21ST PGDIS CONFERENCE

PREIMPLANTATION GENETIC DIAGNOSIS INTERNATIONAL SOCIETY

PGDIS

6-8 May 2024

Kuala Lumpur

Malaysia

PGT and BEYOND...

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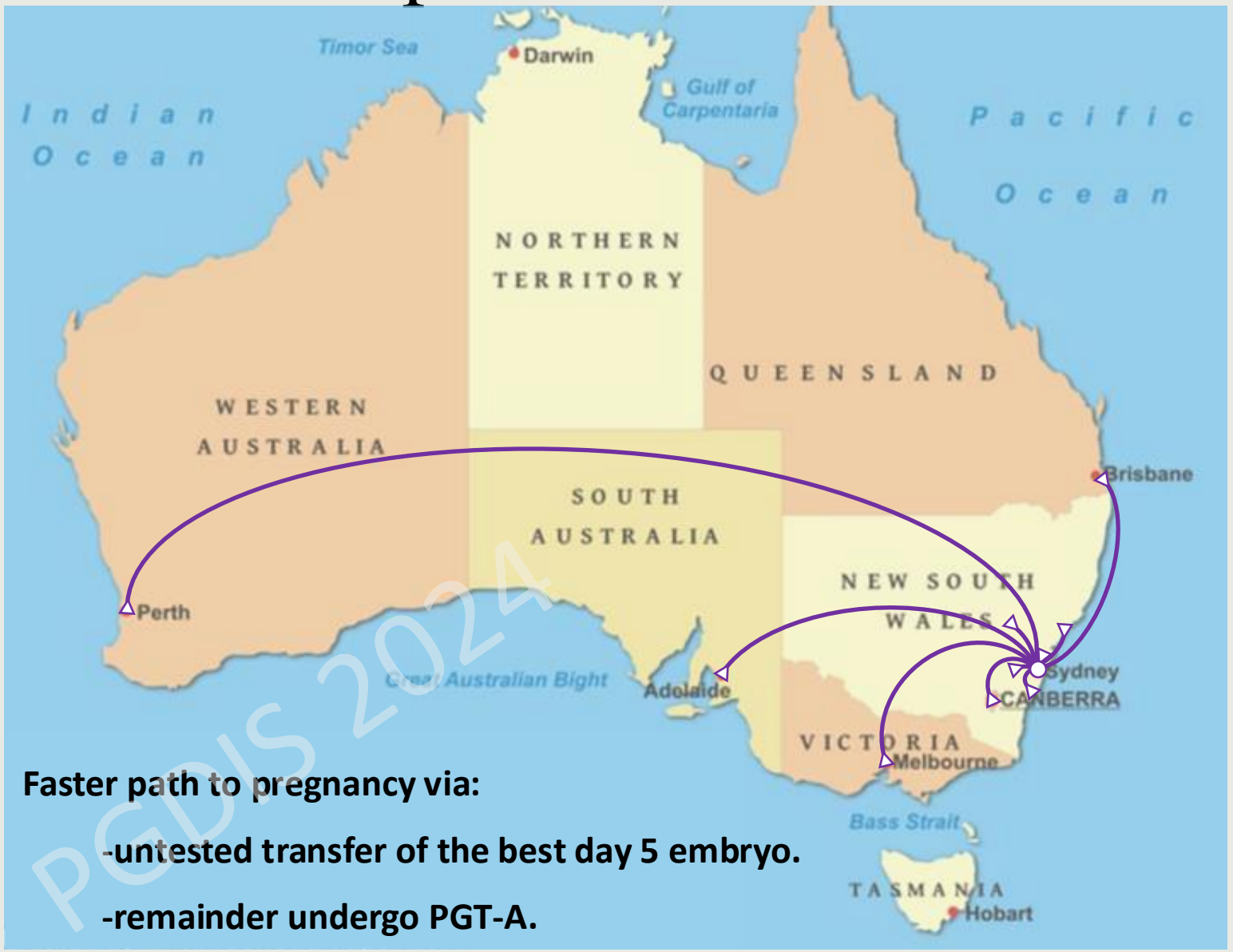
A pragmatic approach to mosaic embryo classification and the minimization of PGT-A false positives.

Steve Grkovic, Dean Morbeck

Part 1: Rapid evolution of mosaic ranking categories



Faster path to pregnancy via:

-untested transfer of the best day 5 embryo.

-remainder undergo PGT-A.

In-house PGT facilitates a close relationship between all stake holders, which has enabled:

- Use of outcome data to rapidly progress through several iterations of a mosaic ranking system.
- Full control over which mosaic details get reported.
- Clinical rebiopsies of suspected false positives.

