Non-invasive PGT-A: What´s next?

Carmen Rubio, PhD

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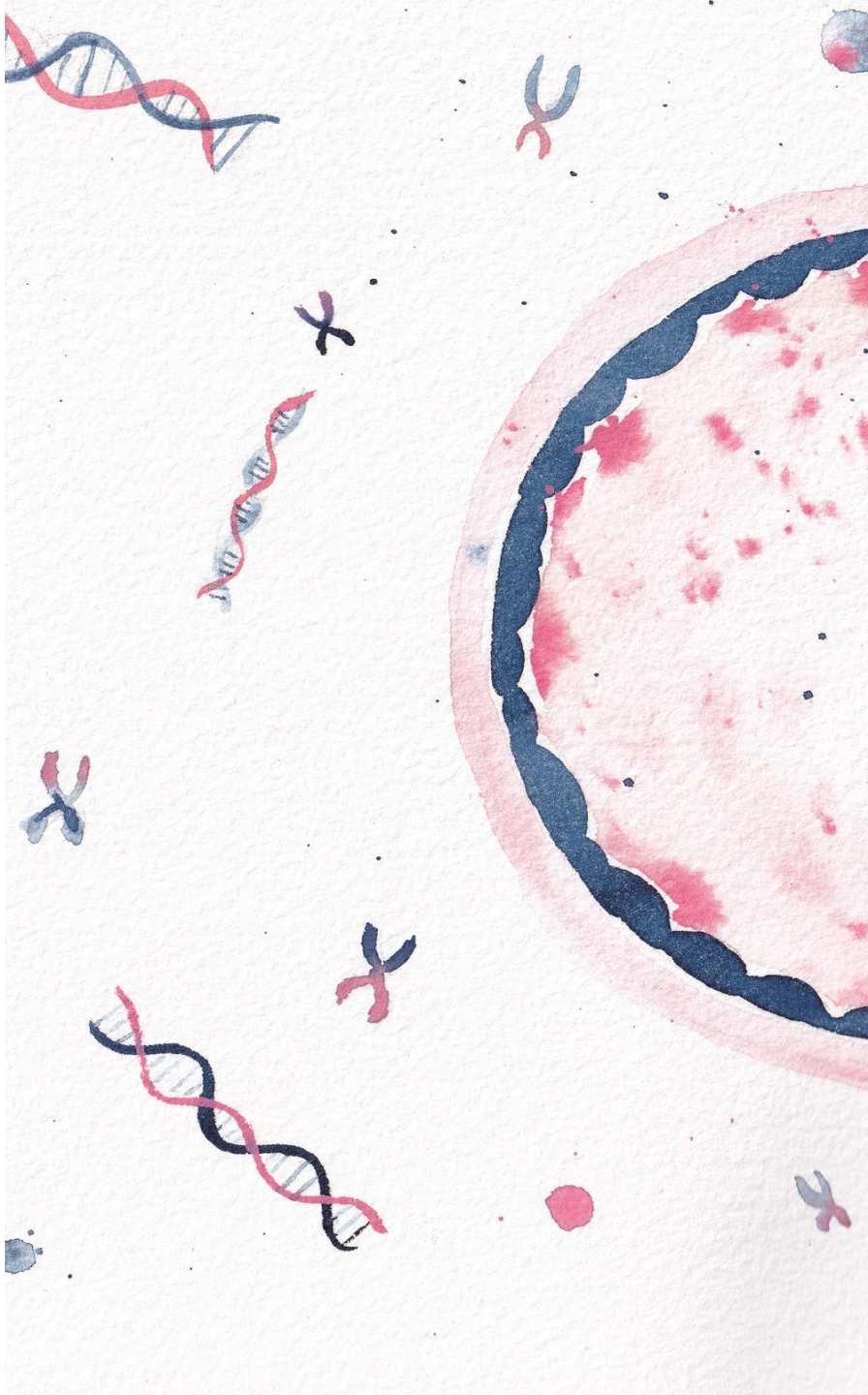
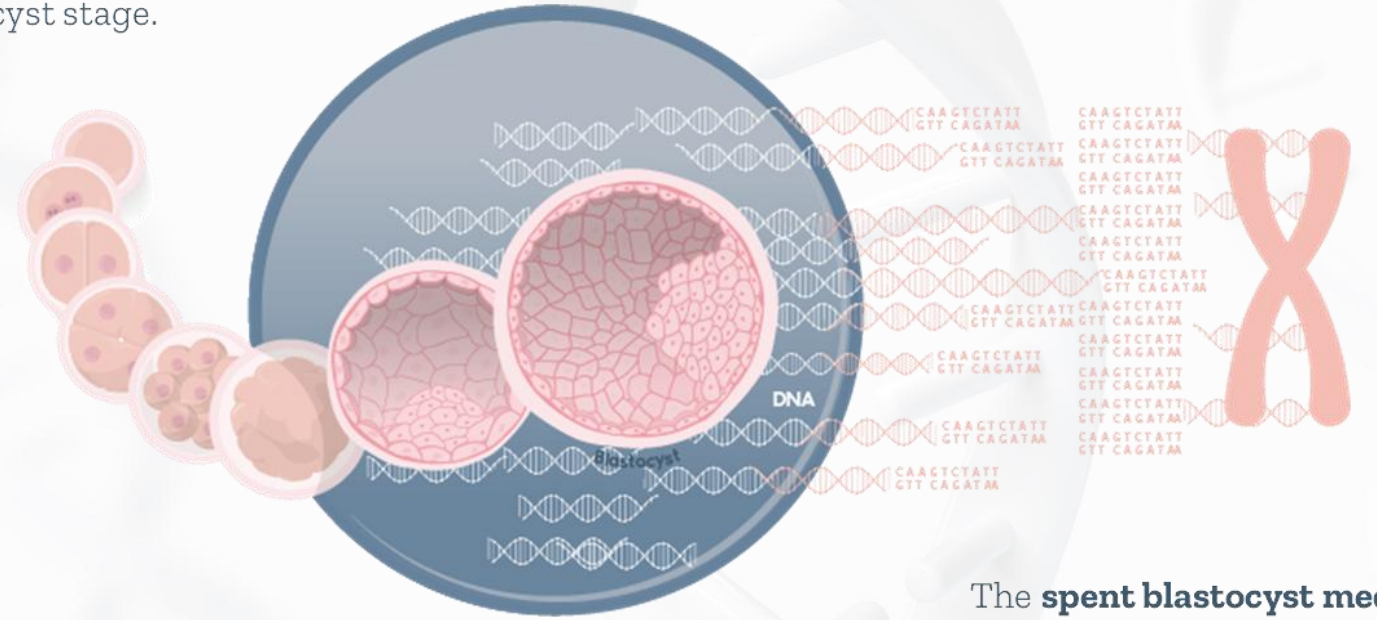


Embryo cell-free DNA is released during embryo development

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.

