Prenatal diagnosis of fetal mosaic aneuploidy: misconceptions and misinterpretations

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Outline

1、Overview

2、Mechanism

3、Uniparental disomy

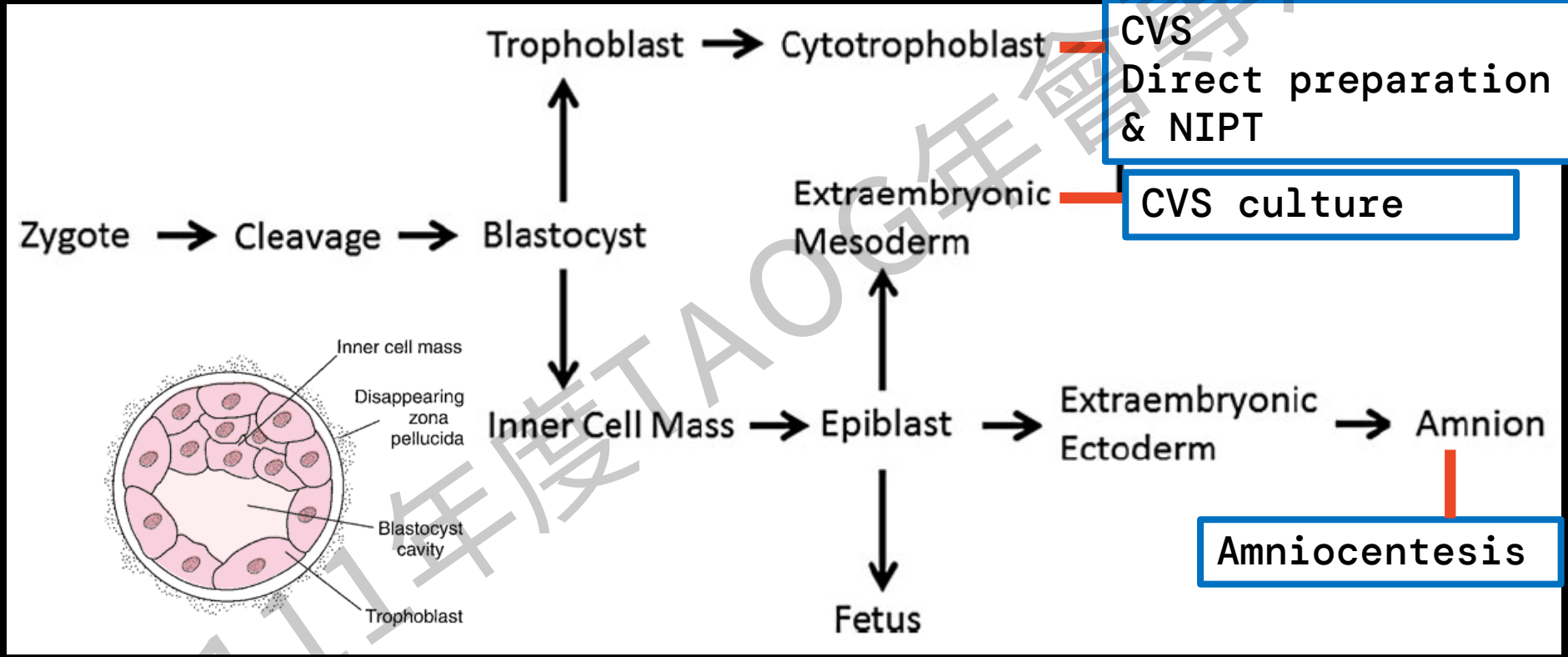
4、Mosaicism in different stages

Overview

Chromosome Mosaicism

- Two or more chromosomally distinct cell lines within an individual arising from a single zygote
- Aneuploidy & structural abnormality
- Genetic diseases, miscarriages and preimplantation embryo wastage, cancer
- General mosaicism: presence of a two or more cell lines throughout the entire organism
- Confined mosaicism: chromosomal mosaicism only present in a particular area (brain, placenta, gonads…)
- Villus tissue, aminocytes→ embryo?

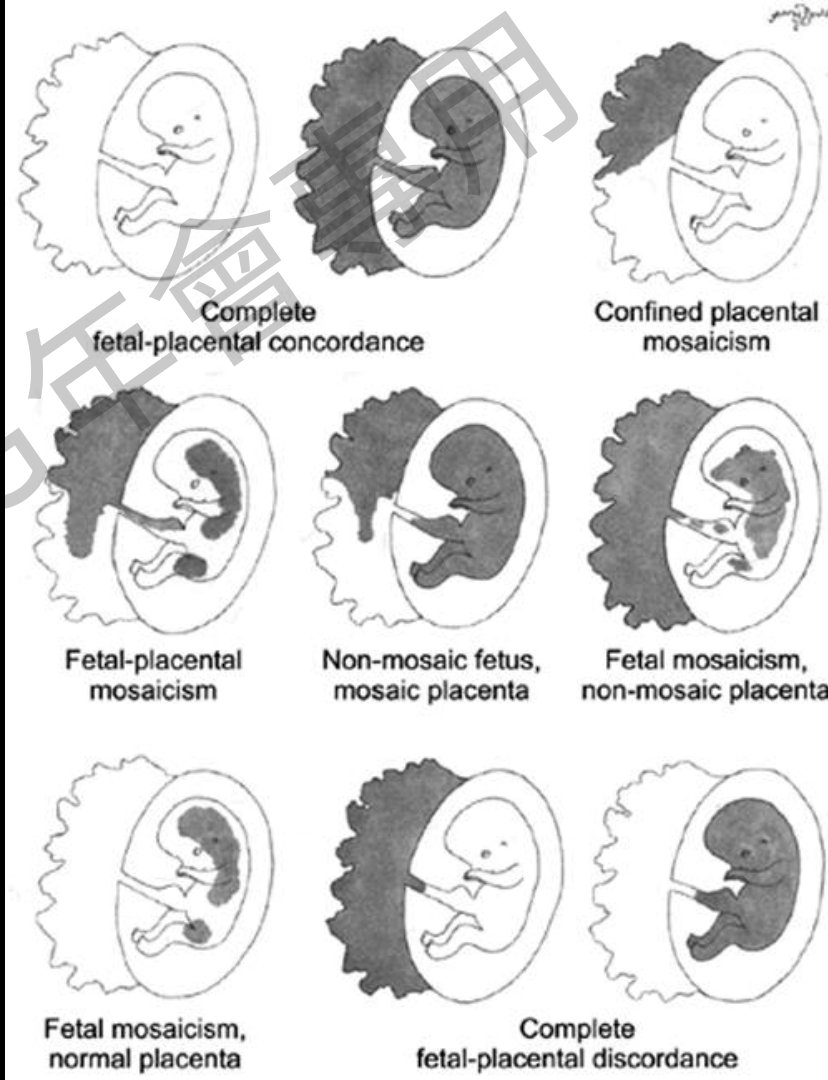
Lineage diagram arising from human embryo differentiation



Human Reproduction Update 2014;20:571-581

Type of mosaicism

1. Confined placental mosaicism
2. True constitutional fetal mosaicism
3. pseudomosaicism



Chromosome Abnormalities and Genetic Counseling, 5th Edition

Pseudomosaicism

chromosome anomalies arisen in culture (artifact)

Flask method	*In situ* method
A. Indications for extensive work-up	A. Indications for extensive work-up
(1) Autosomal trisomy involving a chromosome 2, 5, 8, 9, 12, 13, 14, 15, 16, 18, 20, 21 or 22 (SC, MC)[a]	(1) Autosomal trisomy involving a chromosome 2, 5, 8, 9, 12, 13, 14, 15, 16, 18, 20, 21 or 22 (SC_o, MC_o)[b]
(2) Unbalanced structural rearrangement (MC)	(2) Unbalanced structural rearrangement (MC_o)
(3) Marker chromosome (MC)	(3) Marker chromosome (MC_o)
B. Indications for moderate work-up	B. Indications for moderate work-up
(4) Extra sex chromosome (SC, MC)	(4) Extra sex chromosome (SC_o, MC_o)
(5) Autosomal trisomy involving a chromosome 1, 3, 4, 6, 7, 10, 11, 17 or 19 (SC, MC)	(5) Autosomal trisomy involving a chromosome 1, 3, 4, 6, 7, 10, 11, 17 or 19 (SC_o, MC_o)
(6) 45,X (MC)	(6) 45,X (SC_o, MC_o)
(7) Monosomy (other than 45,X) (MC)	(7) Monosomy (other than 45,X) (SC_o, MC_o)
(8) Marker chromosome (SC)	(8) Marker chromosome (SC_o)
(9) Balanced structural rearrangement (MC)	(9) Balanced structural rearrangement (MC_o)
	(10) Unbalanced structural rearrangement (SC_o)
C. Standard, no additional work-up	C. Standard, no additional work-up
(10) 45,X (SC)	(11) Balanced structural rearrangement (SC_o)
(11) Unbalanced structural rearrangement (SC)	(12) Break at centromere with loss of one arm (SC_o)
(12) Balanced structural rearrangement (SC)	(13) All single-cell abnormalities
(13) Break at centromere with loss of one arm (SC)	

Prenat. Diagn. 19: 1081–1090 (1999)

Why different cell lines exist?