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Evolving mosaic reporting system

June 2016 - June 2018

(Period A June 2016 – May 2018).

Earliest iteration, ranking derived from natural conception data, report all 20-<80%.

June 2018 - January 2022

(Period B July 2018 – June 2020).

Update based on the limited outcome data, literature and guidelines available. Report all mosaicism >40-<80%.

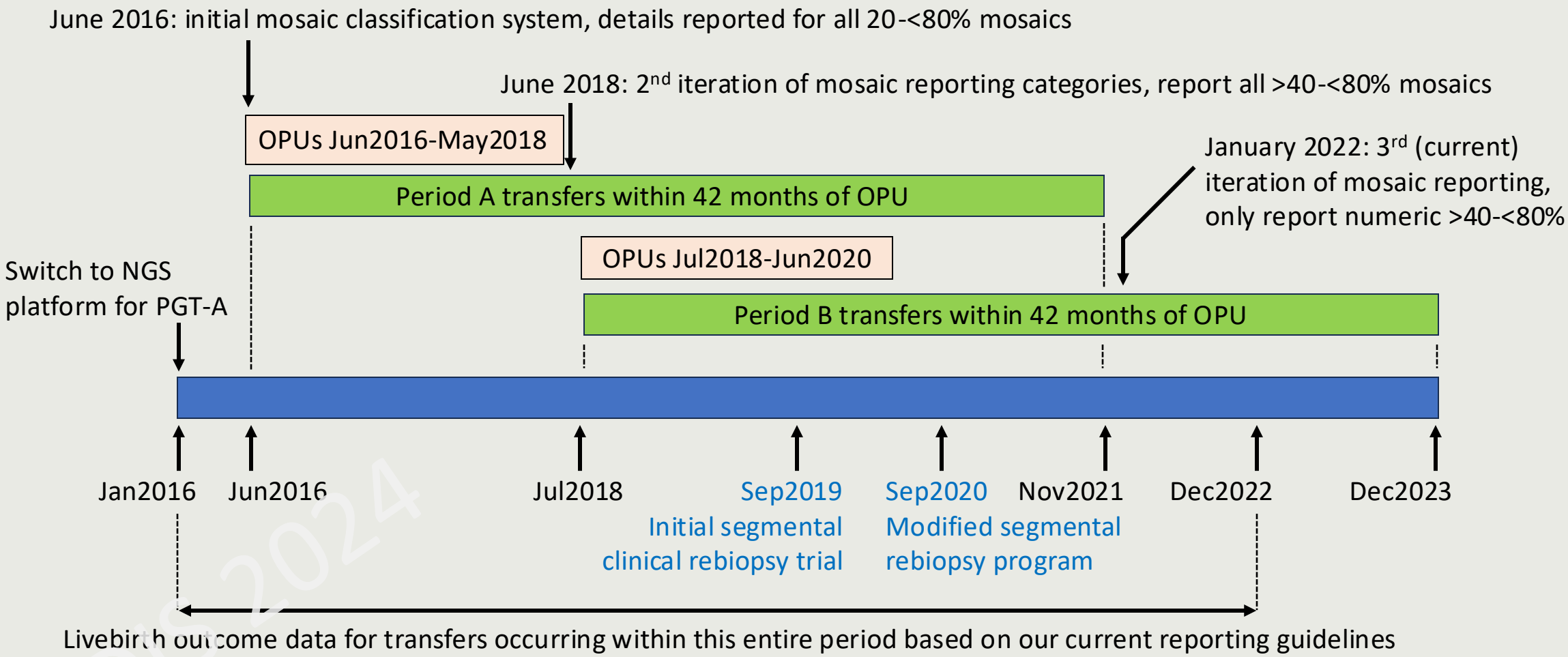
January 2022-Present

Simplified, based on larger cohorts of outcome data. Only reported mosaicism is numeric >40-<80%. Includes rebiopsy of suspected false positives.

Transfer category	Details (current reporting categories)
TR-A1	Clean NAD profiles.
TR-A2	Noisy NAD profiles.
TR-A3	1 or more segmental mosaics 20 - <80% (≥20 MB), mosaic details NOT reported.
TR-B	1 or more whole chromosome mosaics 20% - 40%, mosaic details NOT reported.
TR-C	1 or more whole chromosome mosaics >40% - <80%, mosaic details ARE reported. Mandatory genetic counselling.
PENDING	Suspected false positives requiring rebiopsy prior to transfer. Rebiopsy will classify as ABN, A3, B, or C.

