21ST PGDIS CONFERENCE

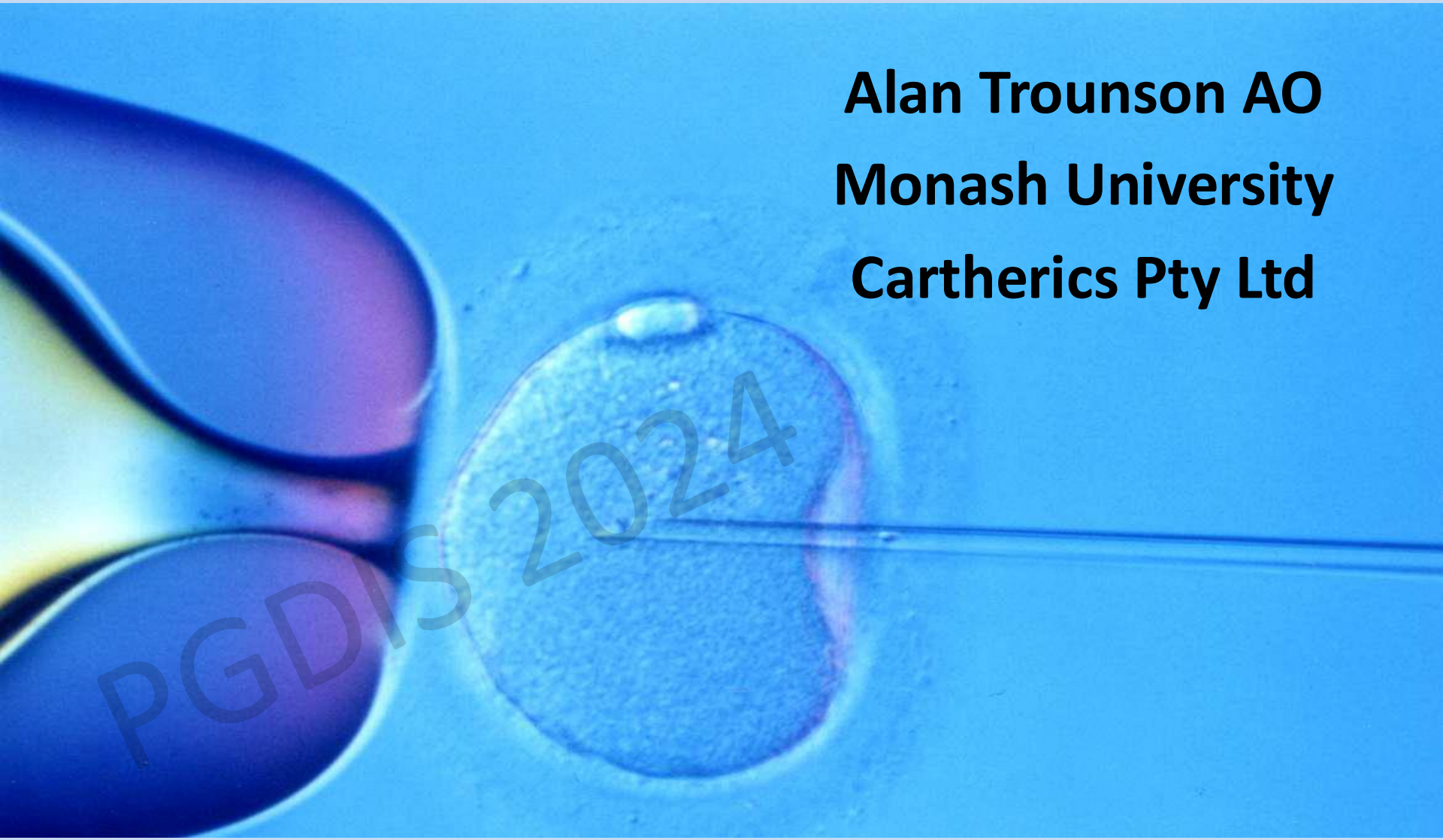
PREIMPLANTATION GENETIC DIAGNOSIS INTERNATIONAL SOCIETY

PGDIS

6-8 May 2024
Kuala Lumpur
Malaysia

PGT and BEYOND...

A Personal View of IVF, PGD and Future of ART as seen by a Scientist



Alan Trounson AO

Monash University

Cartherics Pty Ltd

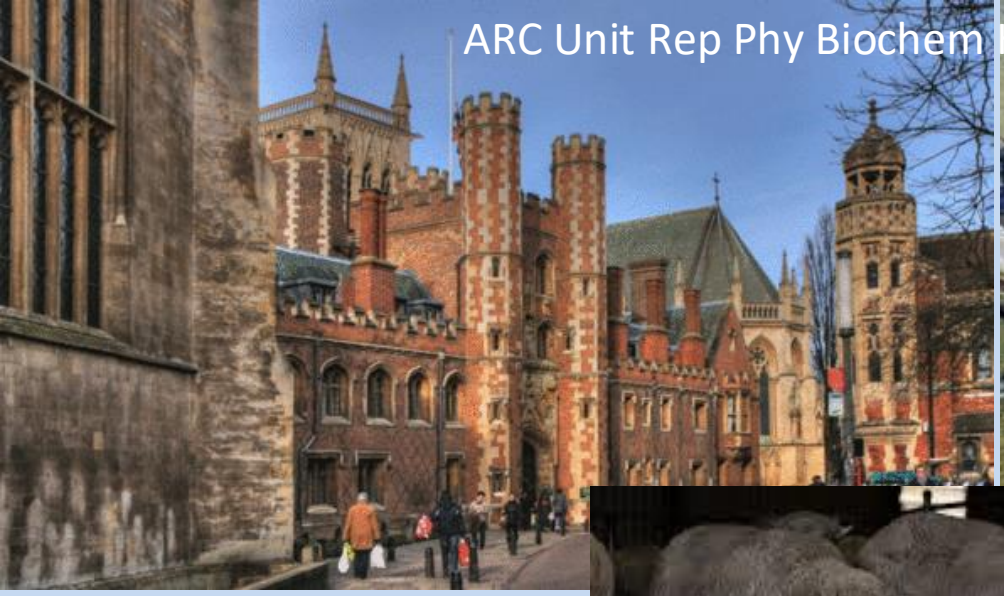
Talk Summary

- How was IVF initiated
  - Superovulation, IVF, ICSI, Culture, Cryopreservation
- Chromatin organization in the preimplantation embryo
- Success and security – efficacy and safety
  - Chromatin instability in preimplantation embryos
  - Mosiacism and PGD-A accuracy
  - Mitachondria DNA Errors
  - Selecting embryos for transfer (PGT-A)
  - Genetic repair using gene editing tools – people or embryos?
- Artificial embryos and where is the border for the sanctity of life
- Gametes from Pluripotent Stem Cells
- Should PGT-A be offered for ART available to all?

How was IVF Developed

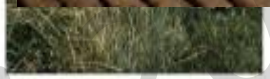
- Superovulation
- IVF
- ICSI
- Culture
- Cryporeservation

ARC Unit Rep Phy Biochem Huntintdon Road Cambridge



a

b



c

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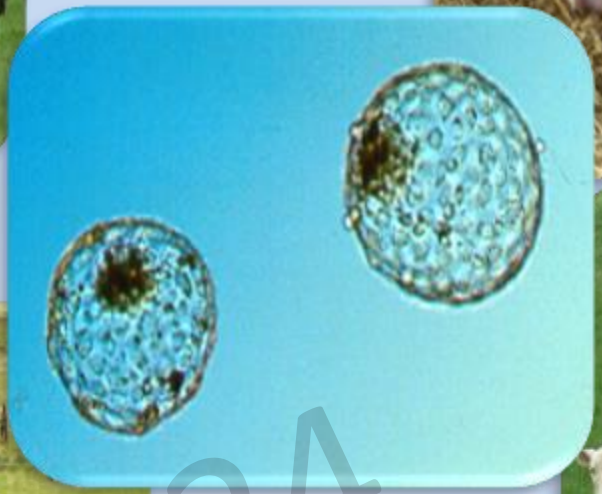
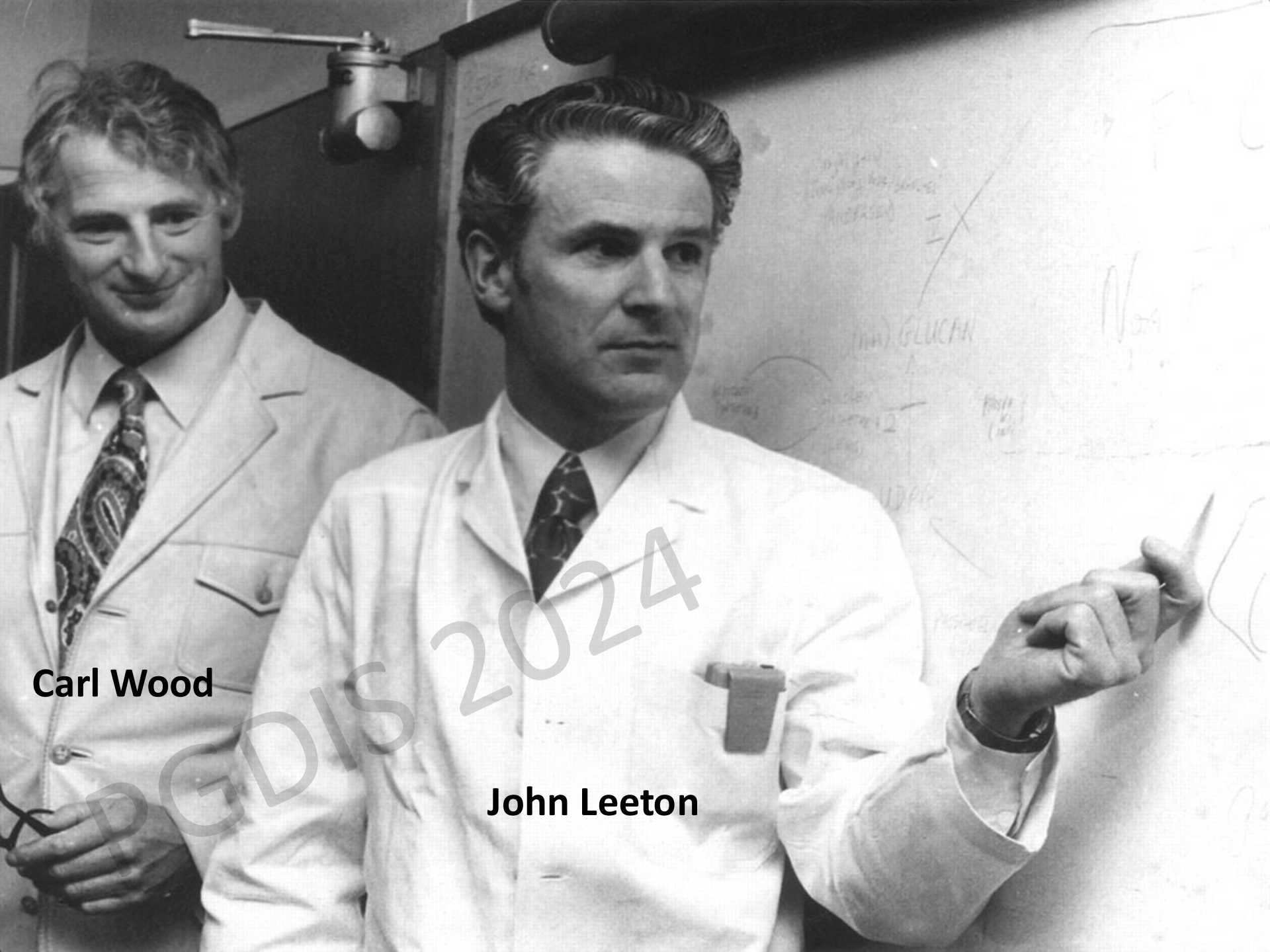
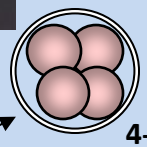
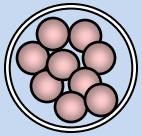
Turning Embryos into Offspring



