2021 position statement on mosaic embryo transfers – behind the scenes controversies

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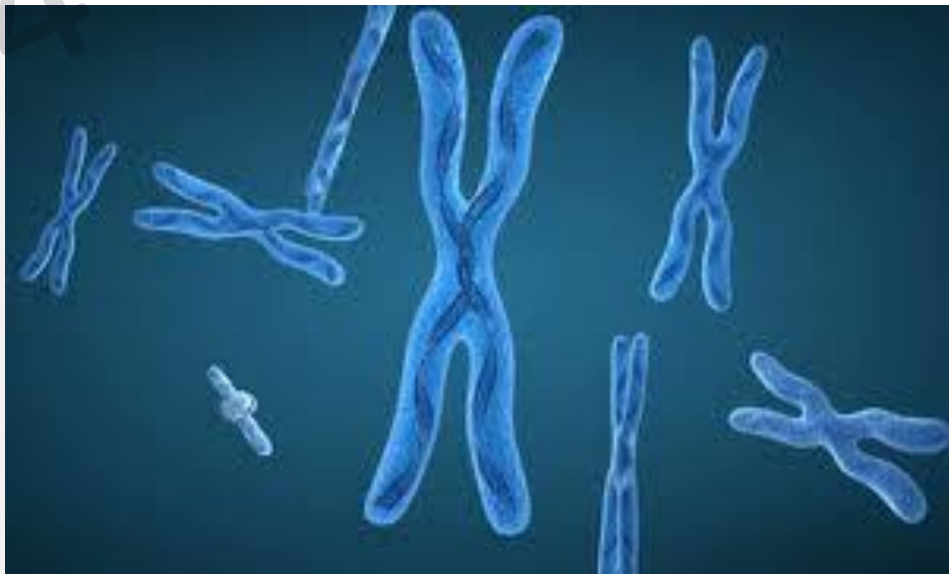


Disclaimer for Position Statement

- Was written as a Position Statement rather than a set of Guidelines
- Pre-submission drafts were reviewed by Members of PGDIS Board
- Based on the peer reviewed literature
  - no other material including unpublished papers, personal communications, networking discussions or individual laboratory results were considered
- Recommendations were crafted consistent with the known biology of preimplantation development

Summary of the published literature at the time

- Levels of mosaicism from TE biopsies evaluated by NGS based PGT-A were highly variable between laboratories
  - from as low as 5% to as high as 30%!
  - at the time, from networking, the general consensus for the mosaics detected was: a third were probably aneuploids, a third were probably euploids and a third probably mosaics

- In most studies of embryo transfers, compared to euploid transfers, mosaic embryos had lower implantation and higher miscarriage rates

- Transfer of mosaic embryos (< 50% mosaicism measurement for many chromosomes) produced good outcomes for the patient
  - babies born with normal karyotypes and no evidence of disease
  - only isolated case reports of adverse outcomes

Structure of Position Statement

- Highlight the new literature published after 2019 Position Statement

- Specific guidance given to
  - laboratories
  - clinics
  - clinicians
  - counselors

- Main recommendations for transfer of mosaic embryos and patient follow up

Key recommendations from the 2021 position statement regarding transfer of mosaic embryos

- If the analyzed cohort contains both euploid and mosaic embryos, euploid embryos should be preferentially transferred

- If there are only mosaic embryos for transfer
  - rather than initiate another IVF cycle, transfer the mosaic embryo after risk evaluation (genetic counselling)

- When more than one mosaic embryo is considered for transfer
  - preferentially transfer “low level” mosaics
  - the level of mosaicism has a higher priority than the chromosome involved
  - choose simple rather than complex mosaics

- If pregnancy ensues after mosaic embryo transfer, recommend prenatal follow up by either amniocentesis or NIPT

Controversies during drafting of the Position Statement - 1

When only mosaic embryos are available for transfer:

Option 1: Do not transfer the mosaic embryo, initiate another IVF cycle?