Data throughout this presentation is organized according to our current categories

Study timeline and changes to mosaic reporting categories



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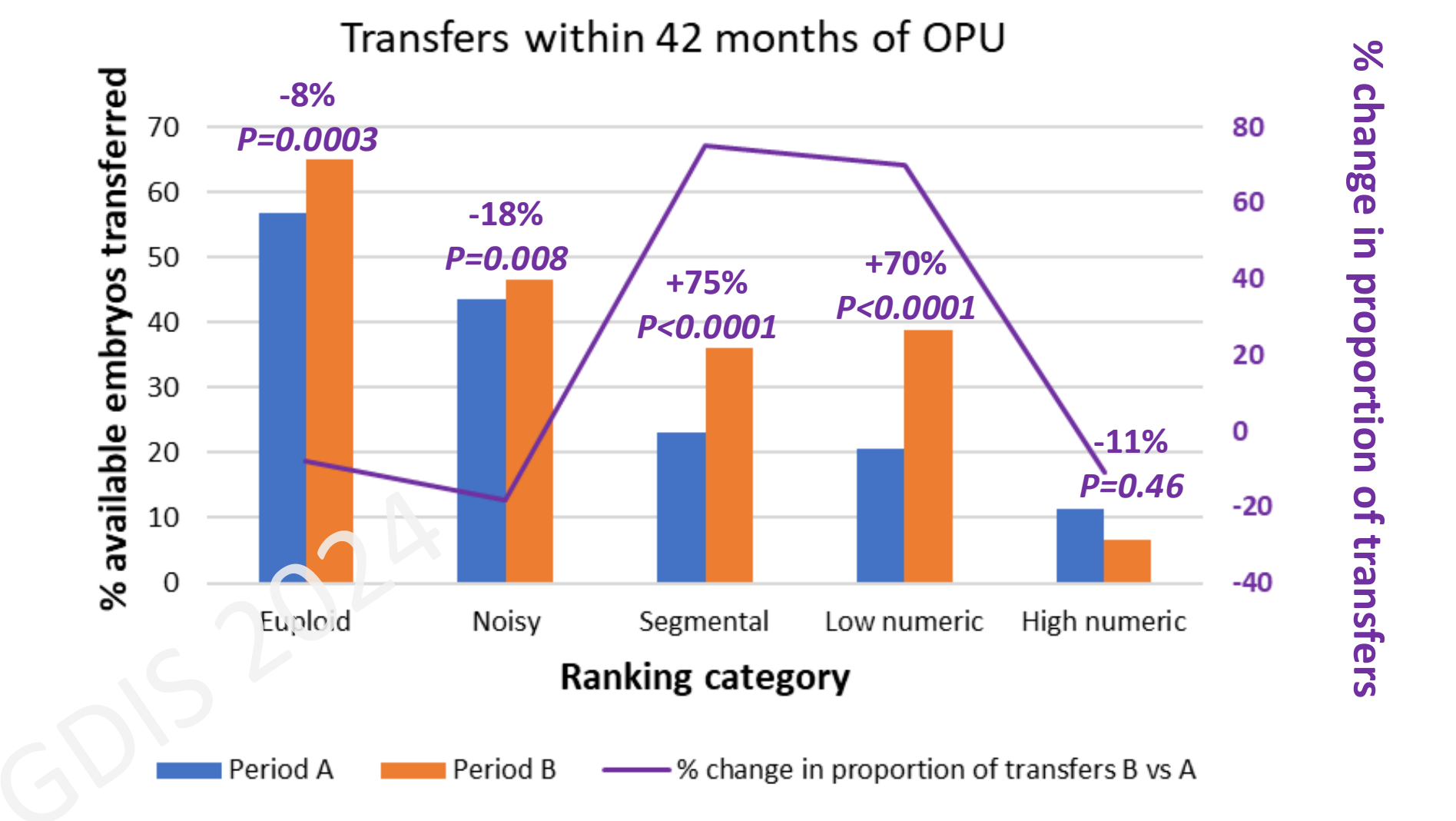
Percentage available embryos transferred within 42 months



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Outcomes based on current mosaic ranking system

Mosaic Category	Age at OPU	# ETed	bHCG	Fetal Heart	Live Birth *P* vs TR-A1	Miscarriage	
						Sub-Clinical	Clinical
TR-A1 NAD (euploid)	35.7	3970	2600 65.5%	2262 57.0%	2154 **54.3%**	13.0%	4.8%
TR-A2 Noisy	36.0	809	491 60.7%	410 50.7%	395 **48.8%** 0.005	16.5% *P* vs A1 0.038	3.7% *P* vs A1 0.32
TR-A3 20-<80% segmental	36.9	540	344 63.7%	295 54.6%	278* **51.5%** 0.23	14.2% *P* vs A1 0.52	5.8% *P* vs A1 0.46
TR-B 20-40% numeric	36.4	618	345 55.8%	278 45.0%	260& **42.1%** <0.0001	19.4% *P* vs A1 0.001	6.5% *P* vs A1 0.22
TR-C >40-<80% numeric	38.0	48	16 33.3%	12 25.0%	11 **22.9%** <0.0001	25.0% na	8.3% na

