Recent developments in niPGT-A

Carmen Rubio, PhD
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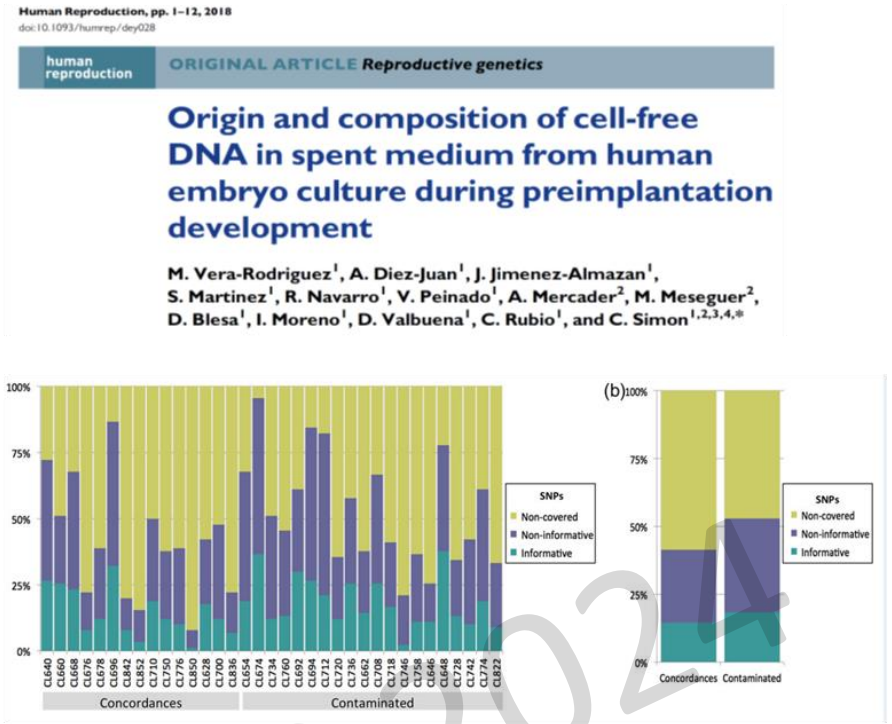
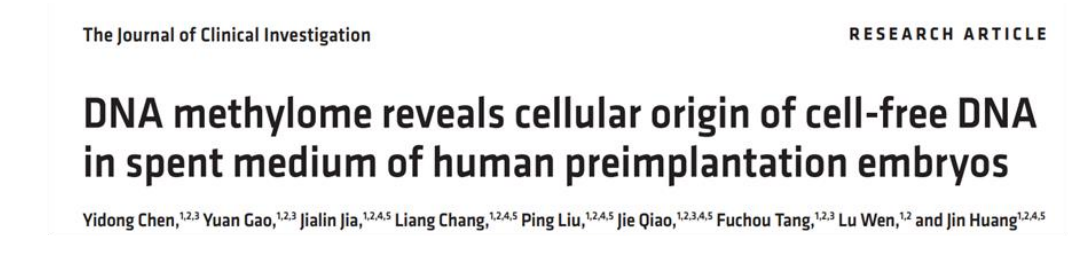
Concordance of cfDNA vs TE biopsies & whole blastocysts

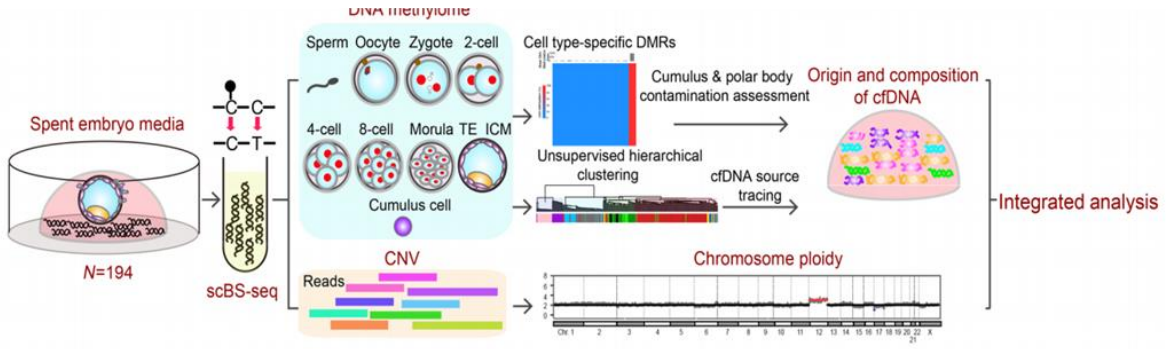
Authors	No. of SBM	Informative media	Ploidy Concordance With TE or WB	False positives	False negatives	Embryo manipulation	Time in culture	WGA method	NGS platform
Xu et al., 2016	42	100%	85.7% (WB)	9.5%	4.8%	D3 Vitrification	D3-D5	MALBAC (Yikon)	NGS (Illumina)
Vera-Rodríguez et al., 2018	56	91.1%	33.3% (TE)	-	66.7%	D3 AH	D3-D5	Sureplex + ReproSeq (Thermo)	NGS (Thermo)
Ho et al., 2018	41	97.6%	65.0% (TE) 45.5% (WB)	-	-	D3 AH vs no AH	D1 to D5	Picoplex (Rubicon)	NGS (Thermo)
Huang et al., 2019	52	92.3	89.1% (TE) 93.7% (WB)			D3 AH + TE biopsy ation D5/6	24h culture after thawing	MALBAC (Yikon)	NGS (Illumina)
Yeung et al., 2019	168	69.0% D5: 55.6% D6: 84.6%	73.3% (TE) D5:				D3-D5 D3-D6	Sureplex (Illumina)	NGS (Illumina)
Rubio et al., 2019	115	93.9% D5: 81.8% D6/7: 98.8%					D4-D5 D4-D6/7	Reproseq (Thermo)	NGS (Thermo)
Rubio et al., 2020[4]	1301	85.2%					D4-D6/7	Reproseq (Thermo)	NGS (Thermo)
Lledo et al., 2020	92	92.4%					-D5/6	MALBAC (Yikon) Sureplex (Illumina)	NGS (Illumina)
Yin et al., 2021	75	78.7%					d after thawing	MALBAC (Yikon)	NGS (Illumina)
Shitara et al., 2021	20	95%					4h for D5 3h for D6	Sureplex (Illumina)	NGS (Illumina)
Hanson et al., 2021	166	62.7% D5: 17.6% D6/7: 74.2%					D5: 24-48h D6: 48-72h D7: 72-96h	MALBAC (Yikon)	NGS (Illumina)
Chen et al., 2021	265	96.6%	74.. 78.1% (				D3-D5/6	MALBAC (Yikon)	NGS (Illumina)
Shi et al., 2022	212	100%	84.4% (TE)			nkage before vitrification	24h cultured after 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)

