NYU Langone Health

PCRS 2025

Exploring the Clinical Utility of Mosaic and Segmental Aneuploid PGT-A Results

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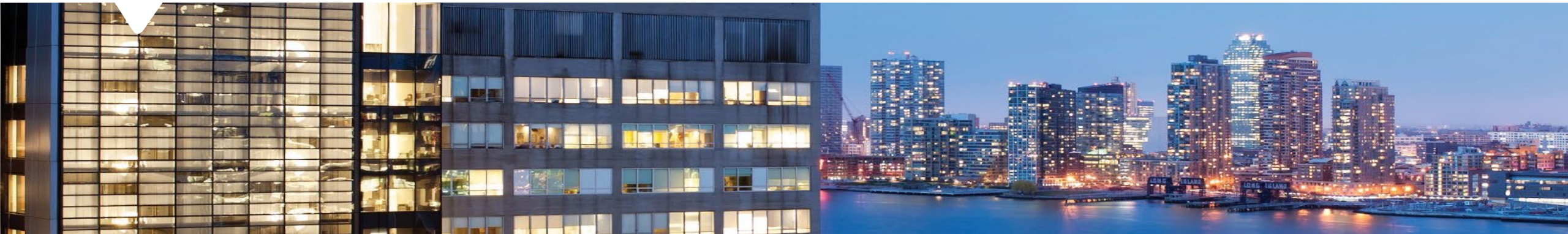
March 20, 2025



Disclosures

Neither I nor members of my immediate family have any actual or potential financial interests to disclose relating to the content of this presentation.

Expected Learning Outcomes

1. Describe how embryonic mosaicism is inferred via preimplantation genetic testing for aneuploidy (PGT-A) and recognize the limitations of this diagnosis.

2. Differentiate between the significance of mosaic and segmental aneuploid results identified in the preimplantation embryo from those identified in the prenatal and postnatal periods.

3. Review current outcome data to rank PGT-A embryos for transfer and maximize positive outcomes.

Purpose & Scope of PGT-A

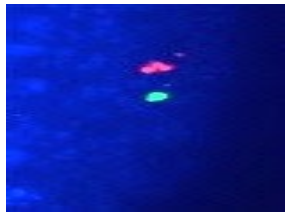
- Purpose: to avoid transfer of embryos that cannot produce a viable pregnancy
- What can current PGT-A platforms detect?

Full monosomies/trisomies	☑
Deletions/duplications >10 Mb	☑
69,XXY and 69,XYY triploid	☑
Deletions/duplications <10 Mb	Depends on platform
69,XXX triploid and 23,X haploid	Depends on platform
Uniparental disomy	Depends on platform
Deletions/duplications <1 Mb	☒
Balanced rearrangements	☒
Mendelian or polygenic disease	☒

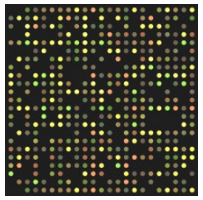
PGT-A is a test for embryo viability, NOT health

Evolution of PGT-A

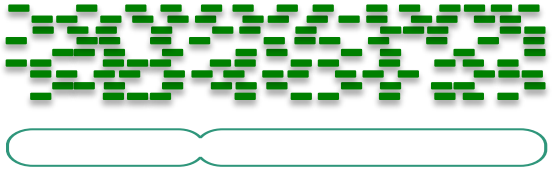
1990s
FISH

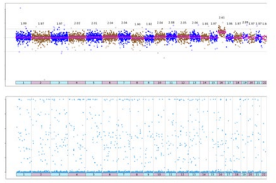


~2010
aCGH, SNP arrays
qPCR

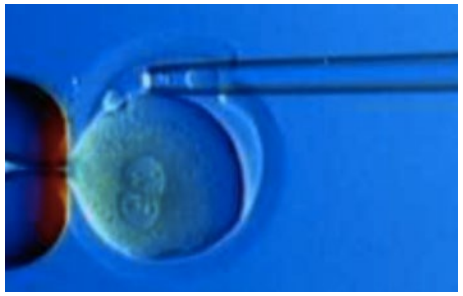


~2015
NGS

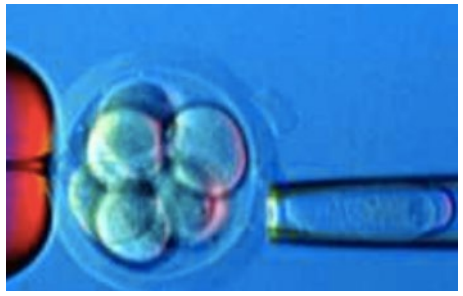




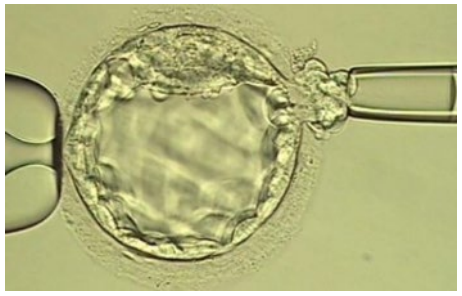
~2019
NGS+SNP



Polar body
mature oocyte, zygote



Blastomere
cleavage-stage



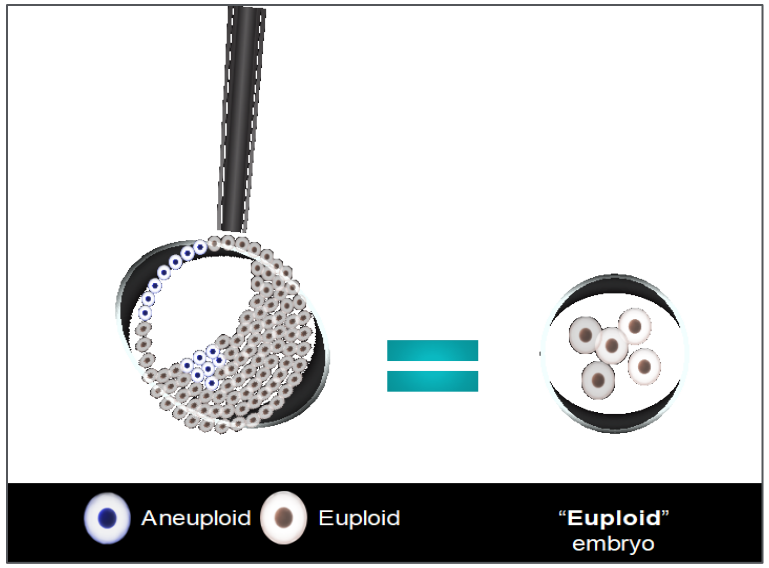
Trophectoderm
blastocyst

As testing becomes more sensitive, line between euploid and aneuploid becoming increasingly blurred

Missing Mosaicism

Mosaicism is a known limitation of euploid results

- ACOG and ASRM recommend that all patients who conceive via PGT-A be offered prenatal screening and diagnosis



Clinical error rates of next generation sequencing and array comparative genomic hybridization with single thawed euploid embryo transfer

Jenna Friedenthal[a,*], Susan M. Maxwell[a], Ashley W. Tiegs[b,c], Andria G. Besser[a], Caroline McCaffrey[a], Santiago Munné[d], Nicole Noyes[a], James A. Grifo[a]

European Journal of Medical Genetics 63 (2020) 103852

1-2% identifiable error rate per transferred euploid embryo

Identifying Mosaicism

Does a mosaic result truly indicate mosaicism?

Does a mosaic biopsy result represent the rest of the embryo?

Does a mosaic result predict reproductive potential?

What is the clinical impact of a mosaic result on an ongoing pregnancy?

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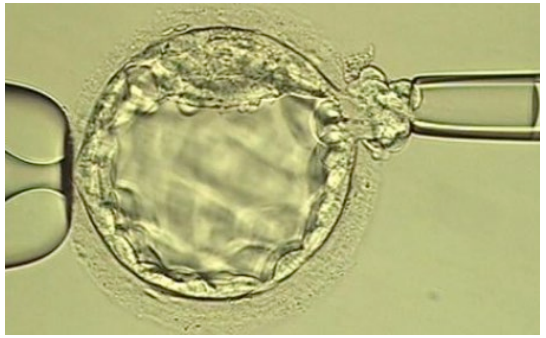
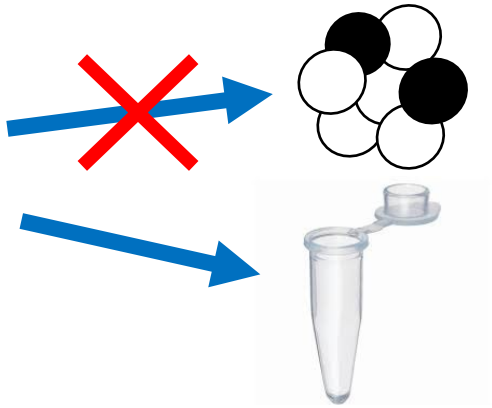
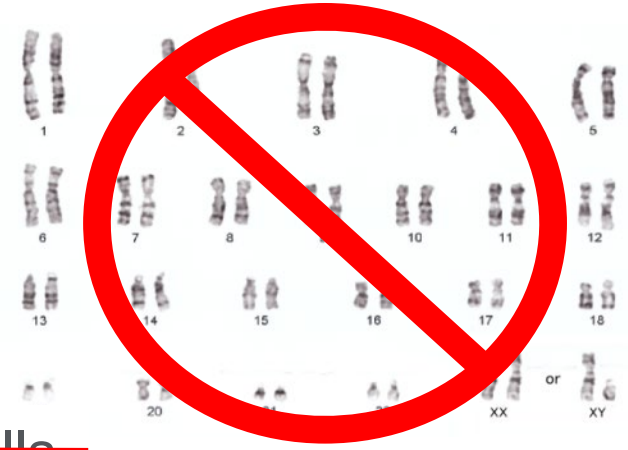
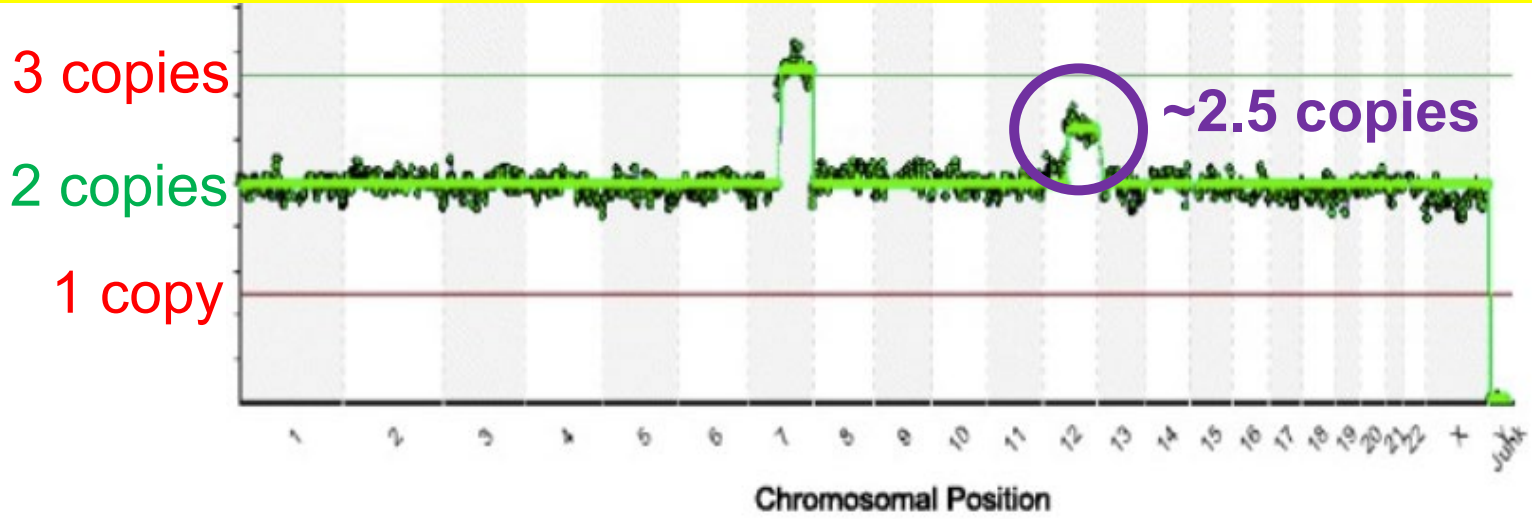






~~Mosaicism = Mix of euploid and aneuploid cells~~

“MOSAICISM” = INTERMEDIATE COPY NUMBER



Does ICN = mosaicism?

Other causes of ICN:

- Statistical variation
- Amplification artifact/noise
- Contamination

Isn't it time to stop calling preimplantation embryos "mosaic"?

Paulson & Treff, Fertil Steril 2020

"…propose abandoning the misleading and inaccurate designation 'mosaic.' … use a more accurate term, 'intermediate copy number.'

"Mosaicism is certainly one possible explanation for intermediate copy number, but there are many other possibilities."