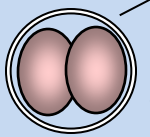






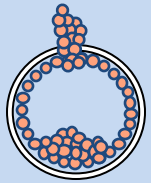
8-cell

4-cell

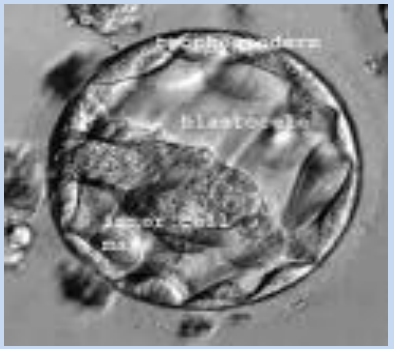


2-cell

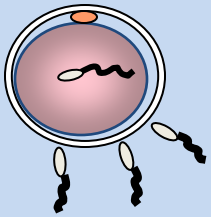
Embryos



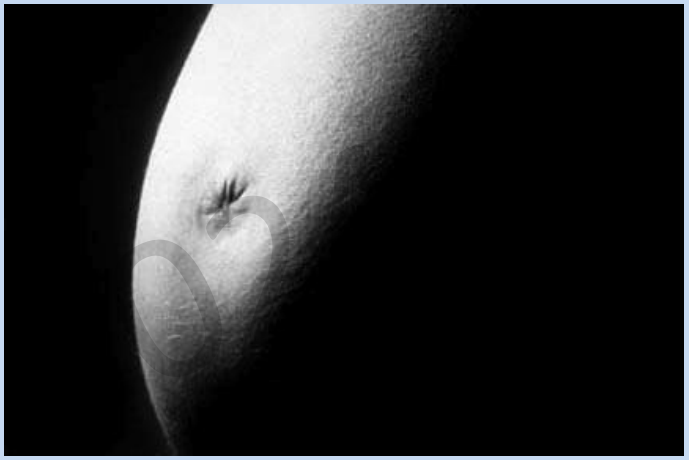
Blastocyst

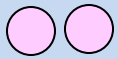


Single embryo - Transfer to patient



IVF



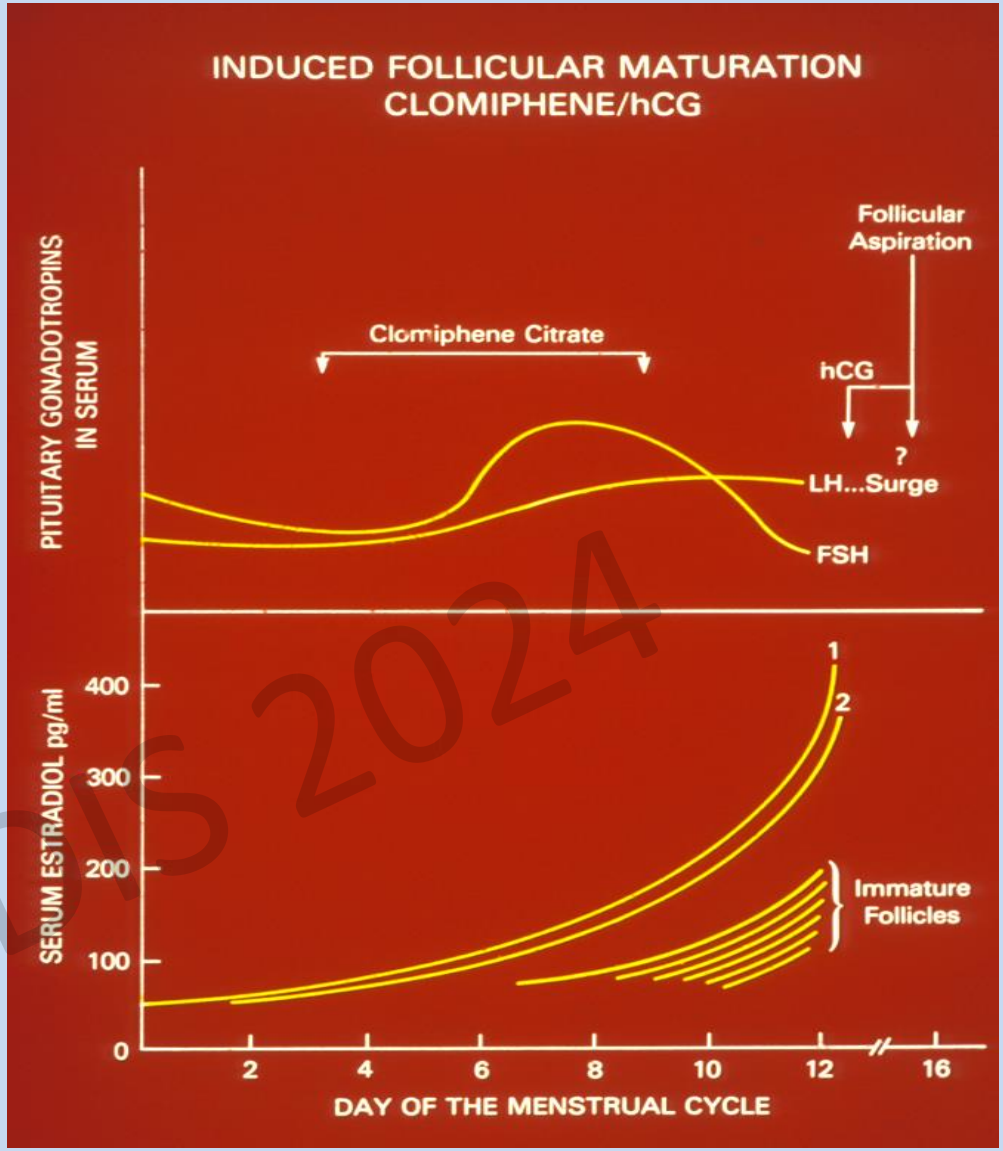


MII eggs

Clomiphene citrate (50-100mg) + hCG

Added HMG/FSH to increase collection of multiple oocytes and pregnancy success

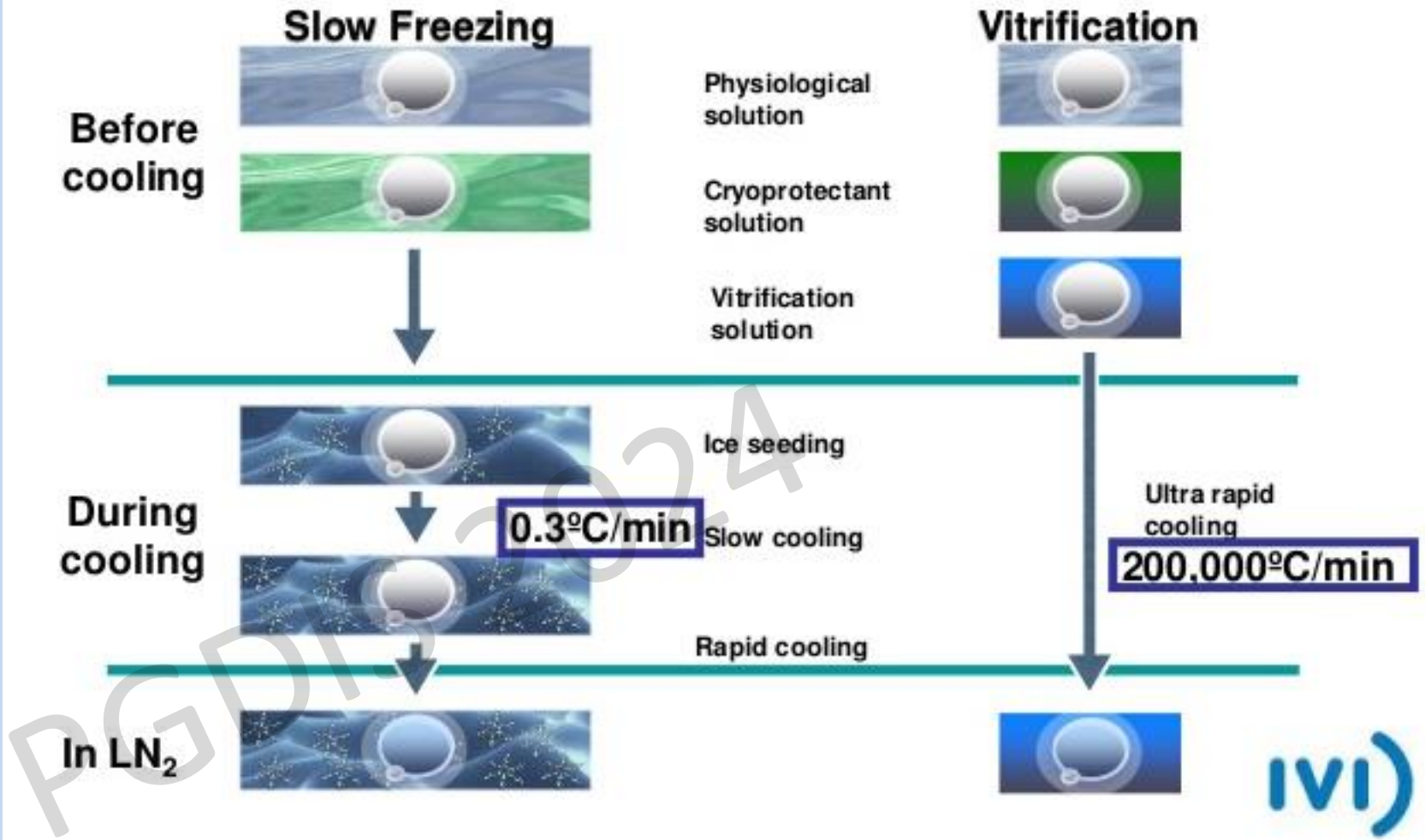
Gentle induction of Follicle Growth



Major Developments in human IVF at Monash

- Vitrification of human oocytes
  Human Repord. 1999
- Human oocyte maturation and IVF
  Fertil. Steril. 1994
- Embryo biopsy in mouse embryos
  Biol. Reprod. 1989
- Subzonal microinjection of human sperm for fertilization
  Fertil. Steril. 1987
- Use of epididymal sperm for IVF
  J,In Vitro Fert. Embryo Transf. 1985
- Egg and embryo donation with or without ovarian function
  Br. Med. J. 1983; Nature 1984
- Freezing human embryos
  Nature 1983
- Growing human IVF to blastocysts in vitro
  J Reprod. Fertil. 1982
- Multiple ovulation / control of timing ovulation for IVF
  Science 1981

