Mosaicism: Causes and Clinical Outcomes

PGDIS 2024

Manuel Viotti[1,2]

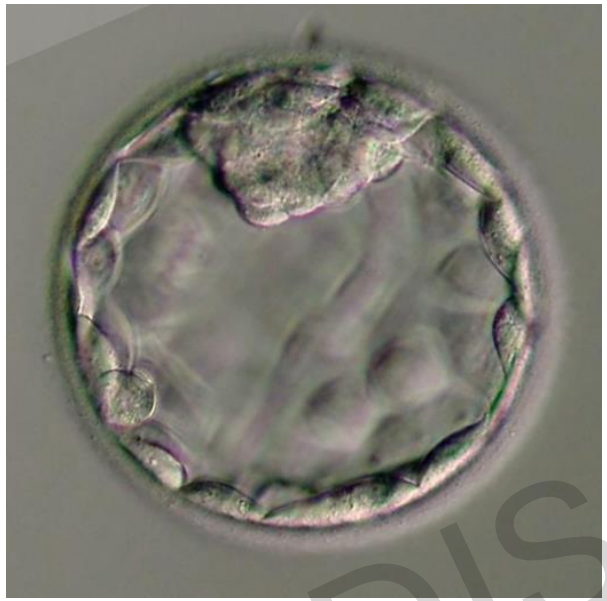
[1] Kindlabs (Kindbody)
[2] Zouves Foundation

Agenda

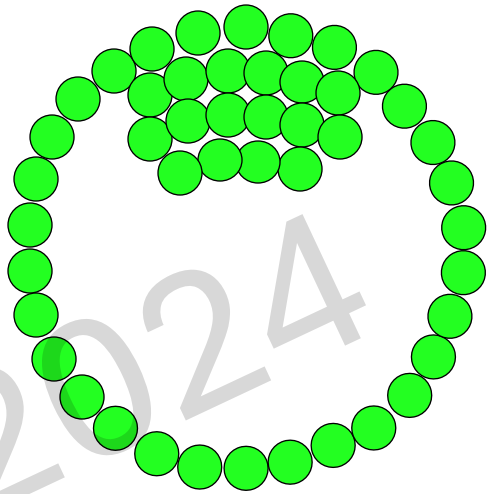
1. Identifying sources of ‘artifactual’ mosaicism

2. Update: Clinical outcomes from IRMET

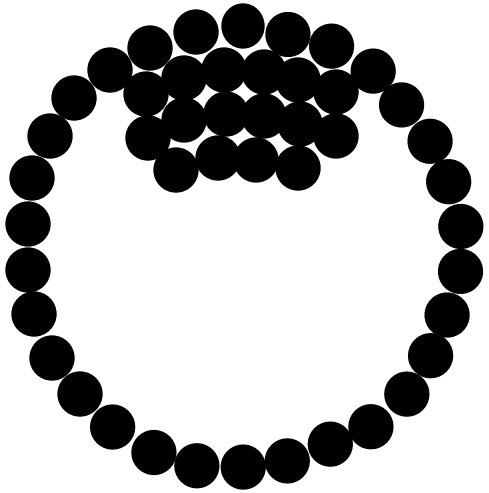
3. Computational modelling of mosaicism



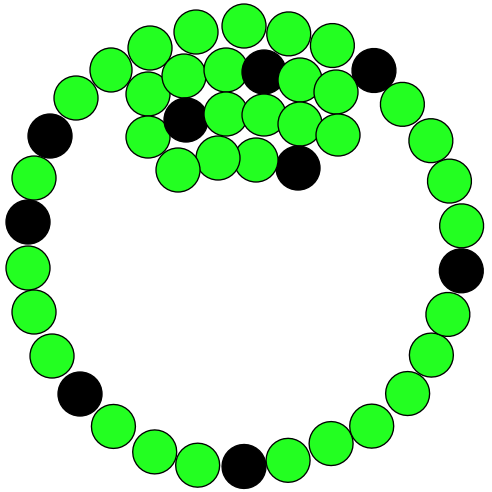
Euploid Embryo



Aneuploid Embryo



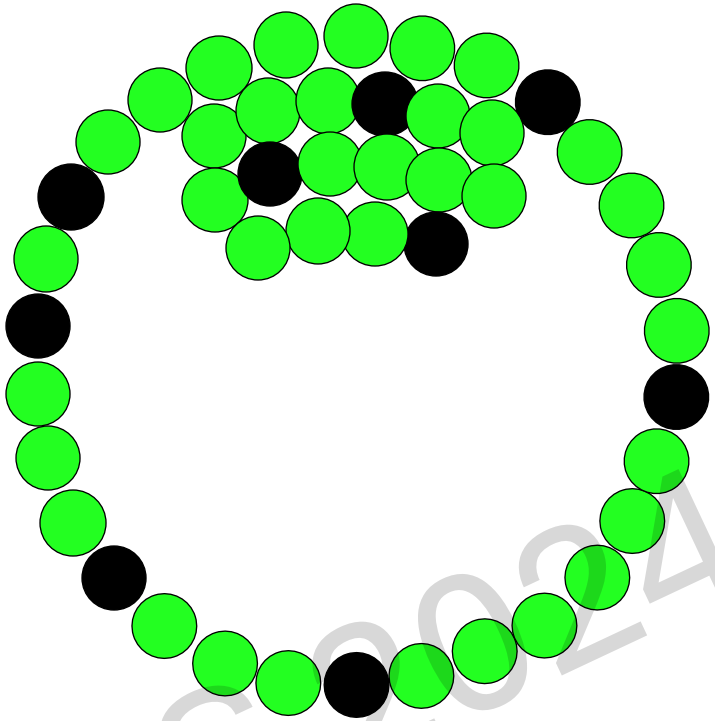
Mosaic Embryo



Aneuploid Cell

Euploid Cell

Chromosomal Mosaicism in Embryos



Aneuploid Cell

Euploid Cell

- Described for over 30 years

- Errors of chromosome segregation in mitotic cell division

- Dynamic, can happen at any time and be corrected

- High incidence

Sources of Mosaicism in Embryos

Endogenous	Induced	Artifactual

Sources of Mosaicism in Embryos

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- Early cell divisions are error prone: faulty mitosis

- ‘Natural’ Baseline-level of mosaicism

- Not age related

- Genetic component

Induced

Artifactual

Sources of Mosaicism in Embryos

sciencemag.org **SCIENCE**

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Common variants spanning *PLK4* are associated with mitotic-origin aneuploidy in human embryos

Rajiv C. McCoy[1], **Zachary Demko**[2], **Allison Ryan**[2], **Milena Banjevic**[2], **Matthew Hill**[2], **Styrmir Sigurjonsson**[2], **Matthew Rabinowitz**[2], **Hunter B. Fraser**[1], and **Dmitri A. Petrov**[1]

[1]Department of Biology, Stanford University, Stanford, California, USA

[2]Natera, Inc., San Carlos, California, USA

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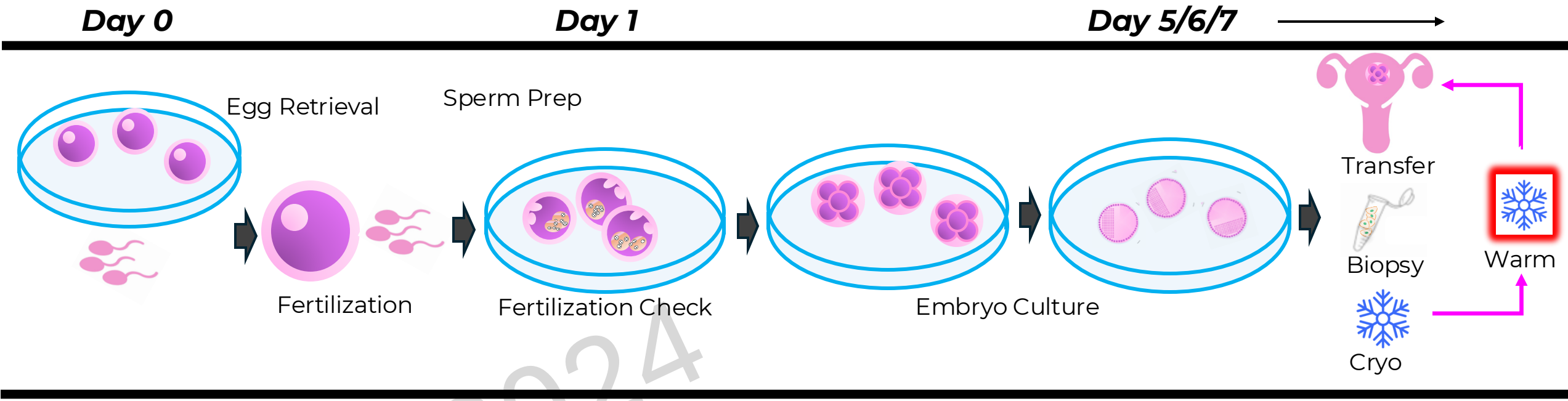
- Exogenous perturbations

Artifactual

Creating Mosaicism by Inducing Mitotic Errors?



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b. Events in the PGT lab

Methods

n= 6,322 clinical TE biopsies collected between Apr ‘23 and Jan ‘24

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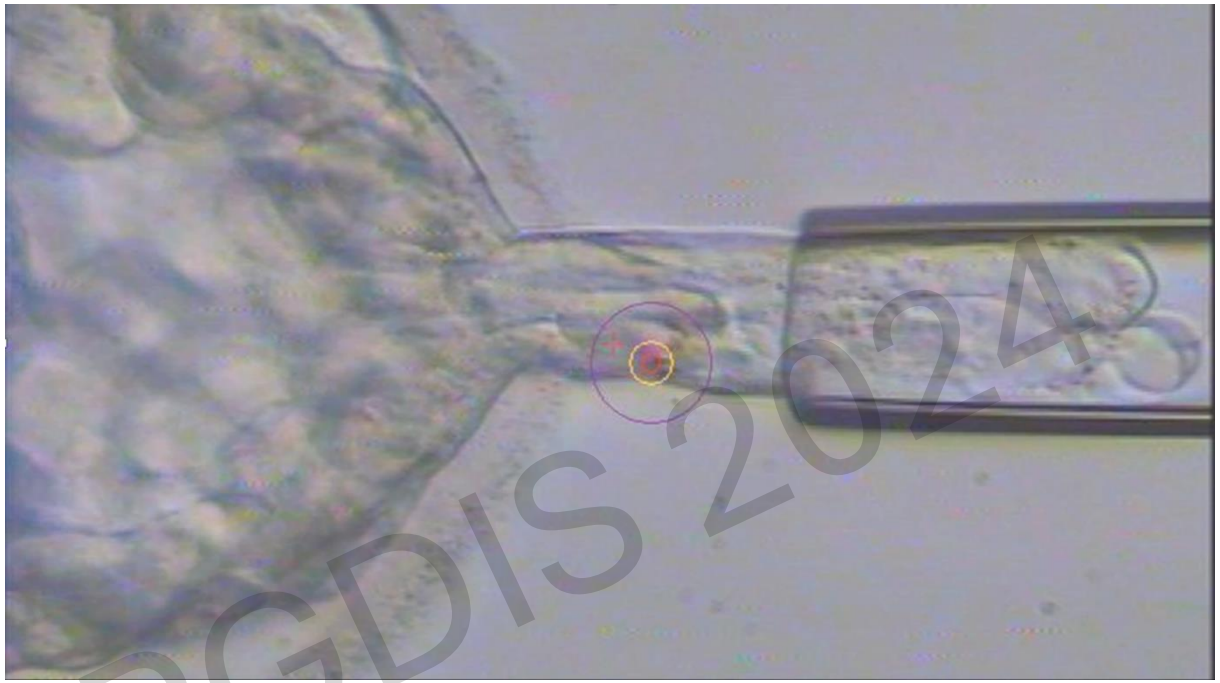
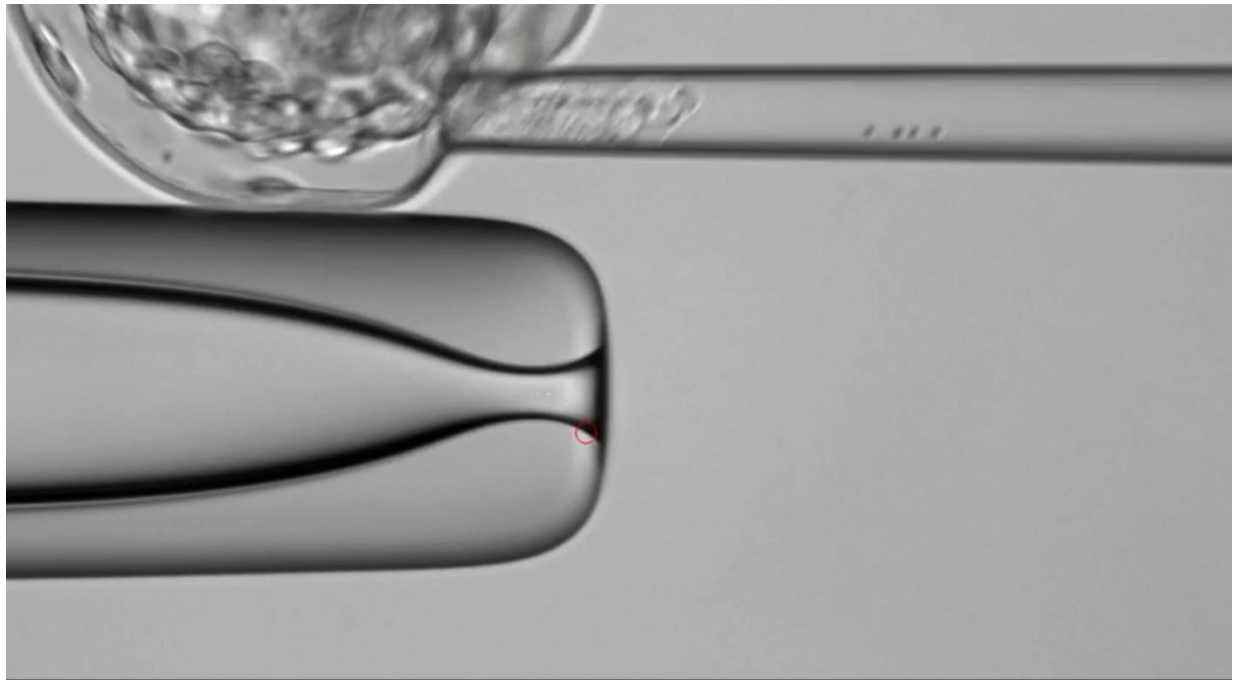
Comparisons

1. Biopsy method ‘Pulling’ vs ‘Flicking’





Yang et al *Fertil Steril* 2020

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2. Pre-Loading tube with media vs. No Pre-Loading

vs.

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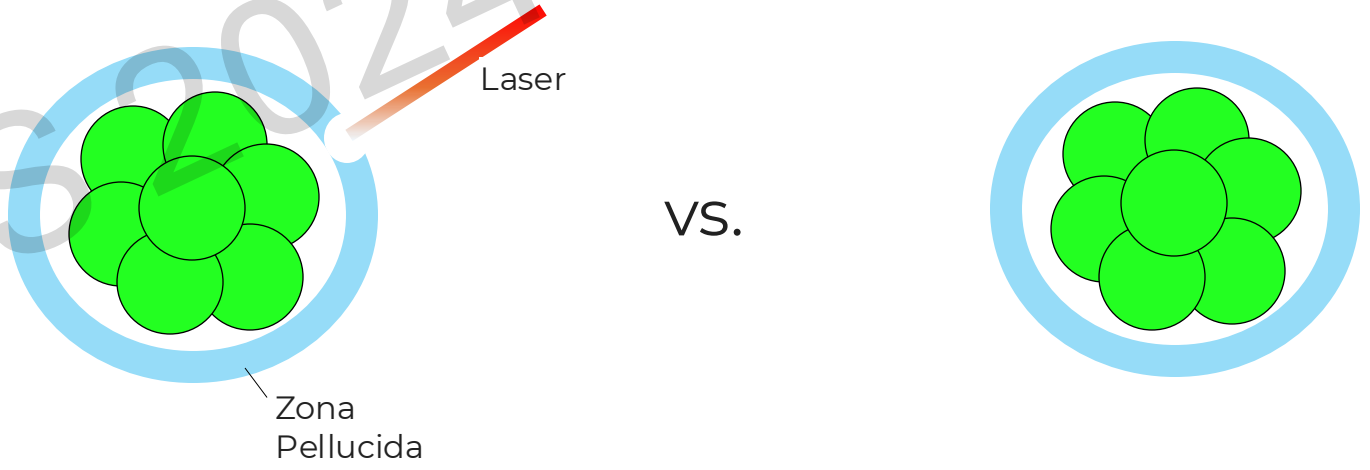
3. Assisted Pre-Hatching Day3/4 vs No Assisted Pre-Hatching



Methods

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Readouts

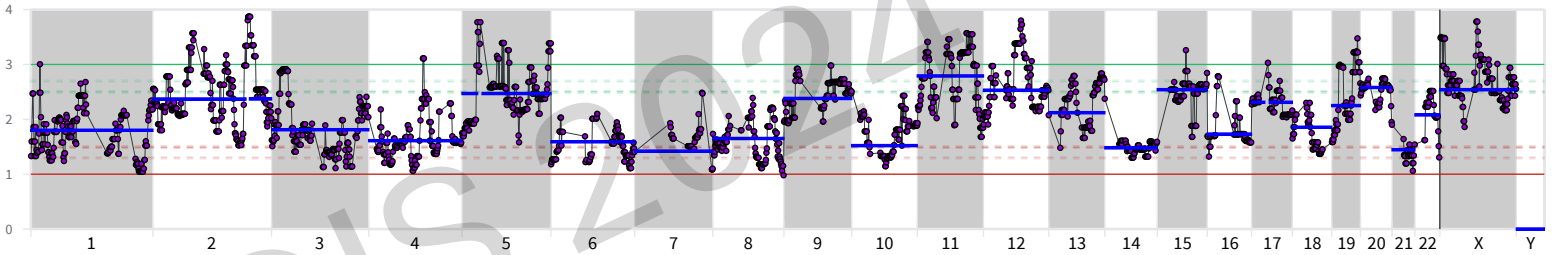
Incidence of Mosaicism

Incidence of
No Result/Inconclusive

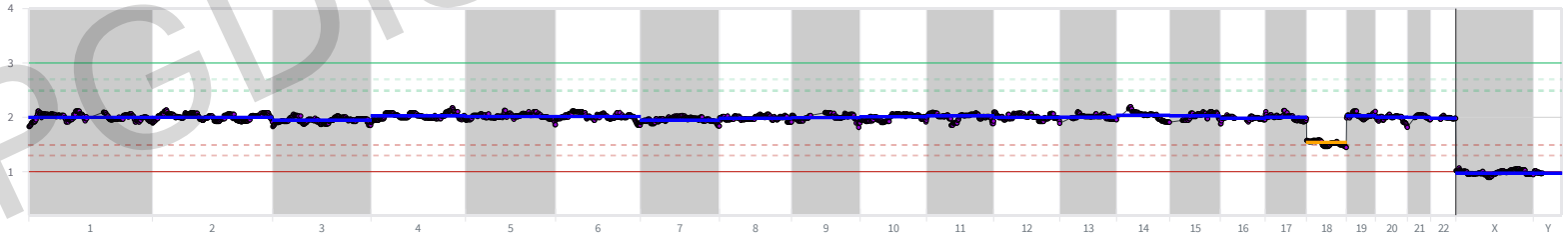
DLRS (technical noise)

[D = |log(CCNi / CCNi+1)|, where i = bin position]
(75th percentile of D –
25th percentile of D) / normalizing constant