Not all PGT-A is equal

Reproductive genetics laboratory may impact euploid blastocyst and live birth rates: a comparison of 4 national laboratories' PGT-A results from vitrified donor oocytes

Jonah Bardos, M.D., M.B.E.,[a,b] Jaclyn Kwal, M.D.,[c] Wayne Caswell, M.S.,[d] Samad Jahandideh, Ph.D.,[e] Melissa Stratton, B.S.,[d] Michael Tucker, Ph.D.,[e] Alan DeCherney, M.D.,[a] Kate Devine, M.D.,[e] Micah Hill, D.O.,[b] and Jeanne E. O'Brien, M.D., M.Sc.[d]

Reproductive outcomes	Laboratory A N (%)	Laboratory B N (%)	Laboratory C N (%)	Laboratory D N (%)	*P* value (between 4 laboratories)	*P* Value (pairwise comparison)
Euploid	661/898 (73.6%)	583/921 (63.3%)	142/233 (60.9%)	314/581 (52.3%)	<0.001	<0.001 all vs. A
Aneuploid	128/898 (14.2%)	303/921 (32.8%)	64/233 (27.4%)	184/581 (31.6%)	<0.001	<0.001 all vs. A
Mosaic	89/898 (9.9%)	26/921 (2.8%)	13/233 (5.5%)	67/581 (11.5%)	<0.001	NS
No call rate	20/898 (2.2%)	9/921 (1.0%)	14/233 (6.0%)	16/581 (2.8%)	<0.001	NS
Live birth rate	143/247 (57.8)	122/230 (53.0%)	31/67 (46.3%)	71/150 (47.3%)	0.14	0.04, A vs. D
Biochemical Pregnancy Loss rate	22/247 (8.9%)	18/230 (0.8%)	5/67 (7.5%)	11/150 (7.3%)	0.50	NS
Miscarriage rate	26/247 (10.5%)	22/230 (9.6%)	7/67 (10.4%)	17/150 (11.3%)	0.80	NS
Induced abortion	2/247 (0.8%)	2/230 (0.9%)	0/67 (0.0%)	0/150 (0.0%)	0.20	NS
Not pregnant	54/247 (21.8%)	66/230 (28.6%)	24/67 (35.8%)	51/150 (34%)	0.1	NS

NS = not significant.

Bardos. Euploidy rate varies by PGT-A lab. Fertil Steril 2022.

Aneuploidy rates, % of cycles with no euploid embryos, and live birth rates differed significantly between platforms

PGT-A Labs: What's the Difference?

'PGT-A is "very, very powerful" when done well... [but] it isn't always done well. Most labs are not doing such rigorous studies, and most companies use commercial tests that aren't as well-validated... **While most PGT-A testing uses the same core technologies, there's variation in exactly how different testing platforms amplify and assess the DNA taken from the biopsied cells.** If a validated PGT-A test used in scientific research is a sports car… many commercially available platforms are like minivans: "They all have four wheels, a steering wheel, and an engine. **But they're different in almost every way**."'

- Dr Richard Scott via Jamie Ducharme, Time Magazine, March 2025

PGT-A Labs: What's the Difference?

Amplification methods | Analysis | Bioinformatics/Reporting

- DNA amplification causes noise/artifact
  - Overall whole genome amp (WGA) is associated with more noise than targeted amp
  - Primary Template Amplification (PTA) is a new WGA method associated with reduced noise

PGT-A Labs: What's the Difference?

Amplification methods

Analysis

- aCGH
- NGS
- SNP
- Combined NGS/SNP

Bioinformatics/Reporting

PGT-A Labs: What's the Difference?

Amplification methods

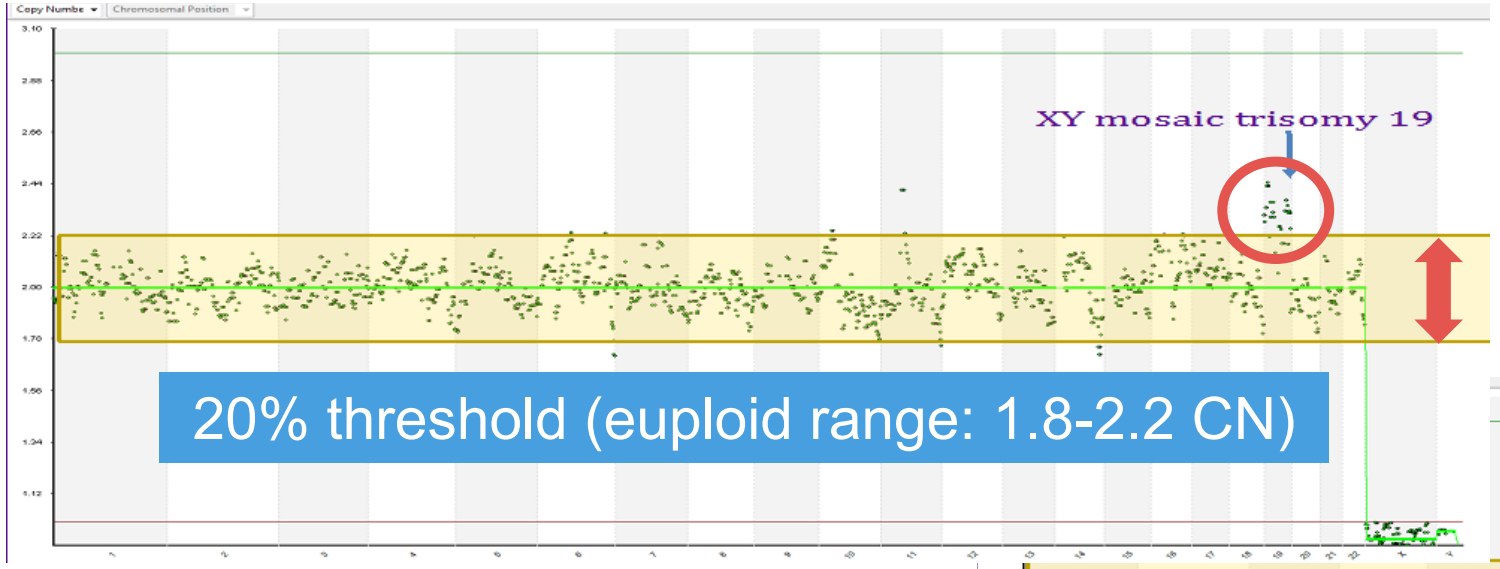
Analysis

Bioinformatics/Reporting

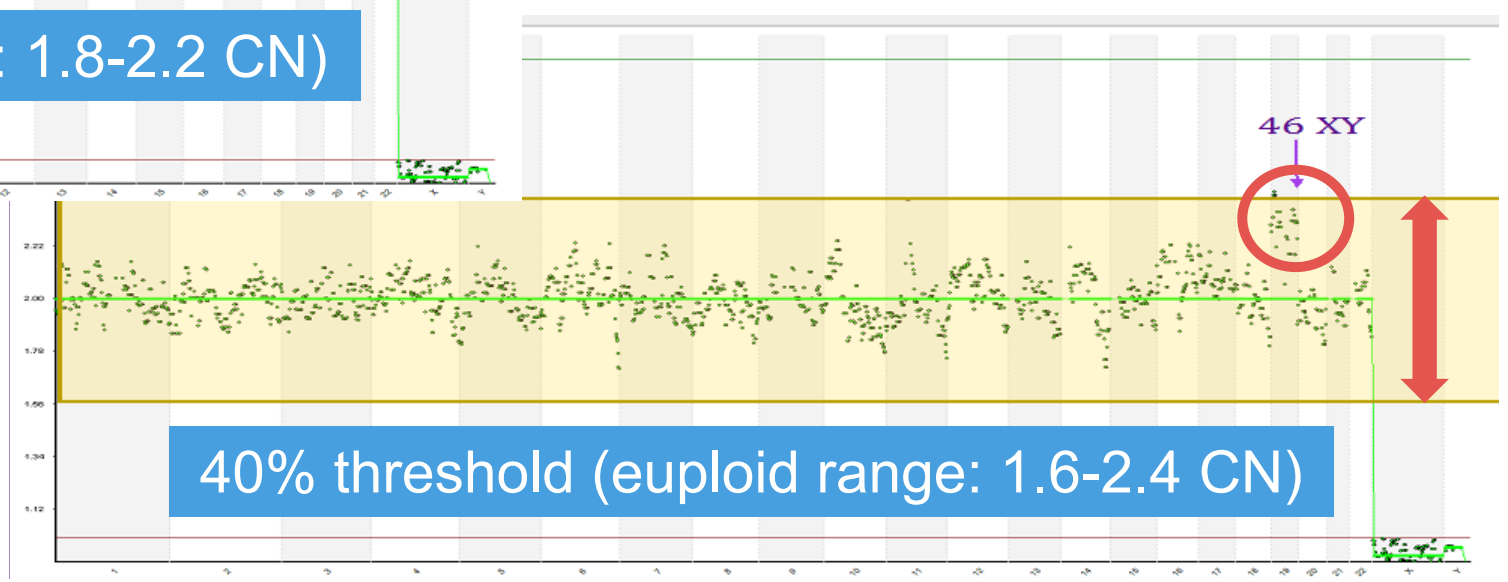
- Normal/abnormal/mosaic thresholds
- Masking approaches
- Opt-in vs opt-out mosaicism models
- Made up terms with variable definitions: complex, chaotic

PGT-A Labs: What's the Difference?

NGS alone relies on a cutoff approach

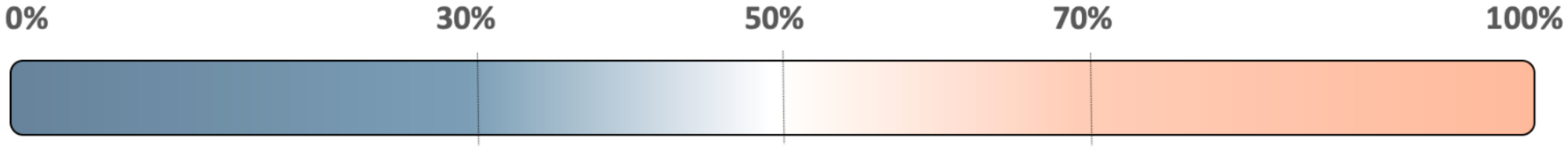


The meaning of euploid/mosaic/aneuploid varies by (arbitrarily) selected thresholds, which can vary by lab (and sometimes by individual chromosome)



PGT-A Labs: What's the Difference?

The meaning of "mosaic" can vary by provider preference



Full mosaic reporting

EUPLOID	LOW MOSAIC	HIGH MOSAIC	ANEUPLOID

No mosaic reporting

30% threshold

EUPLOID	ANEUPLOID

50% threshold
(Coming soon!)

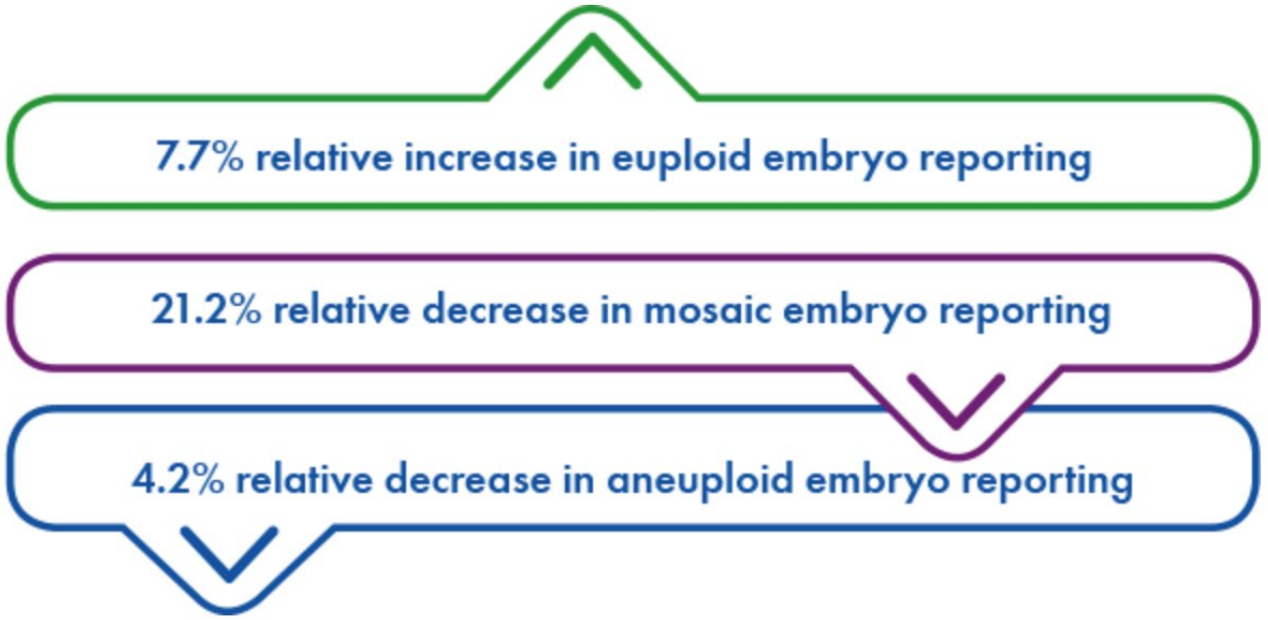
EUPLOID	ANEUPLOID

PGT-A Labs: What's the Difference?

The meaning of "mosaic" can change over time within a lab

More than 1300 extra euploid embryos reported

We have reported an extra 1300+ euploid embryos as a result of the PGTai platform, which previously would have been reported as aneuploid or mosaic.



PGT-A Labs: What's the Difference?

