Thermo)	NGS (Thermo)
Lledo et al., 2020	92	9[illegible]		12.0% or 15.7%	13.3% or 12.0%			MALBAC (Yikon) Sureplex (Illumina)	NGS (Illumina)
Yin et al., 2021	75	78.7%		10.2%	-			MALBAC (Yikon)	NGS (Illumina)
Shitara et al., 2021	20	95%	88.9% (TE)	5.6%	5.6%			[illegible]ureplex (Illumina)	NGS (Illumina)
Hanson et al., 2021	166	62.7% D5: 17.6% D6/7: 74.2%	63.5% (TE) D5: 50.0% D6/7: 64.3%	26.9% D5: 33.3% D6/7: 26.5%	8.7% D5: 16.7% D6/7: 8.2%			MALBAC (Yikon)	NGS (Illumina)
Chen et al., 2021	265	96.6%	74.2% (TE) 78.1% (WB)	14.5% (TE) 16.8% (WB)	11.3% (TE) 5.1% (WB)	N[illegible]		MALBAC (Yikon)	NGS (Illumina)
Shi et al., 2022	212	100%	84.4% (TE)	13.2%	2.4%	Artificial shrinkage b[illegible] vitrification	[illegible] thawing	MALBAC (Yikon)	NGS (Illumina)
Lei et al., 2022	113	98.2%	68.5% (TE)	-	-	D3 AH	D3-D5/6	MALBAC (Yikon)	NGS (Illumina)
Xie et al., 2022	161	91.3% D5: 81%, D6/7: 92%- 100%	75% (TE)	21.5%	3.5%	NO	D4-D5/6	MALBAC (Yikon)	NGS (Illumina)
Sonehara et al., 2022	46	100%	59.1% Low quality (WB) 70.8% High quality (WB)	-	-	NO	D3-D6/7	PG-Seq Rapid (Perkin Elmer)	NGS (Illumina)

Informativity rates on D6:
→ 98.8%

Ploidy concordance TE on D6:
→ 89.1%

Sensitivity & specifity: niPGT-A compared to PGT-A

TE & cfDNA vs. whole blastocysts

F&S, 2021. Fertility battle

Noninvasive preimplantation genetic testing for aneuploidy in spent culture medium as a substitute for trophectoderm biopsy

Carmen Rubio, Ph.D.,[a,b] Catherine Racowsky, Ph.D.,[c] David H. Barad, M.D., M.S.,[d,e] Richard T. Scott Jr., M.D., H.C.L.D.,[f,g] and Carlos Simon, M.D., Ph.D.[h,i]

PRO: Noninvasive preimplantation genetic testing for aneuploidy in spent culture medium will substitute for trophectoderm biopsy *(continued)*

Pro 2. Catherine Racowsky, Ph.D.

Comparison of the performance of niPGT-A and iPGT-A for PGT-A using samples collected following blastocyst culture.

Performance characteristic	niPGT-A (n = 102)	iPGT-A (n = 100)	*P* value
FPR	21.7% (5/23)	62.5% (15/24)	.008
FNR	1.3% (1/79)	0.0% (0/76)	1.000
PPV	94.0% (78/83)	83.5% (76/91)	.034
NPV	95.5% (21/22)	100.0% (18/18)	1.000
Sensitivity	98.7% (78/79)	100.0% (76/76)	1.000
Specificity	78.3% (18/23)	50.0% (12/24)	.069
Concordance for embryo ploidy	94.1% (96/102)	85.0% (85/100)	.039
Concordance for whole chromosome CN	81.4% (83/102)	67.0% (67/100)	.024

Note: The comparison includes 3 studies assessing the performance characteristics of niPGT-A and iPGT-A on whole embryo after the collection of samples (spent medium with or without BF) at the blastocyst stage (culture day 5 to 6 or day 6 to 7). *P* values were derived using the chi-square and Fisher's exact tests, with $P < .05$ considered statistically significant. BF = blastocyst fluid; CN = copy number; FNR = false-negative rate; FPR = false-positive rate; iPGT-A = invasive preimplantation genetic test for aneuploidy; niPGT-A = noninvasive preimplantation genetic test for aneuploidy; NPV = negative predictive value; PPV = positive predictive value; PGT-A = preimplantation genetic testing for aneuploidy.

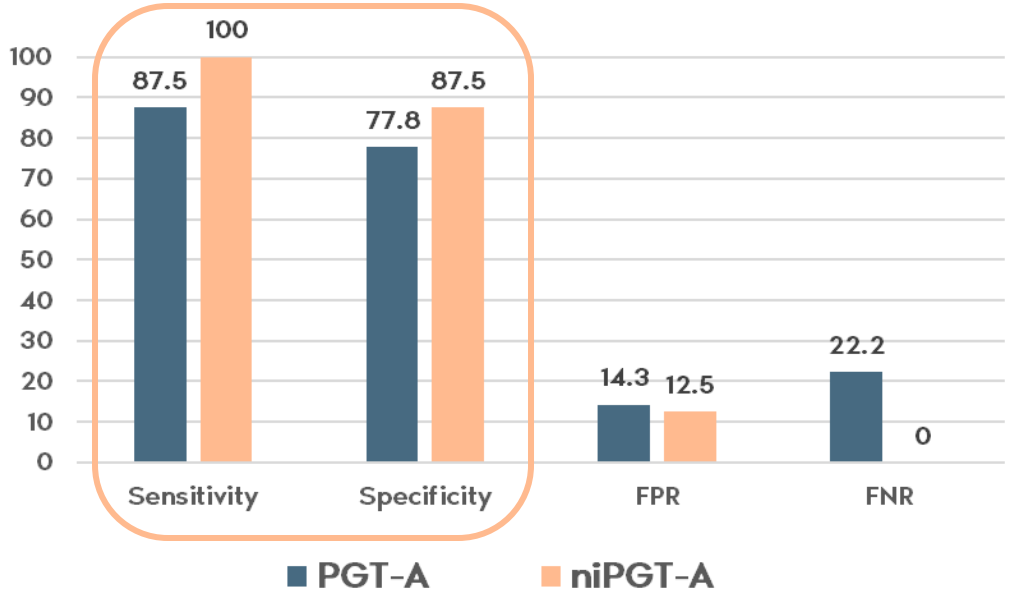
TE & cfDNA vs. day-8/10 outgrowths

PLOS ONE, 2021

RESEARCH ARTICLE

Cell-free DNA in spent culture medium effectively reflects the chromosomal status o embryos following culturing beyond implantation compared to trophectoderm biopsy

Akihiro Shitara*, Kazumasa Takahashi, Mayumi Goto, Harunori Takahashi, Takuya Iwasawa, Yohei Onodera, Kenichi Makino, Hiroshi Miura, Hiromitsu Shirasawa, Wataru Sato, Yukiyo Kumazawa, Yukihiro Terada



N= 10 blastocysts donated for research (D5 + D6)

Igenomix®

Multicenter concordance study: ICM vs. SBM vs. TE

PGDIS 2023

OBSTETRICS

Multicenter prospective study of concordance between embryonic cell-free DNA and trophectoderm biopsies from 1301 human blastocysts

Carmen Rubio, PhD[1]; Luis Navarro-Sánchez, PhD[1]; Carmen M. García-Pascual, PhD; Olcay Ocali, BS; Danilo Cimadomo, PhD; William Venier, MSc; Gerardo Barroso, MD; Laura Kopcow, MD; Mustafa Bahçeci, MD; Marcos Iuri Roos Kulmann, BSc; Lourdes López, MD; Emilio De la Fuente, MSc; Roser Navarro, MSc; Diana Valbuena, MD, PhD; Denny Sakkas, PhD; Laura Rienzi, MSc; Carlos Simón, MD, PhD

The **290 day-6/7 blastocysts** belonged to 164 patients (mean age 37.4 and mostly AMA indication).