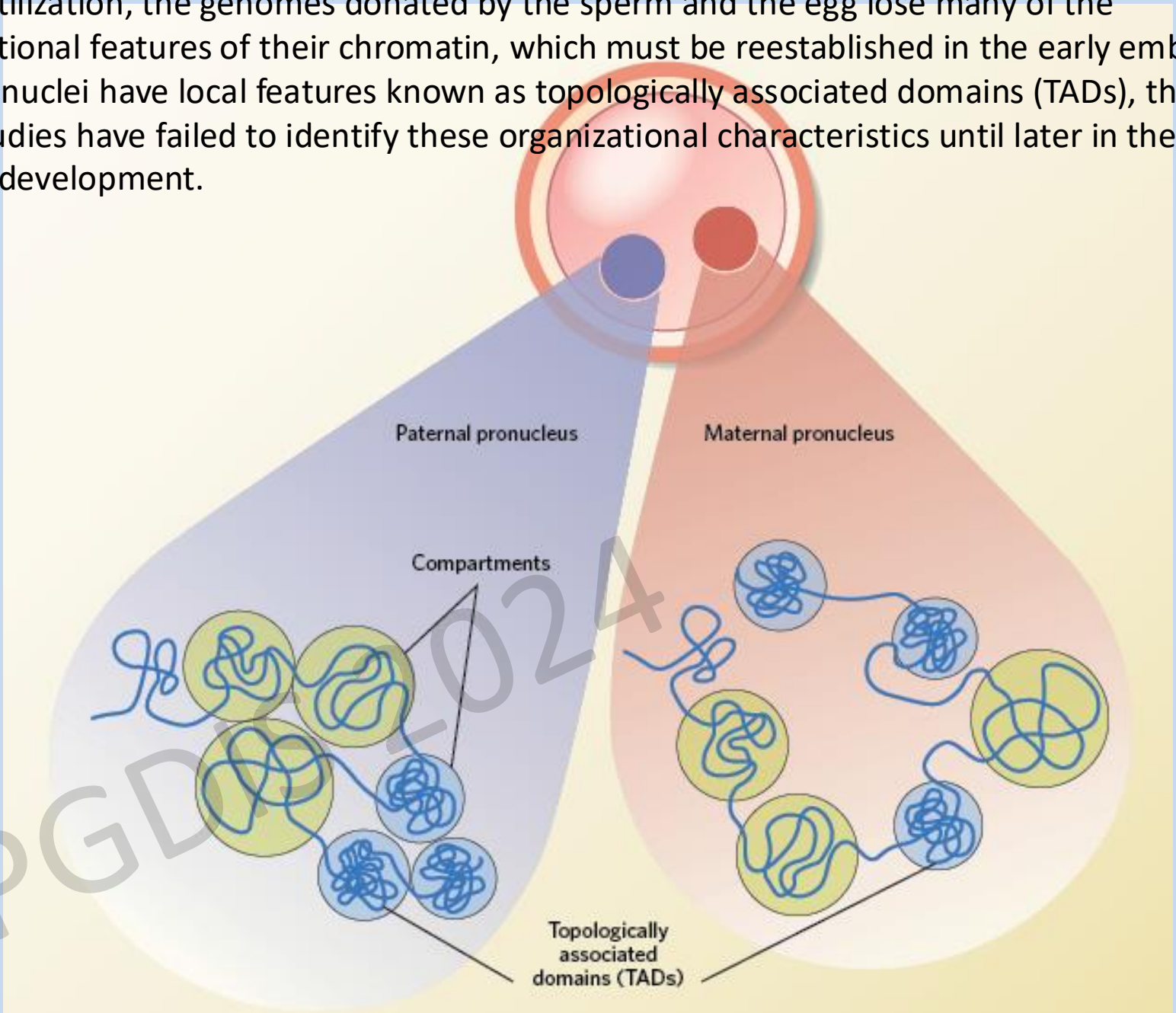
Cryopreservation Techniques



Chromatin Organization in the Preimplantation Embryo

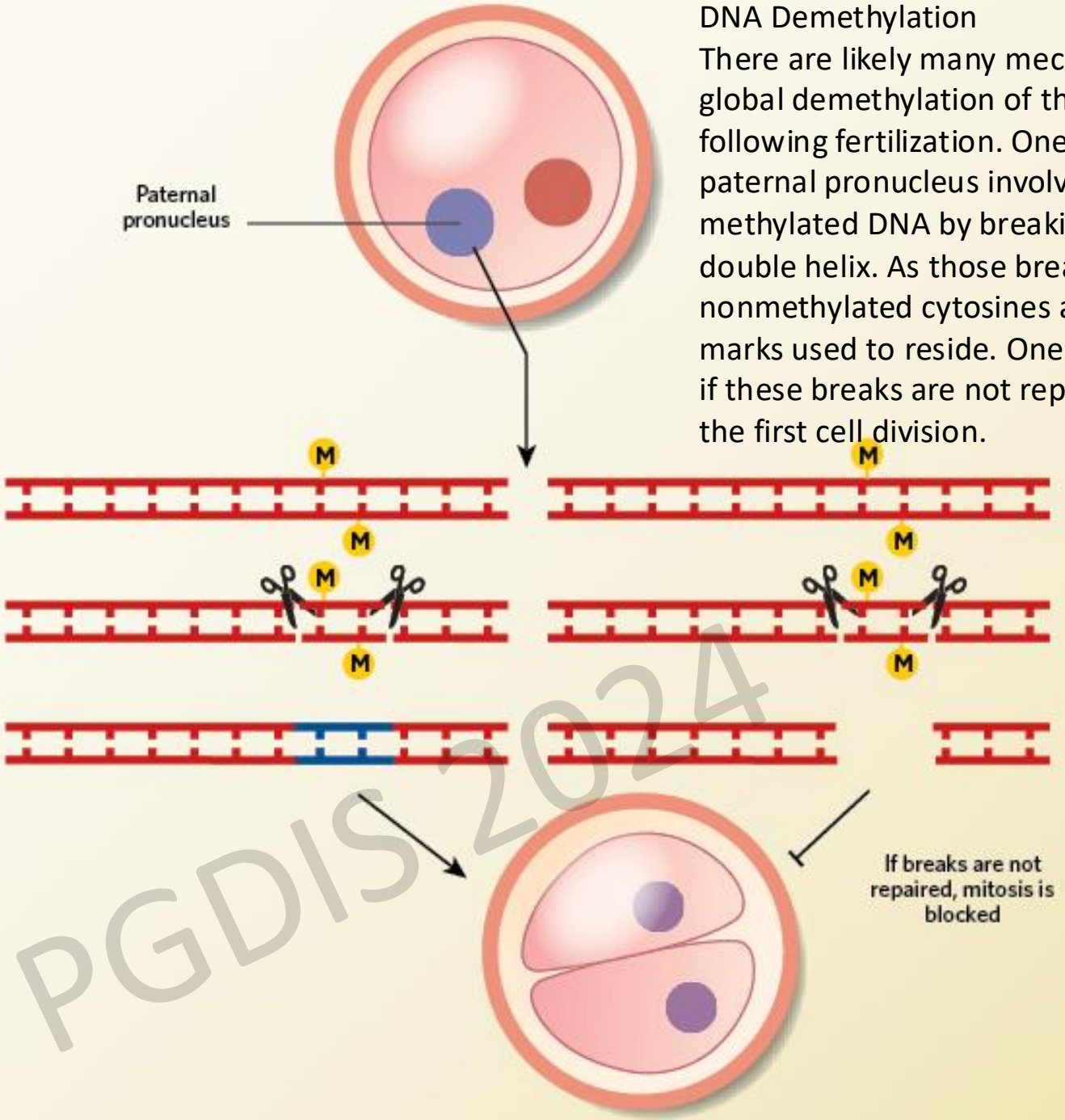
Chromatin Changes

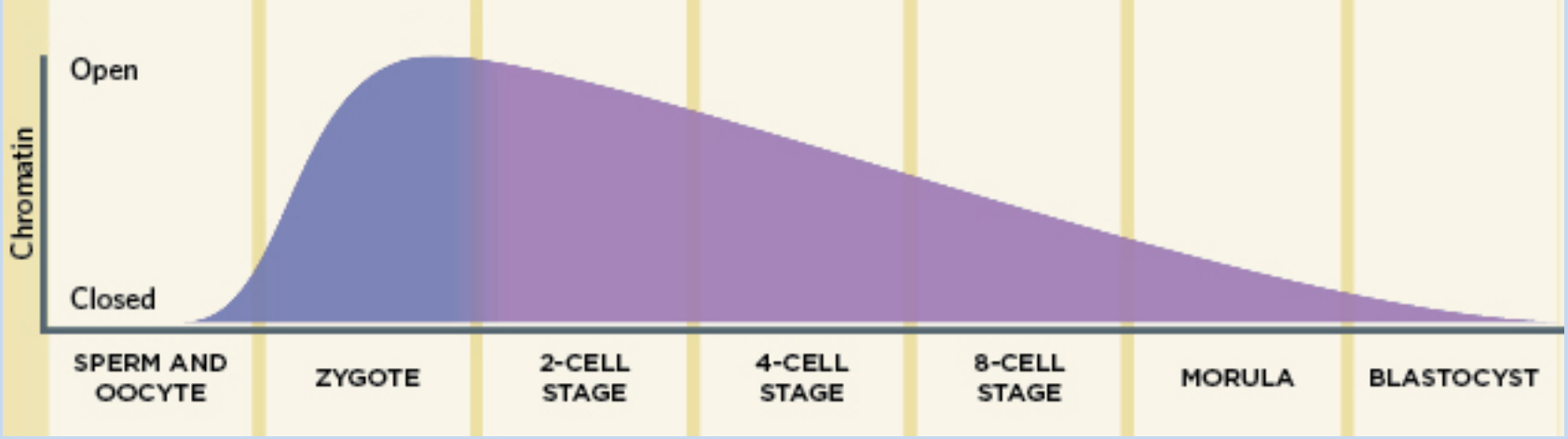
After fertilization, the genomes donated by the sperm and the egg lose many of the organizational features of their chromatin, which must be reestablished in the early embryo. Both pronuclei have local features known as topologically associated domains (TADs), though other studies have failed to identify these organizational characteristics until later in the first week of development.



