Mosaicism & Miscarriages

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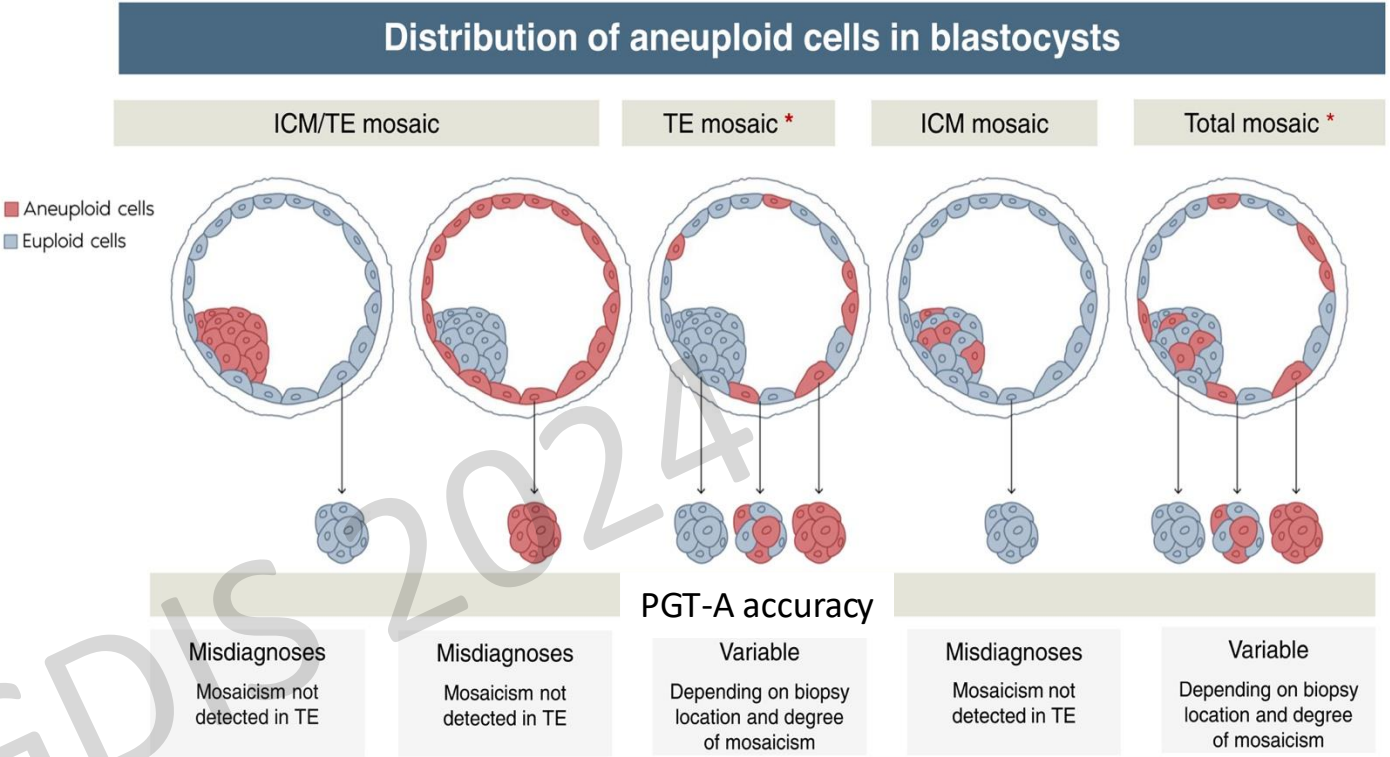
Outline

- Mosaicism: Formation and Types (prenatal aspect)

- Presence of heterogeneously distributed mosaicism
  - a study in first-trimester miscarriage POCs