In combination, the three sample types (embryo cfDNA, ICM and TE) were **informative** in **230** of the blastocysts analyzed.

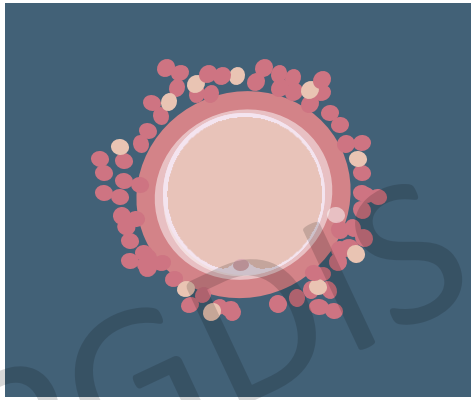
	ploidy concordance	*full concordance	*partial concordance	false negatives	false positives	**PPV	**NPV	specificity	sensitivity
†TE-cfDNA	89.1%	61.7%	27.4%	9.6%	1.3%	0.985	0.353	0.800	0.898
†ICM-cfDNA	87.0%	56.5%	30.4%	7.8%	5.2%	0.939	0.471	0.571	0.911
†ICM-TE	90.0%	70.0%	20.0%	2.2%	7.8%	0.916	0.667	0.357	0.975

†Sample reference for the comparison.
*Ploidy concordance includes both full concordance (when the chromosomal status for all the chromosomes in both samples is the same) and partial concordance (the chromosomal status for some chromosomes might differ between samples, but they are both aneuploid).
**PPV: positive predictive value; NPV: negative predictive value.

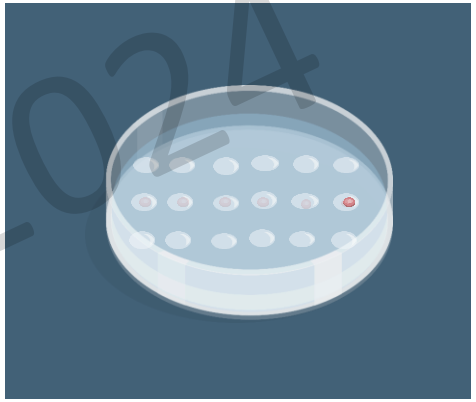
How to improve and standardize niPGT-A results

- ✓ Informativity rate range: 81.8% D5 → 98.8% D6
- ✓ Concordance rate range TE-cfDNA: 76% D5 → 89.1% D6
- ✓ Concordance rate with full frozen blastocyst/ICM: up to 93.7% D6/7

