“How far down the (mi)niPGT path should we go?”

L. Gianaroli, MD, FRCOG

H-Index (Google Scholar): 83
S.I.S.Me.R. Reproductive Medicine Institute, Bologna, Italy

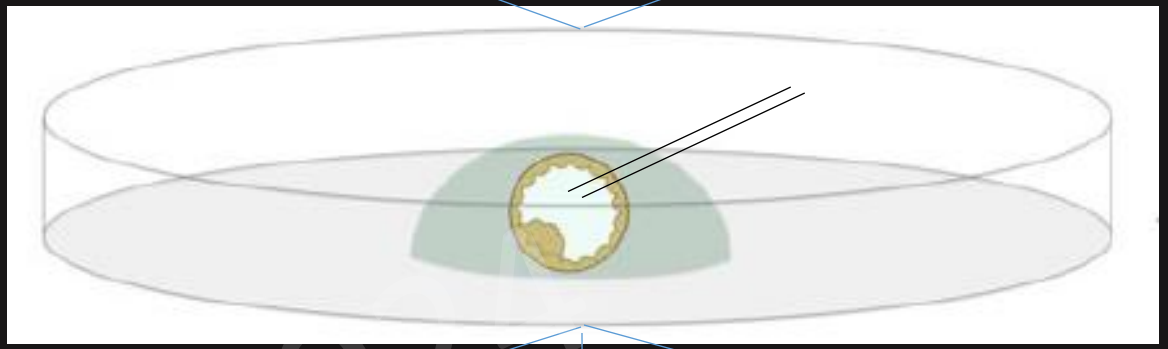
PGDIS 2024 Kuala Lumpur, 7th May 2024

MINIMAL / NON INVASIVE-PGT

A minimal / non-invasive diagnostic test that utilizes cell-free DNA in a blastocyst's spent culture medium to detect chromosomal copy number variations.

Minimal / no risk of causing damage to the embryo

Bypass the issue of TE mosaicism



Simple technique

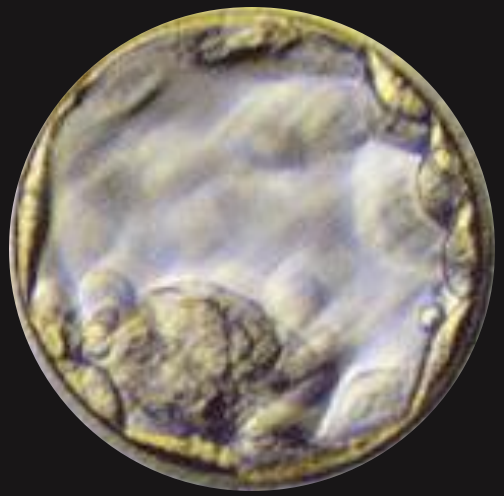
Affordable

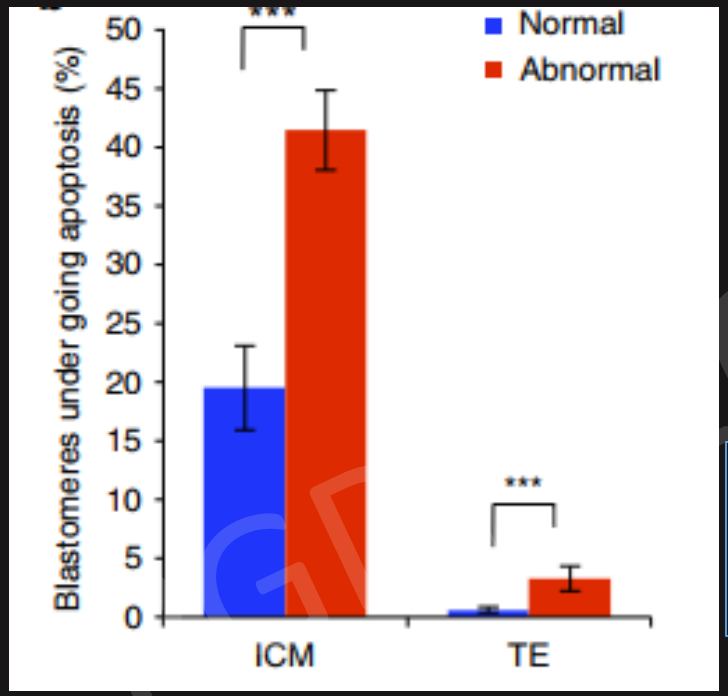
Possibility of extra information about embryo viability

ORIGIN OF CELL FREE DNA

In conventional TE biopsy, possible ICM mosaicism remains undiagnosed

- **The origin of extraembryonic DNA** is unknown, but it is thought to be released in small amounts as a result of **cell death**.
- **DNA fragmentation** is linked to **necrosis and apoptosis, but other mechanisms could be involved**.
- Nearby cells can phagocytose apoptotic cells or shed them into the **blastocoel cavity** or the **surrounding medium**.





In the mouse, frequency of apoptosis in the ICM was significantly higher in the abnormal clone of cells in the effort to eliminate them, while aneuploid TE cells continue to proliferate.

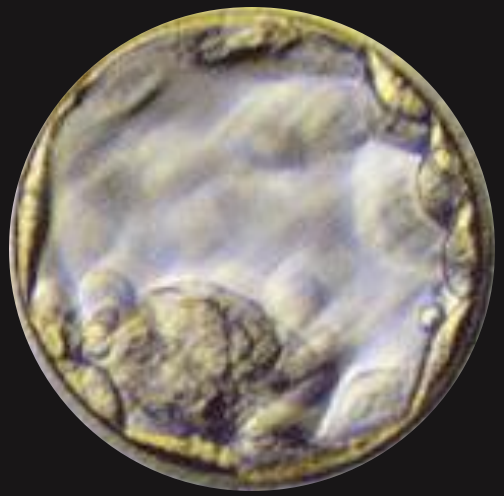
Cell free DNA may be more informative about the lineage that will produce the fetus than TE biopsies, as apoptosis is mainly restricted to the ICM.

Bolton et al., Nat Commun 2016

“The blastocoel fluid”

BLASTOCOEL FLUID

- The blastocoel, which has an average capacity of **4–6 nL**, begins to form on day 4 and fully develops between days 5 and 6.
- The surrounding **monolayer of TE cells** forms a solid barrier separating the blastocoel from the external environment through tight connections.



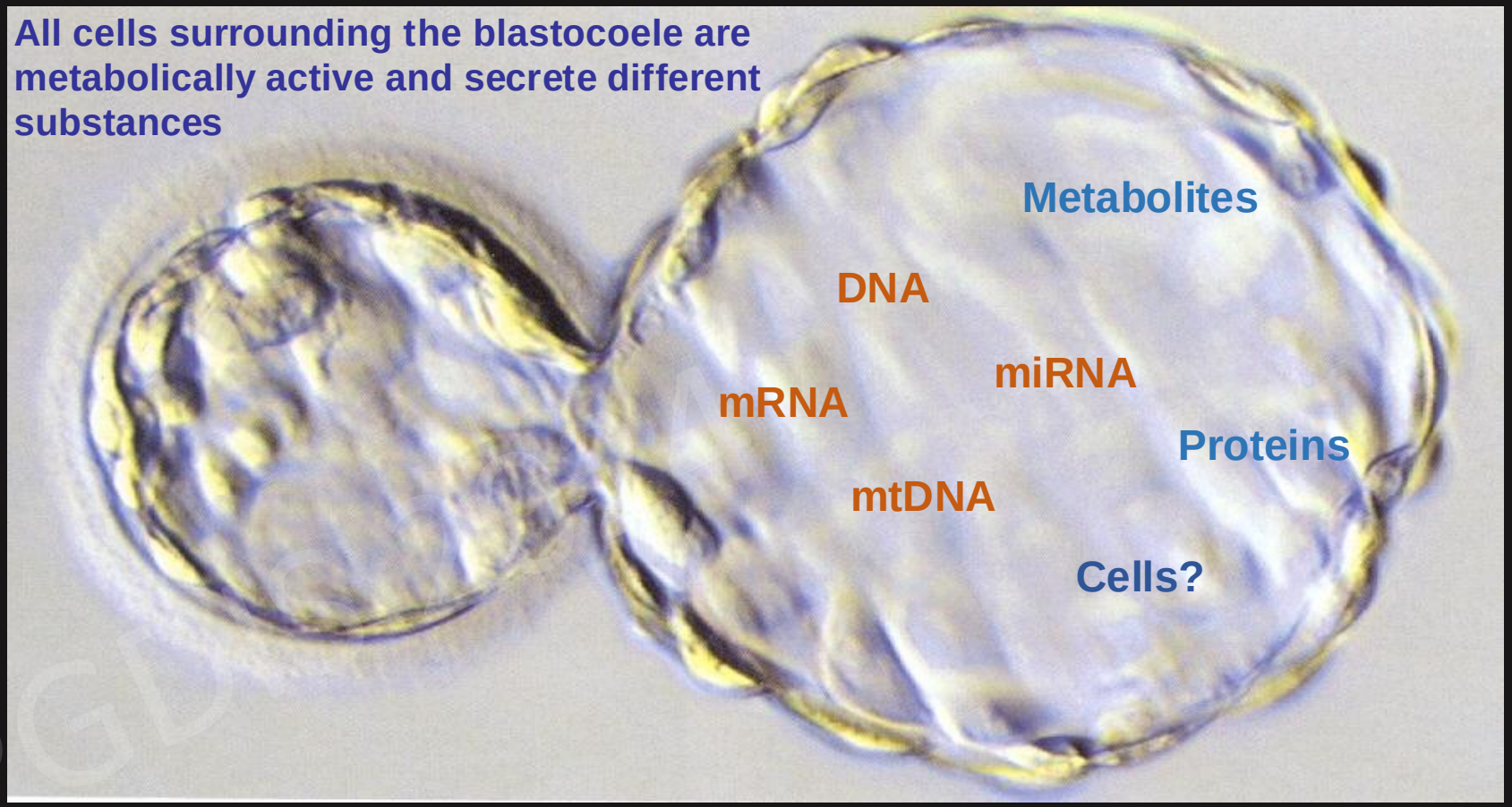
Due to the **absence of impurities** from the culture medium, the blastocoel can provide

- a **pure sample of embryo secretions**

- information on ICM

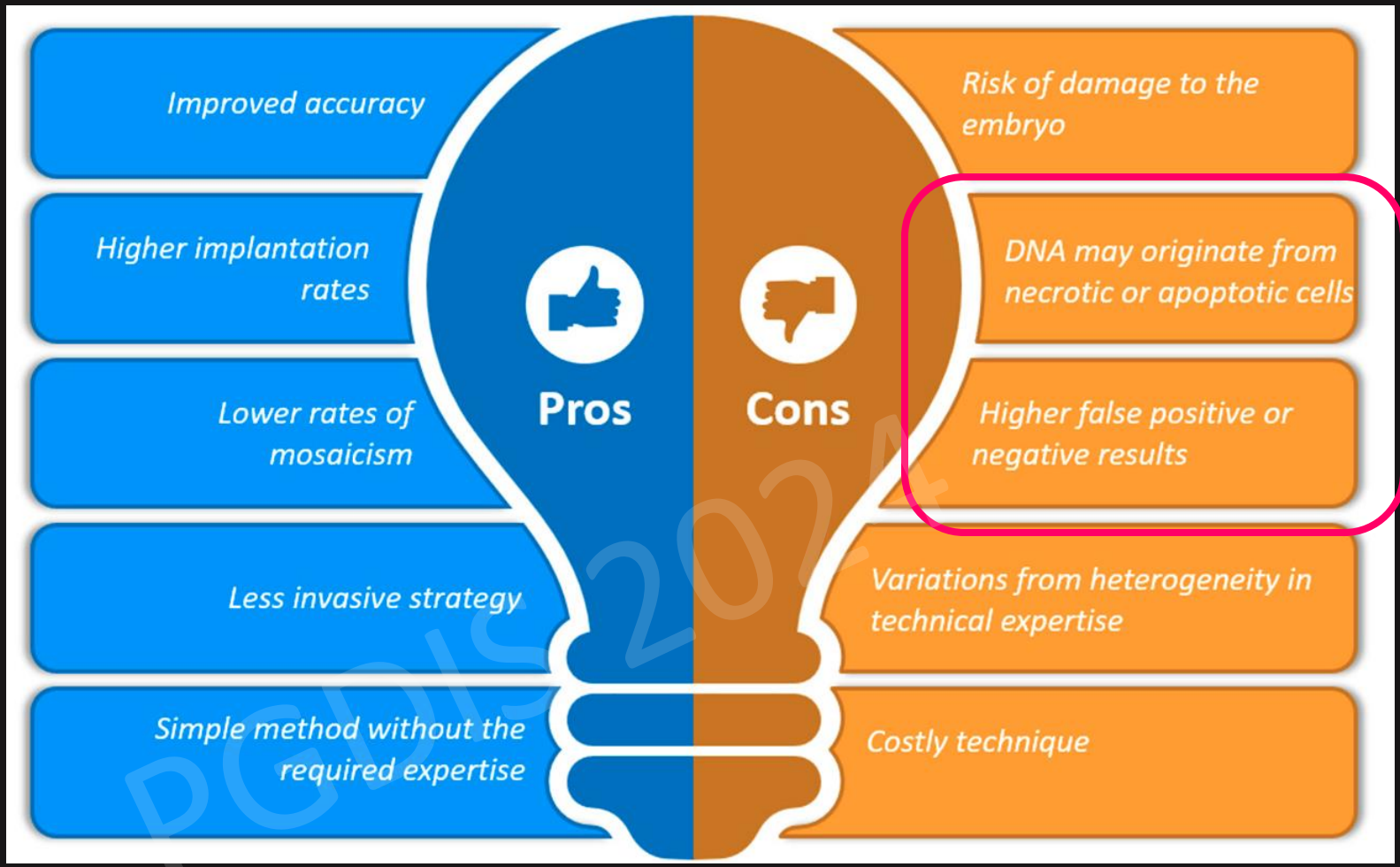
BLASTOCOELIC FLUID

The blastocoelic fluid is the natural environment of blastocyst growth.
The assessment of its composition provides an opportunity to expand the knowledge of embryonic physiology.