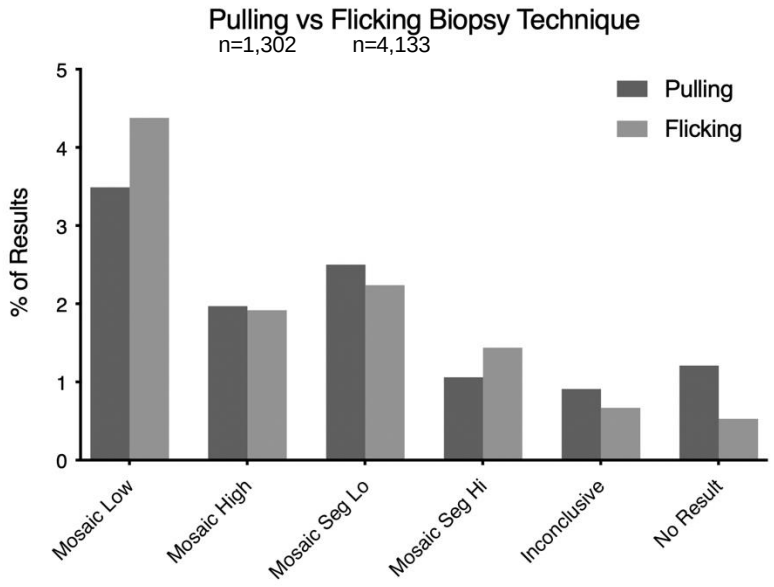
DLRS
0.760

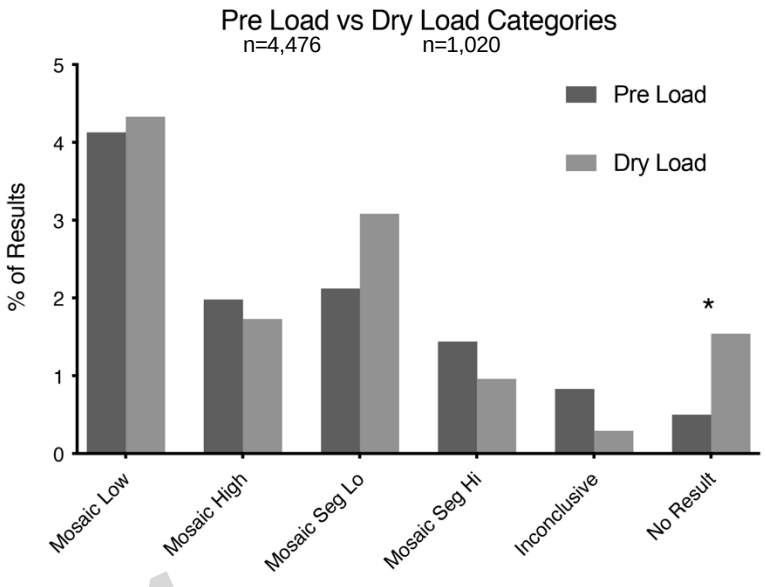


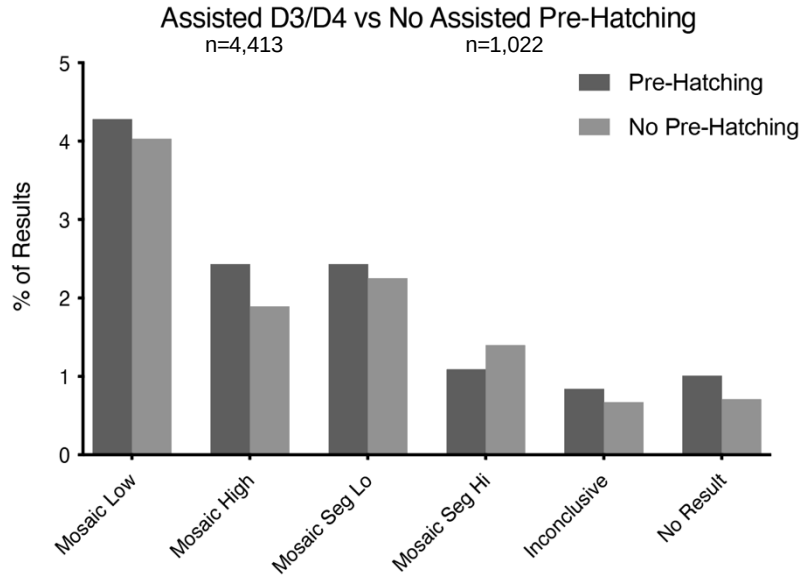
DLRS
0.071

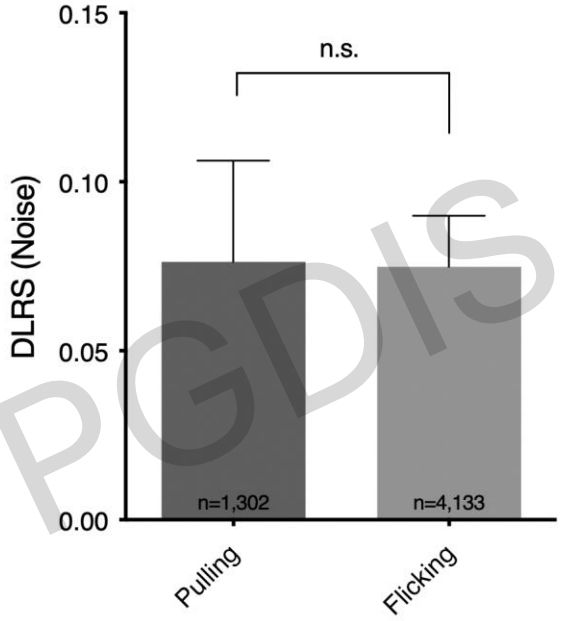


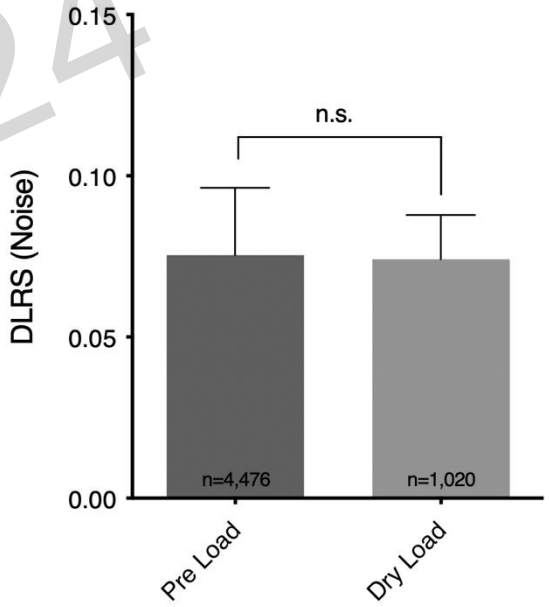
Results from n= 6,322 clinical TE biopsies

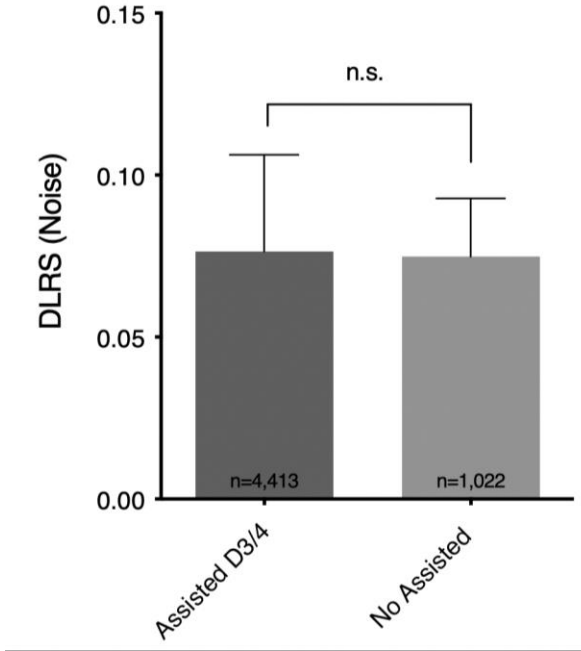












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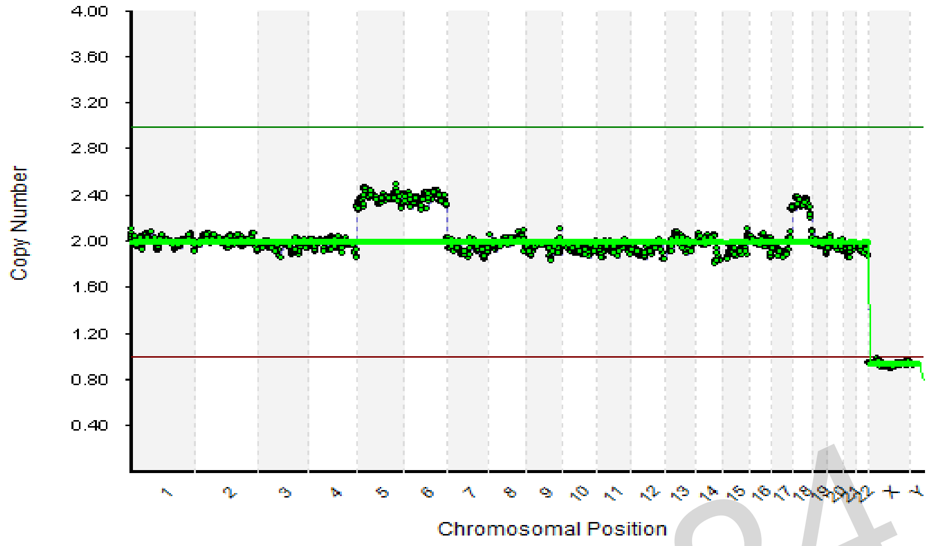
Artifactual

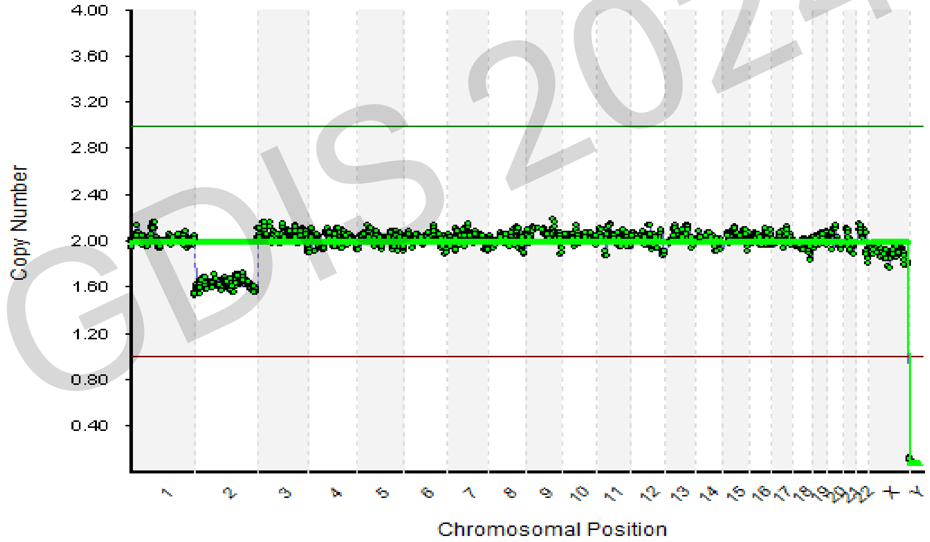
- PGT-A Results indicate mosaicism

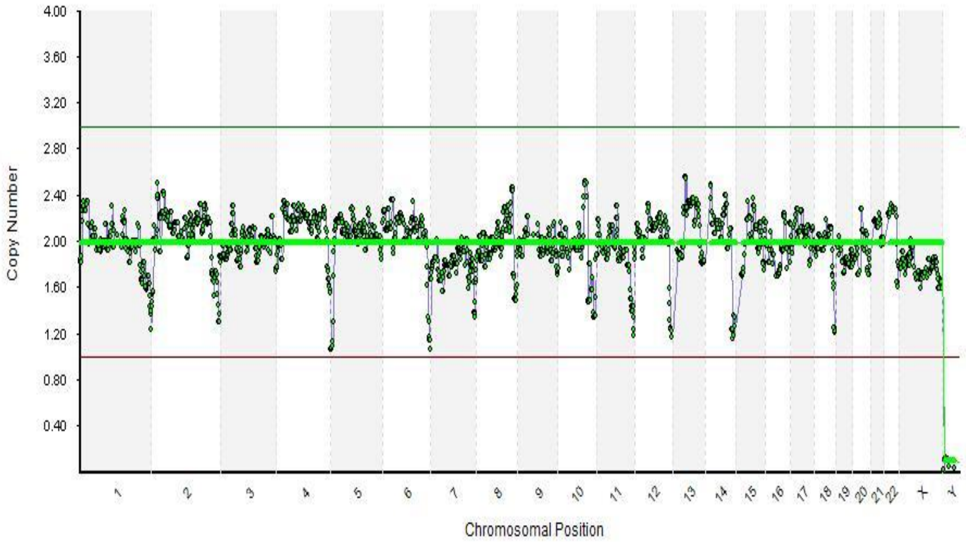
a. Events in the IVF lab (biopsy)

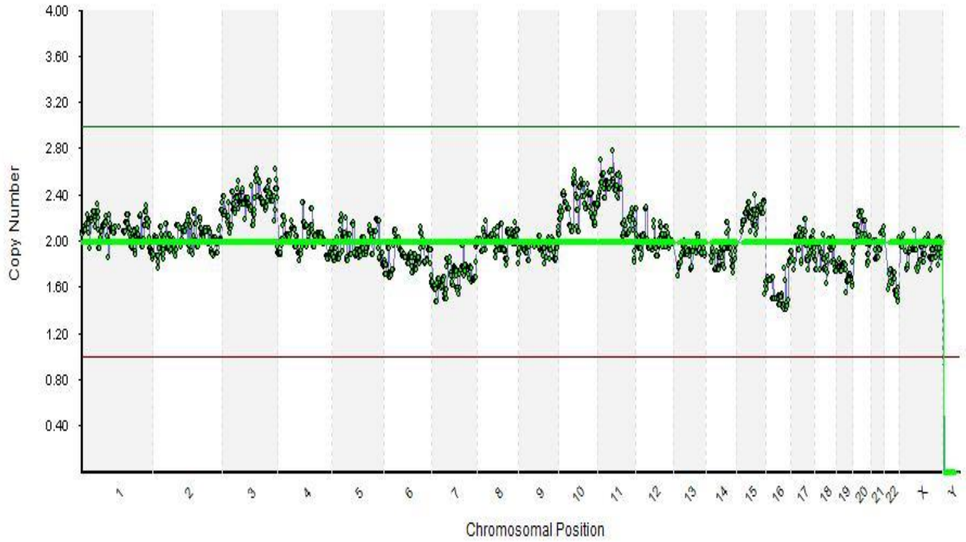
b. Events in the PGT lab

?Mosaicism ≠ Noise?