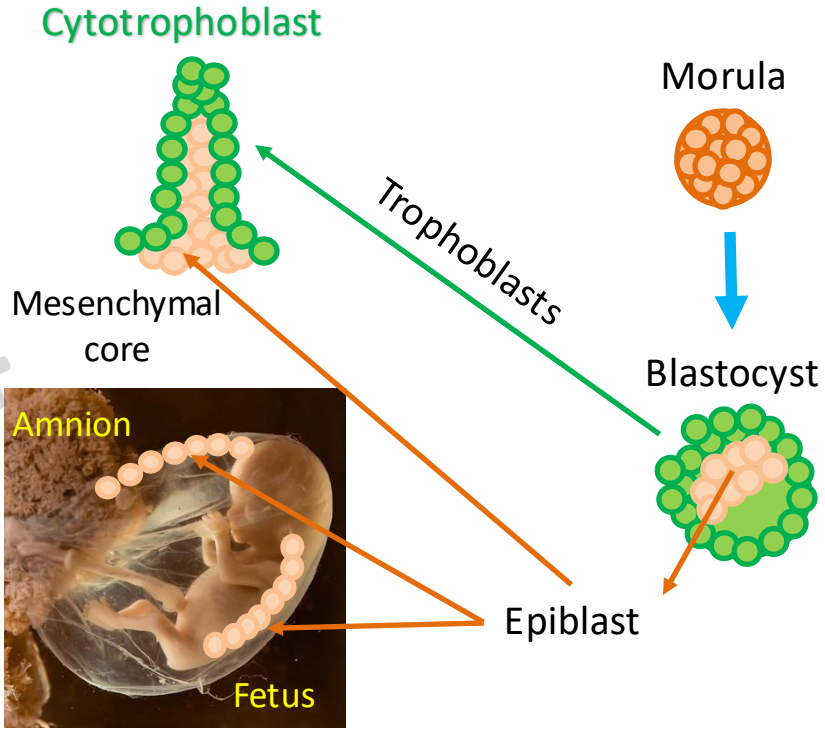
Mosaicism



* Most frequent types of mosaicism

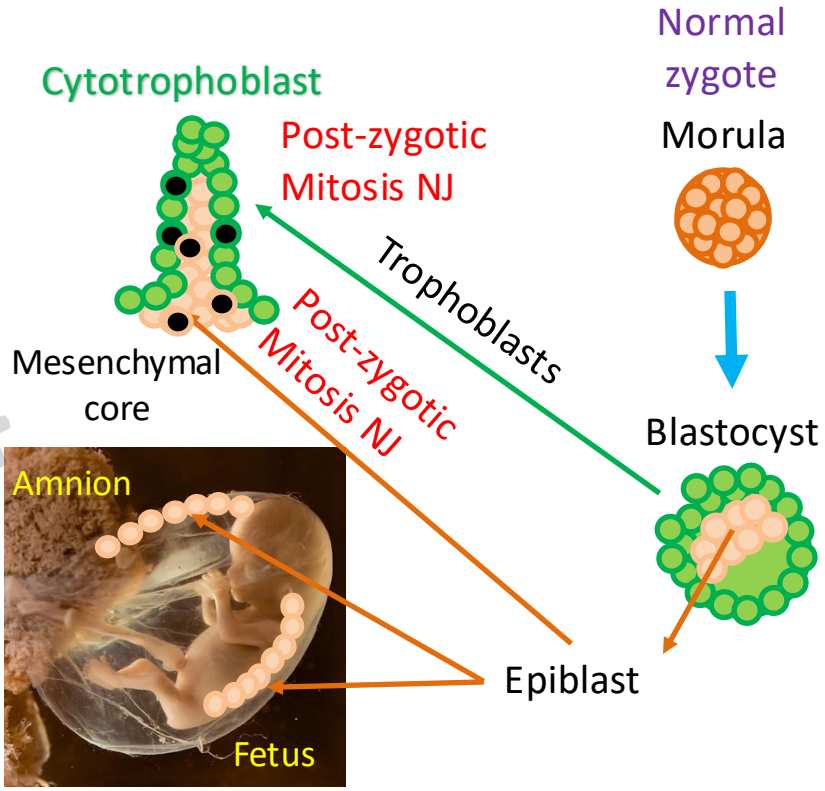
Capalbo et al., HR 2016; Vera-Rodriguez et al, F&S, 2017; Popovic et al., HR 2018

Mosaicism: Formation and Types



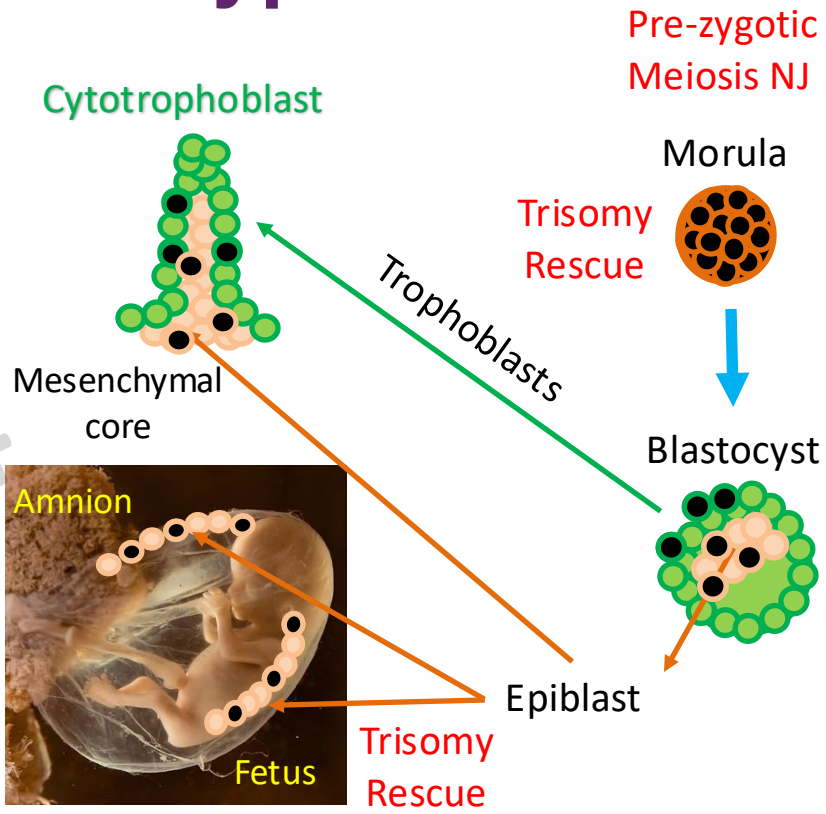
Mosaicism: Formation and Types

	Placental compartment		Fetal compartment	Proport-ion
	Cyto-Tropho-blast	Mesen-chymal core	Amniocytes / blood	(%)
CPM I				
CPM II				
CPM III				
TFM IV				
TFM V				
TFM VI				
CFM				



