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Enhanced clinical utility of a concurrent preimplantation genetic testing by integrating the detection of triploidy and uniparental disomy

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Disclosure of interests

- Department of O&G, CUHK received service fee from PGT result interpretation service
- The CUHK prenatal diagnosis lab. provides diagnostic test support to Basecare in Asia.
- Consultant for Basecare Medical Device Co Ltd (Hong Kong) and INEX (Singapore)

Outline

- Why need a All in one testing (PGT-plus)
- Introduce the concurrent PGT-A/-M/-SR testing platform
- Clinical utility of All in one (PGT-plus) in CUHK

Pan-ethnic expanded carrier screening uncover at risk couples (1% to 20%)

genes

MDPI

Article

Clinical Implementation of Expanded Carrier Screening in Pregnant Women at Early Gestational Weeks: A Chinese Cohort Study

Mengmeng Shi [1,†], Angeline Linna Liauw [1,†], Steve Tong [1], Yu Zheng [1], Tak Yeung Leung [1,2], Shuk Ching Chong [1,2,3], Ye Cao [1,2,3,4], Tze Kin Lau [5], Kwong Wai Choy [1,2,4,5,*] and Jacqueline P. W. Chung [1,*]

Han Brunner

Original Article

Obstetric Medicine

Thalassemia screening by third-generation sequencing: Pilot study in a Thai population

Obstetric Medicine
1–7
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Kuntharee Traisrisilp[1], Yu Zheng[2], Kwong Wai Choy[2] and Pimlak Chareonkwan[3]

Pan-ethnic expanded carrier screening uncover at risk couples

At risk couple 4/103=3.8%

No. couple screened since May 2023	Gene Panel (~300 monogenic diseases)	Chromoseq + Limited karyotyping
Tested	475	471
Affected	94 (19.8%) (some couples may carried more than 1 diseases)	15 (3.2%)
Findings	Significant: 22 (4.6%) • 1*SMA • 1*CAH • 1*Pendred synd • 9*GJB2 + other pathogenic mutation (mild to severe deafness)	Significant: 15 (3.2%) • 1*Robertsonian trans • 4*Reciprocal trans • 3*Mosaic monosomy X • 4*Pathogenic CNV • 3*Clinical signif SV
	• 8*alpha-thal (8 Barts) • 2*beta-thal (2 major)	
	Others: 77 (16.2%) • 6*alpha-thal, 1 beta-thal (intermed) • 26*GJB2; 44*G6PD	

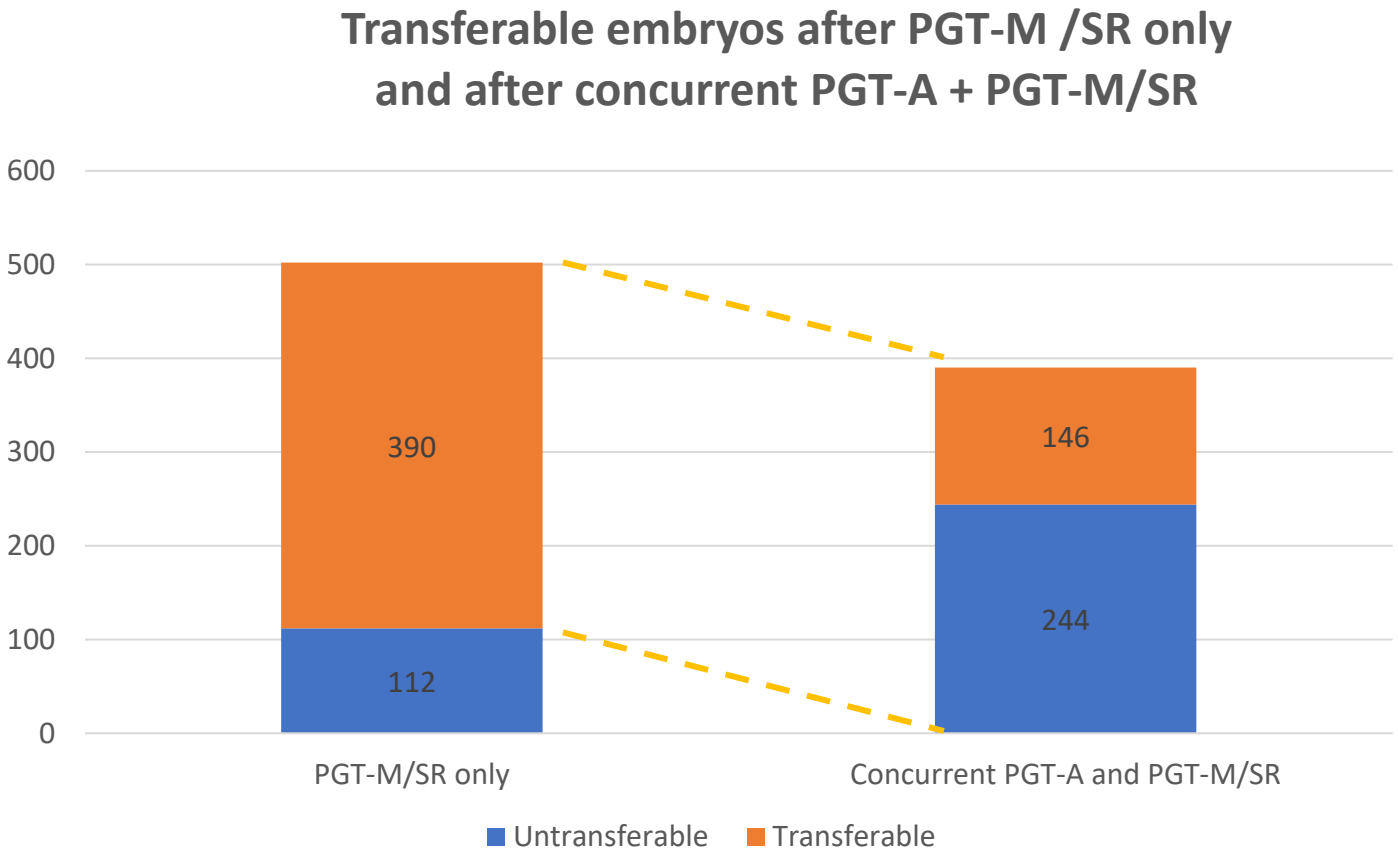
Shi et al. Genes. 2021

A couple with monogenic disorder come for PGT-M…

1. Should we offer concurrent PGT-A for PGT-M/-SR?

2. Why and what should we offer?

Why concurrent PGT: A large proportion of unaffected embryos can be aneuploid.