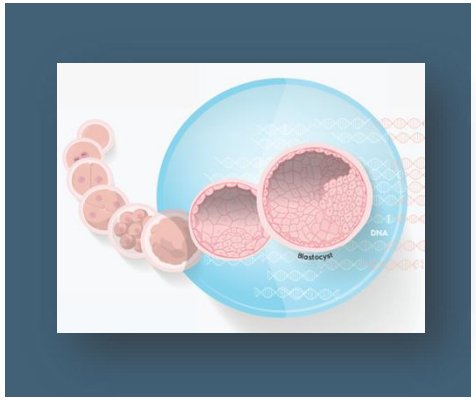
DECREASE CONTAMINATION



LAB PROTOCOL AND EMBRYO CULTURE



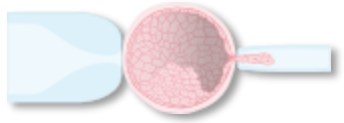
TIMING FOR MEDIA COLLECTION



PRIORITIZATION ALGORITHMS

niPGT-A profiles showing discordant results

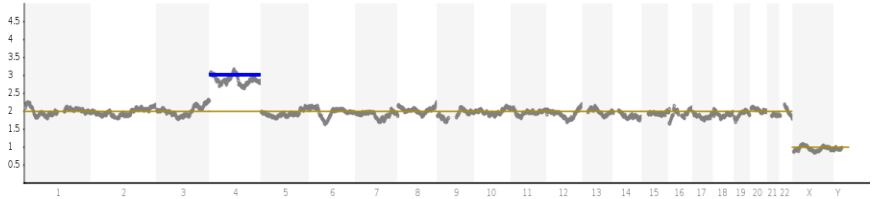
TE biopsy



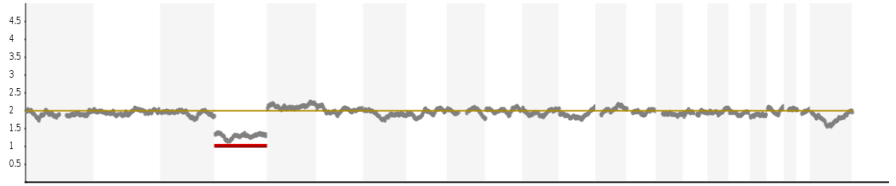
SBM

PB contamination

47, +4, XY

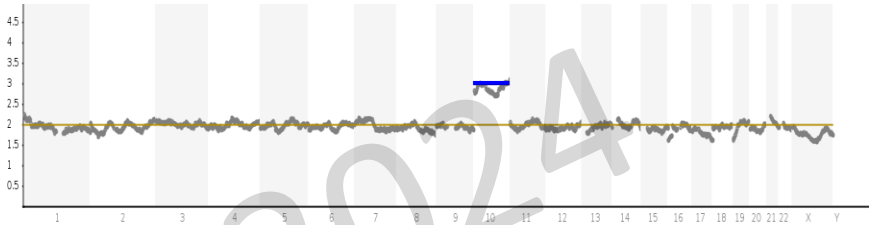


45, -4, XX

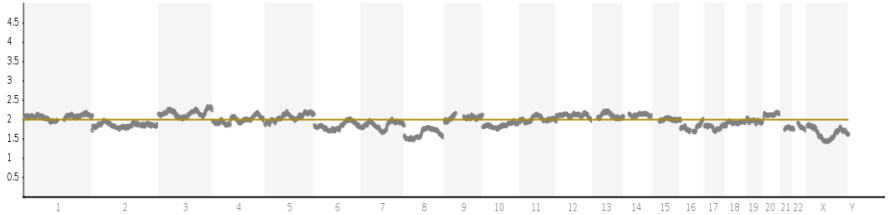


MCC

47, +10,

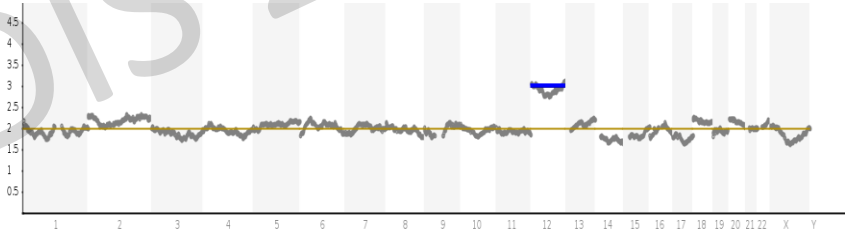


46, XX

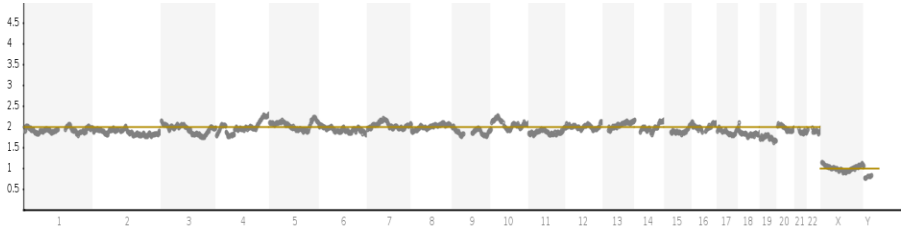


External contamination

47, +12,



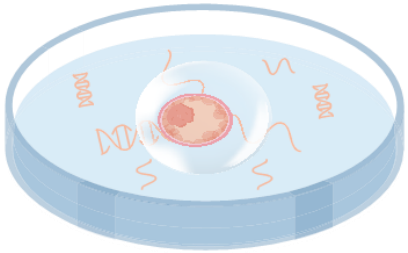
46, XY



Tips to decrease contamination

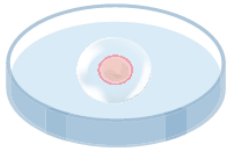
Sources of DNA CONTAMINATION

- **MCC** --> cumulus cells, PBs
- **External**
  - Manipulation of the culture medium
  - Technologist / IVF lab



MCC

Day 4 embryos



Washings

Crucial steps:
Oocyte denudation and embryo serial washes on D4, collection D6

External

Culture media

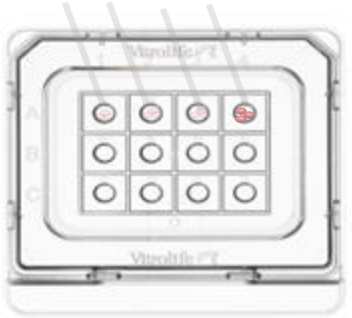
Always use a fresh aliquot and individual capillaries for each embryo during washing and media collection

Technologist/IVF lab procedures ---------→ DNA free hood surfaces Technologist properly covered

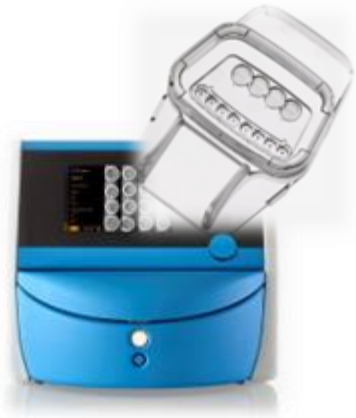
Pre-clinical validations in each lab are crucial