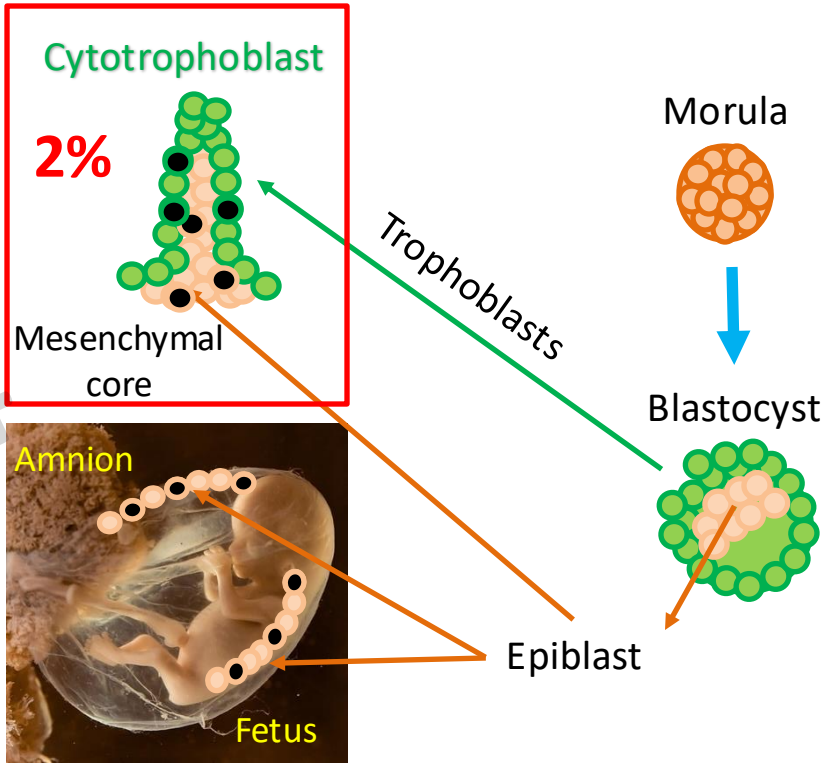
Mosaicism: Formation and Types

	Placental compartment		Fetal compartment	Proport-ion
	Cyto-Tropho-blast	Mesen-chymal core	Amniocytes / blood	(%)
CPM I	Abn	Nor	Nor	40%
CPM II	Nor	Abn	Nor	40%
CPM III				
TFM IV				
TFM V				
TFM VI				
CFM				



Mosaicism: Formation and Types

	Placental compartment		Fetal compartment	Proport-ion
	Cyto-Tropho-blast	Mesen-chymal core	Amniocytes / blood	(%)
CPM I	Abn	Nor	Nor	40%
CPM II	Nor	Abn	Nor	40%
CPM III	Abn	Abn	Nor	7%
TFM IV				
TFM V				
TFM VI	Abn	Abn	Abn	6%
CFM				



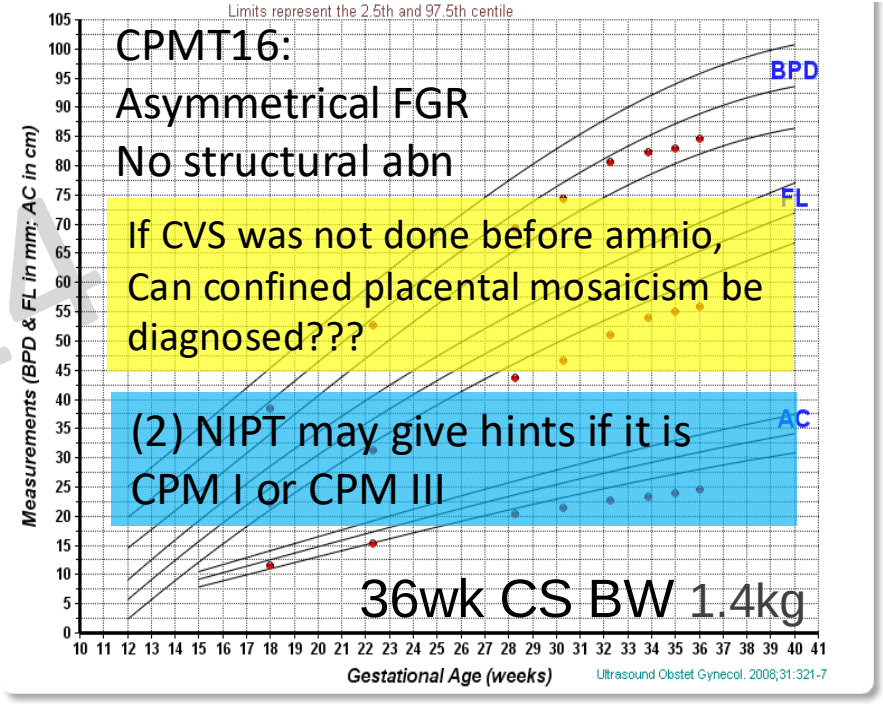
Mosaicism: Types and Testing

	Placental compartment		Fetal compartment	Proport-ion	NIPT	CVS	Amnio
	Cyto-Tropho-blast	Mesen-chymal core	Amniocytes / blood	(%)			
CPM I	Abn	Nor	Nor	40%			
CPM II	Nor	Abn	Nor	40%			
CPM III	Abn	Abn	Nor	7%			
TFM IV	Abn	Nor	Abn	1%			
TFM V	Nor	Abn	Abn	6%			
TFM VI	Abn	Abn	Abn	6%			
CFM	Nor	Nor	Abn	Rare			

Example Mosaic T16 Case

- 1st trim Down screening NT 1.64mm, fbhCG 3.64MoM, PAPPA 0.15MoM
- T21 1:7 ; T18 1:222 ; T13 1:405
- CVS – mos 47XX+16(16) / 46XX(12);
- Amnio - 46XX (=CPMII T16)