Data: from 2015-2021
CUHK PWH PGD Lab

PGT-M only: 77.7% (390/502) are transferrable.

Concurrent PGT-M+PGT-A: only 29.1% (146/502) are transferrable.

Is it possible to combine PGT-M/SR/A in ONE experimental procedure?

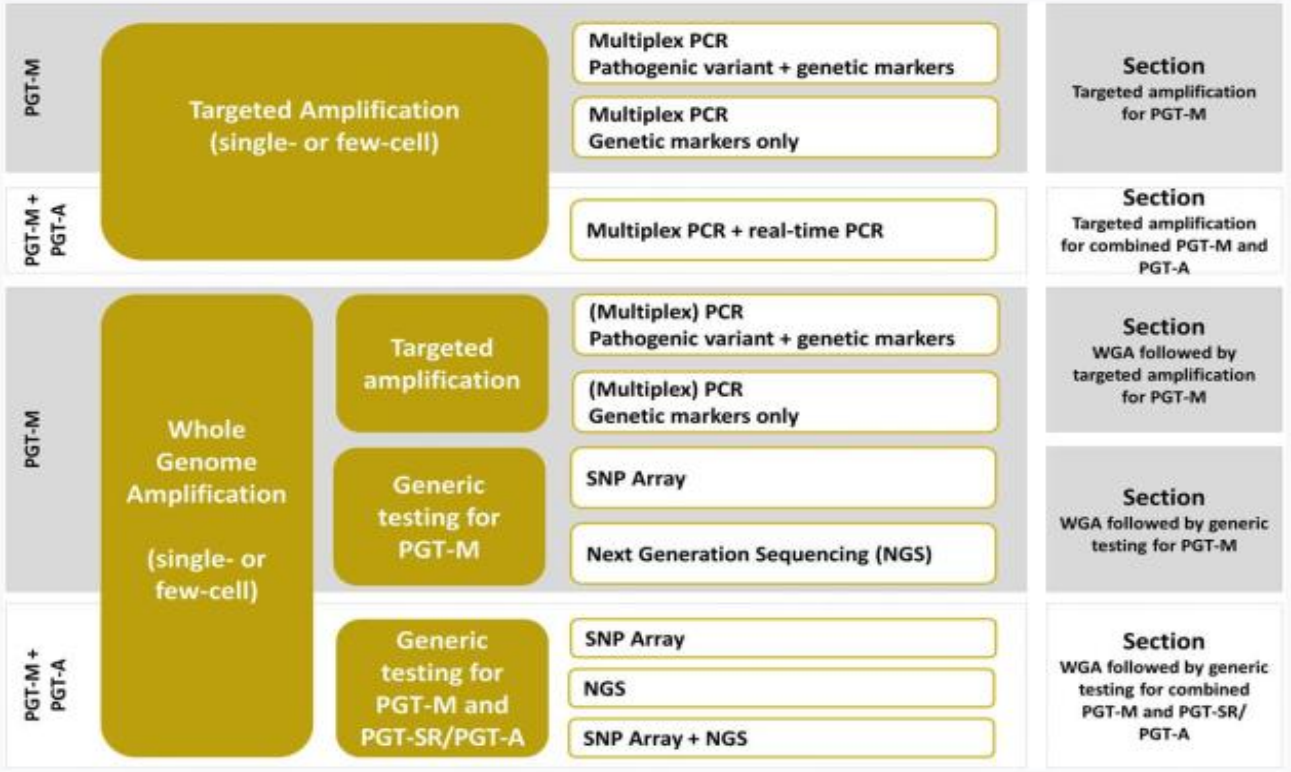
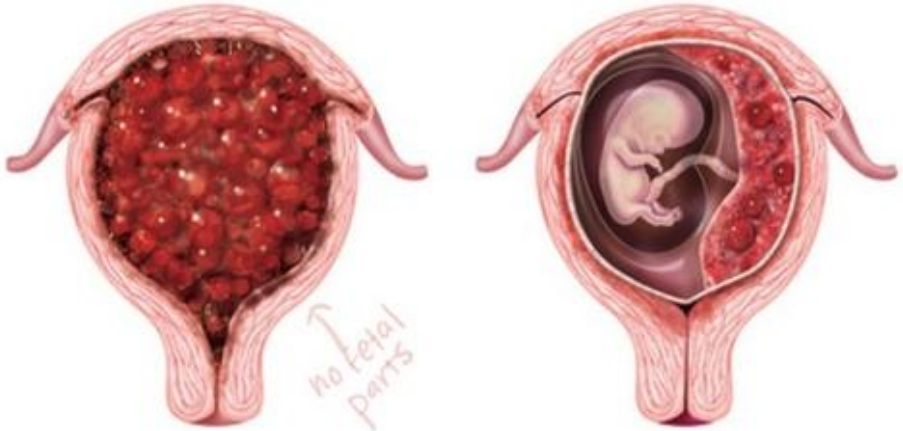


Figure 2 Overview of the testing strategies that can be applied for PGT-M. PGT-M: PGT for monogenic/single-gene defects, PGT-A: PGT for aneuploidy, PGT-SR: PGT for chromosomal structural rearrangements, SNP: single nucleotide polymorphism, NGS: next-generation sequencing.

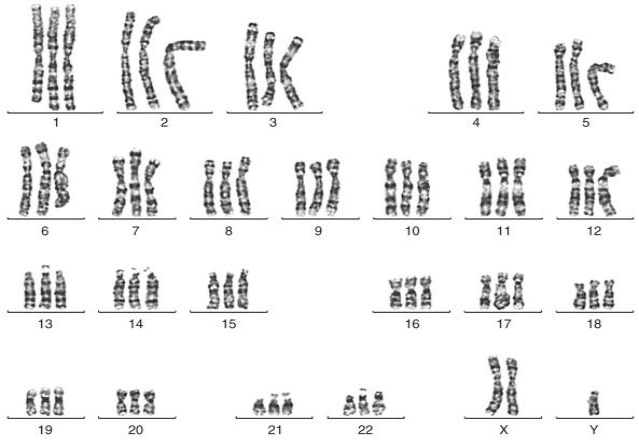
Key issue I:
Triploidy is underappreciated in PGT while important in pregnancy

- **Incidence**: 0.474% in blastocysts [1], ~9% in early pregnancy loss [2].
- **Undetectable** by routine CNV analysis-based NGS platforms, especially 69,XXX.