IVF lab protocol and embryo culture conditions

Day 0/1

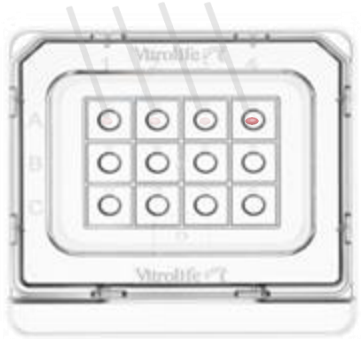


Extra washes after denudation



Culture up to day 4

Day 4



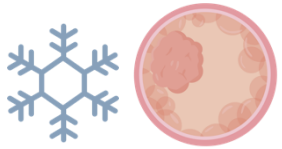
Individual extra washes

Day 4 up to day 6/7

Culture in droplets of 20-25µl

Day 6/7

Check the embryos that have reached the blastocyst stage



Blastocyst vitrification

Media aspiration + freeze the sample at -20°C

NGS analysis

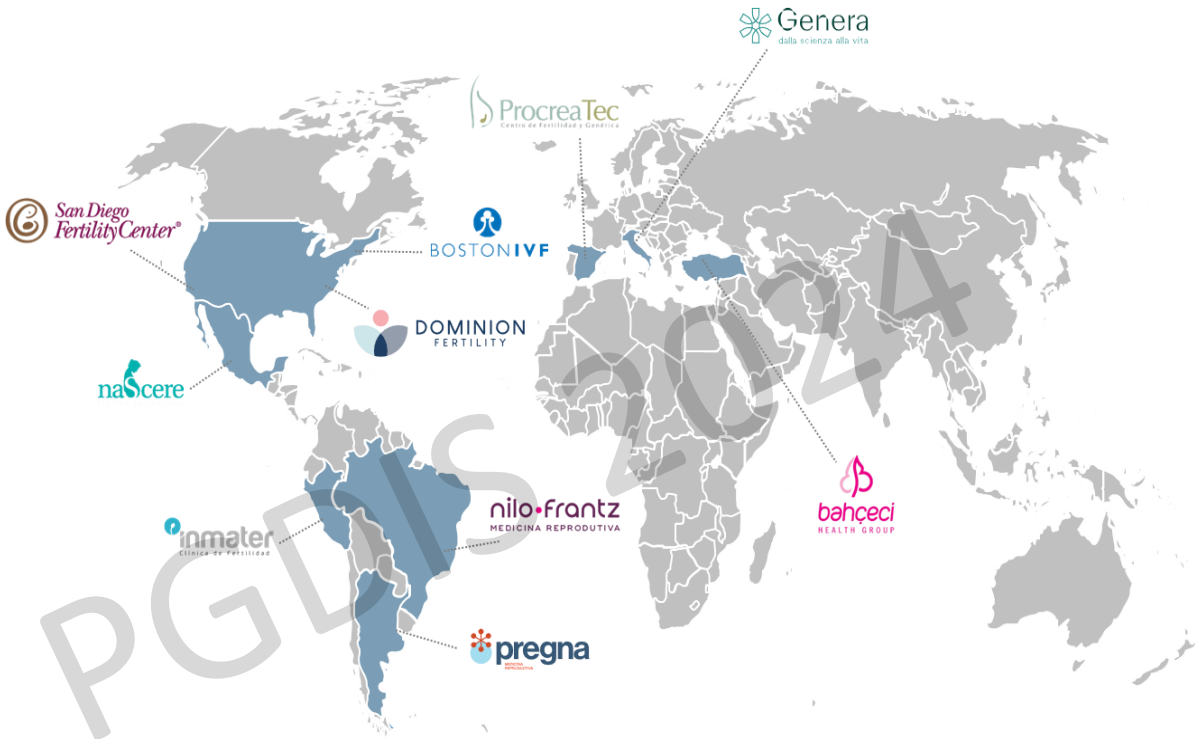
niPGT-A report & prioritization

Impact of culture conditions on concordance rates

American Journal Obstetrics & Gynaecology, 2020

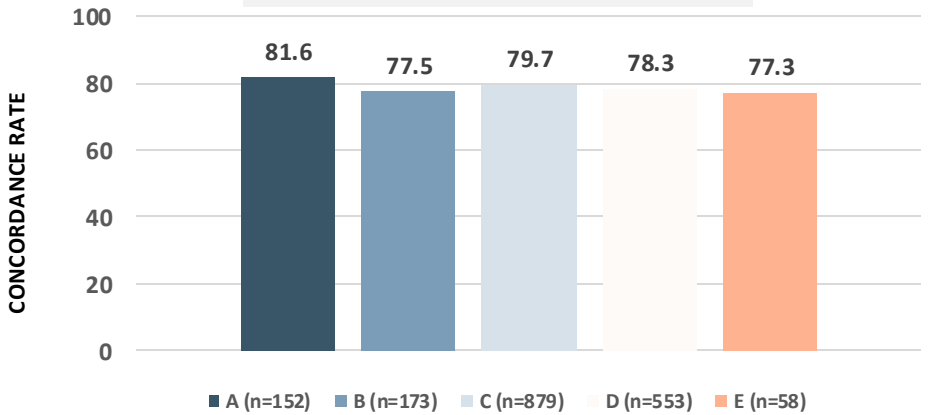
Multicenter prospective study of concordance between embryonic cell-free DNA and trophectoderm biopsies from 1301 human blastocysts

Carmen Rubio, PhD[1]; Luis Navarro-Sánchez, PhD[1]; Carmen M. García-Pascual, PhD; Olcay Ocali, BS; Danilo Cimadomo, PhD; William Venier, MSc; Gerardo Barroso, MD; Laura Kopcow, MD; Mustafa Bahçeci, MD; Marcos Iuri Roos Kulmann, BSc; Lourdes López, MD; Emilio De la Fuente, MSc; Roser Navarro, MSc; Diana Valbuena, MD, PhD; Denny Sakkas, PhD; Laura Rienzi, MSc; Carlos Simón, MD, PhD

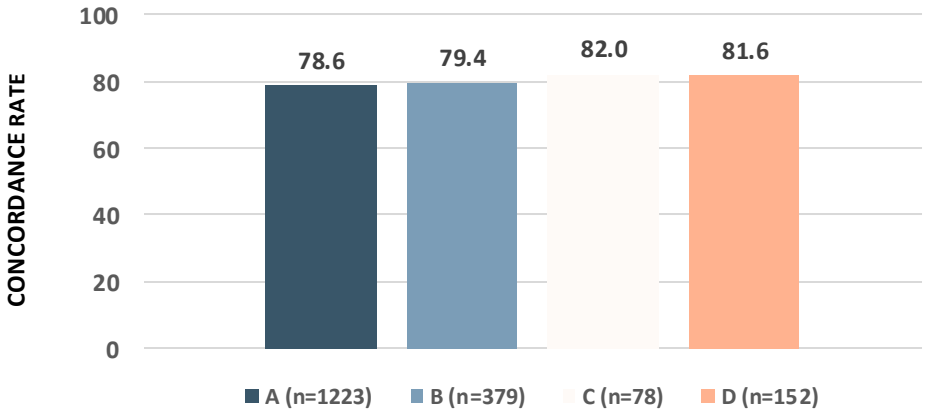


No impact of culture conditions

BENCHTOP INCUBATOR MODEL



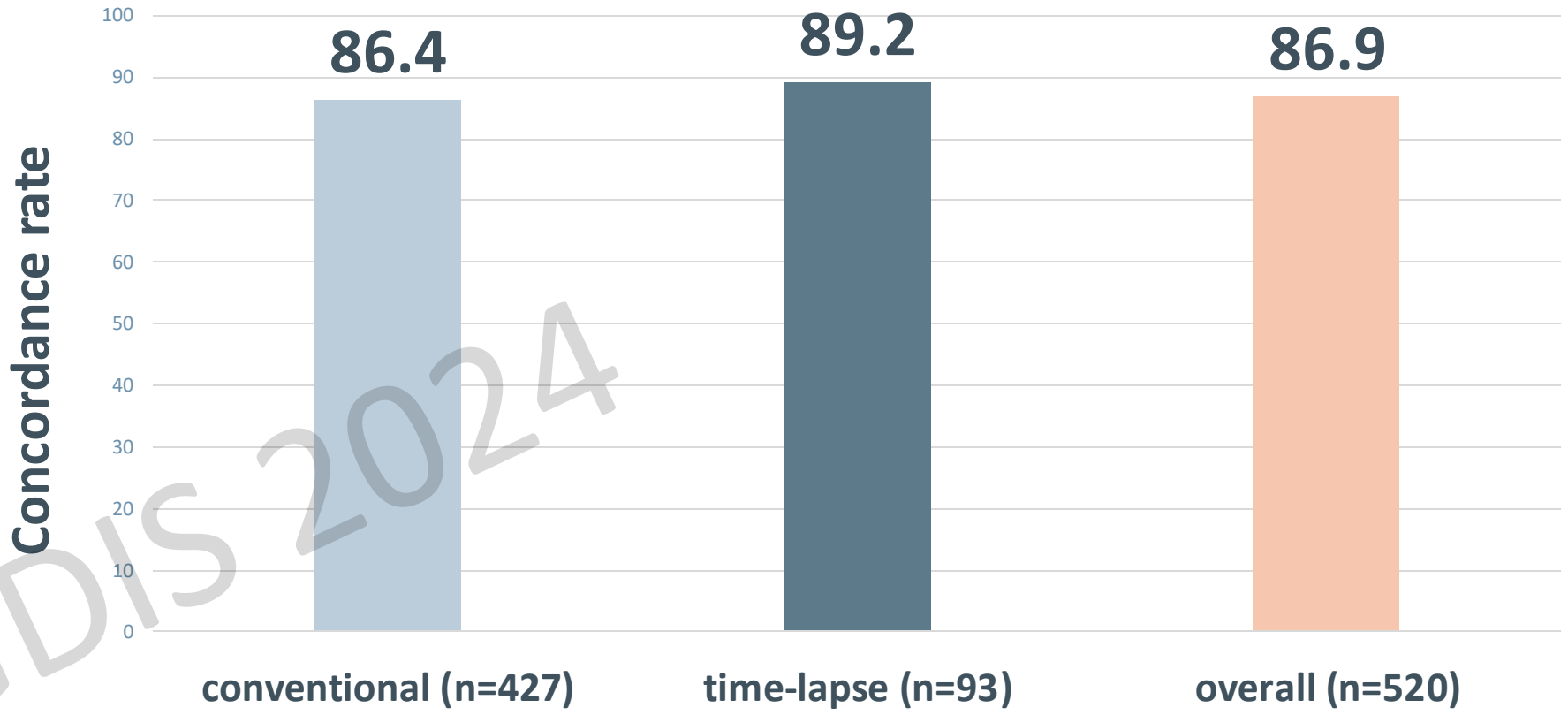
CULTURE MEDIA



Prospective Multicenter Concordance Study (NCT03520933)