BLASTOCOEL FLUID

According to the different reports, the genetic material in the BF is amplified to a great degree of variety, and **significant differences in the concordance rate between BF and TE cells** are also observed.

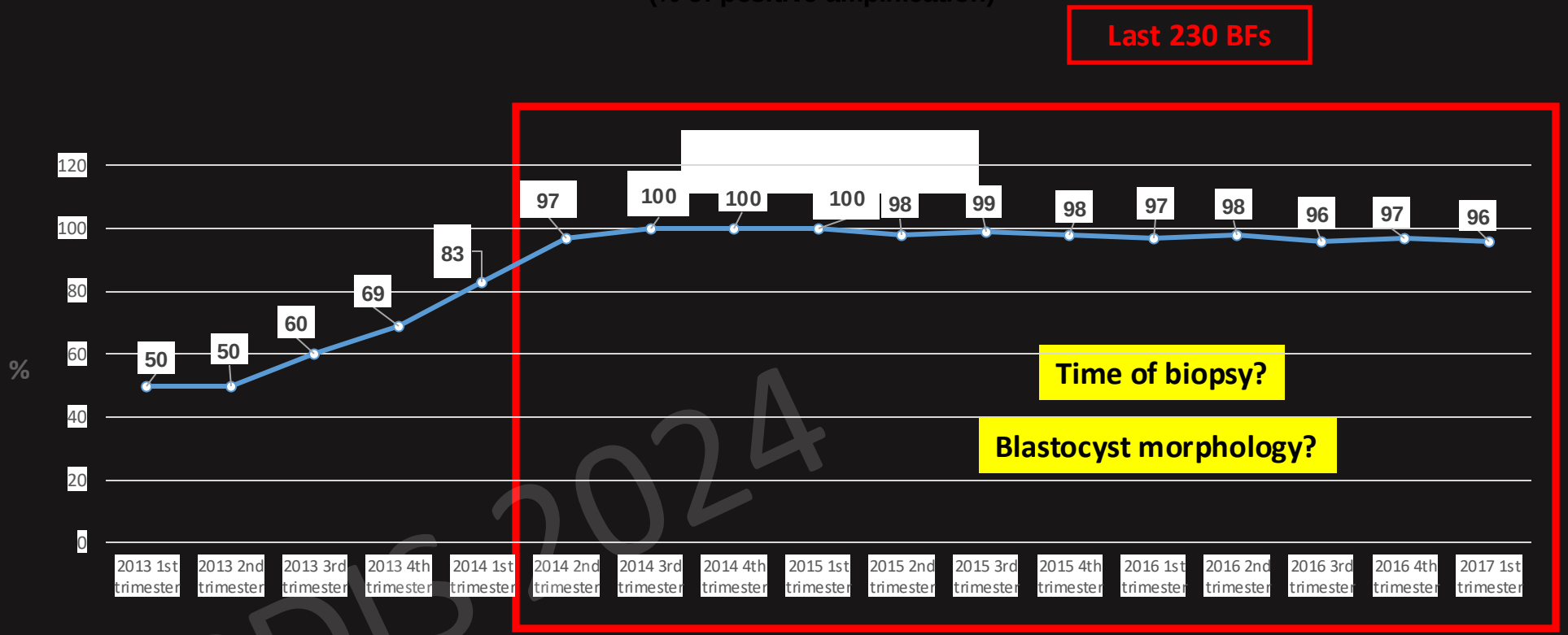


Doubts on the efficiency of BF as a source of DNA for PGT-A

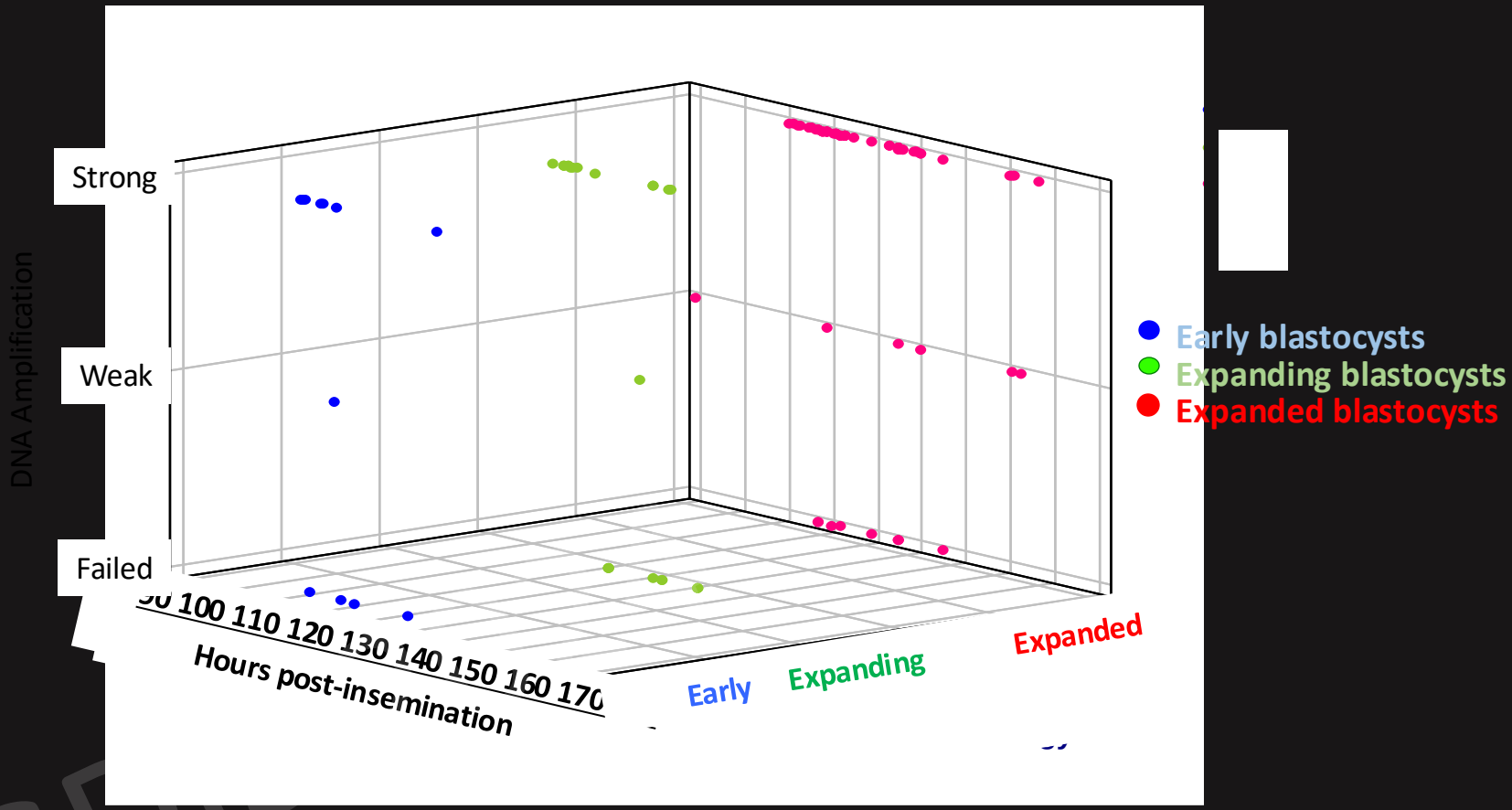
BLASTOCOELC FLUID ASPIRATION

Our experience throughout time on 287 BFs

(% of positive amplification)



DETECTION OF DNA IN THE BLASTOCOELIC FLUID



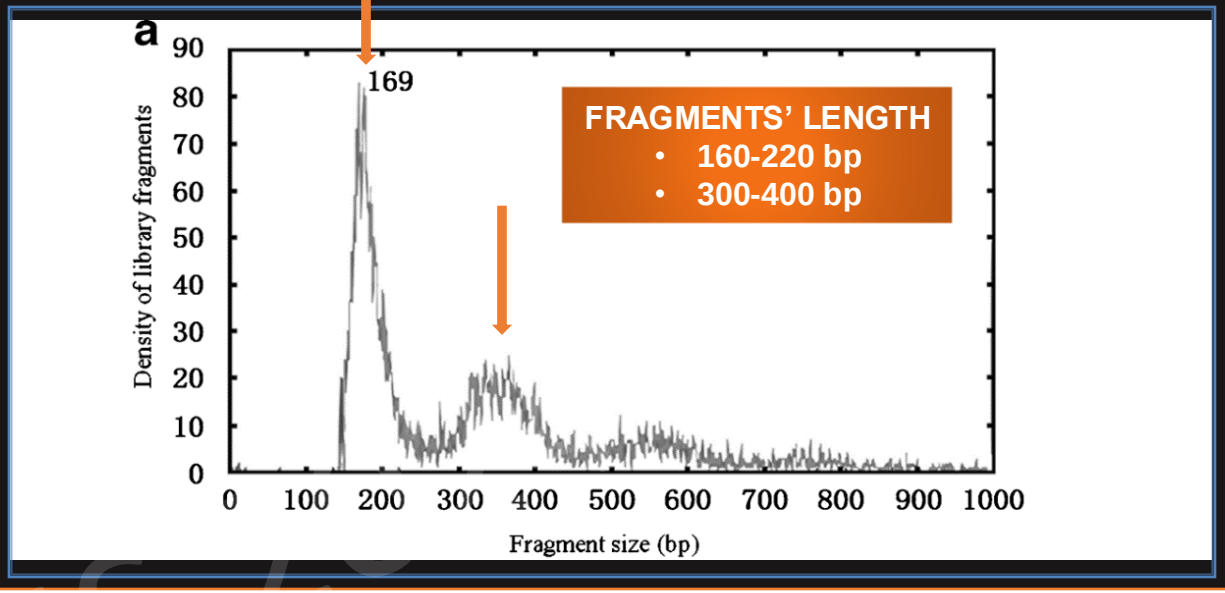
Expanded Day 5 blastocysts have the highest chances of having DNA in the BF

Magli et al. *Fert Ster* 2016

BLASTOCOELIC FLUID ASPIRATION

Where does this DNA come from?

Direct sequencing of 3 pooled blastocoelic fluids



DNA FRAGMENTS' LENGTH SIMILAR TO CIRCULATING PLASMA DNA

TYPICAL LENGHT OF APOPTOTIC FRAGMENTS

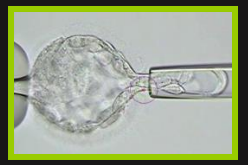
Zhang et al., *J Assist Reprod Genet*, 2016

CONCORDANCE STUDY

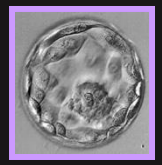
CONCORDANCE PER CHROMOSOME

Polar bodies(n=36)

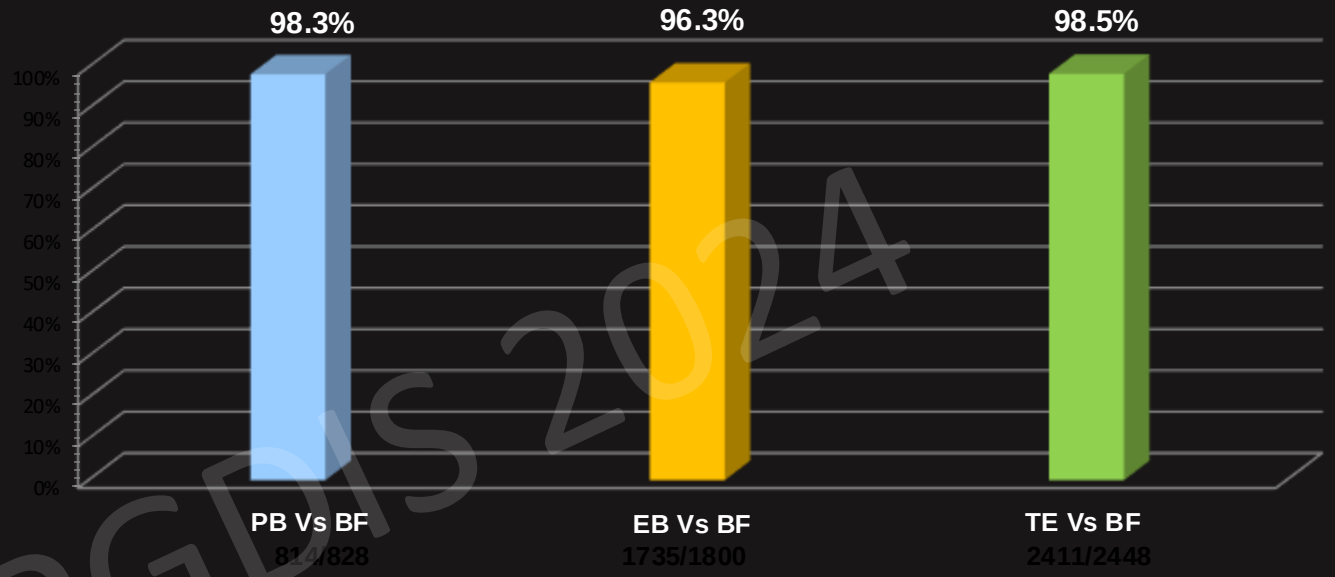
Blastomeres (n=75)



Trophectoderm (n=102*)



Blastocoelic fluid (n=111)



- 2 sample failed amplification
- 7 embryos were transferred

Deoxyribonucleic acid detection in blastocoelic fluid: a new predictor of embryo ploidy and viable pregnancy

M. Cristina Magli, M.Sc., Cristina Albanese, M.Sc., Andor Crippa, Ph.D., Carla Tabanelli, M.D., Anna P. Ferraretti, M.D., and Luca Gianaroli, M.D.

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

Fertil Steril 2019

The group with **negative BF amplification** had **higher clinical pregnancy rates** (77% vs. 37%) when 53 paired embryos were used for IVF.

Negative BF amplification could function as an extra selection factor to help **prioritize embryos** for transfer during IVF.