Option 2: Transfer the embryo understanding the risk and only initiate another IVF cycle if pregnancy not established?

How did we resolve?

- no embryo should be left behind, but acknowledge the risk
  - only adding fuel to fire for the “Do No Harm” group
- at the time, transfer of mosaics had generally favorable outcomes

Controversies during drafting of the Position Statement - 2

How to define cutoff for "low" and "high" level mosaics?

Option 1: Use 50% mosaicism as the cutoff (consistent with ESHRE statement)

OR

Option 2: Consider the noise band set by the laboratory

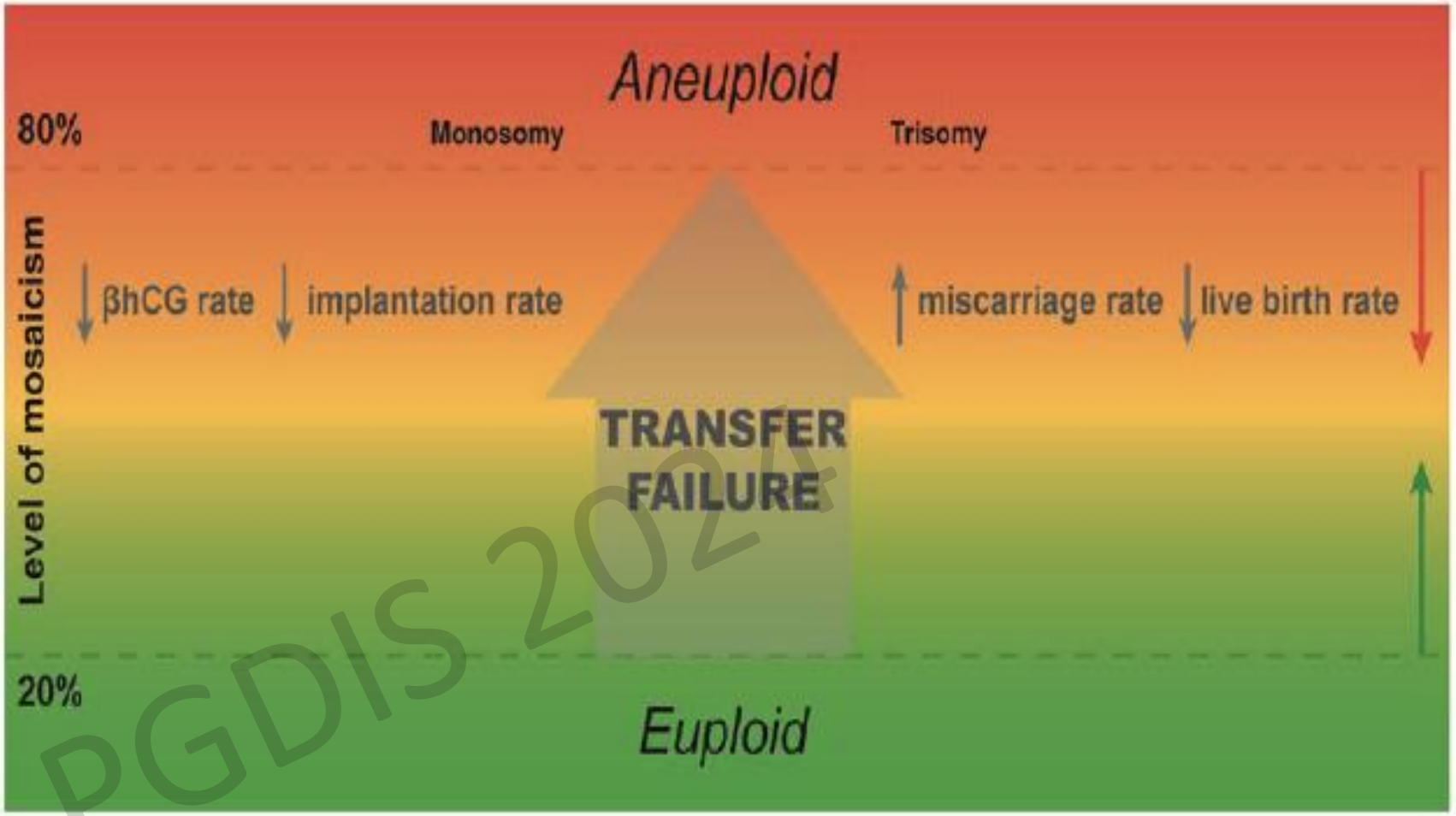
How did we resolve:

- Logical thinking!

- If we define a noise band of 20% to define a euploid (0-20%). then
  - a measurement of 30% could actually be as high as 50% or as low as 10%
  - a measurement of 40% could actually be as high as 60% or as low as 20%
  - a measurement of 50% could actually be as high as 70% or as low as 30%

- If a noise band of 30% is applied, interpretations become broader and absurd!

A logical view on mosaicism



Controversy during publication phase of the Position Statement

- Position Statement submitted to RBM Online

- In contrast to 2019, the Editor assigned to the manuscript required peer review of the document!
  - the article was dragged through three revisions until final acceptance!
  - publication delayed by 4 months!

First key point of contention

Statements need more balance

1. **Experts were given to much freedom to express their personal opinions**

- **Our assessment**
  - the Editor and the two reviewers were also strongly expressing their personal opinions

- **The revision process**
  - both sides were doubling down!
  - rebuttals were tedious and detailed to address reviewer's concerns

- **Outcomes**
  - consensus reached on all points of dispute or clarification
  - in all fairness, the final published document was more balanced
  - in retrospect it would have been beneficial for RBM Online to publish the reviewer's comments and PGDIS rebuttals for general viewing

Second key point of contention

Review of some key publications missed

2. **Authors failed to acknowledge the findings from one of the top European laboratories:**

Capalbo et al, Mosaic preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. Am J Hum Genet, 2021

	GROUP A EUPLOID	GROUP B LOW MOSAIC (20-30% VARIATION)	GROUP C MODERATE MOSAIC (30-50% VARIATION)	ADJ OR (95% C.I. P-VALUE)
TEST SETS, N	484	282	131	
POSITIVE PREGNANCY TEST, % (N)*	55.8% (270/484)	55.0% (155/282)	55.7% (73/131)	**0.98** (0.75-1.27; 0.86)
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)
MONOCHORIAL TWINS DELIVERY, N	1	1	1	
GESTATIONAL AGE, MEAN (95%C.I.)	38.4 (38.0-38.7)	38.2 (37-9-38.6)	38.1 (38.0-38.5)	
BIRTH WEIGHT, MEAN (95%C.I.)	3,286 (3,200-3,371)	3,174 (3,080-3,267)	3,130 (2,950-3,310)	

Second key point of contention

Review of some key publications missed

2. **The authors failed to acknowledge the findings from one of the top European laboratories:**

   Capalbo et al, Mosaic preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. Am J Hum Genet, 2021

- **Our assessment revealed**
  - was a good study that should be acknowledged, but at the time the paper was in print form (not officially published)
  - most alarmingly, the editor was revealed as a coauthor on the paper!

- **Outcome**
  - during revision process, this paper was officially published and findings acknowledged in the Position Statement

External Review of Position Statement by IDNHG-IVF

Journal of Assisted Reproduction and Genetics (2023) 40:817–826
https://doi.org/10.1007/s10815-023-02763-6

REVIEW

Check for updates

A review of the 2021/2022 PGDIS Position Statement on the transfer of mosaic embryos

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Received: 14 December 2022 / Accepted: 22 February 2023 / Published online: 9 March 2023

Abstract
The practice of preimplantation genetic testing for aneuploidy (PGT-A) in association with in vitro fertilization (IVF) since 2016 has been mostly directed by three highly controversial guidance documents issued by the Preimplantation Genetic Diagnosis International Society (PGDIS). Because these documents are so influential on worldwide IVF practice, the most recent one is here the subject of a detailed review, again revealing important misrepresentations and internal contradictions. Most importantly, however, this most recent guidance document still does not prevent the non-use and/or disposal of large numbers of embryos with substantial pregnancy and live-birth potential and, therefore, continues to propagate an IVF practice harmful to many infertile women.