niPGT-A concordance studies vs TE biopsies

Authors	No. of SBM	Informative media	Ploidy concordance TE-SBM	False positives	False negatives	Embryo manipulation	Time in culture	WGA method	PGT-A technique
hamonki et al., 2016	57	96.5%	33.3%	-	-	AH on D3	D3-D5/6	Repli-G (Qiagen)	aCGH (Agilent Technologies)
Feichtinger et al., 2017	22	81.8%	72.2%	5.6%	22.2%	PB biopsy, AH on D3	D0-D5/6	SurePlex (Illumina)	aCGH (Illumina)
Vera-Rodríguez et al., 2018	56	91.1%	33.3%	-	66.7%	AH on D3	D3-D5	Sureplex (Illumina) + ReproSeq (Thermo)	NGS (Thermo)
Ho et al., 2018	41	97.6%	65.0%	-	-	AH on D3 vs no AH	D1 to D5	Picoplex (Rubicon)	NGS (Thermo)
Huang et al., 2019	52	92.3%	**89.1%**	2.2% (1/46)	8.7%	AH on D3, TE biopsy plus vitrification on D5/6	D5-D6; D6-D7 24h culture after thawing	MALBAC (Yikon)	NGS (Illumina)
Yeung et al., 2019	168	69.0% D5: 55.6% D6: 84.6%	73.3% D5: 76% **D6: 71.2%**	12.9% D5: 12% D6: 13.6%	13.8% D5: 12% D6: 15.2%	AH on D3	D3-D5 D3-D6	Sureplex (Illumina)	NGS (Illumina)
Rubio et al., 2019	115	93.9% D5: 81.8% D6/7: 98.8%	78.7% D5: 63% **D6/7: 84%**	13.9% D5: 29.6% D6/7: 8.6%	2.8% D5: 3.7% D6/7: 2.5%	NO	D4-D5 D4-D6/7	Reproseq (Thermo)	NGS (Thermo)
Rubio et al., 2020	1301	85.2%	78.2%	12.4%	8.3%	NO	D4-D6/7	Reproseq (Thermo)	NGS (Thermo)
Lledo et al., 2021	92	92.4%	74.7% or 72.3%	12.0% or 15.7%	13.3% or 12.0%	AH on D3	D3-D5/6	MALBAC (Yikon) or Sureplex (Illumina)	NGS (Illumina)
Shitara et al., 2021	20	95%	88.9%	5.6%	5.6%	Vitrified D5/6 embryos Zona pellucida removed	24h for D5 3h for D6 blastocysts	Sureplex (Illumina)	NGS (Illumina)
Hanson et al., 2021	166	62.7% D5: 17.6% D6/7: 74.2%	63.5% 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%	AH on D3	D5: 24-48h D6: 48-72h D7: 72-96h	MALBAC (Yikon)	NGS (Illumina)
Chen et al., 2021	265	96.6%	74.2%	14.5%	11.3%	NO	D3-D5/6	MALBAC (Yikon)	NGS (Illumina)
Lei et al., 2022	113	98.2%	68.5%	-	-	AH on D3	D3-D5/6	MALBAC (Yikon)	NGS (Illumina)
Xie et al., 2022	161	91.3% D5: 81%, D6: 92%, D7: 100%	75%	21.5%	3.5%	NO	D4-D5/6	MALBAC (Yikon)	NGS (Illumina)
Xu et al., 2023	35	74.3%	58.3%	33.3%	8.3%	Previously vitrified on D3 or D5	D3-D5/6 or D5 +24h	PicoPLEX (Takara)	NGS (Basecare)
Handayani et al., 2024	28	92.9%	30.8%	0%	50%	AH on D4	D0-D5/6	Sureplex (Illumina)	NGS (Illumina)
Takeuchi et al., 2024	35	80.0%	71.4%	21.4%	7.1%	Previously vitrified on D4 or D5. Some also AH. Some ZP removed	D4: 24h D5: 8, 16 or 24h	Reproseq (Thermo)	NGS (Thermo)
Bednarska-Czerwińska et al., 2024	143	99.3%	83.7% D5: 79.7% **D6: 87.5%**	12.8% D5: 20.3% D6: 5.6%	3.5% D5: 0% D6: 6.9%	NO	D4-D5/6	Sureplex (Illumina)	NGS (Illumina)

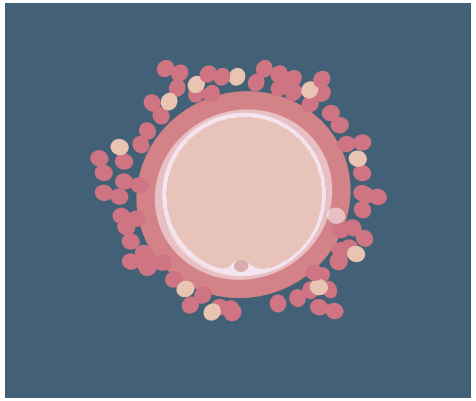
niPGT-A concordance studies vs whole blastocysts

Authors	No. of SBM	Informative media	Ploidy concordance WB-SBM	False positives	False negatives	Embryo manipulation	Time in culture	WGA method	PGT-A technique
Xu et al., 2016	42	100%	85.7%	9.5%	4.8%	Vitrification on D3	D3-D5	MALBAC (Yikon)	NGS (Illumina)
Ho et al., 2018	41	97.6%	45.5%	-	-	AH on D3 vs no AH	D1 to D5	Picoplex (Rubicon)	NGS (Thermo)
Huang et al., 2019	52	**92.3%**	**93.7%**	6.3% (3/48)	-	AH on D3, TE biopsy plus vitrification on D5/6	D5-D6; D6-D7 Cultured for 24h after thawing	MALBAC (Yikon)	NGS (Illumina)
Rubio et al., 2020	81	90.1%	84.4%	6.2 % (4/64)	9.4%	NO	D4-D6/7	Reproseq (Thermo)	NGS (Thermo)
Yin et al., 2021	75	78.7%	89.8%	10.2% (6/59)	-	Biopsy on D5/6 and vitrification	Cultured for 24h after thawing	MALBAC (Yikon)	NGS (Illumina)
Shitara et al., 2021	20	**95%**	**93.8%**	-	6.2%	Vitrified D5/6 Zona pellucida removed	24h for D5; 3h for D6 blastocysts	Sureplex (Illumina)	NGS (Illumina)
Chen et al., 2021	265	96.6%	78.1%	16.8%	5.1%	NO	D3-D5/6	MALBAC (Yikon)	NGS (Illumina)
Shi et al., 2022	212	100%	84.4%	13.2%	2.4%	artificial shrinkage before vitrification	Cultured for 18-24h after thawing	MALBAC (Yikon)	NGS (Illumina)
Sonehara et al., 2022	46	100%	Low: 59.1% High: 70.8%	Low: 13.6% High: 12.5%	Low: 22.7% High: 16.7%	NO	D3-D6/7	PG-Seq Rapid Non-Invasive kit (Perkin Elmer)	NGS (Illumina)
Xu et al., 2023	35	74.3%	61.9%	38.1%	-	Previously vitrified on D3 or D5	D3-D5/6 (n=26) or D5 +24h (n=9)	PicoPLEX (Takara)	NGS (Basecare)
Ardestani et al., 2024	135	81.5%	**92.5%**	7.5%	-	Previously vitrified on D5 or D6. Some with previous TE biopsy	8 or 24h for D5; 8h for D6	Reproseq (Thermo)	NGS (Thermo)
Takeuchi et al., 2024	35	80.0%	75.0%	21.4 %	3.6%	Previously vitrified on D4 or D5. Some also AH. Some ZP removed	D4: 24h; D5: 8, 16 or 24h	Reproseq (Thermo)	NGS (Thermo)

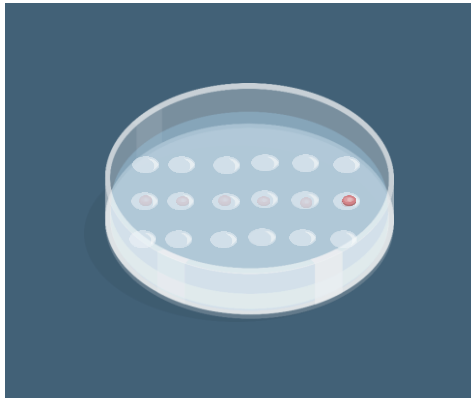
How to improve and standardize niPGT-A results