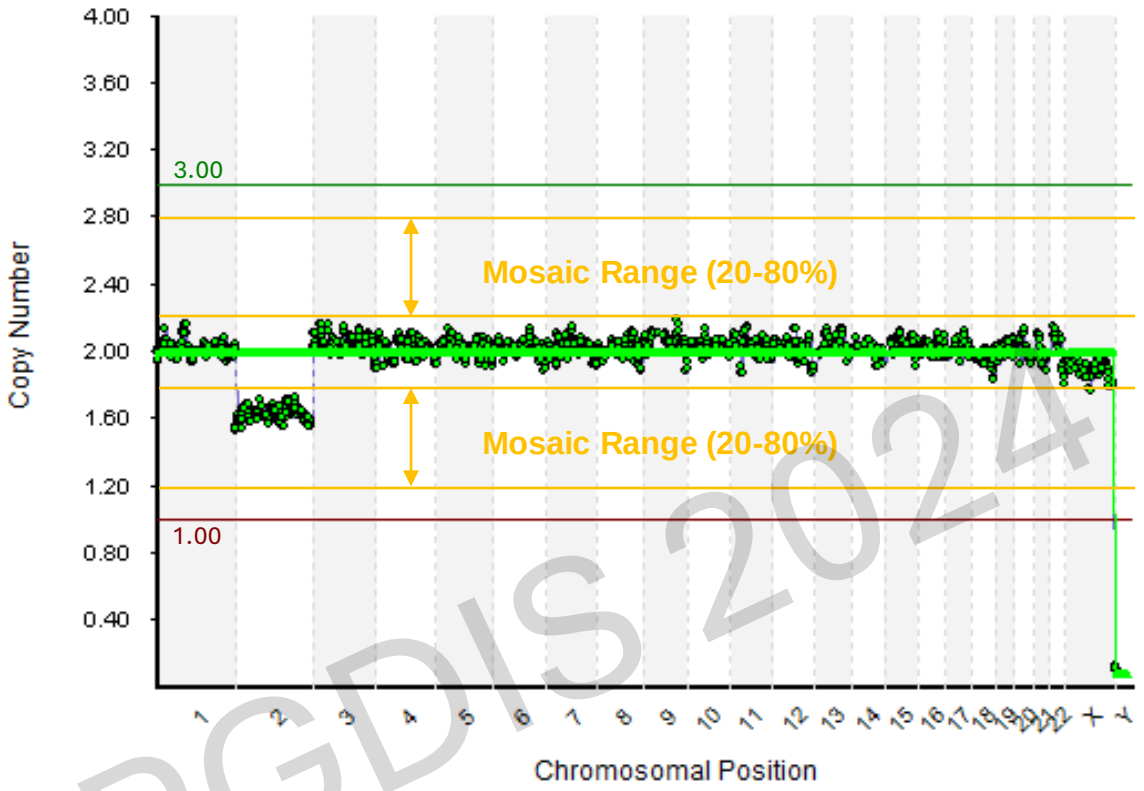


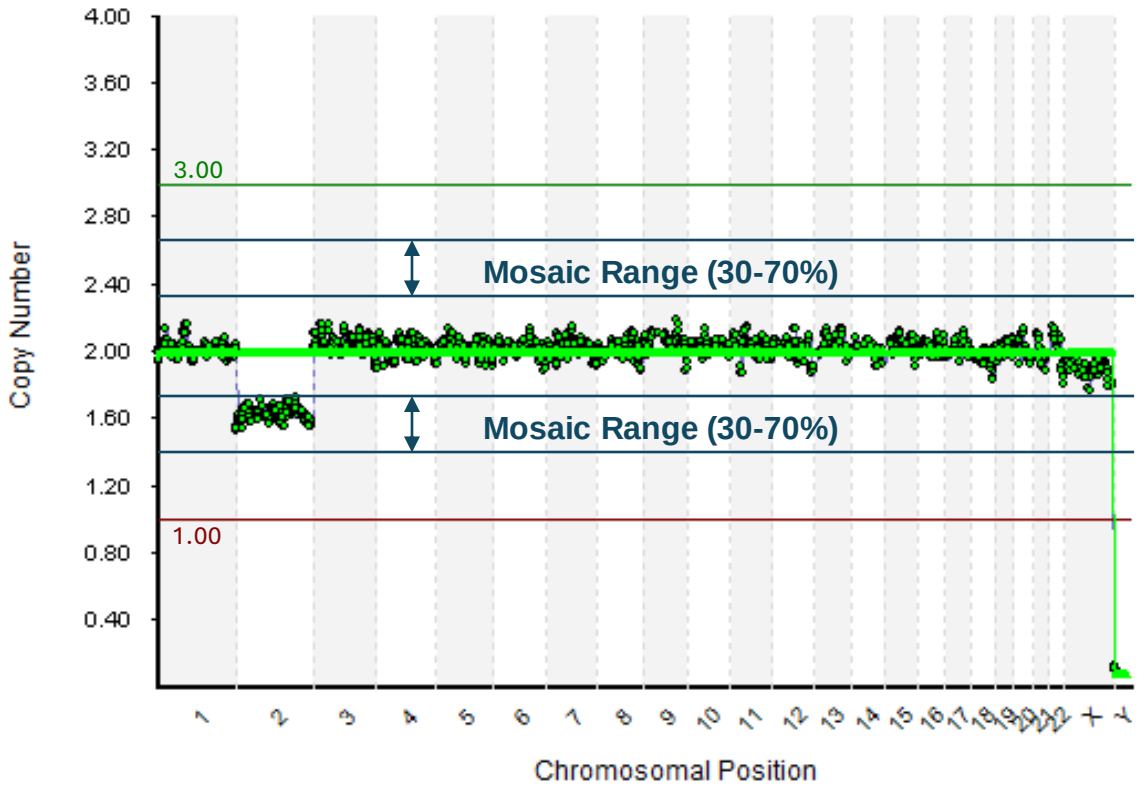




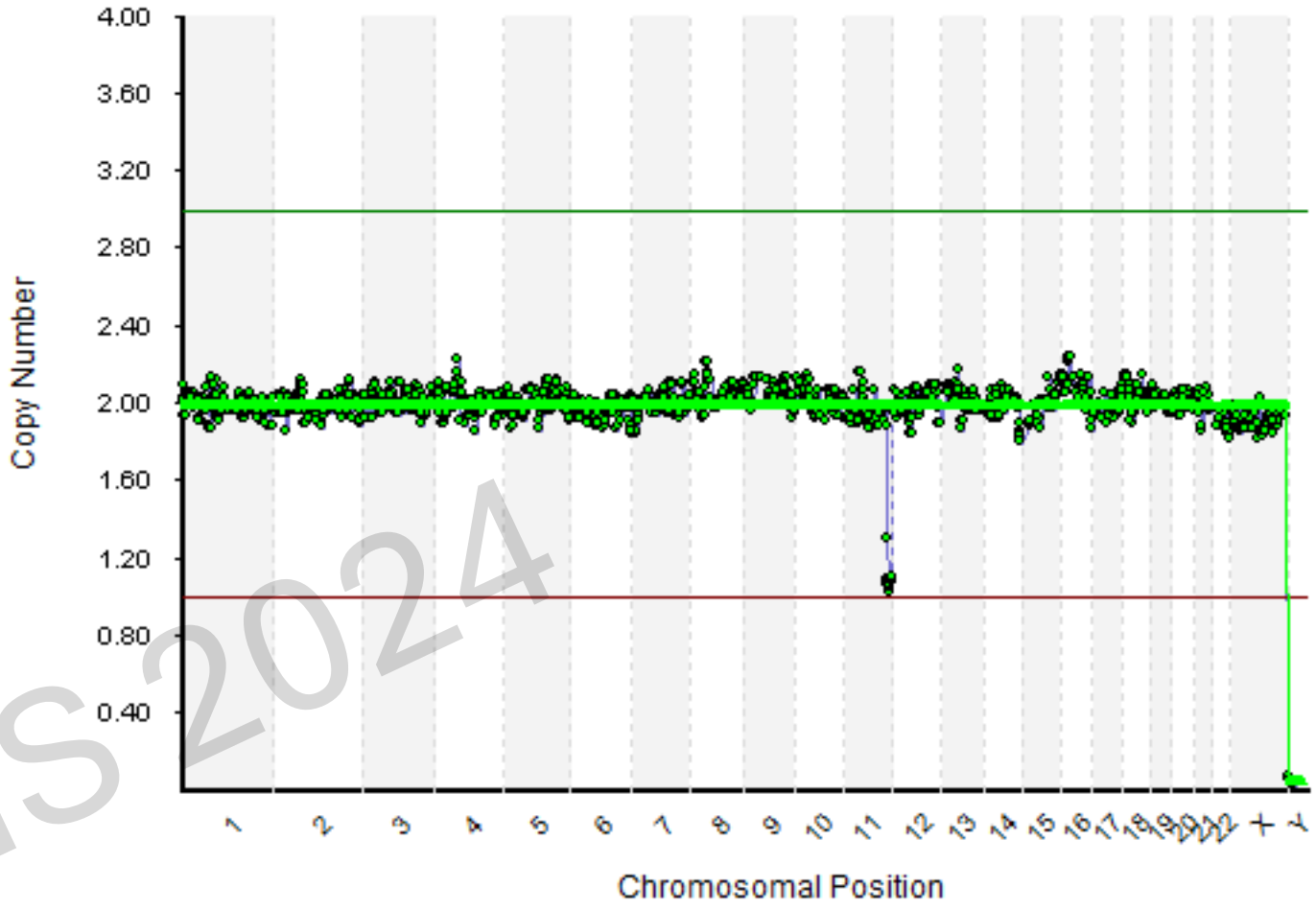


Intermediate Copy Number (ICN) Indicates Mosaicism



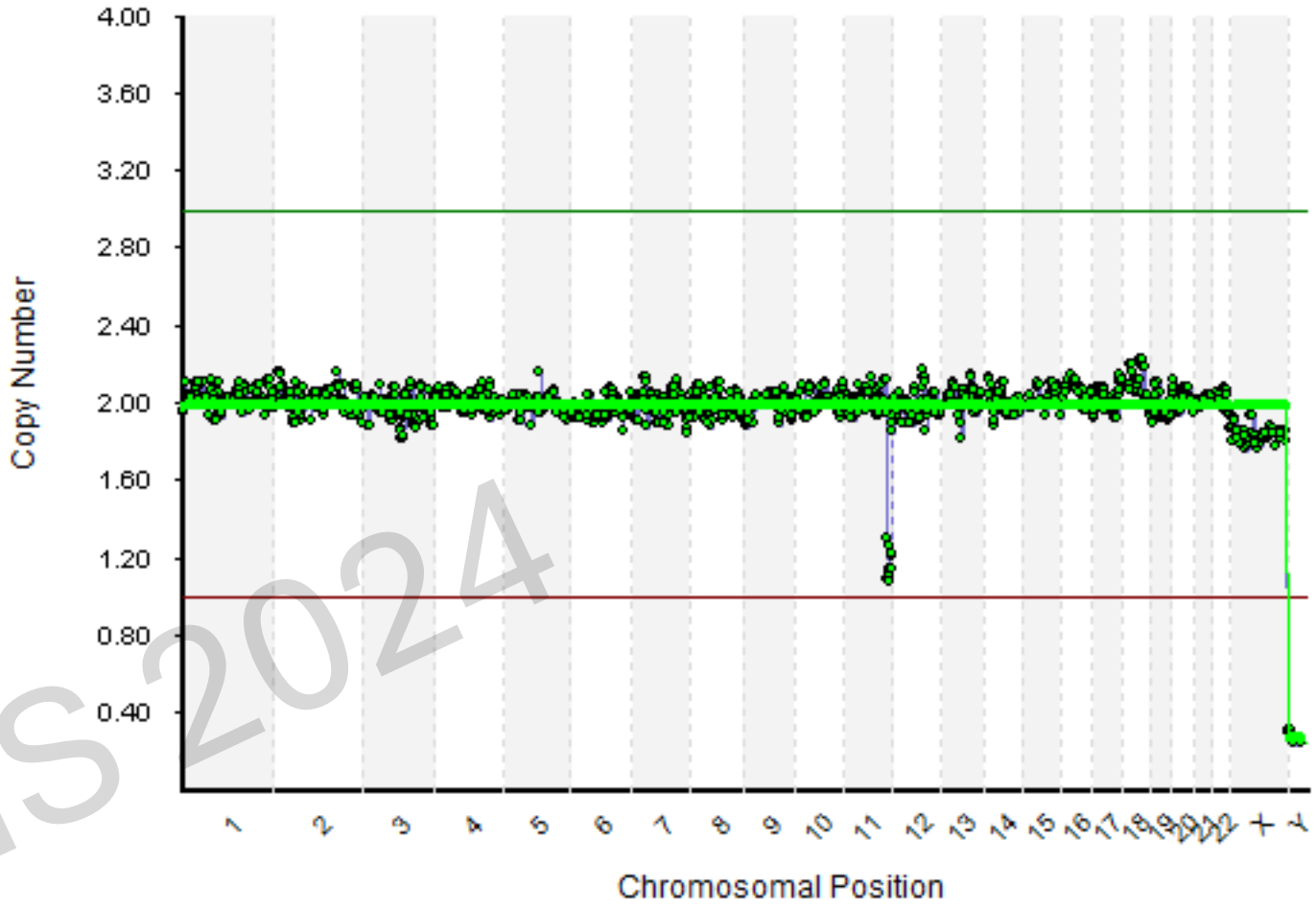


Mixing Experiment



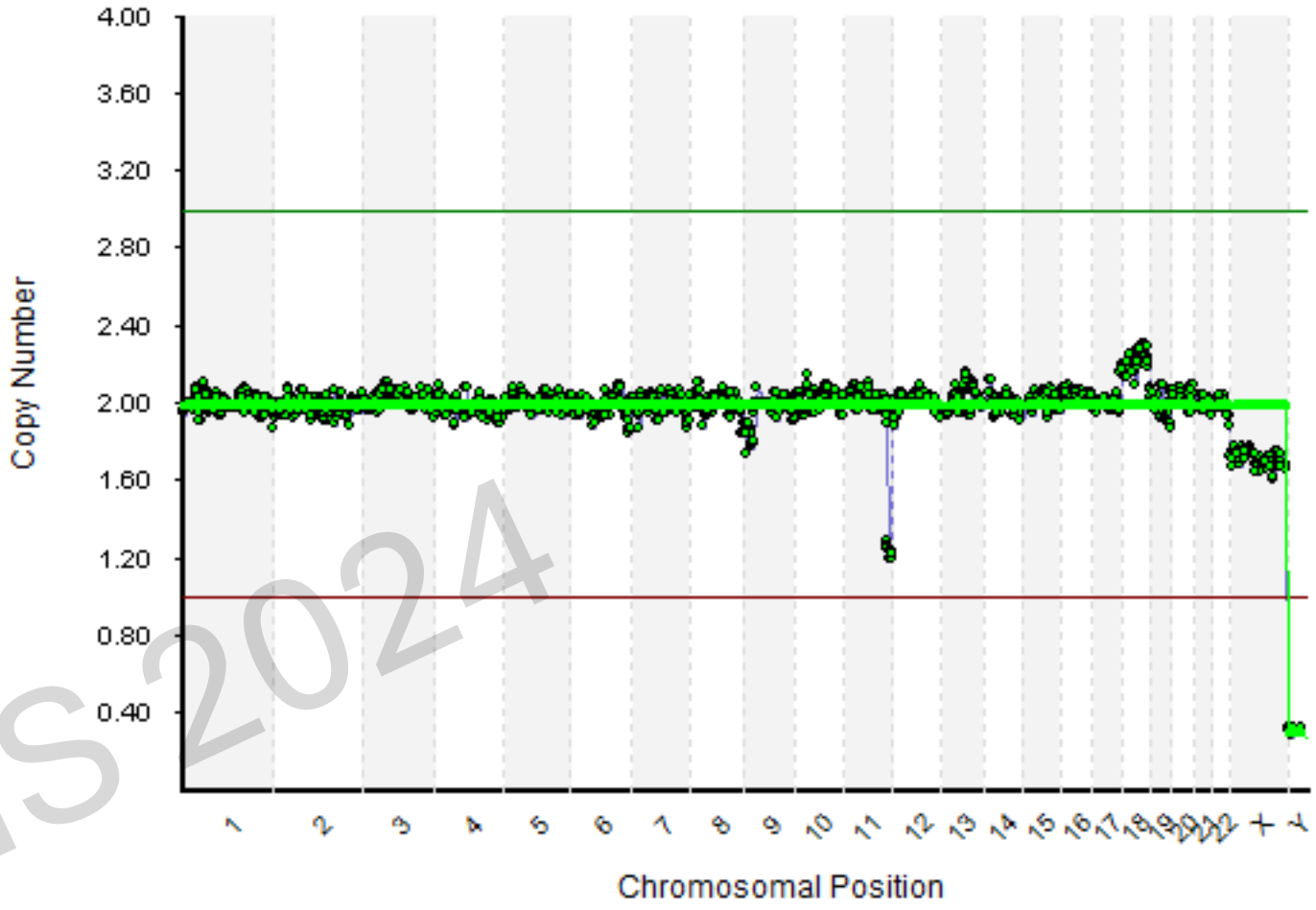
Ratio
10:0

Mixing Experiment



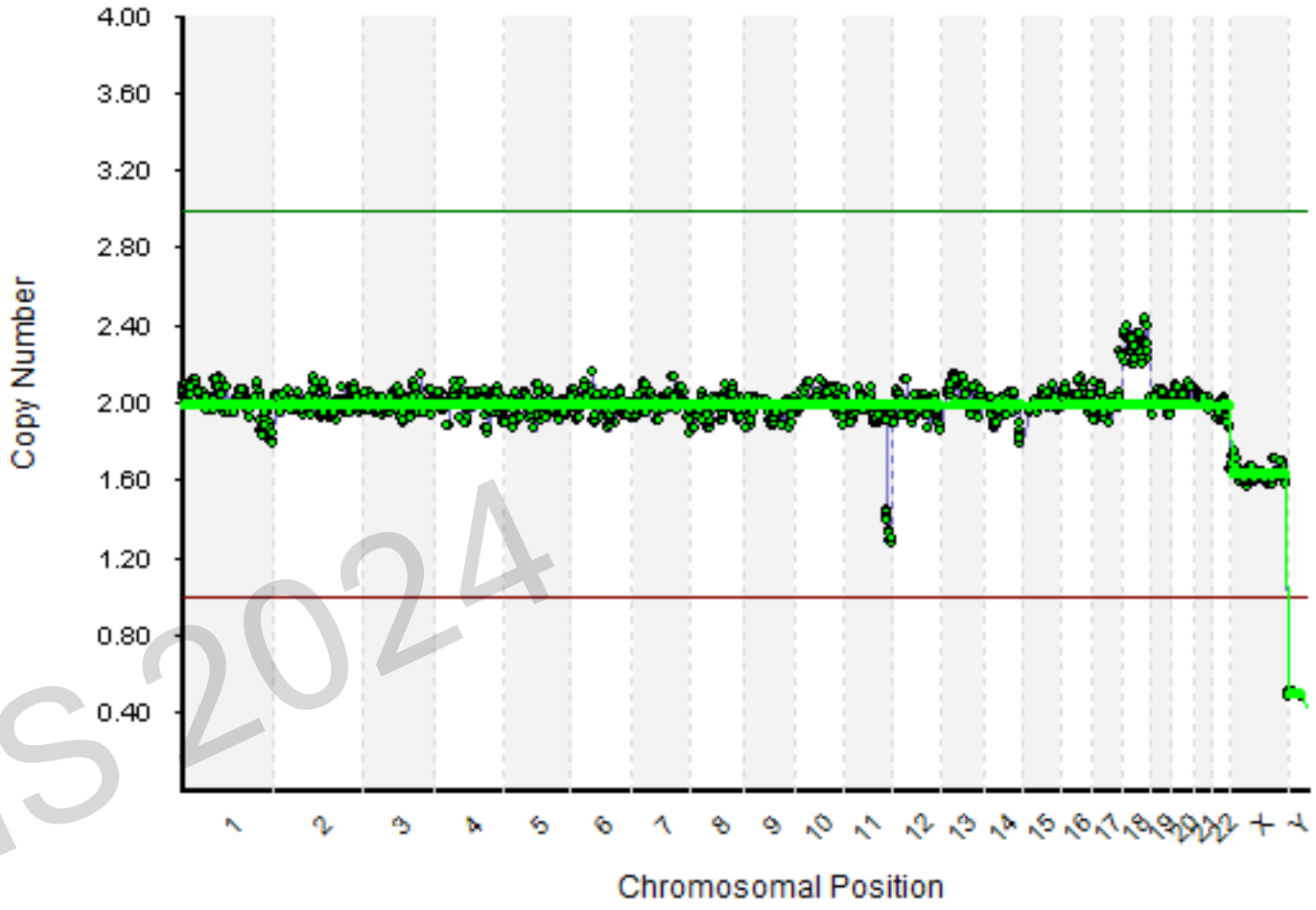
Ratio
9:1

Mixing Experiment



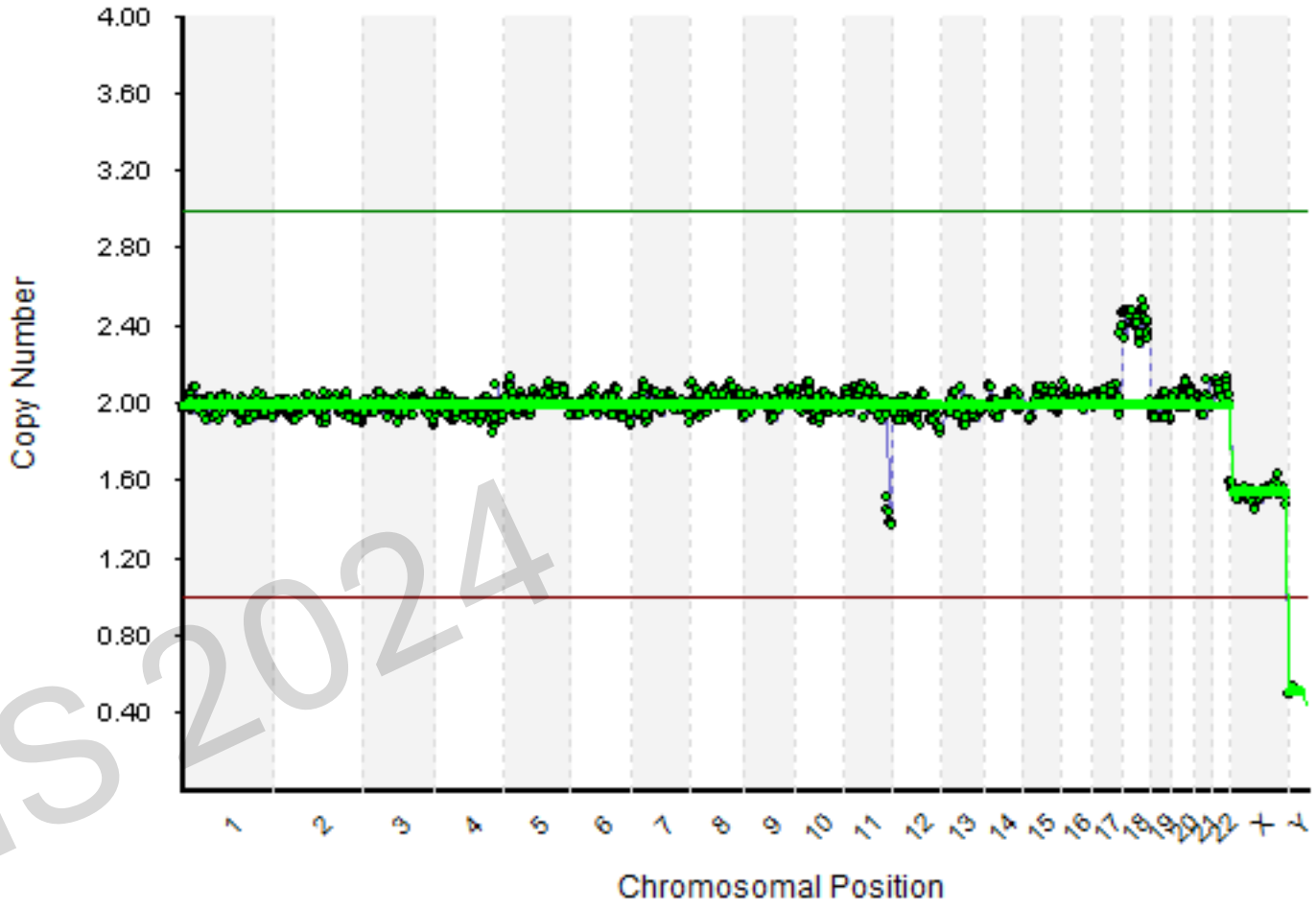
Ratio
8:2

Mixing Experiment



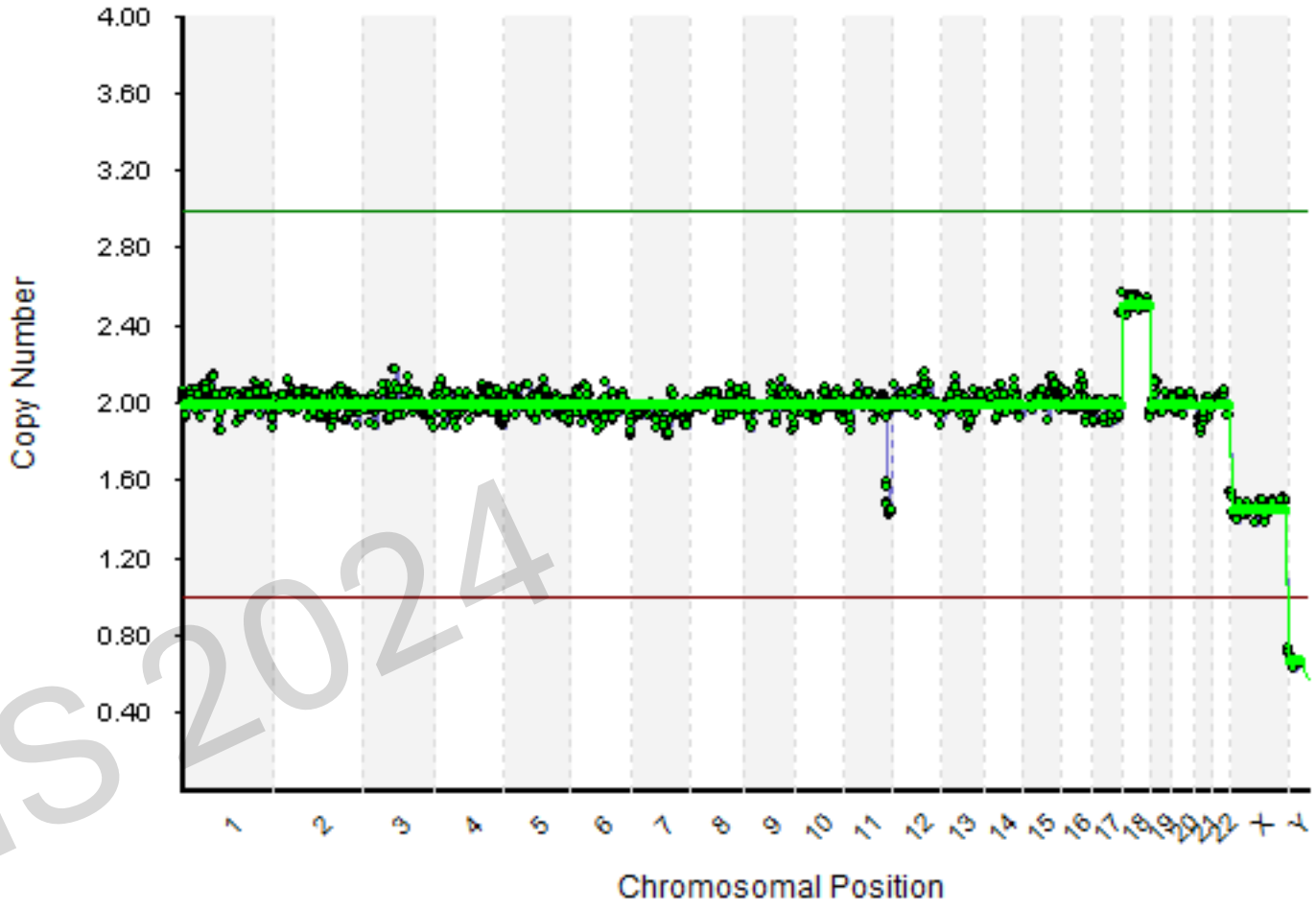
Ratio
7:3

Mixing Experiment



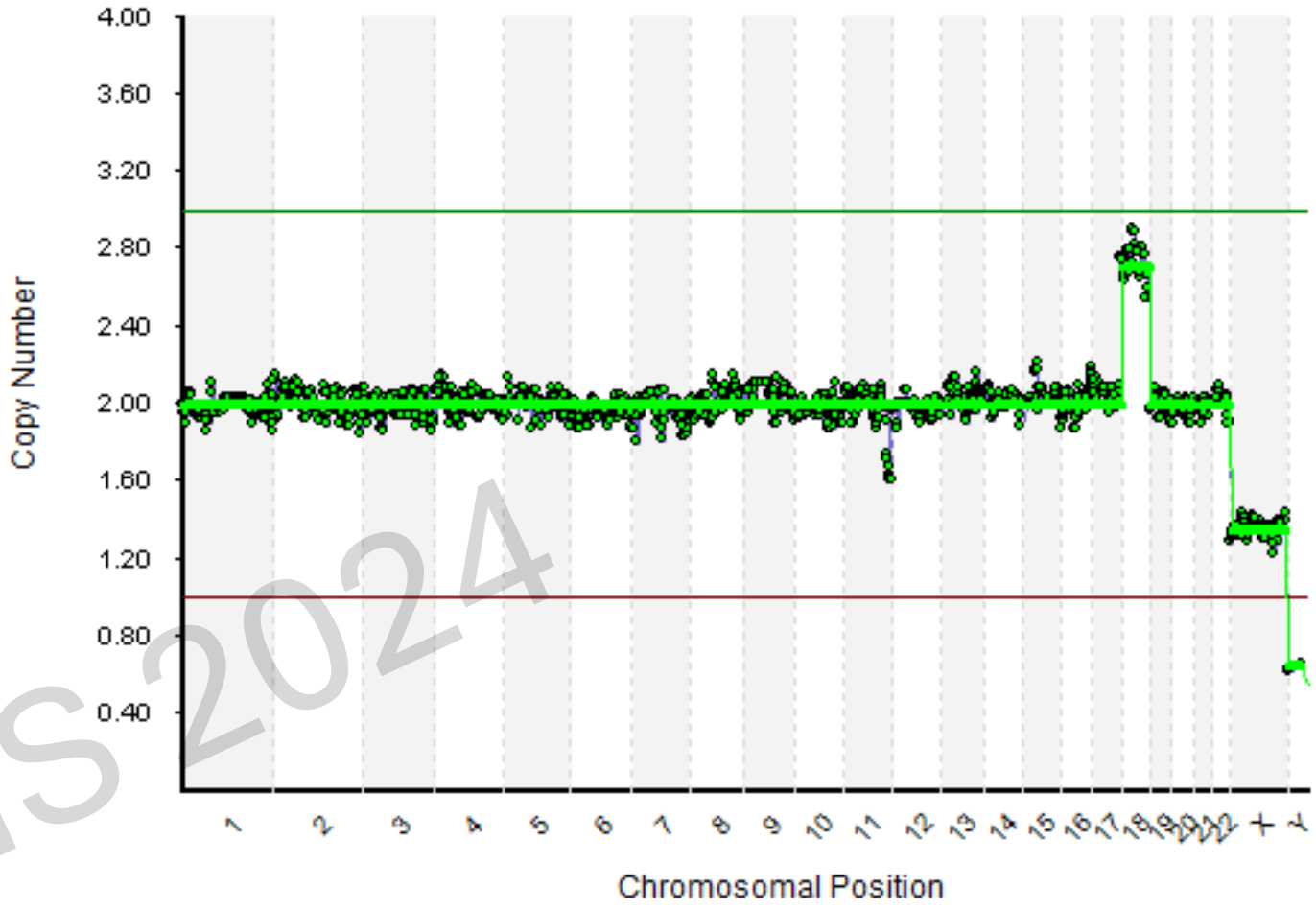
Ratio
6:4

Mixing Experiment