The meaning of "mosaic" can change over time within a lab

"As you know, this analysis was originally completed in 2019 and mosaic reporting was not the standard. Therefore, after a multistep review process, this embryo was initially reported as aneuploid with trisomy 6. As you may imagine, much has changed in the landscape of PGT-A and mosaic reporting, which has become increasingly routine. After reviewing this with an LD, rather than there being an error with the call, under our current algorithm **this embryo would be reported as low mosaic for trisomy 6**. An embryo is reported as "low mosaic" if the copy number variation in the biopsy is between 30% and 50%. "

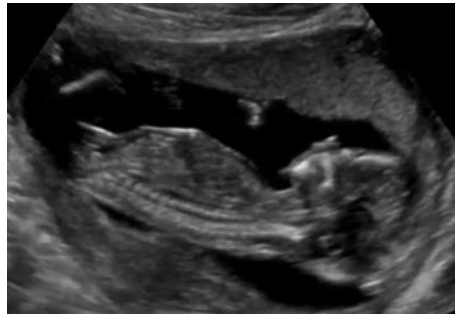
PGT-A Labs: What's the Difference?

Made up terminology can impact outcomes

Result	Sex	Chromosomes Impacted	Interpretation
Aneuploid	XX	???	Complex Abnormal
Aneuploid	XX	+11	Abnormal

Amended on request:

Result	Sex	Chromosomes Impacted	Interpretation
Aneuploid	XX	-5 [mos], -13 [mos], -21 [mos]	Complex Abnormal
Aneuploid	XX	+11	Abnormal



PGT-A Labs: What's the Difference?

JBRA Assisted Reproduction 2023;**27**(3):453-462
doi: 10.5935/1518-0557.20230011

Original article

The importance of standardizing criteria for PGT-A interpretation of blastocysts analyzed by next-generation sequencing

Anna Calull Bagó[1], María Teresa Izaguirre Hernández[1], Patricia Cancino Villarreal[1], Claudia González Ortega[1], Antonio Martín Gutiérrez Gutiérrez[1]

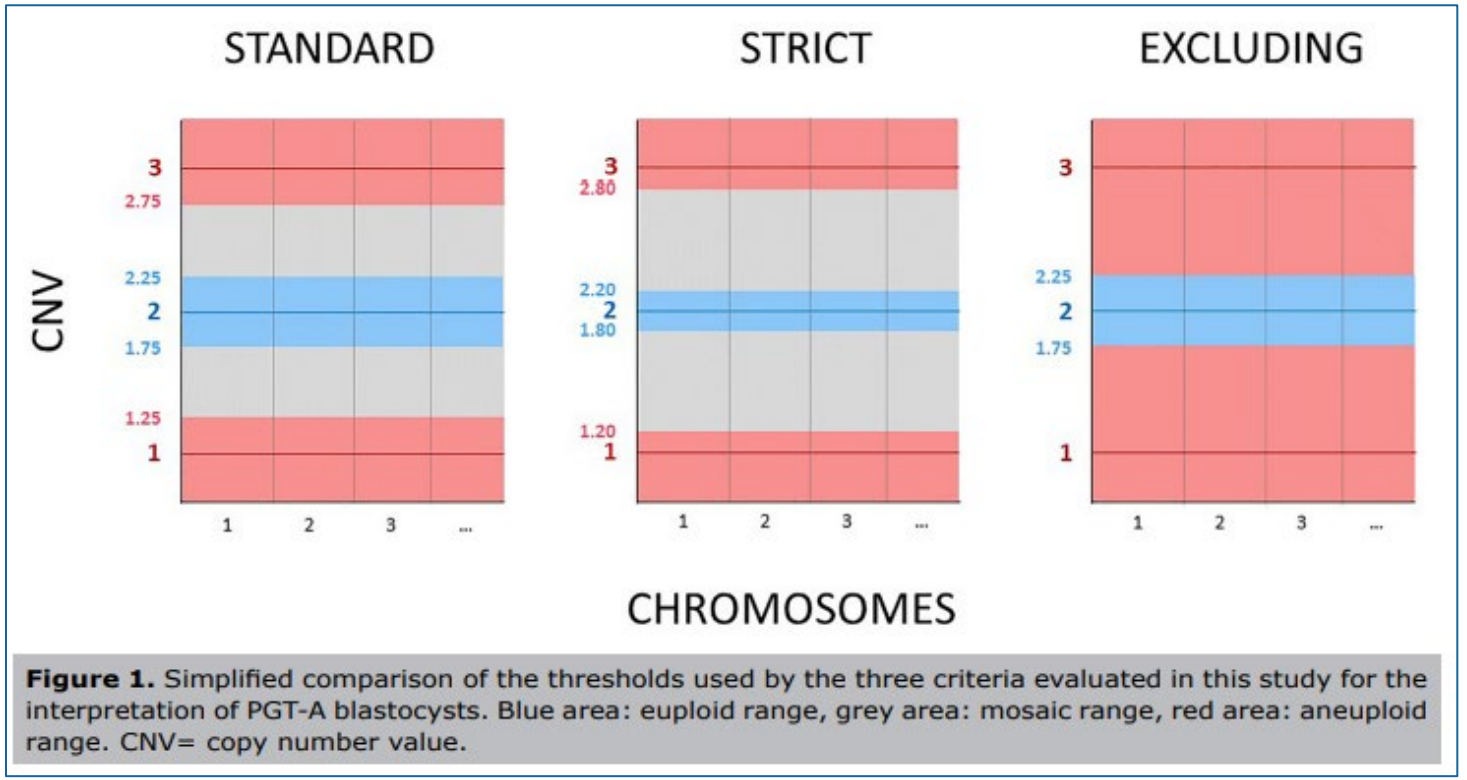


Figure 1. Simplified comparison of the thresholds used by the three criteria evaluated in this study for the interpretation of PGT-A blastocysts. Blue area: euploid range, grey area: mosaic range, red area: aneuploid range. CNV= copy number value.

~1/3 IVF cycles had ≥1 embryo that would have been issued different PGT result using the reporting criteria of an alternative lab

PGT-A Labs: What's the Difference?

Clinical management of mosaic results from preimplantation genetic testing for aneuploidy of blastocysts: a committee opinion

Practice Committees of the American Society for Reproductive Medicine and the Genetic Counseling Professional Group

American Society for Reproductive Medicine, Washington, DC

- Clinicians should understand the prevalence and reporting structure (including the implications of "masking") of mosaic PGT-A results issued by their reference laboratory

Identifying Mosaicism

Does a mosaic result truly indicate mosaicism?

Does a mosaic biopsy result represent the rest of the embryo?

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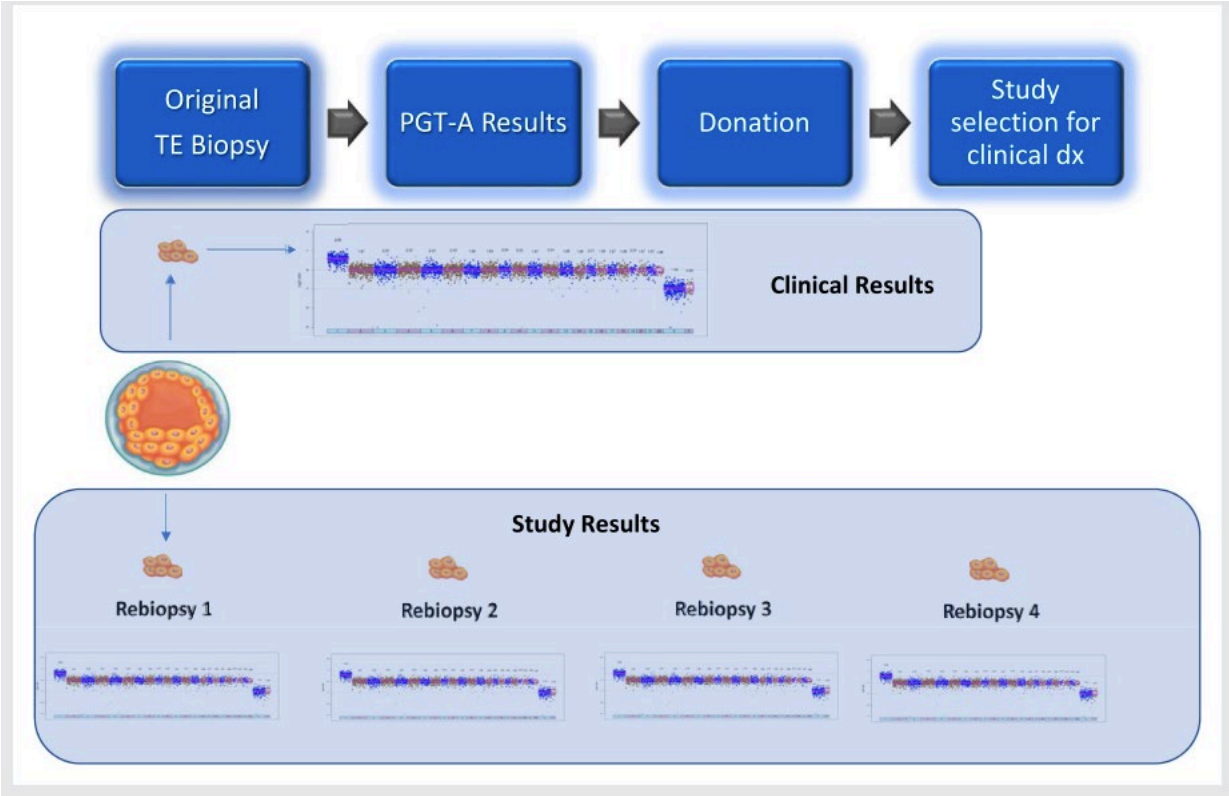
What is the clinical impact of a mosaic result on an ongoing pregnancy?

Does a mosaic biopsy represent the rest of the embryo?

The concordance rates of an initial trophectoderm biopsy with the rest of the embryo using PGTseq, a targeted next-generation sequencing platform for preimplantation genetic testing-aneuploidy

Julia Kim, M.D., M.P.H.,[a,b] Xin Tao, Ph.D.,[c] Michael Cheng, M.S.,[a] Ayesha Steward, M.S.,[a] Vanessa Guo, B.A.,[c] Yiping Zhan, Ph.D.,[c] Richard T. Scott Jr., M.D., H.C.L.D.,[a,b] and Chaim Jalas[c]



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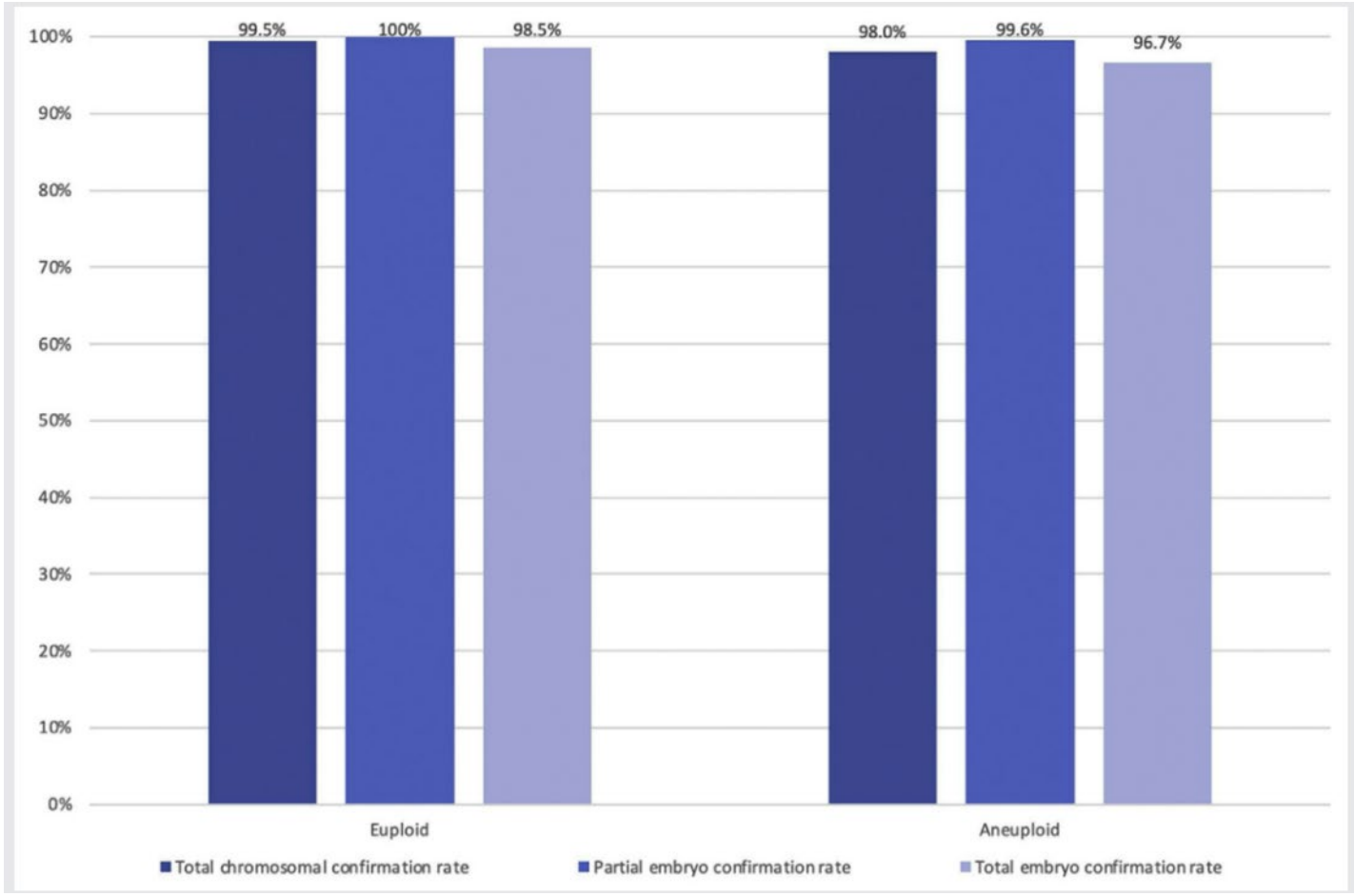