OXFORD human reproduction

Human Reproduction, 2023, 1–9

https://doi.org/10.1093/humrep/dead088

Original Article

Embryology

Failure to detect DNA in blastocoel fluid is associated with a higher live birth rate in both PGT-A and conventional IVF/ICSI cycles

L. Gianaroli, D. Perruzza, C. Albanese, S. Azzena, C. Tabanelli, Anna P. Ferraretti, and M. Cristina Magli *

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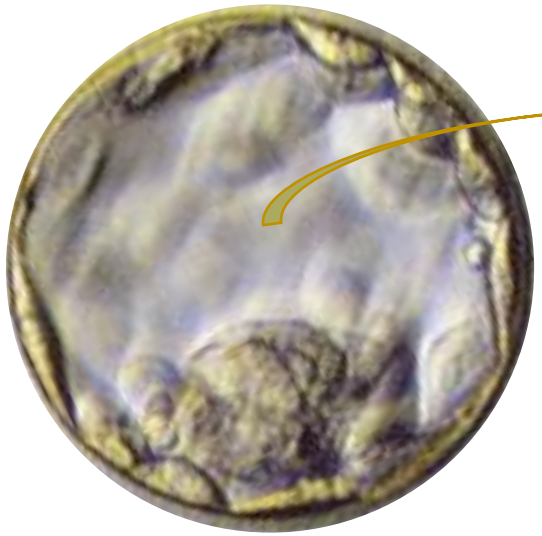
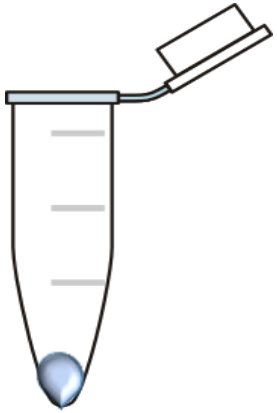
EMBRYO PRIORITIZATION FOR TRANSFER

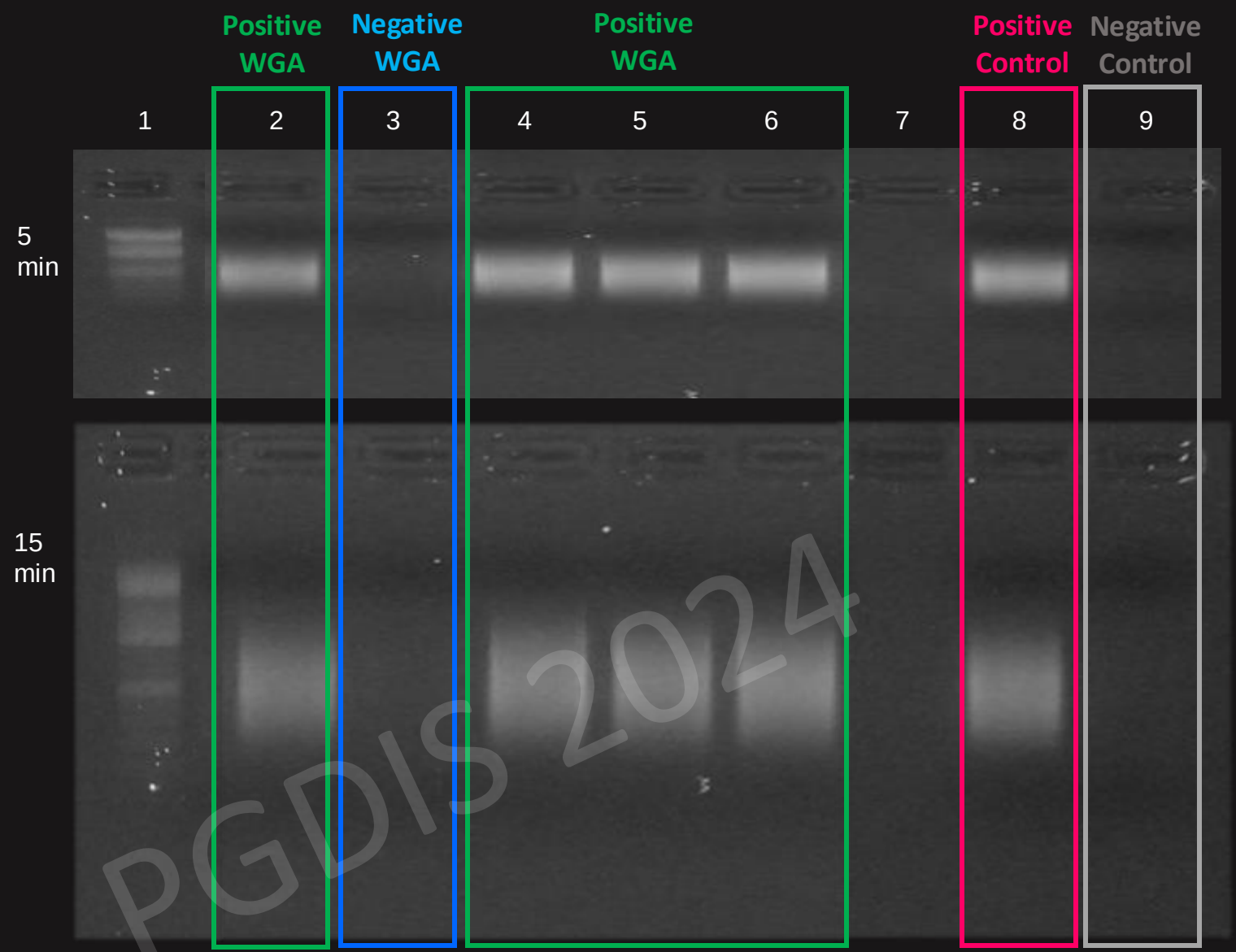


Aspiration of the Blastocoelic Fluid (BF)



Whole Genome Amplification (WGA)

ASSESSMENT OF BF-WGA RESULTS



An aliquot of the amplified product loaded onto a 1.5% agarose gel.

Successful amplification was indicated by a band with an intensity similar to that of the positive control.

In negative amplification, the lane in the agarose gel looked like the negative control.

Magli et al., Fertil Steril 2019

EMBRYO PRIORITIZATION FOR TRANSFER

	PGT-A programme ⇓ only TE-euploid blastocysts transferred	Good prognosis patients ⇓ Low-aneuploid incidence expected	
No. Patients	102	88	*p*
Maternal age (years), mean±SD	37.9±4.6	33.7±5.4	<0.001

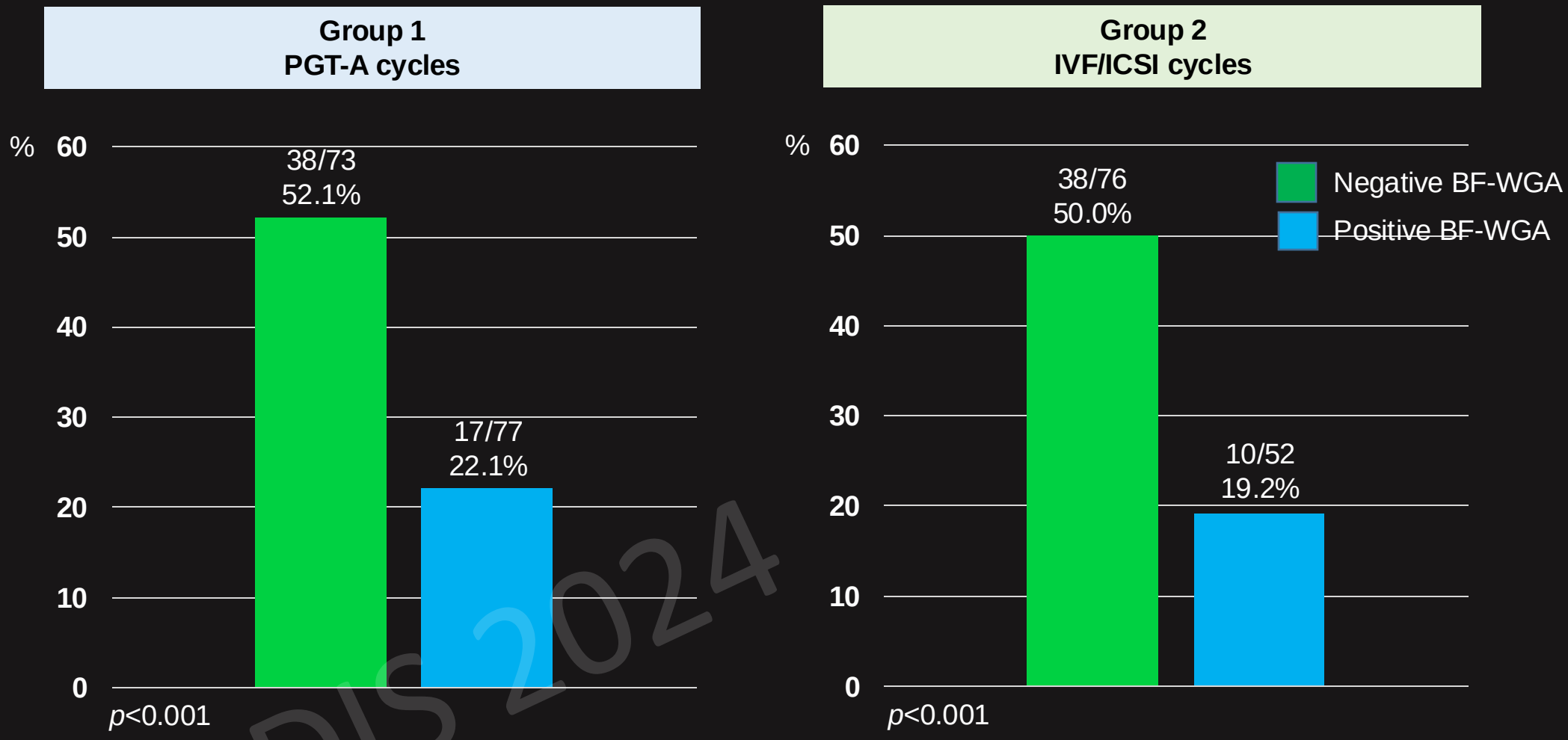
The aim of this prospective study was to verify whether the absence or presence of DNA in the BF as detected by the used WGA analysis could be a valid method to prioritize embryos for transfer both in PGT-A cycles and in conventional IVF cycles.

The primary outcome investigated was the live birth delivery rate (LBR) after the first transfer.

EMBRYO PRIORITIZATION FOR TRANSFER

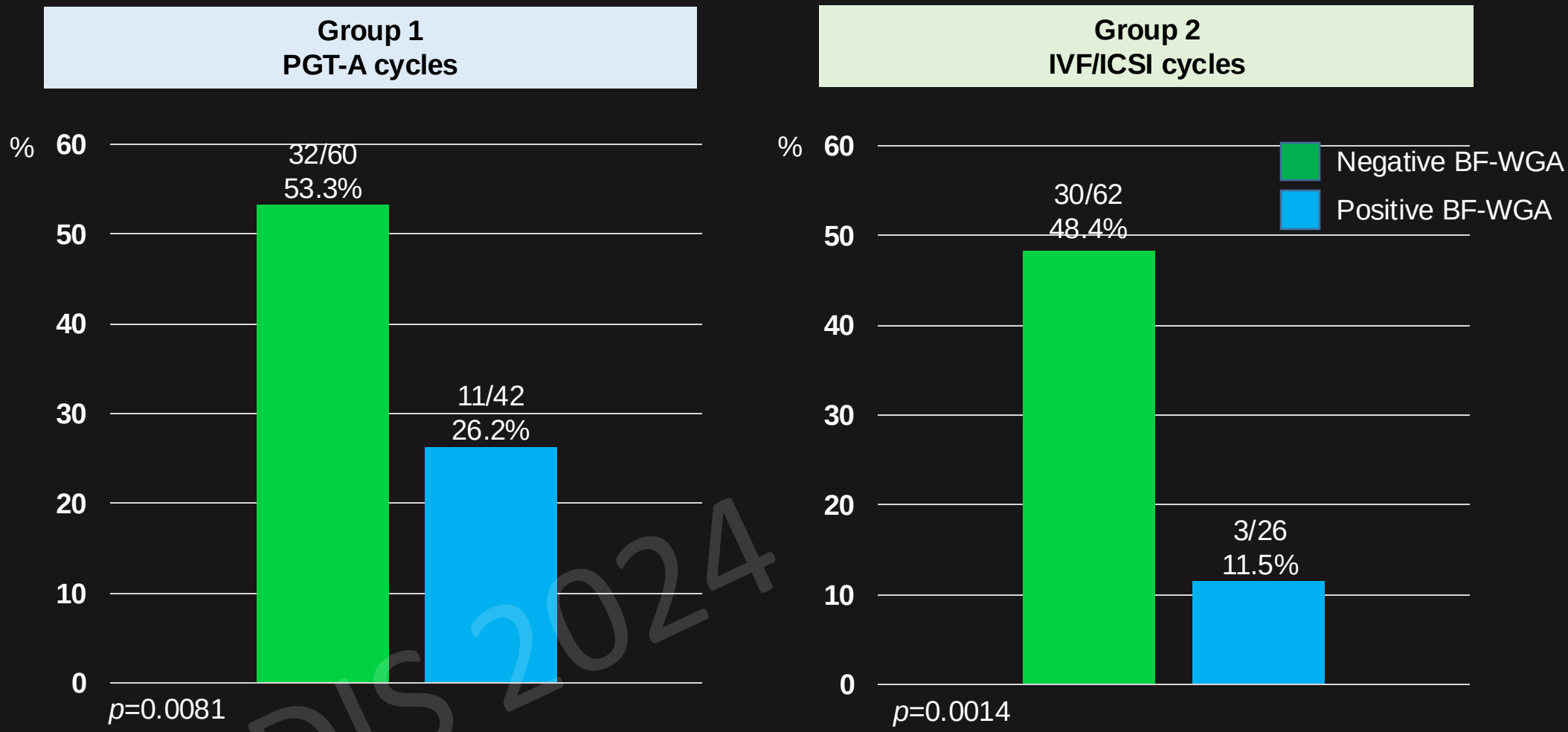
	Group 1 PGT-A		Group 2 Conventional IVF/ICSI
Blastocysts with BF biopsy	534		315
Negative BF-WGA (%)	202 (37.8)	<0.001	175 (55.6)
Positive BF-WGA (%)	332 (62.2)	<0.001	140 (44.4)

LBR PER TRANSFER



In both groups, the LBR was significantly higher after the transfer of negative BF-WGA blastocysts.