Placental mesenchymal dysplaisa

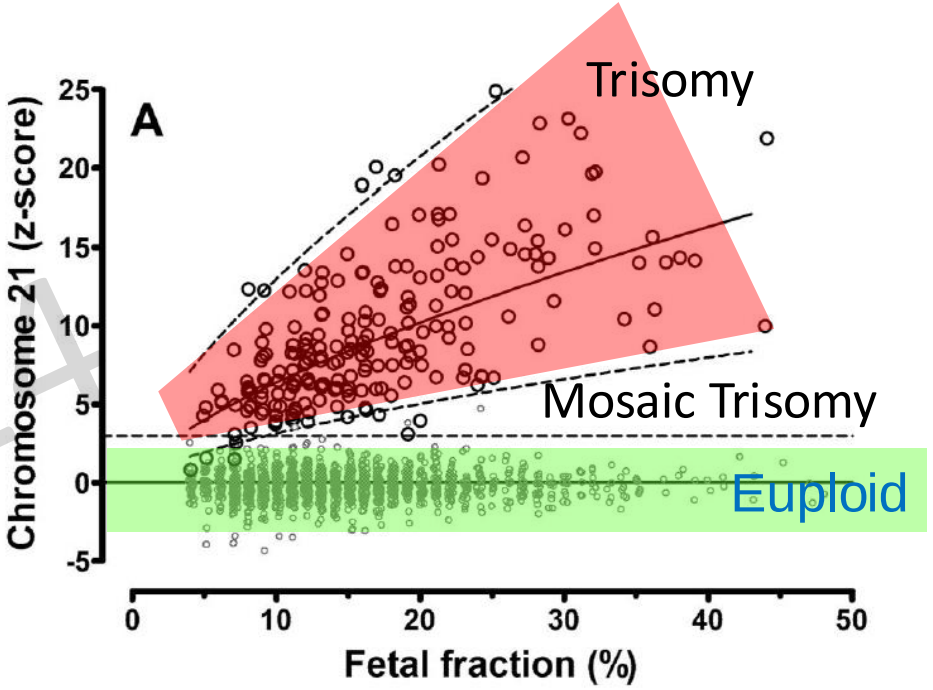


Mosaicism: Types and Testing

	Placental compartment		Fetal compartment	Proport-ion	NIPT	CVS		Amnio	
	Cyto-Tropho-blast	Mesen-chymal core	Amniocytes / blood	(%)		Direct (mixed pred cyto	Culture (Mesen-chymal)	Direct	Culture
CPM I	Abn	Nor	Nor	40%	+ve	+ve	-ve	-ve	-ve
CPM II	Nor	Abn	Nor	40%	-ve	+/-ve	+ve	-ve	-ve
CPM III	Abn	Abn	Nor	7%	+ve	+ve	+ve	-ve	-ve
TFM IV	Abn	Nor	Abn	1%	+ve	+ve	-ve	+ve	+ve
TFM V	Nor	Abn	Abn	6%	-ve	+/-ve	+ve	+ve	+ve
TFM VI	Abn	Abn	Abn	6%	+ve	+ve	+ve	+ve	+ve
CFM	Nor	Nor	Abn	Rare	-ve	-ve	-ve	+ve	+ve

Threshold of Mosaicism detectable by various methods

Methods	Threshold
karyotype	5% (100 cells)
FISH	5-10%
PCR	10%
Low-pass	10%
CMA	10-15%
NIPT	Depend on mosaic level, & fetal fraction



Palomaki et al Genet Med 2011

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Prevalence of mosaicism in first-trimester miscarriage?

Part II: Presence of heterogeneously distributed mosaicism, which was prevalent in first-trimester miscarriage POCs

First-trimester miscarriage: prevalent and multifactorial

Embryonic/fetal genetic abnormalities: ~50% by Karyotyping [1]

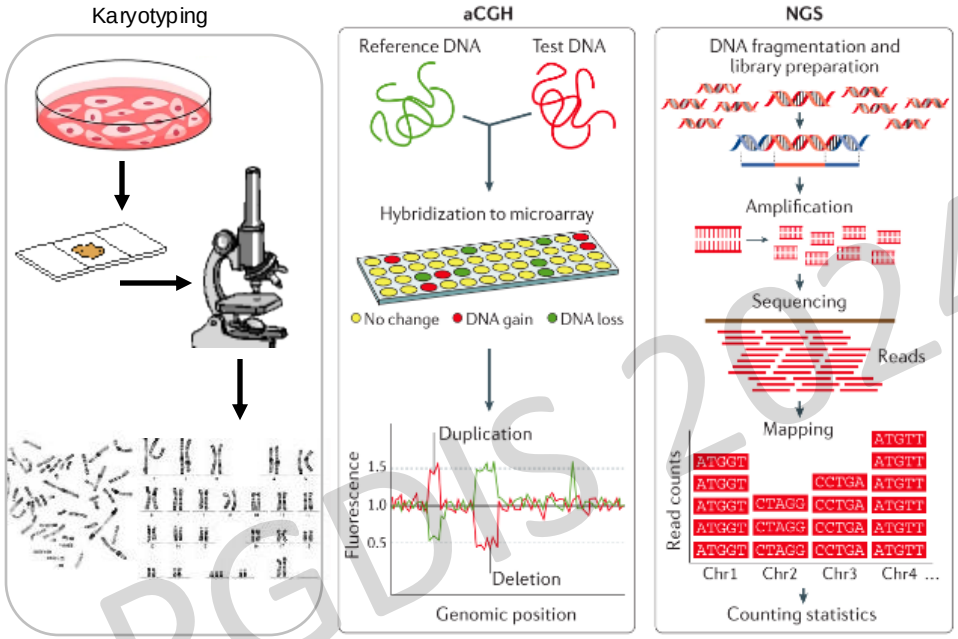
Fetal factor

Products Of Conception (POC)

[1] Zhang T et al., *BJOG*, 2018

Genetic investigation and Low-pass genome sequencing

Wang et al. GIM 2019 (n=1023) [4]



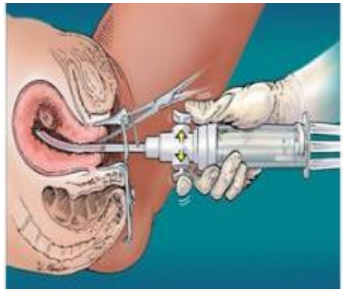
Low-pass genome sequencing (GS)
Low=low coverage (0.25X); pass=high throughput

1) high-throughput: 32-48 samples per flowcell ;
2) high sensitivity in mosaicism detection:
   whole chromosome: 10% [1];
   segments larger than 2.5 Mb: 20% [1];
3) high resolution: 50 kb CNVs [2]
4) cost-effective.