- ✓ 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

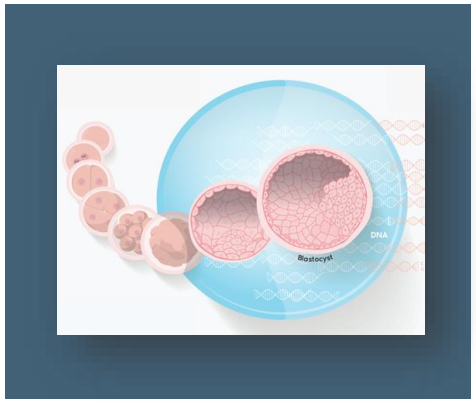
DECREASE CONTAMINATION



LAB PROTOCOL AND EMBRYO CULTURE



TIMING FOR MEDIA COLLECTION



PRIORITIZATION ALGORITHMS

Origin of the cfDNA in the culture medium

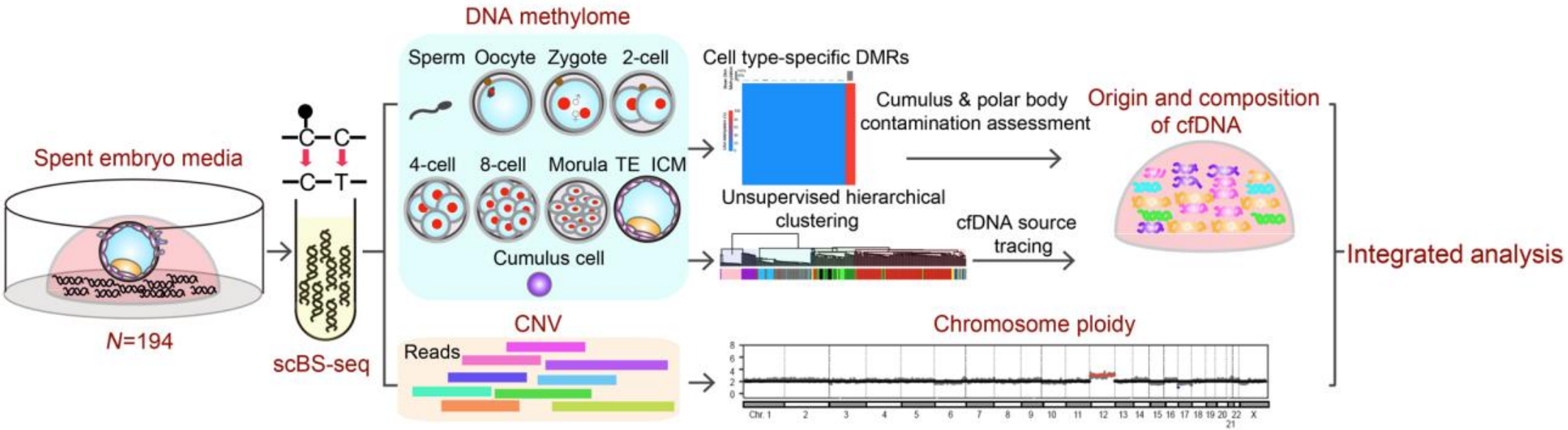
The Journal of Clinical Investigation

2021

DNA methylome reveals cellular origin of cell-free DNA in spent medium of human preimplantation embryos

Yidong Chen,[1,2,3] Yuan Gao,[1,2,3] Jialin Jia,[1,2,4,5] Liang Chang,[1,2,4,5] Ping Liu,[1,2,4,5] Jie Qiao,[1,2,3,4,5] Fuchou Tang,[1,2,3] Lu Wen,[1,2] and Jin Huang[1,2,4,5]

- ✓ Polar body contamination mainly comes from the **second polar body**: →27% SECM with PB contamination (higher or Day-5 than on Day- 6).
- ✓ On Day-6 approximately one-third of samples were positioned with TE and that approximately **two-thirds were positioned with ICM**.

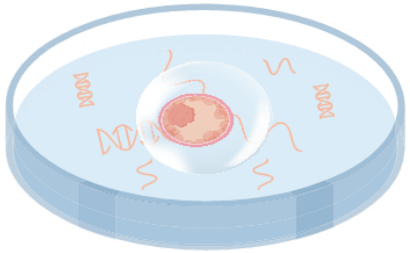


Results regarding **sampling time**→ The amplified DNA amounts were significantly higher in the Day 6 samples than in the day 5 samples samples, with lower contamination with **cumulus cells** observed on Day 6.

Tips to decrease contamination

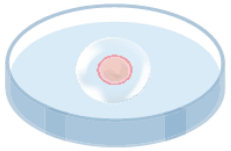
Sources of DNA CONTAMINATION

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



MCC

Day 4 embryos



Washings

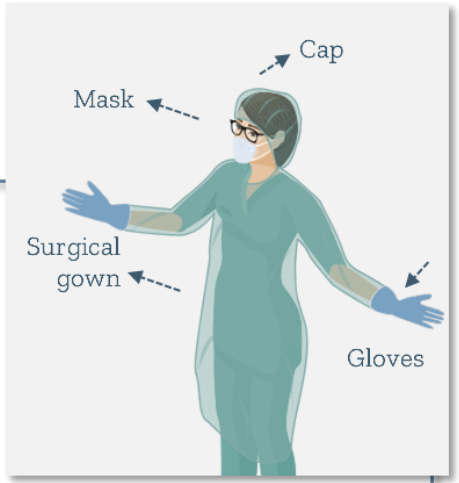
Crucial steps:
Oocyte denudation and embryo serial washes on D4, collection D6

External

Culture media

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

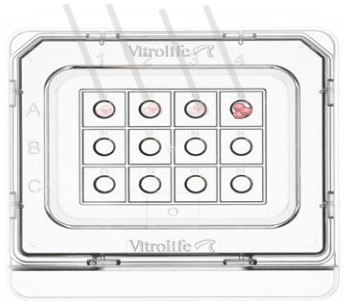
Technologist/IVF lab procedures ---------→ DNA free hood surfaces Technologist properly covered



Pre-clinical validations in each lab are crucial

IVF lab protocol and embryo culture conditions

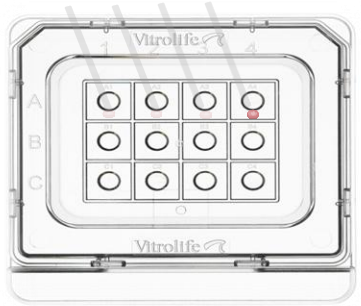
Day 0/1



Extra washes after denudation

Culture up to day 4

Day 4

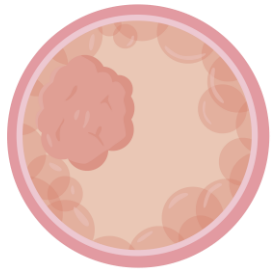


Individual extra washes

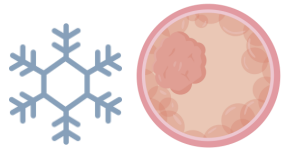
Day 4 up to day 6/7

Culture in droplets of 20-25µl

Day 6/7



Check the embryos that have reached the blastocyst stage



Blastocyst vitrification

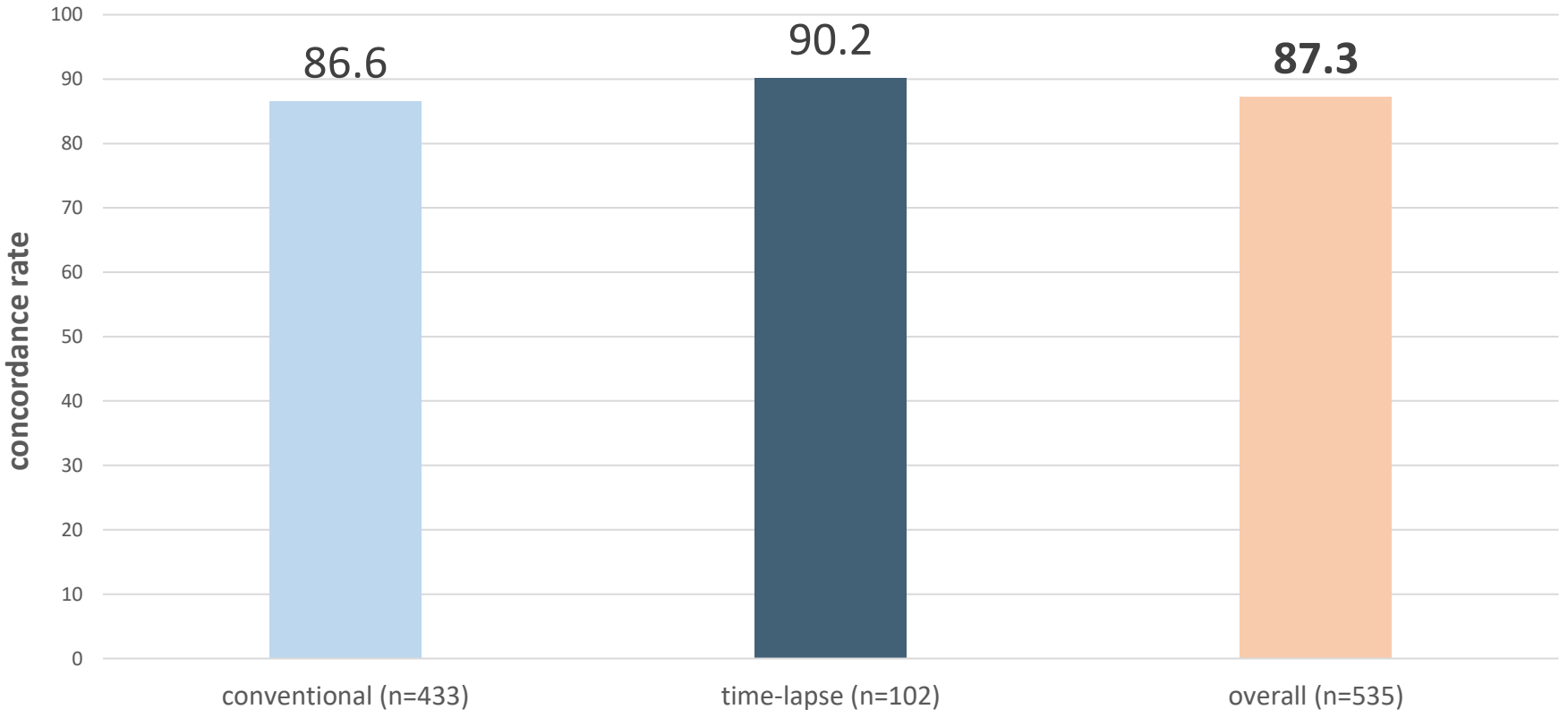
Media aspiration + Freezing at -20°C

NGS analysis

niPGT-A report & prioritization

IVF lab culture and protocol – Fresh Blastocysts

A **validation** must be carried out before starting with clinical cases: to practice and become familiar with the changes and to check that the viability of the embryo is not compromised.