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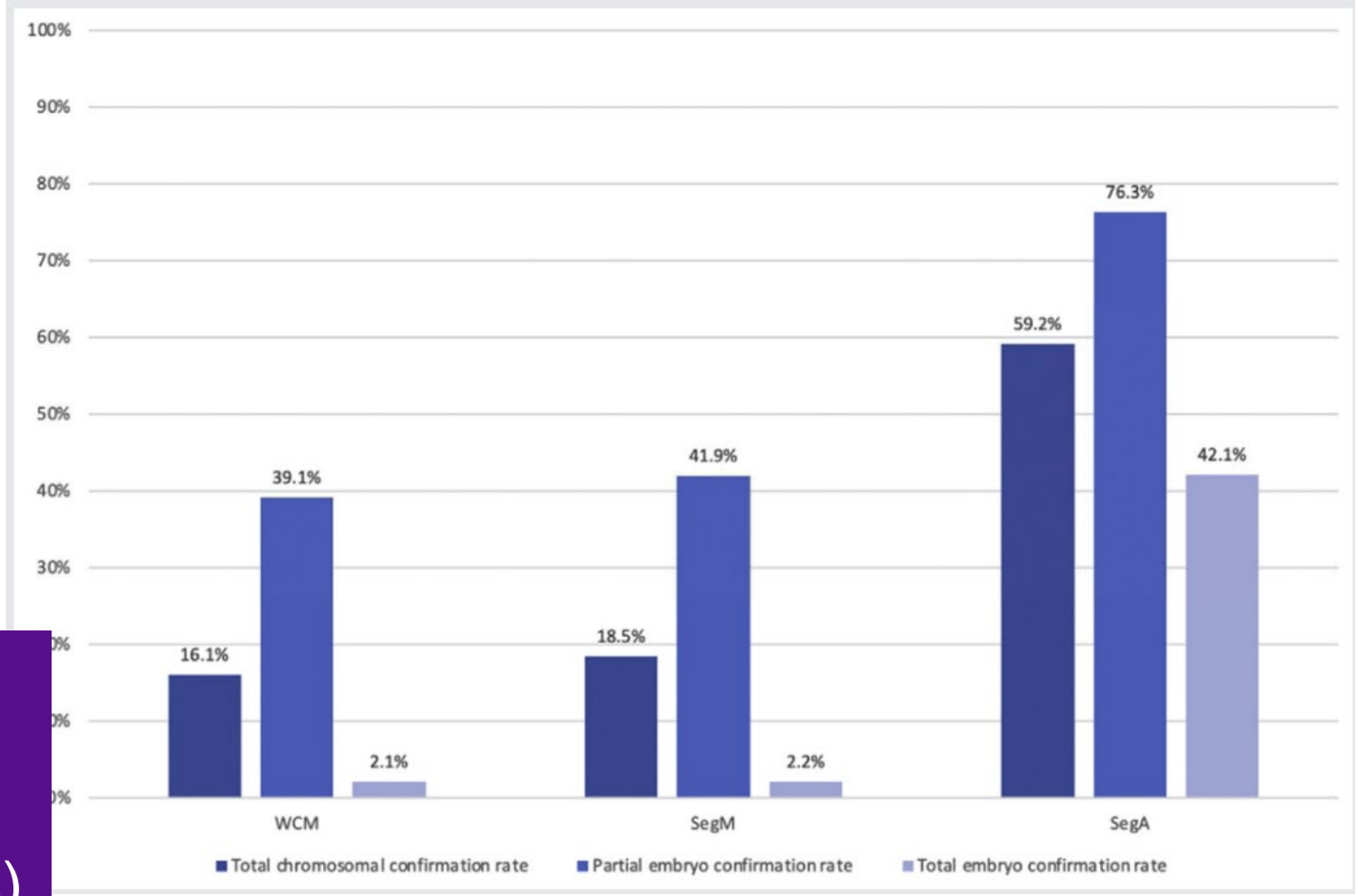

Consistent with likely origin:

- WCA: meiotic
- Mosaicism: mitotic (post-zygotic)

Additional Problem: Detecting Mosaicism

Does a mosaic result truly indicate mosaicism?

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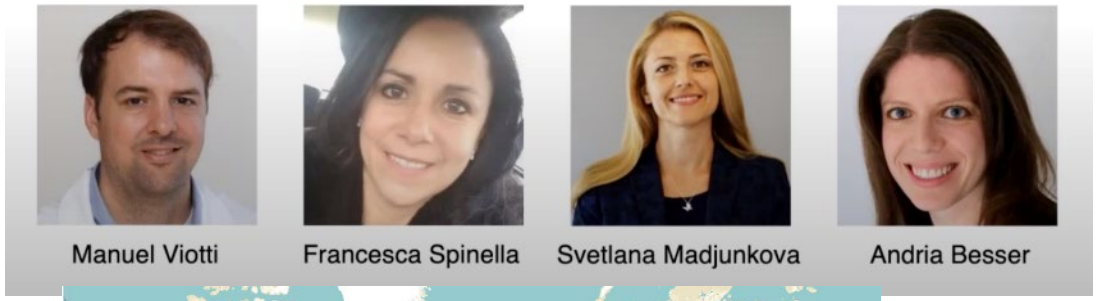
Does a mosaic result predict reproductive potential?

What is the clinical impact of a mosaic result on an ongoing pregnancy?

International Registry of Mosaic Embryo Transfers (IRMET)



501(c)3 non-profit

mosaicregistry@gmail.com

www.irmet.net

Mission is to create a central database for all mosaic ETs, for the purpose of:

- Observing trends with different types of mosaic results
- Adding to the literature with result-specific outcome data

Mosaic Embryo Ranking Tool 2.0

Embryo Type	Stage	ICM	TE	Ranking	
Mosaic Segmental, Low Level	4	B	B	1	Delete
Mosaic, High Level, One/Two Chromosome	5	A	A	2	Add
					Export to Excel...

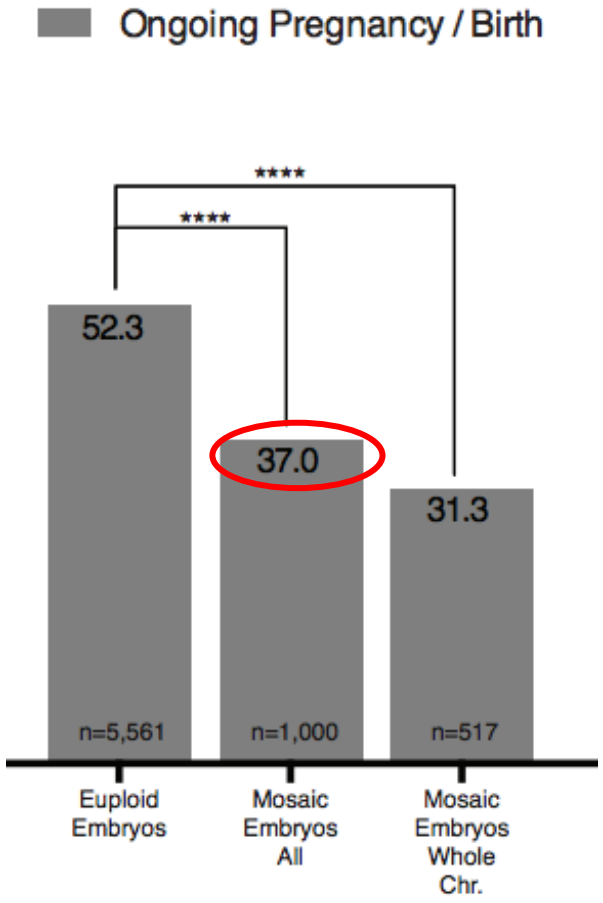
Does a mosaic result predict reproductive potential?

Using outcome data from one thousand mosaic embryo transfers to formulate an embryo ranking system for clinical use

Manuel Viotti, Ph.D.,[a,b] Andrea R. Victor, M.S.,[a] Frank L. Barnes, Ph.D.,[a,b] Christo G. Zouves, M.D.,[a,b] Andria G. Besser, M.S.,[c] James A. Grifo, M.D., Ph.D.,[c] En-Hui Cheng, Ph.D.,[d] Maw-Sheng Lee, M.D., Ph.D.,[d,e] Jose A. Horcajadas, Ph.D.,[f] Laura Corti, M.Sc.,[g] Francesco Fiorentino, Ph.D.,[h] Francesca Spinella, Ph.D.,[h] Maria Giulia Minasi, M.Sc.,[i,j] Ermanno Greco, M.D.,[i,j] and Santiago Munné, Ph.D.[k]

[a] Zouves Foundation for Reproductive Medicine, Foster City, California; [b] Zouves Fertility Center, Foster City, California; [c] New York University Langone Fertility Center, New York, New York; [d] Lee Women's Hospital, Taichung, Taiwan; [e] Chung Shan Medical University, Institute of Medicine, Taichung, Taiwan; [f] Overture Life, New York, New York; [g] IRCCS San Raffaele Scientific Institute, Milan, Italy; [h] Eurofins Genoma Group, Molecular Genetics Laboratories, Rome, Italy; [i] European Hospital, Centre For Reproductive Medicine, Rome, Italy; [j] Villa Mafalda, Center For Reproductive Medicine, Rome, Italy; [k] Cooper Genomics, Livingston, New Jersey

Objective: To study how the attributes of mosaicism identified during preimplantation genetic testing for aneuploidy relate to clinical outcomes, in order to formulate a ranking system of mosaic embryos for intrauterine transfer.
Design: Compiled analysis.
Setting: Multi-center.
Patient(s): A total of 5,561 euploid blastocysts and 1,000 mosaic blastocysts used in clinical transfers in patients undergoing fertility treatment.
Intervention(s): None.
Main Outcome Measure(s): Implantation (gestational sac), ongoing pregnancy, birth, and spontaneous abortion (miscarriage before 20 weeks of gestation).
Result(s): The euploid group had significantly more favorable rates of implantation and ongoing pregnancy/birth (OP/B) compared with the combined mosaic group or the mosaic group affecting only whole chromosomes (implantation: 57.2% vs. 46.5% vs. 41.8%; OP/B: 52.3% vs. 37.0% vs. 31.3%), as well as lower likelihood of spontaneous abortion (8.6% vs. 20.4% vs. 25%). Whole-chromosome mosaic embryos with level (percent aneuploid cells) <50% had significantly more favorable outcomes than the ≥50% group (implantation: 44.5% vs. 30.4%; OP/B: 36.1% vs. 19.3%). Mosaic type (nature of the aneuploidy implicated in mosaicism) affected outcomes, with a significant correlation between number of affected chromosomes and unfavorable outcomes. This ranged from mosaicism involving segmental abnormalities to complex aneuploidies affecting three or more chromosomes (implantation: 51.6% vs. 30.4%; OP/B: 43.1% vs. 20.8%). Combining mosaic level, type, and embryo morphology revealed the order of subcategories regarding likelihood of positive outcome.
Conclusion(s): This compiled analysis revealed traits of mosaicism identified with preimplantation genetic testing for aneuploidy that affected outcomes in a statistically significant manner, enabling the formulation of an evidence-based prioritization scheme for mosaic embryos in the clinic. (Fertil Steril® 2020;■:■–■. ©2020 by American Society for Reproductive Medicine.)
Key Words: IVF, preimplantation genetic testing, Next-Generation Sequencing, embryo, mosaicism



Does a mosaic result predict reproductive potential?