Mechanisms of Mosaicism

1.
Normal conceptus
+ mitotic error
→

Abnormal cell line

2.
Meiotic error→
Abnormal conceptus
+ mitotic rescue→
Normal cell line

Distribution & ratio:

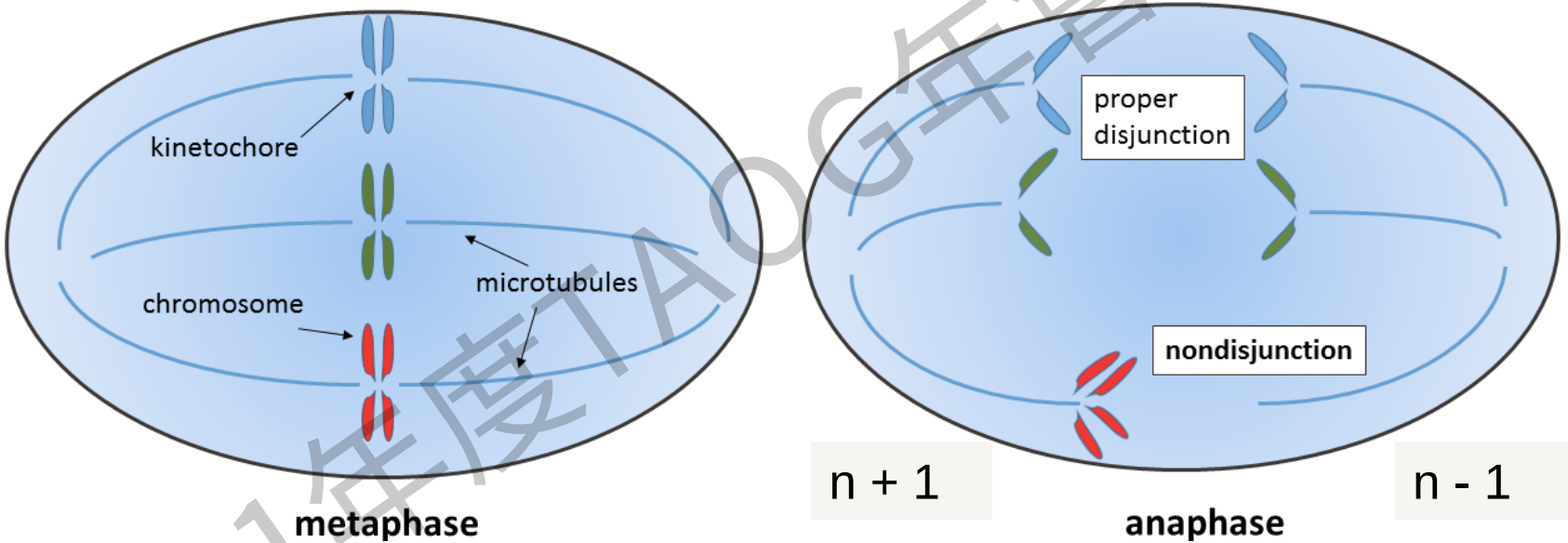
- Time
- Place

→ Phenotype

Mechanism of Chromosome Mosaicism

Nondisjunction

Failure of sister chromatids to separate



Least prevalent mechanism among autosomal aneuploidy

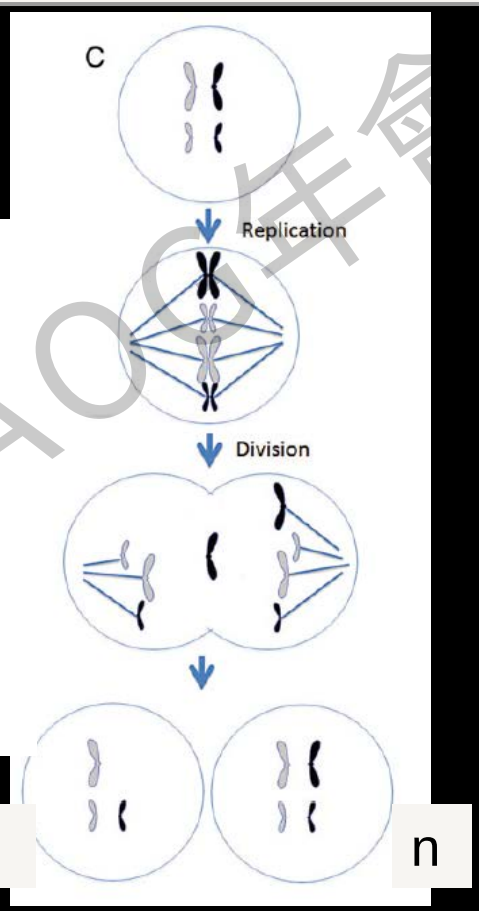
Main mechanism for sex chromosome malsegregation

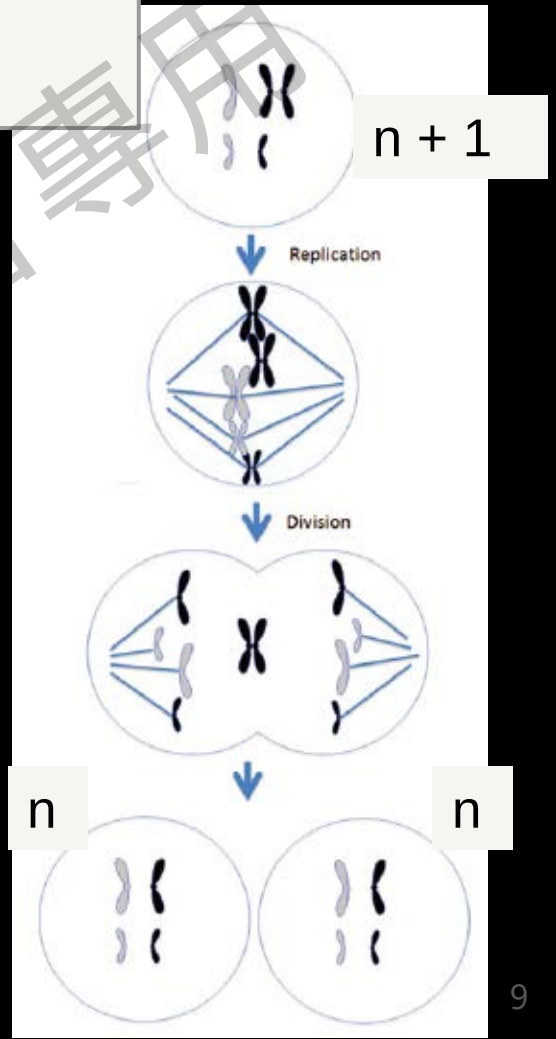
Hum Reprod 2002;17:2362–2367

Mechanism of Chromosome Mosaicism

Anaphase lagging

- A single chromatid fails to attach to the spindle
- Main source of mosaicism in human preimplantaion stage
- Trisomy rescue
- Monosomy rescue (endoduplication)

