21ST PGDIS CONFERENCE

PREIMPLANTATION GENETIC DIAGNOSIS INTERNATIONAL SOCIETY

PGDIS

6-8 May 2024
Kuala Lumpur
Malaysia

PGT and BEYOND...

PREIMPLANTATION DNA METHYLATION SCREENING TO IMPROVE ART OUTCOMES

Jiang Liu, PhD

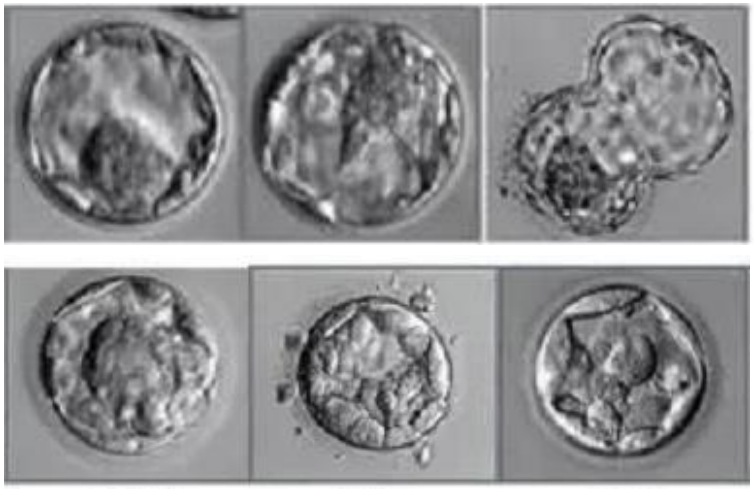
Nyuwa Life Technology
Institute of Biophysics, CAS

Challenges in ART

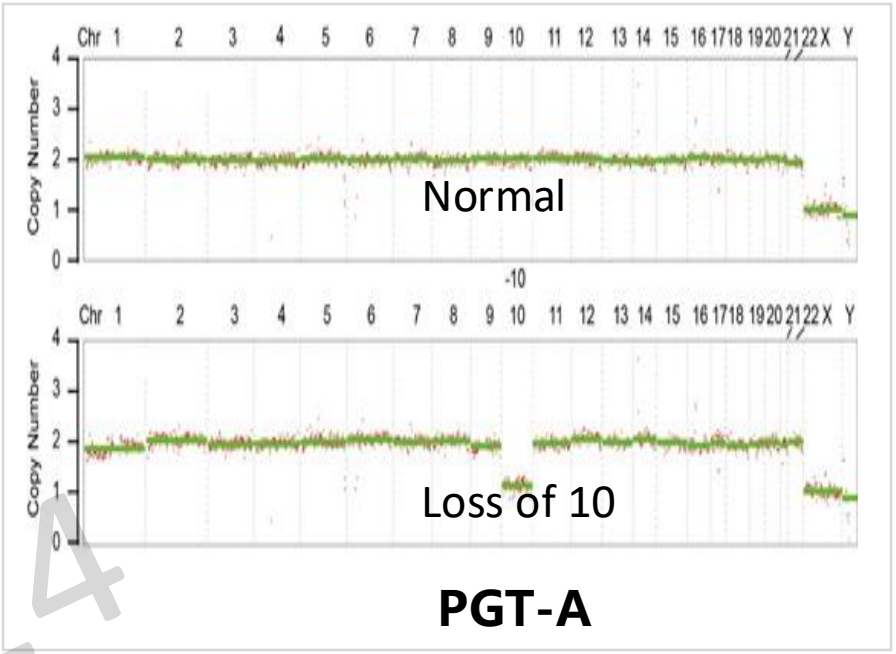
- 1. Low birth rate
- 2. High birth defect

Current major methods in embryo selection

Gardner Morphology Grade + PGT-A



Morphology



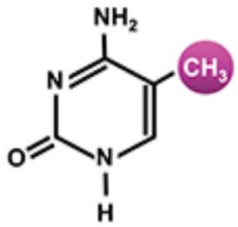
PGT-A

- Gardner Morphology grade aims to evaluate development potential, which relies on experience