ARTICLE

Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial

Antonio Capalbo,[1,*] Maurizio Poli,[1] Laura Rienzi,[2] Laura Girardi,[1] Cristina Patassini,[1] Marco Fabiani,[1] Danilo Cimadomo,[2] Francesca Benini,[3] Alessio Farcomeni,[4] Juliana Cuzzi,[5] Carmen Rubio,[6,7] Elena Albani,[8] Laura Sacchi,[8] Alberto Vaiarelli,[2] Matteo Figliuzzi,[1] Necati Findikli,[9,10] Onder Coban,[11] Fazilet K. Boynukalin,[12] Ivan Vogel,[13] Eva Hoffmann,[13] Claudia Livi,[3] Paolo E. Levi-Setti,[8] Filippo M. Ubaldi,[2] and Carlos Simón[6,7,14,15]

AJHG 2021

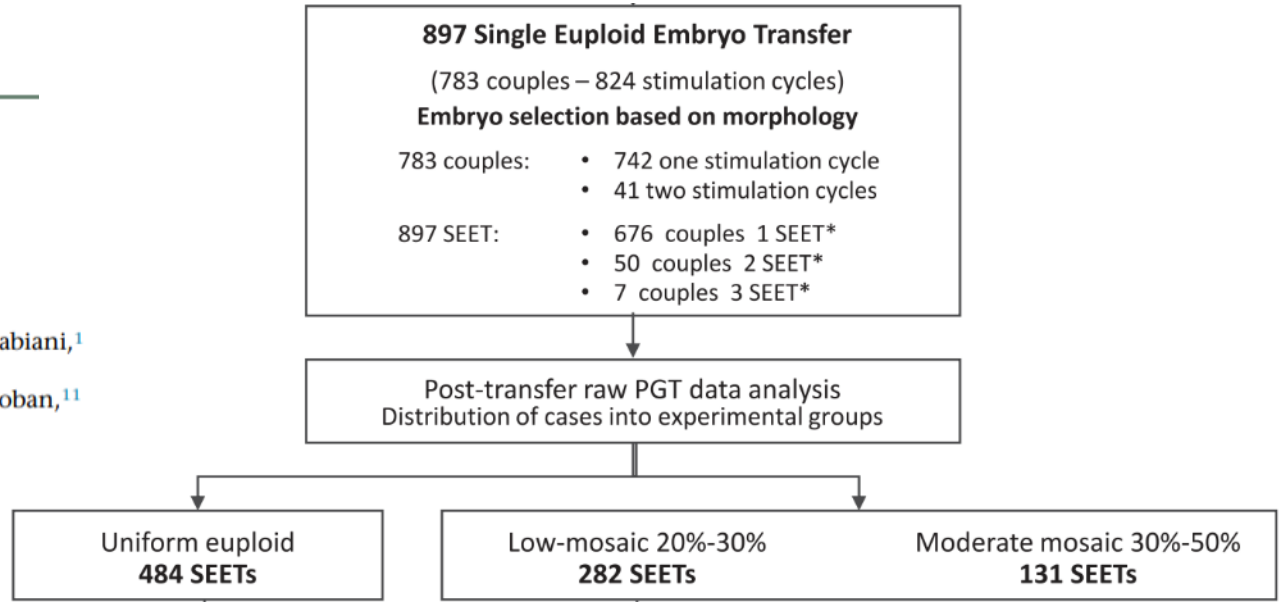


Table 1. Reproductive outcomes of euploid and mosaic embryos

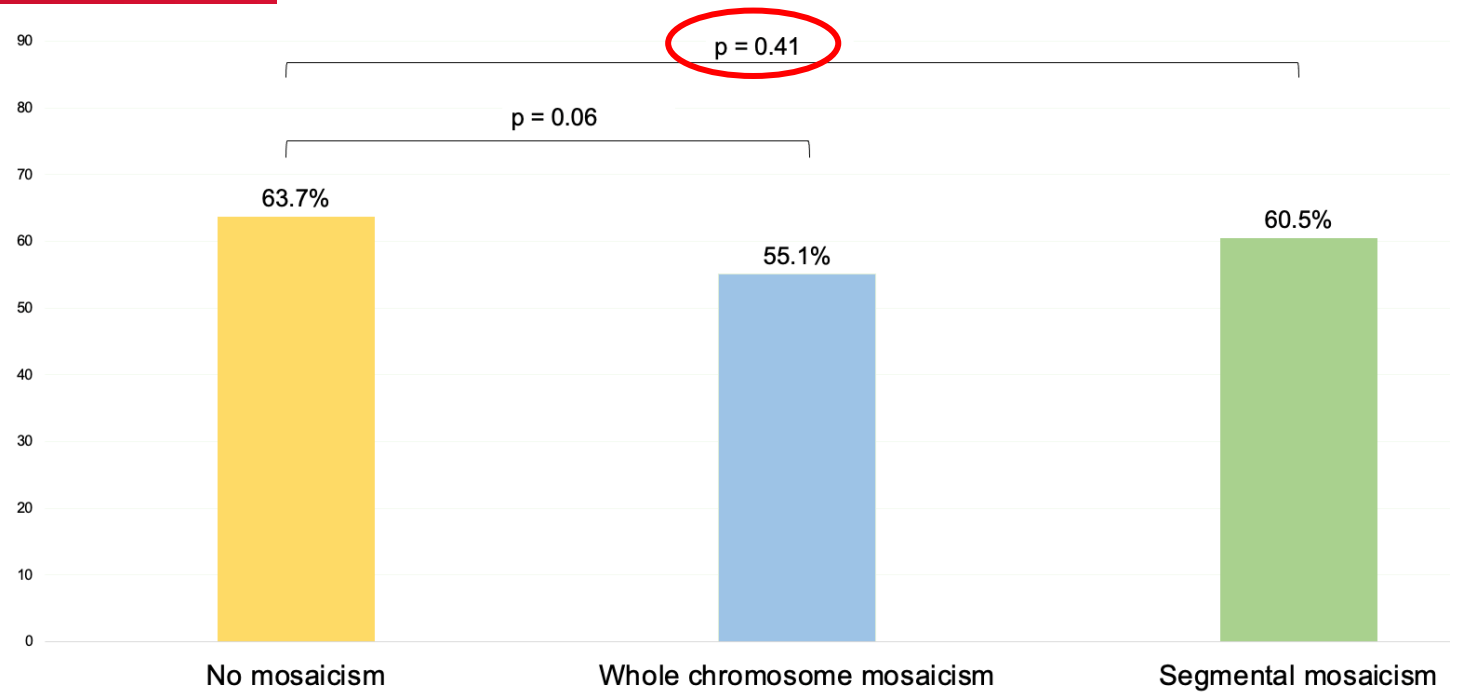
	Group A: Euploid	Group B: Low-grade mosaic (20–30% variation)	Group C: Medium-grade mosaic (30–50% variation)	Adjusted OR (95% CI; p value)
Biochemical pregnancy loss, % (n)	10.7% (29/270)	12.3% (19/155)	13.7% (10/73)	1.18 (0.69–2.02; 0.53)
Miscarriage, % (n)	12.0% (29/241)	11.0% (15/136)	12.7% (8/63)	0.89 (0.50–1.55; 0.69)
Live birth, % (n)	43.4% (210/484)	42.9% (121/282)	42.0% (55/131)	0.97 (0.74–1.26; 0.82)

Does a mosaic result predict reproductive potential?

ABSTRACT | VOLUME 118, ISSUE 4, SUPPLEMENT , E29, OCTOBER 2022

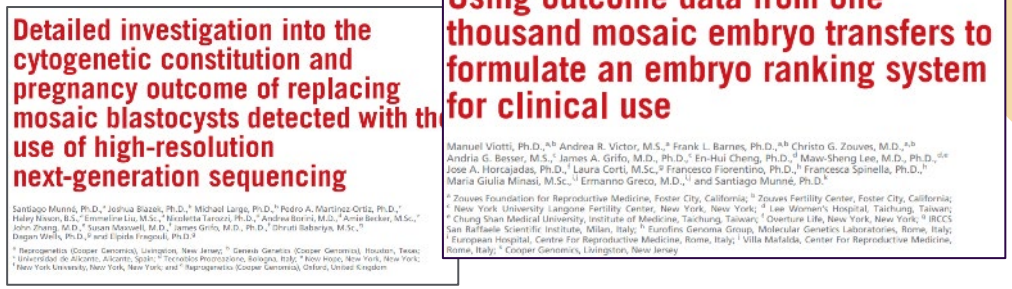
EMBRYOS DIAGNOSED AS PUTATIVE MOSAIC BY THE PGTSEQ PGT-A PLATFORM HAVE A SIMILAR SUSTAINED IMPLANTATION RATE AS THOSE NEGATIVE FOR PUTATIVE MOSAICISM: A BLINDED NON-SELECTION STUDY

Pavan Gill, MD • Yiping Zhan, Ph.D • Christine V. Whitehead, BSN, RN • ... Thomas Molinaro, MD, MSCE • Richard T. Scott Jr., M.D. • Chaim Jalas, N/A • Show all authors



How do we explain these inconsistent outcomes?

UNBLINDED



Detailed investigation into the cytogenetic constitution and pregnancy outcome of replacing mosaic blastocysts detected with th use of high-resolution next-generation sequencing

Using outcome data from one thousand mosaic embryo transfers to formulate an embryo ranking system for clinical use

Manuel Viotti, Ph.D.,[a,b] Andrea R. Victor, M.S.,[a] Frank L. Barnes, Ph.D.,[a,b] Christo G. Zouves, M.D.,[a,b] Andria G. Besser, M.S.,[c] James A. Grifo, M.D., Ph.D.,[c] En-Hui Cheng, Ph.D.,[d] Maw-Sheng Lee, M.D., Ph.D.,[d,e] Jose A. Horcajadas, Ph.D.,[f] Laura Corti, M.Sc.,[g] Francesco Fiorentino, Ph.D.,[h] Francesca Spinella, Ph.D.,[h] Maria Giulia Minasi, M.Sc.,[i] Ermanno Greco, M.D.,[i] and Santiago Munné, Ph.D.[k]

[a] Zouves Foundation for Reproductive Medicine, Foster City, California; [b] Zouves Fertility Center, Foster City, California; [c] New York University Langone Fertility Center, New York, New York; [d] Lee Women's Hospital, Taichung, Taiwan; [e] Chung Shan Medical University, Institute of Medicine, Taichung, Taiwan; [f] Overture Life, New York, New York; [g] IRCCS San Raffaele Scientific Institute, Milan, Italy; [h] Eurofins Genoma Group, Molecular Genetics Laboratories, Rome, Italy; [i] European Hospital, Centre For Reproductive Medicine, Rome, Italy; [j] Villa Mafalda, Center For Reproductive Medicine, Rome, Italy; [k] Cooper Genomics, Livingston, New Jersey

VS

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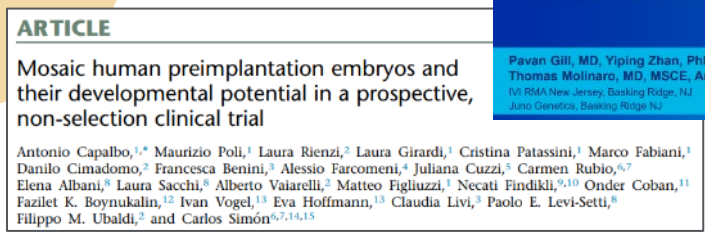


ARTICLE

Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial

Antonio Capalbo,[1,*] Maurizio Poli,[1] Laura Rienzi,[2] Laura Girardi,[1] Cristina Patassini,[1] Marco Fabiani,[1] Danilo Cimadomo,[2] Francesca Benini,[3] Alessio Farcomeni,[4] Juliana Cuzzi,[5] Carmen Rubio,[6,7] Elena Albani,[8] Laura Sacchi,[8] Alberto Vaiarelli,[2] Matteo Figliuzzi,[1] Necati Findikli,[9,10] Onder Coban,[11] Fazilet K. Boynukalin,[12] Ivan Vogel,[13] Eva Hoffmann,[13] Claudia Livi,[3] Paolo E. Levi-Setti,[8] Filippo M. Ubaldi,[2] and Carlos Simón[6,7,14,15]

Embryos diagnosed as putative mosaic by PGTSEQ PGT-A platform have a similar sustained implantation rate as those negative for putative mosaicism: a blinded non-selection study

Pavan Gill, MD, Yiping Zhan, PhD, Christine Whitehead, BSN, RN, Xin Tao, PhD, Marie D Werner, MD, Thomas Molinaro, MD, MSCE, Antonio Capalbo, PhD, Richard T Scott Jr., MD, Chaim Jalas

IVI RMA New Jersey, Basking Ridge, NJ

Juno Genetics, Basking Ridge NJ

ET done <u>with</u> knowledge of mosaic result

- Study population more likely to be enriched with poorer prognosis patients who did not have normal-result embryos to transfer
- Mosaics have lower success rates compared to euploids

ET done <u>without</u> knowledge of mosaic result

- Study design removes bias
- No or minimal difference in successful outcomes between mosaics and euploids

Identifying Mosaicism

Does a mosaic result truly indicate mosaicism?

Does a mosaic biopsy result represent the rest of the embryo?

Does a mosaic result predict reproductive potential?

What is the clinical impact of a mosaic result on an ongoing pregnancy?

What is the clinical impact of a mosaic result?

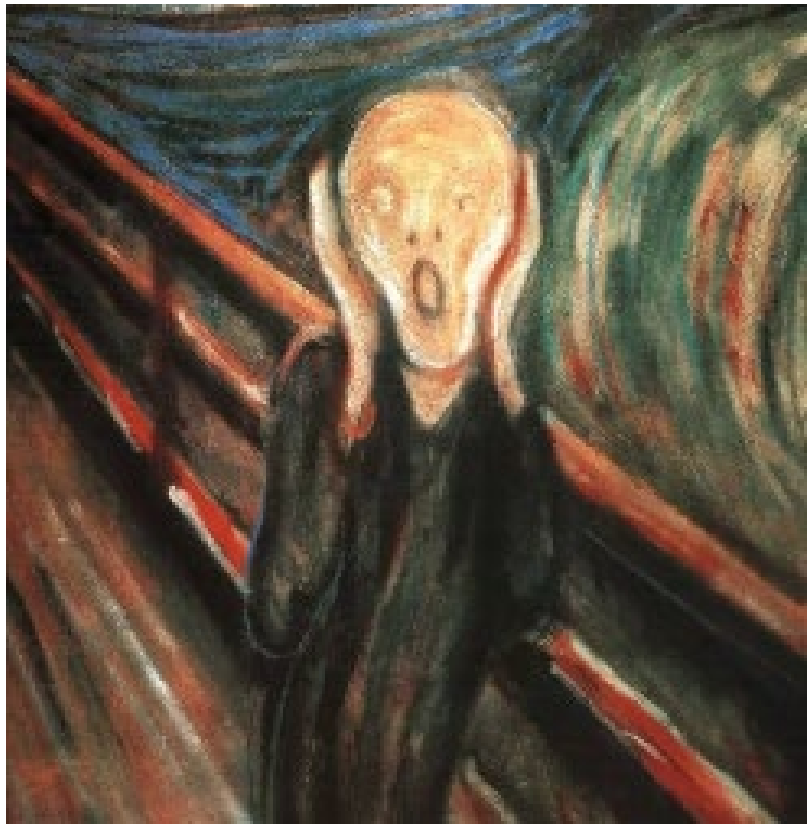
Prenatal/postnatal mosaicism

Fetal mosaicism

- Chromosomal syndrome
- Uniparental disomy

Confined placental mosaicism

- Fetal growth restriction
- IUFD
- Pregnancy complications



What is the clinical impact of a mosaic result?