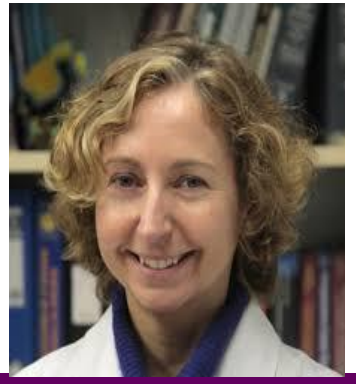
- **Diandric triploidy** leads to partial hydatidiform mole (**PHM**), which is a pre-malignant presentation of gestational trophoblastic disease (GTD) [3].



Triploidy (69,XXY)
not detectable by PGT-A



0.8% (16/1982 TE)

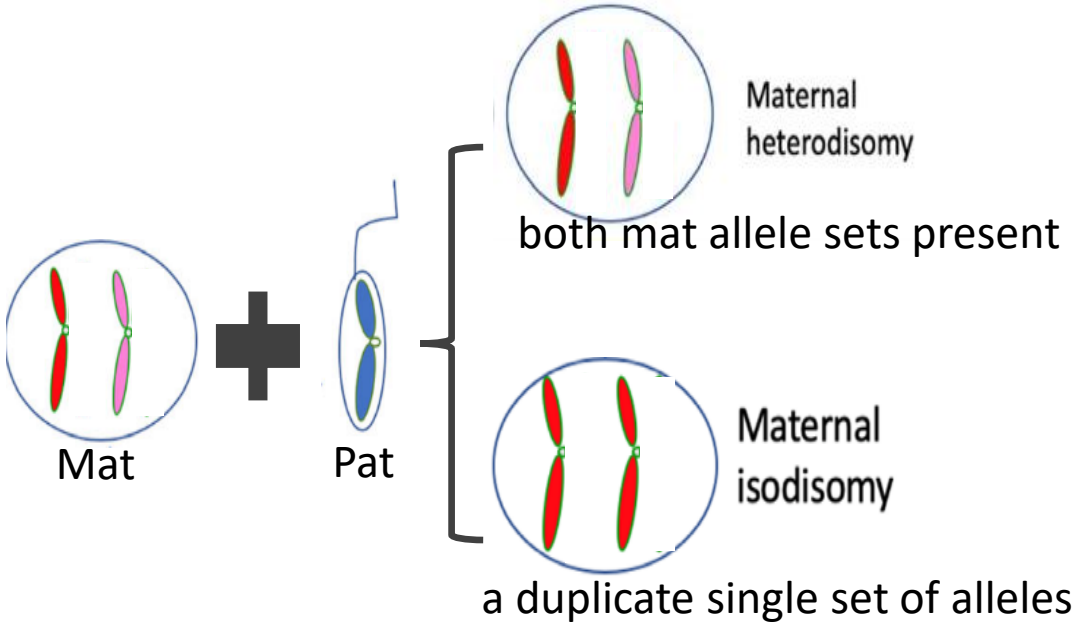


[1] Marin D et al., *Reprod. Biomed. Online*, 2019
[2] T Zhang et al., *BJOG*, 2018
[3] Pan M et al., *J Matern Neonatal Med*, 2021

Key Issue II:
UPD contributes to birth defects and early miscarriages

- **UPD**: two copies of a whole chromosome derived from **the same parent** [1].

- **Incidence**: 3.7%
- (9/241) in morphologically abnormal embryos [2]; 2% (12/610) in euploid POCs [3].
- **Clinical consequence**:
  1) activate a **recessive** diseases [4].
  2) UPD can be a cause of **early miscarriage** if localizeyd to regions with imprinted genes that control embryogenesis and fetal development [4].



[1] P. Benn et.al., *Prenat Diagn*, 2021
[2] Xu et al., Sci. Rep, 2015
[3] Gu et al., J Assist Reprod Genet, 2022
[4] I. Lalou et al., *Eur. J. Obstet. Gynecol*. 2019

We need a PGT platform to detect genome-wide UPD

TABLE 1 Clinical syndromes or phenotypes associated with uniparental disomy

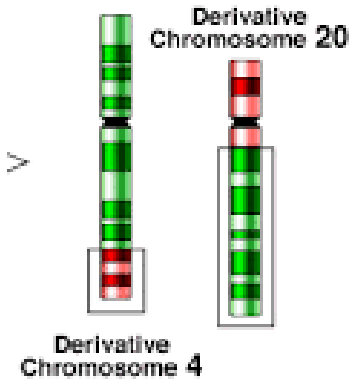
Chromosome region	Disomy	Genes involved	Disorder name	Phenotype	OMIM[a]
6q24.2	Paternal	*PLAGL1*, *HYMA1*	Diabetes mellitus, transient neonatal 1	Transient diabetes mellitus, macroglossia, type 2 diabetes	601410
7q32.2	Maternal	*MEST*	Silver-Russell syndrome 2	Prenatal and postnatal growth restriction, asymmetry, relative macrocephaly	618905
11p15 (mosaic)	Paternal	*H19*, *IGF2*; *CDKN1C*, *KCNQ1*, *KCNQ10T1*	Beckwith-Wiedemann syndrome	Overgrowth, cancer predisposition	130650
11p15 (mosaic)	Maternal	*H19*, *IGF2*	Silver-Russell syndrome 3	Prenatal and postnatal growth restriction, asymmetry, relative macrocephaly	616489
14q32.2	Maternal	*DLK1*, *RTL1*, *DIO3*; *GTL2*, *MEG3*, *MEG8*, *RTL1as*, various ncRNAs, miRNAs, snoRNAs	Temple syndrome	Prenatal and postnatal growth restriction, hypotonia, motor delay, hyperextensible joints, precocious puberty, obesity	616222
14q32.2	Paternal	*RTL1*, *MEG*	Kagami–Ogata syndrome	Skeletal abnormalities, omphalocele, thoracic dysplasia, respiratory failure, developmental delay, facial abnormality	608149
15q11.2-q13	Maternal	*MKRN3*, *MAGEL2*, *NDN*, *SNRPN*, *snoRNAs*	Prader–Willi syndrome	Neonatal hypotonia, failure to thrive, developmental delay, obesity, hypogonadism, behavior problems	176270
15q11.2-q13	Paternal	*UBE3A*	Angelman syndrome	Intellectual disability, ataxia, absent speech, microcephaly, paroxysmal laughter	105830
20	Maternal		Mulchandani–Bhoj–Conlin syndrome	Severe short stature, severe feeding difficulty	617352
20q13.32	Paternal	*GNAS*, *STX*	Pseudohypoparathyrodism, type 1	Hypocalcemia, hyperphosphatemia, osteitis fibrosa cystica.	603233