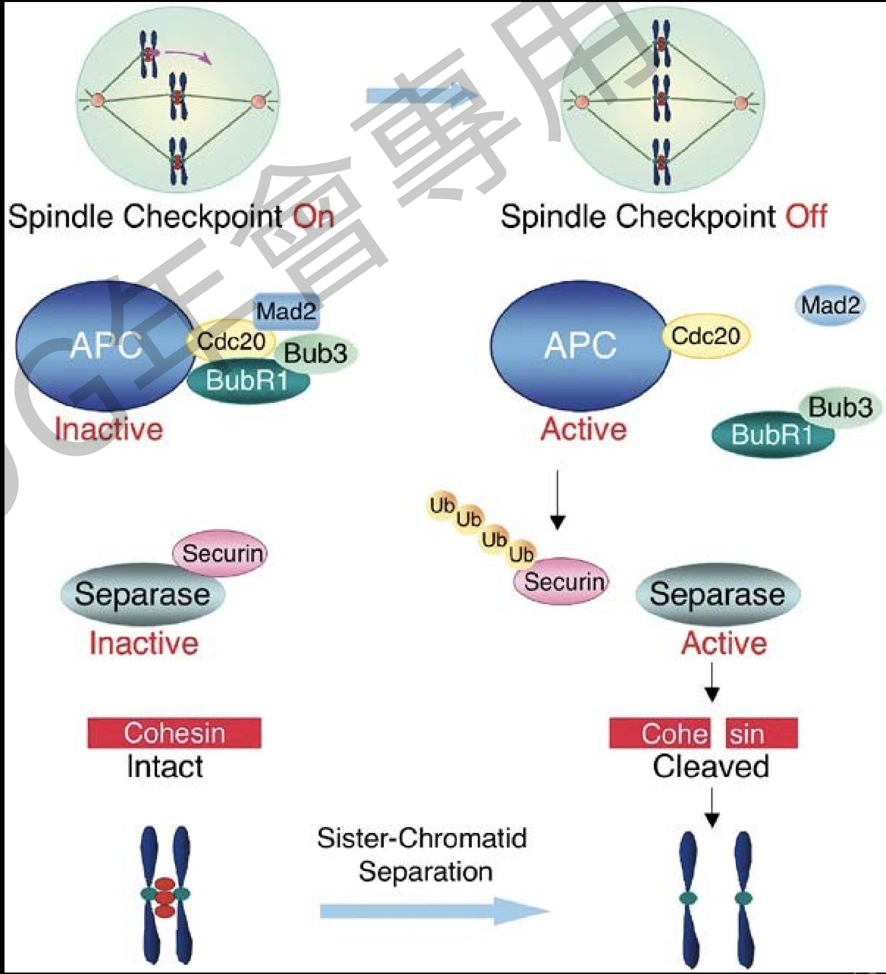




Human Reproduction Update 2020;26(3):313-334

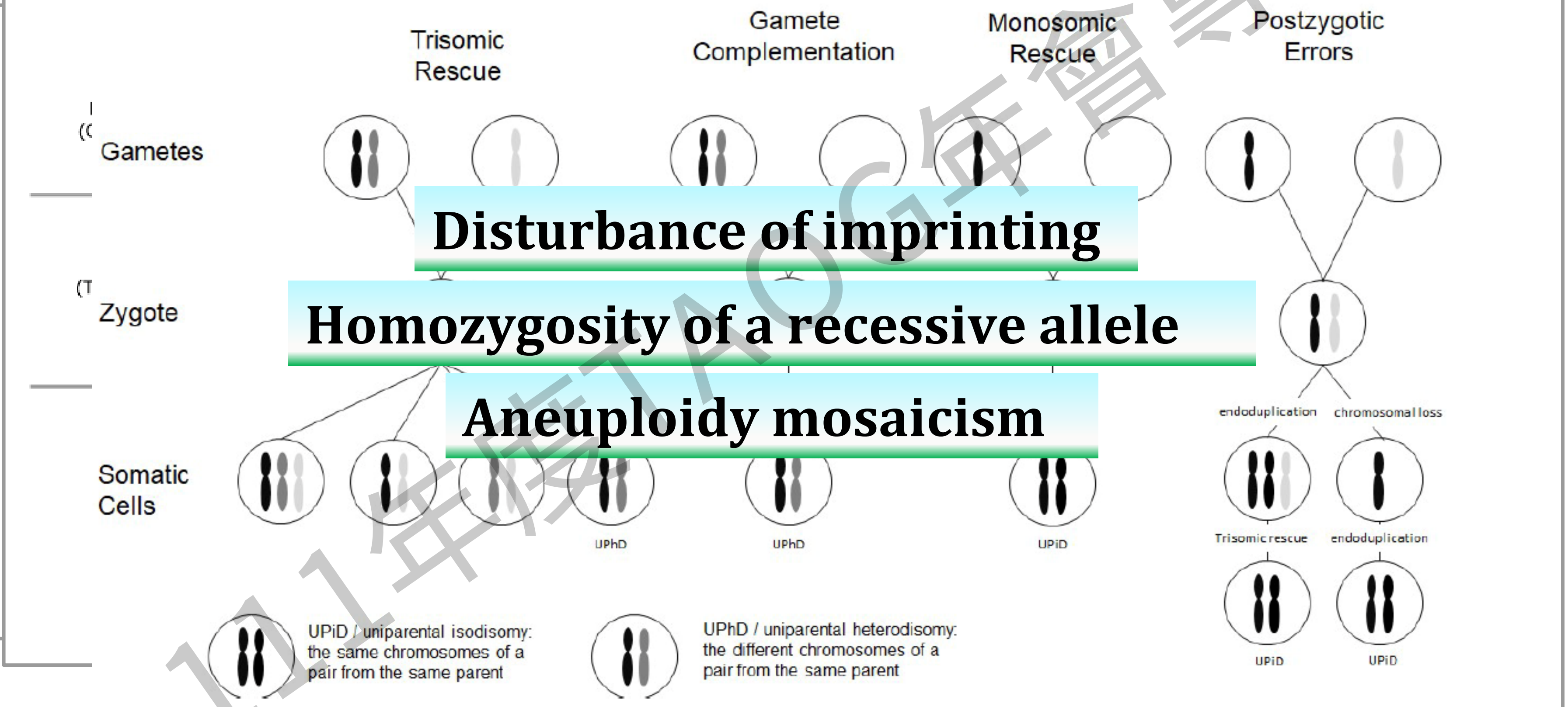
Spindle Assembly Checkpoint

- Protect dividing chromosomes unequally
- Prevent cell dividing until the chromosomes is captured by the mircortubules
- During the first mitotic divisions: this checkpoint seems nonexistent



Oncogene 2004;23(11):2016-2027

Chromosome aneuploidy mosaicism & uniparental disomy



Disturbance of imprinting

Homozygosity of a recessive allele

Aneuploidy mosaicism

Genes. 2020, 11, 1454

Chromosome aneuploidy mosaicism & uniparental disomy

UPD type	Syndrome/Disease	OMIM reference ID	Phenotype
paternal UPD6	Transient neonatal diabete mellitus (TNDM)	#601410	IUGR, neonatal diabetes
maternal UPD7	Silver-Russell	#180860	IUGR/PNGR, dysmorfisms
maternal UPD11	Silver-Russell	#180860	IUGR/PNGR, dysmorfisms
paternal UPD11	Beckwith-Wiedemann	#130650	Overgrowth, dysmorfisms, tumors (or isolated hemihyperplasia)
maternal UPD14	Temple syndrome	*605636 and #176270	IUGR, dysmorfisms
paternal UPD14	Bell-shaped thorax, developmental retardation	#608149	Dwarfisms, dysmorfisms
maternal UPD15	Prader-Willi	#176270	Obesity, dymorfisms, MR
paternal UPD15	Angelman	#105830	MR, dysmorfisms
maternal UPD20	Growth failure, hyperactivity	*139320	IUGR/PNGR
paternal UPD20	Pseudohypoparathyroidism	*139320	Pseudohypoparathyroidism