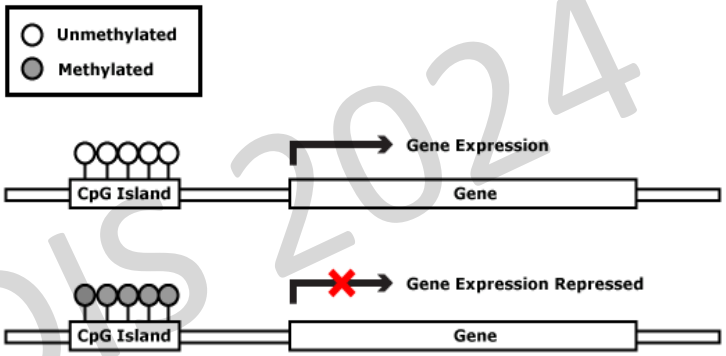
Limitation: There is no molecular biomarker to evaluate development potential

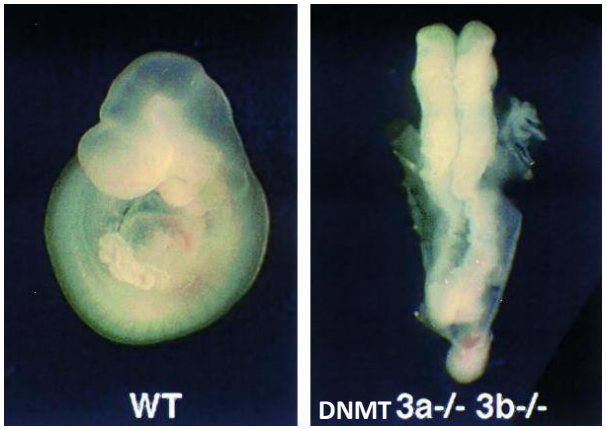
DNA methylation regulates development

- DNA methylation regulates gene expression
- DNA methylation mutation can lead to birth defect or the failure of live birth