LBR AT THE FIRST TRANSFER



In both groups, the LBR at the first transfer was significantly higher after the transfer of negative BF-WGA blastocysts.

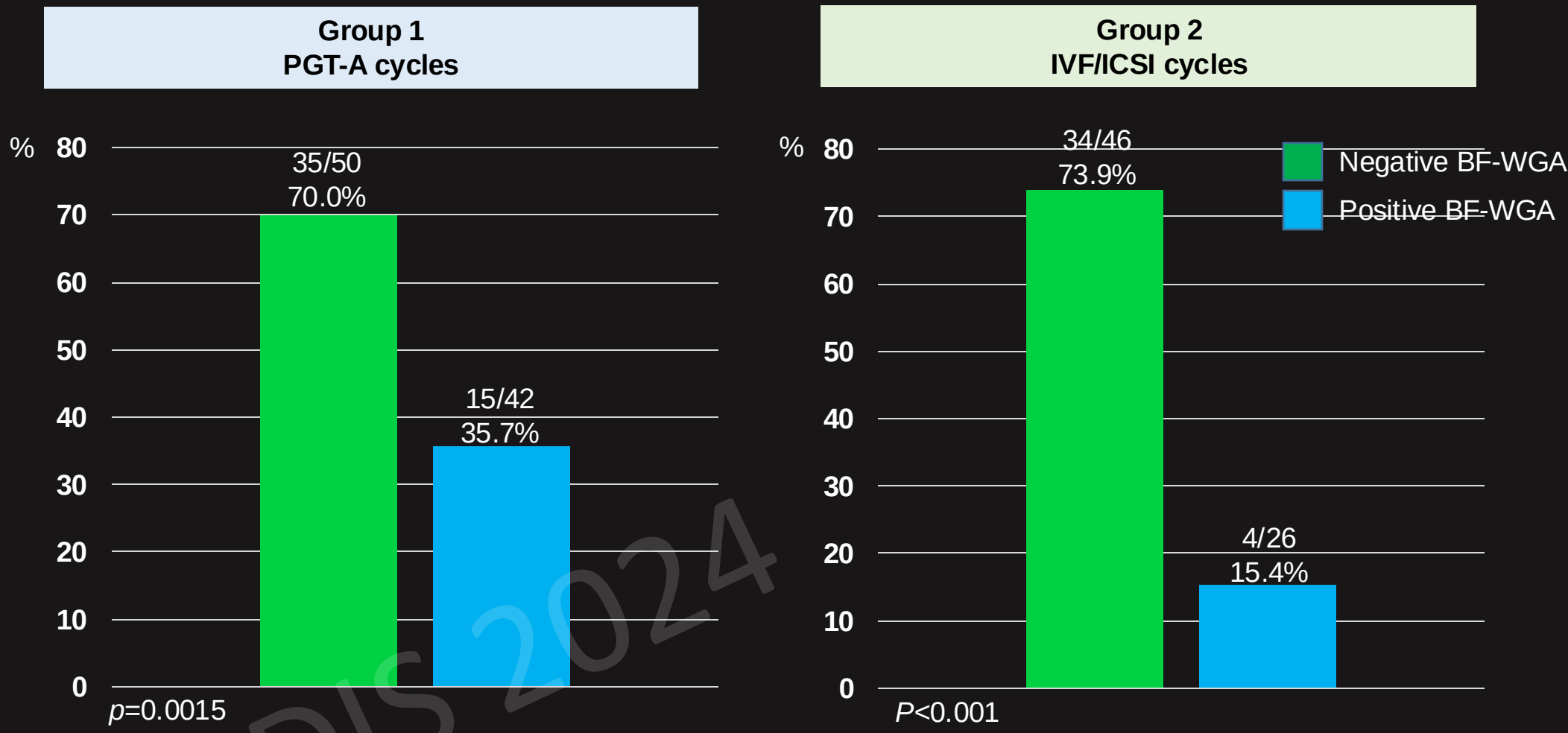
LBR AT THE FIRST TRANSFER

To evaluate the relevant factors determining the clinical outcome, a multiple logistic analysis was performed, in which the primary outcome was the LBR at the first embryo transfer.

After testing the studied variables for their association with the primary outcome, and correcting for possible confounders (maternal and paternal age, number of retrieved oocytes, male factor), the transfer of blastocysts with negative BF-WGA resulted in

- Group 1 (PGT-A): an odds ratio (OR) of 3.52 (95% CI: 1.48–8.88, *p*=0.0057) compared to the transfer of positive BF-WGA blastocysts.
- Group 2 (conventional IVF/ICSI): an OR 6.89 (95% CI: 1.98–32.95, p=0.0056) compared to transfer of positive BF-WGA blastocysts.

LBR PER PATIENT



In both groups, the LBR per patient was significantly higher after the transfer of negative BF-WGA blastocysts.

BF-WGA RESULTS – CLINICAL OUTCOME

	PGT-A	CONVENTIONAL IVF
No. patients	132	114
No. ET	200	180
No. ET with negative WGA	104	111
No. ET with positive WGA	96	69
No. clinical pregnancies with negative WGA (%)	65/104 (62.5)	54/111 (48.65)
	$p < 0.001$	$p = 0.0023$
No. clinical pregnancies with positive WGA (%)	30/96 (31.25)	17/69 (24.6)

SISMER data up to 31.12.2023

HOW TO EXPLAIN THESE FINDINGS?

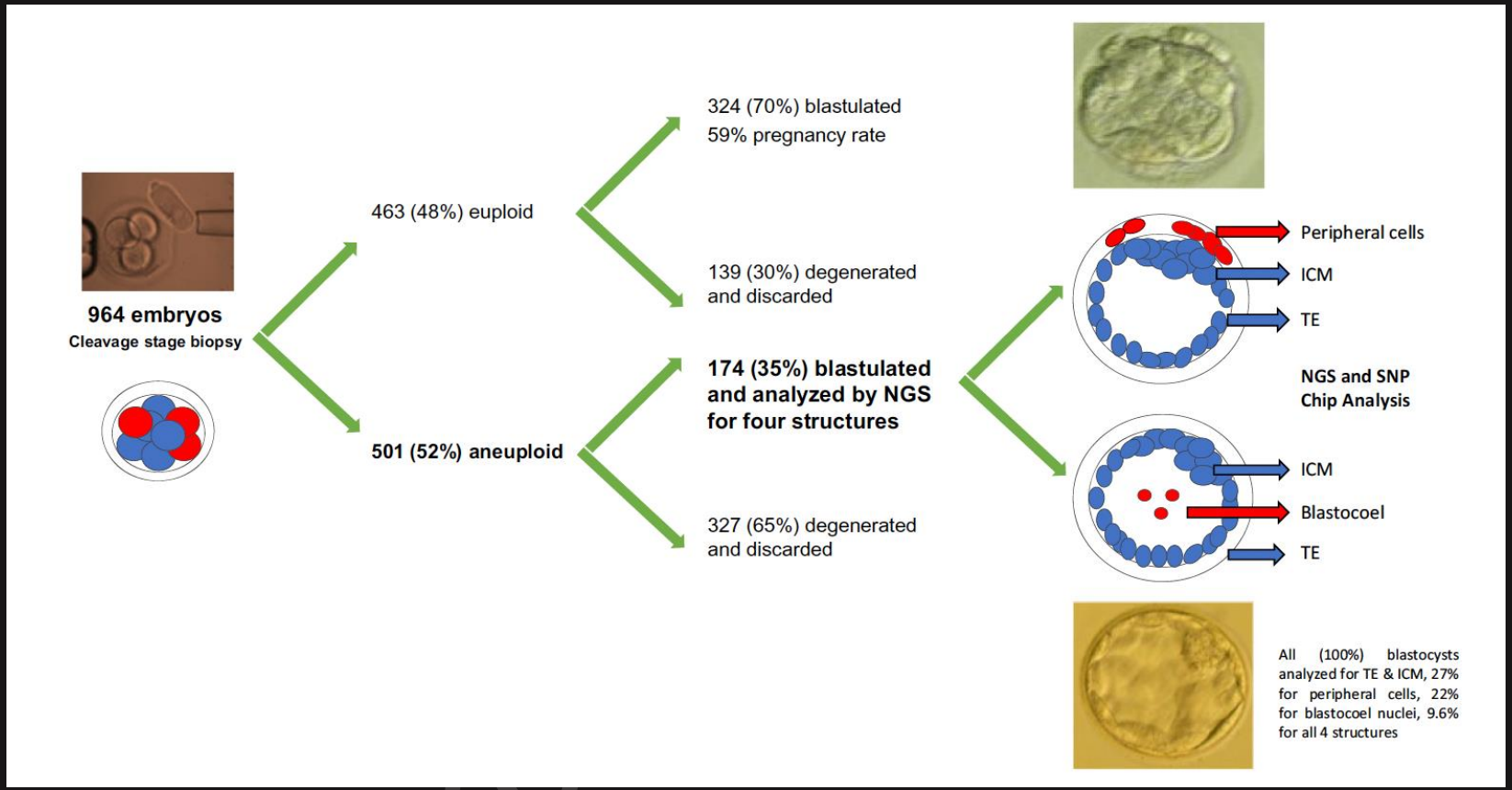
- The presence of DNA in the BF (positive BF-WGA) could be indicative of an abnormal embryo, probably mosaic, that is trying to reach a viable state by marginalizing aneuploid fragments to the periphery of the differentiating embryo.

- The resulting probability of embryo viability would depend on the grade of mosaicism and on the amount of energy required to extrude aneuploid cells into the BF.

  → If this balance is advantageous, the resulting embryo could implant even in case of positive BF-WGA results.

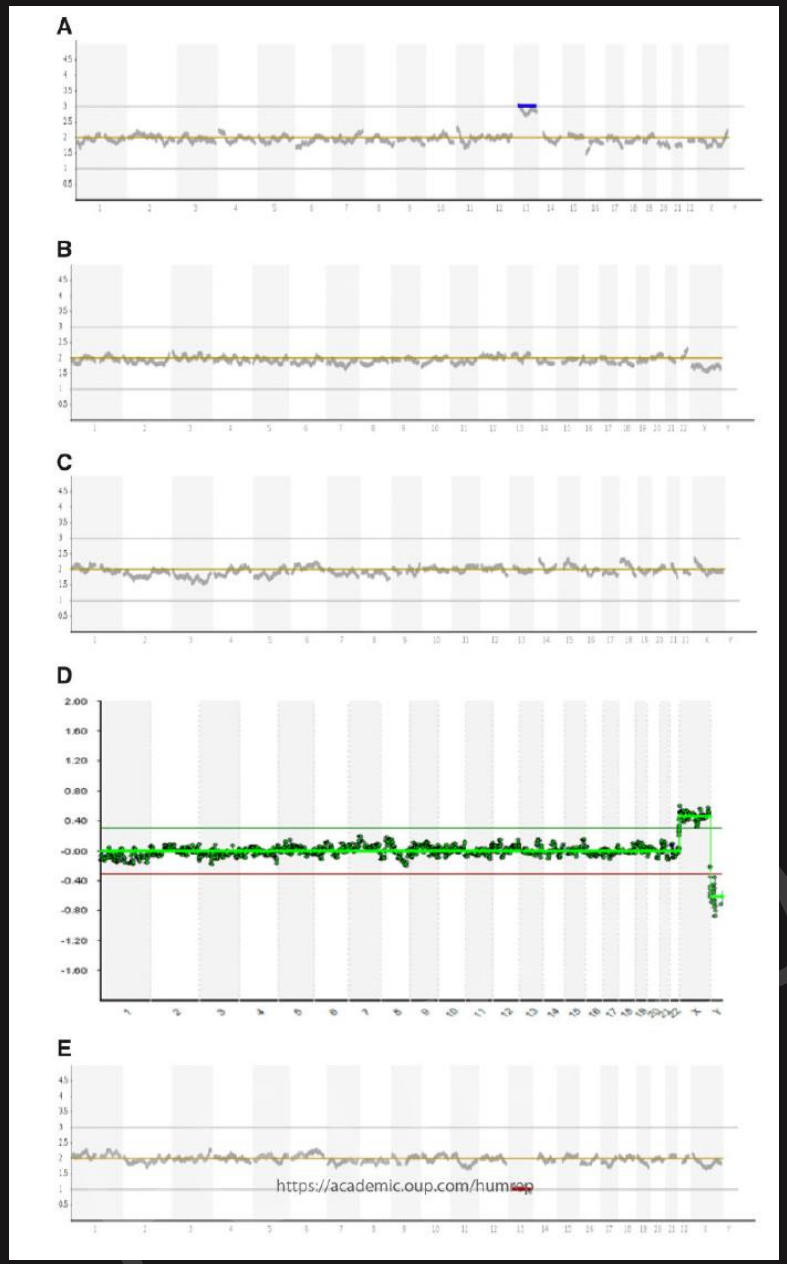
  → Negative BF-WGA could reflect the quiet metabolic state of euploid blastocysts in which necrosis and apoptosis processes are less likely to occur, conferring these blastocysts the highest implantation potential.

HOW TO EXPLAIN THESE FINDINGS?



STUDY QUESTION:

Are chromosome abnormalities detected at Day 3 post-fertilization predominantly retained in structures of the blastocyst other than the ICM, where chromosomally normal cells are preferentially retained?