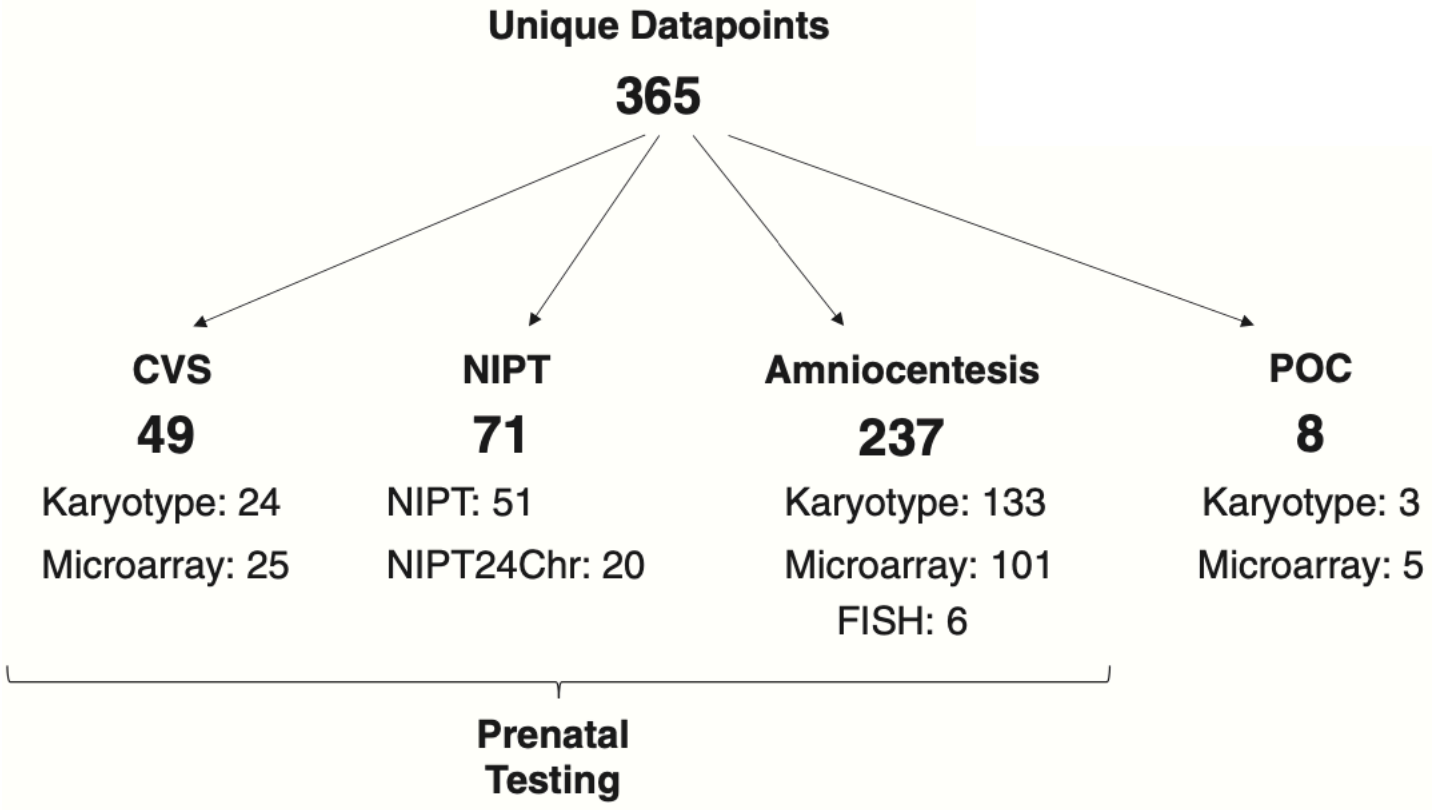
Preimplantation mosaicism

Chromosomal, gestational, and neonatal outcomes of embryos classified as a mosaic by preimplantation genetic testing for aneuploidy

Manuel Viotti, Ph.D.,[a,b] Ermanno Greco, M.D.,[c] James A. Grifo, M.D., Ph.D.,[d] Mitko Madjunkov, M.D.,[e,f] Clifford Librach, M.D.,[e,f,g] Murat Cetinkaya, M.D., Ph.D.,[h] Semra Kahraman, M.D.,[h] Pavel Yakovlev, M.D., Ph.D.,[i] Nikolay Kornilov, M.D.,[i,j] Laura Corti, M.Sc.,[k] Anil Biricik, Ph.D.,[l] En-Hui Cheng, Ph.D.,[m] Ching-Ya Su, M.S.,[m] Maw-Sheng Lee, M.D., Ph.D.,[m,n] Michael D. Bonifacio, M.Sc.,[o] Amber R. Cooper, M.D.,[b] Darren K. Griffin, D.Sc.,[p] Diane Y. Tran, B.S.,[q] Purvi Kaur, B.A.,[q] Frank L. Barnes, Ph.D.,[a,q] Christo G. Zouves, M.D.,[a,q] Andrea R. Victor, Ph.D.,[p,q,r] Andria G. Besser, M.S.,[d] Svetlana Madjunkova, M.D., Ph.D.,[e,f,s] and Francesca Spinella, Ph.D.[l]

Fertil Steril® 2023

2/237 (0.8%)
showed mosaicism in fetus



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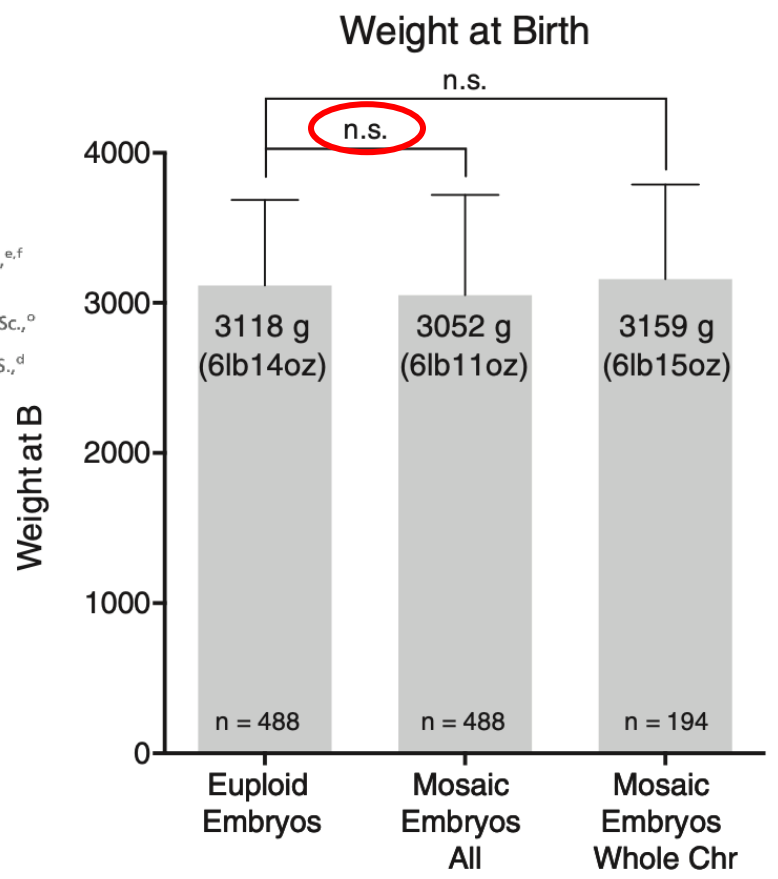
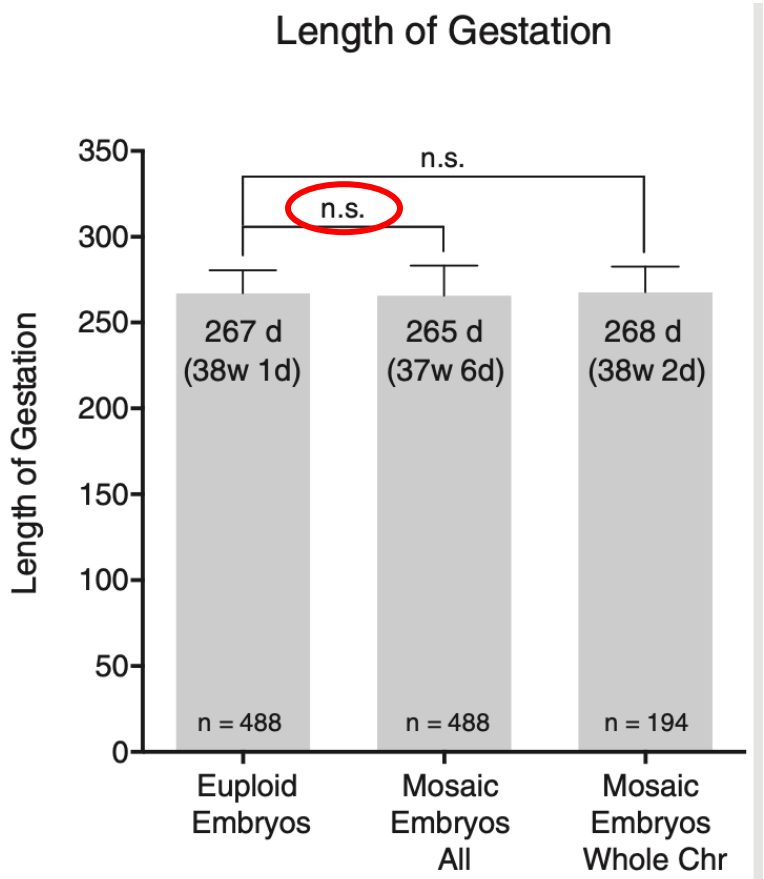
Fertil Steril® 2023





What is the clinical impact of a mosaic result?

Preimplantation mosaicism

RESEARCH LETTER | ARTICLES IN PRESS

Pu

Perinatal and postnatal outcomes up to the third year of life after the transfer of mosaic embryos compared with euploid embryos

Ruth Morales, Ph.D. • Belén Lledó, Ph.D. • José A. Ortiz, Ph.D. • ... Jorge Ten, Ph.D. • Andrea Bernabeu, Ph.D. • Rafael Bernabeu, Ph.D. • Show all authors

Published: May 07, 2024 • DOI: https://doi.or

Postnatal outcomes.

Outcome	Euploid group (n = 115)	Mosaic group (n = 57)	B[a] (95% CI)/OR[b] (95% CI)	*P*
Newborn measures				
Birth weight (g), (mean ± SD)	3,222.5 ± 581.2	3,227.5 ± 530.3	51.310[a] (-110.392; 13.013)	.532
Birth weight <2,500 g, n (%)	10 (8.7)	2 (3.5)	0.072[b] (0.004; 1.462)	.087
Birth weight <1,500 g, n (%)	2 (1.7)	1 (1.8)	0.136[b] (0.002; 11.324)	.377
Birth length (cm), (mean ± SD)	49.9 ± 2.7	50.1 ± 2.7	0.483[a] (-0.245; 1.212)	.192
Birth head circumference (cm), (mean ± SD)	34.5 ± 1.9	34.5 ± 1.9	0.147[a] (-0.525; 0.820)	.664
Apgar score, (mean ± SD)	8.6 ± 2.5	8.9 ± 1.8	0.702[a] (-0.334; 1.738)	.181
Neonatal admission, n (%)	10 (8.7)	5 (8.8)	1.727[b] (0.408; 7.306)	.458
Congenital anomalies, n (%)	10 (8.7)	4 (7.0)	0.836[b] (0.233; 3.005)	.784
Hospital admission, n (%)	6 (5.2)	0 (0.0)	0.000[b] (0.000; —)	.997
Surgical intervention, n (%)	2 (1.7)	0 (0.0)	—	—
Medical hospitalization, n (%)	4 (3.5)	0 (0.0)	—	—
Chronic diseases, n (%)	1 (0.9)	1 (1.8)	1.781[b] (0.079; 40.104)	.610
Age of the child (y), (mean ± SD)	3.48 ± 0.81	2.92 ± 1.32	—	.010

What is the clinical impact of a mosaic result?

Prenatal/Postnatal Mosaicism	≠	Preimplantation Mosaicism
• Diagnosed by seeing different karyotypes in different cell lines • Frequency is stable across clinics/labs • Detected at later developmental stage • Significant risk for a pregnancy/baby		• Suspected based on intermediate copy number value • Frequency is highly dependent on clinic/lab • Detected at early developmental stage • Extremely low risk for a pregnancy

We do not evidence that preimplantation mosaicism impacts risks beyond early embryo viability

What is the clinical impact of a mosaic result?