5mC

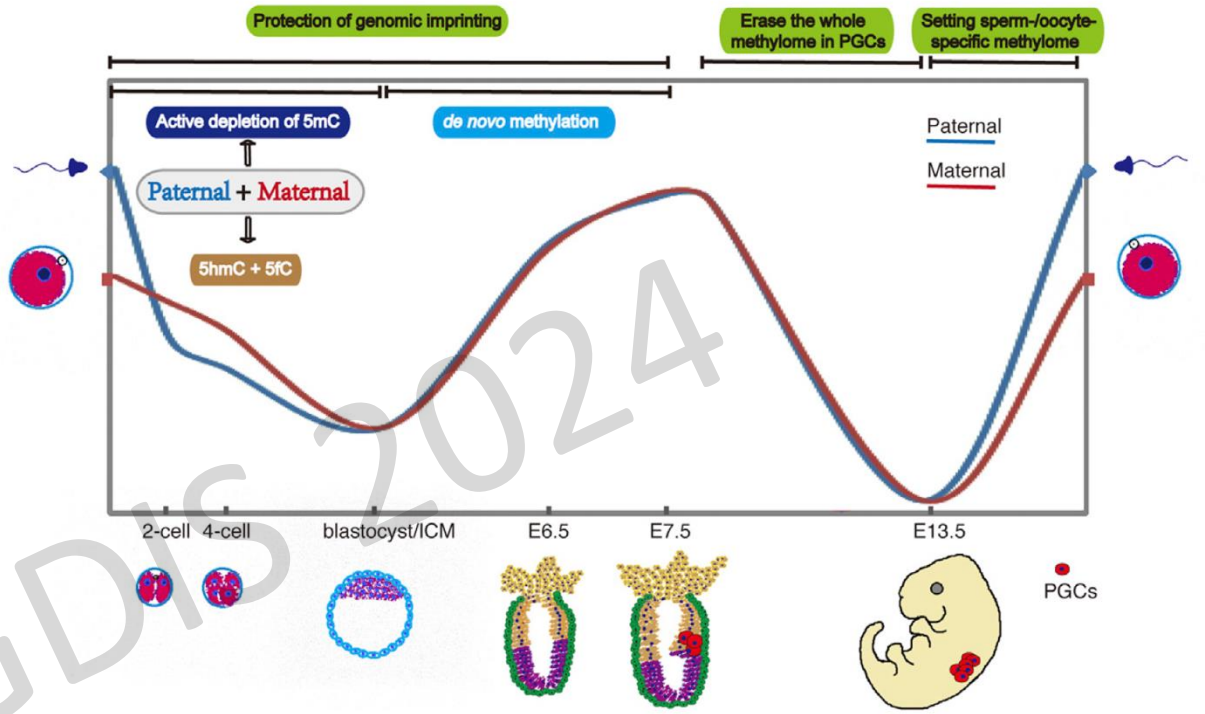




Li et al., 1992

DNA methylation inheritance and reprogramming during mammalian early embryogenosis

About 20% genome of blastocysts maintain DNA methylation before implantation.

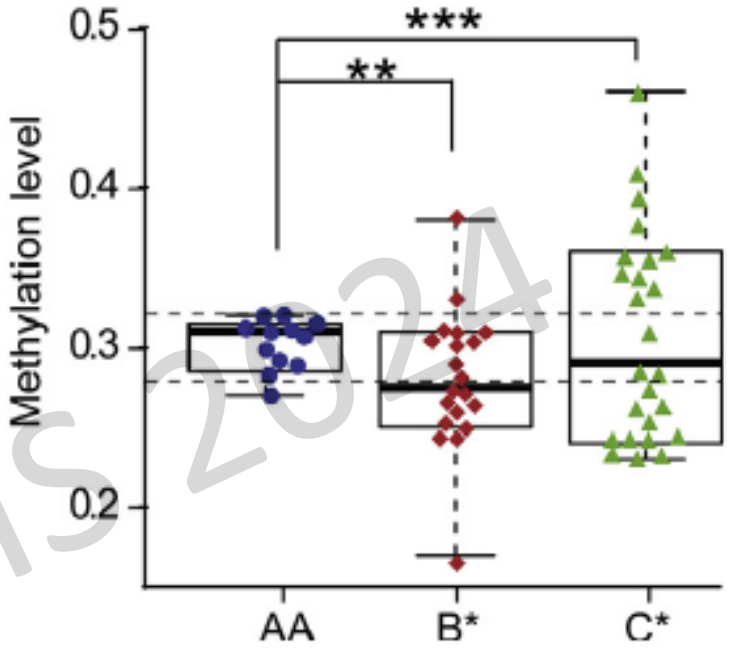


Wang et al. ***Cell,*** 2014

NYUWA LIFE TECHNOLOGY

DNA methylation is a biomarker for embryo selection

The methylation level is quite different among different embryos



J. Genetics and Genomics, 2017; Patent: PCT CN2017/080102

PIMS (Preimplantation DNA Methylation Screening)

- **One PIMS test produce two indexes:**

  1. Chromosome copy number variation (CNV)

  2. Whole genome DNA methylation pattern

Number	Methylation level	CNV
1	0.26	euploidy
2	0.24	aneuploidy: -22(mosaic)(50%)
3	0.55	euploidy
4	0.31	aneuploidy: del(1) (p21.3-p36.33)

J. Genetics and Genomics, 2017; Patent: PCT CN2017/080102

The first clinical trial of PIMS

(An observation trial)

- **Enrollment**:

I. Advanced aged women; Recurrent miscarriage without known reason

II. 2 or more blastocysts with at least BC grade

- **Exclusive**:

I. Abnormal uterus

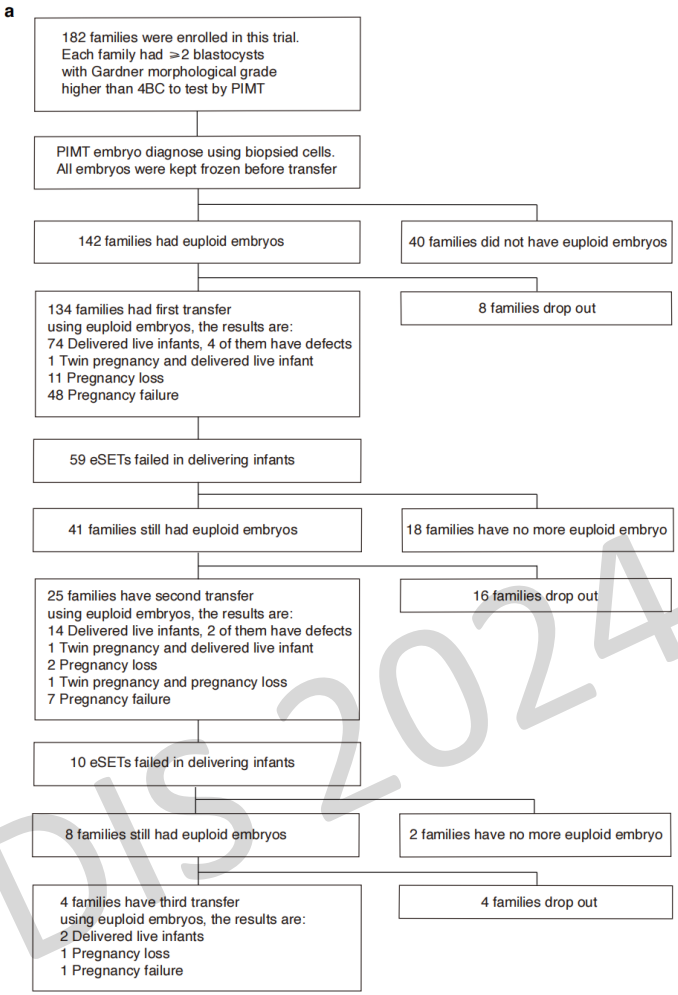
II. Hydrosalpinx

III. Other diseases can affect ART treatment

Cell Research, 2023