Day 3 cleavage-stage blastomere (47, XX, **+13** by NGS)

DNA isolated from the ICM (**46, XX** by NGS)

DNA isolated from the TE (**46, XX** by NGS)

DNA isolated from the peripheral cells (**46, XX** by aCGH)

DNA isolated from the BF (45, XX, **-13** by NGS)

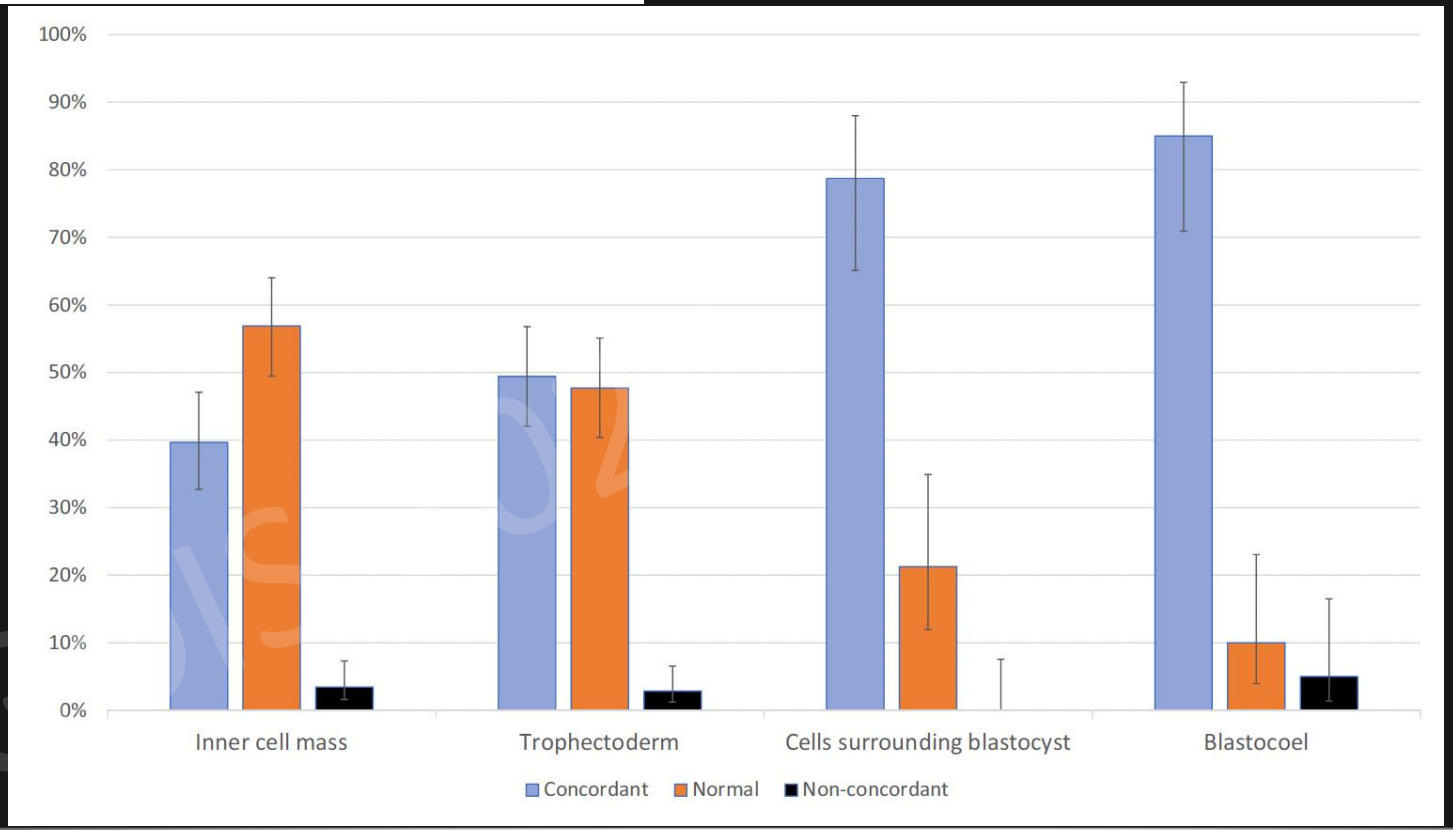
Human Reproduction, Vol.38, No.1, pp. 180–188, 2023
Advance Access Publication on November 9, 2022 https://doi.org/10.1093/humrep/deac238

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

The human embryonic genome is karyotypically complex, with chromosomally abnormal cells preferentially located away from the developing fetus

D.K. Griffin [1,*], P.R. Brezina[2,3], K. Tobler[2,4], Yulian Zhao[2,5], G. Silvestri [1], R.C. Mccoy [6], R. Anchan[7], A. Benner[8], G.R. Cutting[9], and W.G. Kearns[2,8]

In human embryos, aneuploid cells are sequestered away from the ICM, partly to TE, but more significantly to the blastocoel fluid and to peripheral cells surrounding the blastocyst during Day 3 to Day 5 progression.



Self-correction

CONCLUSIONS

- The data from this prospective study identify **BF-WGA as potential minimally invasive tool** for the selection of the most viable embryos in patients undergoing PGT-A or conventional IVF/ICSI cycles.

- The transfer of **negative BF-WGA** blastocysts resulted in a **significantly higher LBR per transfer** and **per patient** and, very important, an improved outcome **at the first embryo transfer**.

- Should this finding be confirmed in a larger dataset, such an easy and cost-effective tool, namely the processing of the BF by WGA, could become a **valuable option to offer patients the highest chances of a term pregnancy in the shortest time possible**.

Failure to detect DNA in blastocoel fluid is associated with a higher live birth rate in both PGT-A and conventional IVF/ICSI cycles.

Gianaroli et al., Hum Reprod 2023

“The spent culture medium”

PubMed®

non invasive preimplantation genetic testing

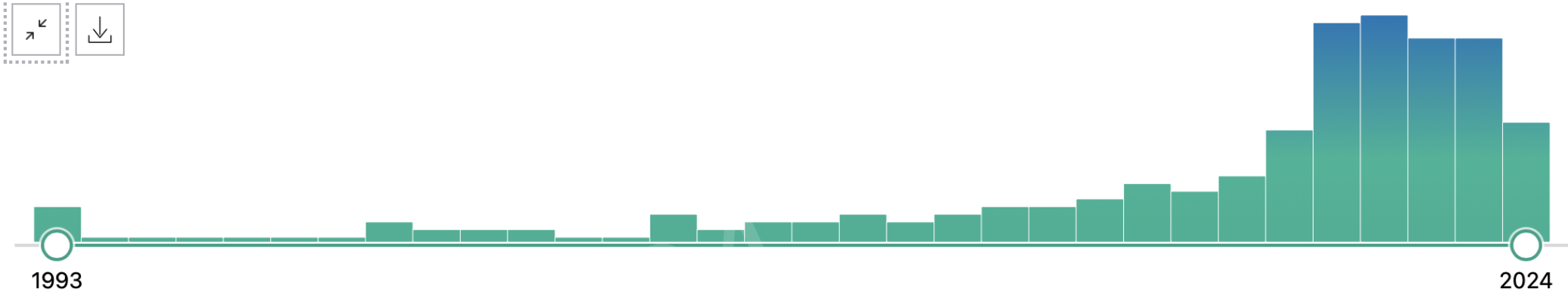
Search

Advanced Create alert Create RSS User Guide

RESULTS BY YEAR

174 results

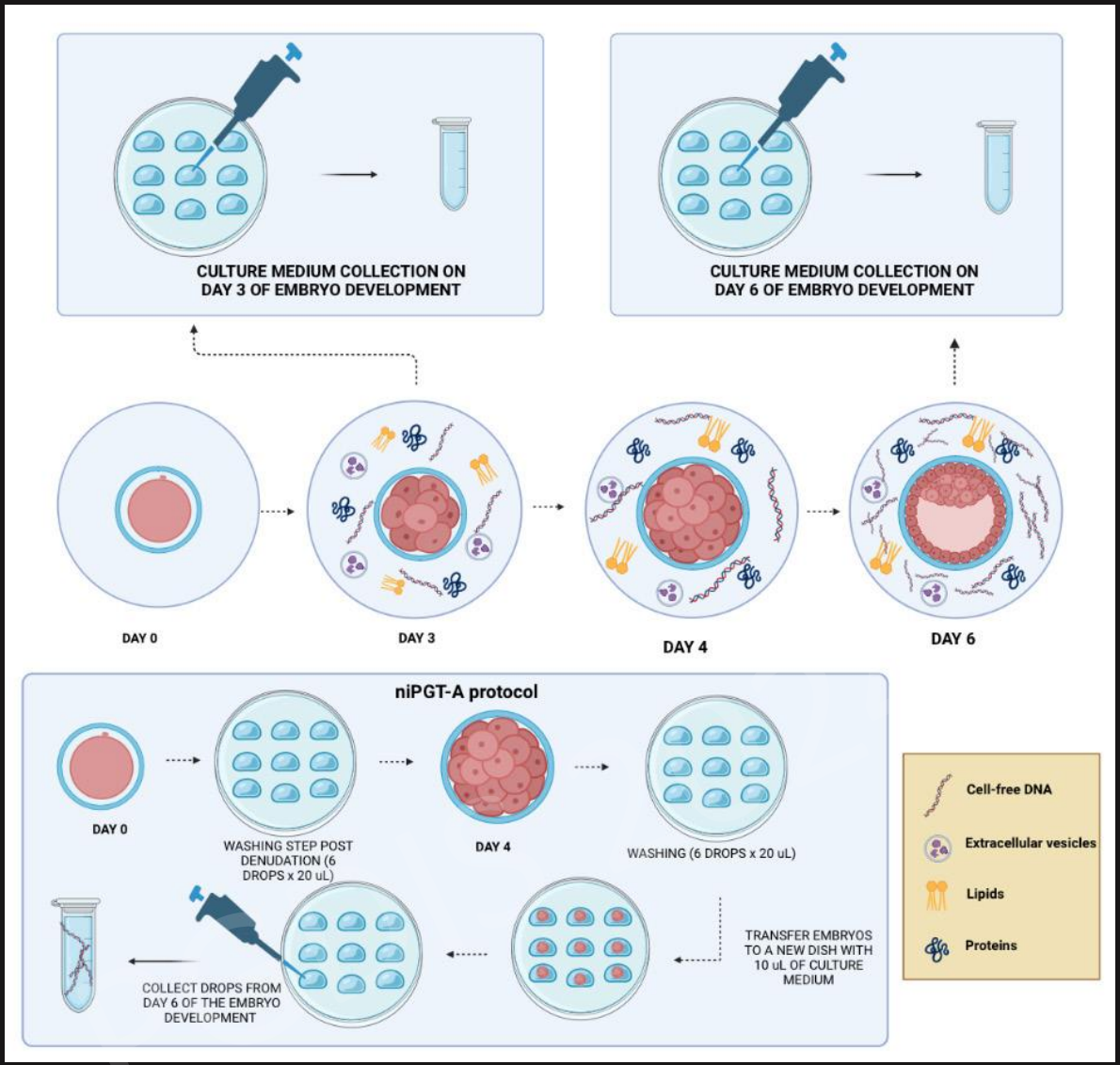
Page 1 of 18



High interest on the topic of niPGT

Possibly in the near future, new strategies and technical approaches (e.g. morphokinetics, proteomics, AI) will provide a solid support to its clinical implementation.

niPGT - SPENT CULTURE MEDIUM



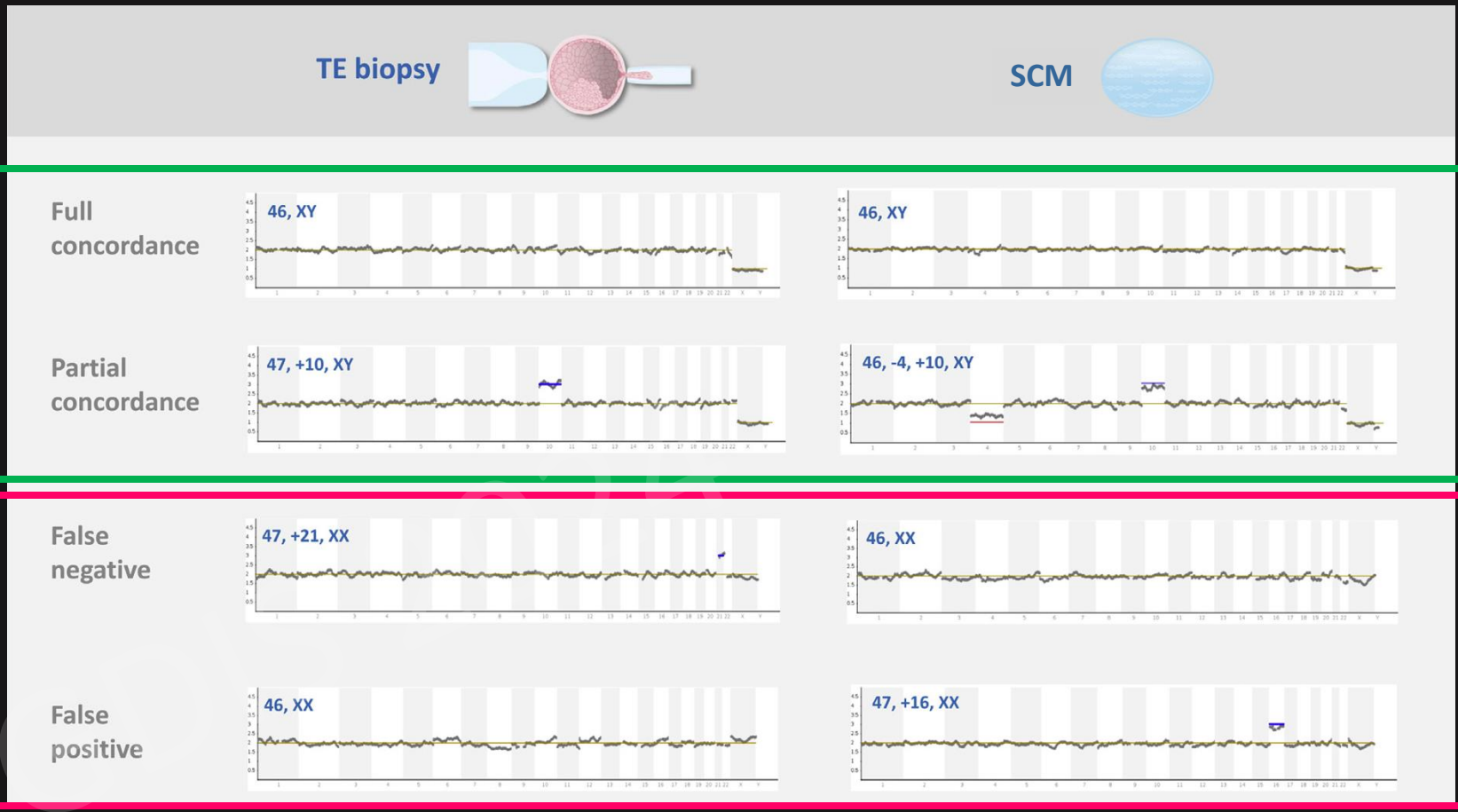
For the success of PGT through SCM analysis, the laboratory protocols have to be adapted:

- **microdrops of 10 µl**
- **extensive and repeated washings to avoid maternal DNA contamination (cumulus cells)**
- **individual cultures using new capillaries for each embryo**
- **medium change on day 4 (usually day 3 for sequential media)**
- **obligatory extension of cultures to day 6**

Chow et al., J Assist Reprod Genet 2024
Del Collado et al., Anim Reprod. 2023

niPGT - SPENT CULTURE MEDIUM

Following these strategies, the concordance rates between SCM and TE biopsy, ICM and whole embryos can be higher than 90%.



niPGT - SPENT CULTURE MEDIUM

Irrespective of this high efficiency, currently

- **niPGT-A based on the analysis of the embryonic cfDNA in the SCM should not be considered a substitute for TE biopsy, but it might help to prioritize for transfer the embryo with higher reproductive potential, better than morphology or morphokinetics.**

- **In this scenario, all embryos could be considered for transfer according to their priority order.**

This could be an attractive option for those patients who want to improve their clinical outcomes without the need for an invasive TE biopsy, and for those patients who do not want to discard embryos.

niPGT - SPENT CULTURE MEDIUM

However,

Not everything is about concordance
(data show that it increases proportionally to the time of embryos in culture)

Embryo viability is of utmost importance
(no damage to the embryo is one of the most driving forces of niPGT)

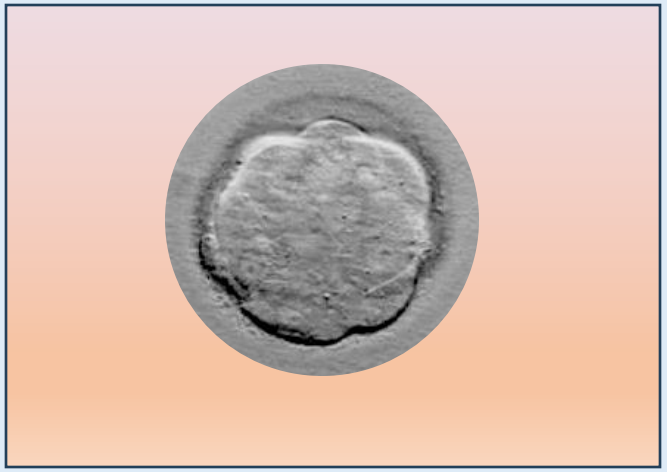
Extension of culture to day 6 / 7 is normally done for slow-cleaving embryos, but forcing it by default could possibly cause damage embryos that would normally be biopsied / vitrified on day 5?

"It is not feasible to prolong an embryo's time in culture once it has reached the mature blastocyst stage. Delaying biopsy and vitrification to ensure adequate DNA amplification with niPGT-A would be clinically irresponsible and would ultimately harm the embryos".