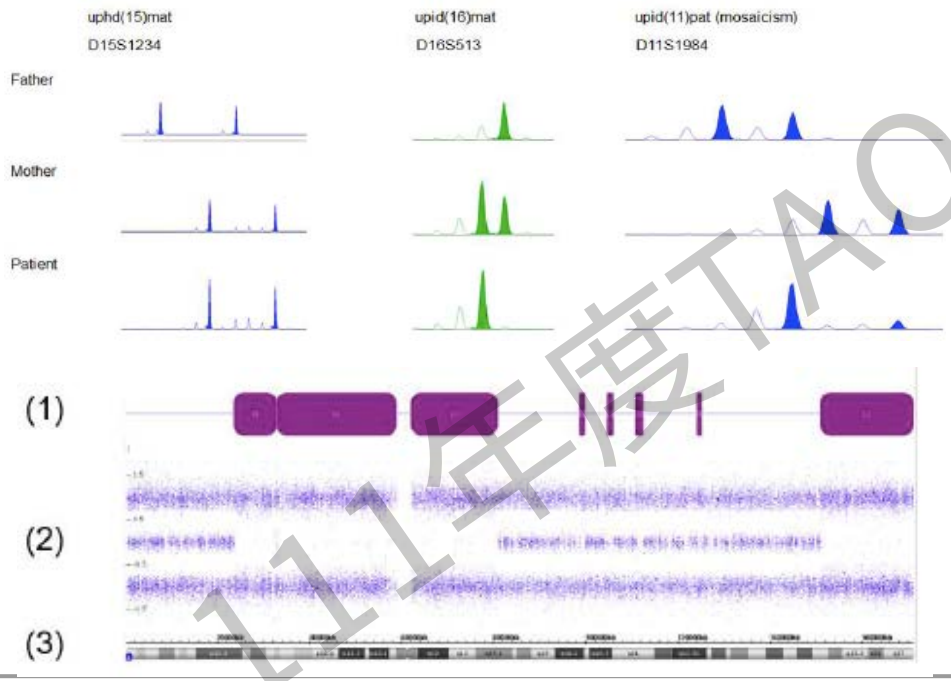
J. Clin. Med. 2014, 3

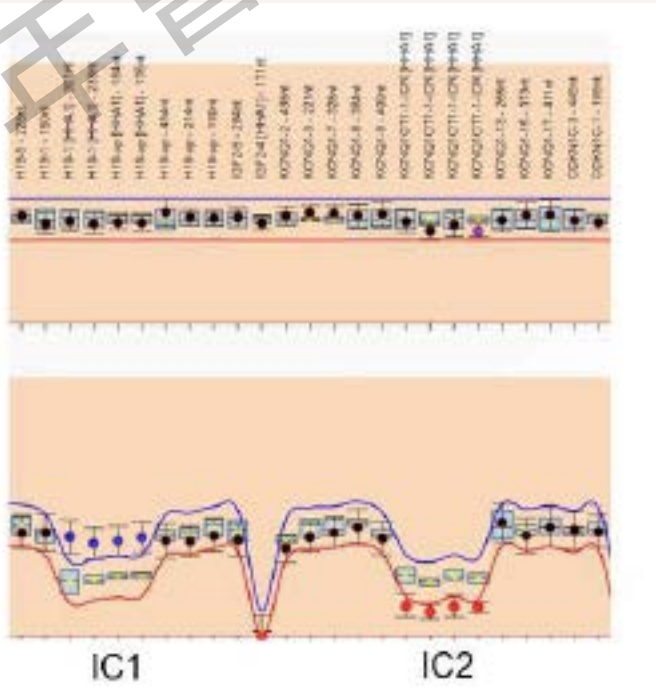
Detection of UPD

Short tandem repeats (STRs) markers

SNP array

MS-MLPA (methylation-specific MLPA)





Genes. 2020, 11, 1454

Mosaicism in Preimplantation and Prenatal Diagnosis

Aneuploidy mosaicism in different stages

Preimplantation embryos	● 2~50% of embryo biopsies ● Pregnancy rates, live birth rates, miscarriage incidence
Cell-free DNA	● Rare autosomal trisomy 1:310
Chorionic villus sampling	● 1~2% of CVS samples ● 87% confined to the placenta, 13% true mosaicism ● CPM for trisomy 16
Amniocentesis	● 0.2% true mosaicism ● Specific chromosome involved ● Not necessarily guarantee a poor outcome

Mosaicism in human preimplantation embryos

Clinical significance

	Clinical Pregnancy	Ongoing Pregnancy/Live Birth	Miscarriage
MET	40.6% [a]	27.1% [a]	33.3%
Euploid Control	59.1%	47.0%	20.5%
Non-PGTControl	48.4% [b]	35.1% [b]	27.4% [b]

[a], $p < 0.05$ between MET and euploid control; [b], $p < 0.05$ between euploid and non-PGT control.

	Clinical Pregnancy			Ongoing Pregnancy/Live Birth			Miscarriage		
	No. of Embryos	p *	p **	No. of Embryos	p *	p **	No. of Embryos	p *	p **
Euploid	281			223			58		
Mosaic level									
<40%	30	0.10	<0.001	21	0.38	<0.001	9	0.64	0.24
≥40%	25		0.17	16		0.04	9		0.08
<50%	47	0.27	<0.001	30	0.07	<0.001	17	0.19	0.02
≥50%	8		0.66	7		0.99	1		1 ***

* p-value were MET is compared within the MET group; ** p-value were MET is compared to euploid; *** chi-square test by Fisher's exact test.

Genes. 2020, 11, 973

Mosaicism in human preimplantation embryos

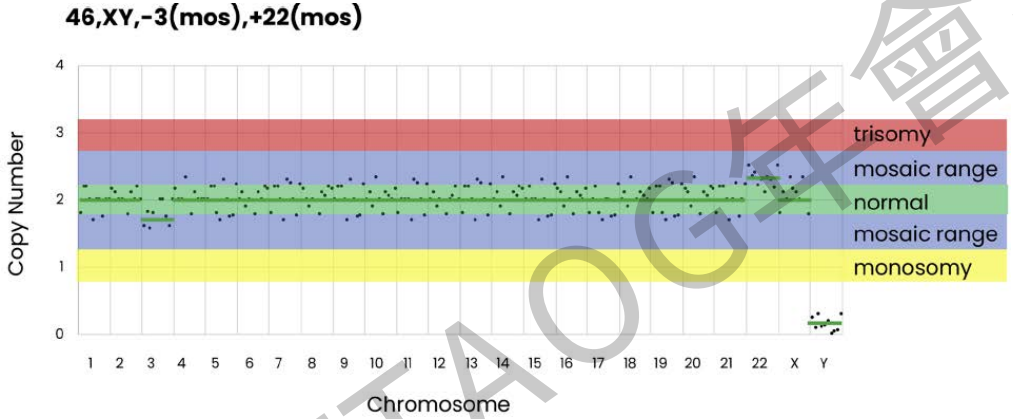
Clinical significance

	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)	

Am J Hum Genet. 2021;108(12):2238-2247

Mosaicism in human preimplantation embryos

Detection



- Cells of biopsy accurately reflect the genome of embryo?
- PGT-A by NGS: intermediate copy number caused by noise/artifact, amplification bias, contamination, mitotic state, variation in biopsy technique, laboratory conditions, laboratory thresholds

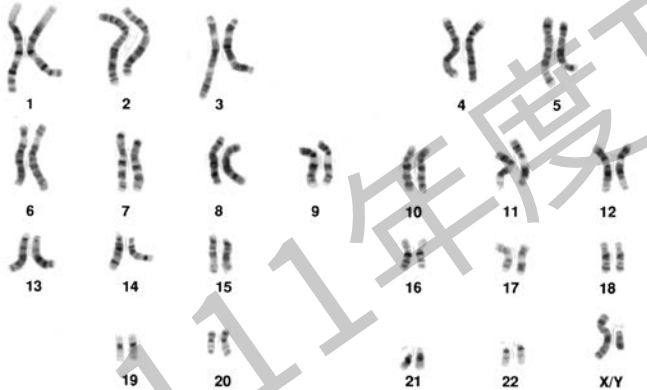
Intermediate copy number cannot predict mosaicism nor outcome