Origin of cfDNA

Sources of contamination and impact according to timing of media collection

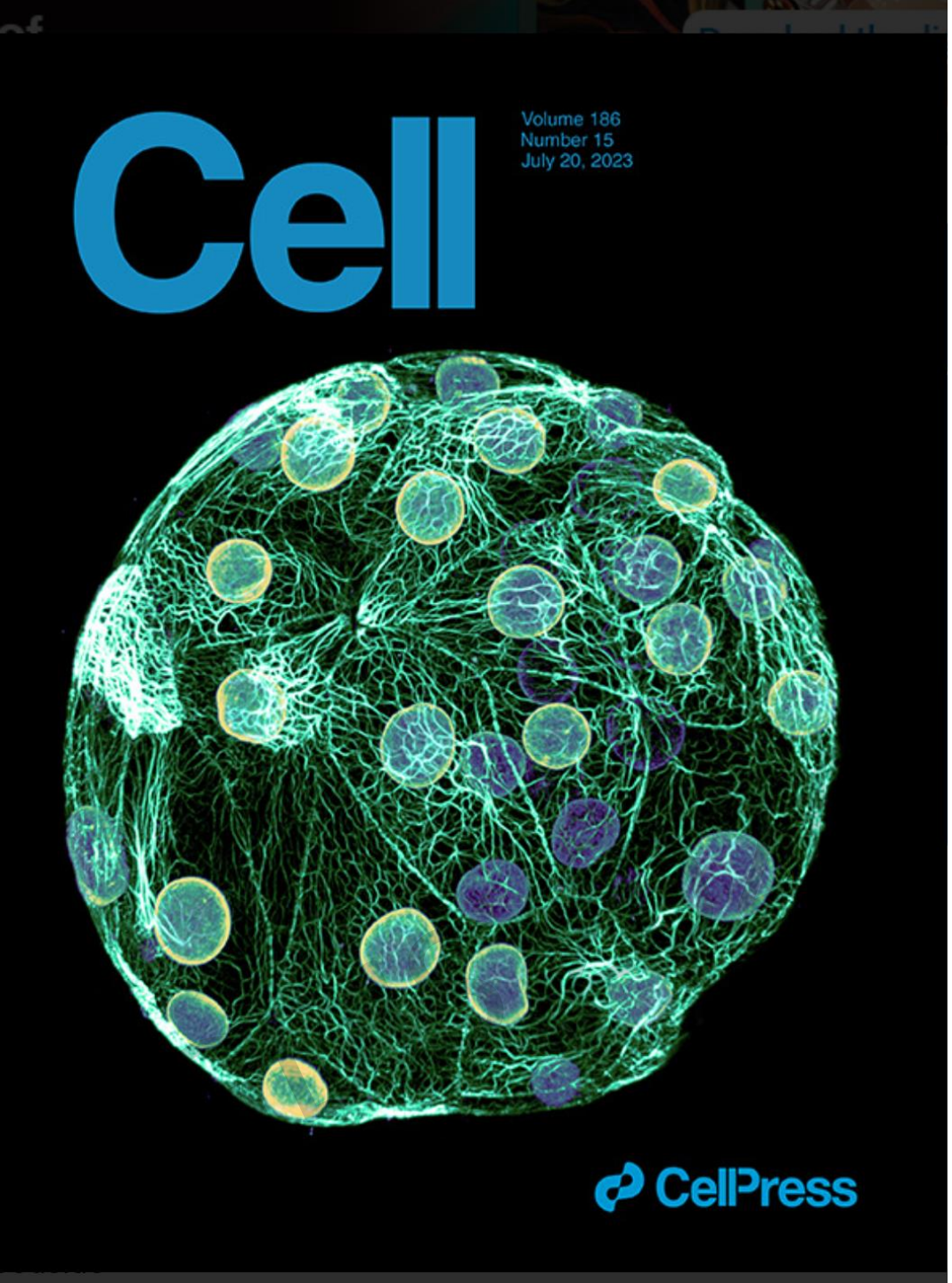






SNPs analysis: poor coverage. Higher concordance with lower contamination

The methylation patterns identified contamination from cumulus cells and polar bodies, higher on D5

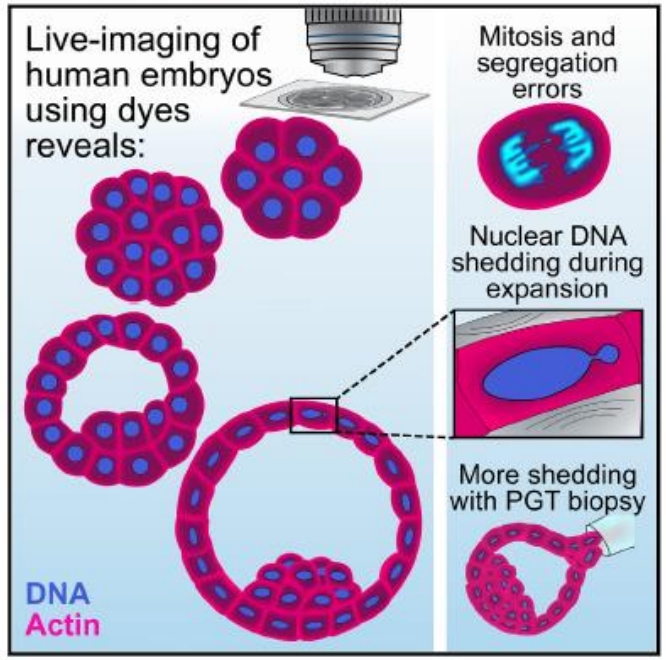


Article

Human embryo live imaging reveals nuclear DNA shedding during blastocyst expansion and biopsy

Ana Domingo-Muelas,[1,2,10] Robin M. Skory,[1,3,10] Adam A. Moverley,[1,4] Goli Ardestani,[5] Oz Pomp,[1] Carmen Rubio,[6] Piotr Tetlak,[1] Blake Hernandez,[1] Eric A. Rhon-Calderon,[1] Luis Navarro-Sánchez,[6] Carmen M. García-Pascual,[6] Stephanie Bissiere,[1] Marisa S. Bartolomei,[1] Denny Sakkas,[5,*] Carlos Simón,[2,7,8,9,*] and Nicolas Plachta[1,11,*]

Graphical abstract



Highlights

- Fluorescent dyes enable live imaging of human embryos without genetic manipulation
- Live imaging reveals differences between human and mouse embryo morphogenesis
- Blastocyst expansion causes trophectoderm cell nuclear budding and DNA shedding
- Mechanical stress from blastocyst expansion or biopsy triggers nuclear DNA loss

Authors

Ana Domingo-Muelas, Robin M. Skory, Adam A. Moverley, ..., Denny Sakkas, Carlos Simón, Nicolas Plachta

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In brief

Live imaging of human embryos unveils differences from mouse development and reveals DNA shedding from trophectoderm cell nuclei associated with mechanical stress from blastocyst expansion and biopsy for preimplantation genetic testing.