Concordance rates in validations according to incubator type

IGX unpublished results



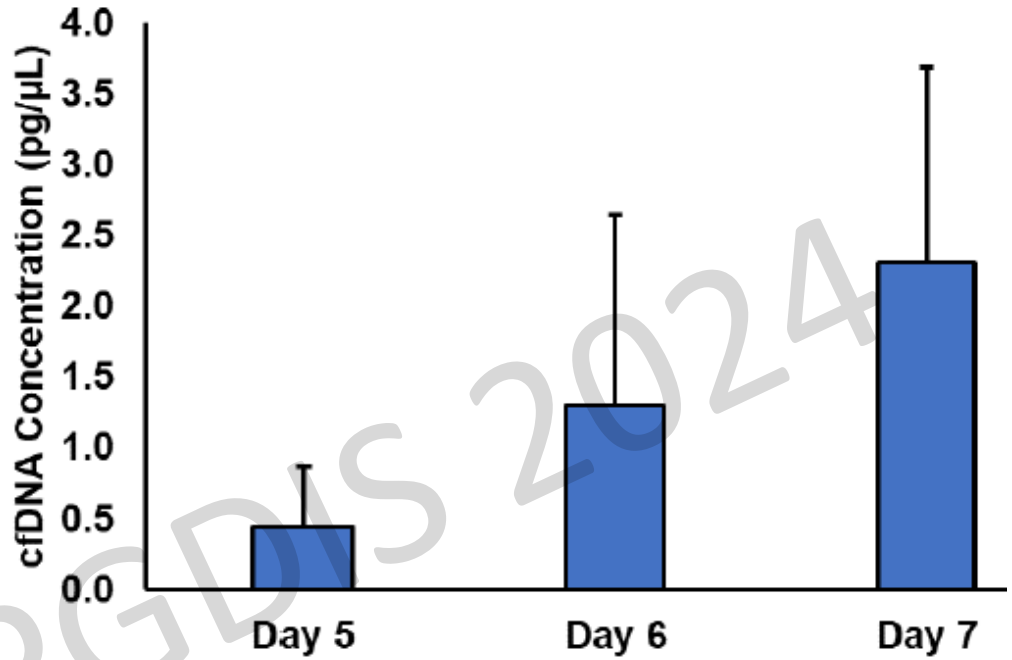
Timing for media collection (fresh blastocysts)

Assessment of cell-free DNA from embryo spent media and its effect on non-invasive PGT-A concordance to conventional PGT-A and calculated copy number noise

Jacob Meyers[1], Julie Laliberté[1], Nao Yasuyama[1], Christopher Sifuentes[1], Wenwen Xiang[1], Karthikeyan Swaminathan[1], Christo Zouves[2], Frank Barnes[2], Isabel Gonzalez[2], Andrea Victor[2], Manuel Viotti[2], Kajal Choudhary[1], Patrick Martin[1], Hui Xu[1] & Andrew Farmer[1]

[1]Takara Bio USA, Inc., Mountain View, CA 94043, USA; [2]Zouves Foundation for Reproductive Medicine, Foster City, CA

ZOUVES FOUNDATION FOR REPRODUCTIVE MEDICINE

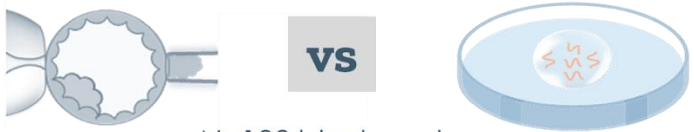


Conclusions

1. The average concentration of cfDNA in spent media is 1.1 pg/µL
2. The concentration of cfDNA in spent media increases with the day of media collection from Day 5 to Day 7.
3. Spent media from aneuploid embryos do not have significantly more cfDNA compared to euploid embryos.
4. cfDNA in spent media is moderately fragmented, which does not vary with day of media collection or between euploid vs aneuploid embryos.
5. Clinical concordance of the Takara Bio niPGT-A compared to conventional PGT-A using VeriSeq PGS is 72%, with a trend of increased niPGT-A vs PGT-A clinical concordance with the day of media collection.
6. The cfDNA input into the niPGT-A assay correlates to the calculated chromosome number noise, measured as DLRS, in the CNV plot, with significant improvement in CCN noise as DNA input increases to 10 pg.
7. The fragmentation score of cfDNA does not affect CCN noise (data not shown).

Meyers J et al. *Fertil Steril*. 2020; **114**(3Suppl.):e422.

Timing for media collection (fresh blastocysts)



N=108 blastocysts

Consistent results with media collected on D6

RESULTS DAY 5

TE BIOPSY vs. SBM

(N=27)

INFORMATIVITY	CONCORDANCE
81.8%	63%

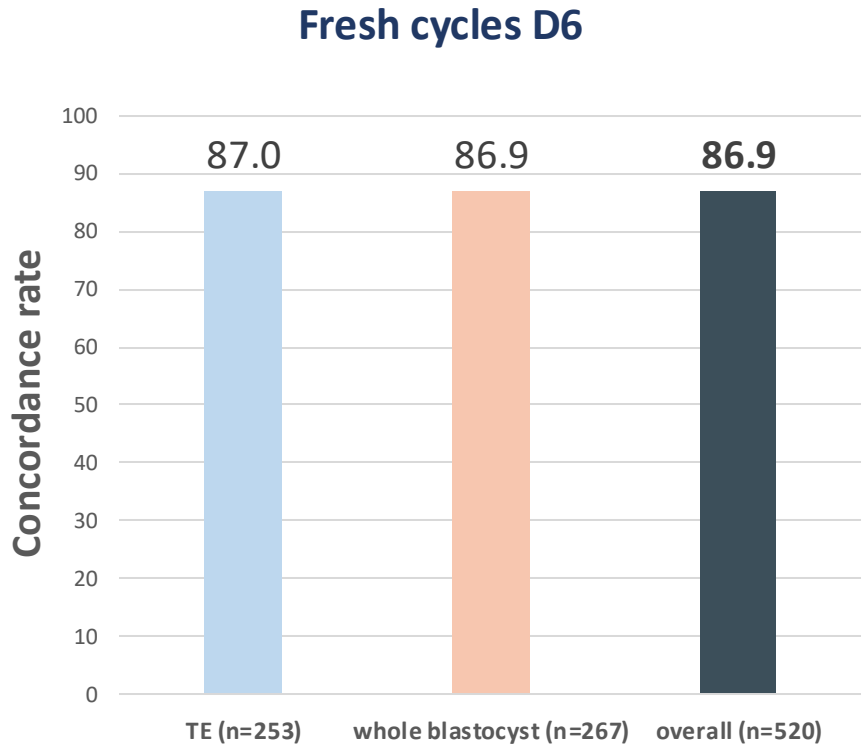
RESULTS DAY 6

TE BIOPSY vs. SBM

(N=81)