NYUWA LIFE TECHNOLOGY

The procedure of PIMS



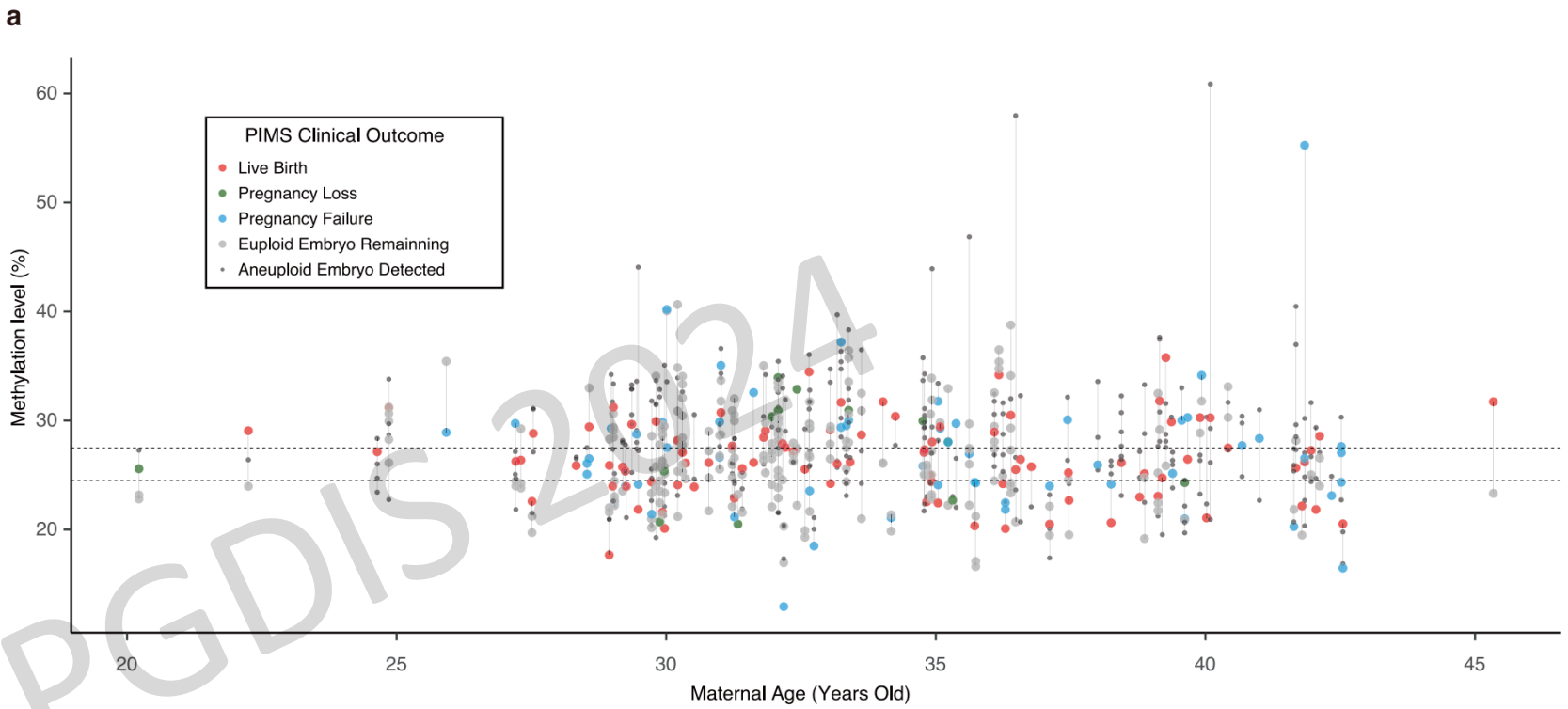
182 families
800 blastocyst tested
163 transferred embryos