Prospective Multicenter Concordance Study (NCT03520933)

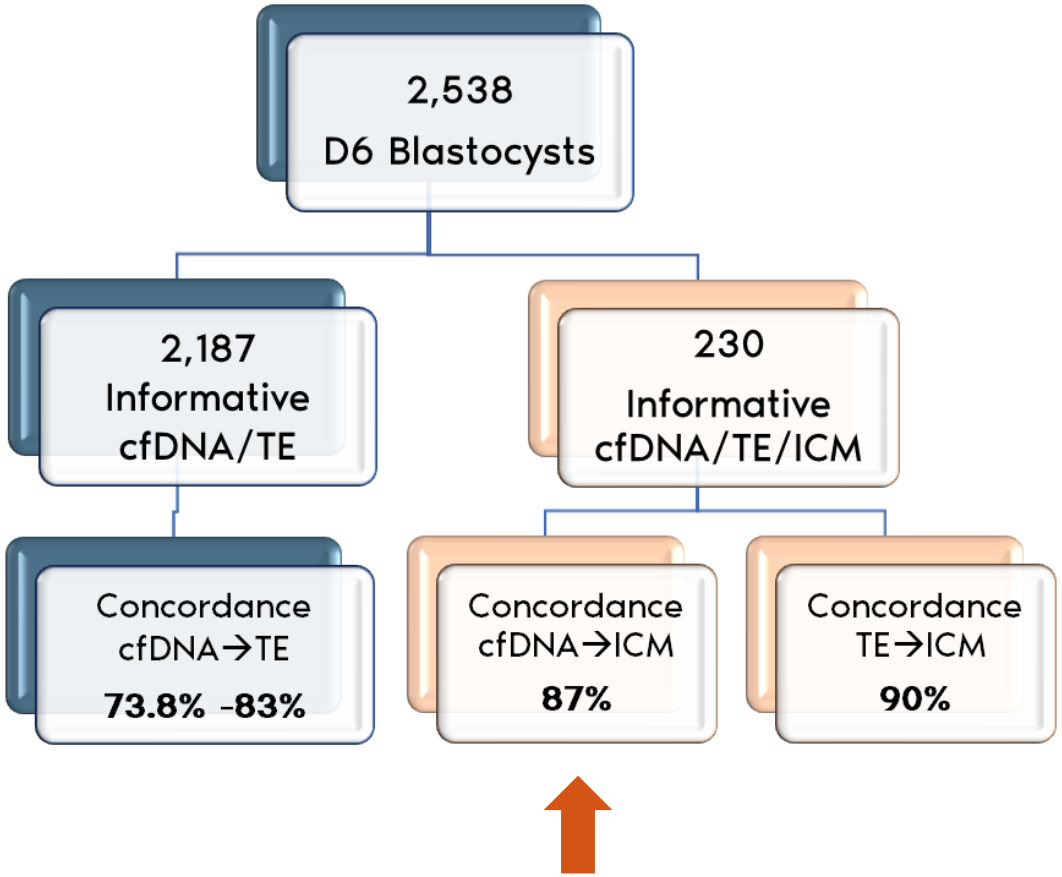
American Journal Obstetrics & Gynaecology, 2020

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

Final Study→ 2,538 blastocysts

Updated unpublished results
Publication in progress



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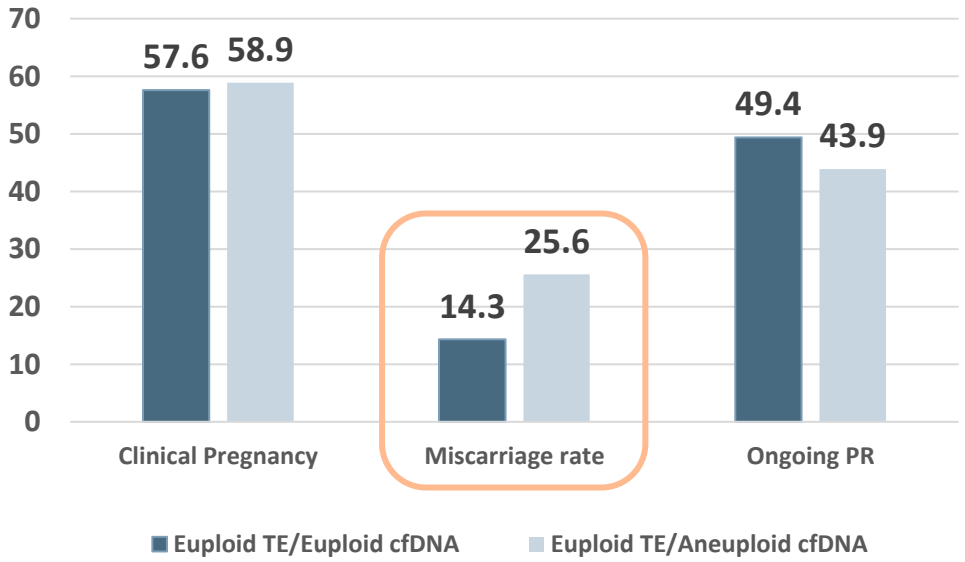
Updated Clinical results 304 SET, unpublished



	Euploid/Euploid	Euploid-Aneuploid
Number of SET	231	73
Mean age (SD)	35.4 (5.0)	34.5 (5.2)

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Prospective Multicenter Concordance Study (NCT03520933)

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

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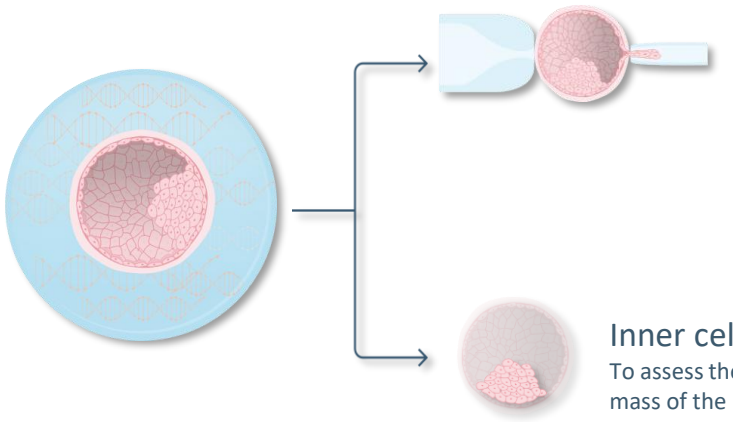
Specific thresholds/chr Aneuploidy calling

Prioritization for Embryo Transfer

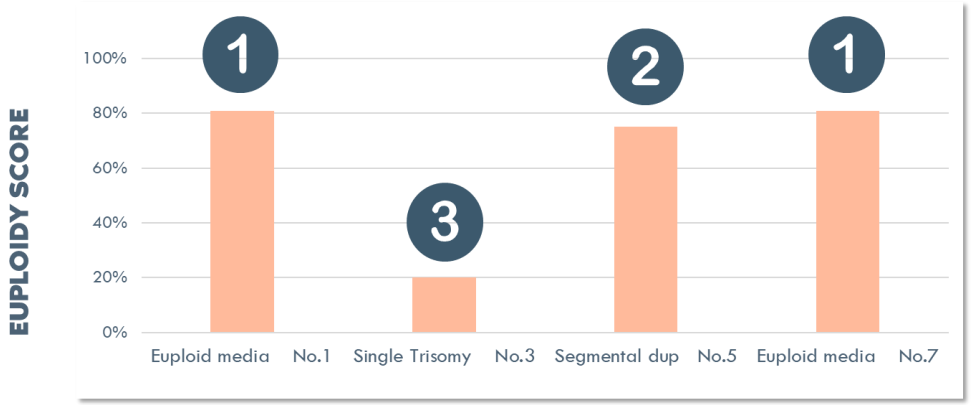


Trophectoderm DNA

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.