Fresh autologous oocytes only, SETs Jan 2016 – Dec 2022

*No significant difference between low- and high-level segmental mosaics

&*P* B vs C = 0.01 (low vs high-level numeric mosaics)

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Part 2: Minimization of PGT-A false positives

Sources of PGT-A false positives

Reconfirmation biopsies: ~ 1% of numeric abnormalities are false positives.

- A tiny fraction (0.3%) of all PGT-A tested embryos
- Follow up testing indicated they were high-level mosaics: Utilization and outcomes are poor

From a dataset of 14705 biopsies:

- 3.2% of embryos classified as ABN solely on the basis of segmental abnormalities.
- 144 (30.8%) gains (duplication)
- 291 (62.3%) losses (deletions)
- 32 (6.9%) both (gain + loss)

Considering their excellent outcomes, false positives arising from segmental mosaicism would be excellent candidates for clinical rebiopsy

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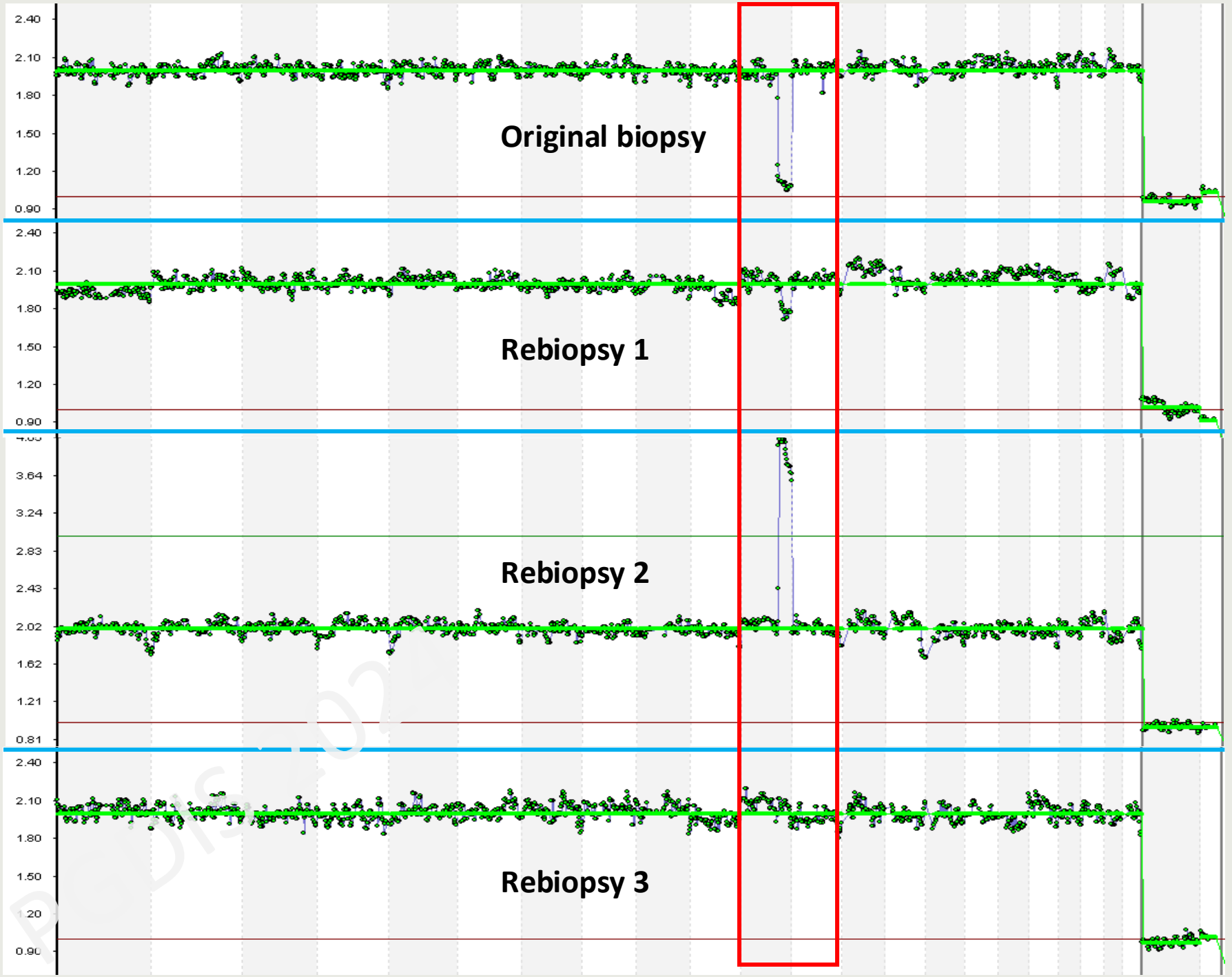