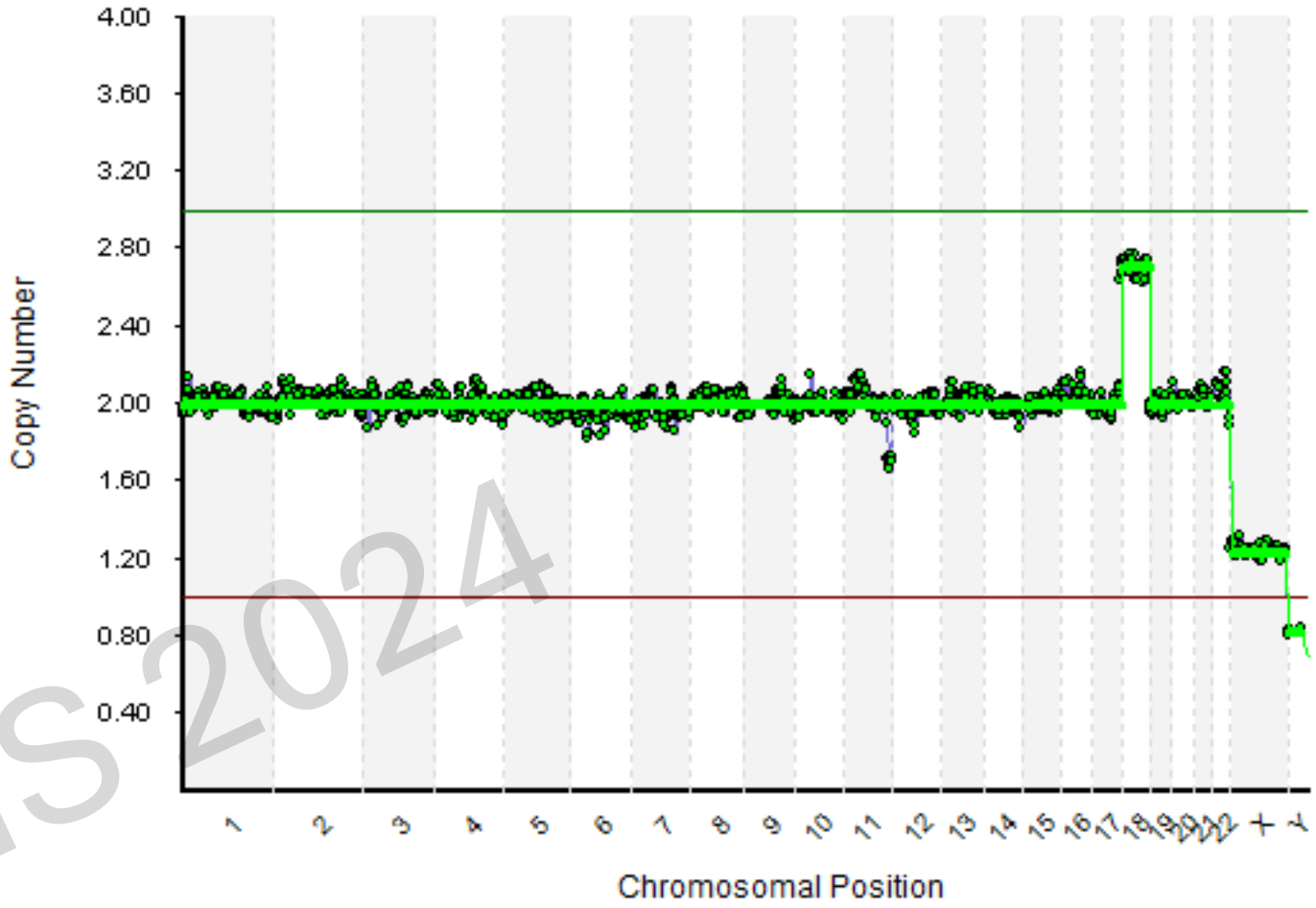
Ratio
5:5

Mixing Experiment



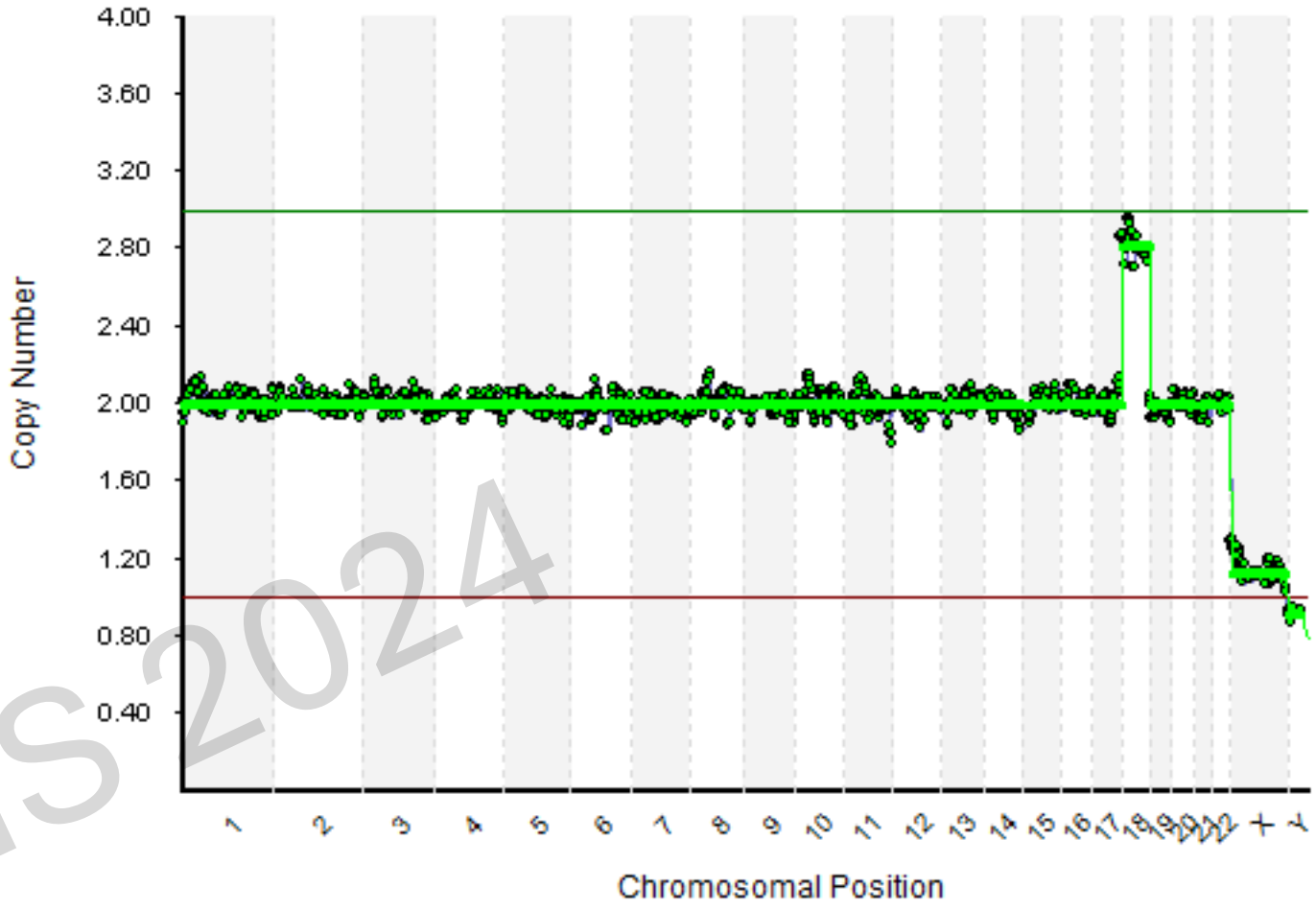
Ratio
4:6

Mixing Experiment



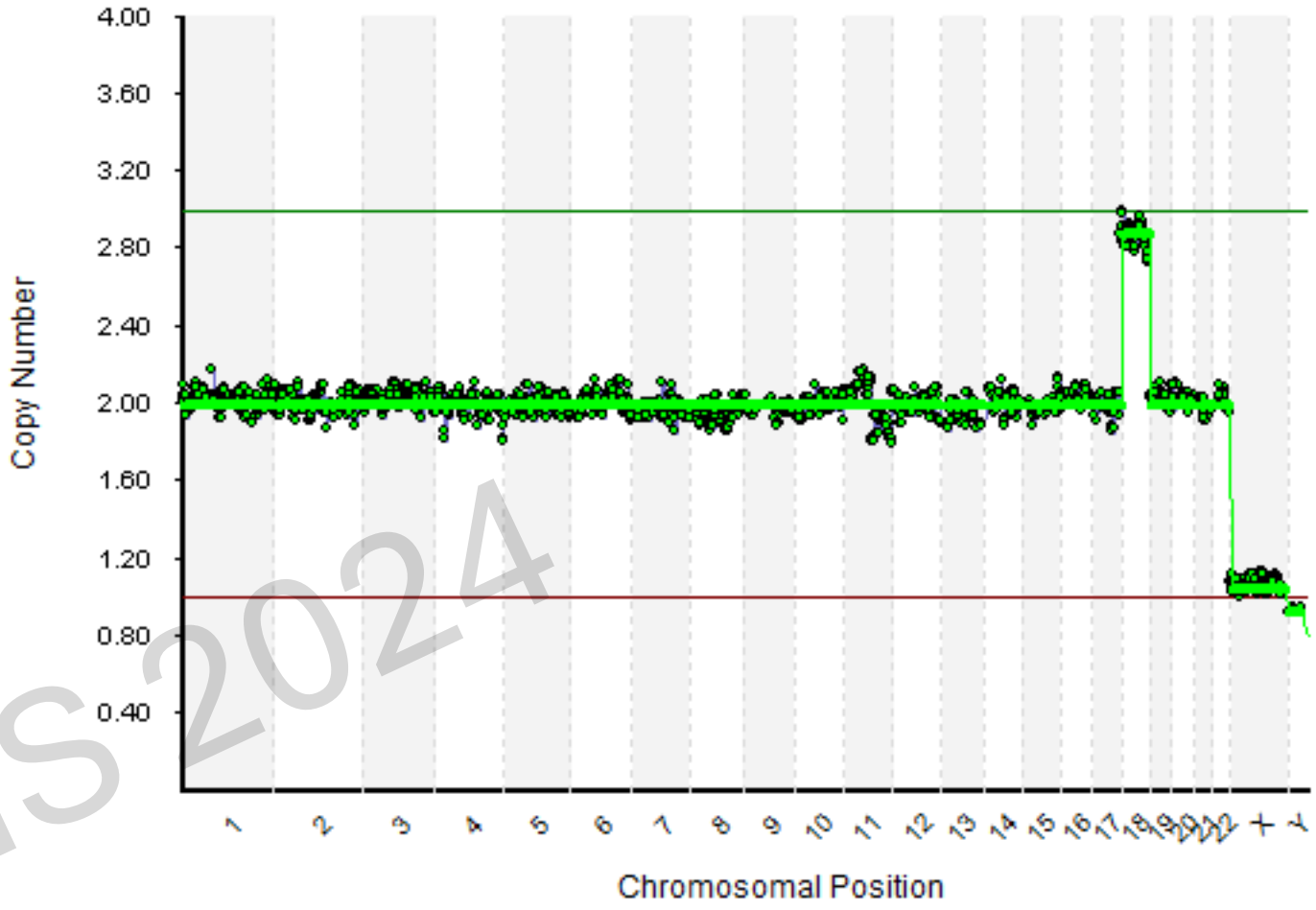
Ratio
3:7

Mixing Experiment



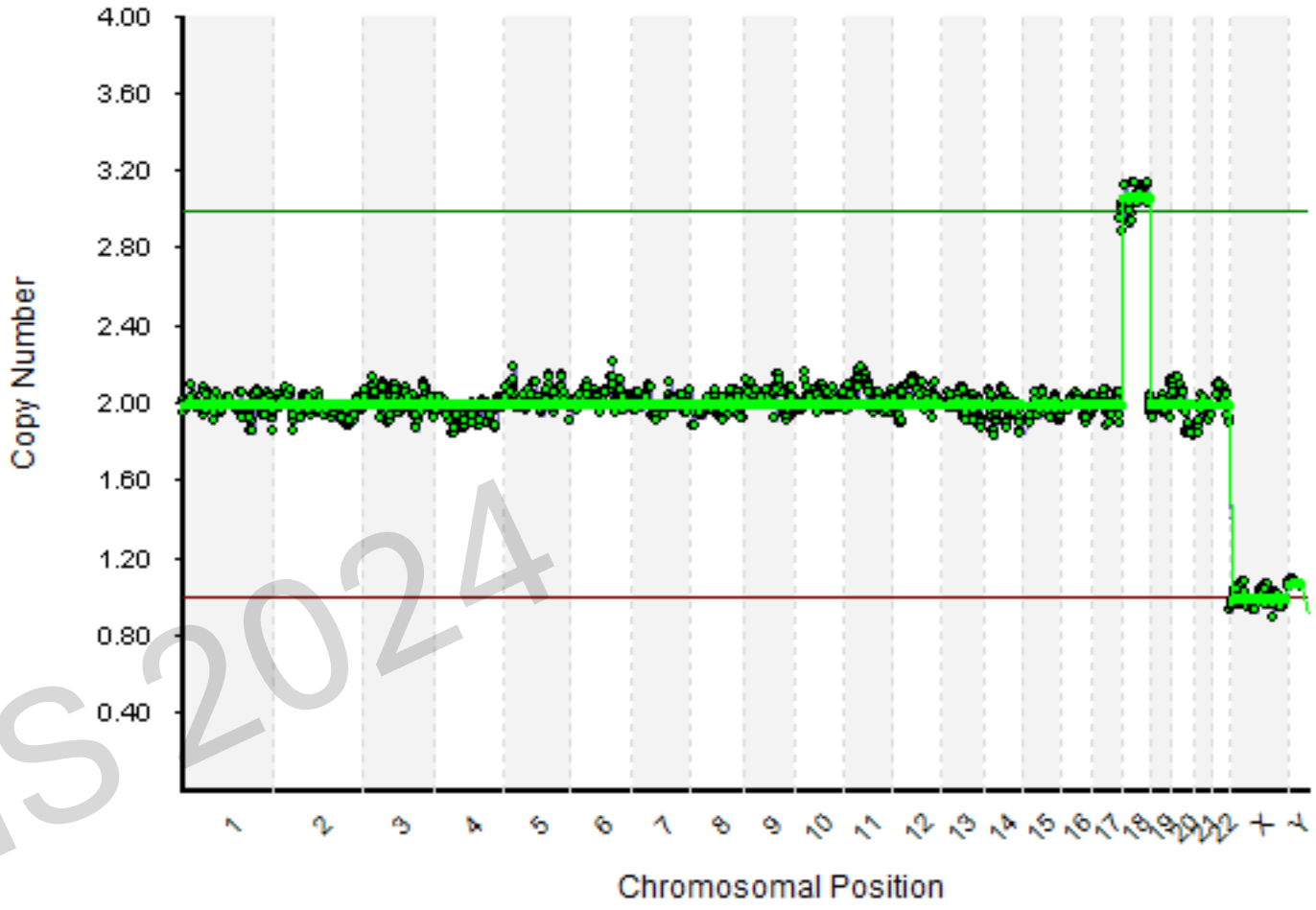
Ratio
2:8

Mixing Experiment



Ratio
1:9

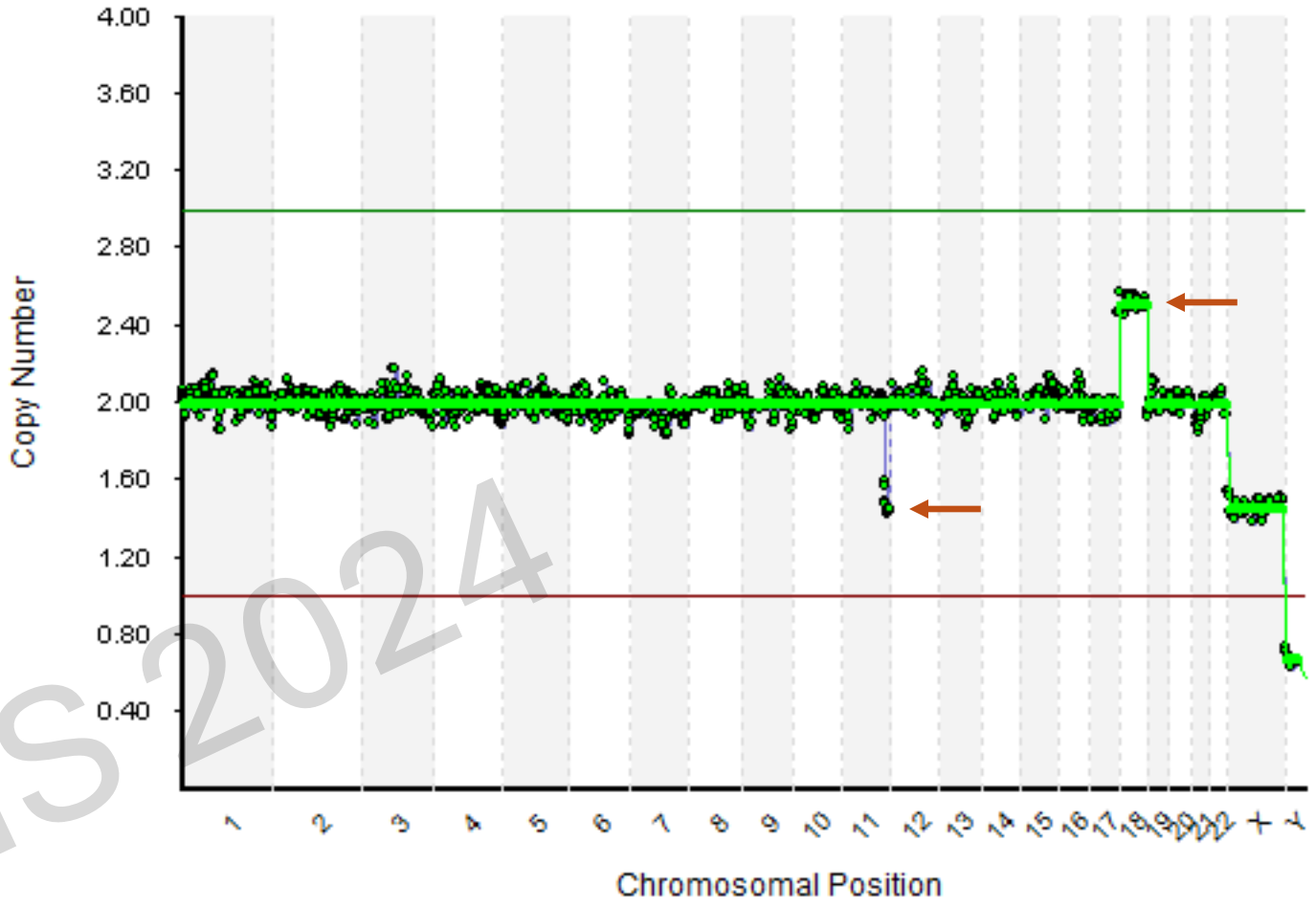
Mixing Experiment



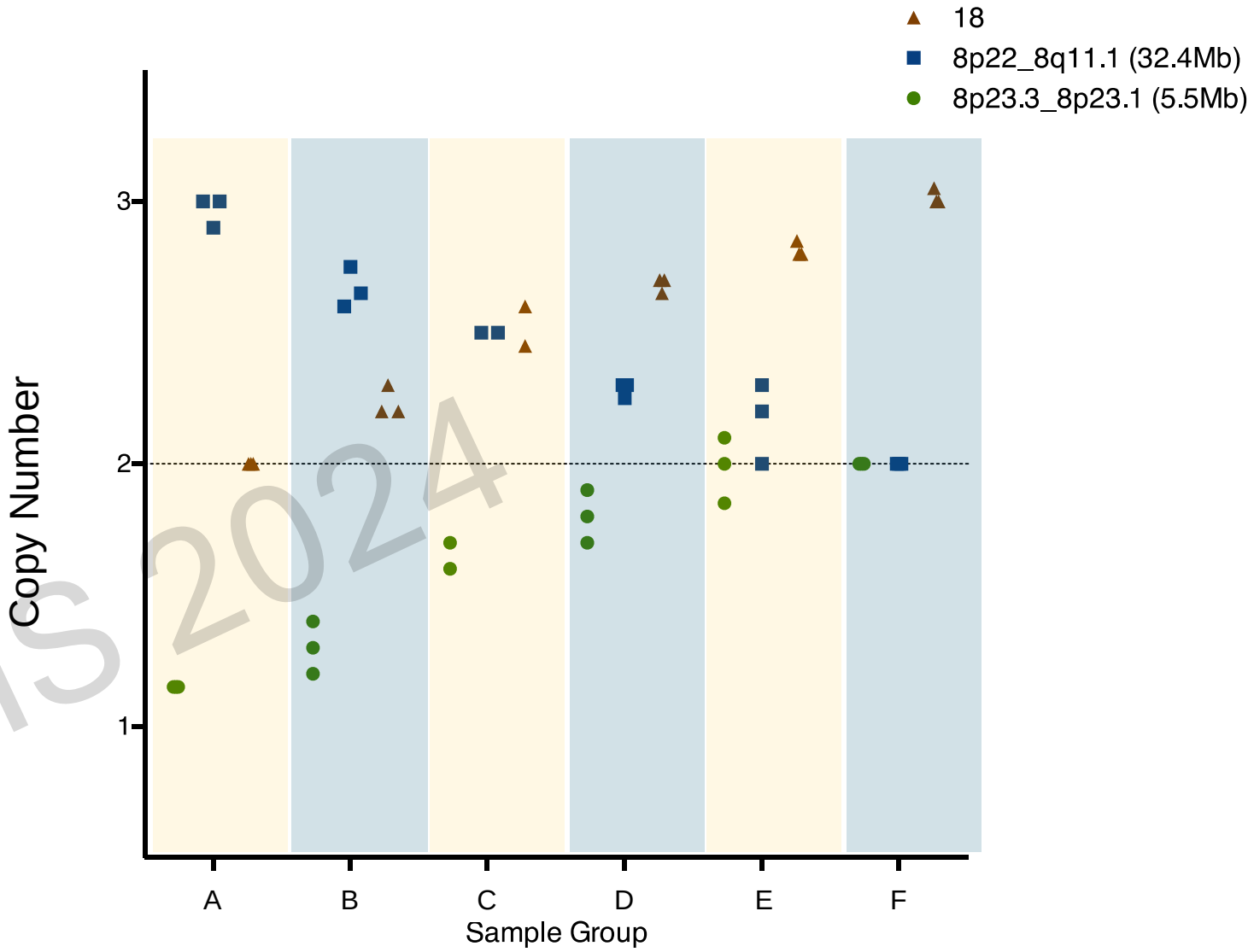
Ratio
0:10

Mixing Experiment

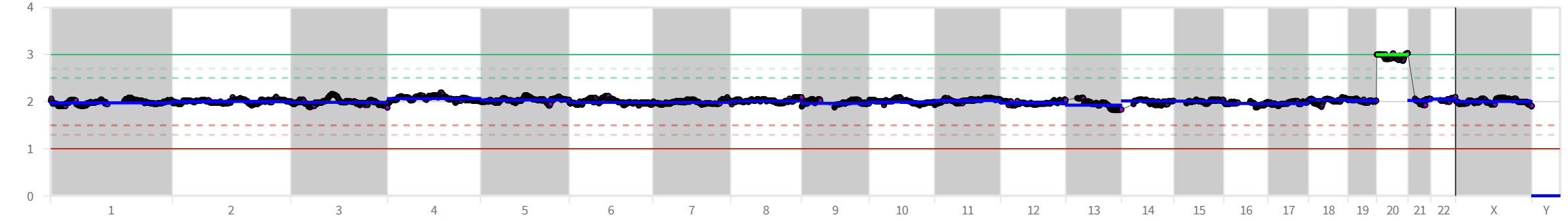
At 50% Mix



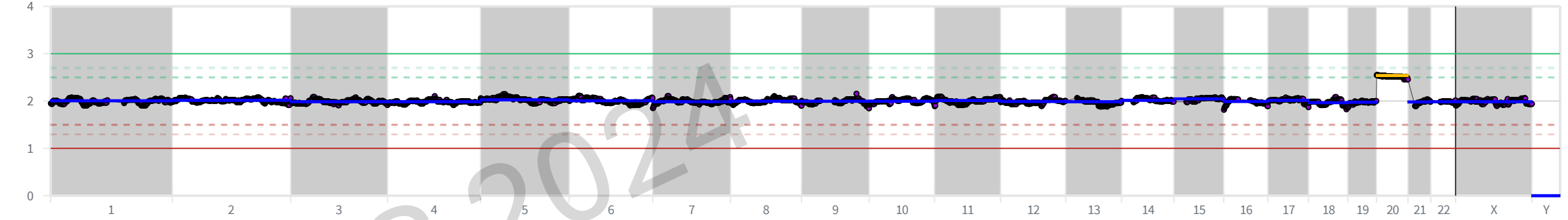
Ratio
5:5


18
8p22_8q11.1 (32.4Mb)
8p23.3_8p23.1 (5.5Mb)
Copy Number
3
2
1
A
B
C
D
E
F
Sample Group