DNA Demethylation

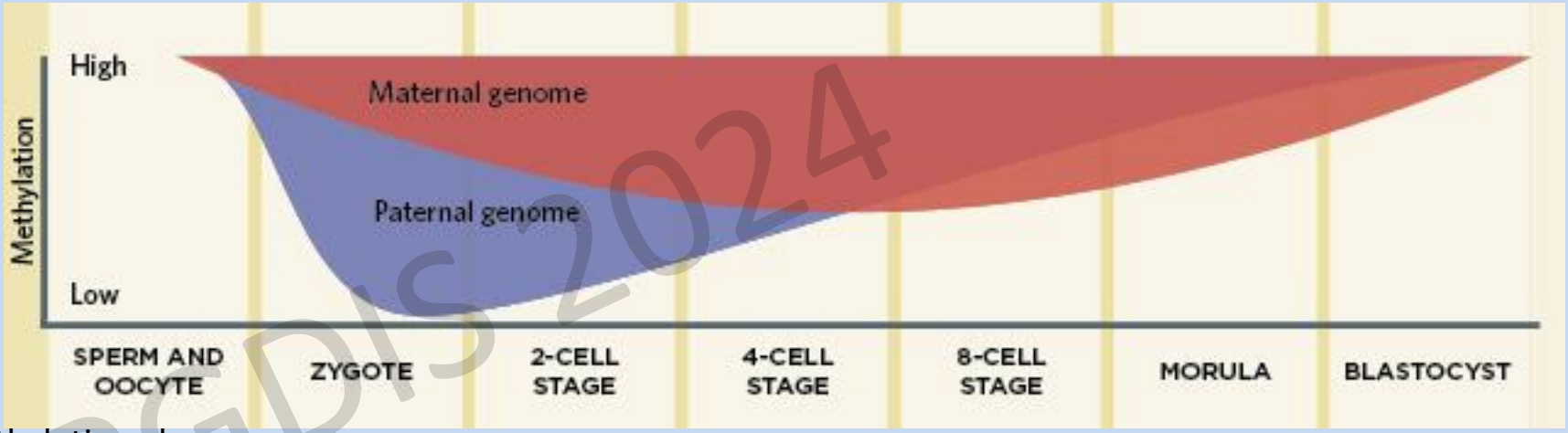
There are likely many mechanisms governing the global demethylation of the zygotic genome following fertilization. One mechanism at play in the paternal pronucleus involves the excision of the methylated DNA by breaking and repairing the double helix. As those breaks are repaired, nonmethylated cytosines are inserted where methyl marks used to reside. One recent study showed that if these breaks are not repaired, the embryo delays the first cell division.





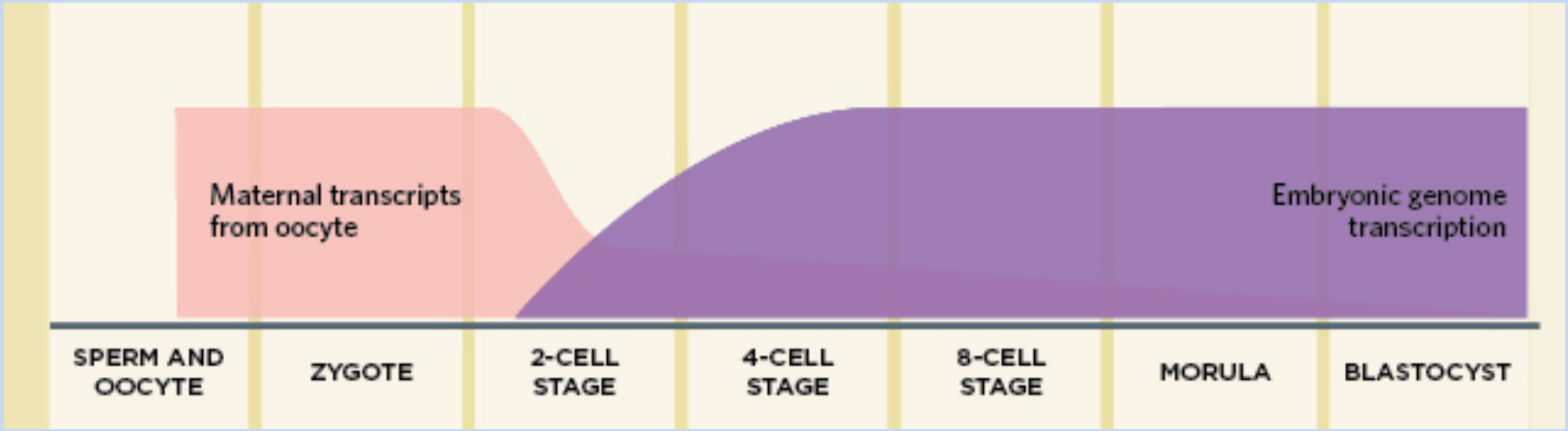
Chromatin changes

In sperm, chromatin is very compact; the overall accessibility of the chromatin in the oocyte, which is still undergoing meiosis, is unclear. Shortly after fertilization, chromatin in both pronuclei undergoes major restructuring, taking on an open configuration before reestablishing local and global organizational features.



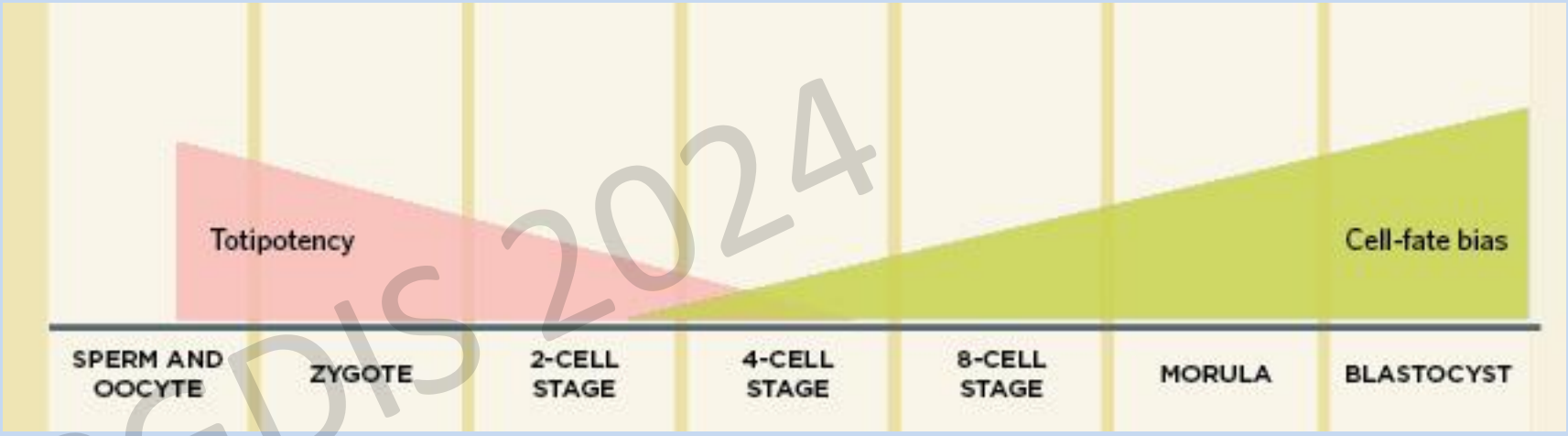
Methylation changes

Following fertilization, the vast majority of methyl marks on the genome are removed. The paternal genome undergoes rapid, active demethylation, while the maternal genome loses its methylation passively over the first couple of cell divisions. Simultaneously, the embryonic genome begins to acquire tissue-specific DNA methyl marks as the cells start to differentiate.



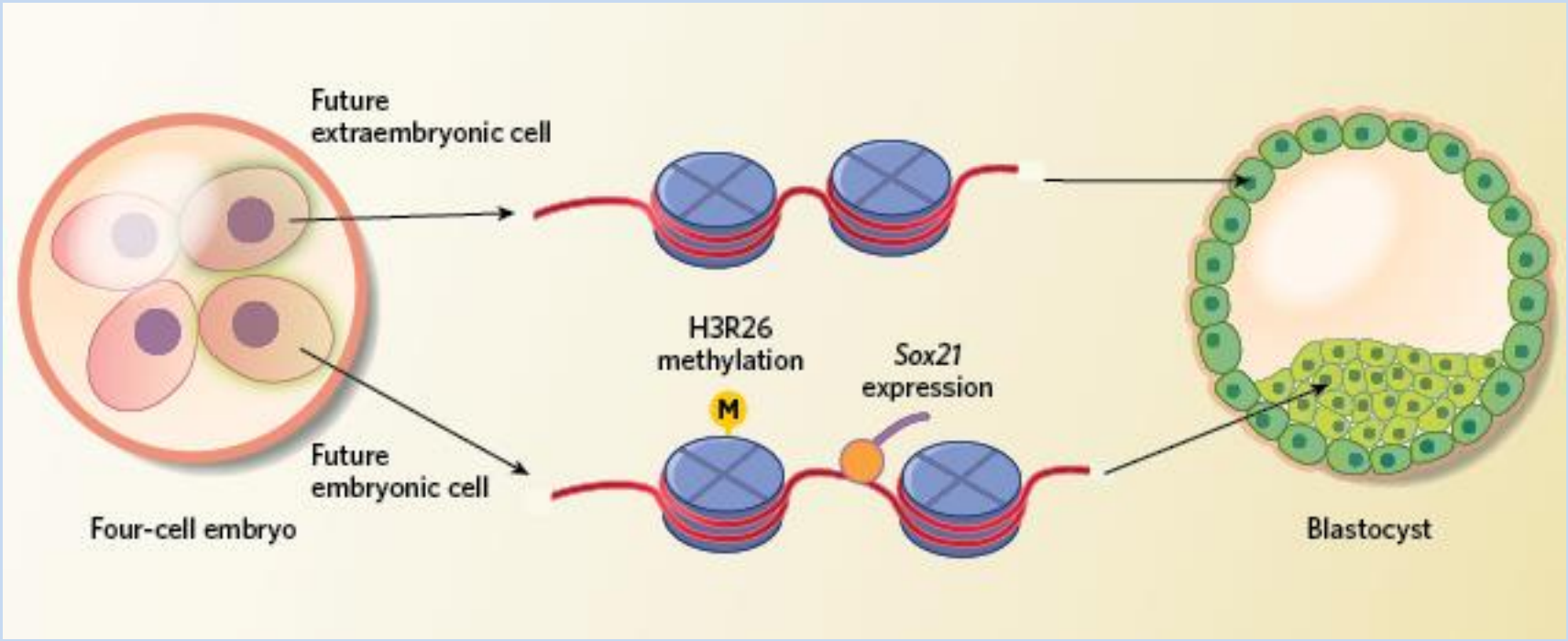
Transcription changes

Messenger RNAs packaged in the oocyte are gradually depleted over the first week of development. Meanwhile, the zygotic genome undergoes multiple rounds of activation, with the genes expressed early on playing key roles in embryonic organization and cell-fate determination.