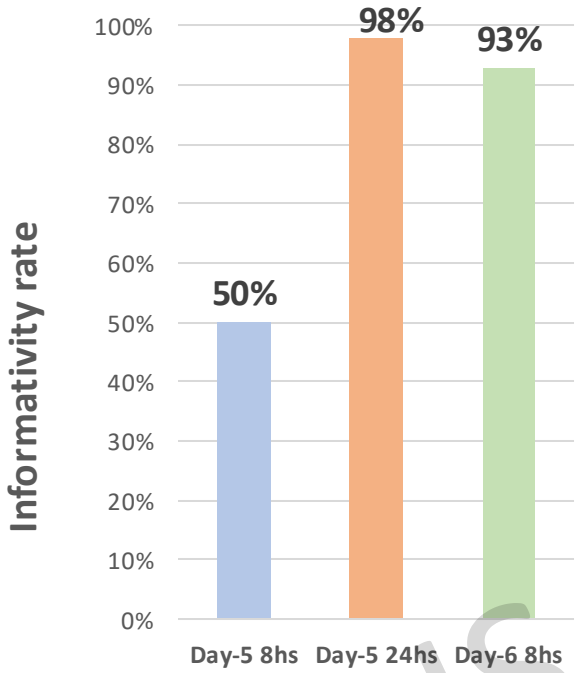
INFORMATIVITY	CONCORDANCE
100%	84%

Rubio et al. *Fertil Steril*. 2019; **112**(3):510-519.



Igenomix unpublished validation results

Timing for media collection (frozen blastocysts)

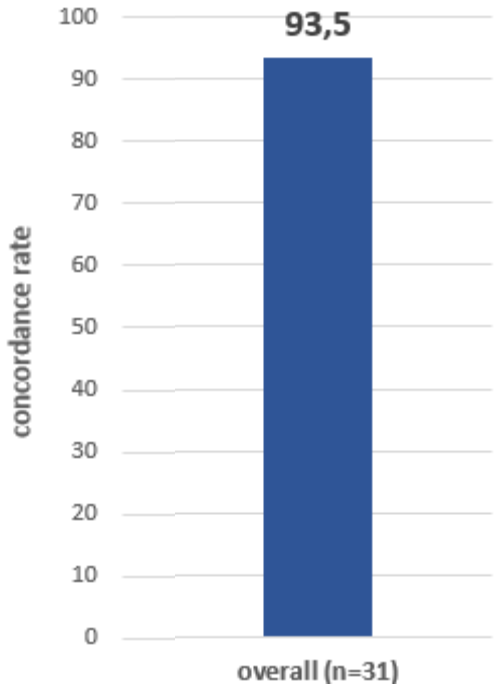


Three timings for medium collection:

- Day 5 → 8 hrs
- Day 5 → 24 hrs
- Day 6 → 6-8 hrs

Ardestani et al., Fertil Steril, Accepted April 2024

	PGT-A + No PGT-A		
	Day-5/8hs	Day-5/24hs	Day-6/8hs
Number of samples	42	40	35
Concordance	**90.5%**	**91.9%**	**90%**
Total	71.5%	78.4%	66.7%
Partial	19%	13.5%	23.3%
False negative	0%	0%	0%
False positive	9.5%	8.1%	10%



Igenomix unpublished validation results

Concerns on timing for media collection

THE IMPACT OF IMPLEMENTING A NONINVASIVE PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (NIPGT-A) PROTOCOL ON OUTCOMES

Olcay Ocali, Pam Jarmuz, Goli Ardestani, Brianna Amaral, Brian French and Denny Sakkas

Boston IVF, Waltham, MA

BOSTONIVF

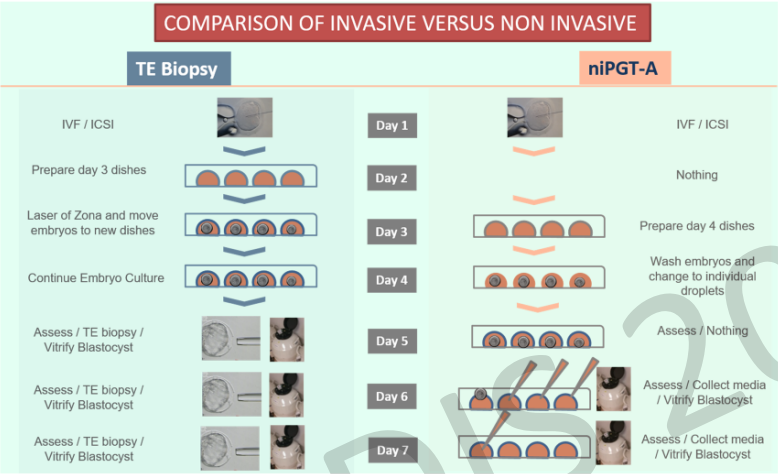


Figure 1. Comparison of the invasive versus non invasive PGT-A protocols. In the current experiments blastocysts were also biopsied after the non invasive PGT protocol.

	niPGT-A	Invasive PGT-A
Number of Patients	83	452
Day and number of Blastocysts Frozen (% blastocysts per 2 pronuclei)		
Day 5	0 (0)	863 (17.7)
Day 6	373 (39.0)	1128 (23.1)
Day 7	36 (3.8)	137 (2.8)
Total	409 (42.8)	2128 (43.6)
Number Euploid (% per biopsied)	237 (57.9)	1223 (57.5)
Number Transfers from PGT cycle	70	292
% Miscarriage Rate	4.3	6.2
% Live Birth Rate	65.7	61.0

Table 1. Clinical outcomes comparing the culture protocols used for invasive versus non invasive PGT-A.