Segmental abnormalities

- Frequently not representative
- Often unstable

Grkovic et al. Challenges in interpreting the relevance of segmental mosaicism detected by NGS. 16th international conference on preimplantation genetics. Valencia, Spain; 2017

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Segmental mosaics: The principal source of false positives

- Victor AR, Tyndall JC, Brake AJ, Lepkowsky LT, Murphy AE, Griffin DK, et al. One hundred mosaic embryos transferred prospectively in a single clinic: exploring when and why they result in healthy pregnancies. Fertility and Sterility. 2019;111:280–93
- Navratil R, Horak J, Hornak M, Kubicek D, Balcova M, Tauwinklova G, et al. Concordance of various chromosomal errors among different parts of the embryo and the value of re-biopsy in embryos with segmental aneuploidies. Molecular Human Reproduction. 2020;26:269–76
- Girardi L, Serdarogullari M, Patassini C, Poli M, Fabiani M, Caroselli S, et al. Incidence, Origin, and Predictive Model for the Detection and Clinical Management of Segmental Aneuploidies in Human Embryos. American Journal of Human Genetics. 2020;106:525–34

Provided us with the confidence to address this issue in a clinical setting

Clinical rebiopsy program of suspected false positives

Patients pre-consent to rebiopsy of ambiguous results at initiation of treatment

All segmental abnormal results: Report as PENDING

PENDING embryo thawed, rebiopsied, refrozen, retested

Report an average of the two results

Euploid or mosaic for original or related abnormality => **Mosaic**

Segmental abnormality confirmed or did not survive=> **Abnormal**

Genea Human Research Ethics Committee approval #GEC0035

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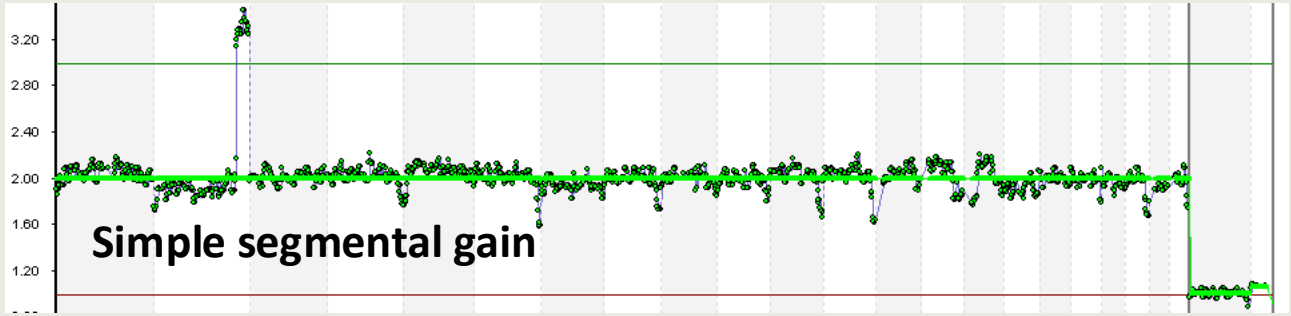
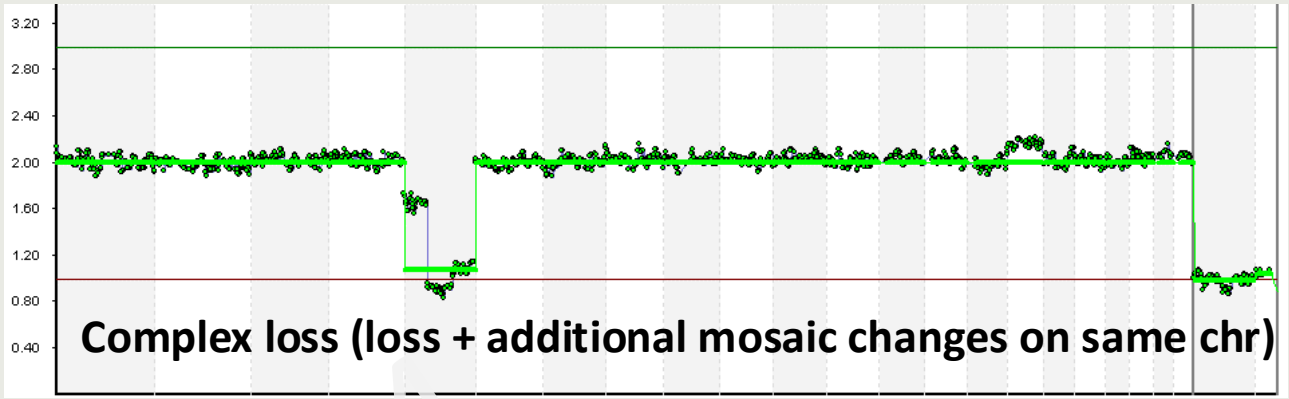
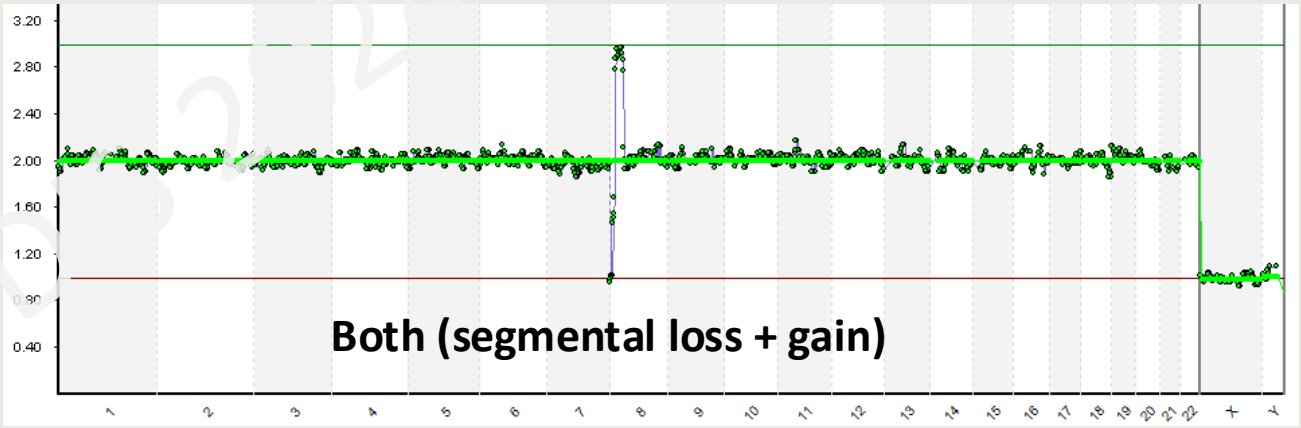
Categories of segmental results







Additional sub-classifications:

Full shift
= gain or loss of >0.95 copies

High-level mosaic shift
= gain or loss of ≥0.8 – ≤0.95 copies

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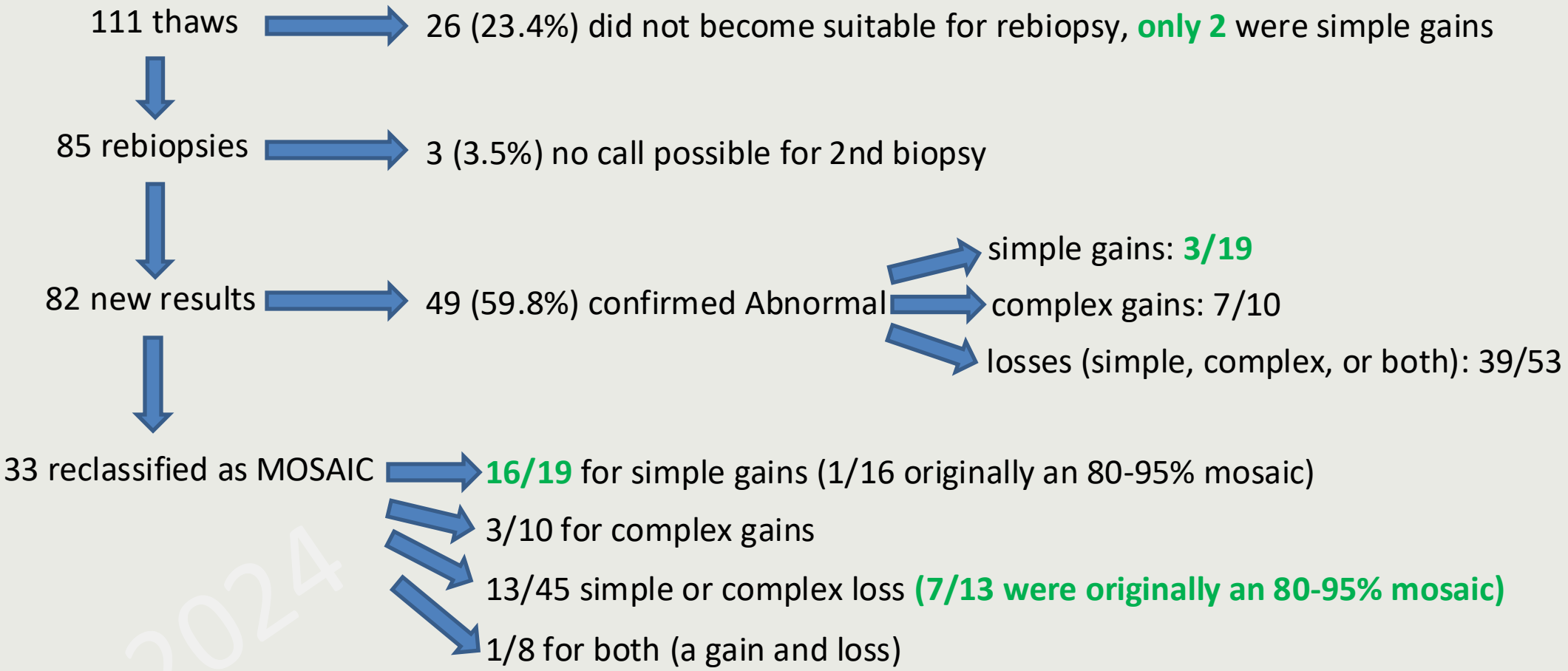
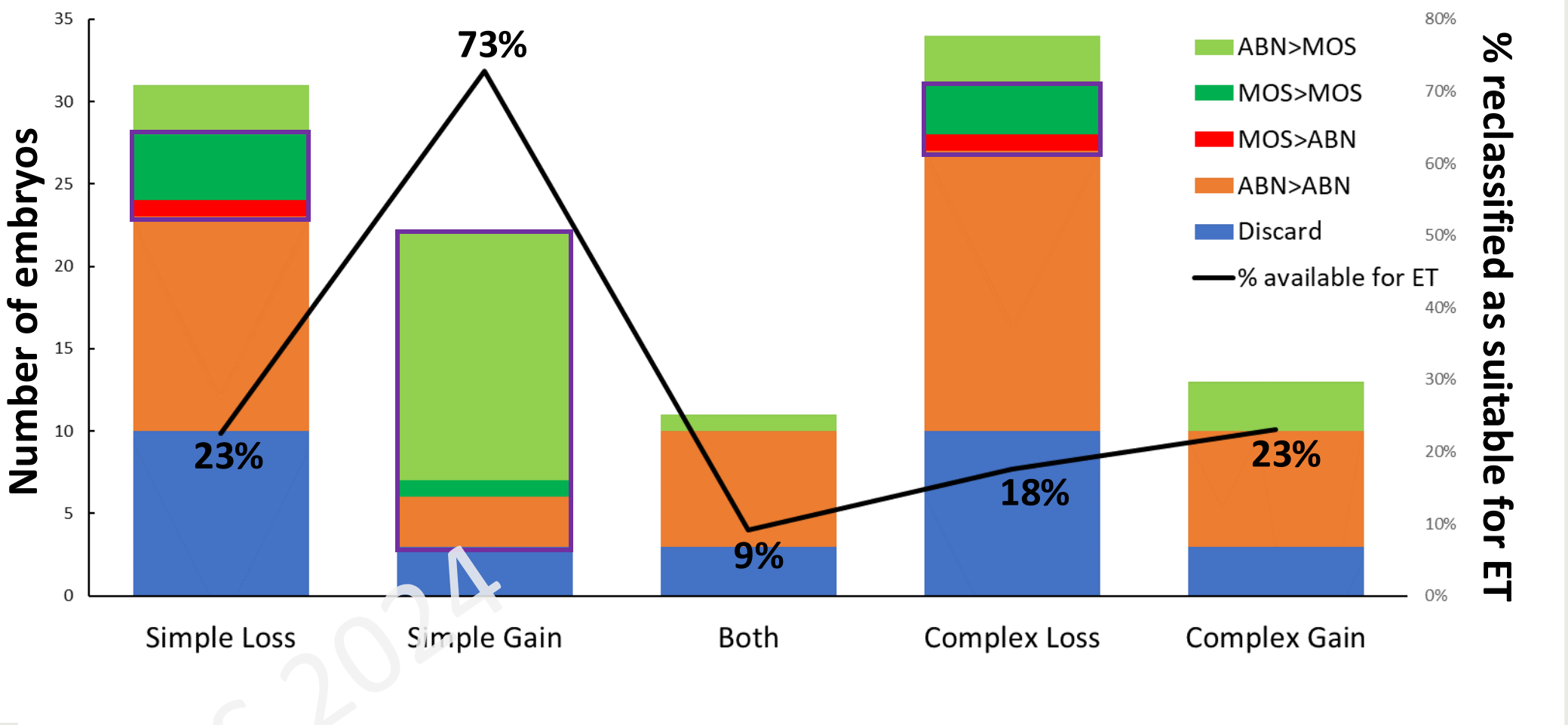
Initial segmental clinical rebiopsy trial