Cell-fate determination

By the four-cell stage, some cells begin to express genes that drive them to become the embryonic lineage that will form the fetus, while other cells begin to express genes associated with the extraembryonic lineage that becomes the placenta.



Cell-Fate Determination

Recent research has shown that cell-fate bias stems from methylation of arginine 26 on histone 3 (H3R26), which lengthens the time certain transcription factors remain on the DNA. Longer binding promotes expression of genes such as Sox21 that drive cells to become the embryonic lineage (blue) that will form the fetus, while cells with shorter binding form the extraembryonic lineage (green) that becomes the placenta.

Success and Security in IVF

- SWOT
- Chromosome instability and mosaicism
- Heterogeneity in embryo development
- Selecting euploid embryos
- Mitachondria DNA errors

Strengths, weaknesses, opportunities and threats analysis of the preimplantation genetic testing for aneuploidies strategy

Alteri etal. Clin Genetics 2019

Strengths	Impact/Benefit
increased implantation rate	to be defined
Decrease miscarriage rate	reduction of medical treatments reduction of distress

Weaknesses	Impact/Risks
3 RCTs in good prognosis patients 1 RCT in AMA patients	to set up clinical procedures based on poor evidence
cumulative IVF success not improved	overtreatment
spectrum of genetic techniques	misdiagnosis
management of mosaicism	decrease in treatment effectiveness

Opportunities	Impact/Benefit
adoption of eSET policy	reduction of multiple pregnancies
reduced time to pregnancy	cost reduction
psychological aspect of healthy care	improvement of patients' management

Threats	Impact/Risks
high cost	patients' dissatisfaction
invasive procedure and not standardized technique	embryo damage
obstetrical and perinatal outcomes: limited data long-term effect: limited data	adverse outcomes

Chromsome instability is common in human cleaveage-stage embryos

Vanneste etal. Nature Med. 2009

- Multiple array based analyses of normal embryos from patients assessed for X-linked disorders, *BRCA2* mutation or familial microdeletion syndromes.
- Segmental imbalances found in 70% of embryos. 40% carried chromosome arm imbalances (due to chromosome breakage or centric fusion)
- 55% of embryos carried terminal segmental imbalances (simple or complex patterns due probably to DNA double strand breaks followed by nondisjunction of the acentric fragment).
- Segmental aneuploidies resulting from breakage-fusion-bridge cycles are as seen in tumors
- The chromosome instability probably occurs in embryos in vivo (30% of conceptuses result in live births; 50% of spontaneous abortions have chromosome imbalances; terminal deletions, duplications and isochromosomes, and mosaics are present at births)
- Given selection against chromosomally abnormal embryos there is probably selection against abnormal blastomeres. Only 9% of embryos were normal diploid in all blastomeres. IVF birth rates per embryo are >20% transferred. Suggests mosaic embryos containing normal blastomeres end up as chromosomally normal fetuses.

Mosaicism is a dominant feature of human preimplantation embryos

Echten-Arends etal. Hum Reprod 2011

Table II Summary of the findings of 36 studies on the chromosomal makeup of human preimplantation embryos.

	All embryos (*n* 5 815)	Developing, cleavage-stage embryos analysed for ≥8 chromosomes (*n* 5 107)
Diploid	177 (22%)	15 (14%)
Mosaic	599 (73%)	77 (72%)
Diploid–aneuploid mosaic	480 (59%)	49 (46%)
% Diploid cells	*(10155/14116) (72%)*	*(151/324) (47%)*
Aneuploid mosaic	119 (15%)	28 (26%)
Other abnormalities	39 (5%)	15 (14%)
Haploid	3 (,1%)	1 (1%)
Polyploid	5 (,1%)	1 (1%)
Aneuploid	18 (2%)	4 (4%)
Monosomy	13 (2%)	3 (3%)
Trisomy	5 (,1%)	1 (1%)
Complex abnormal	13 (2%)	9 (8%)

Table I Classification criteria for the chromosomal makeup of human preimplantation embryos.

Chromosomal makeup	Criteria[a]	FISH examples for X,Y and 18[b]
Diploid	All cells contain two chromosomes for each chromosome pair tested	XX,1818 [7] X,Y,1818 [7]
Mosaic	Not all cells contain the same chromosomal makeup	
Diploid–aneuploid mosaic	A mosaic embryo with one or more diploid cells	XX,1818 [5]/XX,18 [2] XY,1818 [3]/XY,181818 [4]
Aneuploid mosaic	A mosaic embryo without the presence of diploid cells	XX,181818 [3]/XXX,181818 [4] X,18 [1]/X,181818 [4]/X,1818 [2]
Other abnormalities		
Haploid	All cells contain one chromosome for each chromosome pair tested	X,18 [7] Y,18 [7]
Polyploid	All cells contain more than two chromosomes for each chromosome pair tested	XXX,181818 [7] XXYY,18181818 [7]
Aneuploid	All cells contain the same abnormality for one chromosome pair tested	XX,18 [7] XX,181818 [7]
Complex abnormal	All cells contain the same abnormalities for multiple chromosome pairs tested	X,181818 [7] XYY,18 [7]

[a]Embryo should have at least three cells. Criteria can be used for cleavage stage as well as blastocyst stage embryos.
[b]For each category two examples are provided for illustrative purposes. Number between brackets is the number of cells.

Rapid, Regular aCGH or NextGenSequencing

Ma etal. Mol Cytogenetics 2016

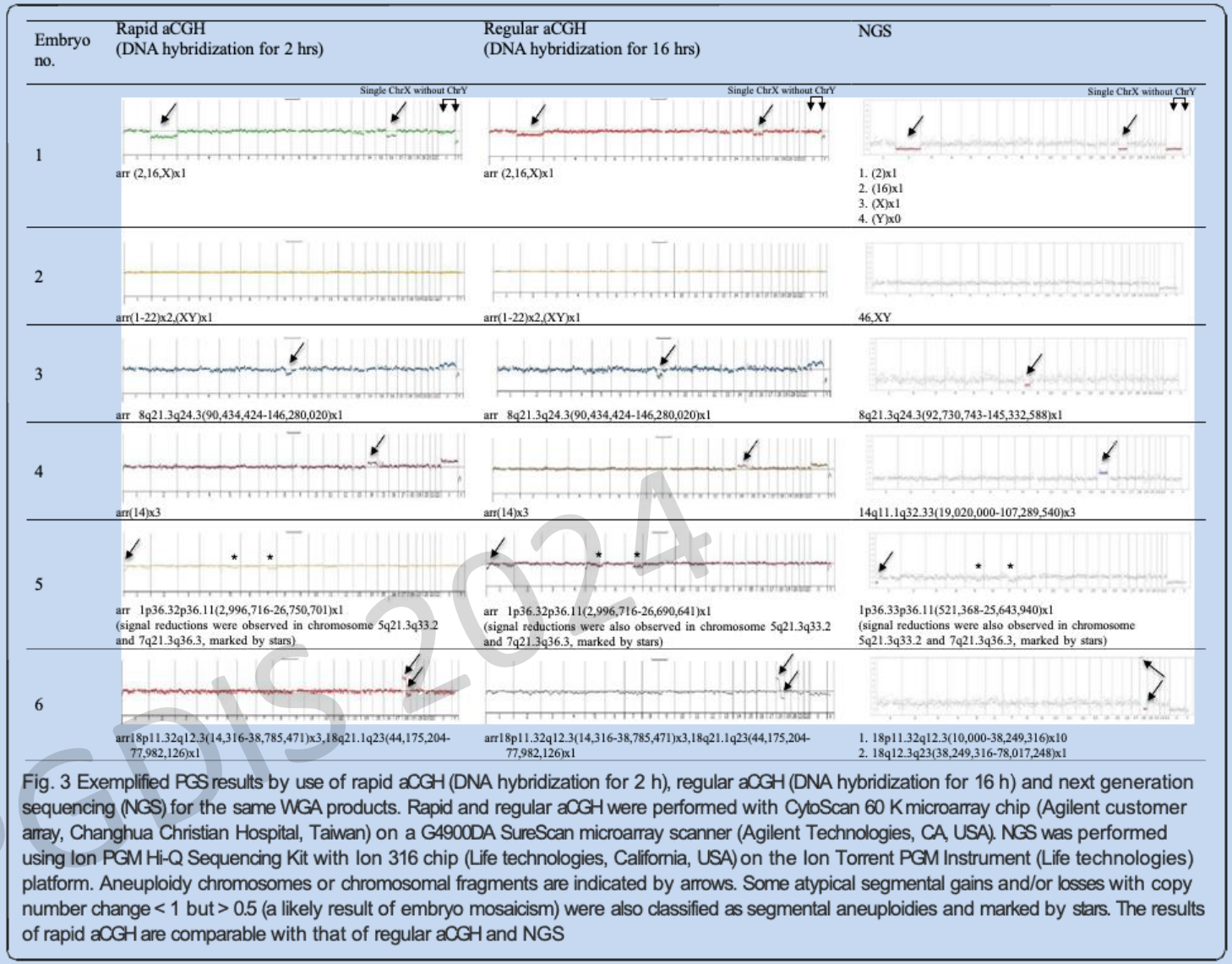
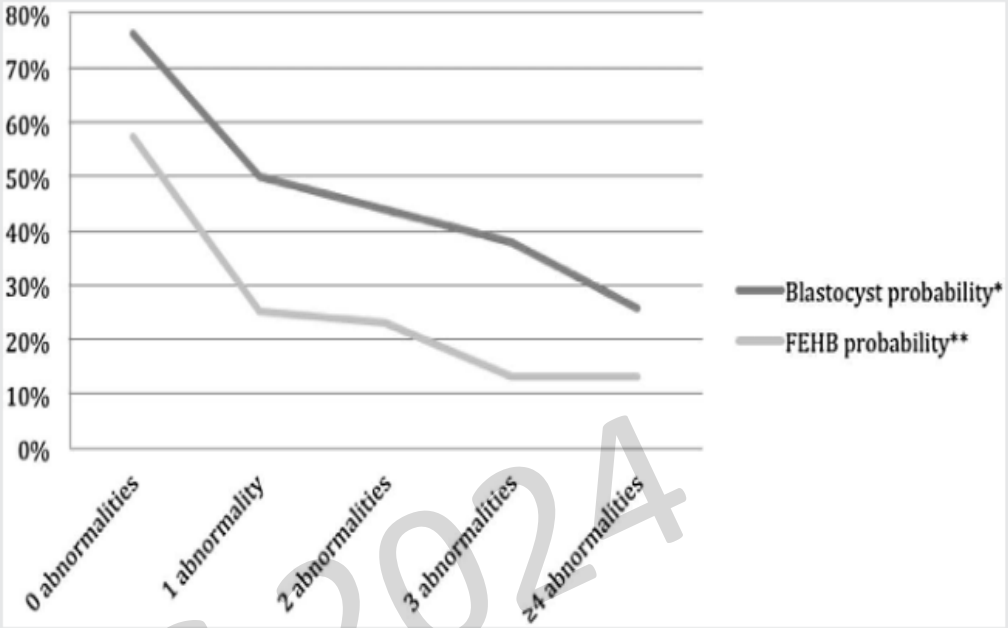