Figures from: Joris Robert Vermeesch et al., Nat. Rev. Genet, 2016
[1] Chau et al. *Hum. Genet*, 2020
[2] Dong et al. *Curr Protoc Hum Genet*, 2017
[3] Dong et al. *Am. J. Hum. Genet*, 2019
[4] Wang et al. *Genet. Med*, 2019

A novel treatment alternative: ultrasound-guided manual vacuum aspiration (USG-MVA)

Jacqueline CHUNG



Advantages :

- Outpatient clinic
- Local anesthesia
- Discharge within the operation day

Intact POC :

- Reduce MCC;
- Facilitate multiple-site sampling ;
- Embryo finding.

ANZJOG

Original Article | Full Access

Efficacy, feasibility and patient acceptability of ultrasound-guided manual vacuum aspiration for treating early pregnancy loss

Jacqueline Pui Wah Chung, Cathy Hoi Sze Chung, Jennifer Sze Man Mak, Tin Chiu Li, Grace Wing Shan Kong

First published: 19 April 2018 | https://doi.org/10.1111/ajo.12811 | Citations: 5

ELSEVIER

The International Journal of Biochemistry & Cell Biology

Volume 147, June 2022, 106226

Ultrasound-guided Manual Vacuum Aspiration is an optimal method for obtaining products of conception from early pregnancy loss for cytogenetic testing

Jacqueline Pui Wah Chung [a,1], Ying Li [a,1], Tracy Sze Man Law [a], Karen Ng [a], Olivia See Yung Chau [a], Kwong Wai Choy [a,b,c,d], David Yiu Leung Chan [a]

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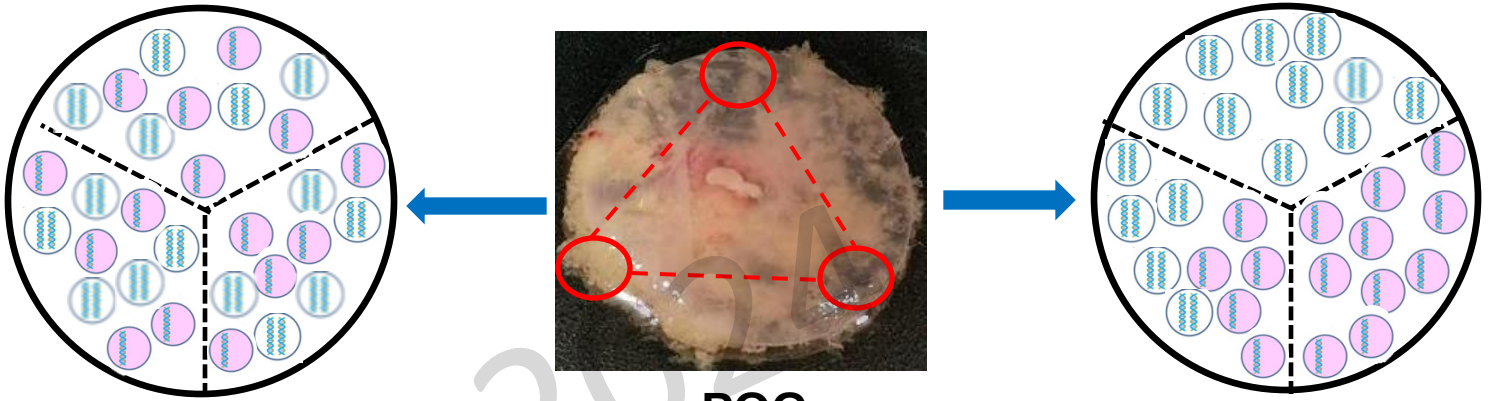
[1] Jacqueline Chung et al., Aust N Z J Obstet Gynaecol, 2019
[2] Jacqueline Chung, Ying Li et al., Int. J. Biochem. Cell Biol., 2022

Hypothesis

Multiple-site sampling increases the mosaicism incidence and diagnostic yield in first-trimester miscarriage

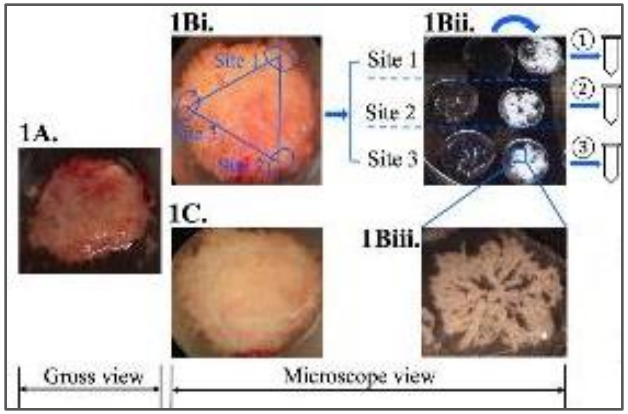


POC

Homogenous mosaicism:
uniform distribution of mosaic cell lines;

Heterogenous mosaicism:
heterogeneity of genetic constitutions within POC;.
requires sampling of multiple sites

Study design and flowchart



Multiple-site sampling under the microscope

DNA extraction & QF-PCR (for triploidy and MCC detection)

Low-pass Genome Sequencing (0.25x)

Bioinformatic analysis & variant interpretation of each site

Consistency between sites?