Nuclear Budding

Nicolás Plachta Lab
Boston IVF

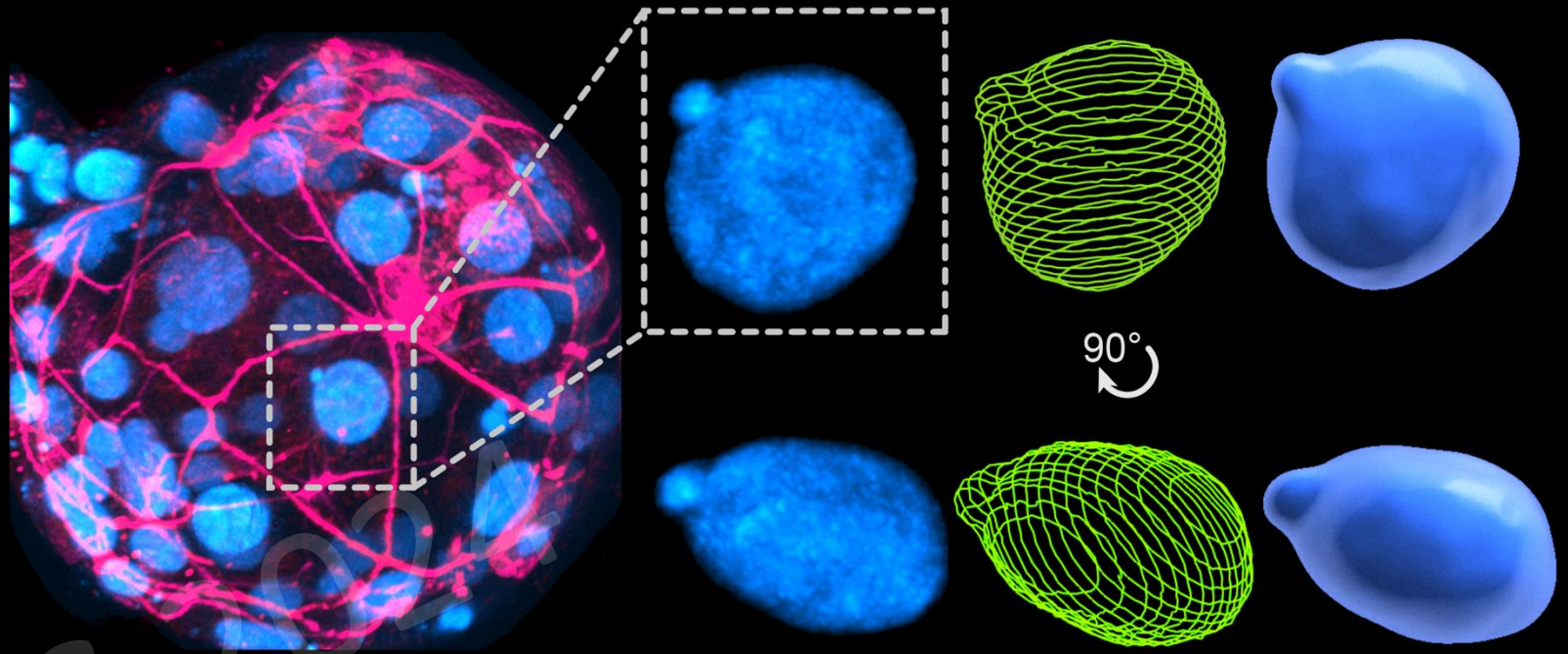
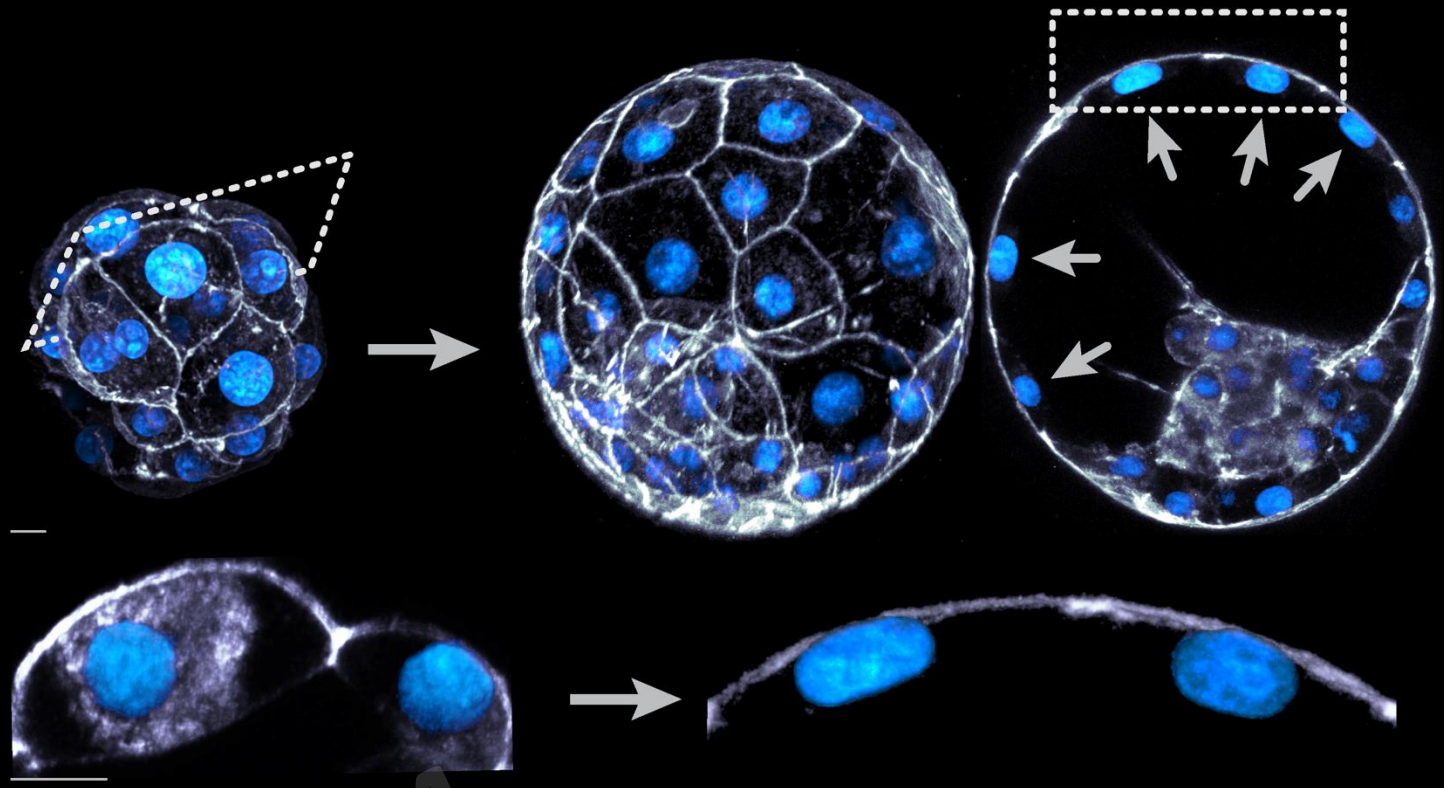


Image credit: Nicolas Plachta (presented at ASRM 2022)

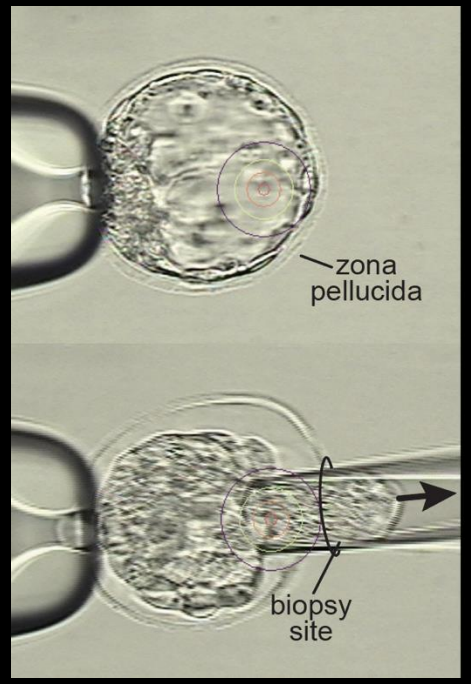


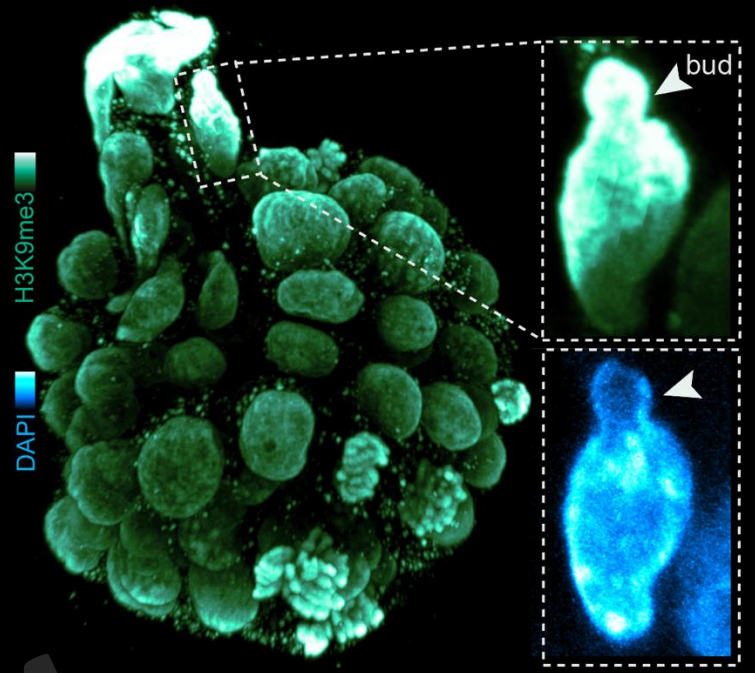
Spherical nuclei

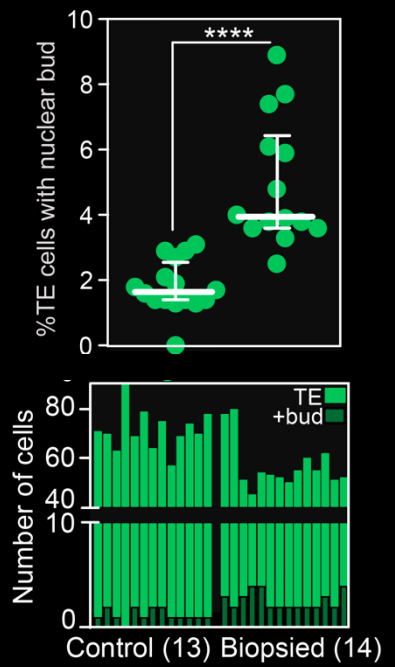
Pressed nuclei

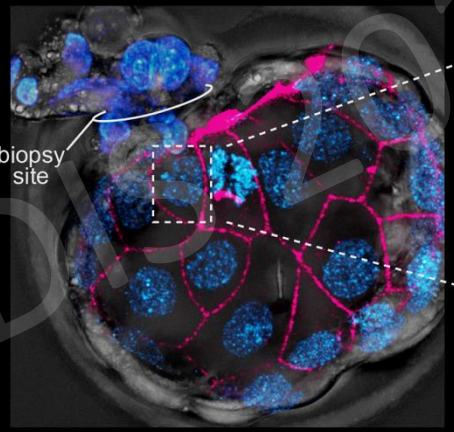
Image credit: Nicolas Plachta (presented at ASRM 2022)