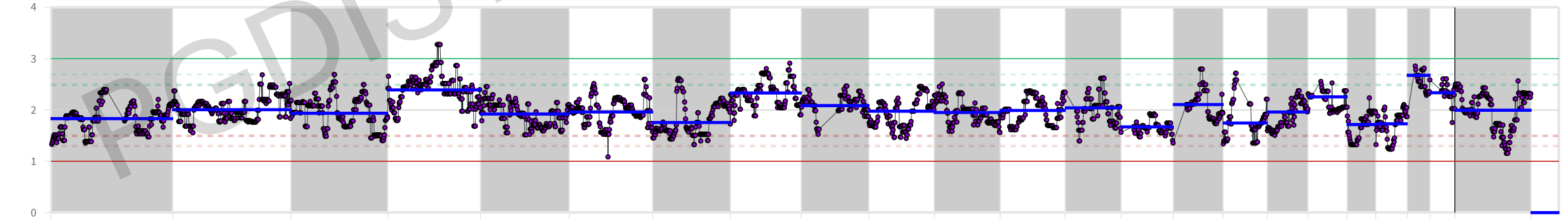
Using Metrics to Distinguish Mosaics from Noise



DLRS
0.069

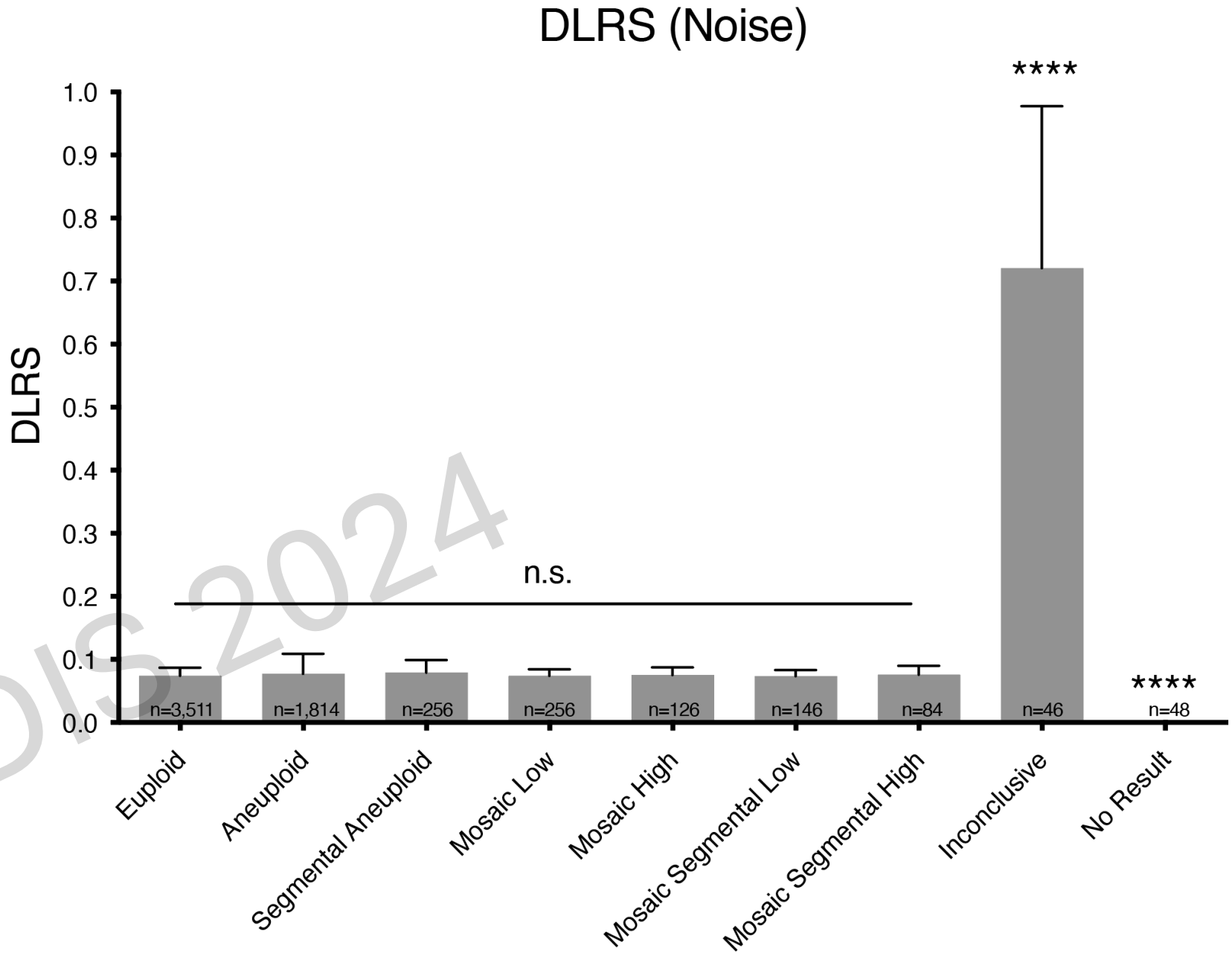


DLRS
0.065



DLRS
0.471

total TE biopsies tested n = 6,322



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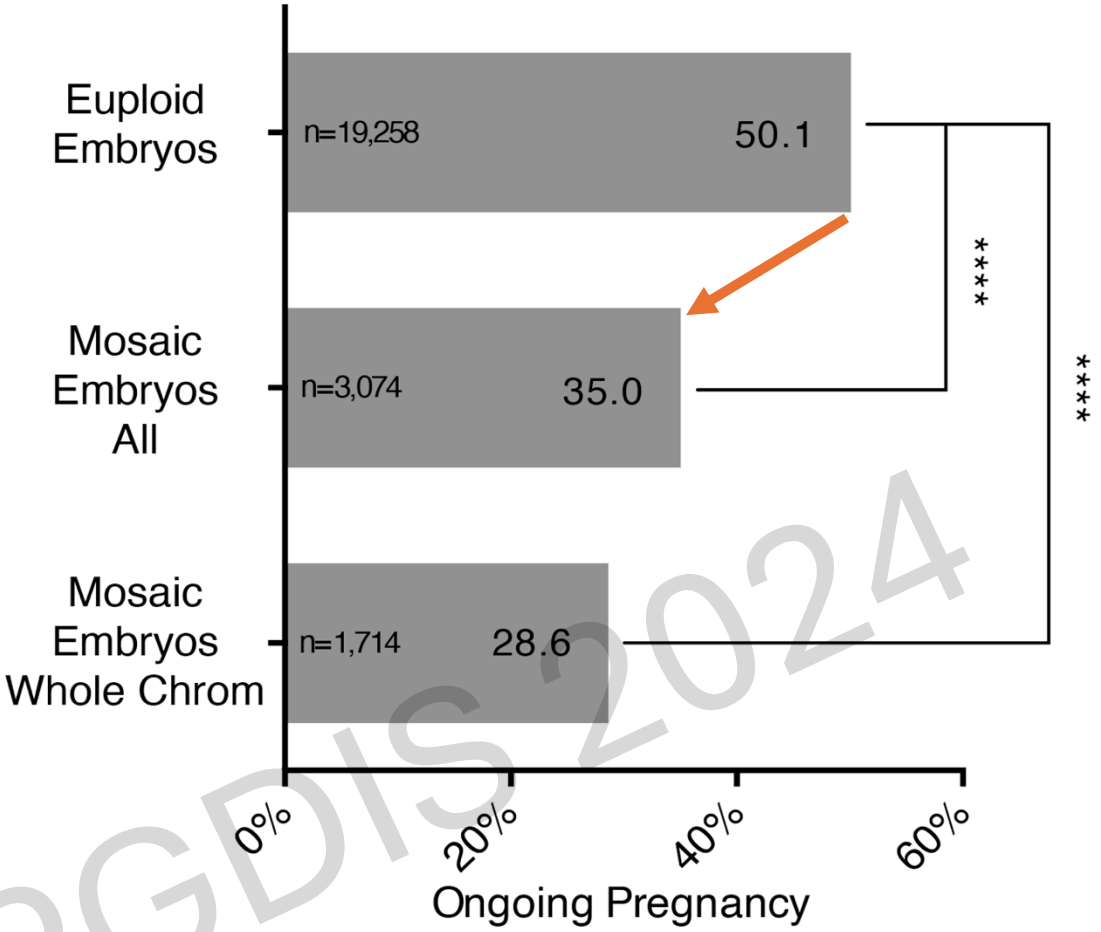
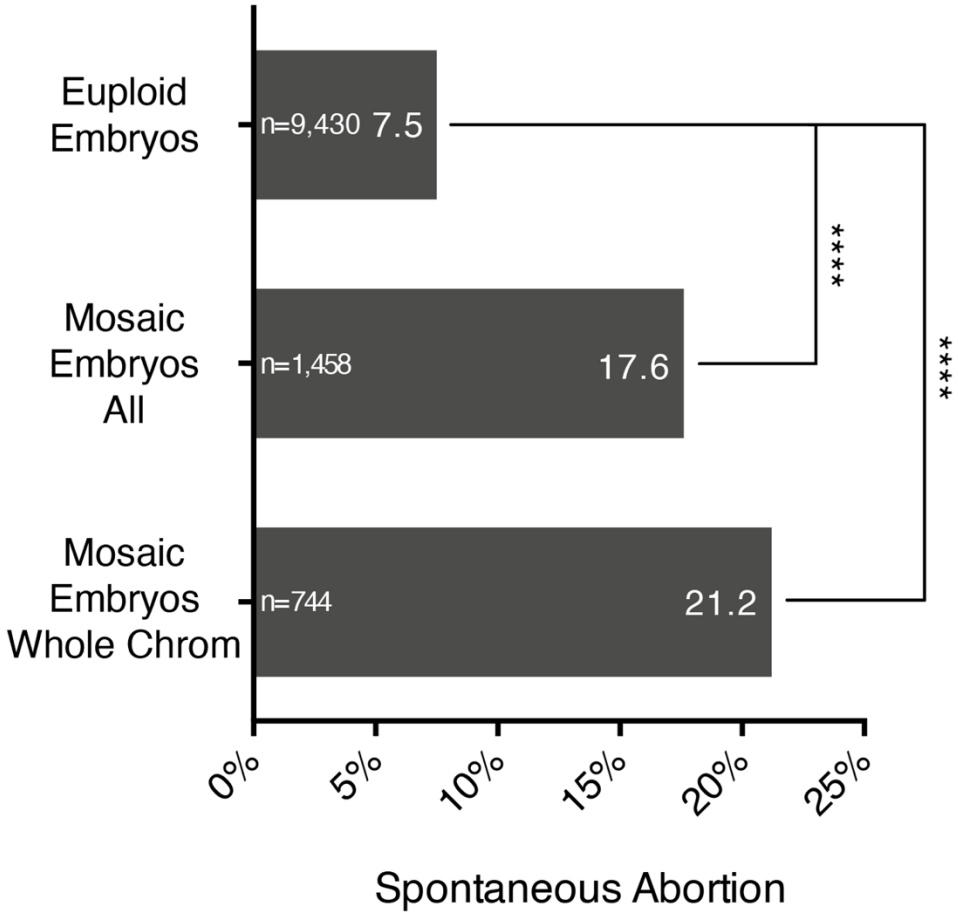
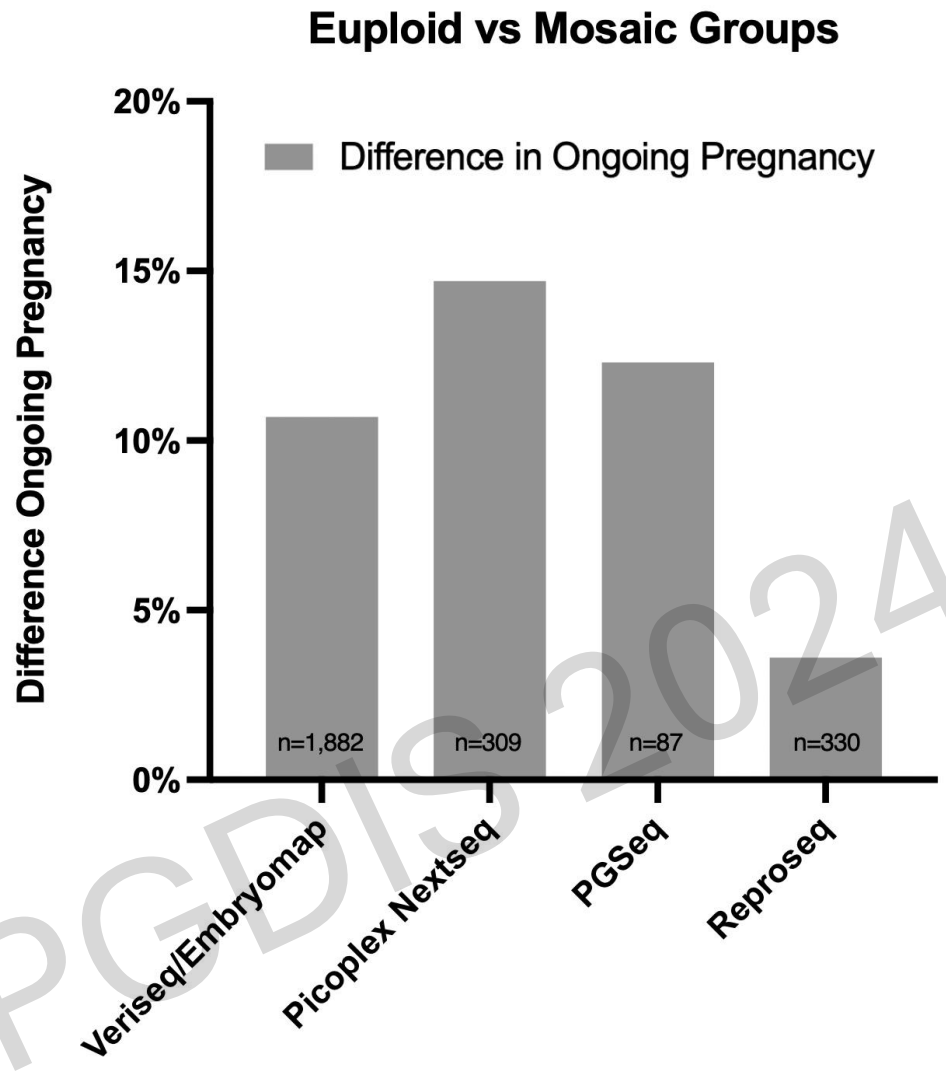
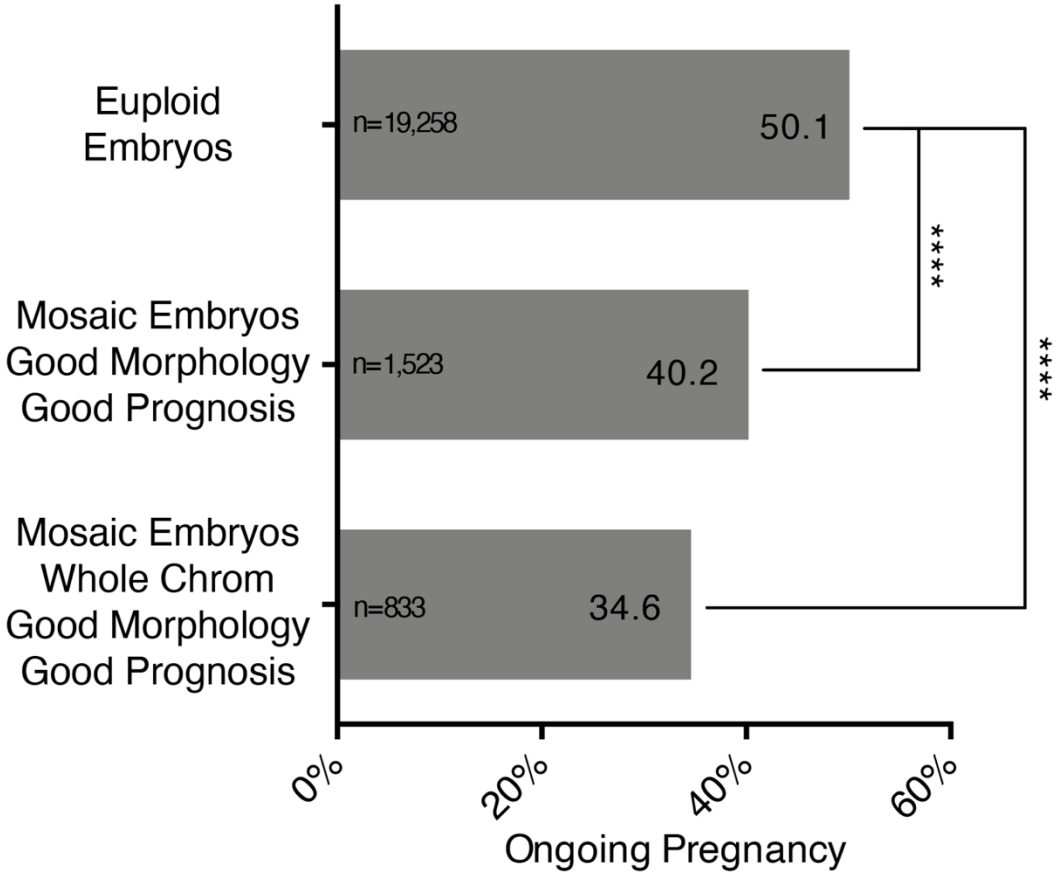
IRMET Update