Yes

Interpretate as the original result

No

Interpretate as macroscopic mosaicism

Yes

Validation & further MCC exclusion

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Results

OXFORD human reproduction

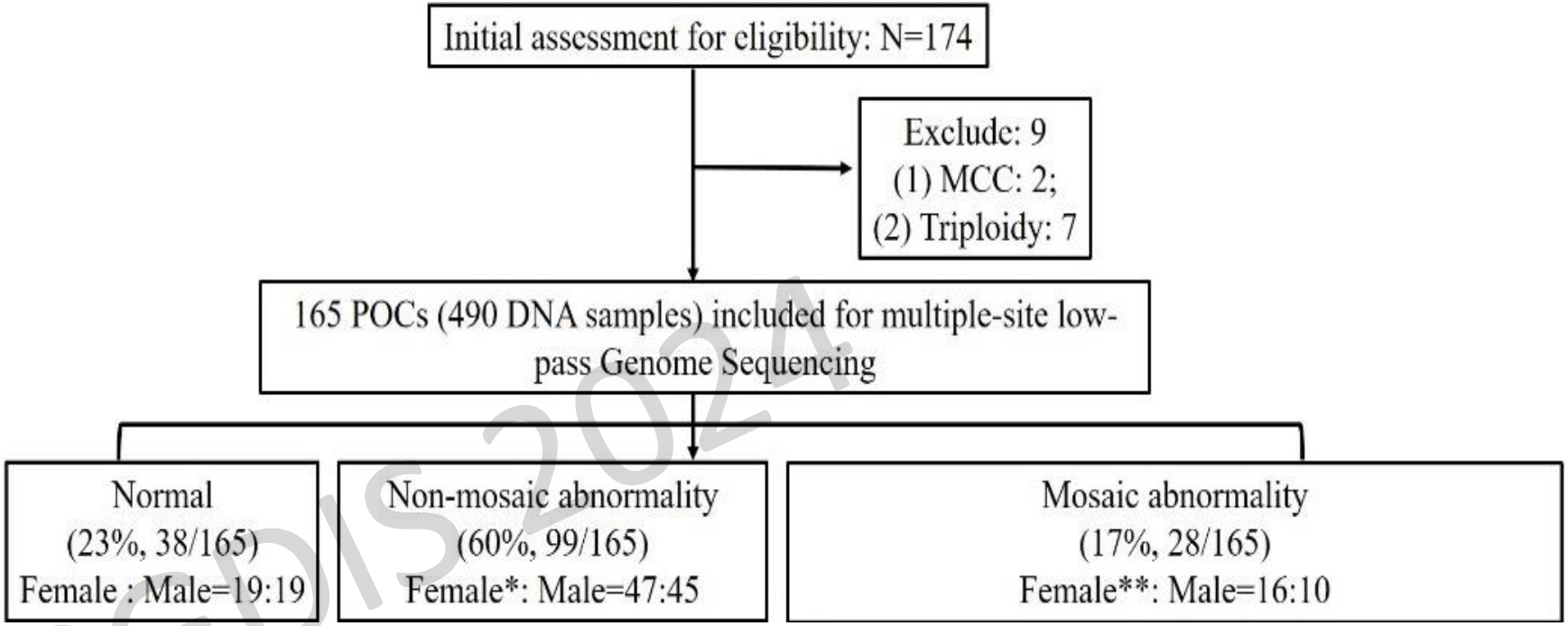
Human Reproduction, 2023, 1–15
https://doi.org/10.1093/humrep/dead090
Original Article

Reproductive genetics

A pilot investigation of low-pass genome sequencing identifying site-specific variation in chromosomal mosaicisms by a multiple site sampling approach in first-trimester miscarriages

Ying Li [1,2,3,†], Matthew Hoi Kin Chau [1,2,3,†], Ying Xin Zhang [1,2,4,5], Yilin Zhao[1,2], Shuwen Xue[1,2], Tin Chiu Li [1], Ye Cao [1,2,3], Zirui Dong [1,2,3], Kwong Wai Choy [1,2,3,6,*], and Jacqueline Pui Wah Chung [1,3,*]

Multiple-site low-pass GS result overview



>77% (117/165)

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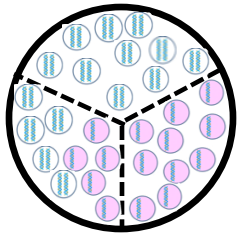
Li Ying et al., *Hum Reprod 2023*

Results 1

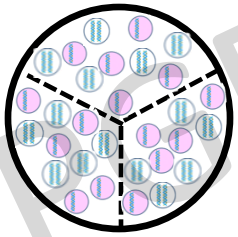
Chromosomal mosaicisms in 17% (28/165) of first-trimester miscarriages, majority heterogenous mosaicisms 75% (21/28)