Fig. 3 Exemplified PGS results by use of rapid aCGH (DNA hybridization for 2 h), regular aCGH (DNA hybridization for 16 h) and next generation sequencing (NGS) for the same WGA products. Rapid and regular aCGH were performed with CytoScan 60 K microarray chip (Agilent customer array, Changhua Christian Hospital, Taiwan) on a G4900DA SureScan microarray scanner (Agilent Technologies, CA, USA). NGS was performed using Ion PGM Hi-Q Sequencing Kit with Ion 316 chip (Life technologies, California, USA) on the Ion Torrent PGM Instrument (Life technologies) platform. Aneuploidy chromosomes or chromosomal fragments are indicated by arrows. Some atypical segmental gains and/or losses with copy number change < 1 but > 0.5 (a likely result of embryo mosaicism) were also classified as segmental aneuploidies and marked by stars. The results of rapid aCGH are comparable with that of regular aCGH and NGS



The probability of blastocyst and fully expanded or hatching blastocyst (FEHB) progression decreases in a linear fashion with an increasing number of aneuploidies. *, **P <.0001.

Vega. Blastulation rates and aneuploidies. Fertil Steril 2014.

Chromosomal Errors in Advanced maternal Age >40

Rodrigo etal BioMed Res Intn 2014

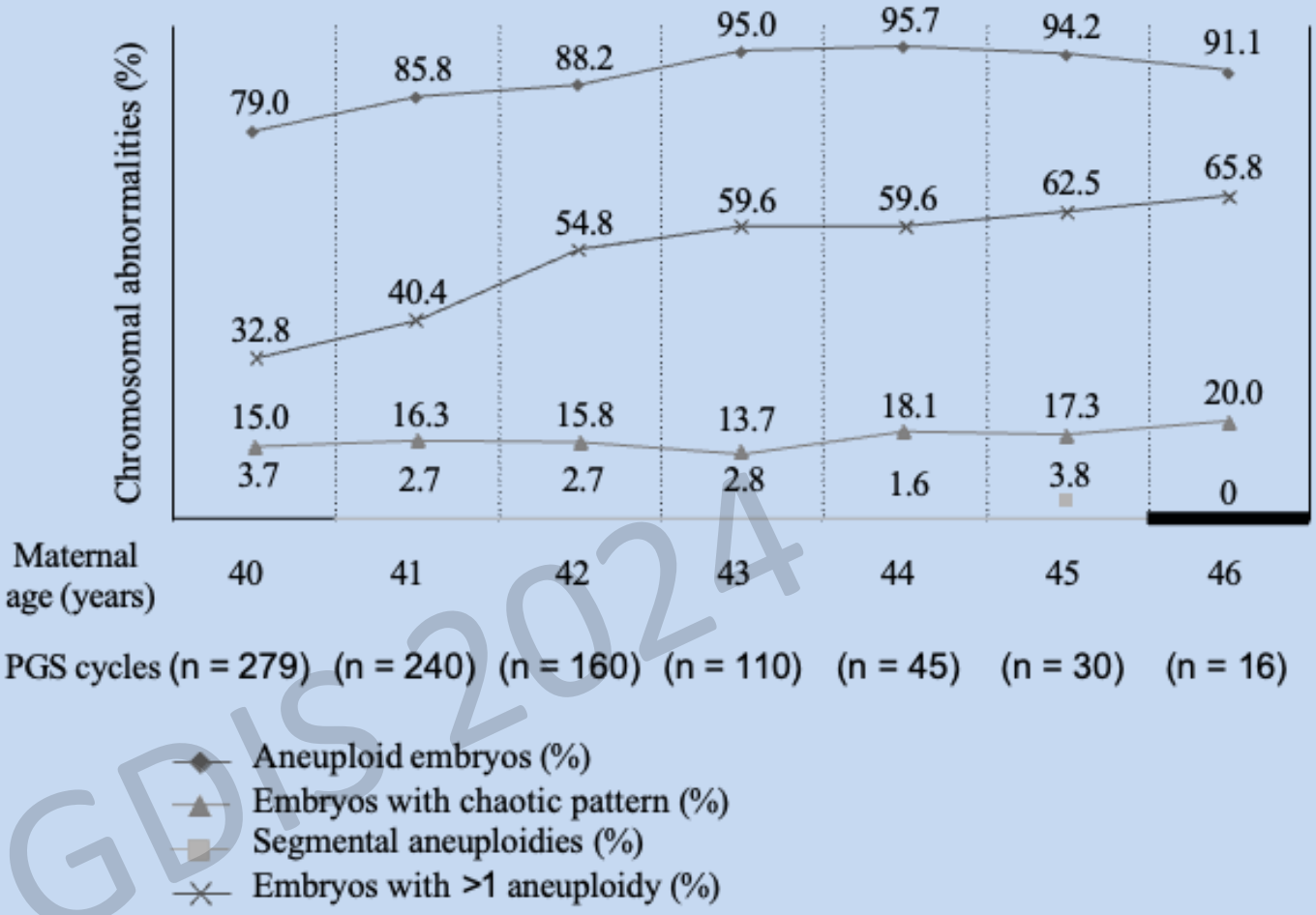


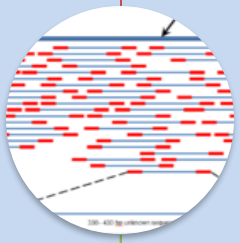
Figure 1: Aneuploidy rates according to maternal age in AMA group.

Selecting Embryos for Transfer

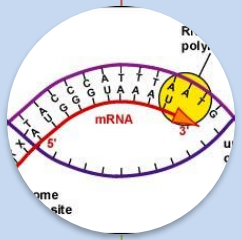
- The rate of mosaicism in human preimplantation embryos prevents accurate determination of developmental potential
- Requires next gen sequencing to detect multiple DNAs
- Correction of mosaicism is likely during development but may persist to birth
- Assay of DNA from extracellular vesicles in culture media has not been scientifically validated
- Data of large multi-centre trials are at best ambivalent for any increased birth rates, or cumulative birth rates following PGT-A
- Normal births have been reported for chromosomally abnormal embryos

Variables that Need to be Accounted For

Bank of viable genetically normal vitrified embryos for transfer into natural cycle