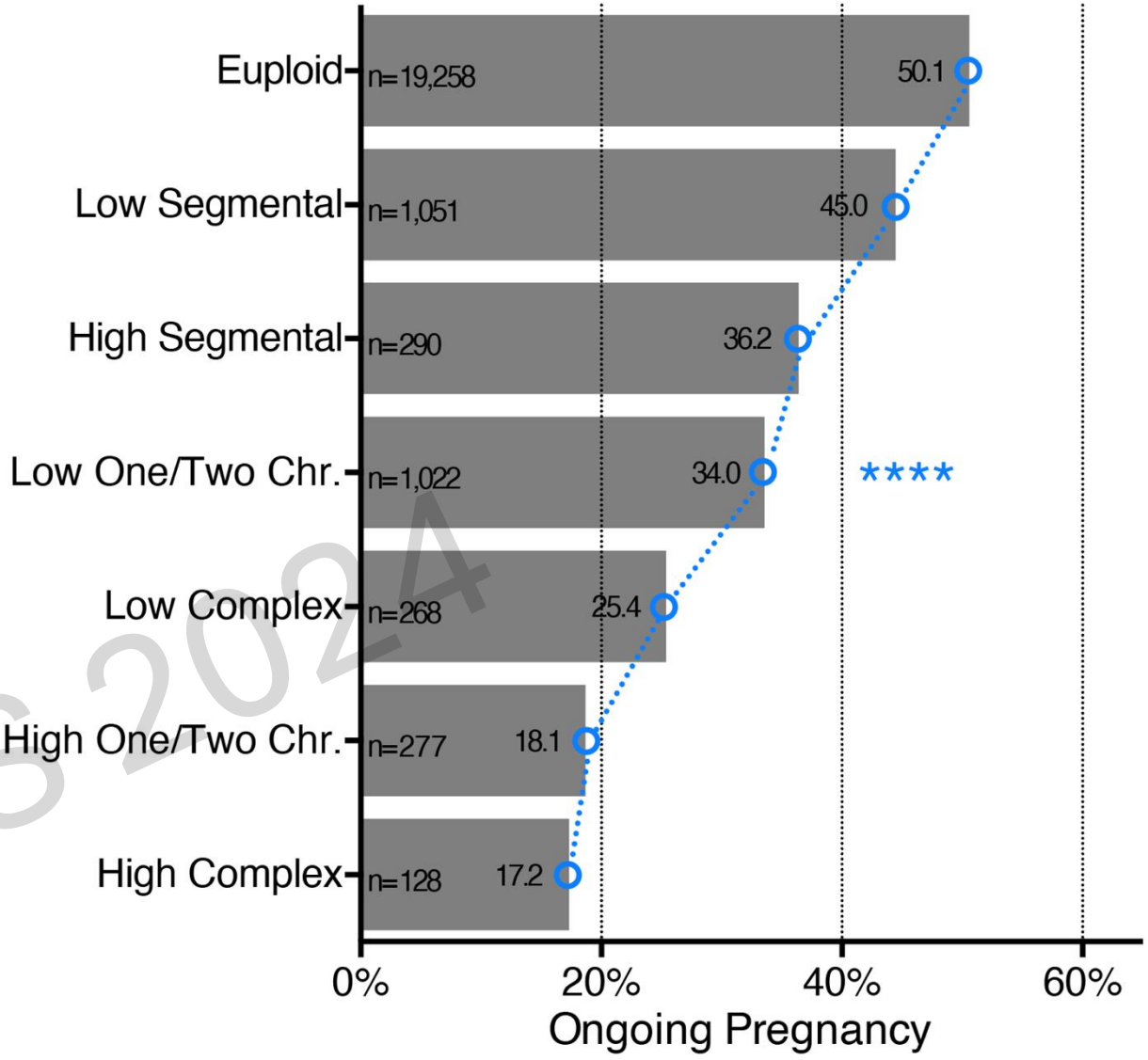


IRMET Update





IRMET Update



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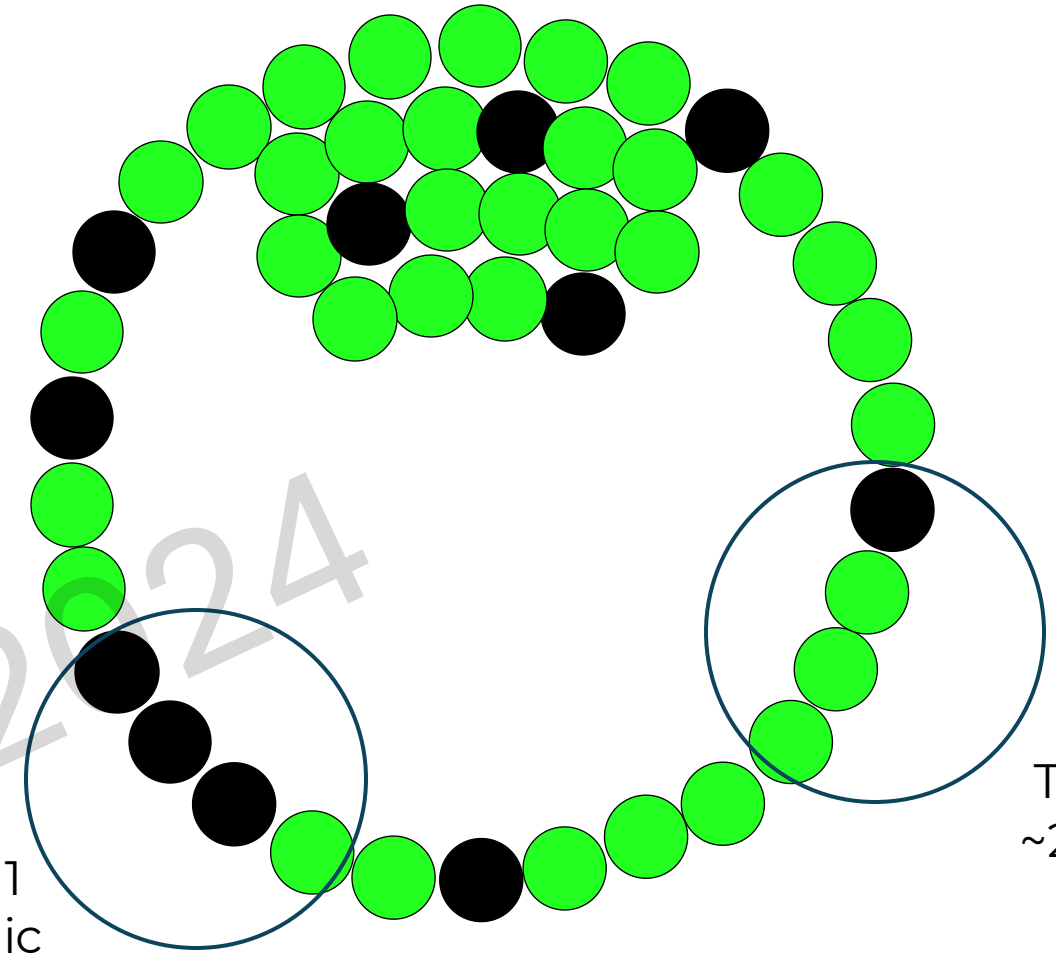
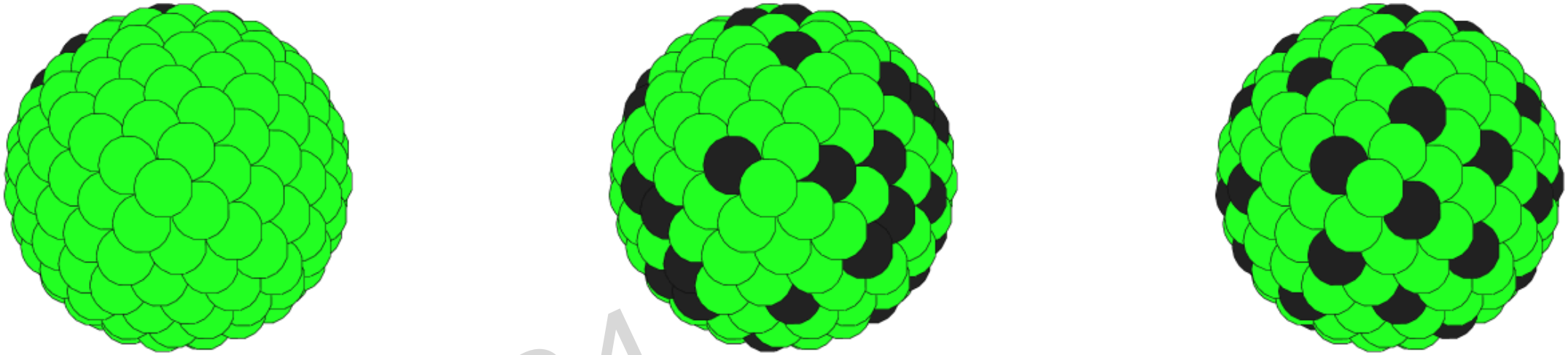
Biopsy Sampling Randomness



TE Biopsy 1
~75% Mosaic

TE Biopsy 2
~25% Mosaic

Virtual Mosaic Embryo



Two variables: the percent aneuploid cells, and the distribution

Aneuploid

Euploid

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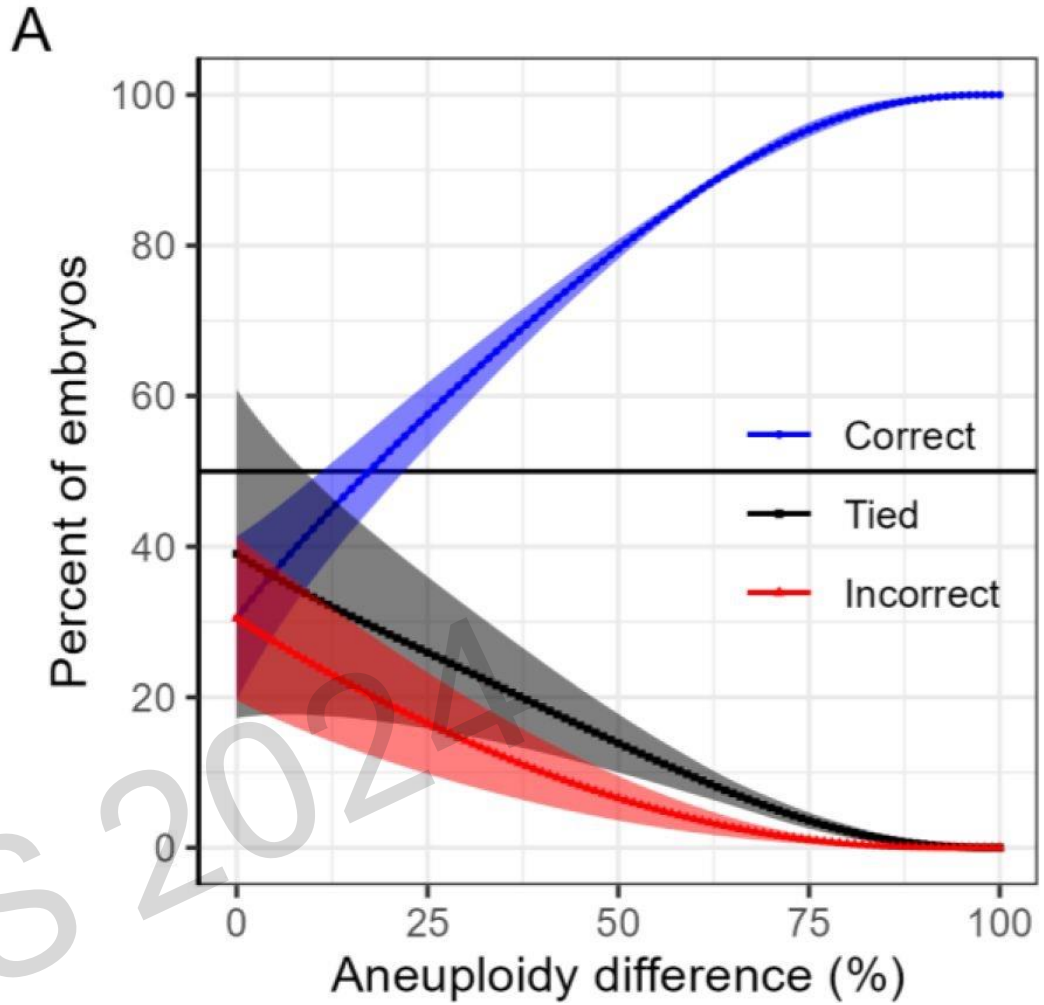
Virtual Mosaic Embryo



'…although the information contained in the biopsy is imperfect, even imperfect information is clinically useful.'

Skinner B, Viotti M, et al. eLife 2024

Acknowledgements

Sources of Artifactual Mosaicism

Kindlabs/Kindbody:

Louise Castillo, **Janan Shahin**, **Lilya Eid**

Christine Wechsberg, Dinura Gunatilake, Malik Chitty, Hiyam Shahin, Mariana Jimenez, Amber Cooper

Mosaic Modeling

University of Essex: Benjamin Skinner

Kent University: Darren Griffin, Peter Ellis

International Registry of Mosaic Embryo Transfers (IRMET)

Zouves Fertility Center: Andrea Victor (&RMALI), Frank Barnes, Christo Zouves

NYU Langone: Andria Besser, Jamie Grifo

Lee Fertility: En-Hui Cheng, Ching-Ya Su, Maw-Sheng Lee

Ospedale San Raffaele Milano: Laura Corti

Clinica Villa Mafalda: Ermanno Greco

Policlinico Città di Udine: Veronica Bianchi

Istanbul Memorial Hospital: Semra Kahraman, Murat Cetinkaya

NGC Moscow/St.Petersburg: Pavel Yakovlev, Nikolay Kornilov

Repromeda: David Kubicek, Miroslav Hornak, Katerina Vesela

Create Toronto: Svetlana Madjunkova, Mitko Madjunkov, Clifford Librach

Genea: Michael Bonifacio, Tamara Mossfield, Rebecca Dickson, Maria Traversa

Eurofins: Francesca Spinella, Anil Biricik

DASA: Juliana Gonçalves

SIMS IVF: Iulian Roman

Kent University: Darren Griffin

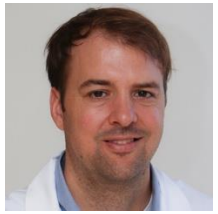
Kindlabs/Kindbody: Amber Cooper

UZ Brussels: Pieter Verdyck, Martine DeRycke

mosaicregistry@gmail.com

www.irmet.net

IRMET Board



Manuel Viotti

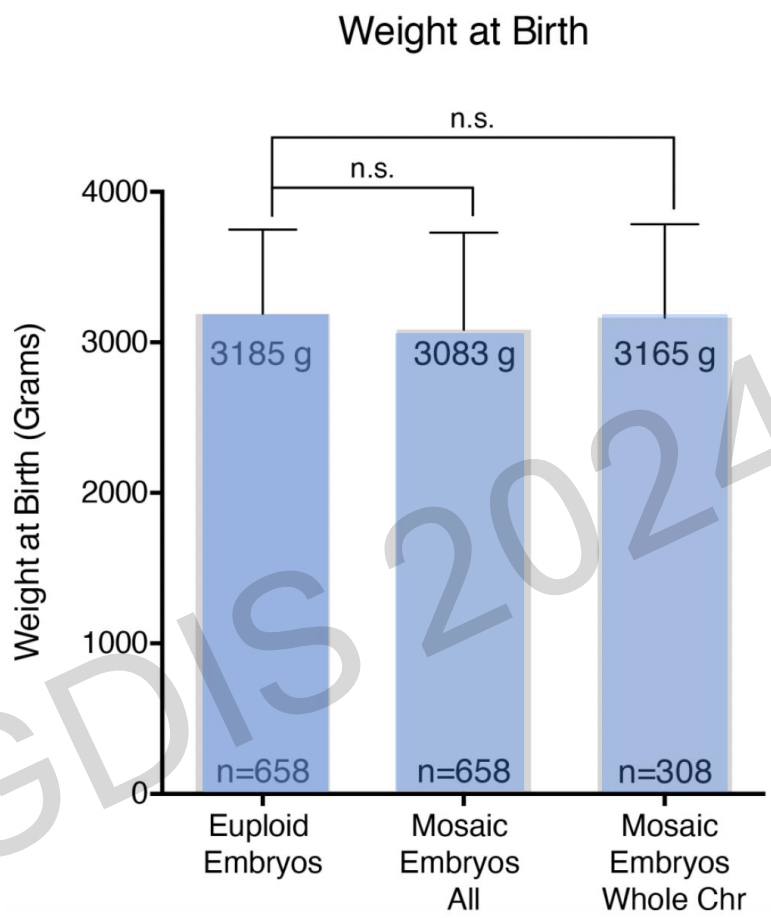
Francesca Spinella

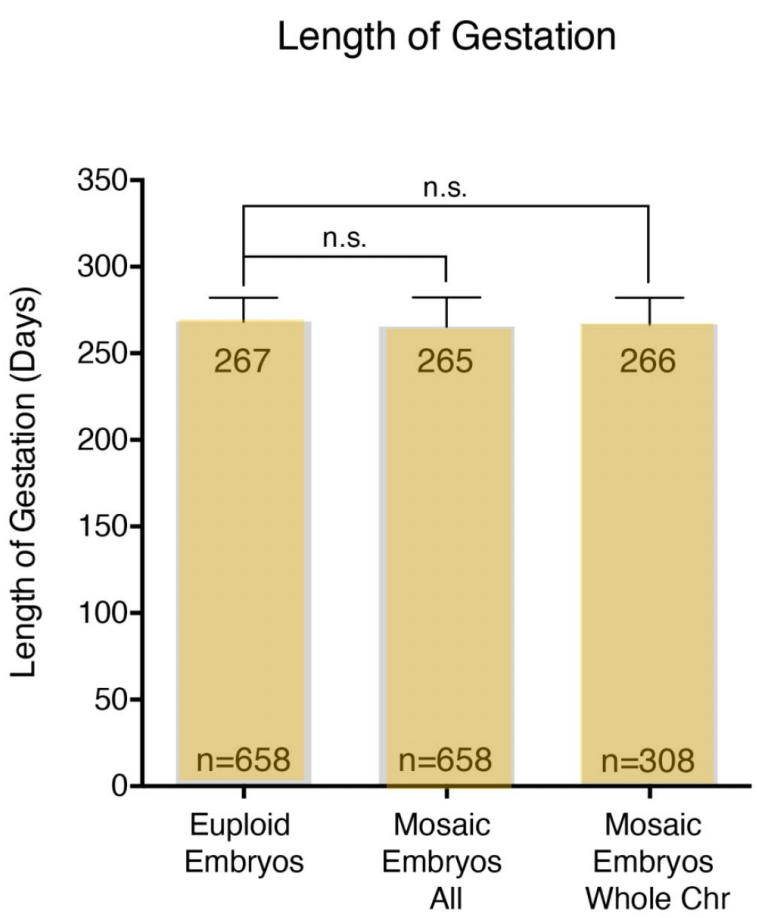
Svetlana Madjunkova

Andria Besser

IRMET Update

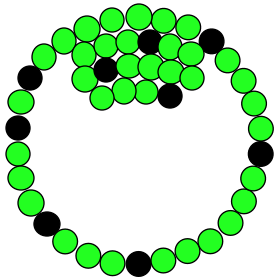
Newborn Data





IRMET Update

625 Mos. Embryo Transfer Pregnancies with Prenatal Testing



7 Cases

Mosaicism PGT-A = Mosaicism in Pregnancy

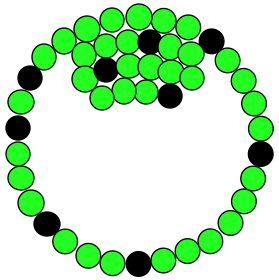
PGT-A	Prenatal Testing	POC	Ultrasound Abnormalities	Outcome
mos -2 (low level)	mos +2 (Amnio)	n/a [*mos -2 in postnatal]	No	Birth
mos +1q,-7,-8,+9,-19,-20,+21 (low level)	mos +21 (CVS+Amnio)	n/a	Yes	Terminated
mos -1p36.33p31.1 (low level)	mos -1p36.33p31.1 (Amnio)	mos -1p36.33p31.1	No	Terminated
mos +21 (low level)	mos +21 (CVS+NIPT)	mos +21	Yes	Terminated
mos +15 (high level)	mos +15 (NIPT)	mos +15 (placenta)	Yes	Terminated
mos +17 (low level)	n/a	mos +17	Yes	Miscarriage
mos +4q32.3q34.3,-Xq27.3q28 (low level)	mos +4q32.3q34.3 (CVS)	n/a	No	Birth

IRMET Update

625 Mos Embryo Transfer Pregnancies with Prenatal Testing

~1.1% persistence of mosaicism

7 Cases



Mosaicism PGT-A = Mosaicism in Pregnancy

PGT-A	Prenatal Testing	POC	Ultrasound Abnormalities	Outcome
mos -2 (low level)	mos +2 (Amnio)	n/a pos		
mos +1q,-7,-8,+9,-19,-20,+21 (low level)	mos +21 (CVS+Amnio)	n/a		d
mos -1p36.33p31.1 (low level)	mos -1p36.33p31.1 (Amnio)	mos		d
mos +21 (low level)	mos +21 (CVS+NIPT)	mos +21	Yes	Terminated
mos +15 (high level)	mos +15 (NIPT)	mos +15 (placenta)	Yes	Terminated
mos +17 (low level)	n/a	mos +17	Yes	Miscarriage
mos +4q32.3q34.3,-Xq27.3q28 (low level)	mos +4q32.3q34.3 (CVS)	n/a	No	Birth

Low level mosaicism

Prenatal testing recommended
Possible strategy:
NIPT (make sure the Chr is tested)
+ Amniocentesis by microarray

Biological Mechanisms:
How does mosaicism arise?

- Altered recombination pattern
- Anaphase lag
- Cell cycle control breakdown
- Centriole dysregulation
- Chaotic divisions
- Chromosome loss
- Chromosome gain
- Chromothripsis
- Cohesin depletion
- Cohesion exhaustion
- Embryo correction
- Endoreplication
- Insufficient crossover maturation
- Inter-chromosomal effect
- Mitotic non-disjunction
- Precocious sister chromatid/dyad separation
- Reverse segregation
- Trisomy rescue
- Weakened centromere cohesion
- Etc, etc, etc……

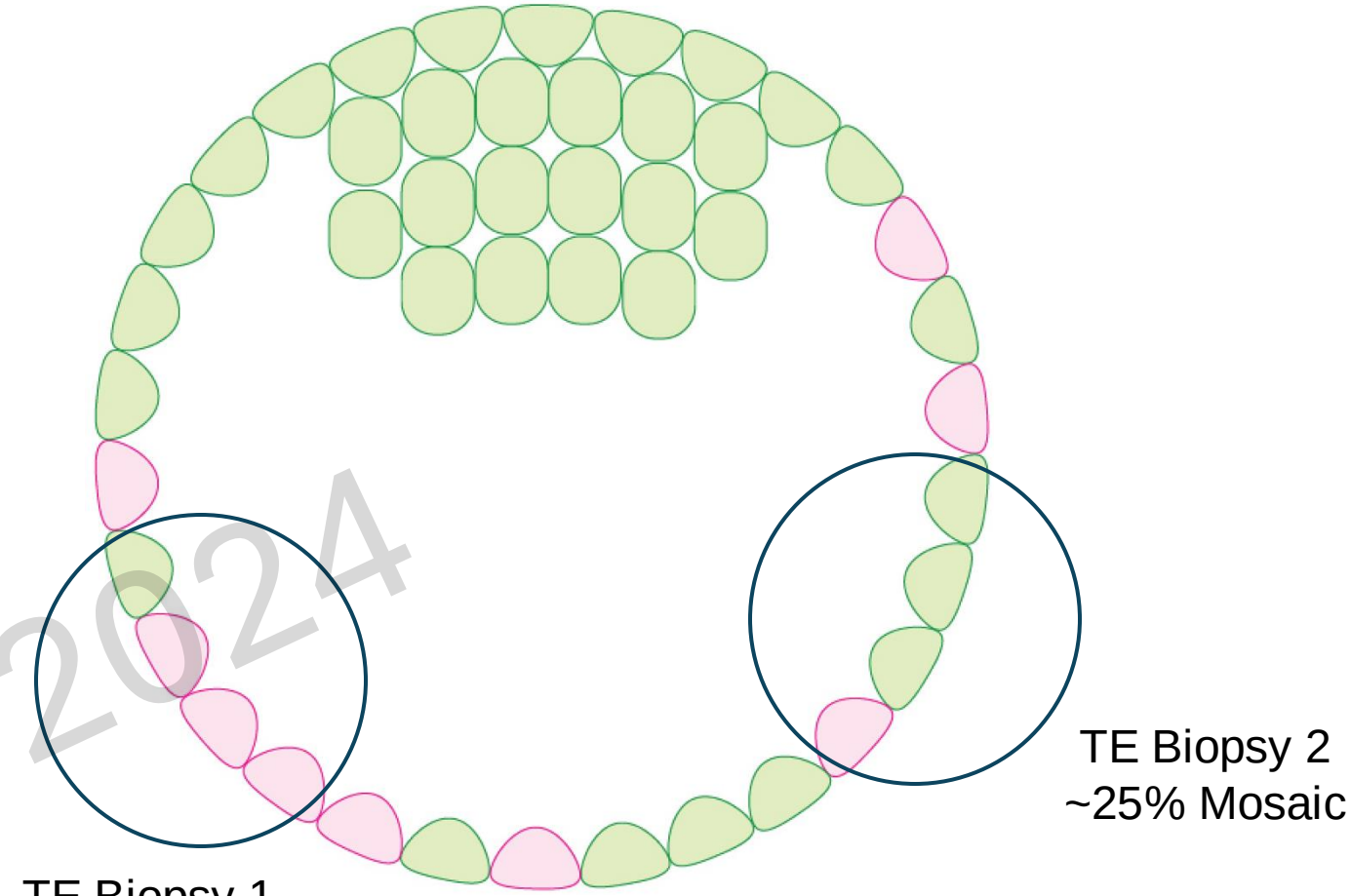
See paper I am reviewing for RBMO (review of mosaicism) has good references to paper talking about what could create mosaicism (technical in the lab etc)

in several studies showing that morphokinetic timing of mosaic embryos fit neither euploid nor aneuploid morphokinetic categories but may overlap with that of euploid and aneuploid embryos (Martin A et l.,. Fertil Steril., 2021). Another s

Also Rajiv and handyside

‘The human embryo is chromosomally complex’ griffin, brezina, etc

Biopsy Sampling Randomness



ICSI vs IVF: Effects on Mosaicism

Journal of Assisted Reproduction and Genetics (2019) 36:153–157
https://doi.org/10.1007/s10815-018-1347-6