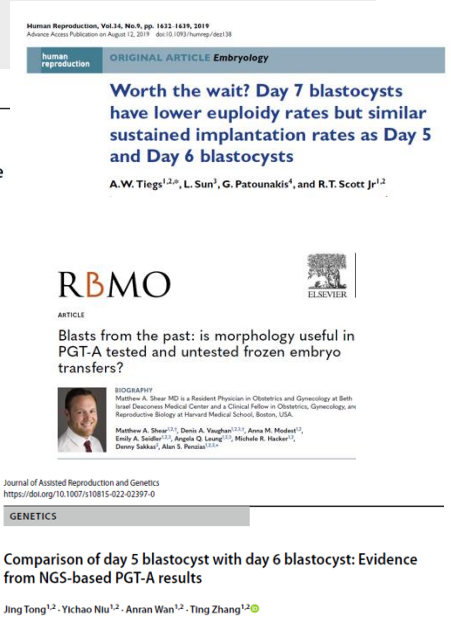
ASRM 2021

Extended culture to day-6, and culture in small microdroplets did not impact live birth rates: 65.7% study group vs. 61.0% conventional PGT-A


Journal of Assisted Reproduction and Genetics
https://doi.org/10.1007/s10815-022-02397-0
GENETICS

Comparison of day 5 blastocyst with day 6 blastocyst: Evidence from NGS-based PGT-A results

Jing Tong[1,2] · Yichao Niu[1,2] · Anran Wan[1,2] · Ting Zhang[1,2]


Human Reproduction, Vol.34, No.9, pp. 1632–1639, 2019
human reproduction
ORIGINAL ARTICLE Embryology

Worth the wait? Day 7 blastocysts have lower euploidy rates but similar sustained implantation rates as Day 5 and Day 6 blastocysts

A.W. Tiegs[1,2,#], L. Sun[1], G. Patounakis[4], and R.T. Scott Jr[1,2]

Comparison of day 5 blastocyst with day 6 blastocyst: Evidence from NGS-based PGT-A results

Jing Tong[1,2] · Yichao Niu[1,2] · Anran Wan[1,2] · Ting Zhang[1,2]

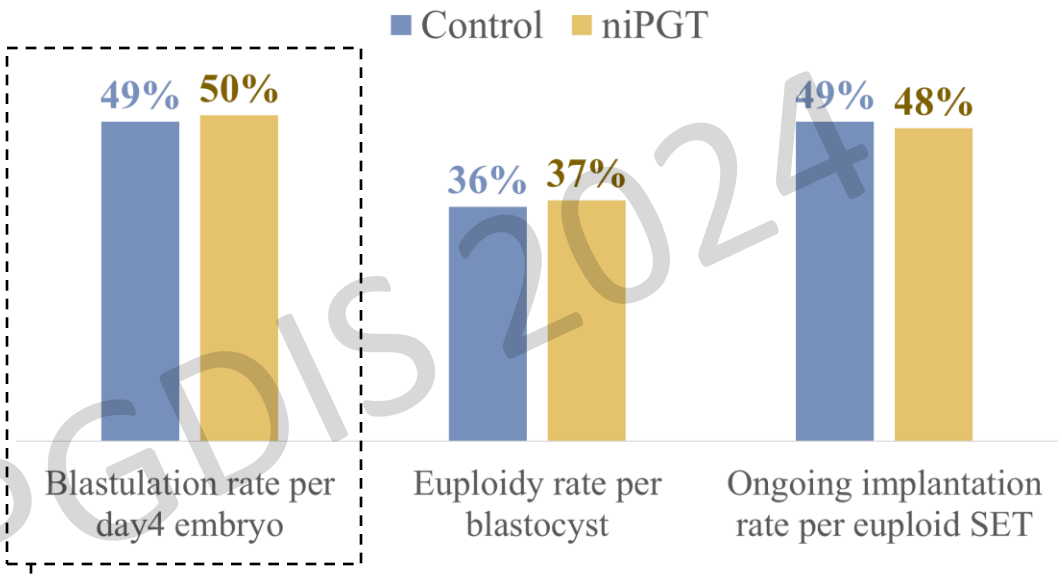
Concerns on timing for media collection

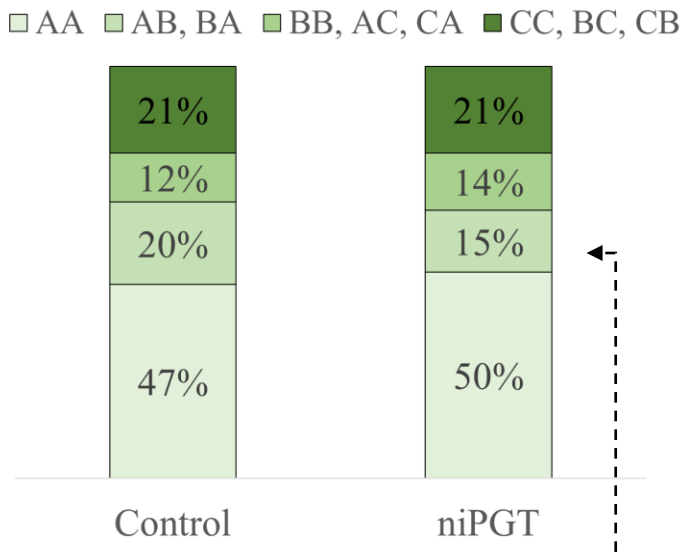
ALPHA 2022

IVF culture media refresh in a reduced volume on day4 aimed at improving non-invasive embryo selection does not affect embryo competence: a prospective analysis of 2605 embryos

R. Maggiulli[1], D. Cimadomo[1], A. Giancani[1], D. Soscia[1], L. Albricci[1], C. Rubio[2], C.M. Garcia-Pascual[2], L. Navarro-Sanchez[2], A. Capalbo[3], C. Simòn[2], F.M. Ubaldi[1], L. Rienzi[1]

This study supports single embryo culture in a reduced volume of media from day-4 till day-6





Diagnostic rules & algorithms

Noninvasive preimplantation genetic testing for aneuploidy in spent medium may be more reliable than trophectoderm biopsy

Lei Huang[a,b], Berhan Bogale[b], Yaqiong Tang[c,d], Sijia Lu[e], Xiaoliang Sunney Xie[a,c,d,1], and Catherine Racowsky[b,1]

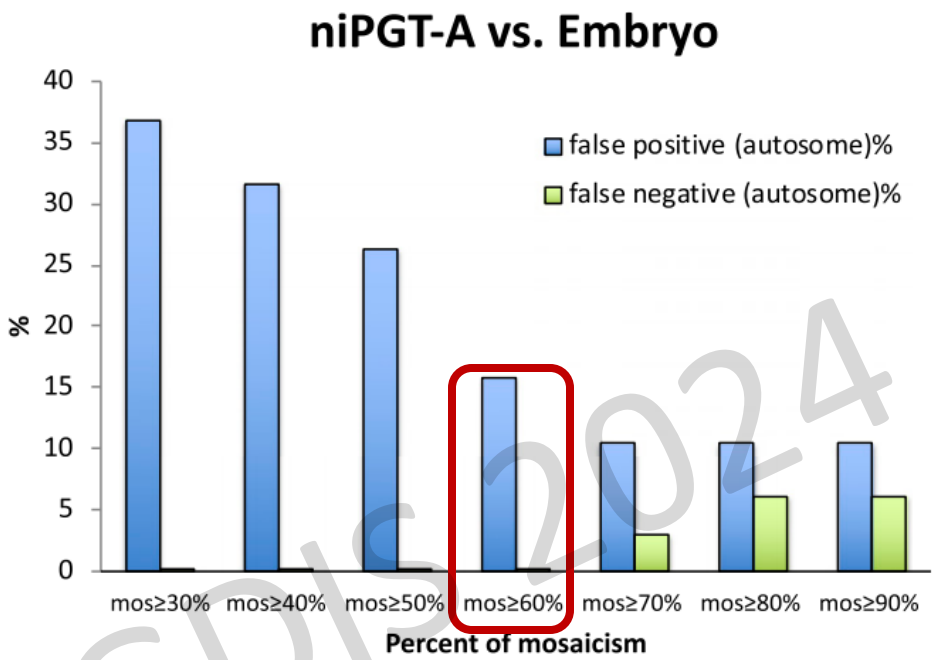


Fig. 4. FPR and FNR as a function of the percent mosaicism in niPGT-A profiles. Sixty percent of mosaicism was set as the threshold for identifying aneuploidy.