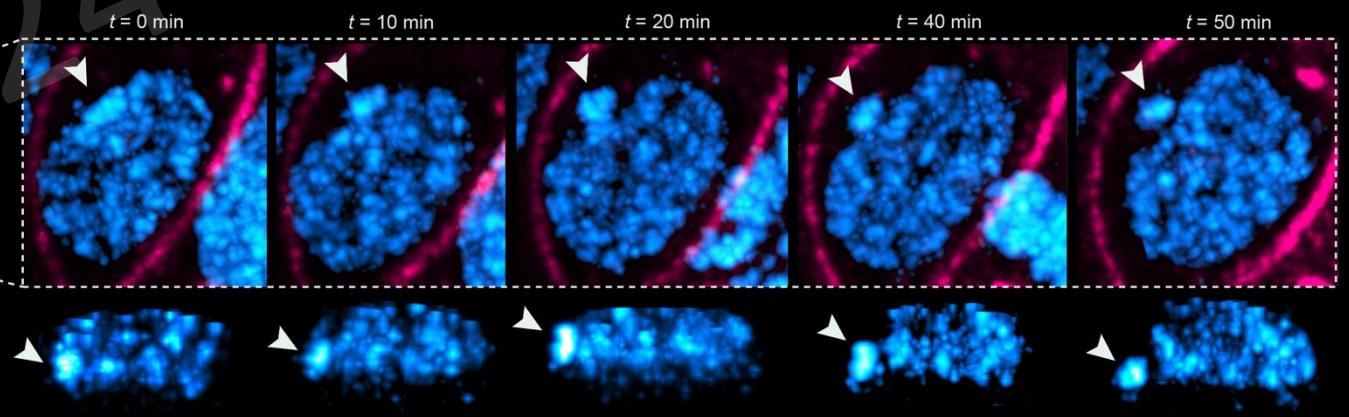
Biopsy triggers DNA shedding











Prospective Multicenter Concordance Study (NCT03520933)

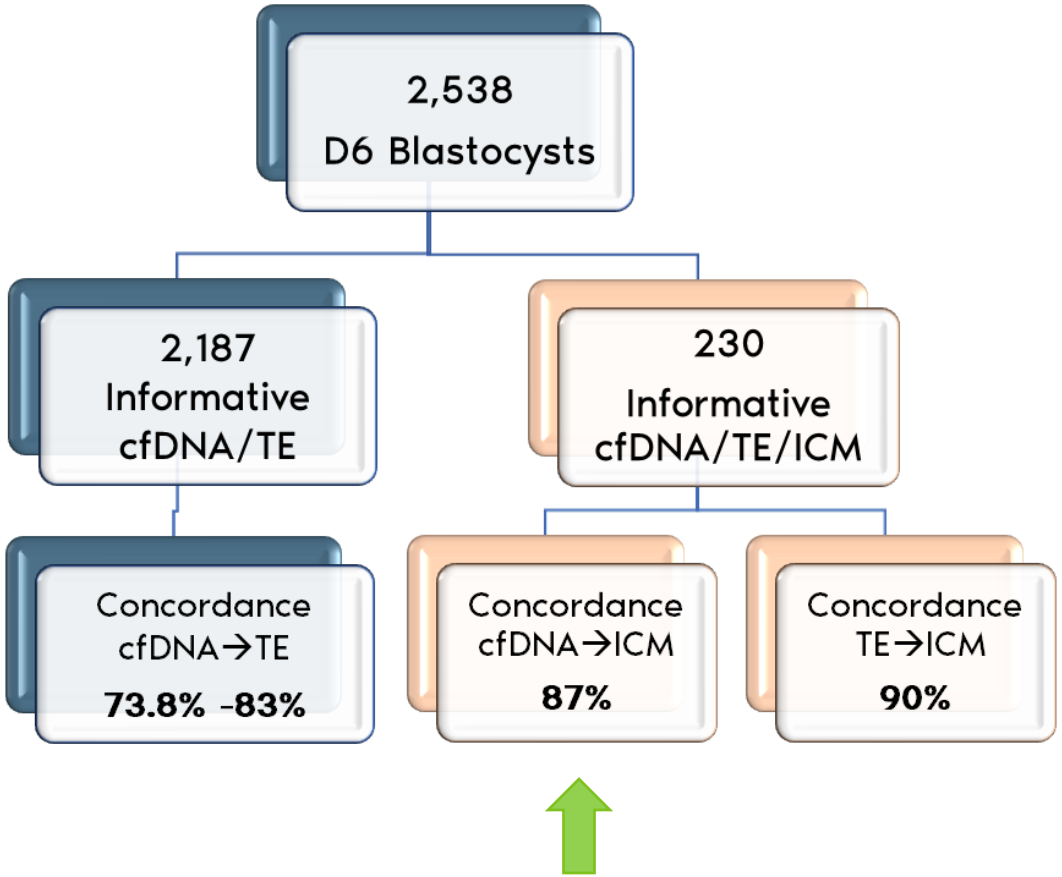
American Journal Obstetrics & Gynaecology, 2020

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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Multivariate analysis of intrinsic and extrinsic factors that can have an impact in the concordance rates

	aOR	95% CI	p-value
Female age	1.03	0.98 - 1.08	0.27
Body mass index	1.00	0.97 - 1.04	0.85
No. previous implantation failures	1.02	0.90 - 1.18	0.73
No. previous miscarriages	1.10	0.93 - 1.31	0.29
No. previous live birth	1.08	0.88 - 1.34	0.49
Oocyte origin (own/donated)	0.73	0.19 - 2.27	0.61
No. MII oocytes	0.96	0.91 - 1.02	0.19
Type of fertilization (ICSI/IVF)	-	-	0.53
No. 2PN	1.04	0.97 - 1.12	0.26
Culture conditions (media-incubator)	-	-	0.67
No. blastocysts analyzed	1.05	0.99 - 1.11	0.14
Embryo Quality (TE, ICM grade)	0.79	0.52 - 1.19	0.26
Expansion degree	-	-	0.68
Day of media collection (D6/7)	1.33	0.34 - 5.34	0.68
No. NGS reads	1.19	1.01 - 1.40	0.04
Media result (euploid/aneuploid)	1.09	0.79 - 1.49	0.61

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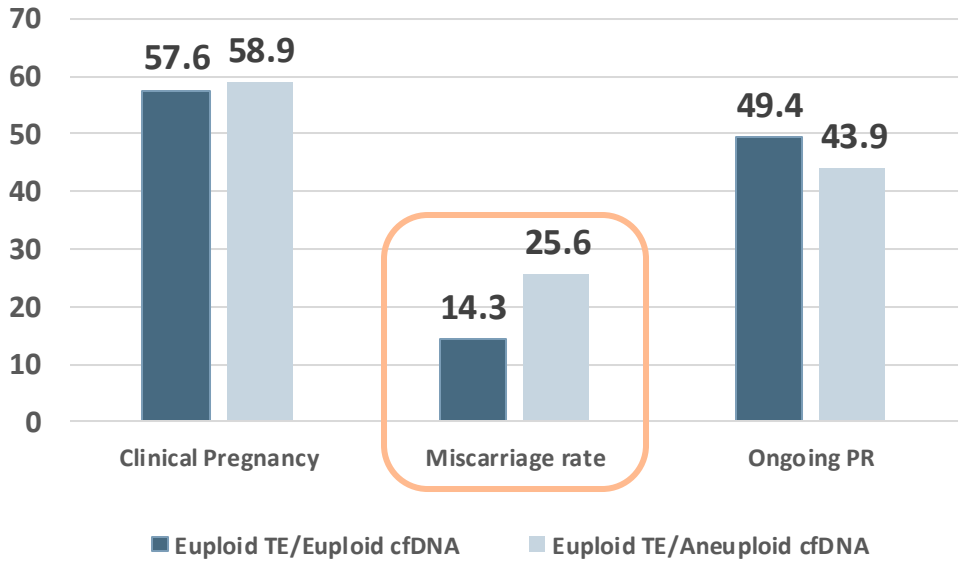
Updated Clinical results 304 SET, unpublished