Cell Research, 2023

NYUWA LIFE TECHNOLOGY

DNA methylation level distribution among different families

Significant differance within one family at different ages

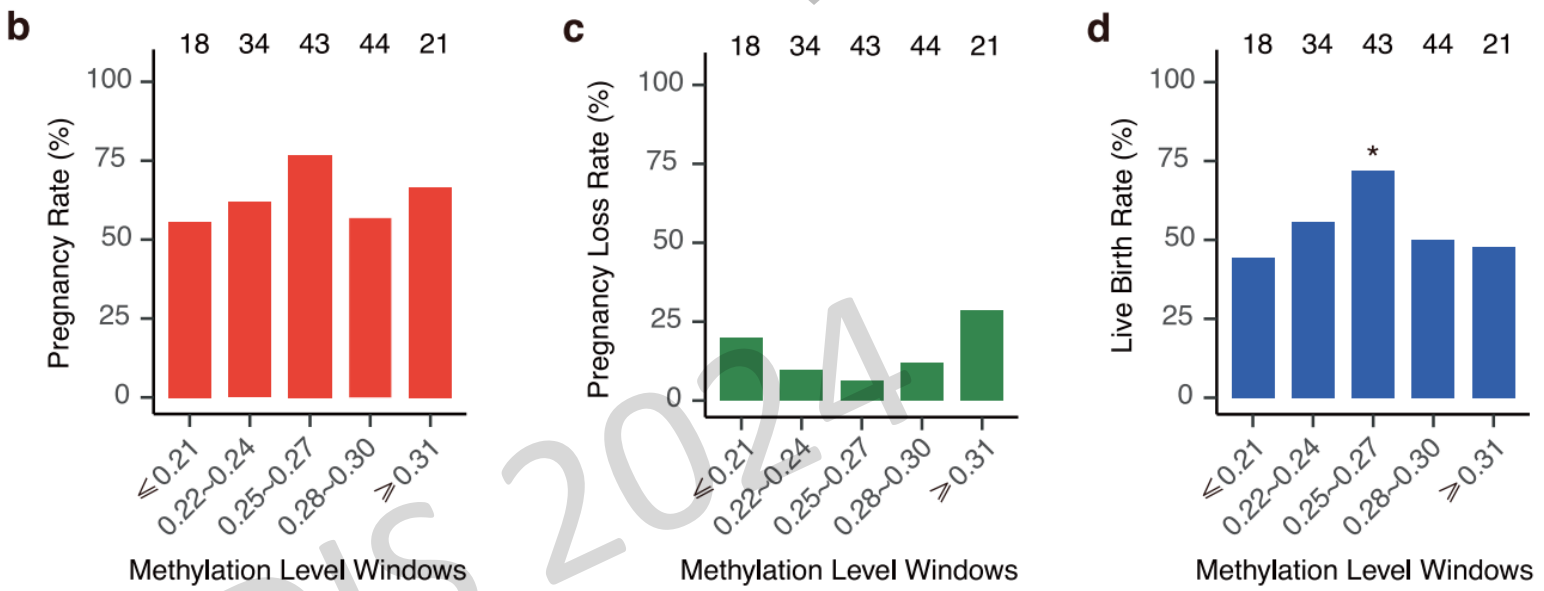


Cell Research, 2023

NYUWA LIFE TECHNOLOGY

DNA methylation level associated with ART outcome

- **Highest pregnant, lowest miscarriage, and highest live birth rates for embryo methylation level within 0.25-0.27 window**
- **Higher live birth rate if methylation level closer to 0.26**



Cell Research, 2023

DNA methylation is the first biomarker to evaluate embryo developmental potential.

A randomized control trial in multiple centers

Enrollment: women from 20-42 years old
Size: 1200 families

Experimental group:
PIMS
Control group:
Younger women: morphology grade
Advanced aged women: PGT-A + morphology grade

More than 20 centers, about 1200 families has enrolled.

Patent of PIMS is granted

Granted in China, Europe, Japan, Korea, etc. al.

NYUWA LIFE TECHNOLOGY

Birth Defects

- 7.9 million (6%) each year, world wide
- 3.2 million disability lifetime

Problem: Only about 1/3 of birth defects can find reason

Currently: We mainly look for the genetic cause

Both genetics and epigenetics can cause birth defects.
It is very limited known about birth defects caused by epigenetics?

Imprinted Gene Disorders

Frequency: 0.14% among nature birth， 0.3% among ART birth （ French national health database)

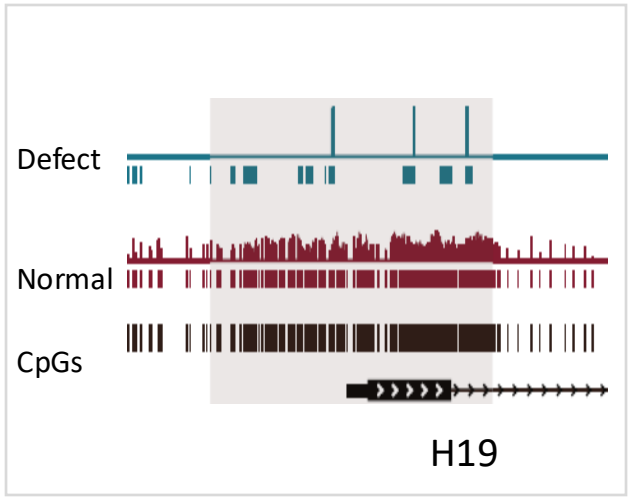
Causes： DNA methylation mutations in imprinted control regions (ICRs) result in abnormal gene expression

Angelman syndrome

Silver-Russells syndrome

Previously： Imprinted gene disorders **cannot** be screened during ART

PIMS can screen imprinted gene disorders



Silver-Russell Syndrome

Cell Research, 2023

PIMS can reduce the rate of birth defect.