Preimplantation mosaicism

False negatives (non-mosaic meiotic aneuploidies) may be the primary risk

Source	PGT-A result	Prenatal/postnatal result	Phenotype
Kahraman, Hum Reprod, 2020	Mosaic -2 (35%)	Amnio: mosaic trisomy 2 (2/100 cells) Postnatal blood: mosaic monosomy 2 (2/100 cells) Postnatal buccal: euploid (400 cells analyzed)	No phenotypic abnormality at birth
Yang, Nat Cell Bio, 2021	Mosaic dup(10)(q11.21-q21.1)	Blood: Non-mosaic 46,XY,dup(10)(q11.21-q11.23)	Coarctation of aorta detected prenatally, "newborn deemed healthy after neonatal correction of the coarctation"
Schlade-Bartusiak, F&S Reports, 2022	Mosaic +15, del20q11.23-qter ("high level")	Blood: Non-mosaic 47,XY,+del(15)(q12q23), maternal UPD(15)	Submucous cleft palate, patent foramen ovale, feeding difficulties
Viotti, PGDIS presentation, 2022	Mosaic +21 (<50%)	NIPT: mosaic trisomy 21 CVS: mosaic trisomy 21 POC: mosaic trisomy 21	Ultrasound anomalies (unspecified)
Greco, Hum Reprod, 2023	Mosaic +1q,-7,-8,+9,-19,-20,+21 (40%)	CVS: mosaic trisomy 21 (80%) Amnio: mosaic trisomy 21 (16%)	Ultrasound anomalies (unspecified)
Greco, Hum Reprod, 2023	Mosaic del1p36.33-p31.1 (40%)	Amnio FISH: mosaic 1p deletion (15%)	Deletion present in 1.5% of brain cells s/p TAB Deletion absent in skin, myocardium, chorion, bone marrow

What is the clinical impact of a mosaic result?

Preimplantation mosaicism

False negatives (non-mosaic meiotic aneuploidies) may be the primary risk

Received: 30 July 2020 | Revised: 2 September 2020 | Accepted: 10 September 2020
DOI: 10.1002/pd.5828

REVIEW

PRENATAL DIAGNOSIS WILEY

Preimplantation genetic testing for aneuploidy: A review of published blastocyst reanalysis concordance data

Diego Marin[1] | Jia Xu[1] | Nathan R. Treff[1,2]

28.4% of “mosaics” were in fact full aneuploids

Journal Pre-proof

Current quantitative methodologies for pre-implantation genetic testing frequently misclassify meiotic aneuploidies as mosaic

Teodora Popa, PhD, Colin Davis, MBBS, Leoni Xanthopoulou, PhD, Evangelia Bakosi, MSc, Chloe He, MSc, Helen O'Neill, PhD, Christian Ottolini, PhD

32.6% of ”mosaic” chromosomal abnormalities showed evidence of meiotic SNP signatures

What is the clinical impact of a mosaic result?

Remember… aneuploid pregnancies also occur after euploid ET!

Clinical error rates of next generation sequencing and array comparative genomic hybridization with single thawed euploid embryo transfer

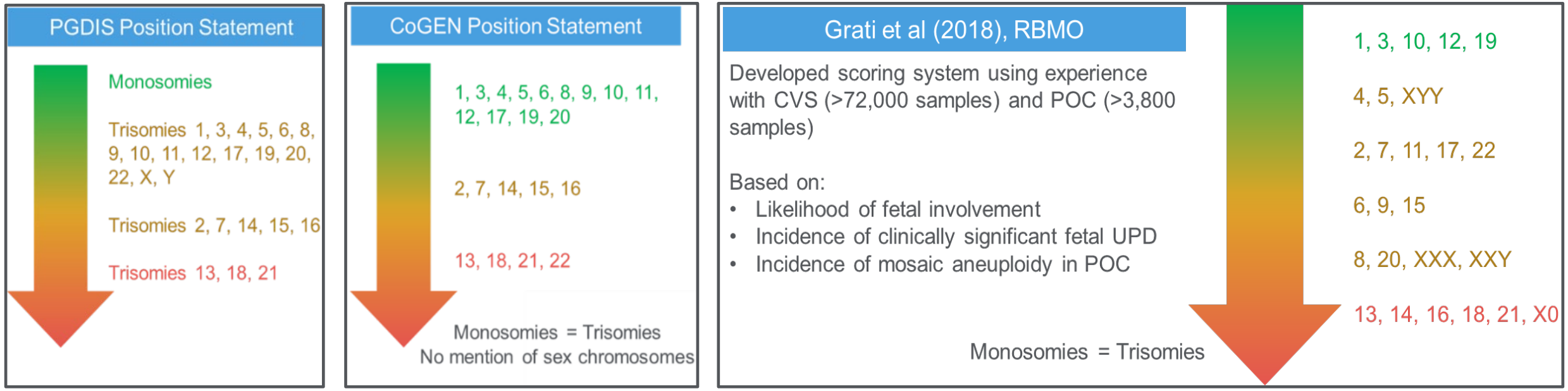
Jenna Friedenthal[a,*], Susan M. Maxwell[a], Ashley W. Tiegs[b,c], Andria G. Besser[a], Caroline McCaffrey[a], Santiago Munné[d], Nicole Noyes[a], James A. Grifo[a]

	Initial PGT-A result	Karyotype[a]	Tissue Type	Outcome
aCGH	46, XY	47, XYY	CVS	Live birth
	46, XX	47, XX + 7	POCs	SAB
	46, XY	47, XY+21	POCs	SAB
	46, XX	46, XX/47, XX+13 (mosaic)	POCs	SAB
			POCs	SAB
			POCs	SAB
	46, XX	69, XXX	POCs	SAB
	46, XY	47, XY +18	Amniotic fluid	ETOP

Is the risk any higher after mosaic ET?

What is the clinical impact of a mosaic result?

Does chromosome # factor in?



Prenatal Mosaicism ≠ Preimplantation Mosaicism

No evidence that these lists can accurately be applied

What is the clinical impact of a mosaic result?

Does chromosome # factor in?

Source	PGT-A result	Prenatal/postnatal result	Phenotype
Kahraman, Hum Reprod, 2020	Mosaic -2 (35%)	Amnio: mosaic trisomy 2 (2/100 cells) Postnatal blood: mosaic monosomy 2 (2/100 cells) Postnatal buccal: euploid (400 cells analyzed)	No phenotypic abnormality at birth
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No consistency in chromosome gain/loss among fetal mosaicism cases

What is the clinical impact of a mosaic result?

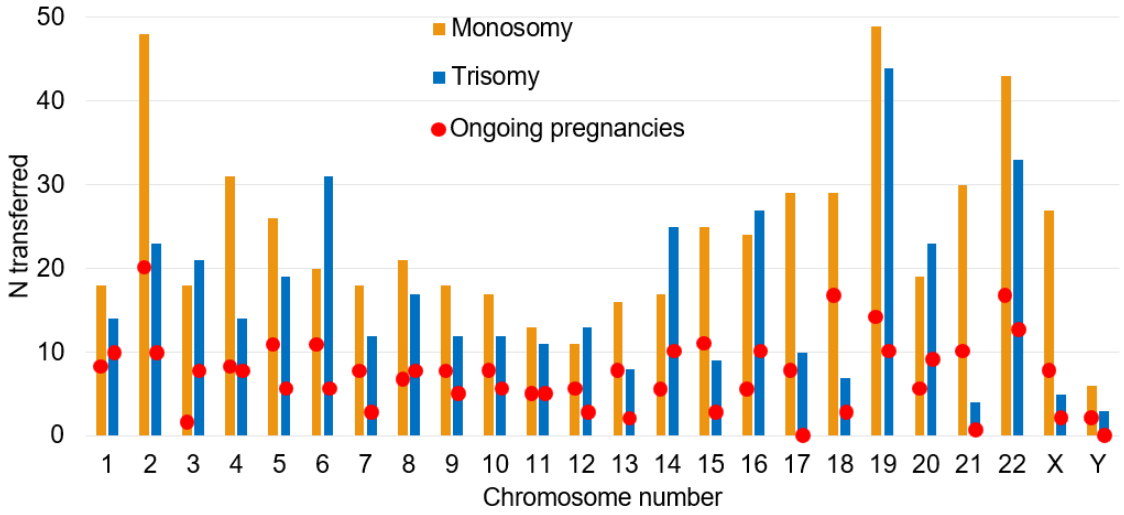
Does chromosome # factor in?

ABSTRACT ONLY · Volume 122, Issue 4, Supplement , E99-E100, October 2024

CAN CHROMOSOME NUMBER PREDICT MOSAIC EMBRYO TRANSFER OUTCOME?

Andria G. Besser, M.S. [1] · Jamie A. Grifo, MD, PHD [2] · Svetlana Madjunkova, M.D., M.SC., PH.D. [3] · Francesca Spinella, PhD [4] · Manuel Viotti, PH.D. [5]

RESULTS: Ongoing Pregnancies by Mosaic Chromosome



CONCLUSIONS

There are currently insufficient data to use chromosome type or number as a selection or ranking criterion in mosaic embryo transfer decisions.

What is the clinical impact of a mosaic result?

Clinical management of mosaic results from preimplantation genetic testing for aneuploidy of blastocysts: a committee opinion

Practice Committees of the American Society for Reproductive Medicine and the Genetic Counseling Professional Group

American Society for Reproductive Medicine, Washington, DC

- **Fetal aneuploidy related to the mosaic PGT-A result is very low (likely <1%)**
- Although categories of mosaic result types may be useful for assessing reproductive potential and prioritizing ET, **it is unclear whether they can be used to predict prenatal and postnatal risks accurately**
- **As with all pregnancies**, genetic counseling and prenatal testing should be offered to patients who conceive after MET in accordance with ACOG and ACMG guidelines

Segmental Aneuploidy Can Be Mosaic or Non-Mosaic

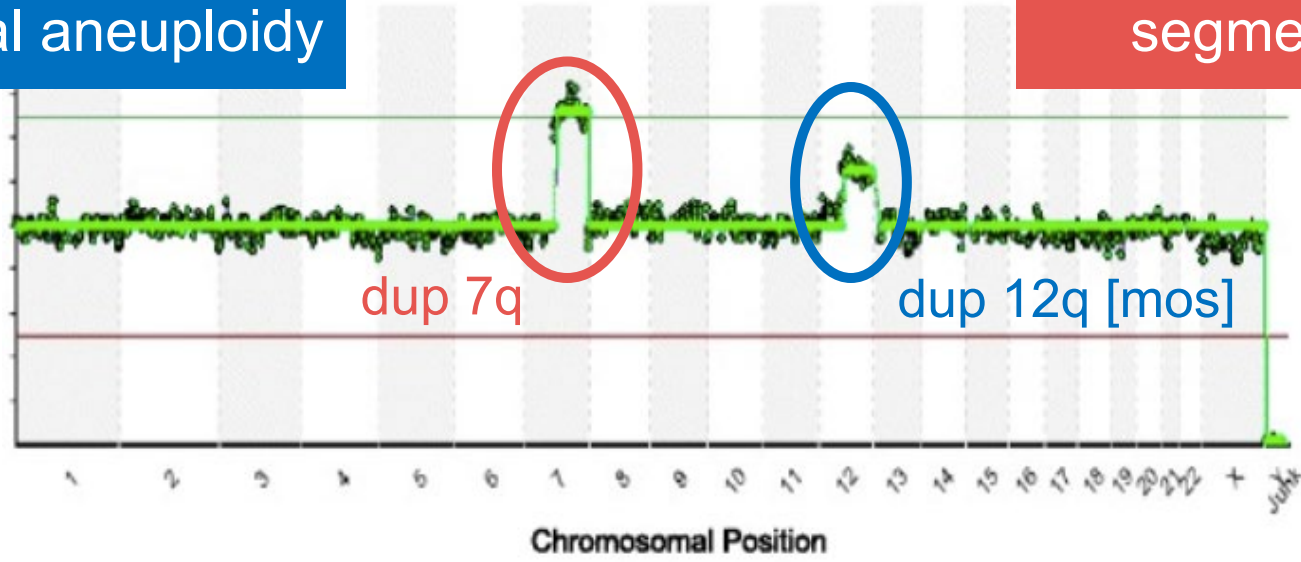
Chromosomal deletion or duplication
AKA partial monosomy/trisomy

Segmental Mosaic

Segmental Aneuploid (NON-mosaic)

Intermediate copy number for the segmental aneuploidy

Full copy number for the segmental aneuploidy



Typically reported as <u>abnormal</u> by lab

Identifying Segmental Aneuploidy

Does a SA result truly indicate SA?

Does a SA biopsy result represent the rest of the embryo?

Does a SA result predict reproductive potential?

What is the clinical impact of a SA result on an ongoing pregnancy?

Does a SA result truly indicate SA?

- Remember WGA introduces amplification bias/artifact
- Copy number status can be impacted by different genomic regions in S phase of cell cycle

Can result in false-positive identification of aneuploidy

Impact for segmental >>> whole chromosome

Does a SA biopsy result represent the rest of the embryo?

Reduced ICM/TE concordance compared to WCA

Many SAs are **mitotic** (post-zygotic) errors and **mosaic** within the whole embryo **even if the biopsy does not show mosaicism**

Concordance rate of SA findings in PGT-A.

		Concordance rate of SA in PGT-A		
Reference	**Platform**	**Embryo stage**	**Concordance rate**	**Absolute values**
Chuang et al., 2018 (47)	NGS	Blastocyst	55.50%	5/9
Popovic et al., 2019 (48)	NGS	Outgrowth 12 dpf	38.46%	5/21
Victor et al., 2019 (49)	NGS	Blastocyst	44.40%	4/9
Lawrenz et al., 2019 (50)	NGS	Blastocyst	16.70%	1/6
Navratil et al., 2020 (46)	NGS	Blastocyst	36.80%	14/38
Girardi et a., 2020 (24)	NGS	Blastocyst	32.10%	17/53
Sachdev et al., 2020 (51)	NGS	Blastocyst	0	0/12
Kim et al., 2021 (11)	NGS	Blastocyst	21.30%	36/196
Mean concordance rate	-	-	30.66%	-

dfp = day post fertilization; NGS = next-generation sequencing; PGT-A = preimplantation genetic testing for aneuploidy; SA = segmental aneuploidy.