GENETICS

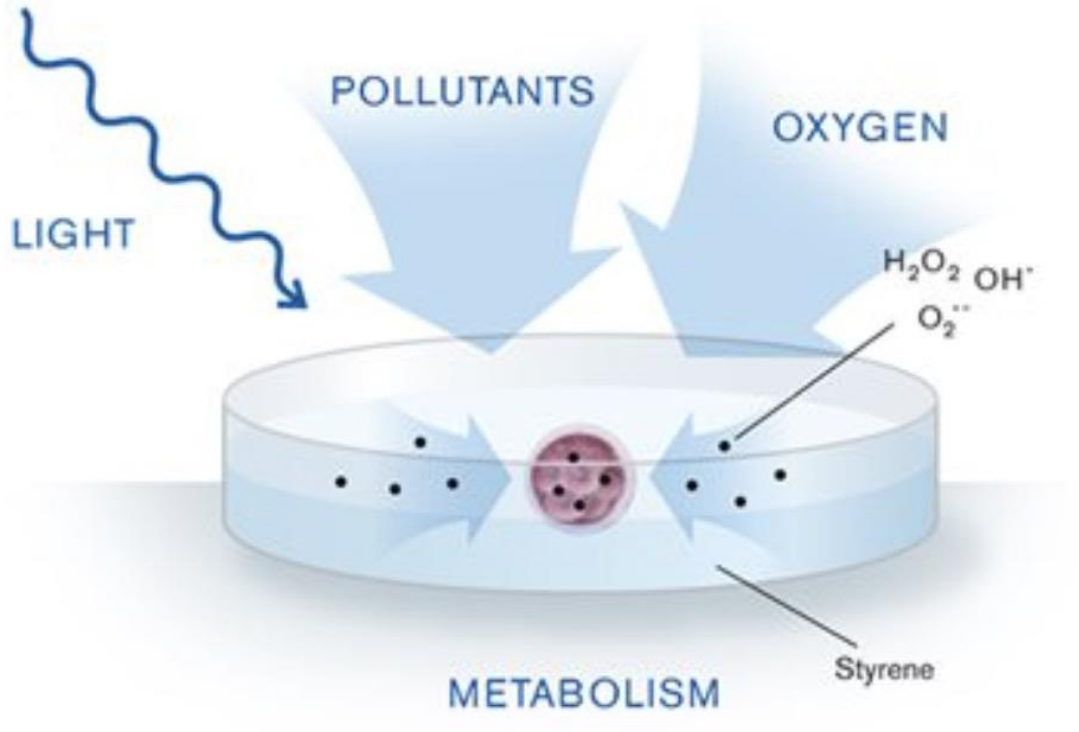
Minimizing mosaicism: assessing the impact of fertilization method on rate of mosaicism after next-generation sequencing (NGS) preimplantation genetic testing for aneuploidy (PGT-A)

Katherine L. Palmerola[1,2] · Sally F. Vitez[2] · Selma Amrane[1,2] · Catha P. Fischer[3] · Eric J. Forman[1]

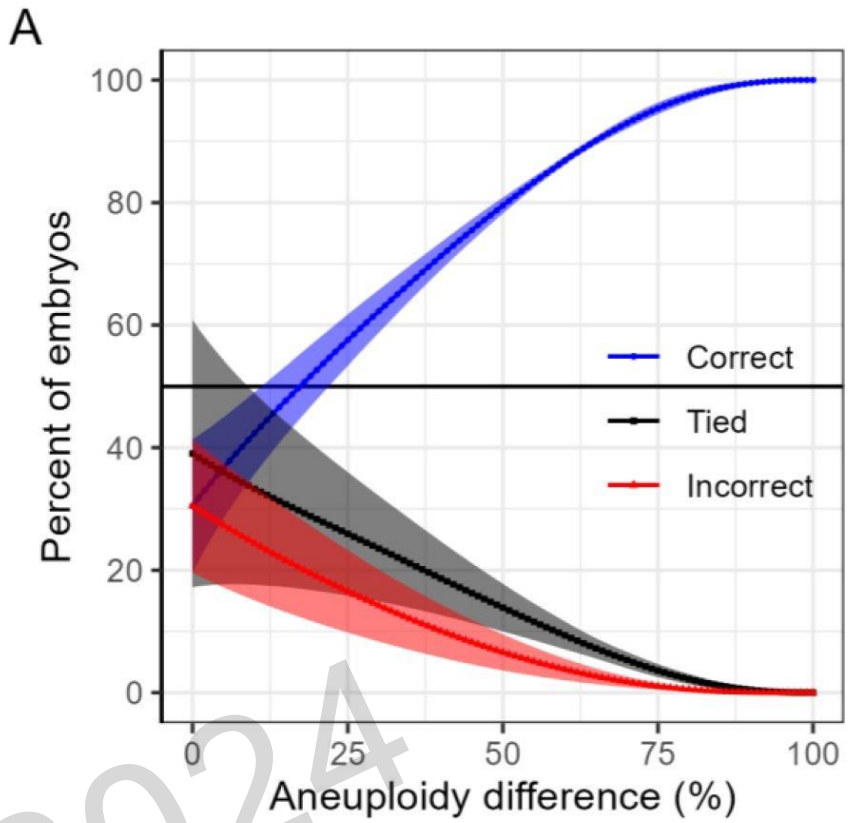
Primary outcome			
NGS PGT-A diagnosis	Conventional insemination (251 blastocysts)	ICSI (724 blastocysts)	*p* value*
Euploid	70 (27.9)	217 (30.0)	0.59
Aneuploid	104 (45.4)	312 (43.1)	0.70
Mosaic	65 (25.9)	151 (20.9)	0.12
No result	11 (4.4)	45 (6.2)	0.36

Variability

- Embryo-to-embryo
- Patient-to-patient
- Clinic-to-clinic



www.vitrolife.com



Skinner B, Viotti M, et al. eLife 2024

If we are trying to rank two embryos using biopsies, there are three possibilities. We get the order right, we get the order wrong, or there is a tie.
The greater the real difference in aneuploidy between the two embryos, the more likely we are to rank them correctly (that's the blue line in panel A).

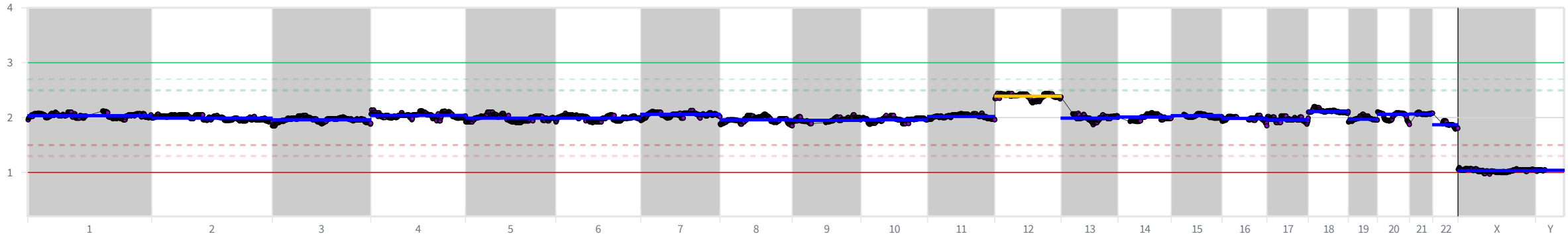
What happens when there is very little difference in aneuploidy between embryos? According to A, we will get it right less than half the time, because there are many ties.
But where we have a tie and no other embryo characteristics to use in preference, we are flipping a coin to rank the embryos. Half the ties will be correct ranking, half incorrect ranking.
If we distribute half the ties to 'correct' and half to 'incorrect', we get panel B.
This shows that as long as there is some difference in aneuploidy between the embryos, we are more likely than chance to rank the embryos correctly.

The biopsies are no longer *accurate*; they do not reflect the true level of aneuploidy in the embryo. However, they still correctly *rank* the embryos from less aneuploid to more aneuploid. Selecting the embryo with the lowest number of aneuploid cells in the biopsy for transfer is still the most sensible decision.

This allowed us to resolve the paradoxical utility of trophectoderm biopsy for PGT-A via a simple maxim: ***although the information contained in the biopsy is highly imperfect, even imperfect information is clinically useful***.

Skinner, Viotti, et al. eLife 2024

66-0224KL13RH-3_S66



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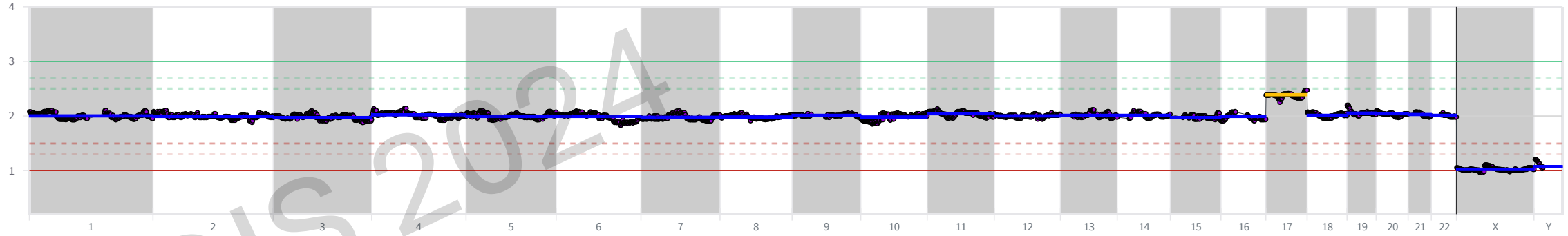

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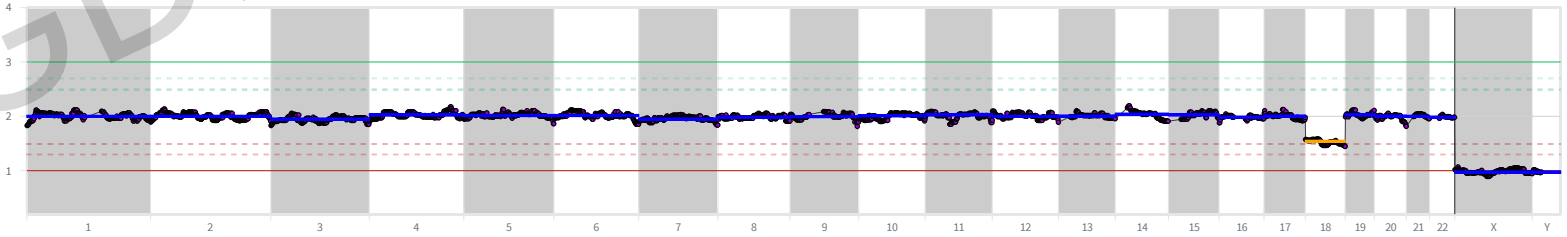

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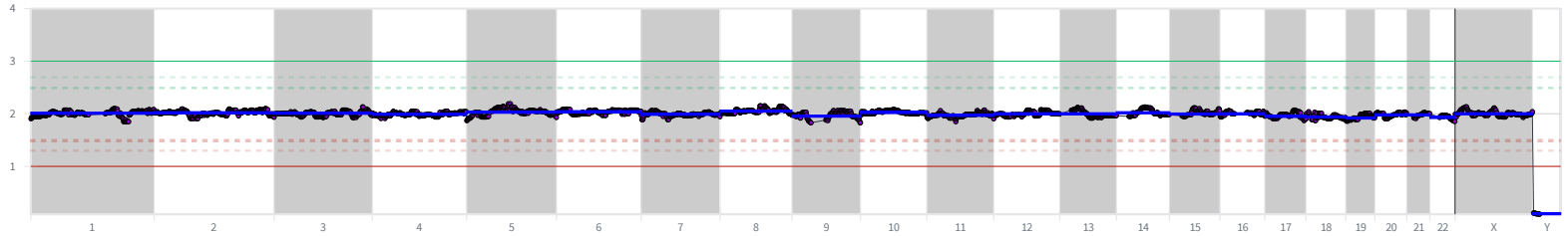

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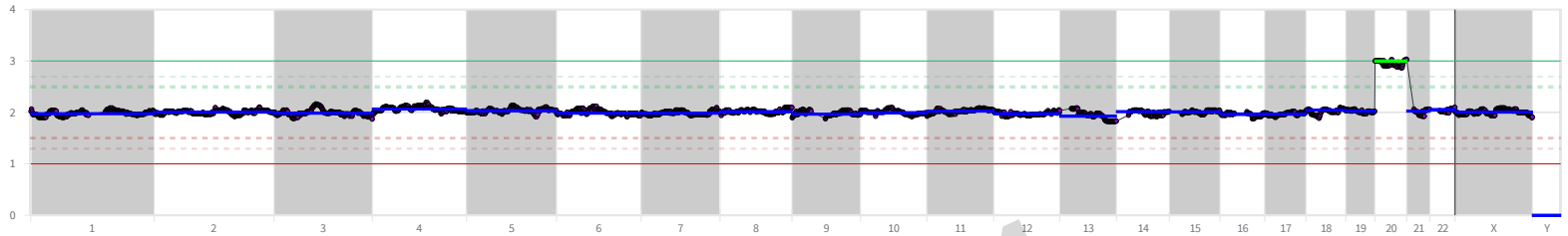

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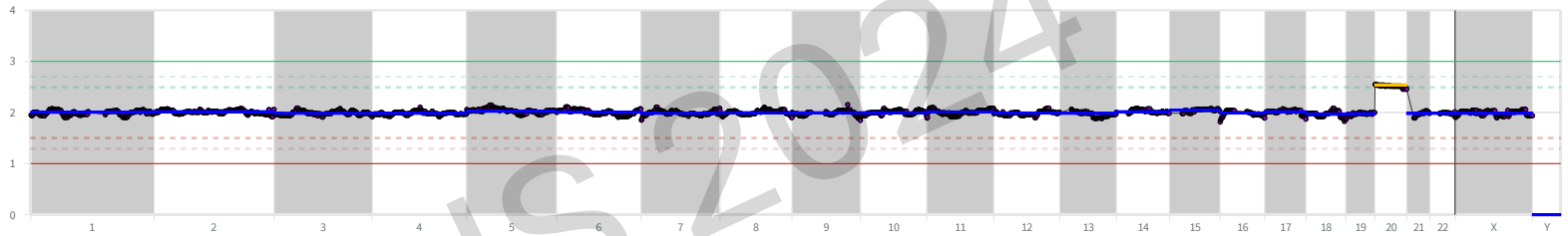

Y-axis re-scaled

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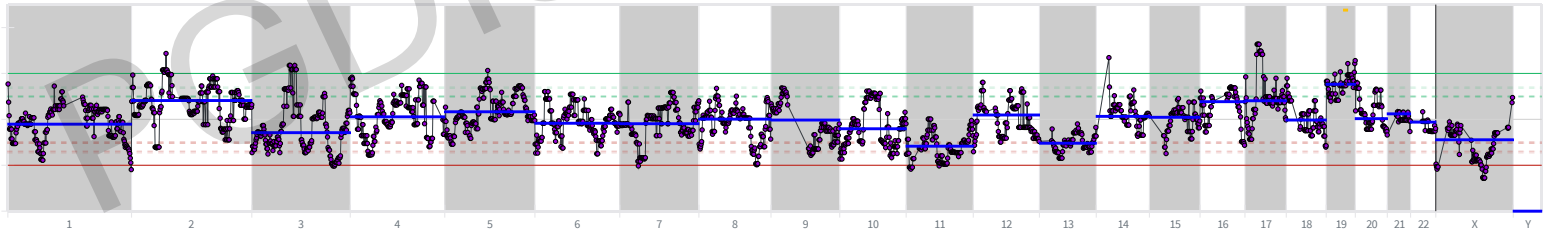

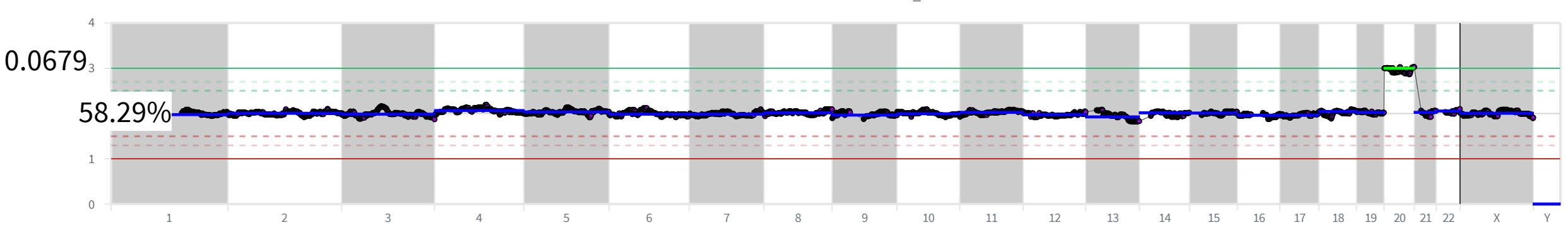

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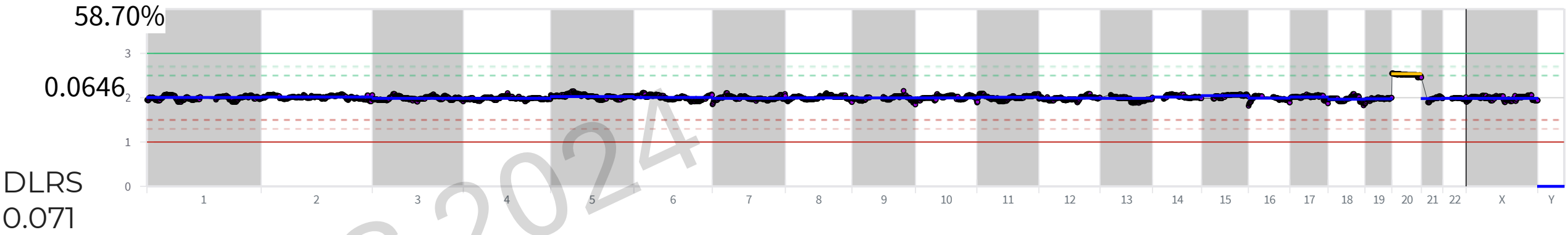

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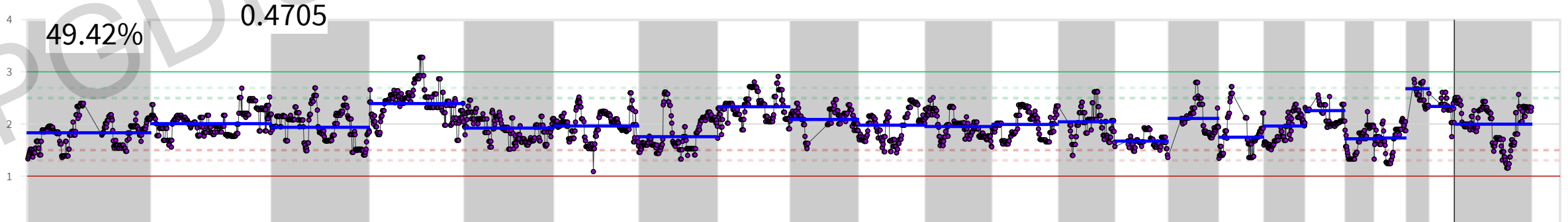
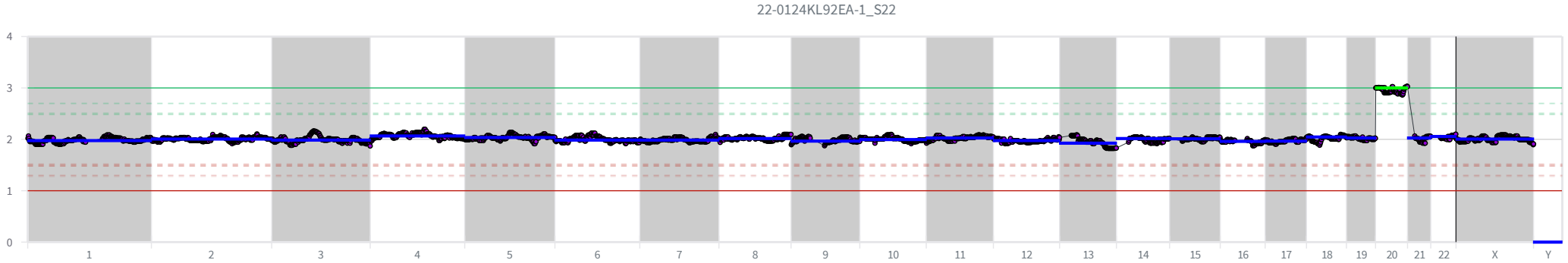