[1] P. Benn et.al., *Prenat Diagn*, 2021
[2] Xu et al., Sci. Rep, 2015
[3] Gu et al., J Assist Reprod Genet, 2022
[4] I. Lalou et al., *Eur. J. Obstet. Gynecol*. 2019

Preimplantation genetic testing for structural rearrangements by genome-wide SNP genotyping and haplotype analysis: a prospective multicenter clinical study

Shuo Zhang,[a,b,c,aa] Yuan Gao,[d,e,f,g,h,i,aa] Xiaohong Wang,[j,aa] Qing Li,[k,aa] Jichun Tan,[l,m,aa] Bo Liang,[n,aa] Ming Gao,[d,e,f,g,h,i,aa] Junping Wu,[a,b,c] Xiufeng Ling,[o] Jiayin Liu,[p] Xiaoming Teng,[q] Hong Li,[r] Yun Sun,[s,t] Weidong Huang,[u] Xianhong Tong,[v] Caixia Lei,[a] Hongchang Li,[d,e,f,g,h,i] Jun Wang,[j] Shaoying Li,[k] Xiaoyan Xu,[l,m] Junqiang Zhang,[o] Wei Wu,[p] Shanshan Liang,[q] Jian Ou,[r] Qiongzhen Zhao,[u] Rentao Jin,[v] Yueping Zhang,[a,s] Chenming Xu,[a,w] Daru Lu,[x,y] Junhao Yan,[d,e,f,g,h,i] Xiaoxi Sun,[a,b,c] Kwong Wai Choy,[z] Congjian Xu,[a,b,c,w,*] and Zi-Jiang Chen[d,e,f,g,h,i,s,t,**]

[a]Shanghai Ji Ai Genetics & IVF Institute, Obstetrics and Gynecology Hospital of Fudan University, Shanghai, 200011, China



Rates of euploid embryos:

30.94% in reciprocal translocation group

51.79% in Robertsonian translocation group

47.26% in inversion carriers

57.63% in insertions

- SNP-haplotyping method is highly accurate, and can be applied universally to different BCR types

PGT-SR: Important to detect non-carrier embryos

To prevent the transmission of BCRs to their offspring can reduce the same associated risks of infertility when reaching reproductive age