Picchetta. Segmental aneuploidies in embryos. Fertil Steril Sci 2023.

Does a SA biopsy result represent the rest of the embryo?

Journal of Assisted Reproduction and Genetics (2022) 39:1313–1322
https://doi.org/10.1007/s10815-022-02487-z

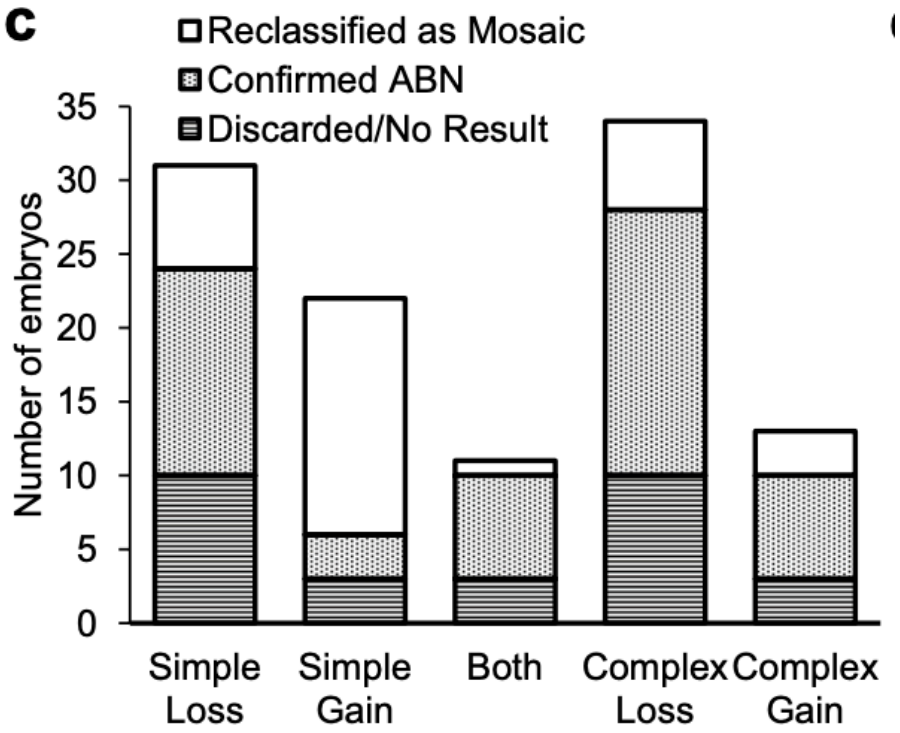
GENETICS

Clinical re-biopsy of segmental gains—the primary source of preimplantation genetic testing false positives

Steve Grkovic[1] · Maria V. Traversa[1] · Mark Livingstone[1] · Steven J. McArthur[1]

Received: 26 January 2022 / Accepted: 31 March 2022 / Published online: 23 April 2022

84% of duplications were euploid on re-biopsy, compared to 33% of deletions



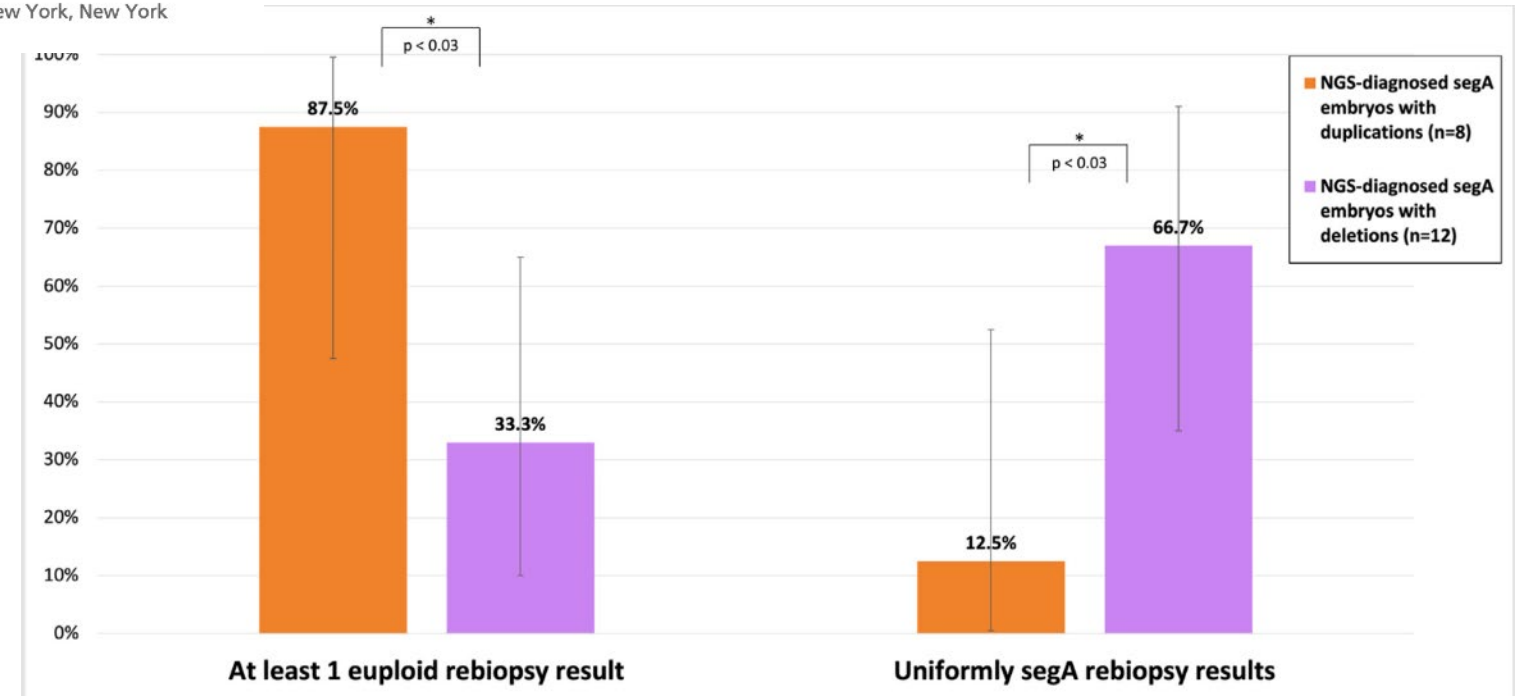
Does a SA biopsy result represent the rest of the embryo?

Blinded rebiopsy and analysis of noneuploid embryos with 2 distinct preimplantation genetic testing platforms for aneuploidy

Sarah Druckenmiller Cascante, M.D., Andria Besser, M.S., C.G.C., Hsiao-Ling Lee, M.S., Fang Wang, Ph.D., Caroline McCaffrey, Ph.D., and James A. Grifo, M.D., Ph.D.

Department of Obstetrics & Gynaecology, New York University Langone Prelude Fertility Center, New York, New York

Only 12% of duplications were present in the entire embryo, compared to 67% of deletions



Reproductive Potential/Clinical Impact of Non-Mosaic SAs

Journal of Assisted Reproduction and Genetics
https://doi.org/10.1007/s10815-024-03282-8

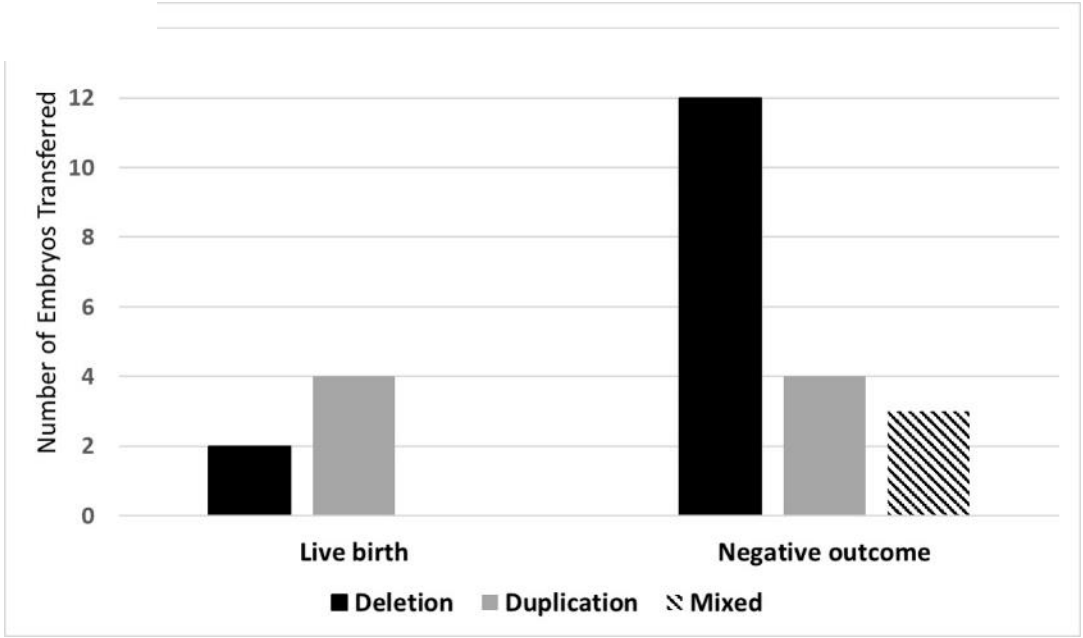
ASSISTED REPRODUCTION TECHNOLOGIES

Healthy live births achieved from embryos diagnosed as non-mosaic segmental aneuploid

Andria Besser[1] · Emily Weidenbaum[2] · Julia Buldo-Licciardi[2] · Caroline McCaffrey[1] · James Grifo[1] · Jennifer Blakemore[1]

Live birth rate: 6/25 (24%)

All reported healthy, 3 with normal prenatal diagnosis (3 untested)



Reproductive Potential/Clinical Impact of Non-Mosaic SAs

In prenatal/postnatal samples,
del/dups are often clinically significant

Chromosome Disorder Guides - English	Hide —
Chromosome 1	
1p Interstitial Deletions	View
1p36 Deletions	View
1q Duplications	View
1q21.1 Microdeletions	View
1q21.1 Microduplications	View
1q23.3 microdeletions	View
1q4 deletions from 1q42 and beyond	View
GATAD2B-associated neurodevelopmental disorder (GAND) GATAD2B syndrome	View
MPPH Syndrome	View
Supernumerary Ring Chromosome 1	View
Urofacial syndrome (Ochoa syndrome)	View
Chromosome 2	
2p Deletions	View
2p Duplications	View
2p15p16.1 Microdeletion Syndrome	View
2p16.3 (NRXN1) deletions	View
2q Duplications	View
2q13 Microdeletions	View
2q13 Microduplications	View
2q23.1 Microdeletion Syndrome	View
2q24.3 Microdeletions	View
2q32 Deletions And Microdeletions	View
2q33.1 Deletions And Other Deletions Between 2q31 And 2q33	View
2q37 Deletion Syndrome	View
2q37 Deletions In Adults And Adolescents	View

Unique
Understanding Chromosome & Gene Disorders

PGT-A has ~10 Mb resolution;
del/dups above this size are rare in LB

Summary

Clinical utility of reporting mosaic results is questionable

- **Mosaicism reporting does not consistently improve pregnancy rates**
  - Can decrease pregnancy rates if selecting against better morphology embryos due to mosaicism
  - Some types of mosaic results have more consistent impact on reproductive potential, but need to be distinguished from full aneuploids for meaningful interpretation
- **Embryos with mosaic results do not seem to present a greater risk to ongoing fetus than euploids**
  - Can unnecessarily eliminate embryos due to unfounded anxiety
  - Can lead to unnecessarily prenatal procedures with additional risks, costs, and burden on healthcare system

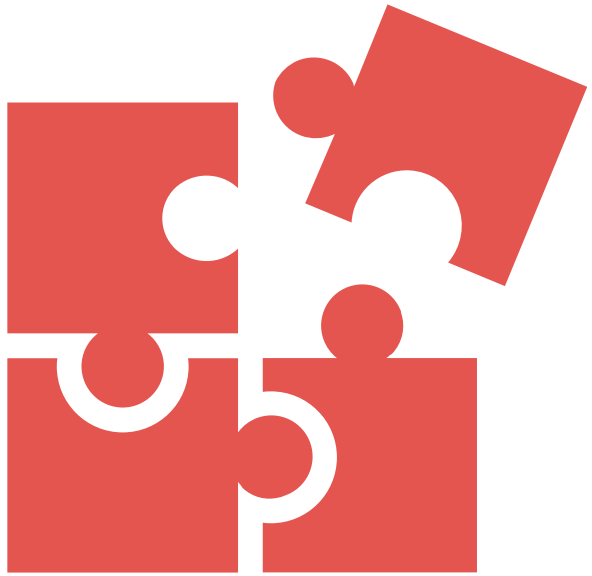
Summary

There appears to be some clinical utility of reporting segmental aneuploid results, however more data is needed

- Many of these are full meiotic abnormalities
- Reproductive potential appears to be clearly impacted
- Risks to ongoing pregnancies are likely low due to size of segmental aneuploidies
  - How low remains TBD

The Missing Puzzle Piece: Clinical Validation

- Determines **predictive values (PPV and NPV)**
- Enables this statement: "*The chance that an embryo with X result from our lab will result in X outcome*"
- **Evidence-based counseling cannot happen without it!**
- Gold standard is a **blinded "non-selection" study**
- Vast majority of labs have NOT done this for PGT-A
- **Why are we not demanding this for our patients?**



THANK YOU!