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

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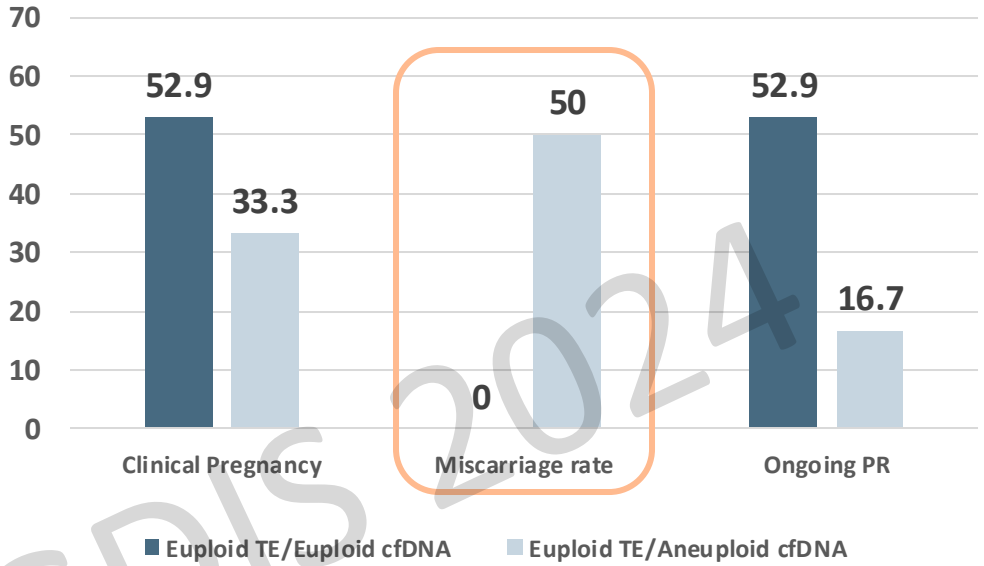
Prospective Multicenter Concordance Study (NCT03520933)

Fertility &Sterility , 2019

Embryonic cell-free DNA versus trophectoderm biopsy for aneuploidy testing: concordance rate and clinical implications



	Euploid/Euploid	Euploid-Aneuploid
Number of SET	17	12
Mean age (SD)	37.5 (2.5)	37.4 (2.3)

American Journal Obstetrics & Gynaecology, 2020

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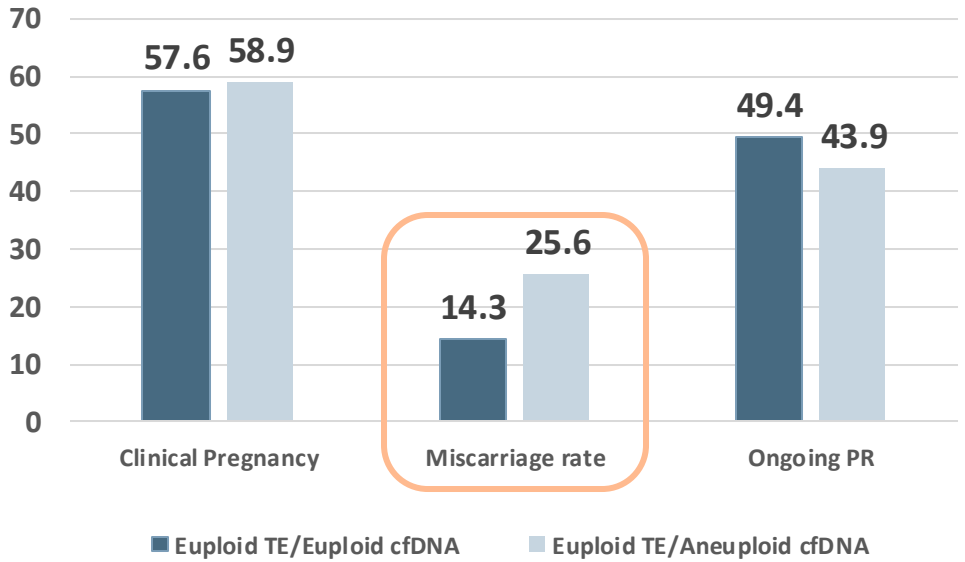

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Number of SET	231	73
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Prospective Multicenter Concordance Study (NCT03520933)

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

OBSTETRICS

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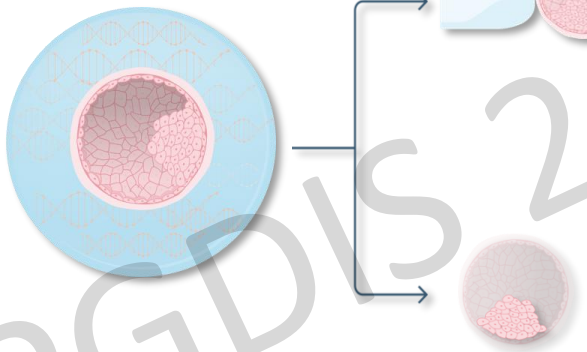
Specific thresholds/chr Aneuploidy calling