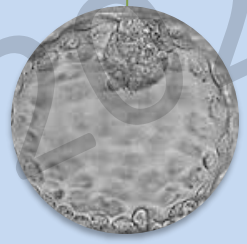


Next Gen Sequ for chromosomal euploidy and DNA integrity

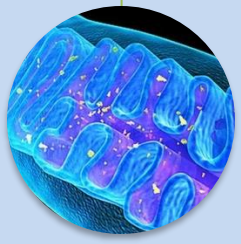


Viable embryonic transcriptome

Optimized culture conditions



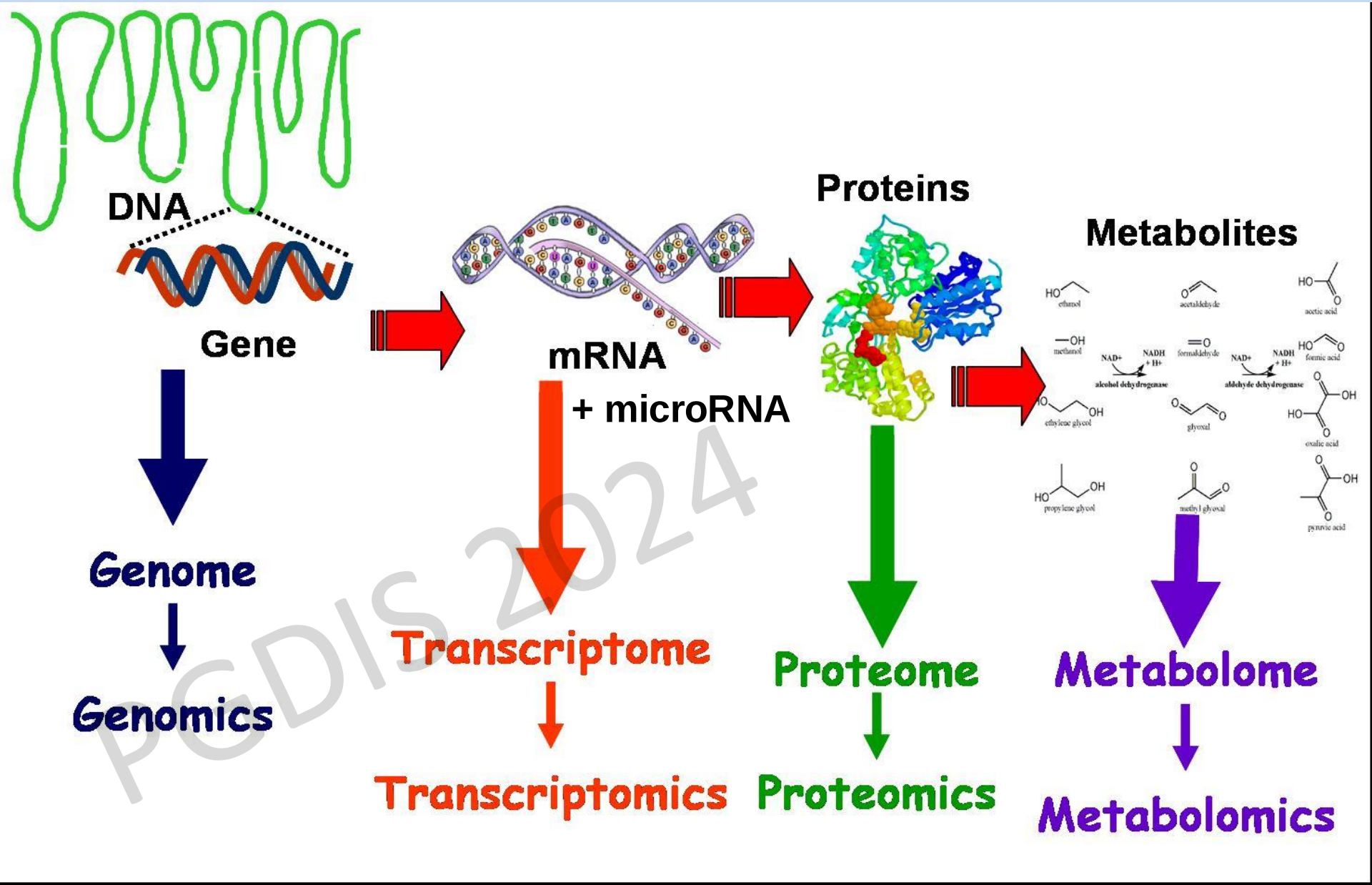
Developmental morphometry

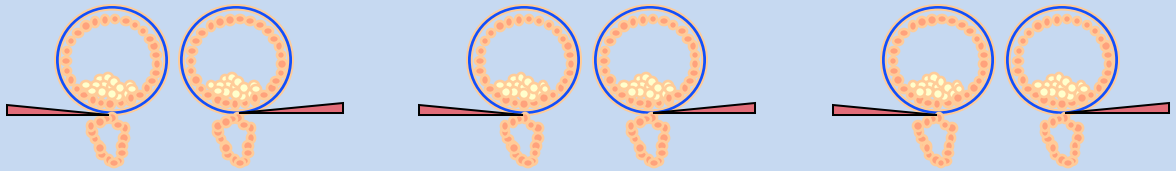


Mitachondrial vitality

Batch Embryo Collection Cycles to Build a Bank of Normal Viable Embryos

Applying "Omics"





1 2 3 4 5 6

Blastocyst transfer

Blastocyst transfer

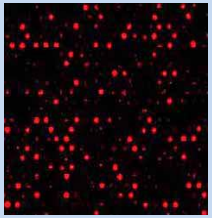
Blastocyst transfer

Group 1
All implanted

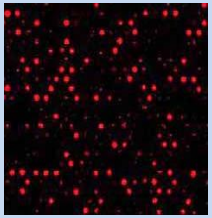
Group 3
None implanted

Group 2
Some implanted

Cord blood



Microarray A



Microarray B

Comparison

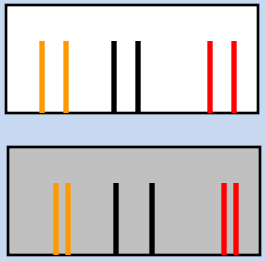
Identification of polymorphic transcripts

cDNA & DNA fingerprinting of sibling blastocysts & cord blood

Subtraction
A - B

5

6



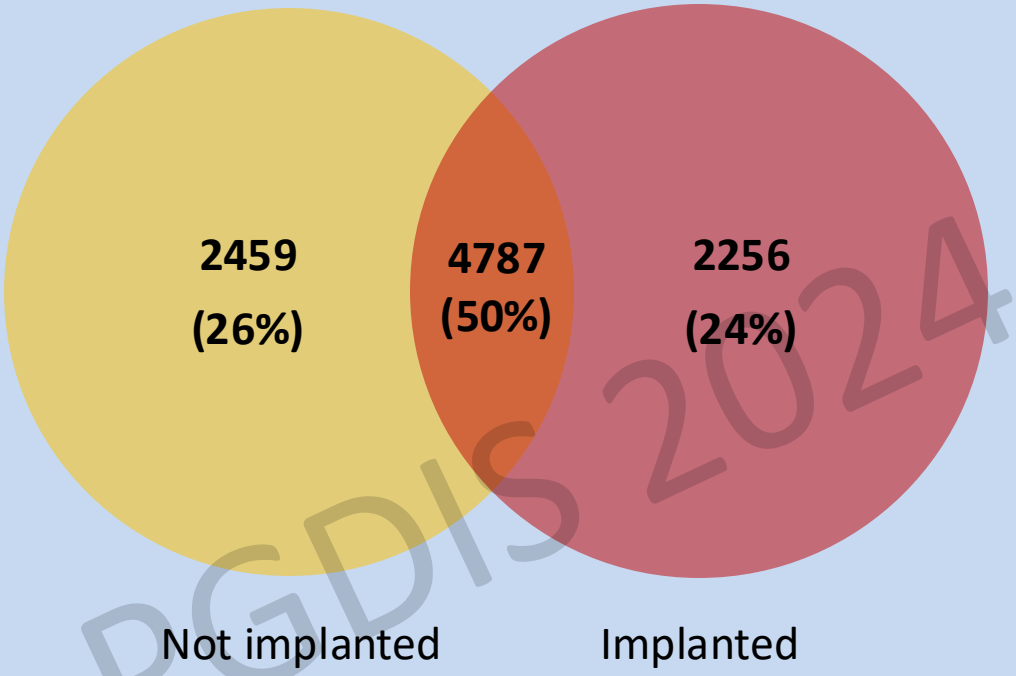
Viable

'Viable' Gene Expression Profile

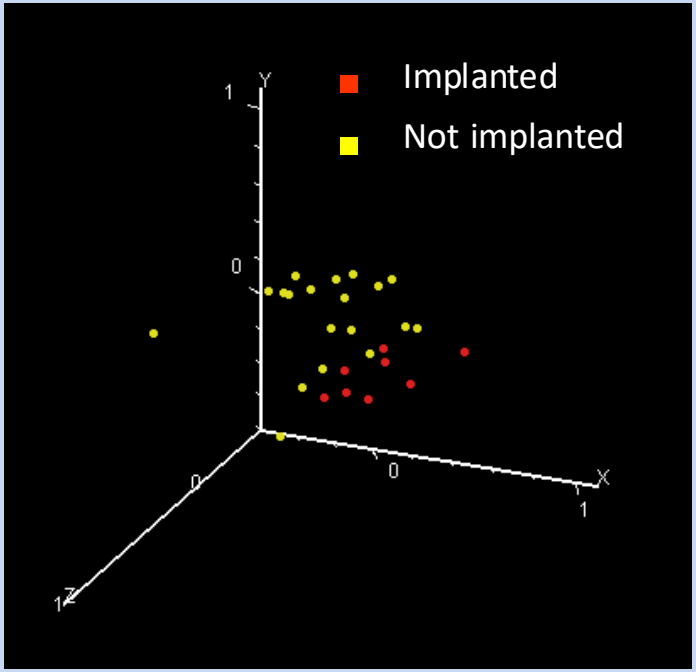
Microarray Validation

Gene analysis of ‘implanted’ versus ‘not implanted’ embryos

Genes associated with viability



Principal components analysis



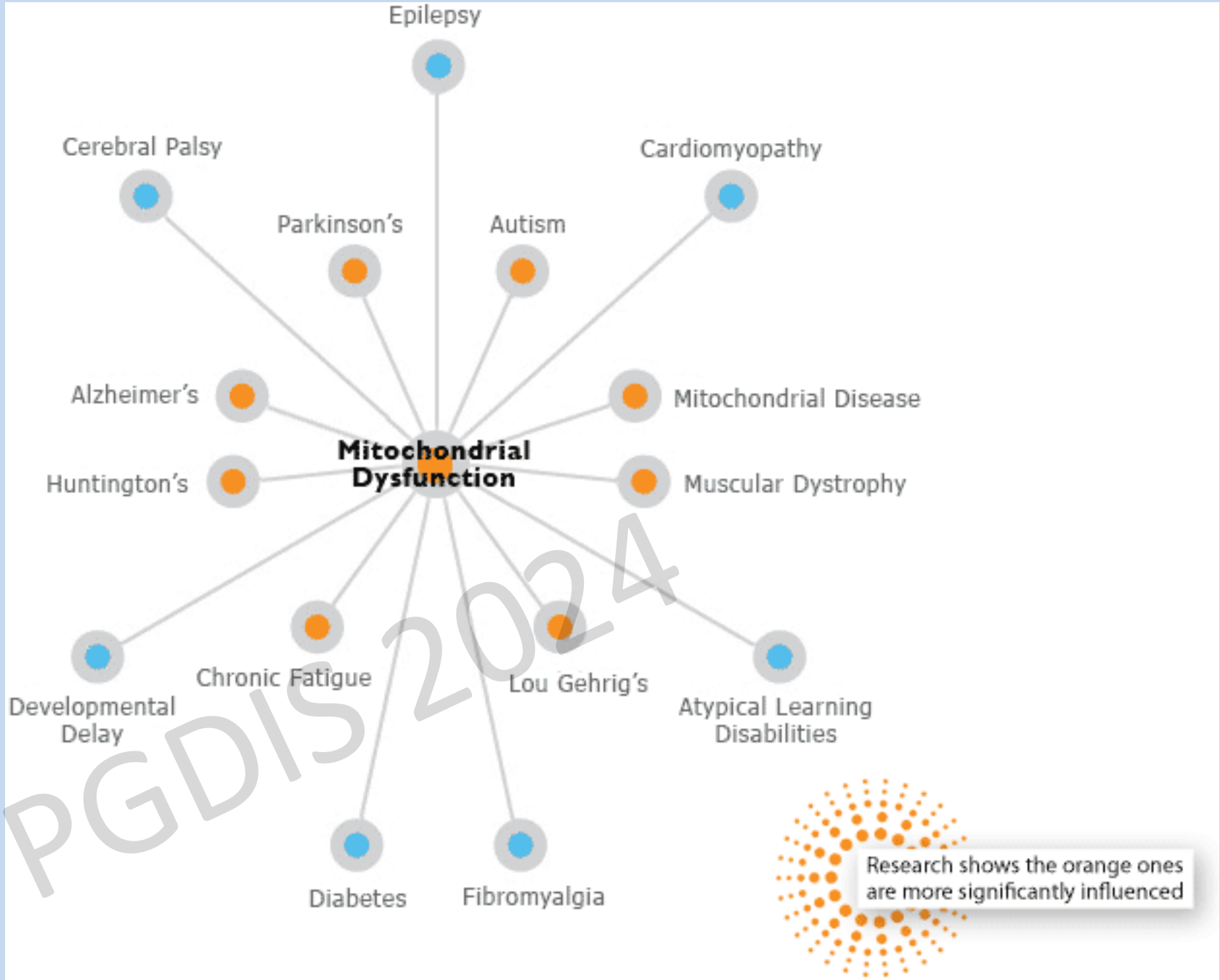
Major Themes in 2412 Genes Unique to Viable TE