Fertil Steril 2021;116:1205–11

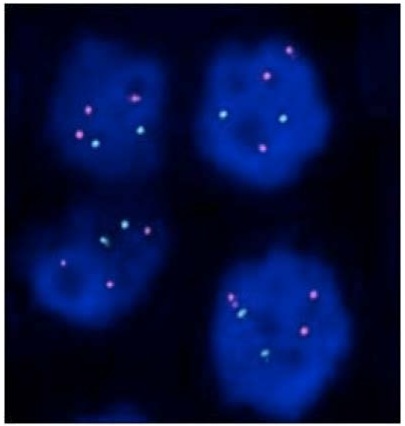
Mosaicism Identified Through Amniotic Fluid Samples

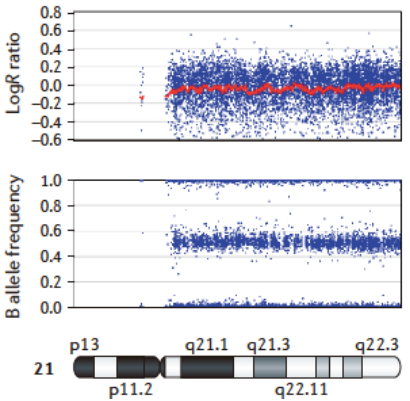
- Amniotic fluid cells:
- 0.2% true mosaicism
- 0.76% level II mosaicism
- High level ultrasound is necessary

Long-term culture



Direct preparation





Mosaicism Identified Through Amniotic Fluid Samples

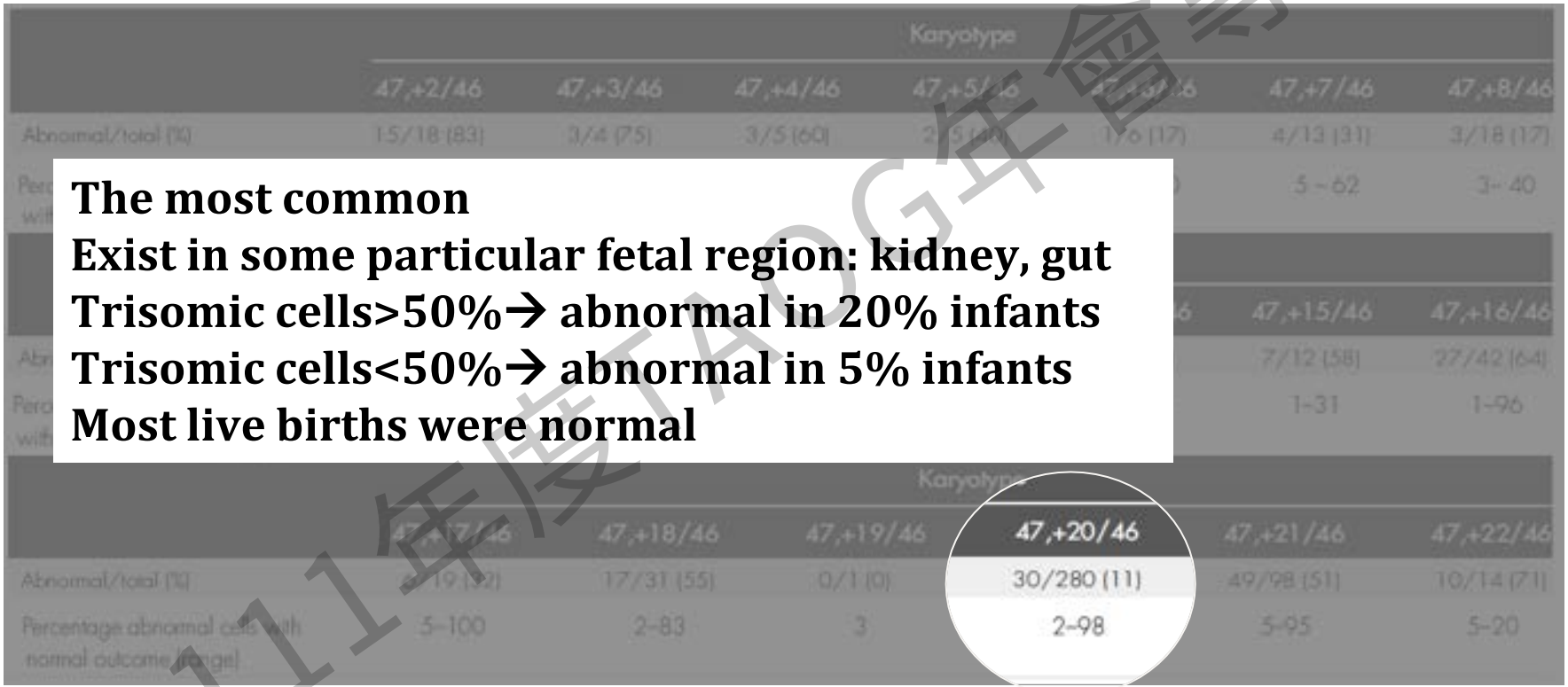
	Karyotype						
	47,+2/46	47,+3/46	47,+4/46	47,+5/46	47,+6/46	47,+7/46	47,+8/46
Abnormal/total (%)	15/18 (83)	3/4 (75)	3/5 (60)	2/5 (40)	1/6 (17)	4/13 (31)	3/18 (17)
Percentage abnormal cells with normal outcome (range)	4 – 16	5	8 – 10	7 – 81	6 – 10	5 – 62	3– 40

	Karyotype						
	47,+9/46	47,+11/46	47,+12/46	47,+13/46	47,+14/46	47,+15/46	47,+16/46
Abnormal/total (%)	19/30 (63)	0/4 (0)	6/28 (21)	10/26 (38)	3/7 (43)	7/12 (58)	27/42 (64)
Percentage abnormal cells with normal outcome (range)	7–84	3–27	2–64	7–82	10–44	1–31	1–96

	Karyotype					
	47,+17/46	47,+18/46	47,+19/46	47,+20/46	47,+21/46	47,+22/46
Abnormal/total (%)	6/19 (32)	17/31 (55)	0/1 (0)	30/280 (11)	49/98 (51)	10/14 (71)
Percentage abnormal cells with normal outcome (range)	5–100	2–83	3	2–98	5–95	5–20

Prenatal Diagnosis 2015, 35, 841–847

Mosaicism Identified Through Amniotic Fluid Samples



The most common
Exist in some particular fetal region: kidney, gut
Trisomic cells>50%→ abnormal in 20% infants
Trisomic cells<50%→ abnormal in 5% infants
Most live births were normal

Prenatal Diagnosis 2015, 35, 841–847

Mosaicism Identified Through Amniotic Fluid Samples

	Karyotype						
	47,+2/46	47,+3/46	47,+4/46	47,+5/46	[illegible]	47,+7/46	47,+8/46
Abnormal/total (%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	3/18 (17)
Percentage abnormal cells with normal outcome (range)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	3–40

	Karyotype						
	47,+9/46	47,+11/46	47,+12/46	47,+13/46	47,+14/46	47,+15/46	47,+16/46
Abnormal/total (%)	19/30 (63)	0/4 (0)	6/28 (21)	10/26 (38)	3/7 (43)	7/12 (58)	27/42 (64)
Percentage abnormal cells with normal outcome (range)	7–84	27	2–64	7–82	10–44	1–31	1–96

	Karyotype					
	47,+17/46	47,+18/46	47,+19/46	47,+20/46	47,+21/46	47,+22/46
Abnormal/total (%)	9/19 (47)	17/31 (55)	0/1 (0)	30/280 (11)	49/98 (51)	10/14 (71)
Percentage abnormal cells with normal outcome (range)	5–100	2–83	3	2–98	5–95	5–20

Very high risk of abnormality
Only 4 normal live birth (5% to 13% mosaicism)