About 10% of human blastocysts have DNA methylation mutations for at least one ICR.

(Unpublished data)

NYUWA LIFE TECHNOLOGY

Methylation mutation of ICRs in aborted tissue

ICRs	ICRs（计数）	比例
NESP-AS/GNAS-AS1	15	6.98%
MEST	6	2.79%
H19	4	1.86%
IGF1R	3	1.40%
SNURF	3	1.40%
GRB10	3	1.40%
L3MBTL	3	1.40%
ZNF331	2	0.93%
GNAS-XL	2	0.93%
INPP5F	2	0.93%
KvDMR1	2	0.93%
PLAGL1	2	0.93%
MCTS2P/HM13	2	0.93%
PEG10	2	0.93%
DIRAS3	1	0.47%
总计	52	24.19%

About 25% of aborted tissue have ICR issue. (from more than 200 patients)

(Unpublished data)

Clinical practice for imprinted gene disorders screened by PIMS

Case number	CNV	Methylation level	ICR	Suggested Transfer Order
RC01230530001	euploid	0.201	/	2
RC01230530002	euploid	0.263	/	1
RC01230530003	euploid	0.254	SNURF (7/0) p-value=0.00815	3
RC01230530004	aneuploid	0.24		

The third embryo has good global methylation level, but methylation mutation in SNURF region.

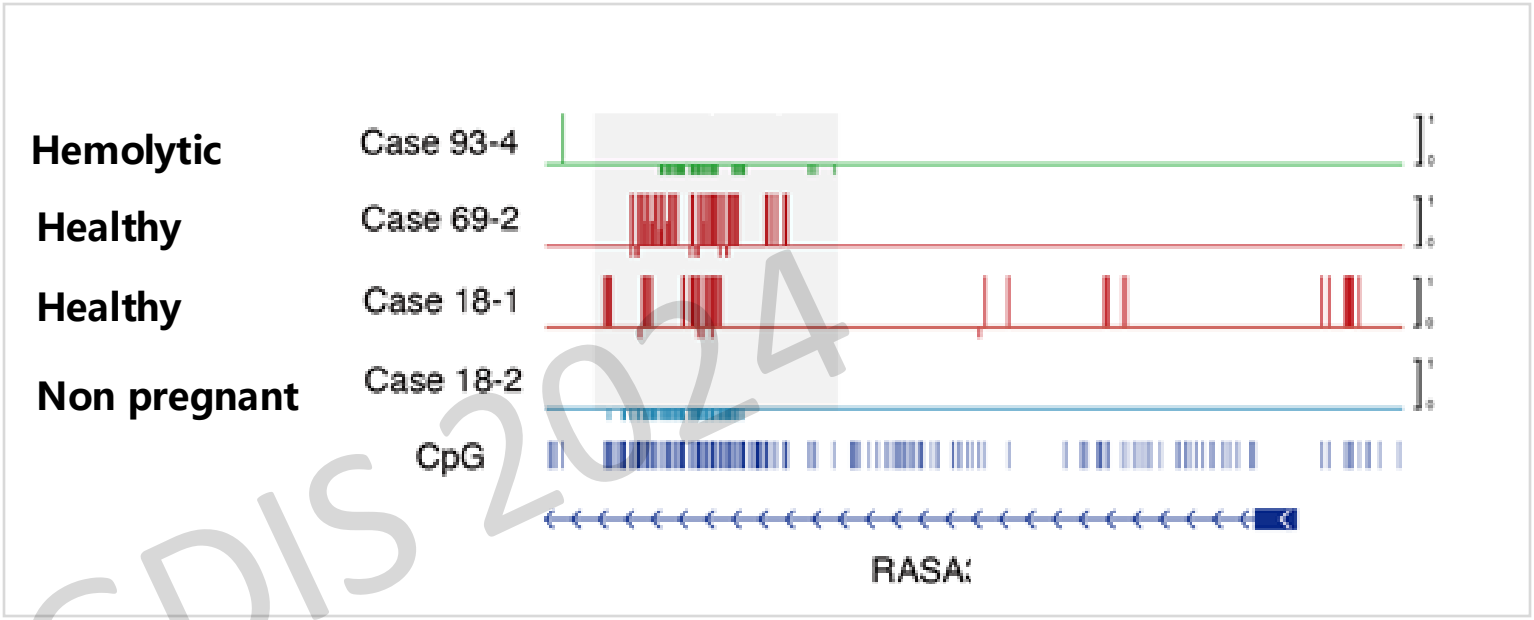
SNURF methylation mutation results in Angelman syndrome.