Most simple gains and many high-level mosaic losses were reclassified as mosaic

Grkovic et al. Journal of Assisted Reproduction and Genetics 2022;39:1313-22

Modification of PENDING rebiopsy protocol



Any 80-95% segmental shift:
80% reclassified as mosaic

Samples rebiopsied by modified protocol

- 70% of embryos reclassified as mosaic would be targeted by modified protocol
- 75% of remaining embryos confirmed abnormal or discarded
- Greatly improved cost/benefit: More sustainable protocol

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Results to date from modified rebiopsy protocol

	Simple gains (≥80%)	Losses (80-95% shifts)	Total, new protocol	Original protocol	Unknown rebiopsies
Number thawed (% of total thaws)	75 (73.5%)	27 (26.5%)	102	111	95
Did not become suitable for re-biopsy (% thaws)	4 (5.3%)	1 (3.7%)	**4.9%**	26.1%	5.3%
New result obtained (% thaws)	71 (94.7%)	26 (96.3%)	**95.1%**	73.9%	94.7%
ABN result (% of new results)	21 (29.6%)	11 (42.3%)	**33.0%**	62.2%	30%
Reclassified as suitable for ET (% of new results)	**50 (70.4%)**	**15 (57.7%)**	**67.0%**	**37.8%**	**70%**
Mosaic change related to original abnormality observed in reclassified embryo (% of reclassified)	7 (14.0%)	8 (53.3%)	23.1%	33.3%	na

- Simple gains: A lower % of changes related to original biopsy finding vs losses
  > Indicates a lower % of abnormal cells vs losses
- Yield of modified rebiopsy protocol equivalent to Unknown (failed) rebiopsies

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Livebirth outcomes of segmental vs unknown rebiopsies

	Segmental rebiopsies	Unknown rebiopsies	Euploid single biopsy
Available for transfer	137	63	-
% PGT-M or PGT-SR	5.8%	44.0%	-
Transferred	20 (14.6%)	38 (60.3%)	3970
bHCG positive	6 (30.0%)	16 (42.1%)	2600 (65.5%)
Fetal heart	5 (25%)	14 (36.8%)	2262 (57.0%)
Livebirths/ongoing	**5 (25%)**	**14 (36.8%)**	**2154 (54.3%)**

Rebiopsies are 2nd choice for transfer due to the ~ 1/3 reduction in livebirths

- Segmental rebiopsies are mandatory: High proportion are PGT-A patients with alternative embryos
- Unknown rebiopsies: High proportion are PGT-M/-SR patients who require a result, or risk adverse PGT-A patients who elect to rebiopsy with intention to transfer

  > Will take considerable time to accumulate outcome data for segmental rebiopsies

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Conclusions

It is possible to limit many of the potential downside risks associated with PGT-A by:

- Increase utilization of low-risk mosaics by ranking, rather than reporting details

  >No significant increase in adverse outcomes for low-risk mosaics

  >An estimated ~40 additional livebirths/year across our clinics

- Focus on minimizing sources of false positives with good healthy livebirth potential

  >Consider the future transfer of segmental gains without rebiopsy?

- Implicit in our approach is a small increase in the risk of false negatives

  >Acceptable trade off to maximize utilization of PGT-A tested embryos.

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THANK YOU

steve.grkovic@genea.com.au

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Girardi et al. 2020: Confirms most false positives are gains

- 53 embryos with an original segmental ABN call thawed for rebiopsy
- 1x confirmed ICM and 3x additional TE biopsies obtained

Study concluded that a 2nd TE biopsy is highly predictive of ICM status

Girardi et al. data was reanalyzed: Separated into gains and losses:

TE original biopsy result	# of embryos	All 4x TE = ABN	≥1/4 TE = NAD	ICM = ABN	ICM = NAD
Segmental loss	27	**17 (63%)**	10 (37%)	**20 (74%)**	7 (26%)
Segmental gain	26	3 (12%)	**23 (88%)**	7 (27%)	**19 (73%)**

Majority of gains reclassified as mosaic and had a NAD ICM: Confirmed our data

Girardi et al. American Journal of Human Genetics 2020;106:525-34