Prenatal Diagnosis 2015, 35, 841–847

Mosaicism Identified Through Amniotic Fluid Samples

	Karyotype						
	47,+2/46	47,+3/46	47,+4/46	47,+5/46	[illegible]	47,+7/46	47,+8/46
Abnormal/total (%)	15/18 (83)	3/4 (75)	3/5 (60)	[illegible]	1/6 (17)	4/13 (31)	3/18 (17)
Percentage abnormal cells with normal outcome (range)	4 – 16	5	8 – 10	7 – 81	6 – 10	5 – 62	3– 40

	Karyotype						
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]5/46	47,+16/46
Abnormal/total (%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] (58)	27/42 (64)
Percentage abnormal cells with normal outcome (range)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1–96

	Karyotype					
	47,+17/46	47,+18/46	47,+19/46	47,+20/46	47,+21/46	47,+22/46
Abnormal/total (%)	6/19 (32)	17/31 (55)	0/1 (0)	30/280 (11)	49/98 (51)	10/14 (71)
Percentage abnormal cells with normal outcome (range)	5–100	2–83	3	2–98	5–95	5–20

Very high risk of abnormality
Only 3 normal live birth (2% to 20% mosaicism)

Prenatal Diagnosis 2015, 35, 841–847

Mosaicism Identified Through Amniotic Fluid Samples

	Karyotype						
	47,+2/46	47,+3/46	47,+4/46	47,+5/46	47,+6/46	47,+7/46	47,+8/46
Abnormal/total (%)	15/18 (83)	3/4 (75)	3/5 (60)	2/5 (40)	1/6 (17)	4/13 (31)	3/18 (17)
Percentage abnormal cells with normal outcome (range)	4–16	5	8–10	7–8	6–10	5–62	3–40

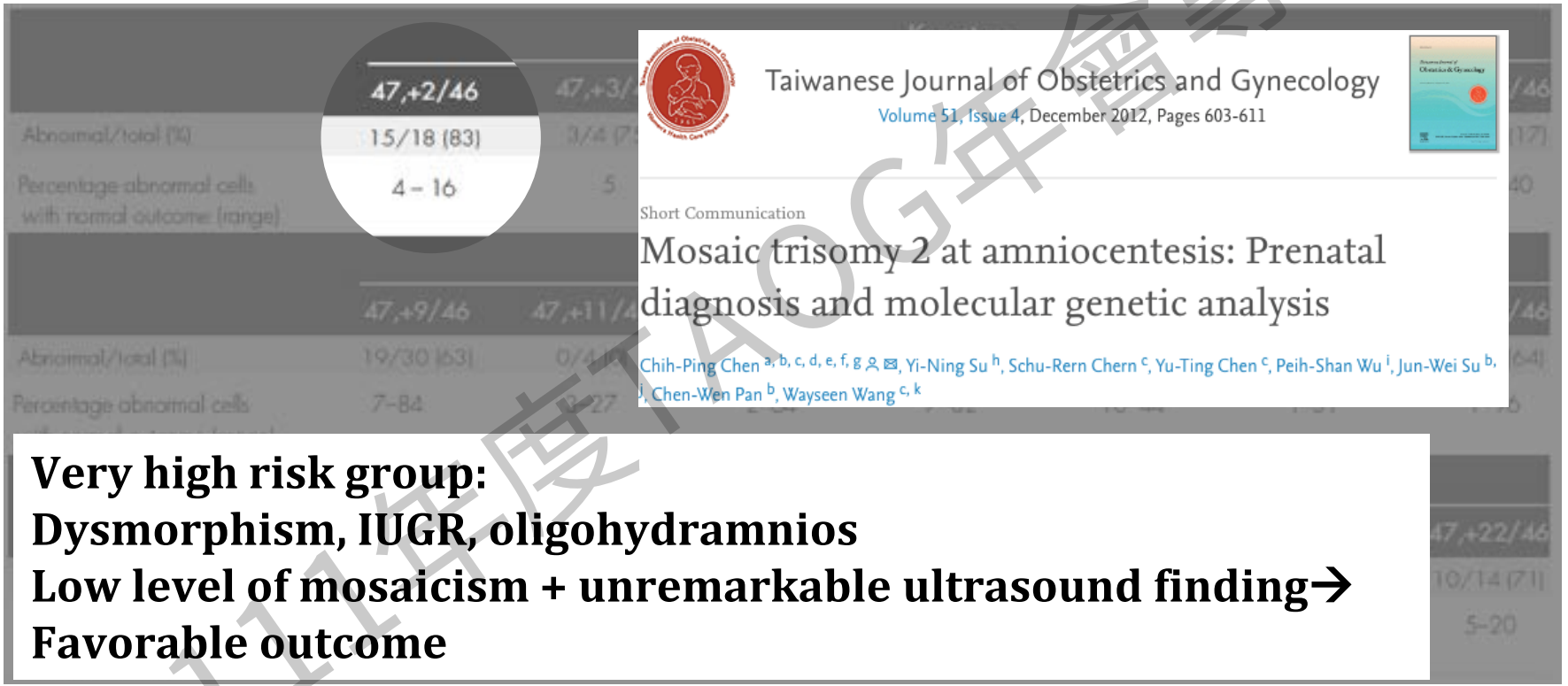
	Karyotype						
	47,+9/46	47,+11/46	47,+12/46	47,+13/46	47,+14/46	47,+15/46	47,+16/46
Abnormal/total (%)	19/30 (63)						27/42 (64)
Percentage abnormal cells with normal outcome (range)	7–84						1–96

	Karyotype					
	47,+17/46	47,+18/46	47,+19/46	47,+20/46	47,+21/46	47,+22/46
Abnormal/total (%)	4/19 (32)	17/31 (55)	0/1 (0)	30/280 (11)	49/98 (51)	10/14 (71)
Percentage abnormal cells with normal outcome (range)	5–100	2–83	3	2–98	5–95	5–20

Very high risk for Down syndrome

Prenatal Diagnosis 2015, 35, 841–847

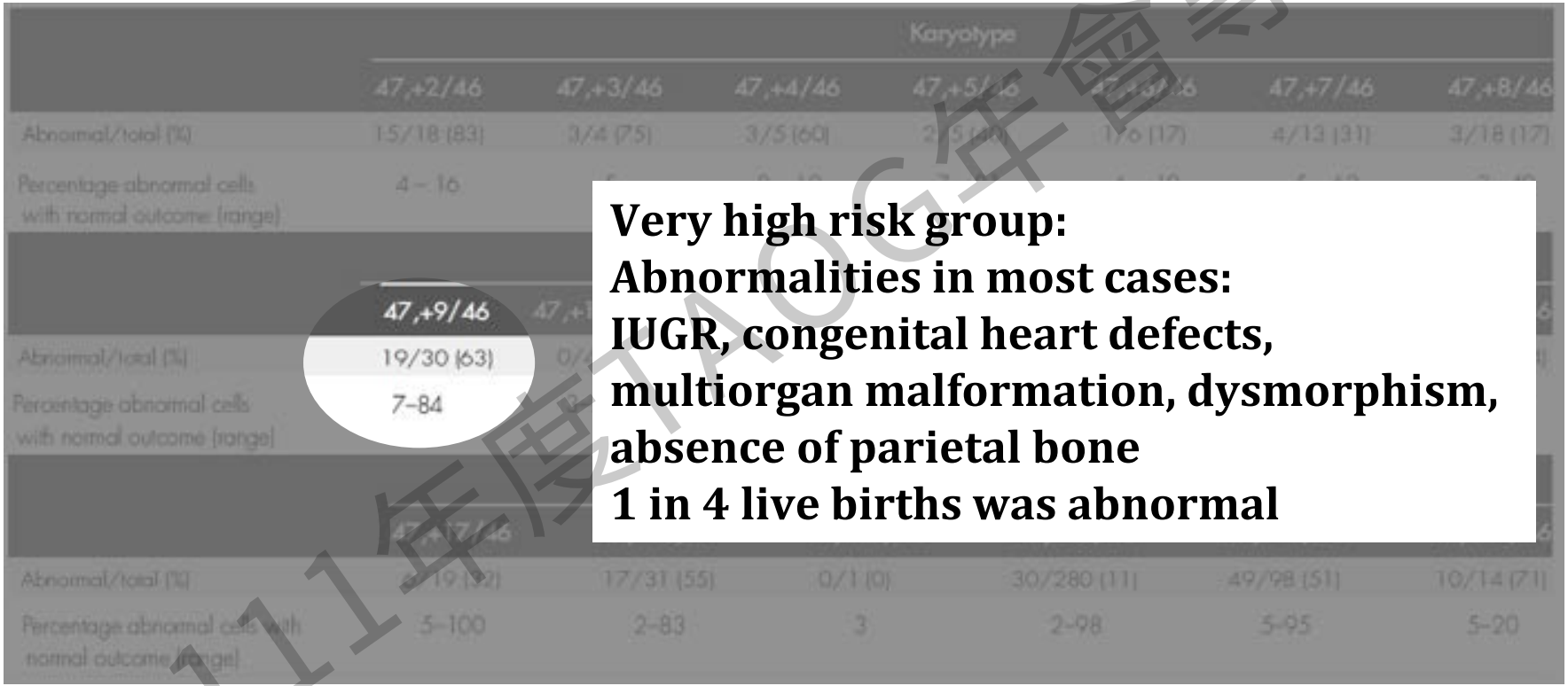
Mosaicism Identified Through Amniotic Fluid Samples