Prioritization for Embryo Transfer

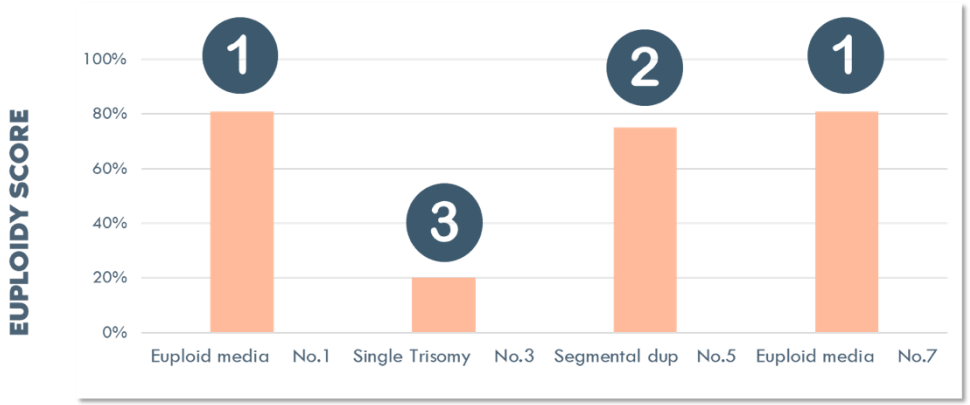


Trophectoderm DNA

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

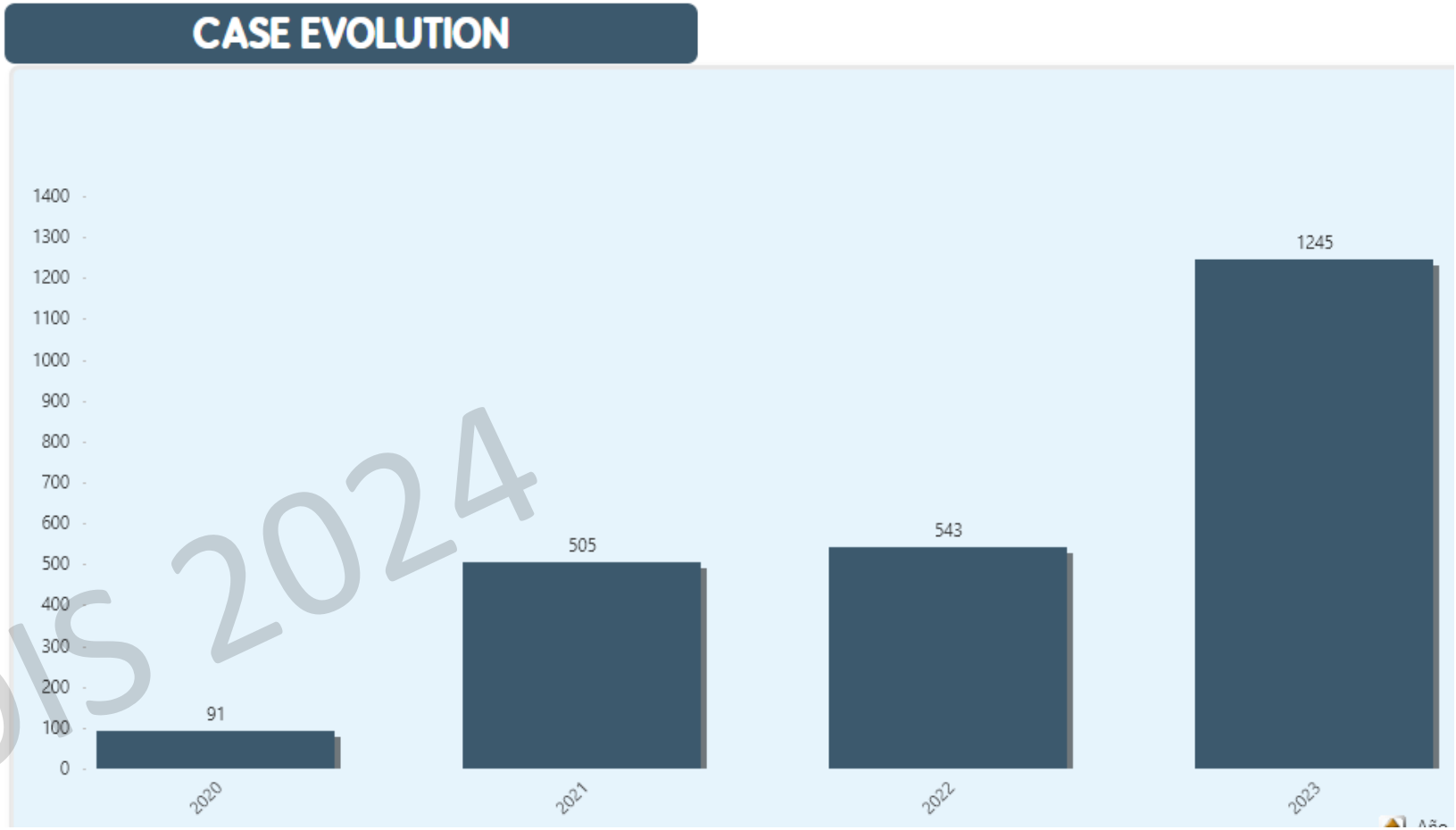
Inner cell mass DNA

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

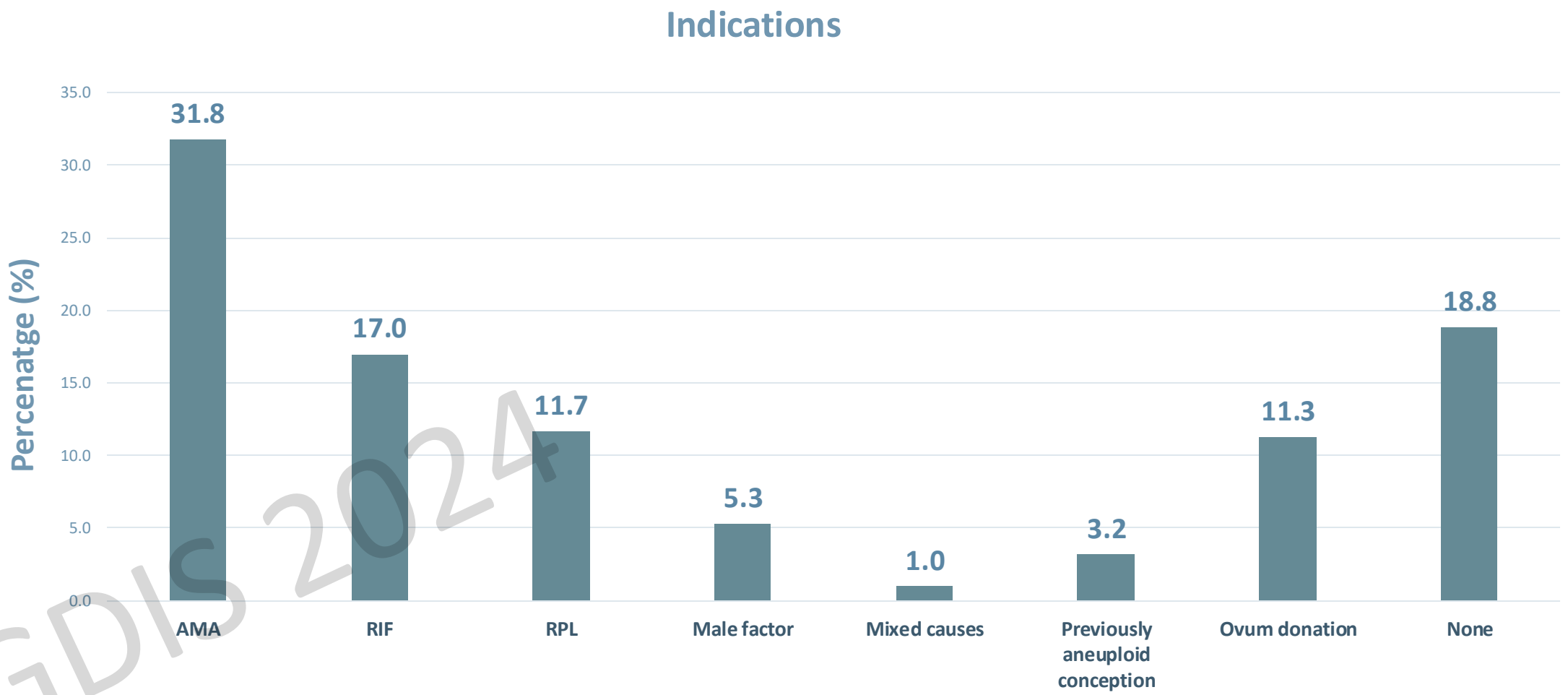


Clinical experience 2020-2023

TOTAL CASES including 2024=2,649 (8,198 SBM)