Keywords In vitro fertilization (IVF) · Preimplantation genetic testing for aneuploid (PGT-a) · Guidelines · Embryo mosaicism

IDNHG-IVF mission statement:

Worldwide not-for-profit organization of physicians and embryologists founded to protect the integrity of IVF practice.

How did this review article evolve?

- RBM Online put the “non reviewed” version of the Position Statement online March 22, 2022 and was eventually published in July 2022 after revision

- In the meantime IDNHG-IVF wanted to submit a timely review and based their review on the “non-reviewed” online Position Statement
  - IDNHG-IVF were unaware that our submission was in a review process!
  - IDNHG-IVF paper was submitted to RBM Online, but rejected

- IDNHG then submitted to JARG and their review published in July 2023
  - strangely some of their critique of the position statement was based on statements made in both the “non reviewed” version and the final published version
  - in total there were over 40 criticisms of the Position Statement 🤦🤦

Some criticisms from IDNHG-IVF review

1. IDNHG-IVF

- "The Position Statement lacks transparency, contains important misrepresentations and internal contradictions, further enhancing rather than defusing the current confusion surrounding the diagnosis of mosaicism"

Response:

- IDNHQ wanted to clearly stamp their authority on the issue to influence the reader before the statements were discussed and argued
- Their statements were
  - all centered around their belief that PGT-A offers no significant benefit to the patient and does harm
  - Goes against sound scientific evidence
- PGT-A is a screening test, not a diagnostic test

Some criticisms from IDNHG-IVF review

2. IDNHG-IVF

- "The Position Statement literature review was biased and did not reference the significant publications from IDNHG-IVF"

Response:

- Most papers published from IDNHG-IVF offer negative opinions on PGT-A and were not directly relevant to the transfer of mosaic embryos

- The scientific work published in some of their papers simply lack good study designs and results have been misinterpreted or mis-represented and do not fit well with the known biology

Some criticisms from IDNHG-IVF review

3. IDNHG-IVF

- "The Position Statement still avoids an outright endorsement of the transfer of even mosaic embryos and therefore re-emphasizes that aneuploid embryos still should go unused and/or be discarded"

Response:

- We said that if only a mosaic embryo was available for transfer, it can be transferred, with an understanding of the risk

- Never implied aneuploids should not be used or discarded
  - just weigh up the risks!

Some criticisms from IDNHG-IVF review

4. IDNHG-IVF

- "In regard to bias against transfer of high level mosaics or aneuploids, surprisingly, good pregnancy and live birth rates following transfers of PGT-A abnormal embryos speak for themselves"

Response:

- At the time, the IDNHG-IVF did elude to data on the transfer of abnormal embryos (mosaics and aneuploids)

- Data was in the manuscript Yang et al, Depletion of aneuploid cells in human embryos and gastruloids, Nat Cell Biol 2021, but was buried in the Supplementary data!

Good outcomes from transfer of 32 “abnormal” embryos?