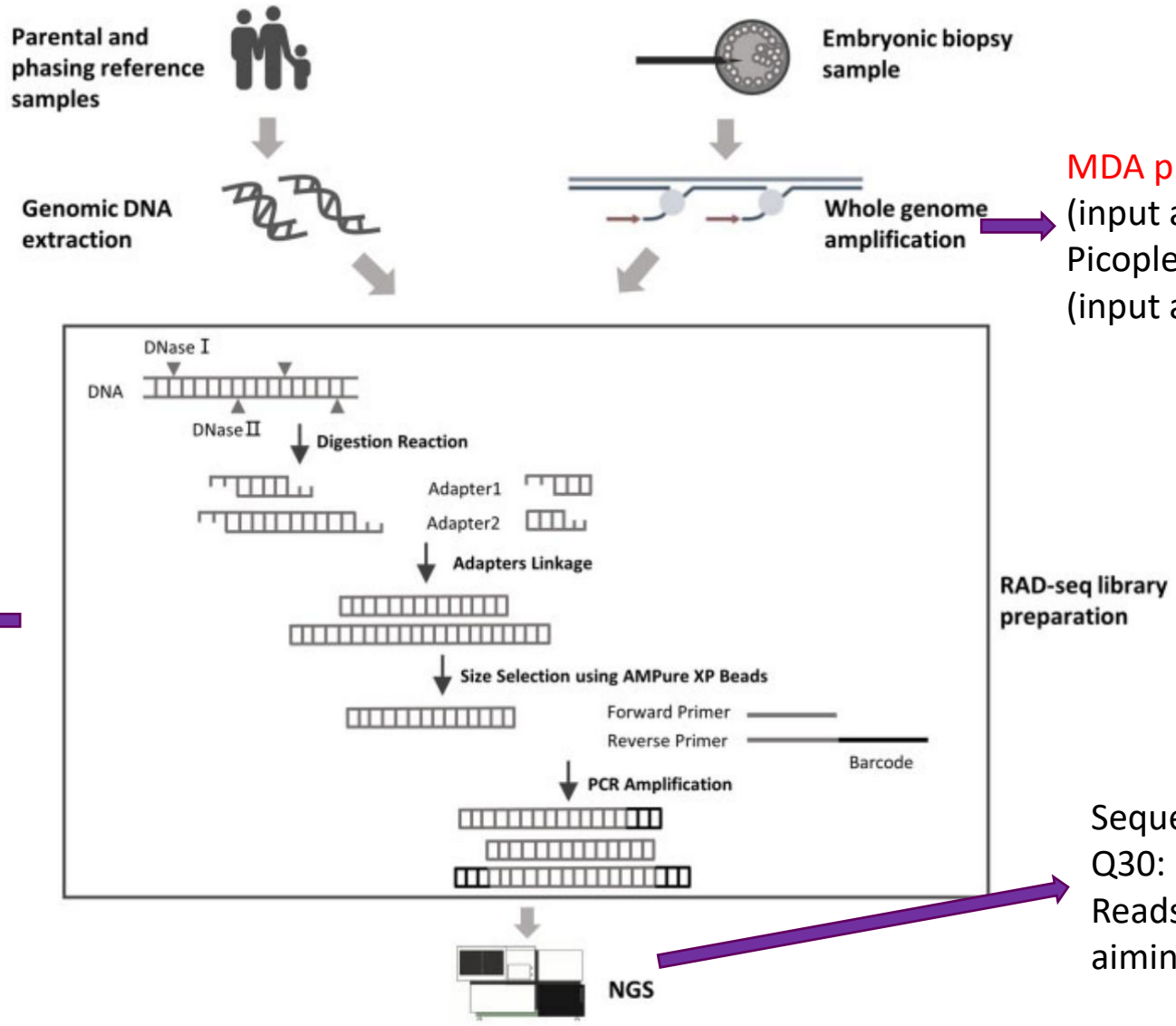
Euploid

Rearrangement type	Unbalanced rearrangements	De novo aneuploidies[a]	Complex abnormalities[b]	Non-carrier embryos	Carrier embryos	Total embryos
Reciprocal translocation	2657 (41.52%)	881 (13.77%)	882 (13.78%)	1029 (16.08%)	951 (14.86%)	6400
Robertsonian translocation	209 (18.25%)	249 (21.75%)	94 (8.21%)	289 (25.24%)	304 (26.55%)	1145
Inversion	17 (11.64%)	56 (38.36%)	4 (2.74%)	35 (23.97%)	34 (23.29%)	146
Insertion translocation[c]	9 (15.25%)	9 (15.25%)	7 (11.86%)	14 (23.73%)	20 (33.90%)	59
Total	2892 (37.32%)	1195 (15.42%)	987 (12.74%)	1367 (17.64%)	1309 (16.89%)	7750

[a]These de novo aneuploidies included 401 mosaic embryos with whole or segmental chromosomes. [b]The complex abnormalities result was defined as a combination of unbalanced rearrangements and one or more of the following features: monosomy, trisomy, segmental aneuploidy, or chromosomal mosaic. [c]For the small sample size in insert translocation subgroup, bias of carrier and non-carrier distribution was inevitable compared to the theoretical 50:50.

Table 2: The PGT-SR results of tested blastocysts.

All in One PGT (PGT-M/SR + PGT-A + PGT-HLA): PGT-Plus platform



MDA product: ~10 kb, conc > 29.4 ng/ul
(input amount: 500ng in 17ul mix; min: 200ng)
Picoplex product and gDNA: conc > 11.8 ng/ul
(input amount: 200ng in 17ul mix; min: 100ng)

QC standard:
conc > 0.6ng/ul
(by qubit)

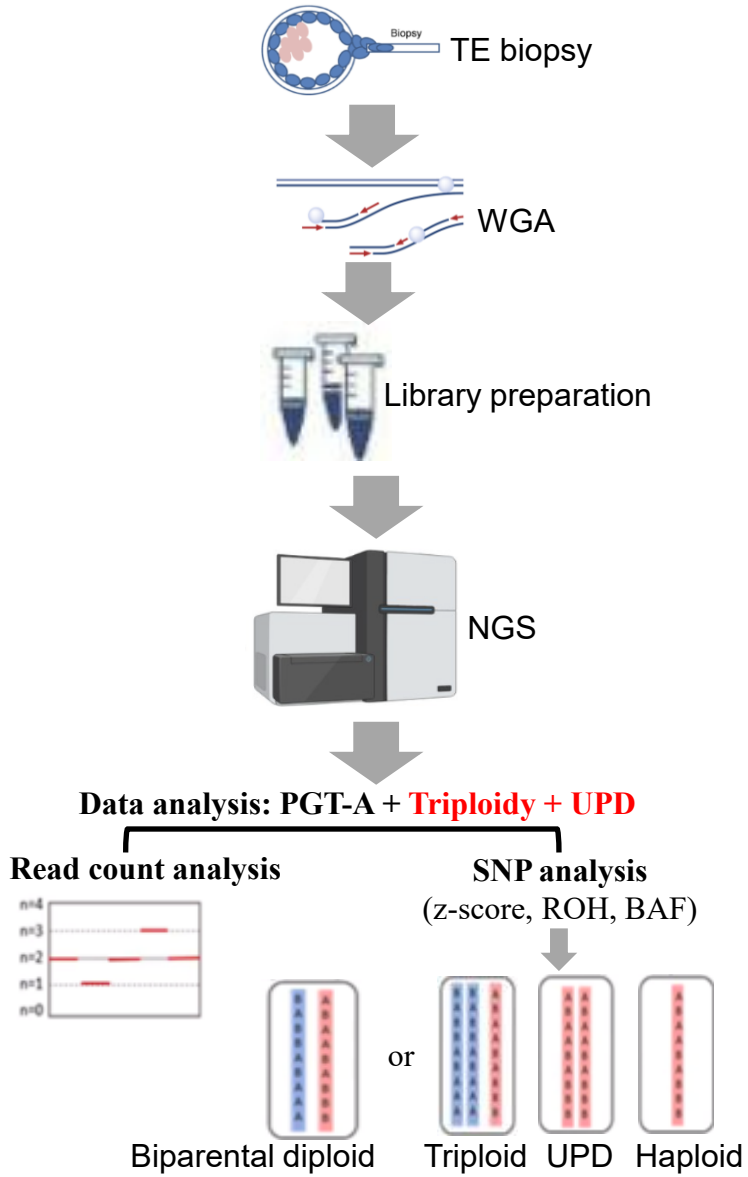
RAD-seq library preparation

Genea BIOMEDX

Sequencing platform: MGI2000 (PE100).
Q30: > 85%.
Reads amount: > 400 M per lane.
aiming a > 80M paired-end reads/sample