Heterogeneity



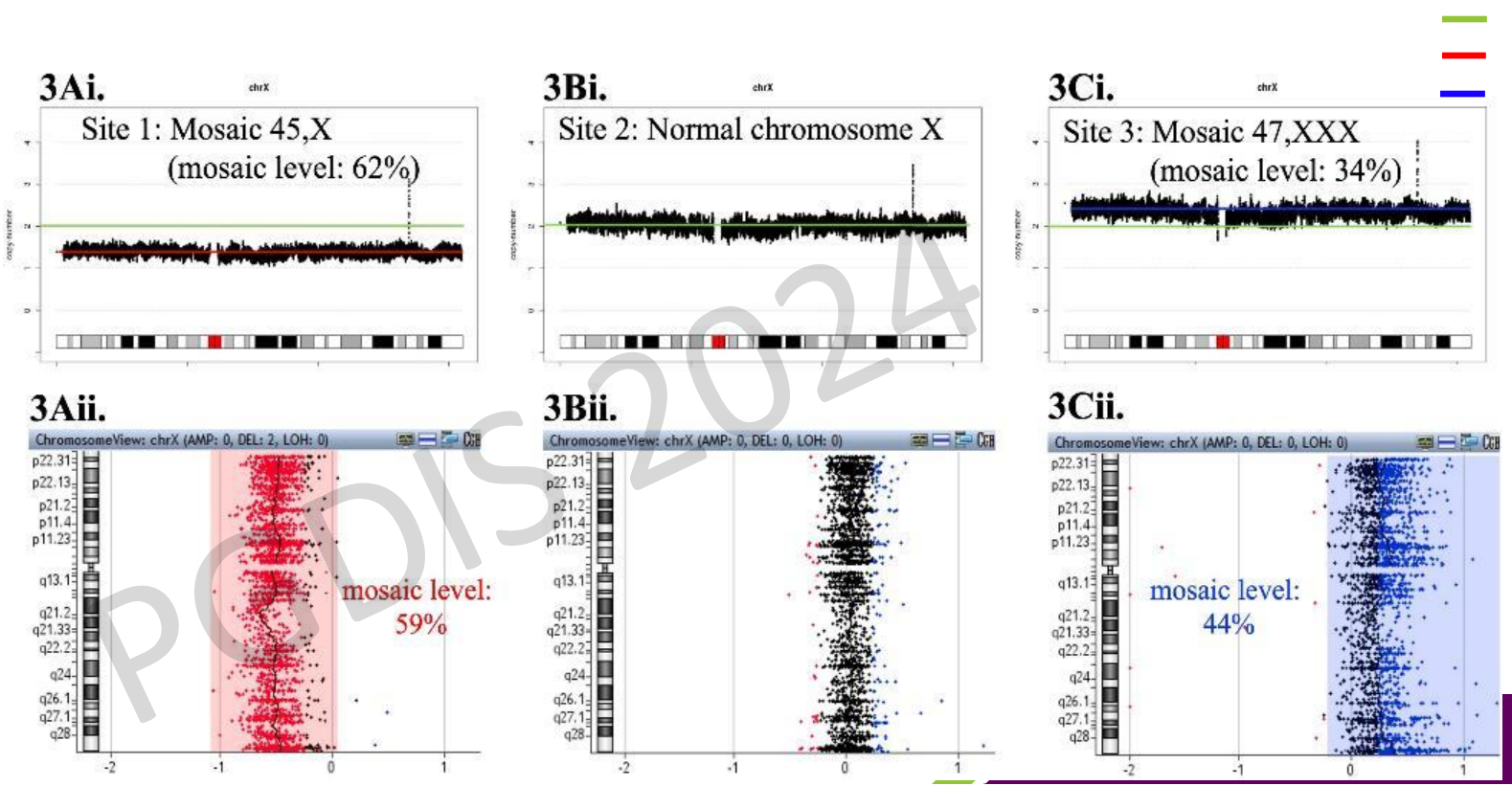
Heterogenous mosaicism	Case number
Mosaic aneuploidy	11
Multiple mosaic aneuploidy	1
Mosaic CNV	4
Multiple mosaic CNV	3
Mosaic aneuploidy/CNV plus non-mosaic abnormality	2

Uniformity

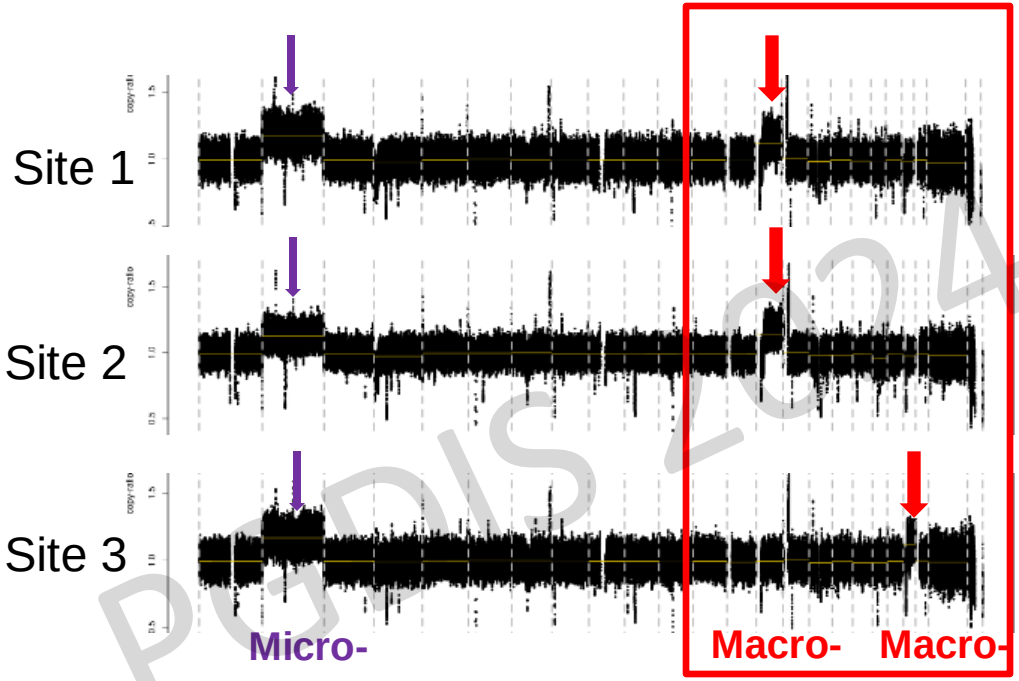


Homogenous mosaicism	Case number
Mosaic aneuploidy	5
Mosaic CNV	2
Mosaic aneuploidy/CNV plus non-mosaic abnormality	2