Supplementary Table 1. IVF cycles leading to pregnancy

# Patient	AGE	GRAVIDITY	PARITY	ORIGINAL EMBRYO Biopsy, result	Laboratory	Method	Bx Day	Morphology Score[a]	# Embryo	# Embryo	outcome - detail
1	42	1	0	47 XY, +12 [mos]	ReproGenetics	CGH	6	5BC	2	Live Birth	46, XY
				45 XX, -22			5	5CC			
2	35	2	0	45, XY, - 6 [mos]	ReproGenetics	NGS	6	5BB	1	Live Birth	46, XY
3	41	2	1	46, XY, del (15) (q24.1-qter)	ReproGenetics	NGS	5	5AA	2	Live Birth	46, XY
				43, XX, - 2, - 14, - 18			5	3AB			
4	41	1	0	46, XX, del (15) (pter-q21.1) [mos]	Cooper Genomics	NGS	5	5BB	3	Live Birth	46, XX
				47, XX, + 19 [mos]			5	5BC			
				46 XY, del (5) (pter-q22.3) [mos], dup (20) (pter-p11.23)			5	5BC			
5	36	0	0	46, XY, dup (10) (q11.21-q21.1) [mos]	Cooper Genomics	NGS	6	6AA	3	Live Birth	46 XY, dup (10) (q11.21 - 11.23)
				44, XY, - 11, - 22			6	5BB			
				44, XY, del (2) (q23.3-qter), - 17, - 22			6	2BB			
6	36	1	1	47, XX, + 14 [mos]	INGENOMIX	NGS	6	3BB	2	SAB[b]	No Genetic Analysis
				45, XX, - 11			5	3BC			
7	39	0	0	47 XX, + 9 [mos]	ReproGenetics	CGH	6	4BB	2	SAB	46, XX
				48, XY, +11[mos], + 22 [mos]			6	4BB			
8	44	1	0	47, XX, + 15	Invitae	NGS	5	4BB	2	SAB	47, XX, + 15
				46, XY, dup (8) (q24.2q24.3)			5	3BB			
9	38	2	1	47, XX, + 12	NATERA Spectru...	NGS	5	3BB	2	SAB	47, XX, + 12
				45, XX, - 22			6	4AA			
10	27	3	3	44 XY, -7, -13	ReproGenetics	aCGH	5	5CC	1	Not Pregnant	
11	45	1	0	47 XY, +14, +21, -16	Progenesis	NGS	6	5BB	3	Not Pregnant	
				49 XY, +18, +19, +21			6	5BB			
				50 XX, Complex			6	4BB			
12	42	4	1	45 XX, -21	EmbryoVu	NGS	6	5AB	1	Not Pregnant	
13	44	0	0	48 XX, +1, +19	Fertility, Genetics	CCS	5	5AB	3	Not Pregnant	
				44 XX, -15, -18			6	4BB			
				46 XY, -11, +16			5	3BB			
14	44	2	1	50 XX, Complex	ReproGenetics	NGS	3	Early Blast	2	Not Pregnant	

Summary of transfer outcomes from 32 "abnormal" embryos

- 6 mosaics transferred (level of mosaicism was unknown)
  - 3 livebirth with normal karyotype
  - 2 miscarriages
  - 1 livebirth with multiple congenital abnormalities

- 26 aneuploids transferred (simple and complex)
  - 1 livebirth with normal karyotype
  - 2 miscarriages
  - 23 not pregnant

- Harm has been done!

Where do we go from here?

- We need the data for any adverse outcomes of mosaic embryos – suppressed?
- Laboratories need to standardize and improve procedures to reduce incidence of mosaicism to 10% or less review embryology and technical aspects
- For mosaic embryos identified by NGS and selected for transfer
  - before transfer use SNP analysis of genome amplified products to determine likelihood of a true or false mosaic?
- Use (i) SNP arrays or (ii) NGS CNV test with SNPs as the standard test?
  - PTA based test with targeted SNP reads has reduced mosaics down to 7%
- Once revised protocols and better methods are widely adopted, we will be in a better position to conduct more meaningful studies to assess the outcomes of mosaic embryos transfers
  - follow fate and do further testing of mosaic failures and successes

Only then, we will be in a better position to consider putting together another PGDIS Position Statement on transfer of mosaic embryos