Hanson et al., Fertil Steril 2021

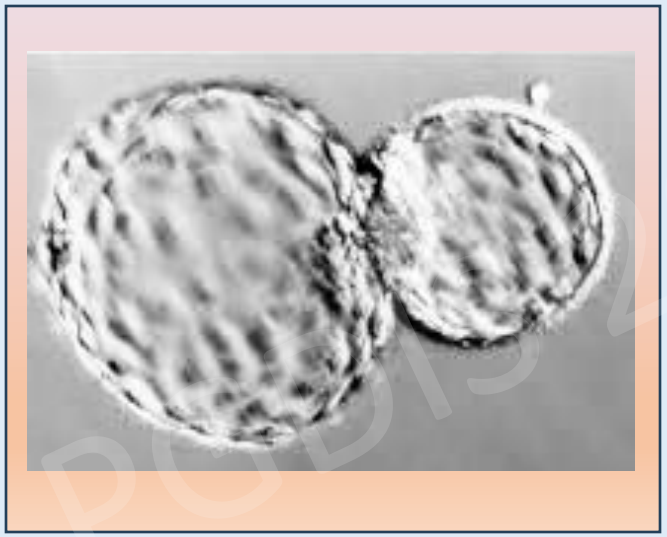
CULTURE TO DAY 6 FOR niPGT

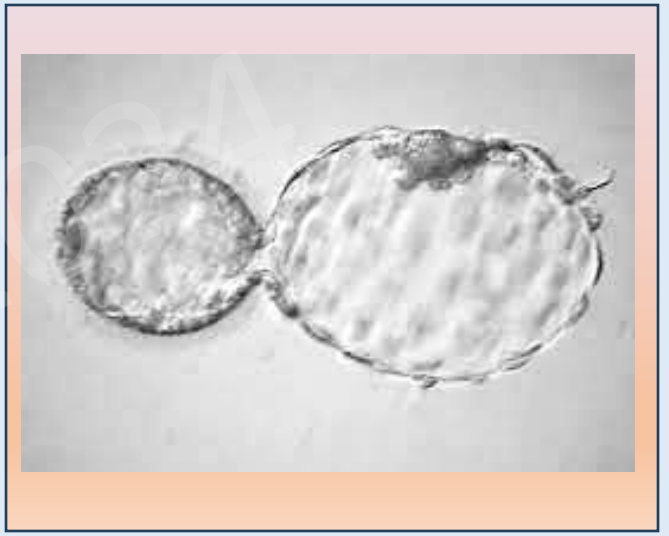
Day 4

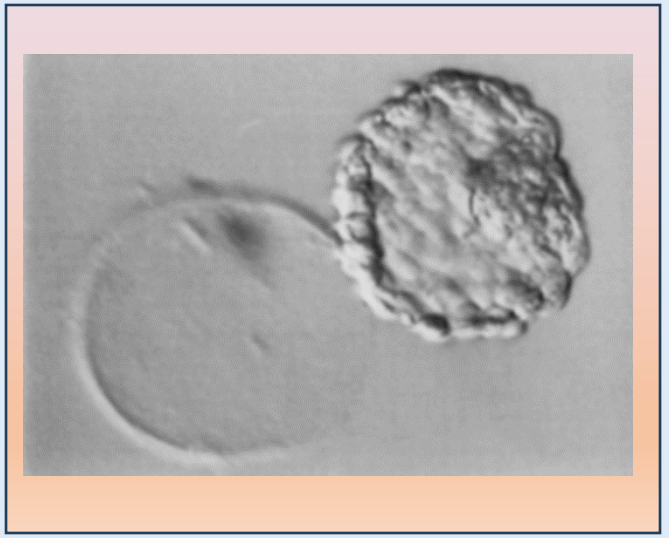


47.4% of embryos cultured to day 6 where half hatched or fully hatched

Day 6



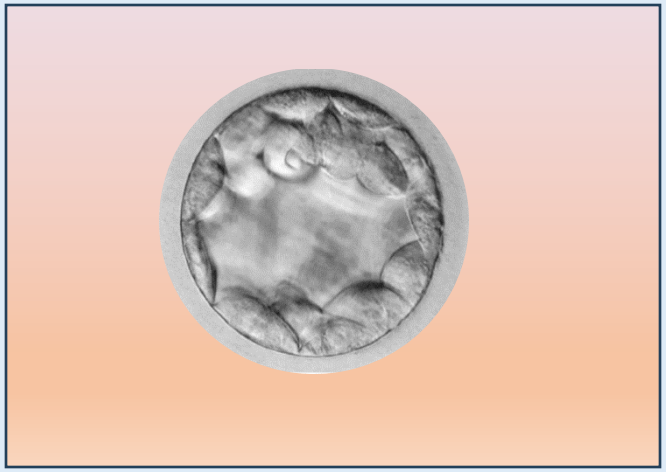




SISMER data 2022-2024

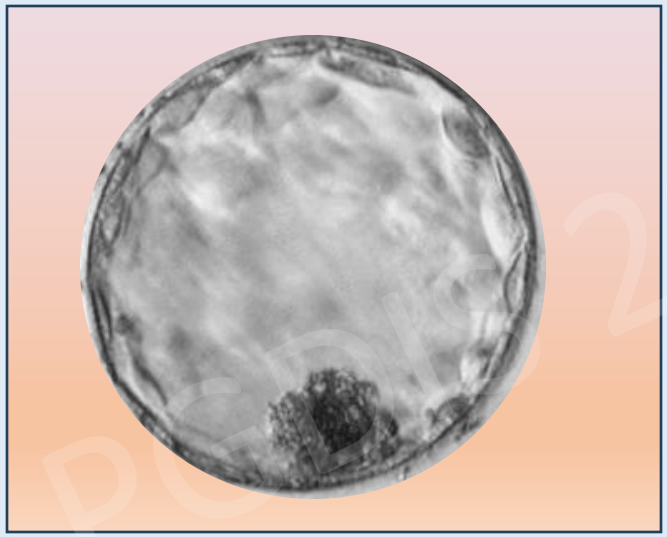
CULTURE TO DAY 6

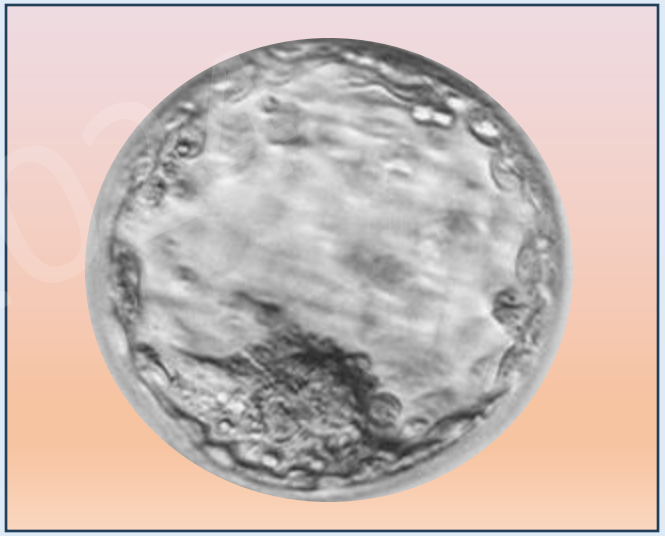
Day 4

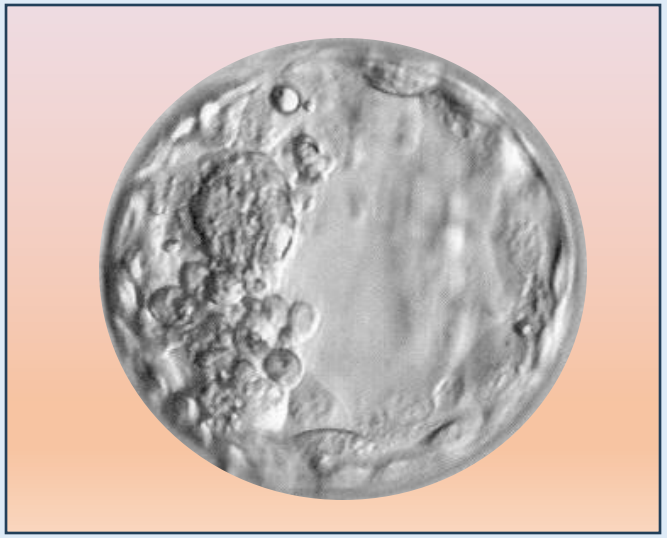


15.5% of embryos cultured to day 6 had some sign of degeneration

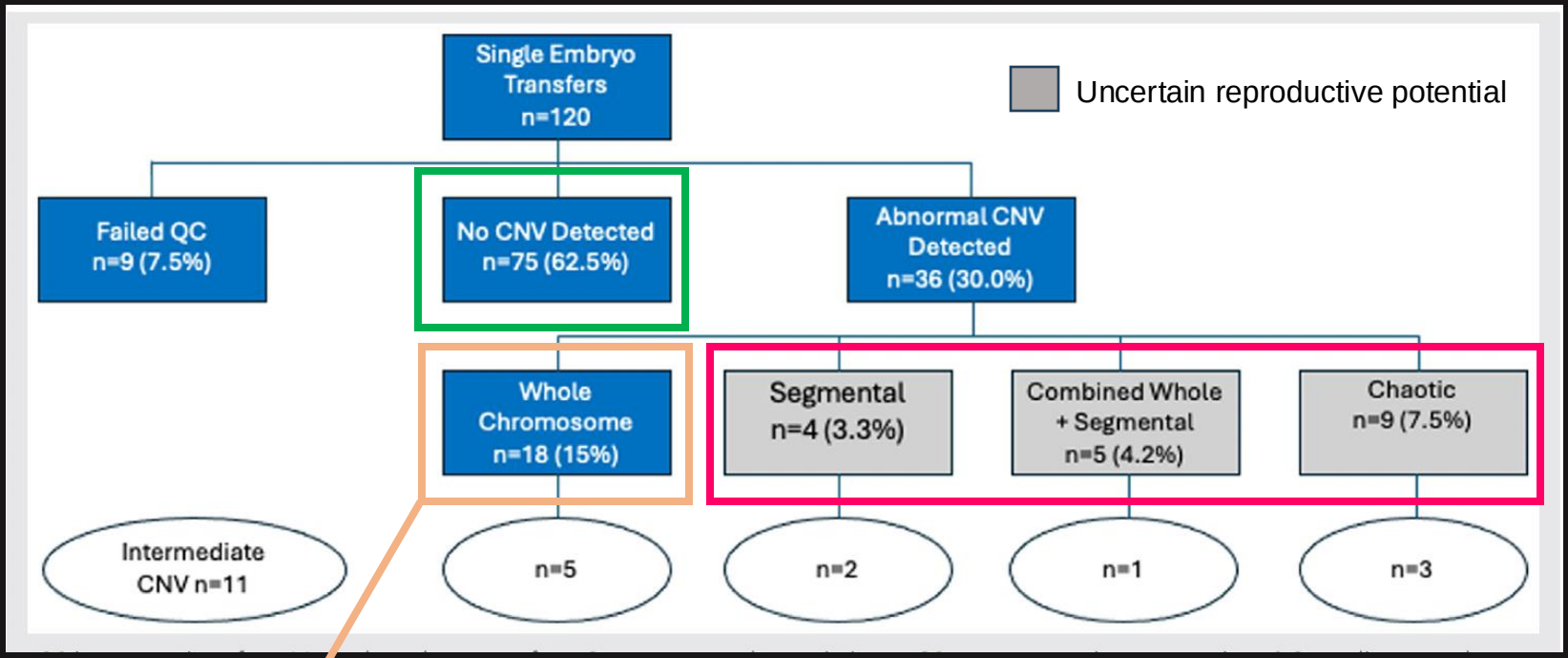
Day 6







SISMER data 2022-2024



94.4% PPV

High Relative Risk for non-viability

57.3% sustained implantation rate

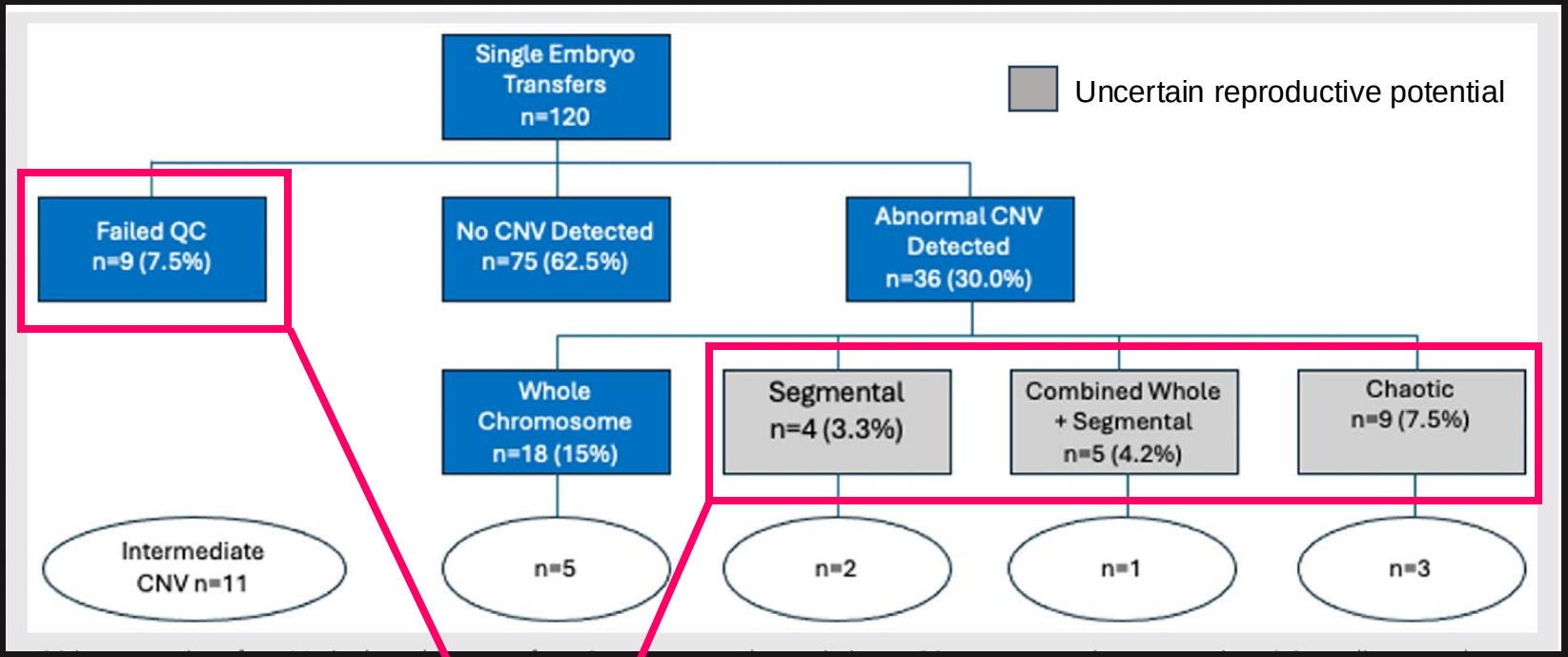
5.6% sustained implantation rate

77.8% sustained implantation rate

Non-selection pilot study

Embryo selection was performed according to the conventional grading, blinded to niPGT-A results.

After clinical outcomes were established, spent culture media samples were analyzed.



Non-selection pilot study

Unintended discarding of viable embryos

22.5% With no reportable result

66% sustained implantation rate

Sex was confirmed **74.1%** of cases

↓

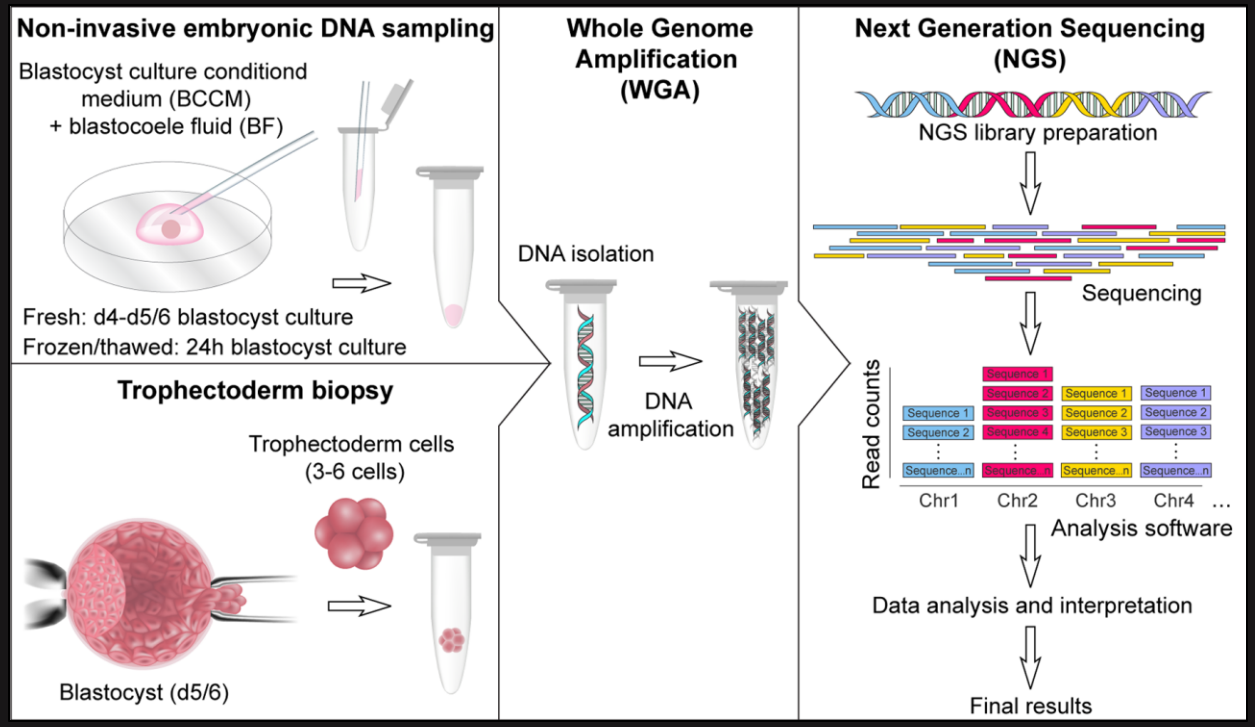
14 male fetuses were misdiagnosed

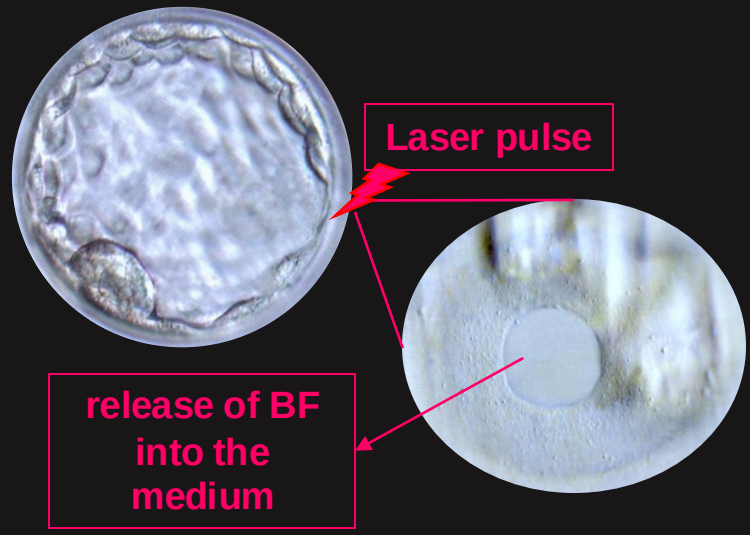
↓

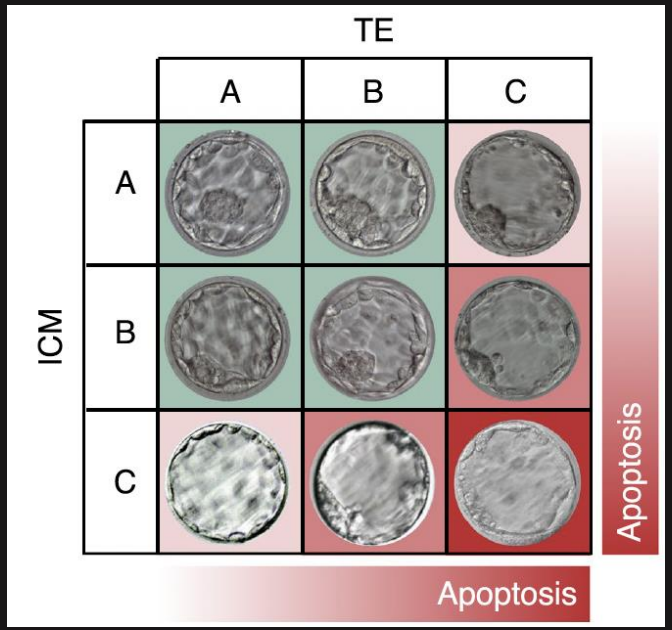
Maternal DNA contamination remains an issue

niPGT validation is needed to provide the best clinical information in consideration of its technologic limitations

“Blastocoel fluid +
spent culture medium”







The overall concordance rate per sample for euploidy/aneuploidy status between miPGT-A and TE biopsy samples was 97.8% (88/90).

The concordance was not different between good 47/48 (97.9%) and moderate/low quality blastocysts 41/42 (97.9%).

“mi/niPGT
General considerations”

niPGT – A CRITICAL VIEW

Evidence	Conclusions

Lawsuit against Monash IVF over embryo testing method

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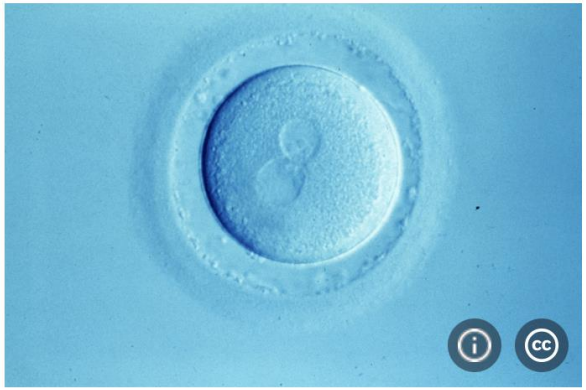


Hundreds of people across Australia are suing the major IVF clinic, Monash IVF, over the destruction of potentially viable embryos.

Between May 2019 and October 2020, Monash IVF offered a new, non-invasive, embryo genetic screening test that has since been suspended. The world-first method had a high false-positive rate, meaning that viable embryos may have been destroyed. The test was used up to 13,000 times and thousands of people could be affected.

Michel Margalit, lead lawyer on the case, said 'I think that there is a real possibility that many, many people will question whether or not they have lost their ability to have children because of this inaccurate testing.'

Instead of procuring an embryo biopsy, the new non-invasive method involved collecting a sample from the liquid surrounding the embryo. Monash IVF told patients that the results of invasive and non-invasive testing were identical in 95 percent of cases. When genetic abnormalities were identified, patients had the choice of saving, destroying or donating the embryos for medical research. The majority of people who were informed that their embryo was abnormal chose not to keep it for use in treatment.

'What we've found out now is [the non-invasive method] perhaps didn't go through the usual robust testing systems that you'd usually expect, which would include publication of the trial data when testing this type of technology, and peer review,' Margalit said.