Impact of extending embryo culture up to day-6

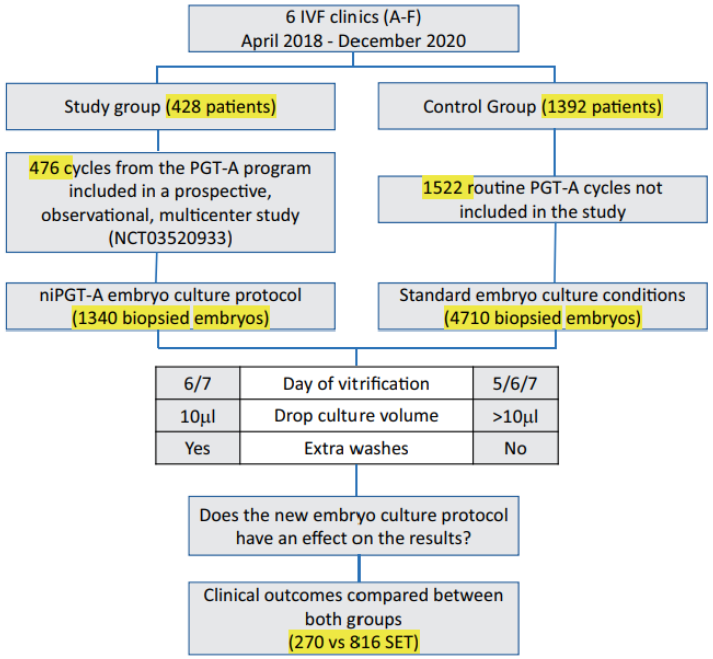
OXFORD human reproduction

Human Reproduction, 2024, 00(0), 1–8
https://doi.org/10.1093/humrep/deae156
Original Article

Embryology

The impact of implementing a non-invasive preimplantation genetic testing for aneuploidies (niPGT-A) embryo culture protocol on embryo viability and clinical outcomes

Denny Sakkas[1,*,†], Luis Navarro-Sánchez[2,*,†], Goli Ardestani[1], Gerardo Barroso[3], Claudio Bisioli[4], Kubra Boynukalin[5], Danilo Cimadomo[6], Nilo Frantz[7], Laura Kopcow[4], Gabriella Mamede Andrade[7], Bilgen Ozturk[5], Laura Rienzi[6], Ariane Weiser[3], Diana Valbuena[2], Carlos Simón[8,9,10], and Carmen Rubio[2]



Clinic A→ All embryos in study group with modified culture conditions and all extended culture to D6.

Table 3. Clinical outcomes after single embryo transfer (SET) of euploid embryos comparing standard culture versus non-invasive PGT-A culture conditions in Clinic A.

Clinic A	Control group Days 5, 6, and 7	Control group Days 6 and 7	Study group Days 6 and 7
Number of SET	265	148	64
Number of positive hCG (%)	198 (74.7%)	111 (75.0%)	49 (76.6%)
Number of clinical pregnancies (%)	180 (67.9%)	100 (67.6%)	44 (68.8%)
Number of miscarriages (%)	15 (8.3%)	10 (10.0%)	2 (4.5%)
Number of live births (%)	165 (62.3%)	90 (60.8%)	42 (65.6%)

Differences were not significant when comparing standard versus non-invasive culture.

Clinics B-F → All embryos in study group with modified culture conditions, only slow ones cultured to D6.

Table 4. Clinical outcomes after single embryo transfer (SET) of euploid embryos comparing standard culture versus non-invasive PGT-A culture conditions in Clinics (B–F*).

Rest Clinics B–F	Control group Day 5	Study group Day 5	Control group Day 6	Study group Day 6
Number of SET	284	63	244	129
Number of positive hCG (%)	205 (72.2%)	45 (71.4%)	156 (63.9%)	73 (56.6%)
Number of clinical pregnancies (%)	197 (69.4%)	42 (66.7%)	137 (56.2%)	62 (48.1%)
Number of miscarriages (%)	25 (12.7%)	5 (11.9%)	28 (20.4%)	8 (12.9%)
Number of ongoing pregnancies* (≥12 weeks) (%)	172 (60.6%)	37 (58.7%)	109 (44.7%)	54 (41.9%)

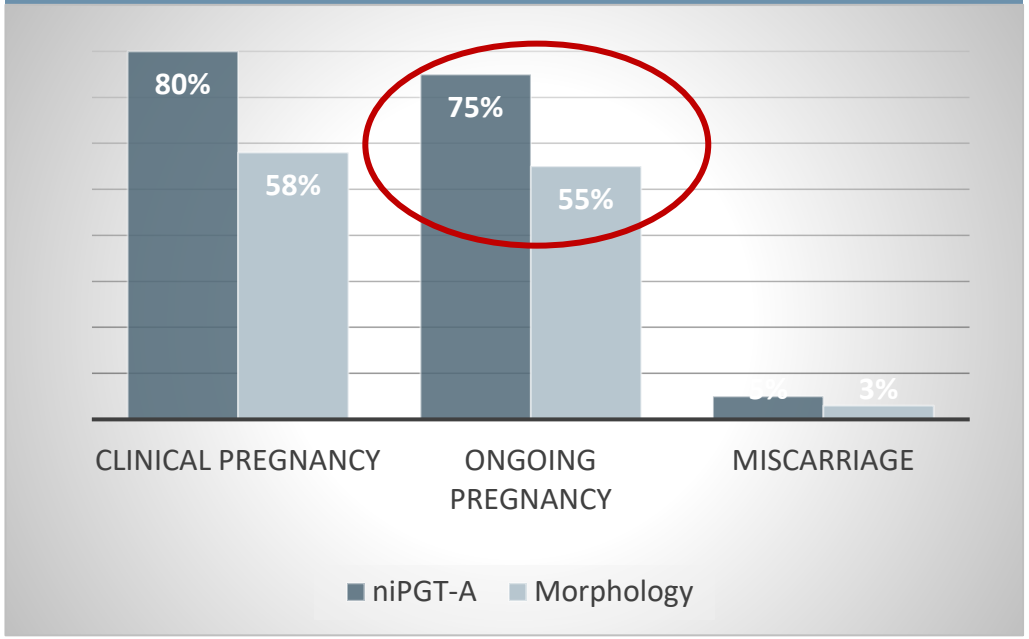
Differences were not significant when comparing standard versus non-invasive culture.
* The majority of ongoing pregnancies were followed up to live birth. Only 19 clinical pregnancies were lost to follow up after 12 weeks (15 in control and 4 in the study group).

No differences among groups, for modified culture conditions and/or extended culture to day-6.

Clinical experience 2020-2023 comparing with morphology

Yosu Franco, Ruber International (Spain).
ESHRE 2022

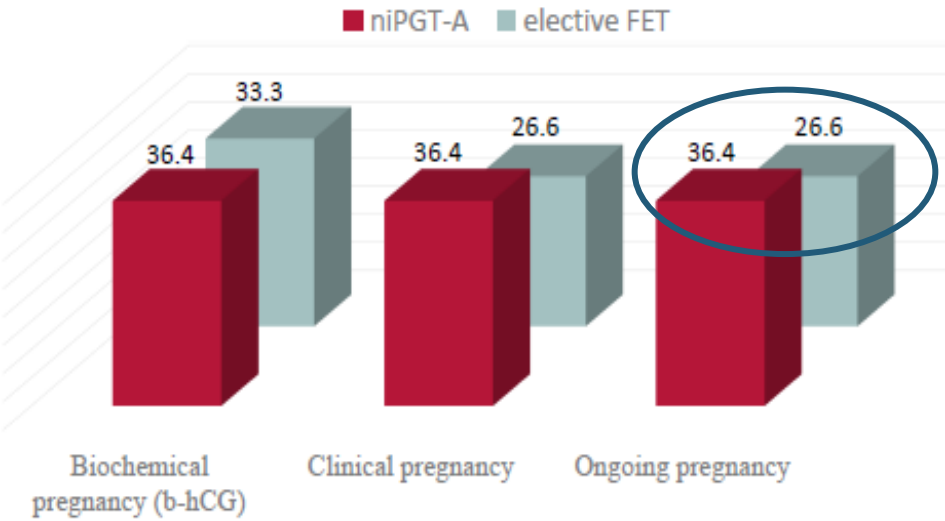
High ongoing pregnancy rate with EMBRACE in good prognosis patients < 38yrs vs. morphology embryo selection



RedLara 2023

First cases of niPGT-A performed. Better clinical outcomes for niPGT-A when compared to morphology, but not statistically significant results due to small sample size.