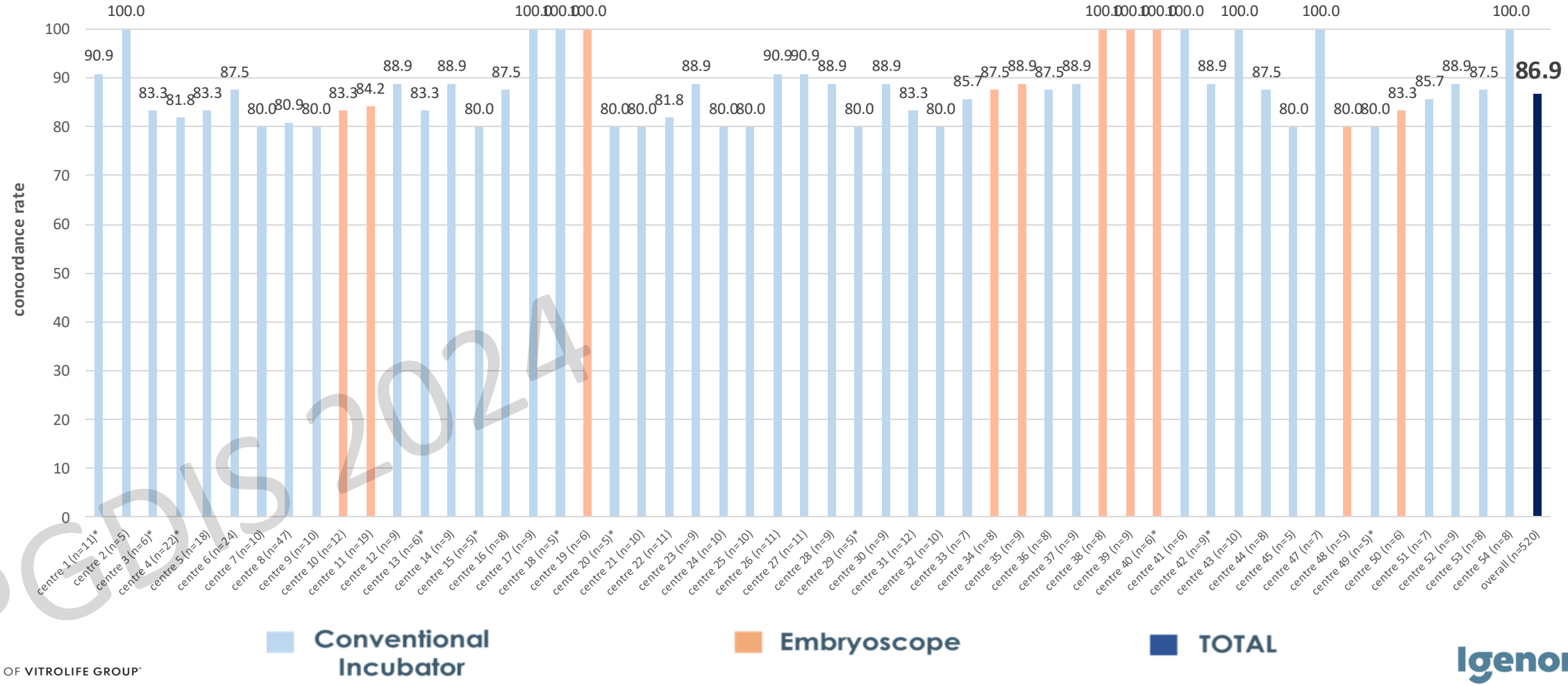


Clinical experience 2020-2023



Clinical experience 2020-2023

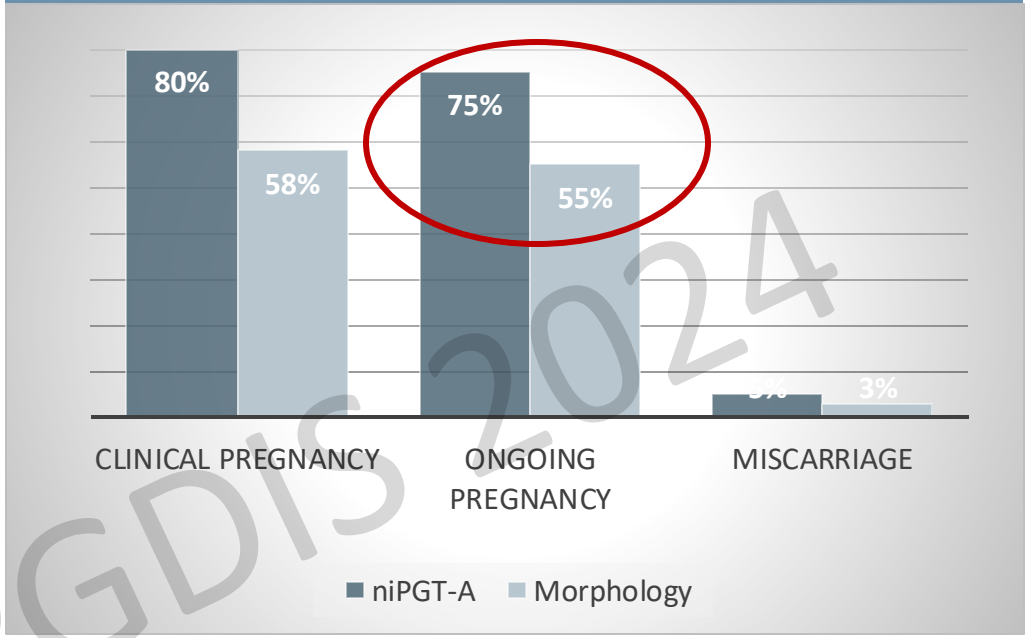
Pre-clinical validations (dry-runs)



Clinical experience 2020-2023 comparing with morphology

Yosu Franco, Ruber International (Spain).
ESHRE 2022

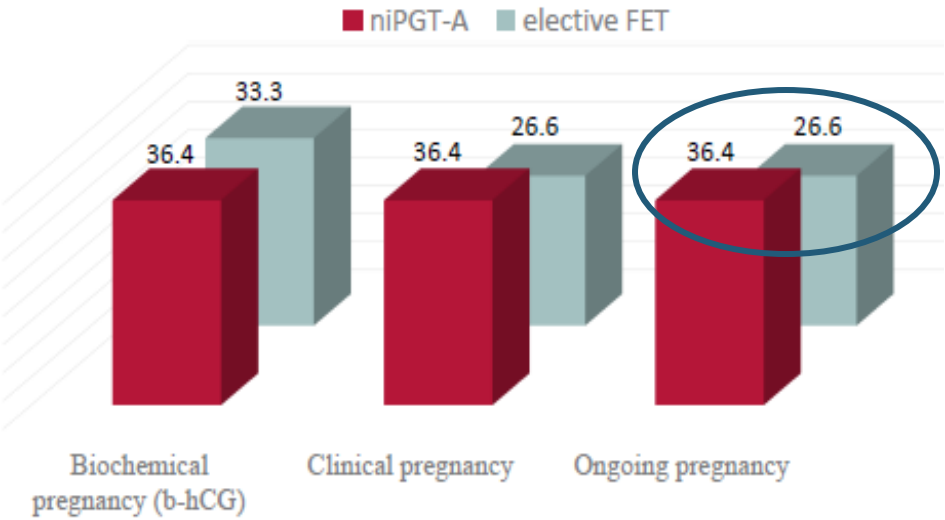
High ongoing pregnancy rate with EMBRACE in good prognosis patients < 38yrs vs. morphology embryo selection



RedLara 2023

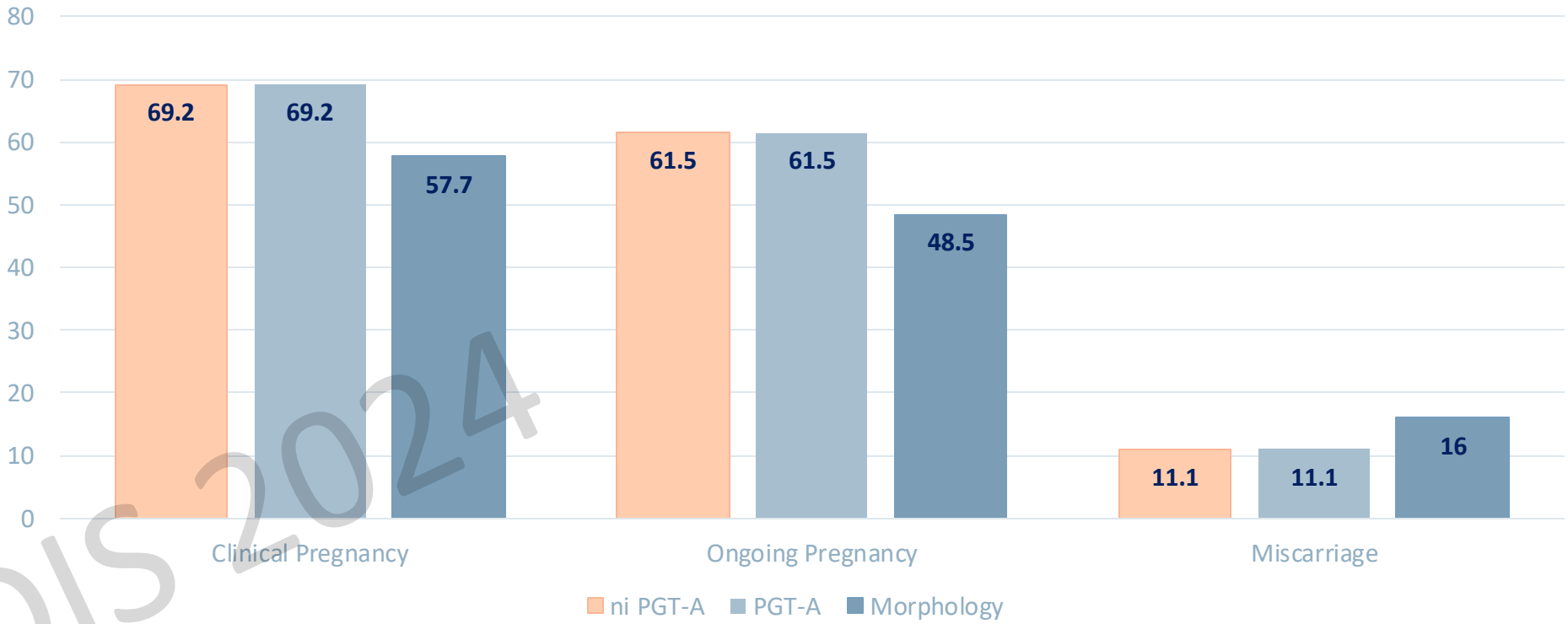
First cases of niPGT-A performed. Better clinical outcomes for niPGT-A when compared to morphology, but not statistically significant results due to small sample size.