Monash IVF are offering free treatment to the affected patients; however, this is not feasible for some. Those affected are seeking compensation for the trauma caused and the lost opportunity to have genetically related children, in a multi-million-dollar class action. 'It's an age and time game for a lot of people in IVF,' Margalit added, 'We will be fighting for the rights of these women who have placed their trust in the hands of a medical provider and unknowingly have had devastating consequences.'

miPGT / niPGT – A COMPARISON

	Blastocoel fluid	Spent culture medium

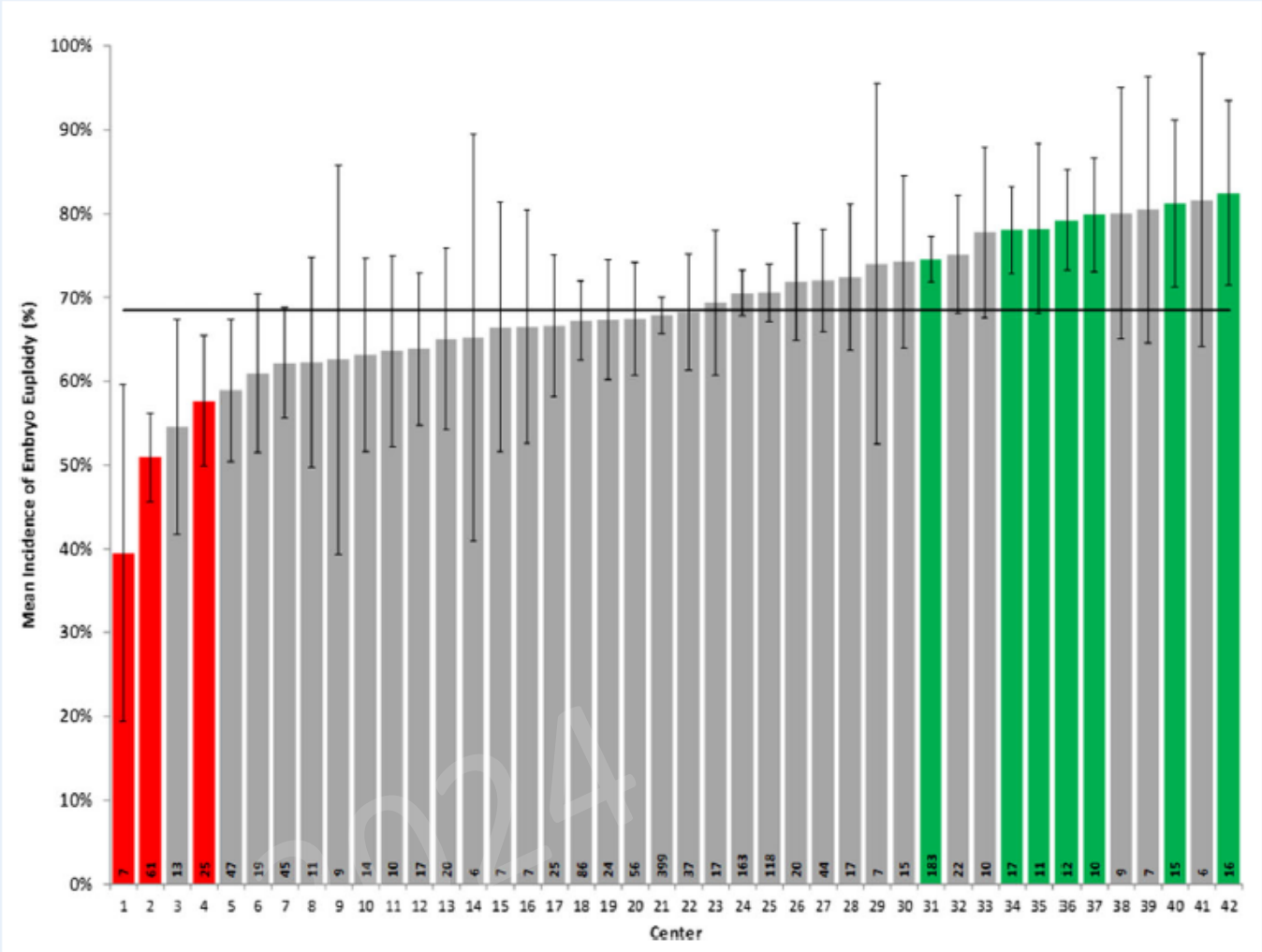
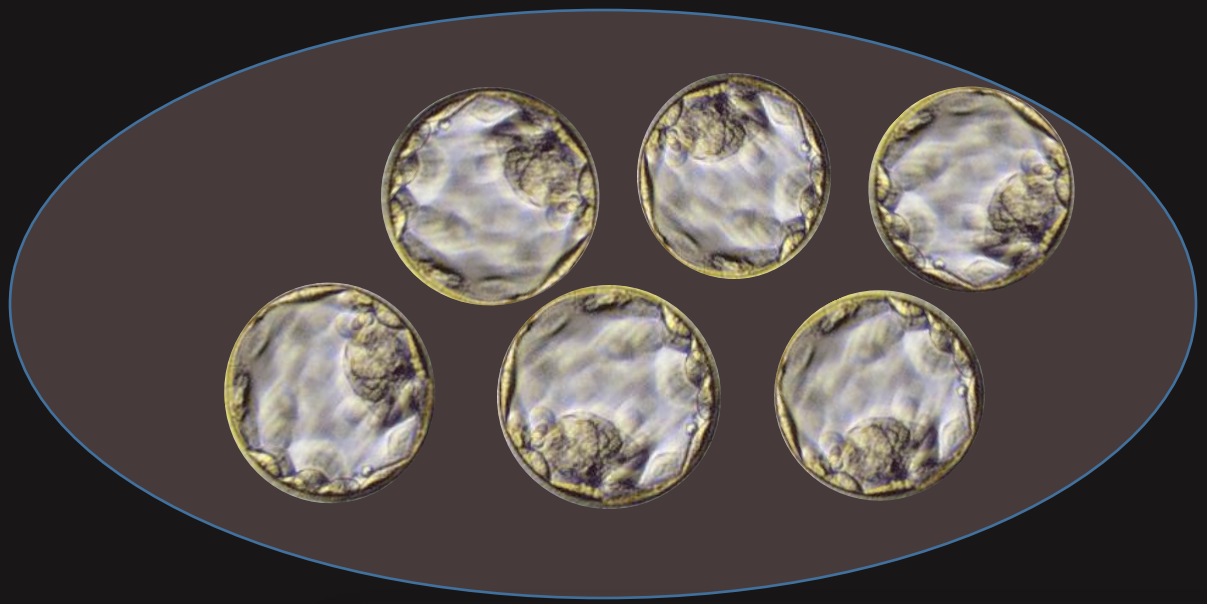


Figure 1 Mean incidence of embryo euploidy (%), calculated for individual centers, weighting each donor cycle equally regardless of the number of embryos per cycle. The mean incidence of donor cycle euploidy for each center is displayed as a shaded bar (error bars represent ±95% confidence limits for the mean value). The overall mean incidence of euploidy for all donors from all centers (68.5%) is displayed as a bold horizontal black line. The number of donor cycles is displayed at the bottom of each center's bar. Centers with an incidence of euploidy that was significantly different from the overall mean incidence of euploidy using Wilcoxon signed-rank test are shaded in red (significantly lower euploidy) or in green (significantly higher euploidy). Centers with an incidence of euploidy that was not significantly different from the overall mean incidence of euploidy are shaded in gray. Center ID number represents the center's ranking from lowest to highest incidence of euploidy.

EMBRYO PRIORITIZATION FOR TRANSFER



Any strategy to go beyond PGT-A and morphology ?

Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with a poor prognosis: identification of the categories for which it should be proposed

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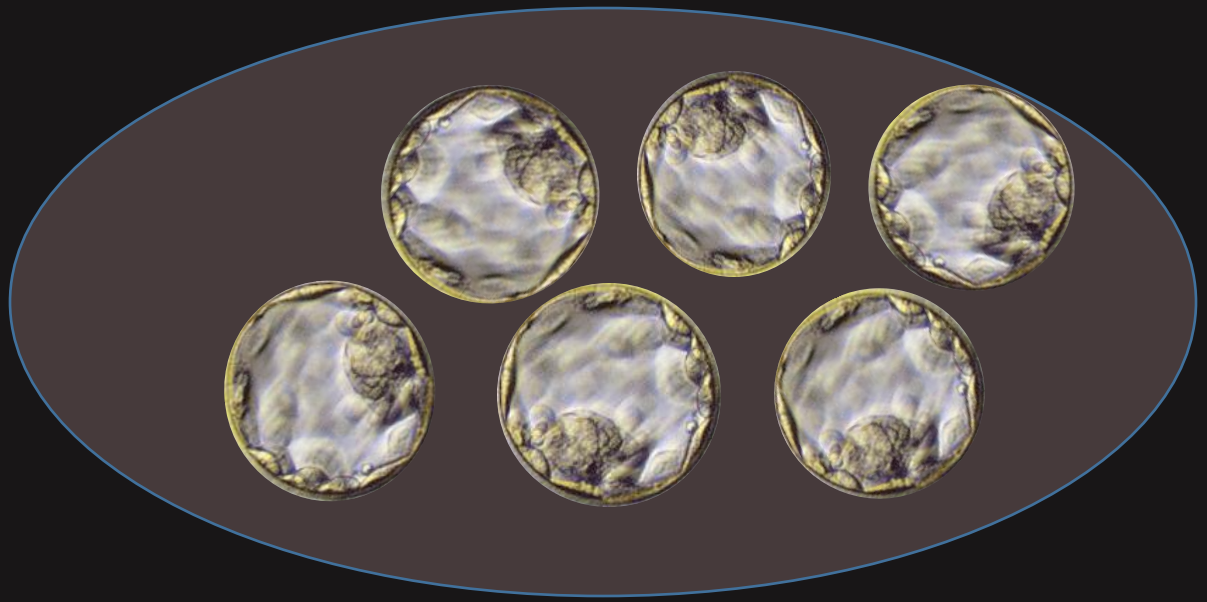
S.I.S.ME.R., Bologna, Italy; and Saint Barnabas Medical Center, Livingston, New Jersey

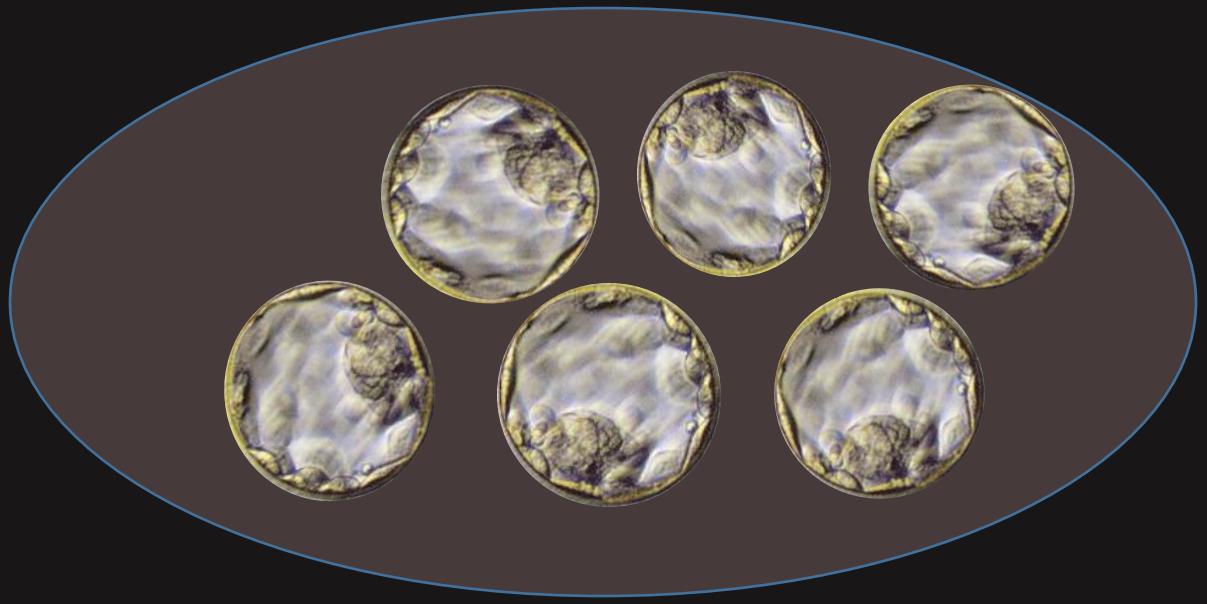
EMBRYO PRIORITIZATION FOR TRANSFER



Any strategy
to go
beyond
PGT-A
and
morphology
?

GLUCOSE? AMINOACIDS (TURNOVER?) PROTEIN(S? FREE FATTY ACID?

EMBRYO PRIORITIZATION FOR TRANSFER



Any strategy to go beyond PGT-A and morphology ?

GLUCOSE? AMINOACIDS (TURNOVER?) PROTEIN(S? FREE FATTY ACID?

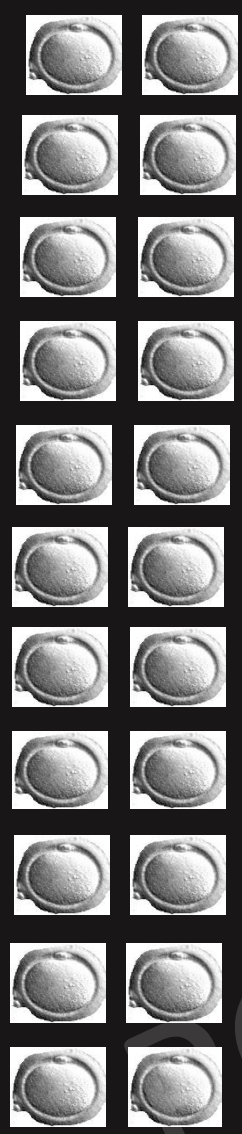
METABOLITES-METABOLOME-METABOLOMICS

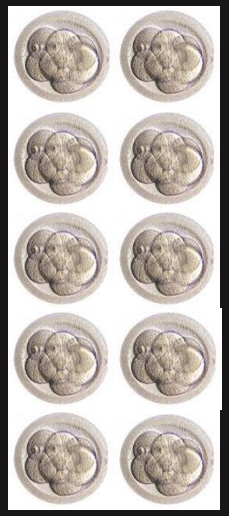
RECURRENT **I**MPLANTATION **F**AILURE

OR

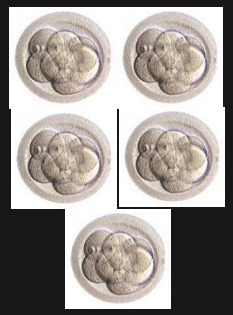
RECOGNITION OF **I**GNORANCE & **F**AILURE

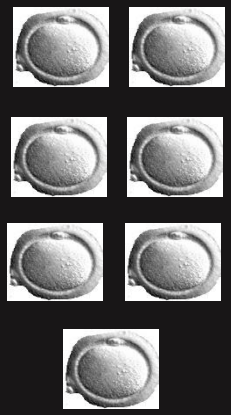
≤ 35 years





> 35 years





EMBRYO COMPETENCE: LOOKING BEYOND EUPLOIDY

WHY	WHAT	WHERE

CONCLUSIONS
(Don’t take these messages home….take them to your clinic!)

- In nature RIF exists and it is normal
- Why should it be considered differently in ART?
- Strategies to reduce RIF cannot be «one fits all»
- The only proven strategy to decrease the incidence of RIF is the drastic reduction of drop out
- RIF criteria should be defined in each ART clinic for different sub-groups of patients and they should be used as KPI
- ART clinic effort is useless without patients’ committment.

CONCLUSIONS

(Don’t take these messages home….
…..take them to your clinic or to your lab)