Xie P et.al., *Hum Reprod*, 2022

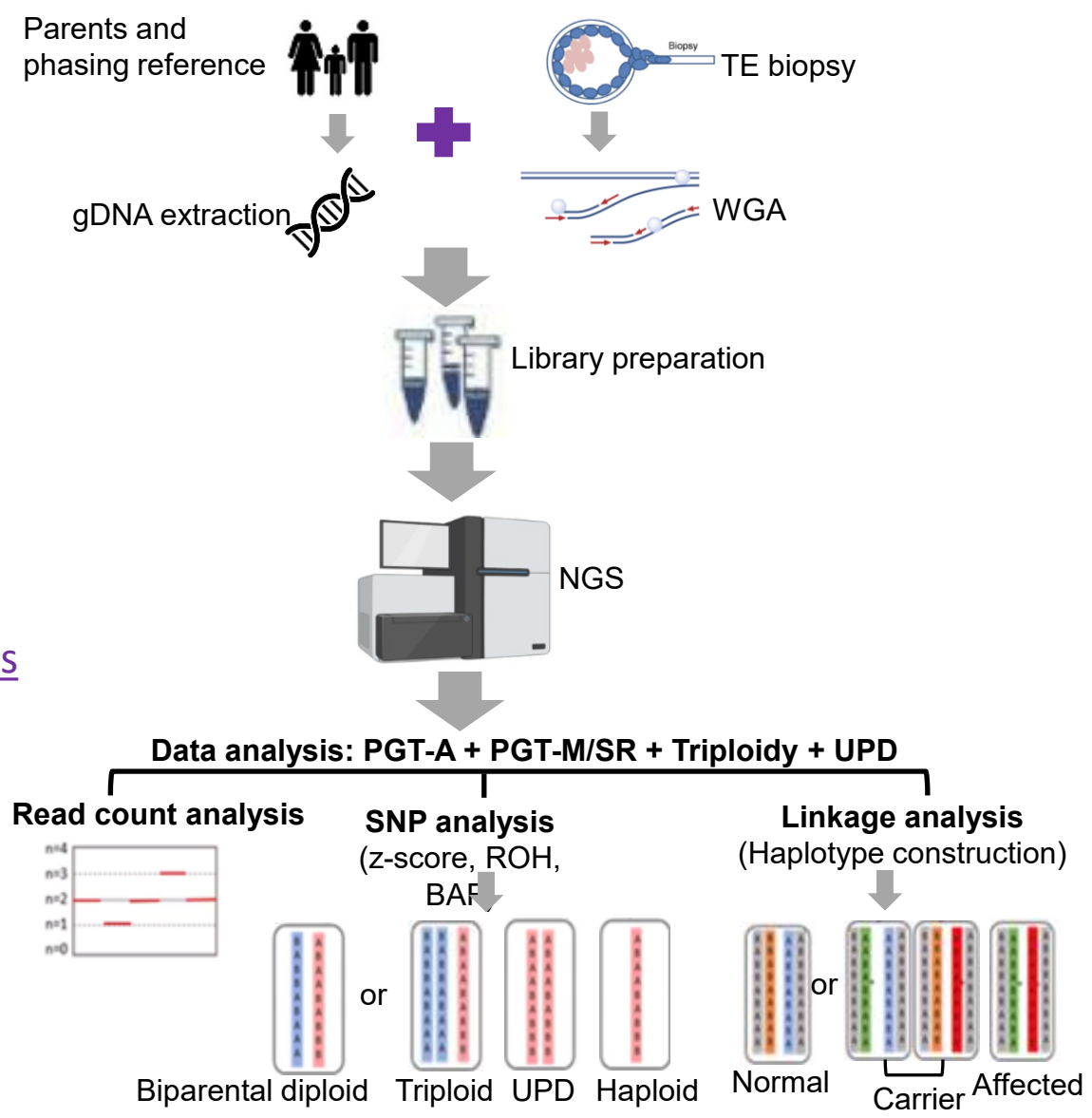
Advantage of All in One PGT platform



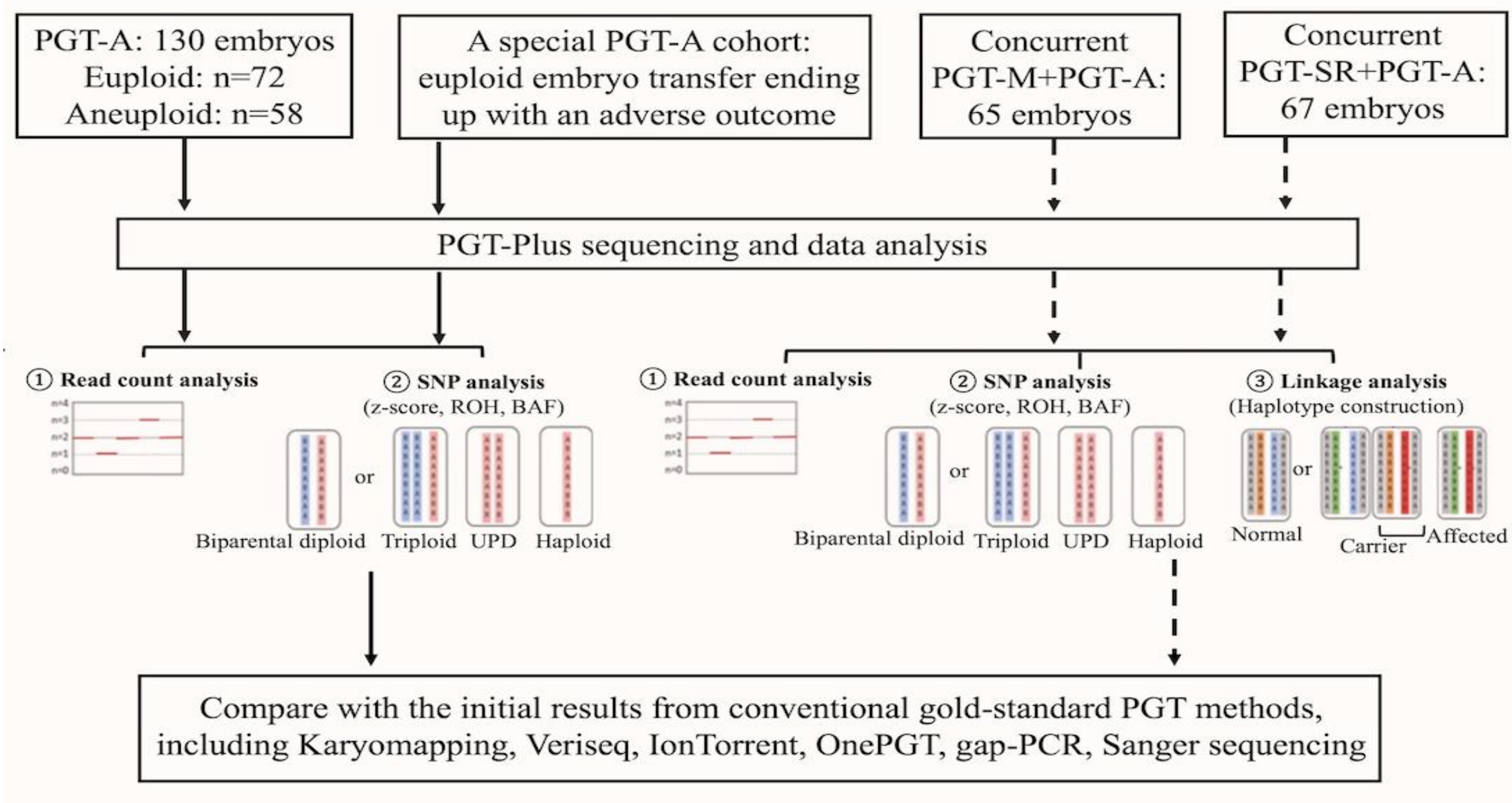
PGT-A only

Vs

All in One PGT-Plus testing



Phase II: Retrospective clinical validation (leftover WGA products)

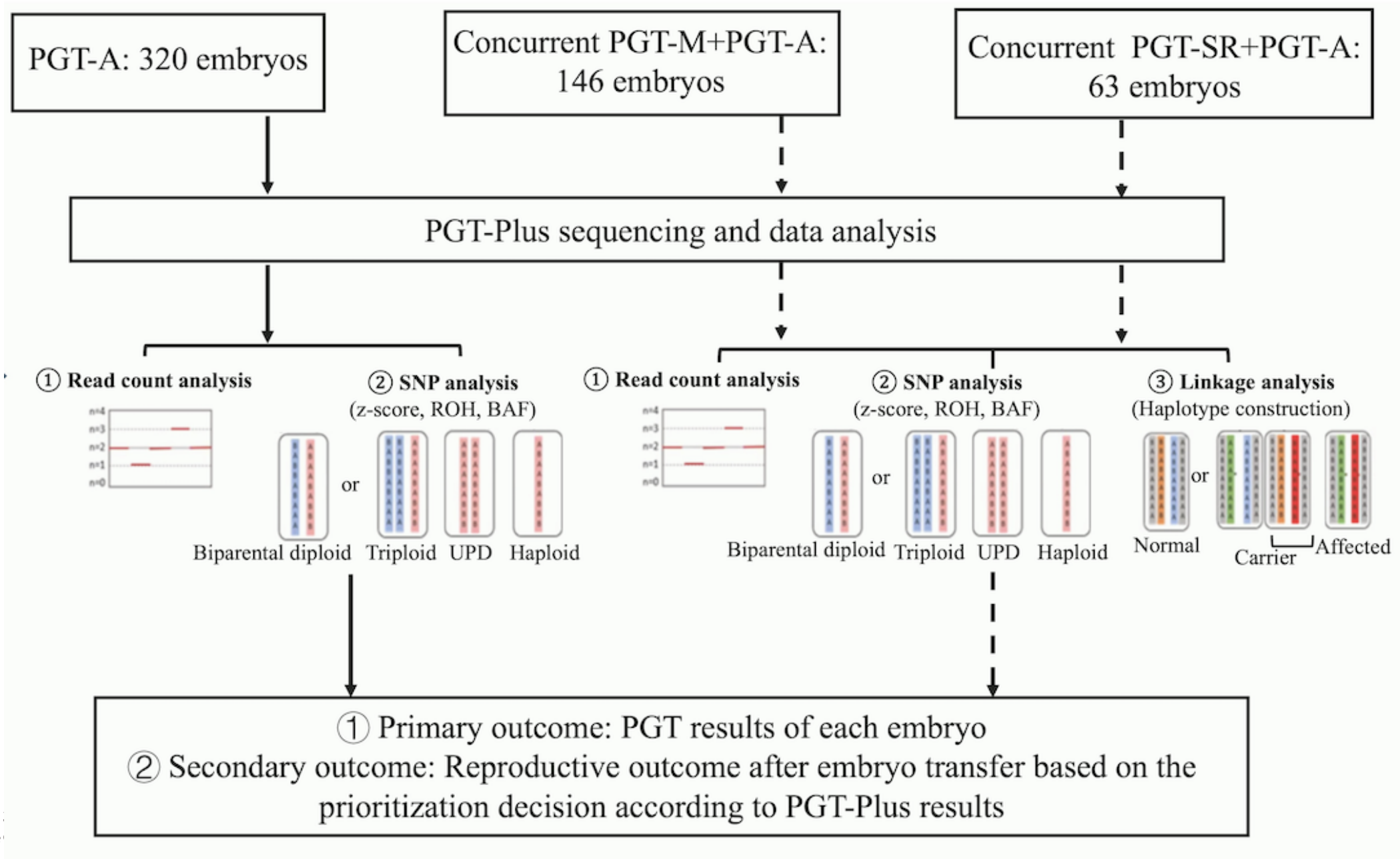


Retrospective cohort: Triploidy and UPD contributed 2.7% (7/262)

Table 1. Significant roles of triploidy and UPD in various sample cohorts

	PGT category	Sample size		Triploidy		UPD	
		No. of embryos	No. of cycles	No. of embryos	No. of cycles	No. of embryos	No. of cycles
Phase II: Retrospective clinical validation	PGT-A	130	67	2	2	2 (gwUPD)	2
	PGT-M+A	65	8	0	0	2 (gwUPD)	2
	PGT-SR+A	67	10	0	0	1 (gwUPD)	1
Subtotal-1		262	85	2 (0.8%)	2 (2.4%)	5 (1.9%)	5 (5.9%)

Phase III: Prospective clinical diagnostic implementation



Prospective study: Triploidy and UPD (1.3%)

Table 1. Significant roles of triploidy and UPD in various sample cohorts

	PGT category	Sample size		Triploidy		UPD	
		No. of embryos	No. of cycles	No. of embryos	No. of cycles	No. of embryos	No. of cycles
Phase III: Prospective diagnostic implementation	PGT-A	320	84	2	2	1 (UPD18)	1
	PGT-M+A	146	28	1	1	2 (gwUPD)	2
	PGT-SR+A	63	11	0	0	1 (gwUPD)	1
Subtotal-2		529	123	3 (0.6%)	3 (2.4%)	4 (0.8%)	4 (3.3%)

Li Y *et al* unpublished data

Conclusion for All in One PGT Testing (PGT-Plus)….