Example of heterogenous mosaicism



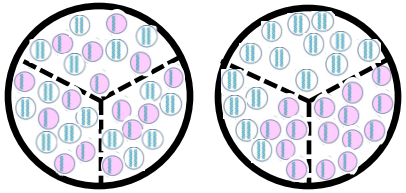
Co-existence of two mosaicism subtypes



Mosaic T2 (35%)
Mosaic T14 (23%)
Normal chr21

Mosaic T2 (25%)
Mosaic T14 (28%)
Normal chr21

Mosaic T2 (33%)
Normal chr14
Mosaic T21 (23%)



Collectively, the case has **heterogenous** mosaic trisomy 14 and 21, but **homogenous** mosaic trisomy 2.

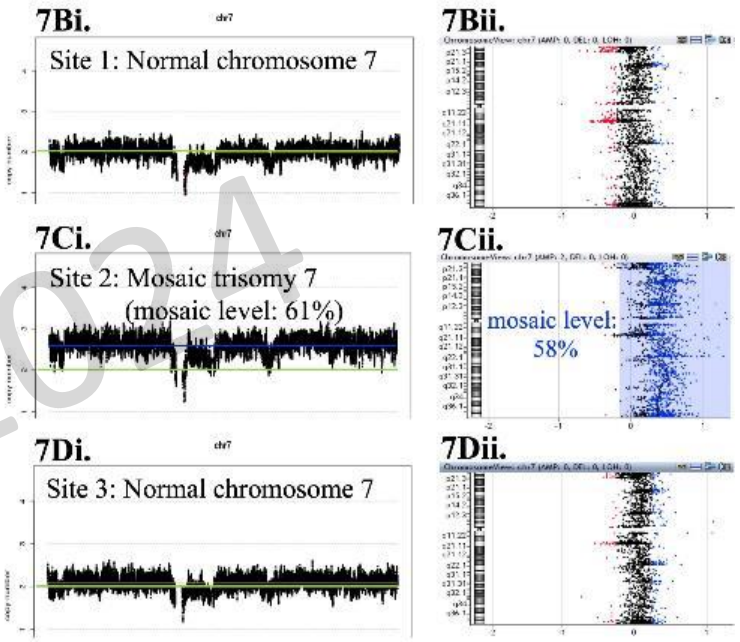
Cross-platform comparison karyotyping vs multiple-site low-pass GS (n=71)

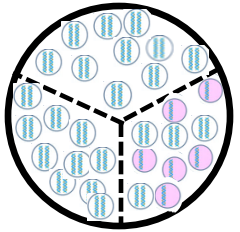
Karyotyping result	Multiple-site low-pass GS result	Case number (%)
Normal	Normal	12 (16.9%)
Abnormal	Abnormal	40 (56.3%)
Abnormal	Abnormal with additional complexities	12 (16.9%)
Culture failure	Yield a diagnosis	4 (5.6%)
Normal	Abnormal	3 (4.2%)

26.8%

26.8% of Karyotyping results were revised by multiple-site low-pass GS.

Case example: false-negative karyotyping results due to heterogenous mosaicism



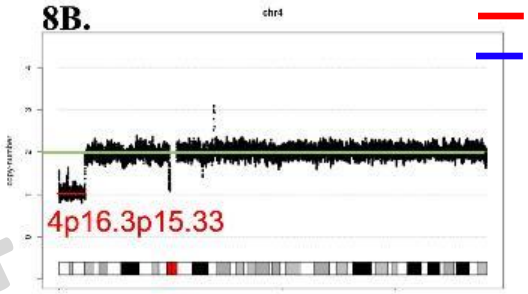


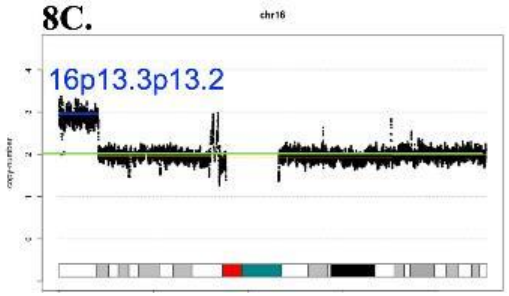
Collectively, the case has mosaic trisomy 7 at one site

Normal reference 正常对照

Copy-number gain 重复

Case example: false-negative by karyotyping due to resolution limit





Normal reference 正常对照
Copy-number loss 缺失
Copy-number gain 重复

Li Ying et al., *Hum Reprod 2023*

Take home message…

- Review the formation and types of mosaicisms (CPM & TFM)

- Genetic abnormalities were detected in 77% (127/165) of 1st trimester miscarriage POCs by low-pass genome sequencing 。
- Specifically, 17.0% (28/165) had either heterogeneously distributed mosaicism (75%; 21/28) or homogeneously distributed mosaicism (25%, 7/28) 。
- Heterogeneously distributed mosaicism (12.7%; 21/165), is prevalent in first-trimester miscarriage POCs.
- Does this reflect the frequently observed PGT-A mosaicism in preimplantation embryos?

THANK YOU

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Collaborative Research Fund

UGC 大學教育資助委員會 University Grants Committee

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PGT and BEYOND...