Can DNA methylation mutation lead to other birth defects?

In the past, no way to find the relationship between birth defect and DNA methylation mutation.

PIMS provides the way to identifying the cause of birth defects by methylation mutations

Methylation mutations in RASA region can lead to the failure of birth live or hemolytic disease of newborn.



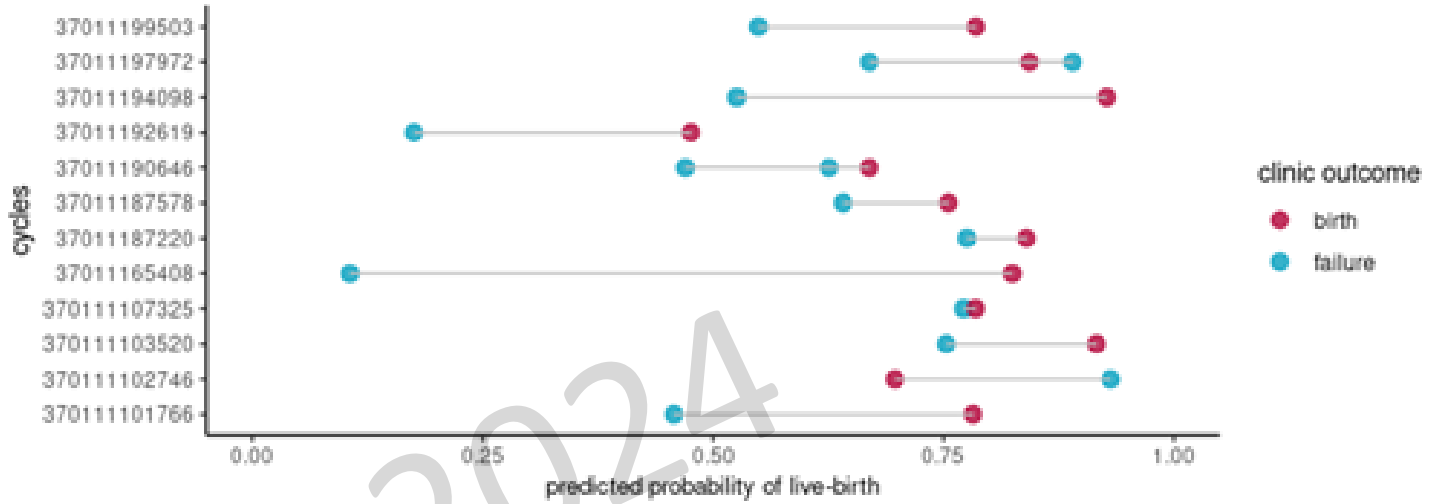
We have proved that DNA methylation mutation in RASA is the cause of hemolytic disease of newborn by DNA methylation engineer.

(Unpublished data)

NYUWA LIFE TECHNOLOGY

Artificial Intelligent of PIMS

- From Big Data, we find many important methylation regions associated with live birth and birth defect
- Use regions, instead of the average methylation level



With more and more data collected from clinics, AI model can be better and better.

AI can improve birth rate, and decrease birth defect rate

PIMS Summary

- One test, two index: Cover PGT-A result, Replace morphology grade
- Improve ART outcome
- Find new cause for birth defect
- For the first time, in screening epigenetic disease, and decrease the rate of birth defects

Let us Work Together to Improve PIMS!

THANK YOU

PGT and BEYOND...