❖For PGT-A:

- PGT-Plus can detect all abnormalities that are also reported by current PGT-A platforms.
- More importantly, PGT-plus euploid embryo for transfer would be able to avoid embryo which turned out to be triploidy or whole genome-wide UPD.

❖For PGT-M:

- PGT-Plus has consistent haplotyping results when using conventional methods (karyomapping, gap-pcr, etc) as a reference.

❖For PGT-SR:

- PGT-Plus can detect unbalanced translocations; Additionally, PGT-Plus enables the identification of translocation carriers among those balanced ones.

Take home message for All in One PGT ….

- Based on our prospective study (N= 529)

  0.6% of human preimplantation embryos are triploidy and
  0.8% are whole genome-wide UPD.

These ‘euploid’ embryo transfer would be otherwise prevented if conventional PGT methods could exclude triploidy and whole genome-wide UPD.

- PGT-Plus enables a more comprehensive abnormality profile detection, thus can be applied as a comprehensive concurrent PGT solution, enabling the detection of **PGT-M/SR with PGT-A, triploidy, parental origin identification, and AOH/UPD** within a single assay.

One-stop flexible PGT-Plus

- **Preimplantation Genetic Testing for Aneuploidies (PGT-A)**

- Gains or losses of chromosomes (aneuploidy) and large chromosome segments of ≥4 million base pairs (Mb) in size
- ≥30% chromosomal mosaicism
- Triploidy & Uniparental isodisomy (isoUPD)

- **Preimplantation Genetic Testing for Monogenic Disorders (PGT-M)**

- Detection of targeted monogenic disorders

- **Preimplantation Genetic Testing for Structural Rearrangements (PGT-SR)**

- Reduces the likelihood of transferring an embryo with unbalanced chromosomal rearrangement
- Balanced translocation carrier can be detected

Advantages of PGT

- Improve implantation rate in those with recurrent implantation failure
- Reduce the subsequent miscarriage risk in couples with recurrent pregnancy loss
- Reduce risk of birth defects
- Reduce risk of multiple pregnancies by single embryo transfer (SET)

- Against known familial/targeted genetic mutations and structural rearrangements for intrauterine transfer.

Limitations 技術局限性

- PGT-A: It cannot detect sub-microscopic abnormalities less than 4 Mb. In addition, mosaicism may lead to the PGT-A result not being representative of the embryo. 無法檢測小於4Mb的亞顯

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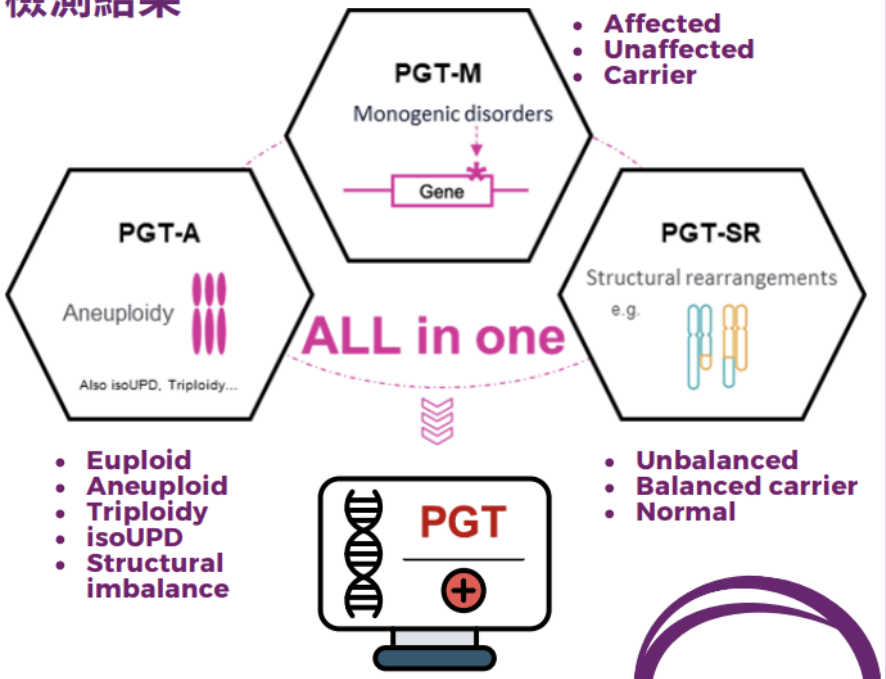
Possible results
檢測結果



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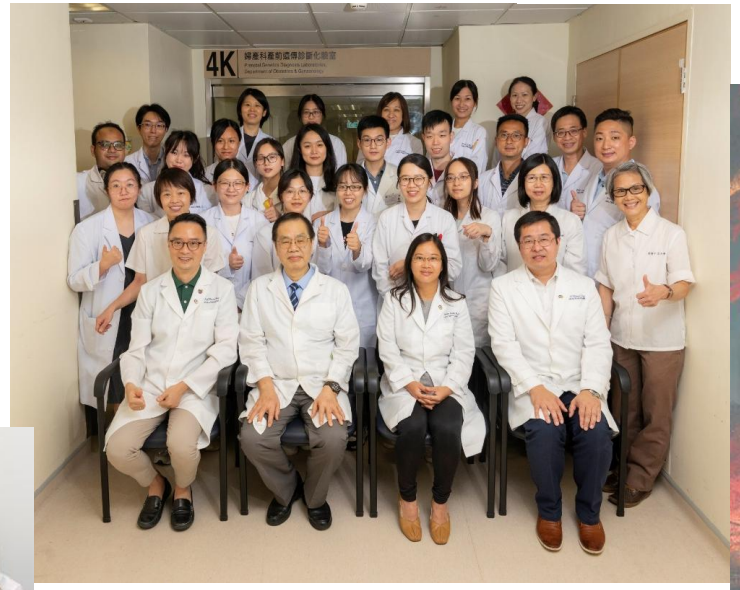

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