Very high risk group:
Dysmorphism, IUGR, oligohydramnios
Low level of mosaicism + unremarkable ultrasound finding→ Favorable outcome

Prenatal Diagnosis 2015, 35, 841–847

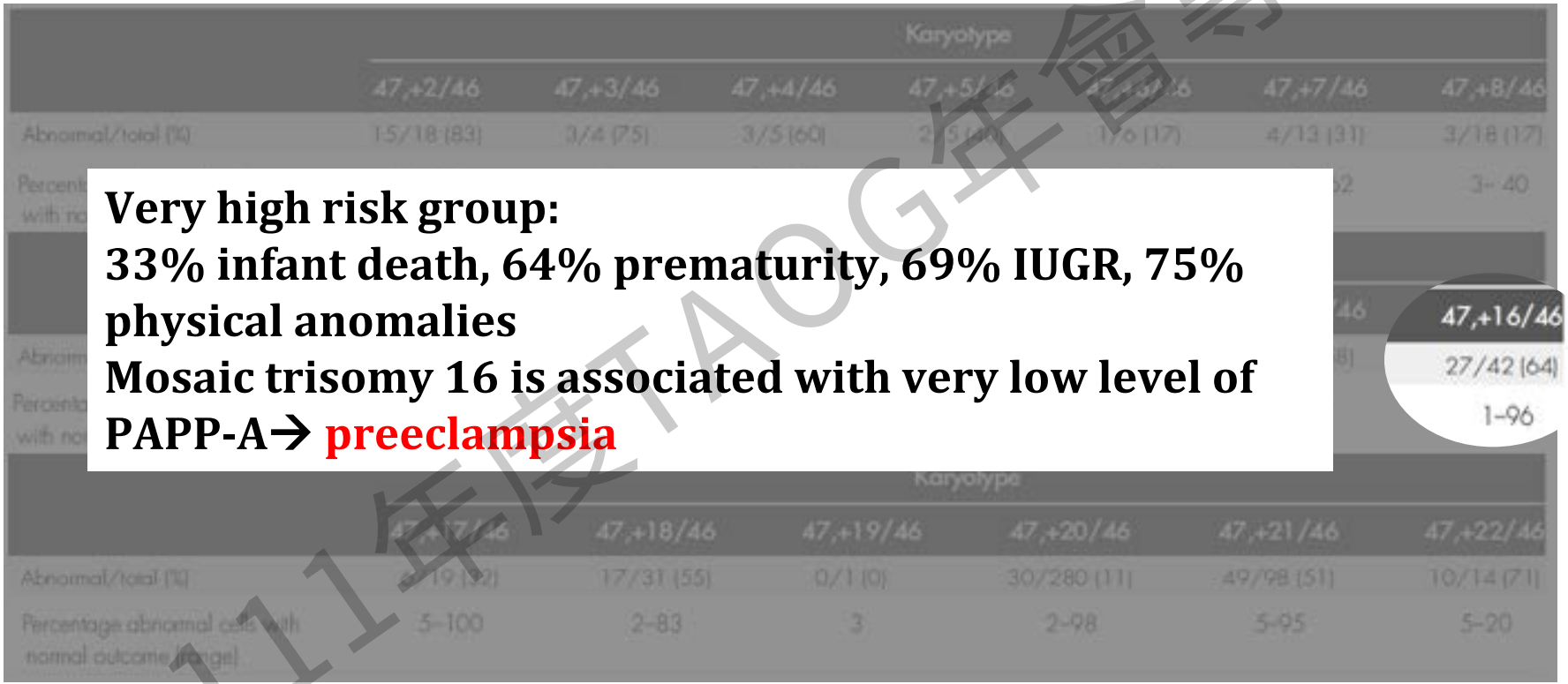
Mosaicism Identified Through Amniotic Fluid Samples



Very high risk group:
Abnormalities in most cases:
IUGR, congenital heart defects, multiorgan malformation, dysmorphism, absence of parietal bone
1 in 4 live births was abnormal

Taiwanese Journal of Obstetrics & Gynecology 59 (2020) 786e78
Prenatal Diagnosis 2015, 35, 841–847

Mosaicism Identified Through Amniotic Fluid Samples



Very high risk group:
33% infant death, 64% prematurity, 69% IUGR, 75% physical anomalies
Mosaic trisomy 16 is associated with very low level of PAPP-A→ preeclampsia

Prenatal Diagnosis 2015, 35, 841–847

Mosaicism Identified Through Amniotic Fluid Samples

	Karyotype						
	47,+2/46	47,+3/46	47,+4/46	47,+5/46	47,+6/46	47,+7/46	47,+8/46
Abnormal/total (%)	15/18 (83)	3/4 (75)	3/5 (60)	2/5 (40)	1/6 (17)	4/13 (31)	3/18 (17)
Percentage abnormal cells with normal outcome (range)	4 – 16	5	8 – 10	7–8	6 – 10	5 – 62	3– 40

	Karyotype						
	47,+9/46	47,+11/46	47,+12/46	47,+13/46	47,+14/46	47,+15/46	47,+16/46
Abnormal/total (%)	19/30 (63)	0/4 (0)	6/28 (21)	10/26 (38)	3/7 (43)	7/12 (58)	27/42 (64)
Percentage abnormal cells with normal outcome (range)	7–84	7–27	2–64	7–82	10–44	1–31	1–96

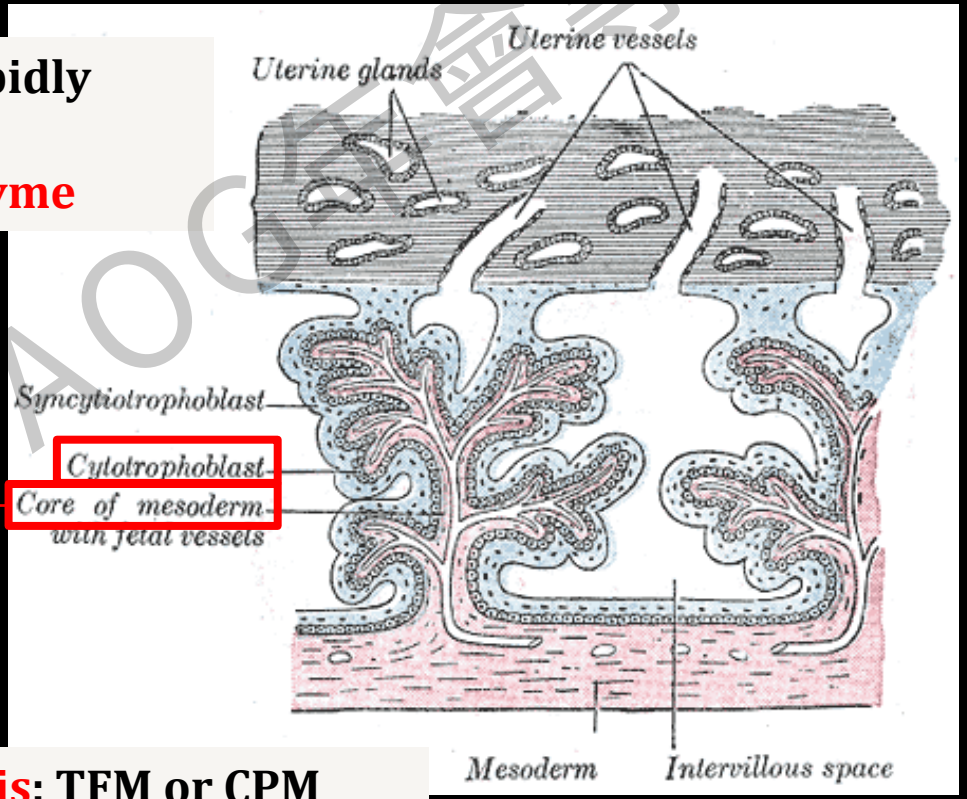
	Karyotype					
	47,+17/46	47,+18/46	47,+19/46	47,+20/46	47,+21/46	47,+22/46
Abnormal/total (%)	4/19 (32)	17/31 (55)	0/1 (0)	30/280 (11)	49/98 (51)	10/14 (71)
Percentage abnormal cells with normal outcome (range)	5–100	2–83	3	2–98	5–95	5–20