Quantifying Noise in Clinical Biopsies

DLRS
0.760



DLRS
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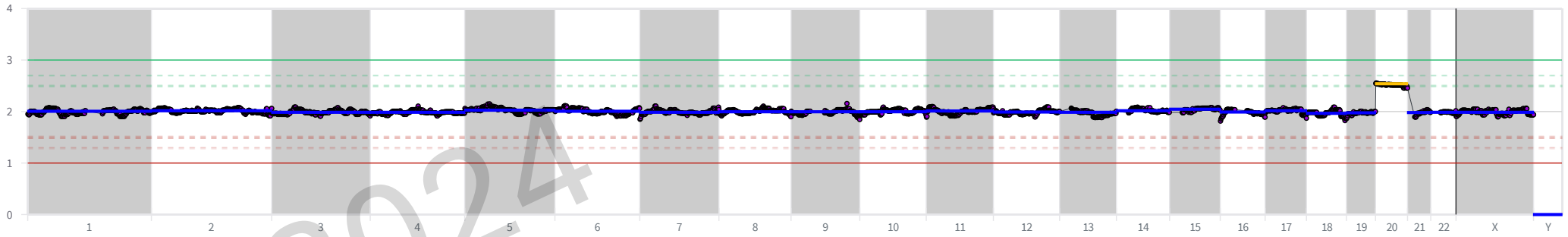
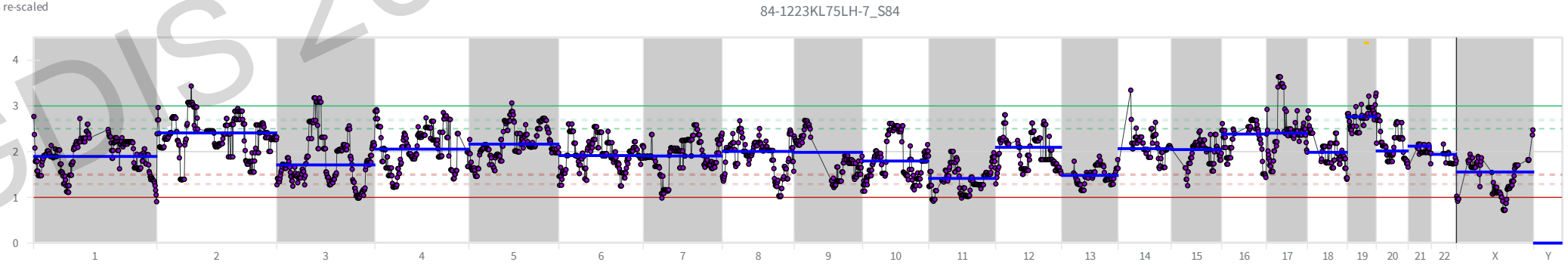
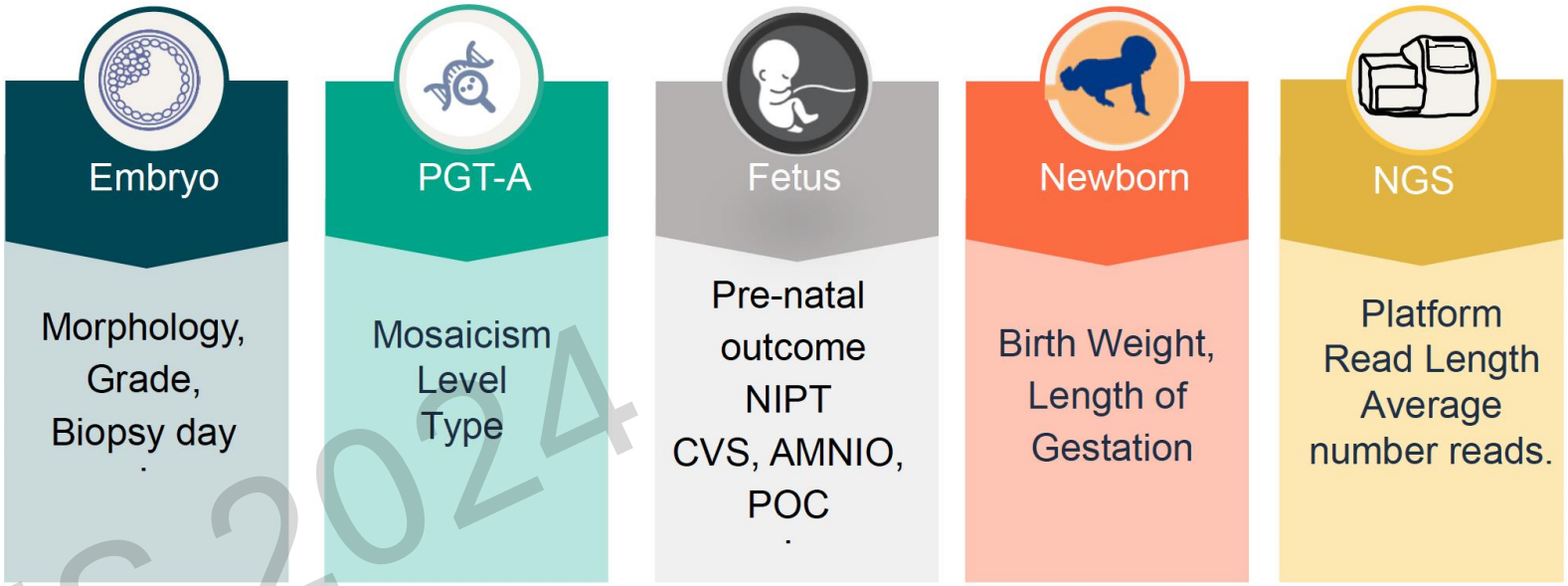
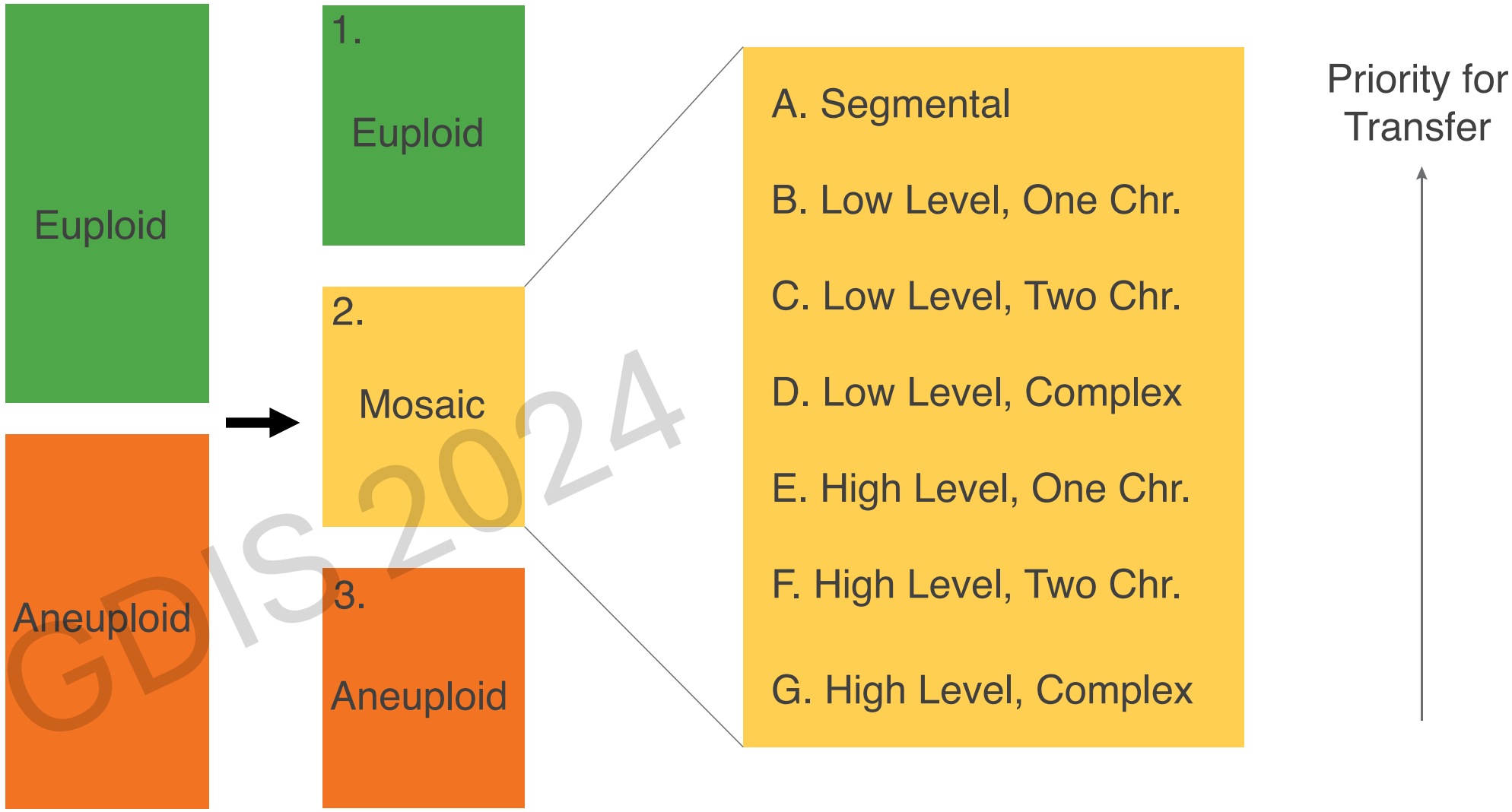


Y-axis re-scaled




Embryo
Morphology,
Grade,
Biopsy day
PGT-A
Mosaicism
Level
Type
Fetus
Pre-natal
outcome
NIPT
CVS, AMNIO,
POC
Newborn
Birth Weight,
Length of
Gestation
NGS
Platform
Read Length
Average
number reads.

Embryo Ranking System



Identifying Mosaic Embryos:

Artifactual mosaic results

Human Reproduction Open, pp. 1–18, 2022
https://doi.org/10.1093/hropen/hoac044

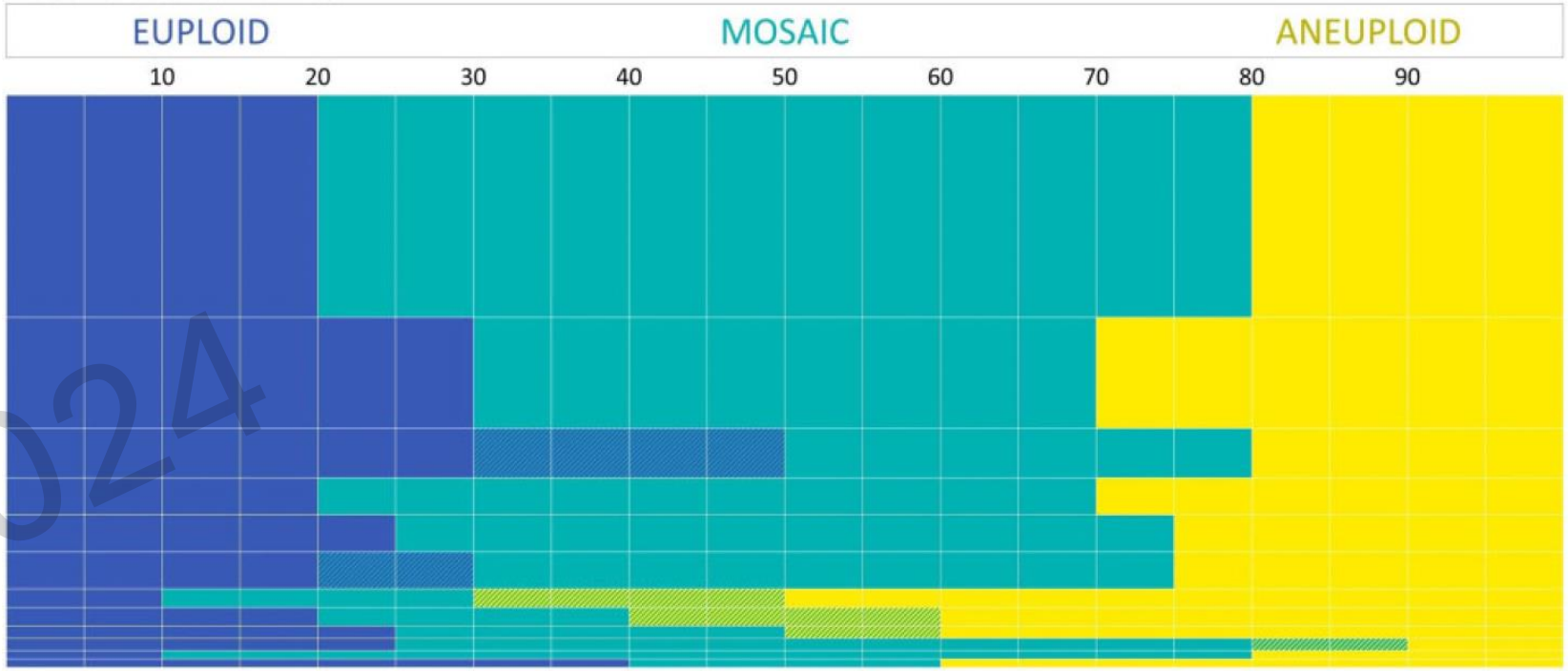
human reproduction open | ESHRE PAGES

ESHRE survey results and good practice recommendations on managing chromosomal mosaicism†

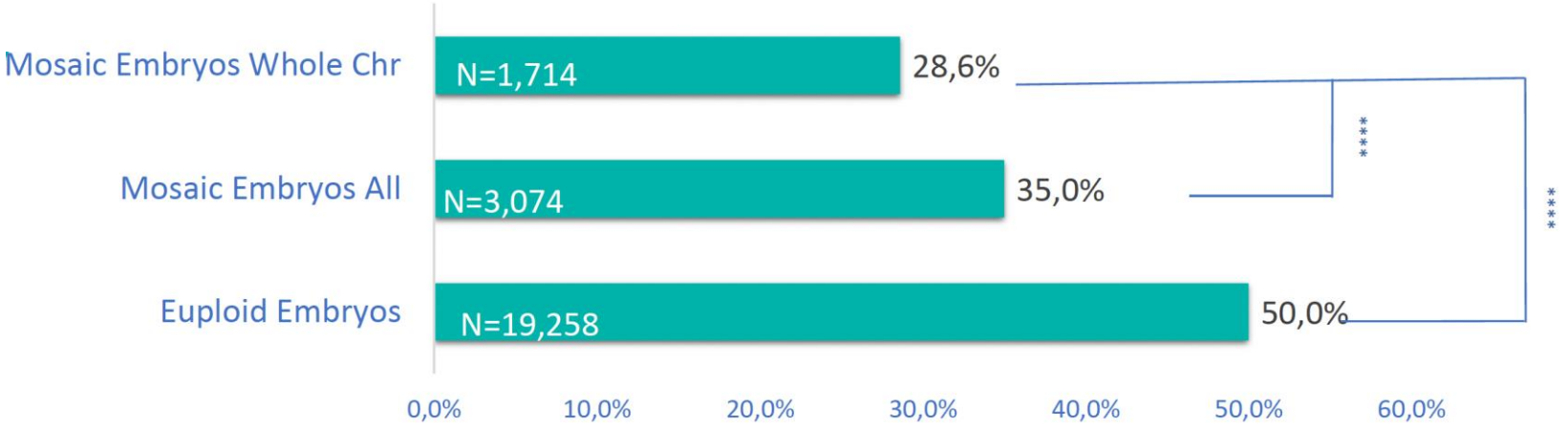
ESHRE Working Group on Chromosomal Mosaicism, Martine De Rycke [1,*], Antonio Capalbo[2], Edith Coonen[3,4], Giovanni Coticchio [5], Francesco Fiorentino [6], Veerle Goossens [7], Saria Mcheik [7], Carmen Rubio[8], Karen Sermon [9], Ioannis Sfontouris [10], Claudia Spits [9], Joris Robert Vermeesch [11], Nathalie Vermeulen [7], Dagan Wells[12,13], Filippo Zambelli,[14] and Georgia Kakourou[15]

[1]Centre for Medical Genetics, UZ Brussel, Reproduction and Genetics, Vrije Universiteit Brussel (VUB), Brussels, Belgium [2]JUNO Genetics, Rome, Italy [3]Departments of Clinical Genetics and Reproductive Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands [4]GROW School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands [5]9.baby, GeneraLife IVF, Bologna, Italy [6]Rome, Italy [7]ESHRE Central Office, Strombeek-Bever, Belgium [8]PGT-A Research Igenomix, Valencia, Spain [9]Research Group Reproduction and Genetics, Vrije Universiteit Brussel, Brussels, Belgium [10]Hygeia IVF Embryogenesis, Athens, Greece [11]Laboratory for Cytogenetics and Genome Research, Department of Human Genetics, KU Leuven, Leuven, Belgium [12]Nuffield Department of Women's & Reproductive Health, John Radcliffe Hospital, University of Oxford, Oxford, UK [13]Juno Genetics, Oxford, UK [14]Eugin Group, Barcelona, Spain [15]Laboratory of Medical Genetics, National & Kapodistrian University of Athens, Choremio Research Laboratory, "Aghia Sophia" Children's Hospital, 11527 Athens, Greece

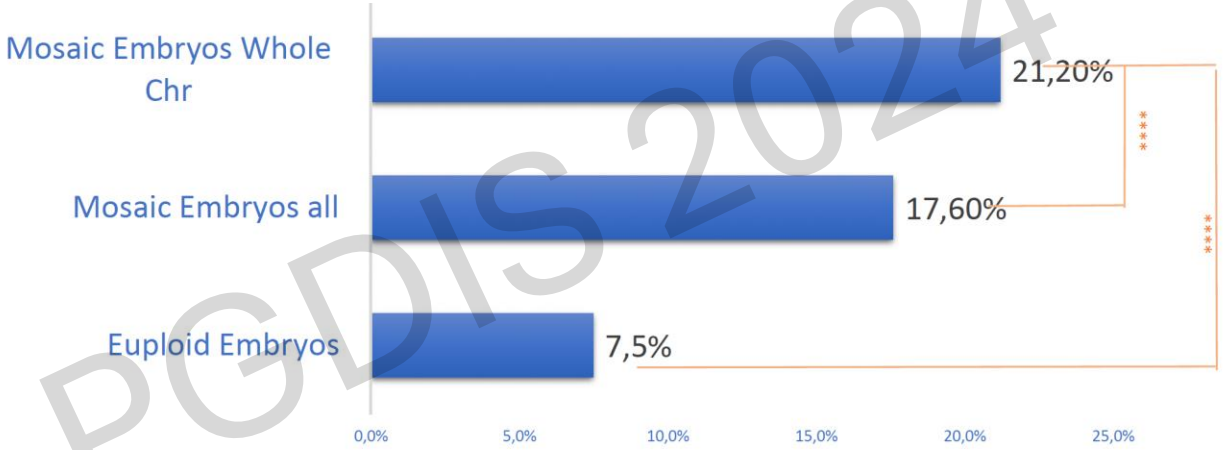
Range of mosaicism (% abnormal cells) considered diagnostically indicative of an aneuploid, euploid or mosaic embryo.



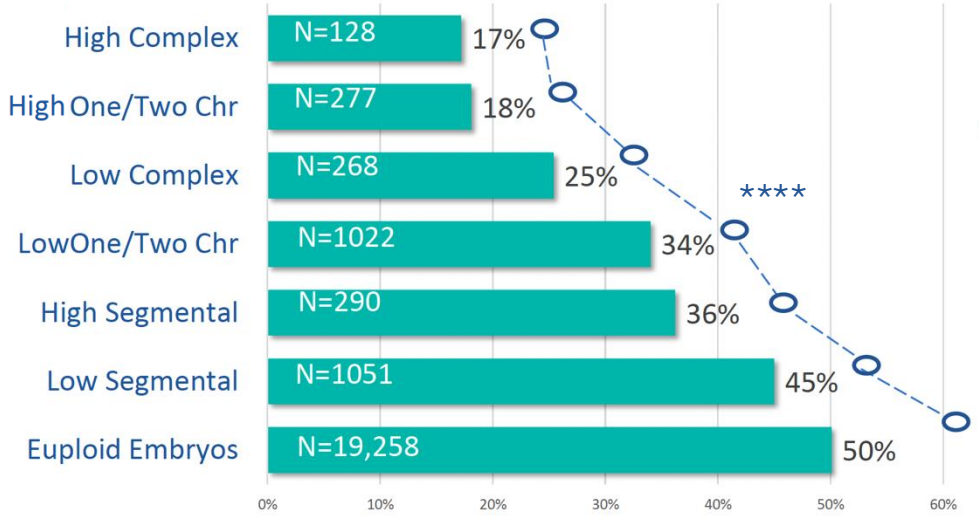
IRMET Update



Ongoing Pregnancy/Birth rate

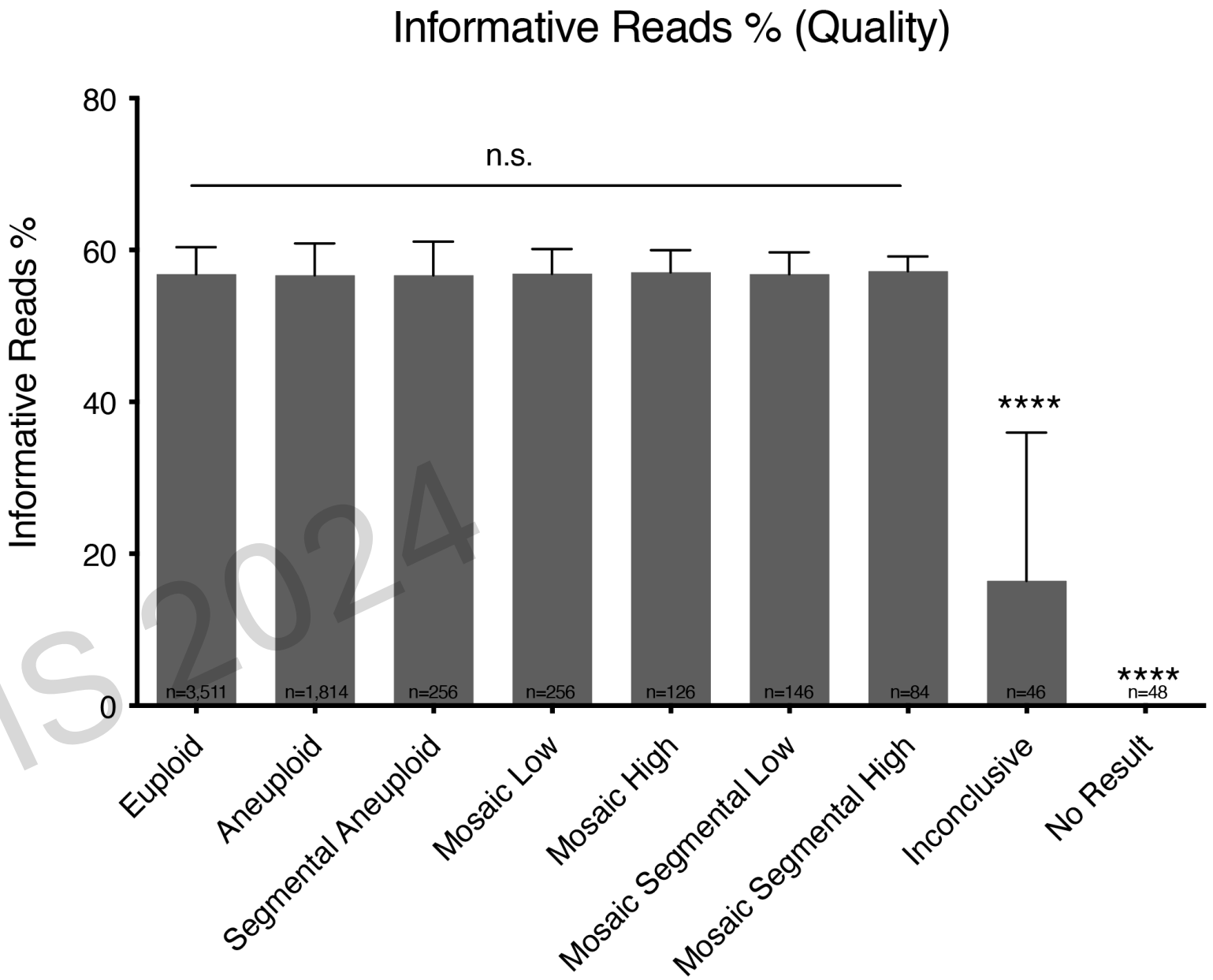


Spontaneous Abortion rate



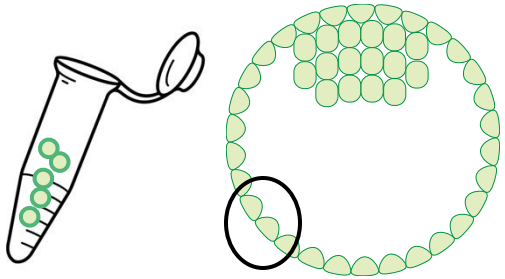
Ongoing Pregnancy rate

total TE biopsies tested n = 6,322

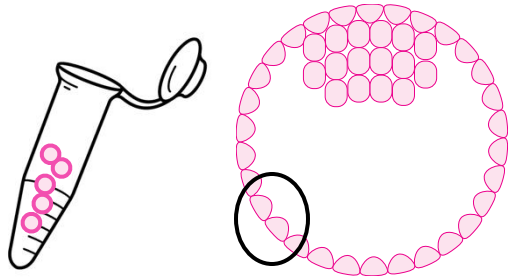


Identifying Mosaic Embryos:

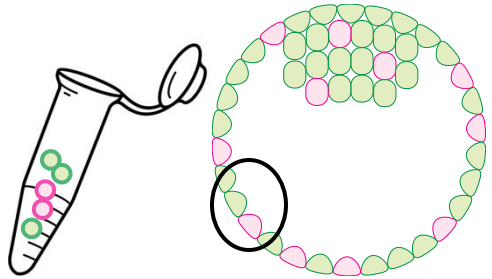
Artifactual mosaic results



Euploid



Aneuploid



Mosaic