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



Clinical experience 2020-2023 comparing with PGT-A

High ongoing pregnancy rate with EMBRACE in good prognosis patients



Franco et al. *Hum Reprod*. 2021; **36**: P–560

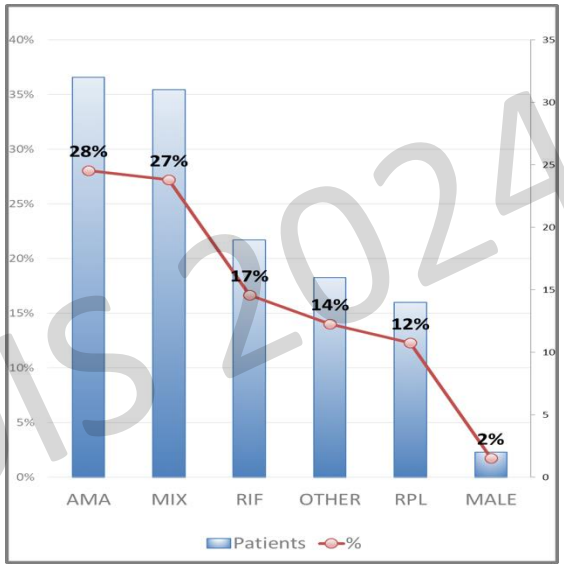
Clinical experience 2020-2023 comparing with PGT-A

Bartłomiej Wojtasik, 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

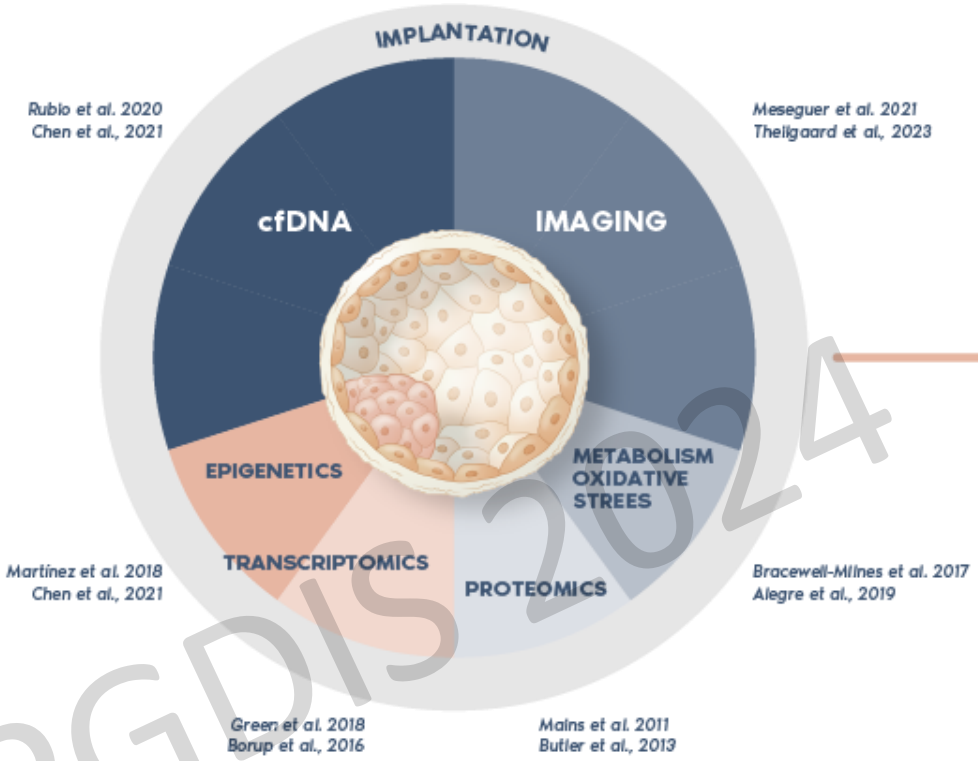
Distribution of indications



	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%**

Holistic View of Embryo Viability

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



Artificial intelligence

Algorithm

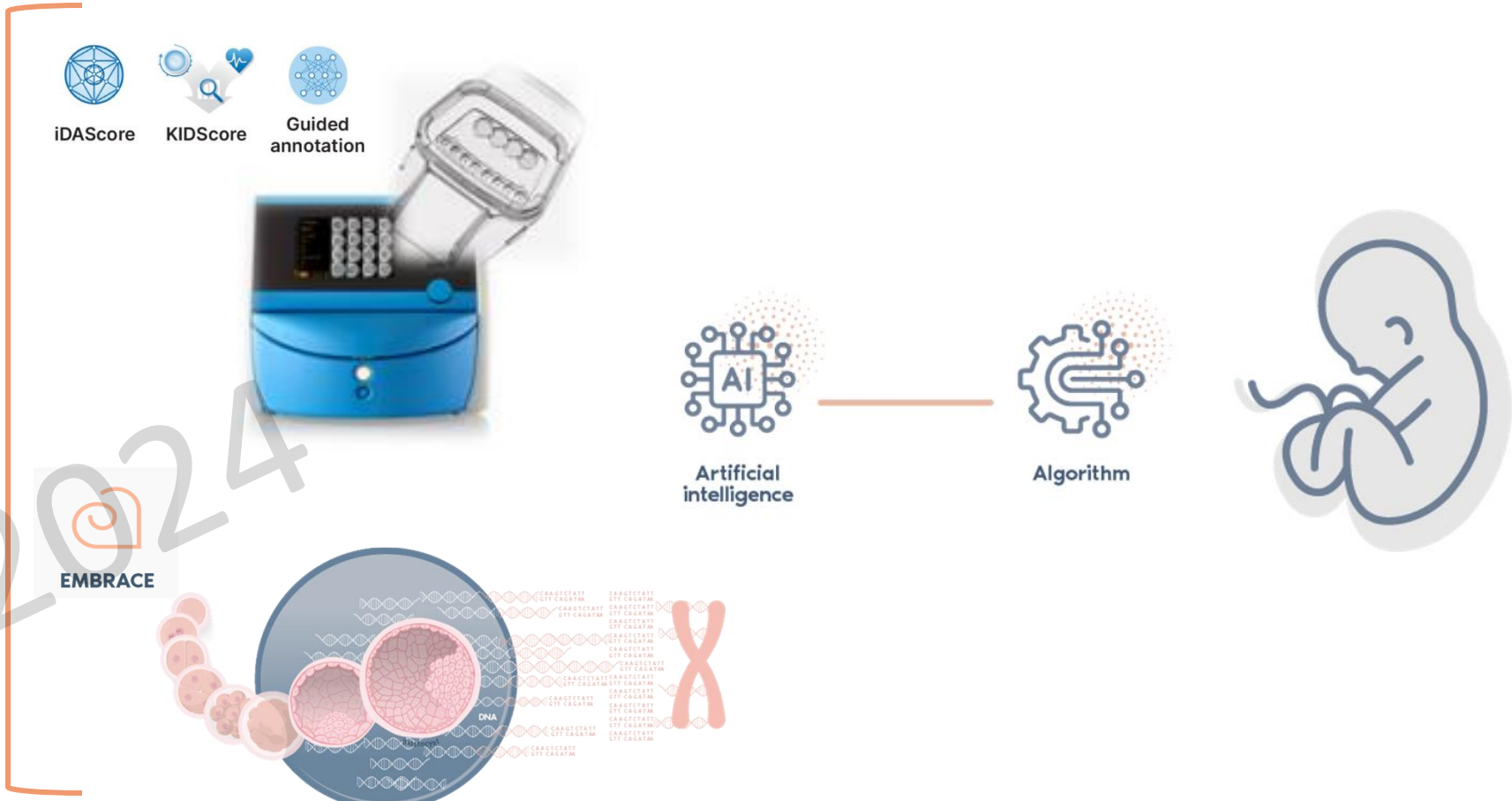
Bioinformatic analysis

Holistic View of Embryo Viability

Comprehensive embryo evaluation combining EmbryoScope & niPGT-A

Multicenter clinical study

>200 patients
>800 embryos
8 clinics



Acknowledgements

Igenomix R&D team

Embryo Research team
Medical and clinical studies team
Bioinformatics team

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Nicolás Plachta Lab Upenn University

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