Figure 1: Results of the comparison of the niPGT-A group versus elective FET



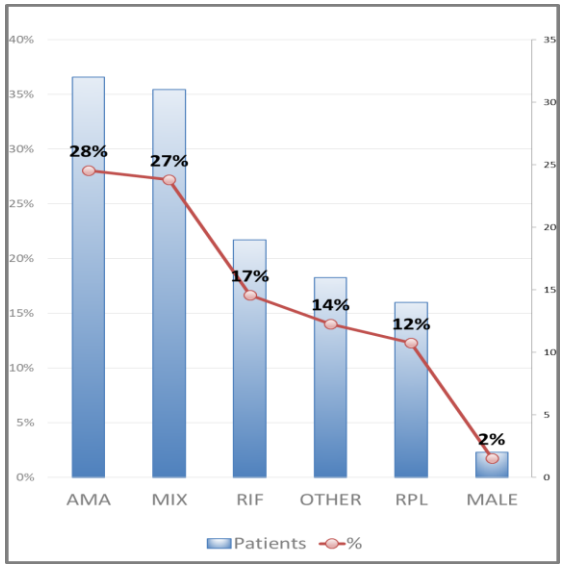
Clinical experience 2020-2023 comparing with PGT-A

Invimed (Poland). Embrace Users Meeting ESHRE 2023

Several indications

- Profile: **Poor prognosis → like PGT-A**
- > 200 EMBRACE cases up to date
- ~ 70% of patients with IVF failure history
- Average ~2,5 IVF cycles and ~ 1 IUI per patient

Distribution of indications



	PGT-A	EMBRACE
No.	201	128
Average age	38,2	36,2
Average COCs	12,70	12,30
Embryos	1285	896
Average	6,39	7,00
Blastulation rate %	**69,0%**	**67,0%**
D5 good and fair	**20,4%**	**24,5%**
Analysed embryos	504	317
Average	2,51	2,48
Informative	488	297
Informativity rate %	**96,8%**	**93,7%**
Patients with euploid	138	92
%	**69%**	**72%**
No. Euploid embryos	240	156
%	**49,2%**	**52,5%**
sFET	**PGT-A**	**EMBRACE**
FET	160	78
CP	70	34
CPR %	**43,8%**	**43,6%**

Non-selection studies in niPGT-A

SEMINAL CONTRIBUTIONS

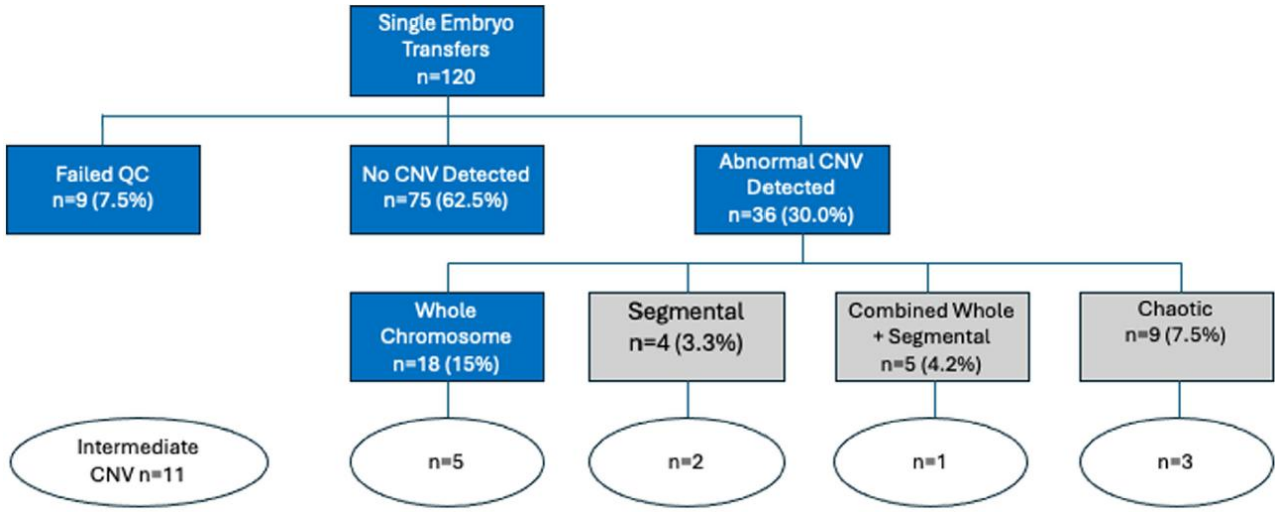
Check for updates

A pilot study to investigate the clinically predictive values of copy number variations detected by next-generation sequencing of cell-free deoxyribonucleic acid in spent culture media

Gary Nakhuda, M.D.,[a] Sally Rodriguez, Sc.M., C.G.C.,[b] Sophia Tormasi, B.Sc., T.S.,[b] and Catherine Welch, M.B.A., T.S.[b]

[a] Olive Fertility Centre, Vancouver British Columbia, Canada; [b] Sequence46, Los Angeles, California

Good Prognosis patients



Patient and cycle characteristics.

	Median	IQR
Age	32	30–34
Partner age	34	32–37.8
Oocytes retrieved	16	11–23
M2 oocytes	13	8–18
2PN	10	7–14
Blastocysts vitrified	6	3–9

IQR, interquartile range.

Nakhuda. Study of predictive values of niPGT-A. Fertil Steril 2024.

Clinical outcomes stratified by NGS interpretation.

NGS interpretation (n = 120)	Implantation %(n)	Clinical pregnancy % (n)	Sustained implantation, NPV % (n)	Total SAB % (n)
Failed QC (9)	66.7 (6)	66.7 (6)	66.7 (6)	0
No CNV (75)	78.6 (59)	64 (48)	57.3 (43)	27.1 (16)
Abnormal CNV (36)	63.9 (23)	44.4 (16)	41.2 (15)	34.7 (8)
Abnormal CNV stratified				
Whole chromosome (18)	50 (9)	11.1 (2)	5.6 (1)	88.9 (8)
Segmental (4)	100 (4)	100 (4)	100 (4)	0
Combined (5)	80 (4)	80 (4)	80 (4)	0
Chaotic (9)	66.7 (6)	66.7 (6)	66.7 (6)	0

NGS, next-generation sequencing; NPV, negative predictive value; SAB, spontaneous abortion; QC, quality control.

Nakhuda. Study of predictive values of niPGT-A. Fertil Steril 2024.

Non-selection studies in niPGT-A

Cinical Outcomes according to blinded niPGT-A results

Similar Female
Mean age (<40yrs)

Transfer of Euploid blastocysts

Euploidy Score
81%

Clinical PR: 45.7
LBR: 38.3

Transfer of Aneuploid Blastocysts

Segmentals*

Euploidy Score
71%

Clinical PR: 75.0%
LBR: 75%

Clinical PR: 46.4%
LBR: 39.2%

Whole Aut Chr

Euploidy Score
24%

Clinical PR: 30.6%
LBR:19.4%

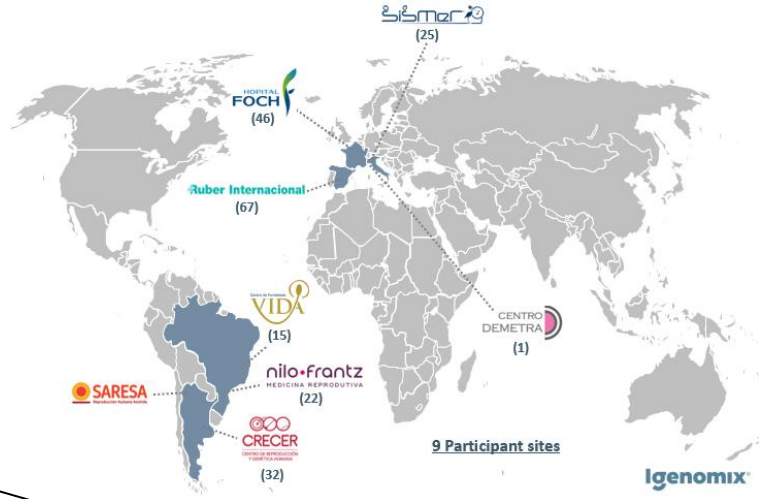
Monosomy X*

Euploidy Score
36%

Clinical PR: 33.3%
LBR: 0%

Clinical PR:
30.8%
LBR: 17.9%

Preliminary results Interim Analysis
(8 centres, 160 SETs) NCT04000152 (IGX promoted study)



Igenomix

niPGT-A in clinical practice: Where are we now?