Human Reproduction, pp. 2020–2034, 2021
doi:10.1093/humrep/deab080

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

Non-invasive preimplantation genetic testing for putative mosaic blastocysts: a pilot study

Xinyuan Li [1,2,3,†], Yan Hao[1,2,3,†], Dawei Chen[1,4,5], Dongmei Ji[1,4,5], Wanbo Zhu[6], Xiaoqian Zhu[1,4,5], Zhaolian Wei[1,4,5], Yunxia Cao[1,4,5], Zhiguo Zhang [1,2,3,*], and Ping Zhou [1,2,3,*]

Raw data→ LM: 20-50%
Adjusted→ Abnormal >50%

Table III Comparison of the diagnostic parameters of SCM and TE re-biopsy.

			Concordance	Sensitivity	Specificity	FPR	FNR	PPV	NPV	PLR	NLR	DOR
Raw	SCM (n = 27)	Per overall ploidy	74.4%	100%	69.7%	30.3%	0	37.5%	100%	3.3	0	–
		Per single chromosome	96.2%	100%	96.1%	3.9%	0	20%	100%	25.8	0	–
	Re-biopsy (n = 27)	Per overall ploidy	82%	100%	78.8%	21.2%	0	46.2%	100%	4.7	0	–
		Per single chromosome	98.0%	77.8%	98.1%	1.9%	22.2%	28%	99.8%	40.1	0.2	176.8
Adjusted	SCM (n = 27)	Per overall ploidy	87.2%	83.3%	84.9%	15.2%	16.7%	50%	96.6%	5.5	0.2	28
		Per single chromosome	98.8%	88.9%	98.9%	1.1%	11.1%	44.4%	99.6%	82.4	0.1	733.6
	re-biopsy (n = 27)	Per overall ploidy	85%	100%	81.8%	18.2%	0	50%	100%	5.5	0	–
		Per single chromosome	98.3%	77.8%	98.5%	1.5%	22.2%	33.3%	99.8%	51.5	0.2	228.3

Sensitivity, (true positives)/(true positives + false negatives); Specificity, (true negatives)/(true negatives + false positives); FPR, false positive rate = (false positives)/(true negatives + false positives); FNR, false negative rate = (false negatives)/(true positives + false negatives); PPV, positive predictive value = (true positives)/(true positives + false positives); NPV, negative predictive value = (true negatives)/(true negatives + false negatives); PLR, positive likelihood ratio = Sensitivity/FPR; NLR, negative likelihood ratio = FNR/Specificity; DOR, diagnostic odds ratio = (true positives * true negatives)/(false positives * false negatives).

Diagnostic rules & algorithms

Rubio et al. *AJOG*. 2020; **223**(5):751.e1-751.e13.

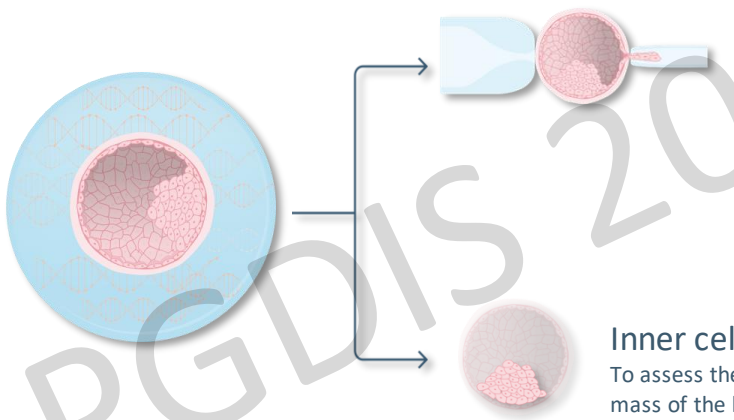
OBSTETRICS

Multicenter prospective study of concordance between embryonic cell-free DNA and trophectoderm biopsies from 1301 human blastocysts

Carmen Rubio, PhD[1]; Luis Navarro-Sánchez, PhD[1]; Carmen M. García-Pascual, PhD; Olcay Ocali, BS; Danilo Cimadomo, PhD; William Venier, MSc; Gerardo Barroso, MD; Laura Kopcow, MD; Mustafa Bahçeci, MD; Marcos Iuri Roos Kulmann, BSc; Lourdes López, MD; Emilio De la Fuente, MSc; Roser Navarro, MSc; Diana Valbuena, MD, PhD; Denny Sakkas, PhD; Laura Rienzi, MSc; Carlos Simón, MD, PhD

Specific thresholds/chr Aneuploidy calling

Prioritization for Embryo Transfer

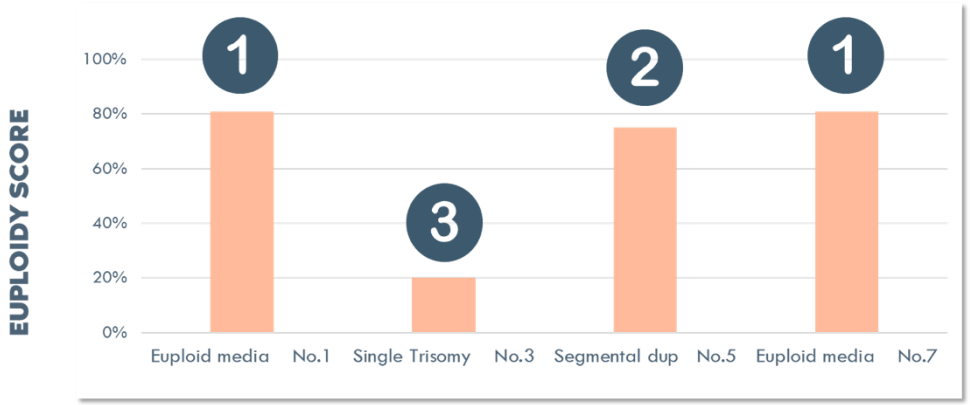


Trophectoderm DNA

To evaluate the concordance and reproducibility of testing embryo cell-free DNA versus TE biopsies from day 6 and day 7 human blastocysts,

Inner cell mass DNA

To assess the concordance rates with the inner cell mass of the blastocysts donated for research.



Prospective Multicenter Concordance Study (NCT03520933)

Acknowledgements

Igenomix R&D team

Embryo Research team
Medical and clinical studies team
Bioinformatics team

Carlos Simón Foundation

Igenomix®
PART OF VITROLIFE GROUP®

Igenomix®