Prenatal Diagnosis 2015, 35, 841–847

Mosaicism Identified Through Chorionic Villus Samples

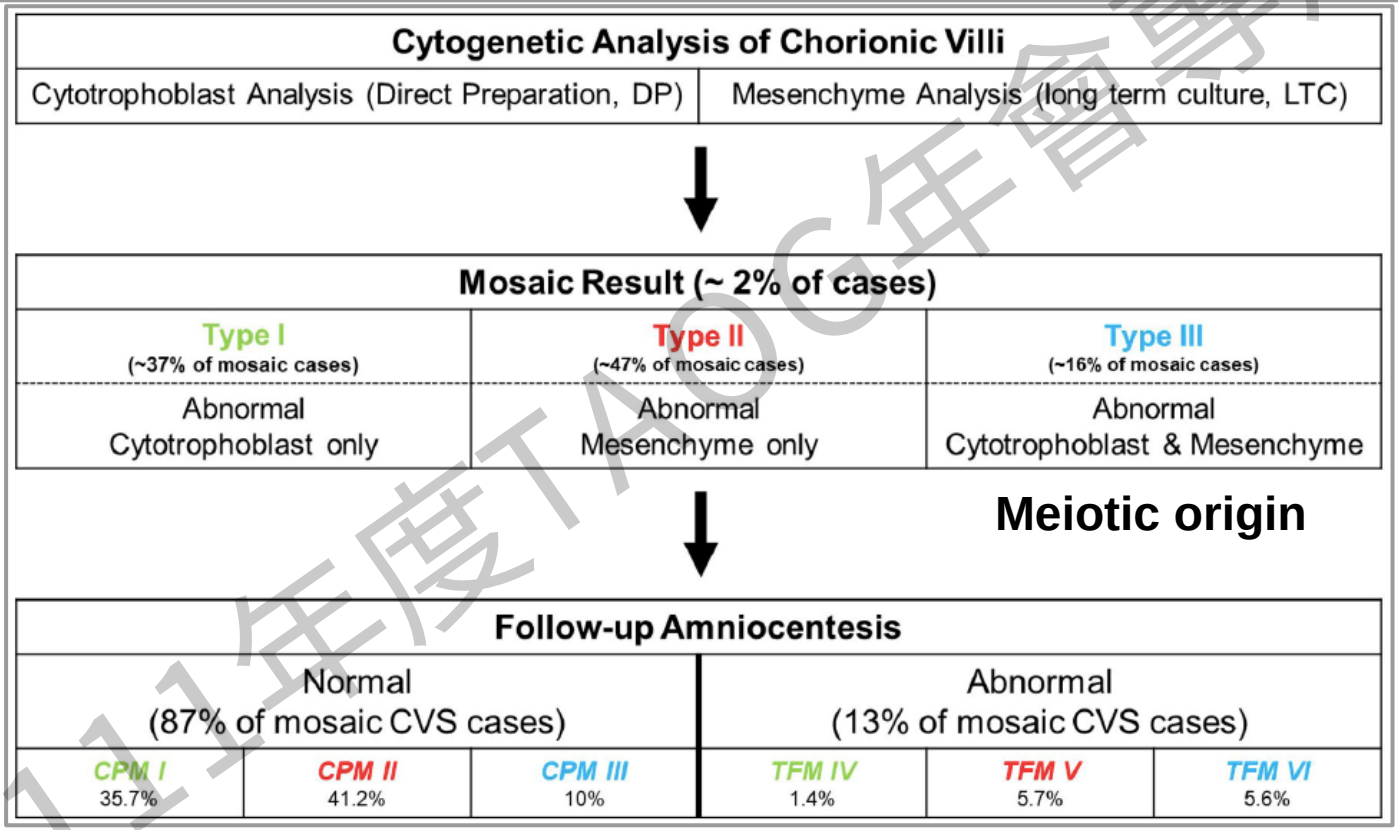
Direct preparation (DP): reflect rapidly dividing **cytotrophoblast**
Long-term culture (LTC): **mesenchyme**

More closely related to fetus



Mosaicism from CVS→ amniocentesis: TFM or CPM

Mosaicism Identified Through Chorionic Villus Samples



Ultrasound Obstet Gynecol 2019; 54: 458–467

Mosaicism Identified Through Chorionic Villus Samples

Confined Placenta Mosaicism

Incompatible with life trisomy: CPM→ more cell counts & FISH study & CMA on uncultured cells

birthweight, NICU rate, hypertensive disorder, preterm birth, Apgar score: no difference

Exception:

Trisomy 16 CPM→ fetal malformation, IUFD, IUGR, preeclampsia, preterm birth↑

Genet Med. 2020;22(2):309–316
Genet Med. 2020;22(2):446–447
Prenatal Diagnosis. 2018;38:1103–1110

Mosaicism & Non-invasive Prenatal Testing Using Cell-free DNA

- Derived from both the mother and apoptotic trophoblasts
- Non-invasive prenatal testing: sometimes shows apparent mosaicism
  - Incompatible with viability: strongly suspected
- Rare Autosomal Trisomy (RAT):
  - overall incidence 1: 310
  - 1:91 in high risk group; 1:556 in general risk group
  - Most common 16, 22, 15
- primary outcomes:
  - 90~94% CPM
  - RAT in high proportion: miscarriage
  - Viable pregnancy but confirmed RAT in amniocentesis: variable phenotypes

Study	Fetal loss	Confirmed abnormality	Abnormal phenotype	Significant UPD	FGR or low birth weight	Apparently normal live birth
Fiorentino (2017)	7	3	0	1	0	6
Pertile (2017)	26	5	1	1	2	14
van Opstal (2018)	0	3	5	0	8	9
Scott (2018)	6	2	5	0	6	9
Wan (2018)	2	1	0	0	Not known	18
Chatron (2019)	0	0	0	1	3	6
Total	41	14	11	3	19	62
% (95% CI) of cases with known outcome (n=151)	27.2 (20.7-34.7)	9.3 (5.6-15.0)	7.3 (4.1-12.6)	2.0 (0.7-5.7)	14.6 (9.6-21.7)	41.1 (33.5-49.0)
Rate in all women tested (1/n)	1,753	5,135	6,536	23,964	2,975ø	1,160

Ultrasound Obstet Gynecol. 2019;54(4):458–467.

Take Home Message

- True fetal mosaicism is rare but doesn't always cause poor outcome
- Chromosome mosaicism is found in amniocentesis→ consider direct preparation for FISH or CMA
- Chromosome 6, 7, 11, 14, 15, 20→ exclude UPD
- Proportion, distribution and specific chromosome involved of mosaicism→ phenotype
- High-level ultrasound is necessary

Thanks for Your Listening