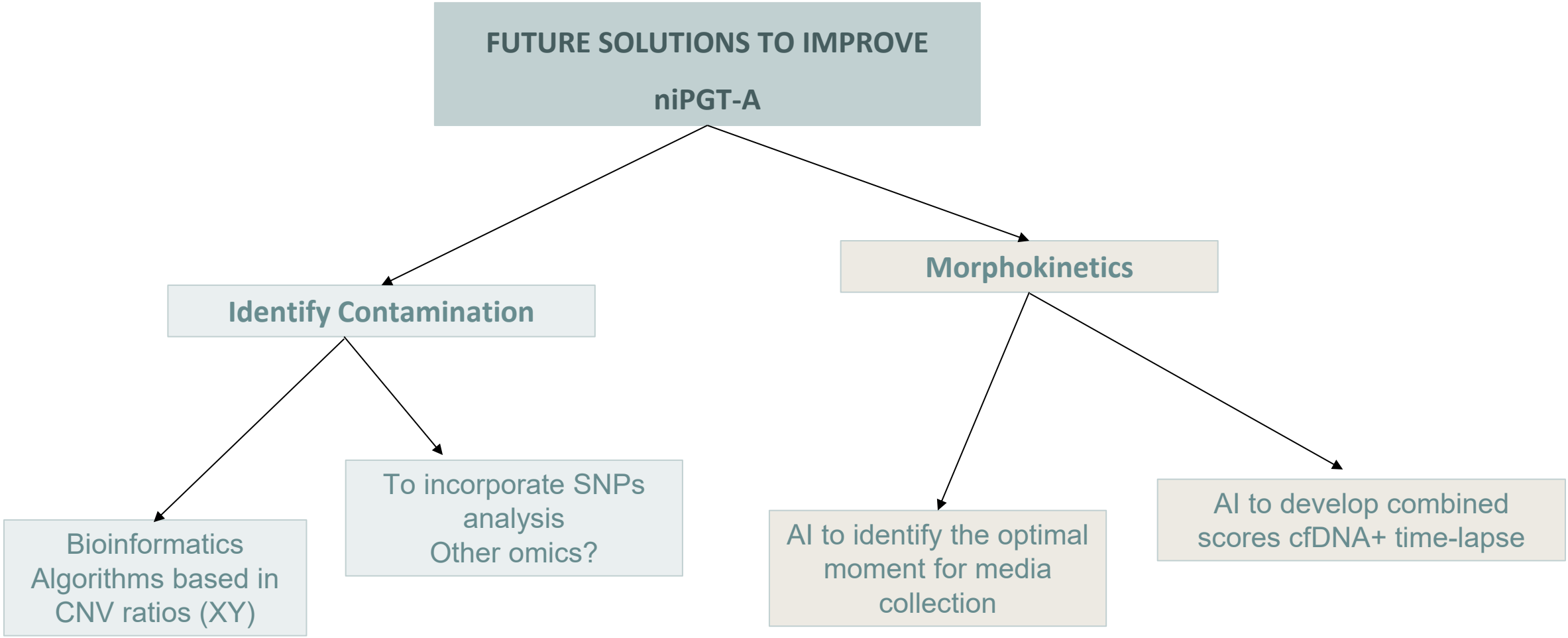
niPGT-A Prioritization Test

- *Improved informativity and concordance results*
- *Optimized laboratory workflow*
- *Low DNA yield in some embryos. When to collect the medium in these cases.*
- *Presence of non-embryonic DNA in some samples*

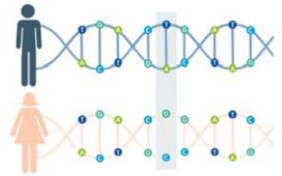
niPGT-A Diagnostic Test

- *Need to improve informativity and concordance*
- *Room for more automation in the laboratory workflow to avoid contamination*
- *Personalization of timing for media collection*
- *Detection of non-embryonic DNA*

What´s next? How to move from prioritization toward diagnosis?

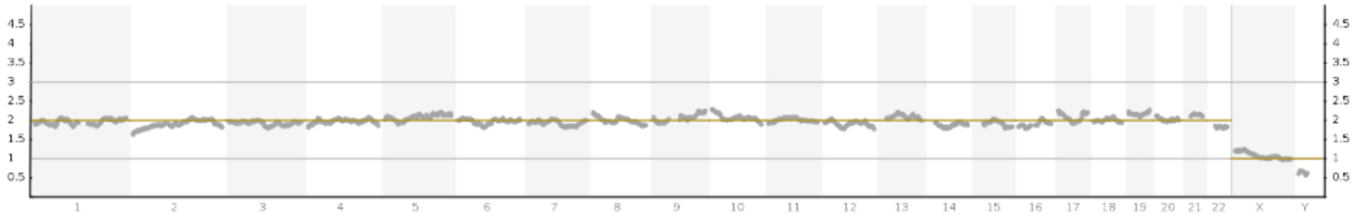


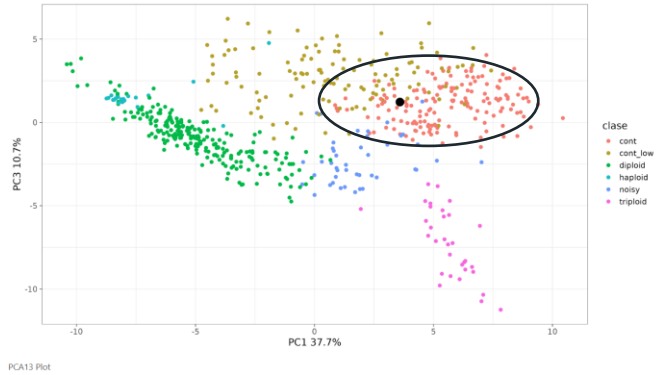
How SNPs can help to identify contamination in SBM



Proprietary algorithm for data analysis (v1.0)

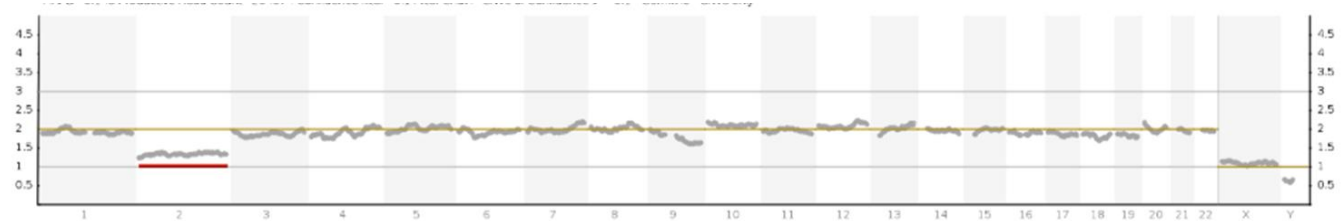
Euploid SBM

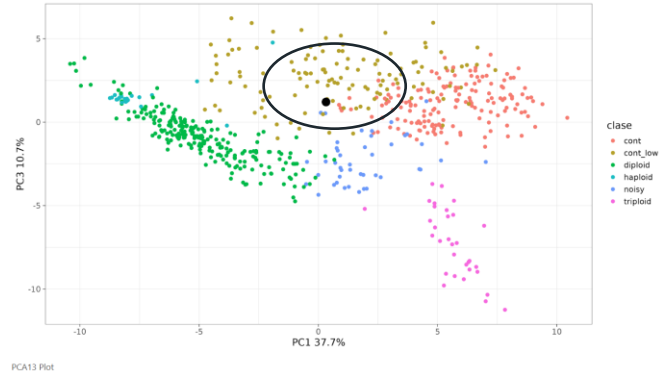




Low contamination

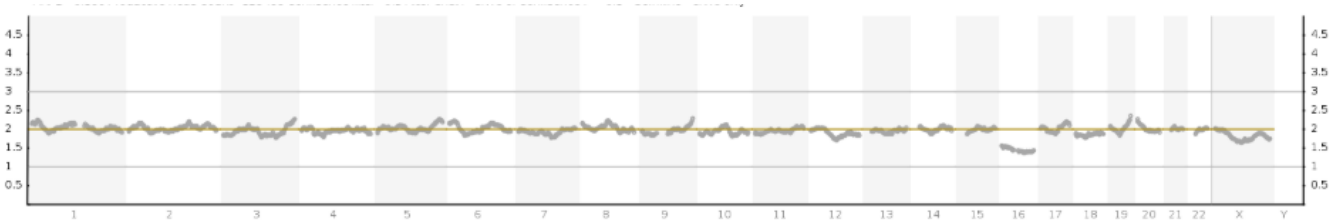
High mosaic -2 → Monosomy 2

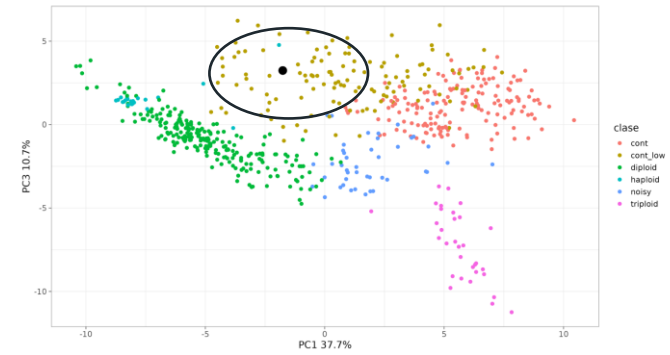




Low contamination

Low mosaic -16 → Monosomy 16





Low contamination

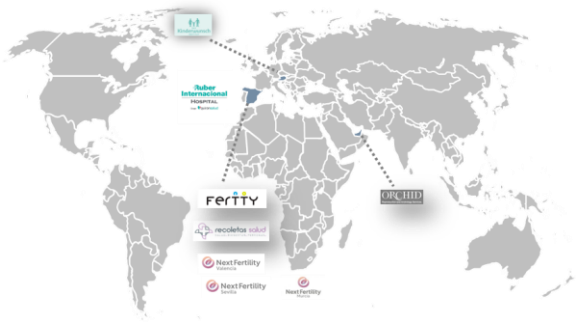
Igenomix®

Synergies between niPGT-A and Morphokinetics

Comprehensive embryo evaluation combining EmbryoScope & niPGT-A

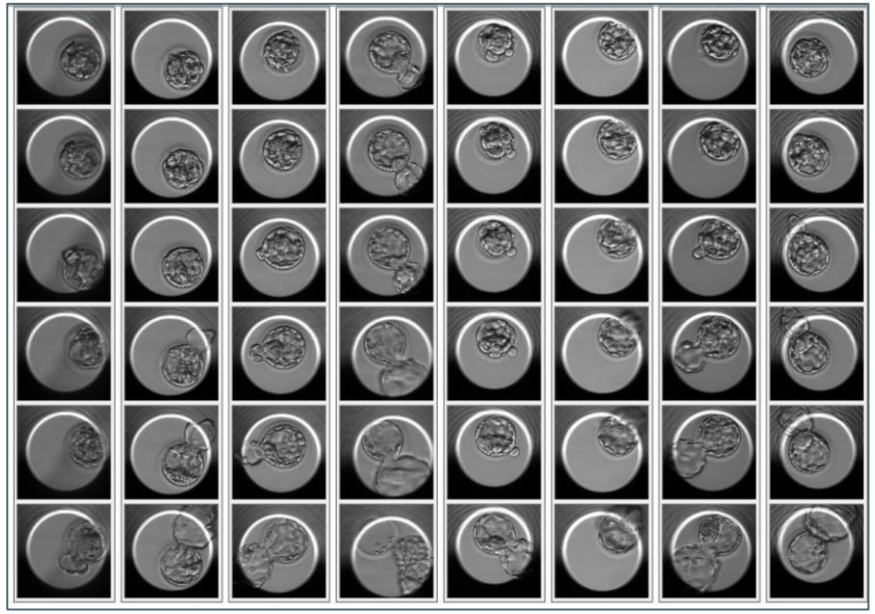
Multicenter clinical study

>200 patients
>800 embryos
8 clinics

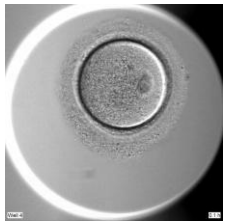


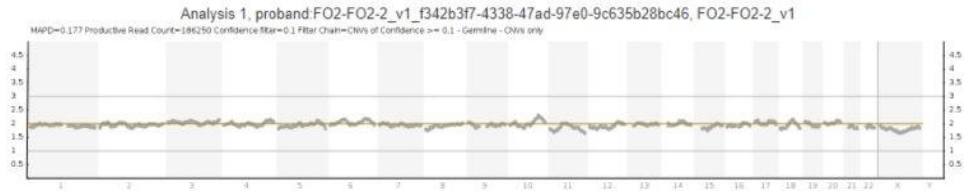
Time-lapse – To determine readiness for media collection

Early results



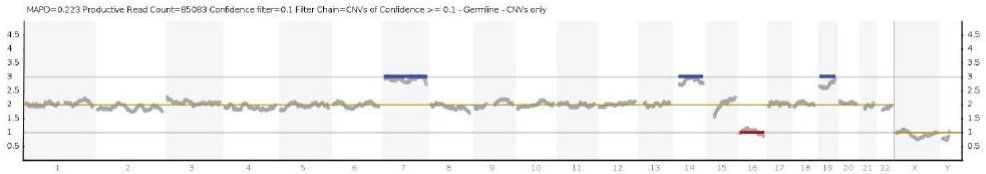
Euploid





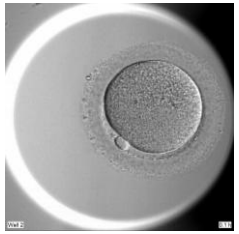
iDAScore D5 6,4

Aneuploid



iDAScore D5 4,6

No-DNA detected



iDAScore D5 2,2

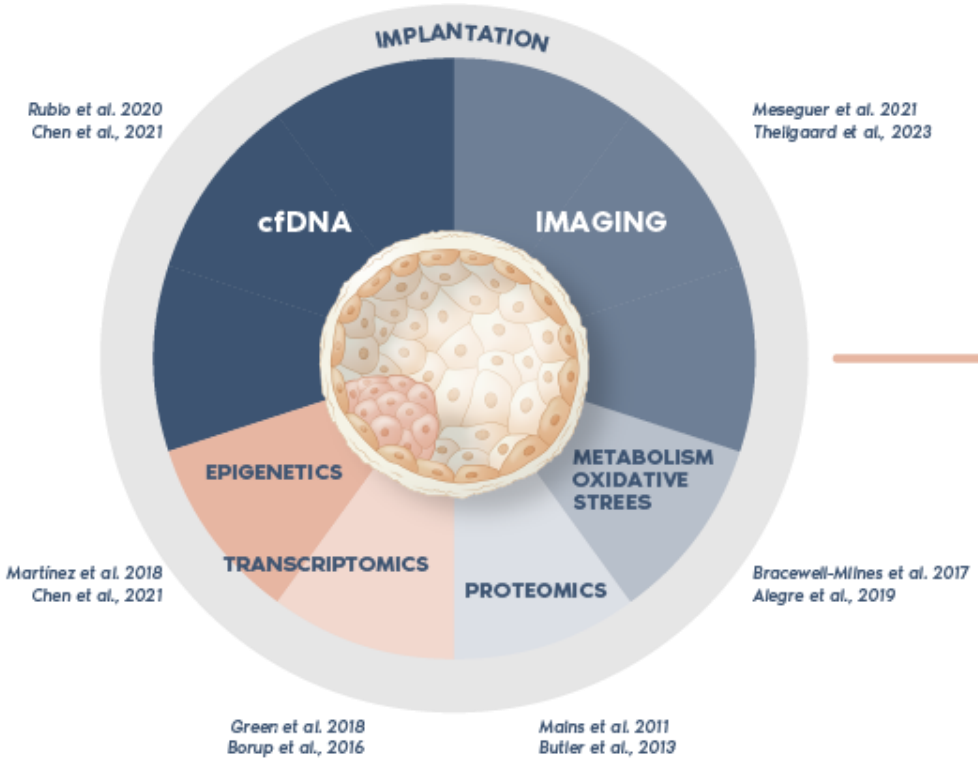
iDAScore D6 7,1

Wait. Collect medium 12hrs later

Igenomix®

Holistic View of Embryo Viability

Non-invasive approaches for embryo assessment: combining imaging & genetics



Bioinformatic analysis

THANK YOU !

Research & Development Team