	Subcategory	P value
Cell adhesion	Homophilic cell adhesion	1.2×10^{-4}
	Calcium-independent cell adhesion	1.3×10^{-2}
	Neuron adhesion	3.5×10^{-2}
	Calcium-dependent cell adhesion	4.1×10^{-2}
Cell communication	Cell–Cell signalling	
	Synaptic transmission	1.7×10^{-4}
	Nerve ensheathment	1.4×10^{-2}
	Signal transduction	
	Adenylate cyclase activation	1.0×10^{-2}
	G-protein signalling	1.4×10^{-2}
	Transmembrane receptor protein tyrosine kinase activation	1.9×10^{-2}
	Acetylcholine receptor signalling	1.9×10^{-2}
	Glutamate signalling pathway	3.0×10^{-2}
	Cell surface receptor linked signal transduction	3.3×10^{-2}
	Activation of MAPK activity	4.1×10^{-2}
Cellular metabolic process	Positive regulation of interleukin-13 biosynthesis	2.4×10^{-3}
	Positive regulation of interleukin-6 biosynthesis	8.5×10^{-3}
	Alanyl-tRNA aminoacylation	1.9×10^{-2}
	Cyclic nucleotide metabolism	3.5×10^{-2}
Response to stimuli	Defense response to bacteria	5.6×10^{-3}

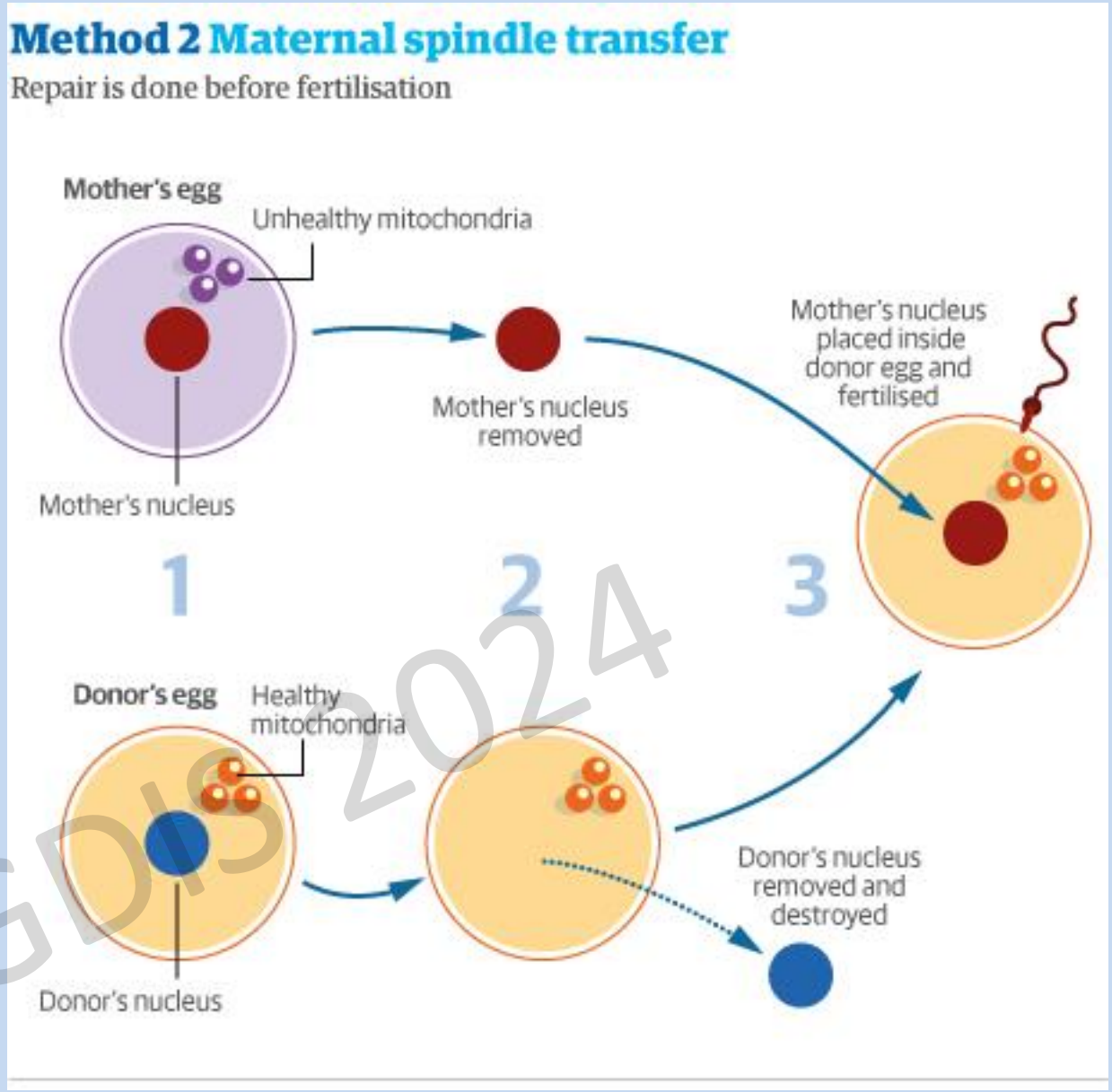
Mitachondrial Diseases

Can mitachondrial DNA errors be corrected?

Inherited Mitochondrial Disease



DNA Manipulation

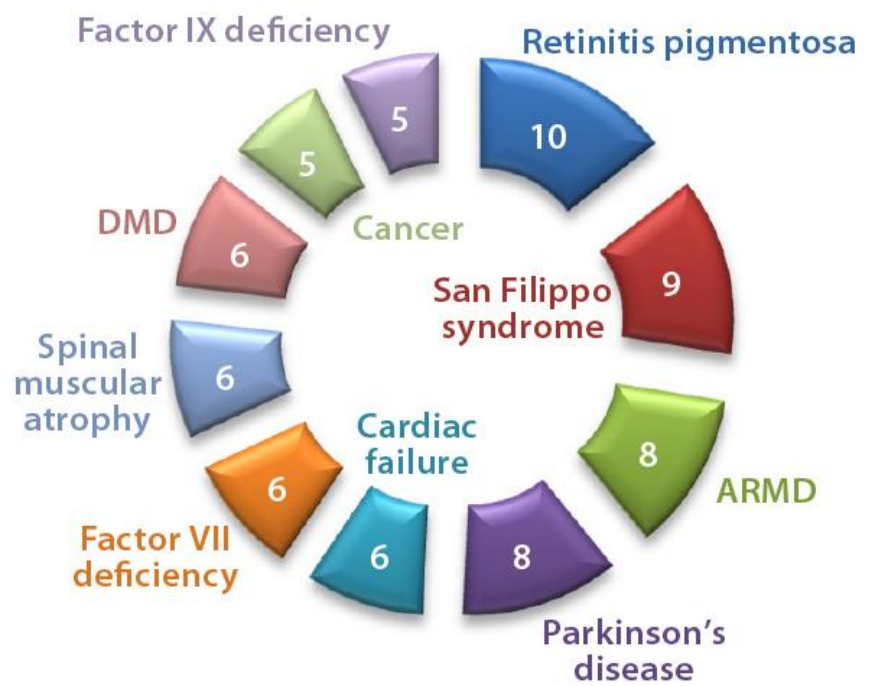


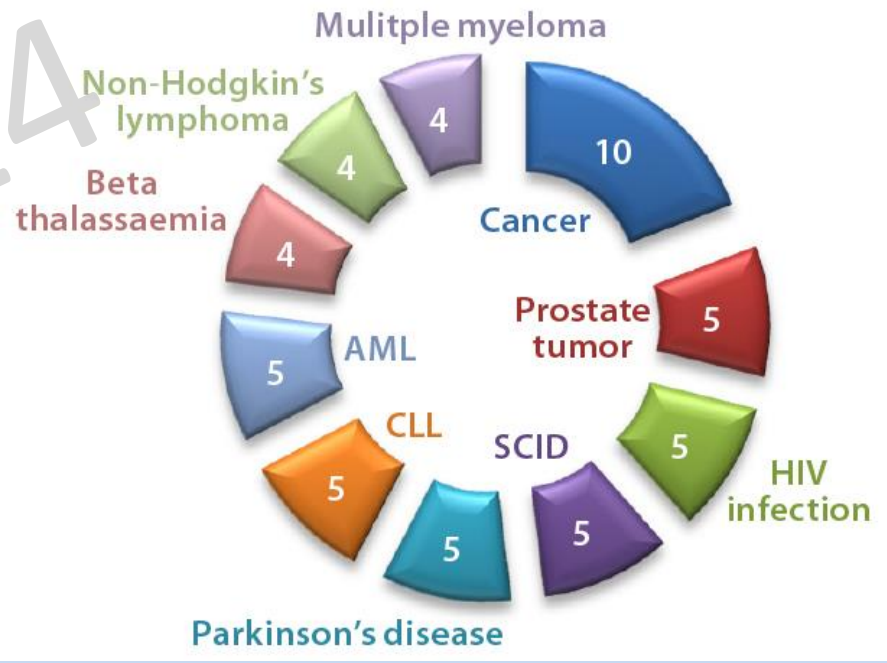
Mitochondrial transfer

- Less than 5 babies born in UK – HFEA Report May 2023
- Carry over donor mt can become dominant in monkey experiments – likely outcome in human studies – is 20% hetroplasmic mtDNA mutations acceptable? Arguably risk is too high.
- Detection of new pre-mt in early stage embryo models – may complicate nuclear (spindle) transfer if not identified and excluded from transfer.

Gene Editing to Correct Genomic Errors

Why Edit Embryos?

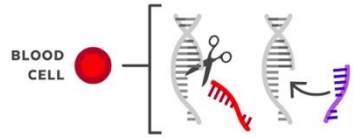
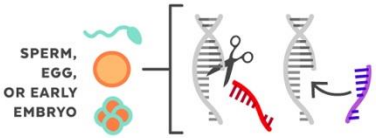
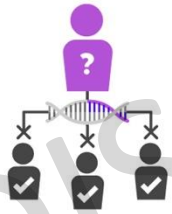
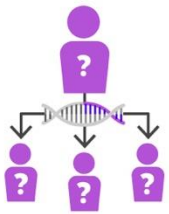




Gene Editing Embryos

- He Jiankui of Southern University of Science and Technology in Shenzhen China was sentenced to three years jail for worlds first gene edited babies. (The Scientist 3.1.2020)
- He altered the *CCR5* gene using CRISPR-cas9 to prevent the children contracting AIDS (mutant *CCR5* associated with resistance to HIV)
- No adverse effects on three children reported
- Criticism is based on risks of unintended genetic effects that would be inhertited

Gene Editing - Human Medicine

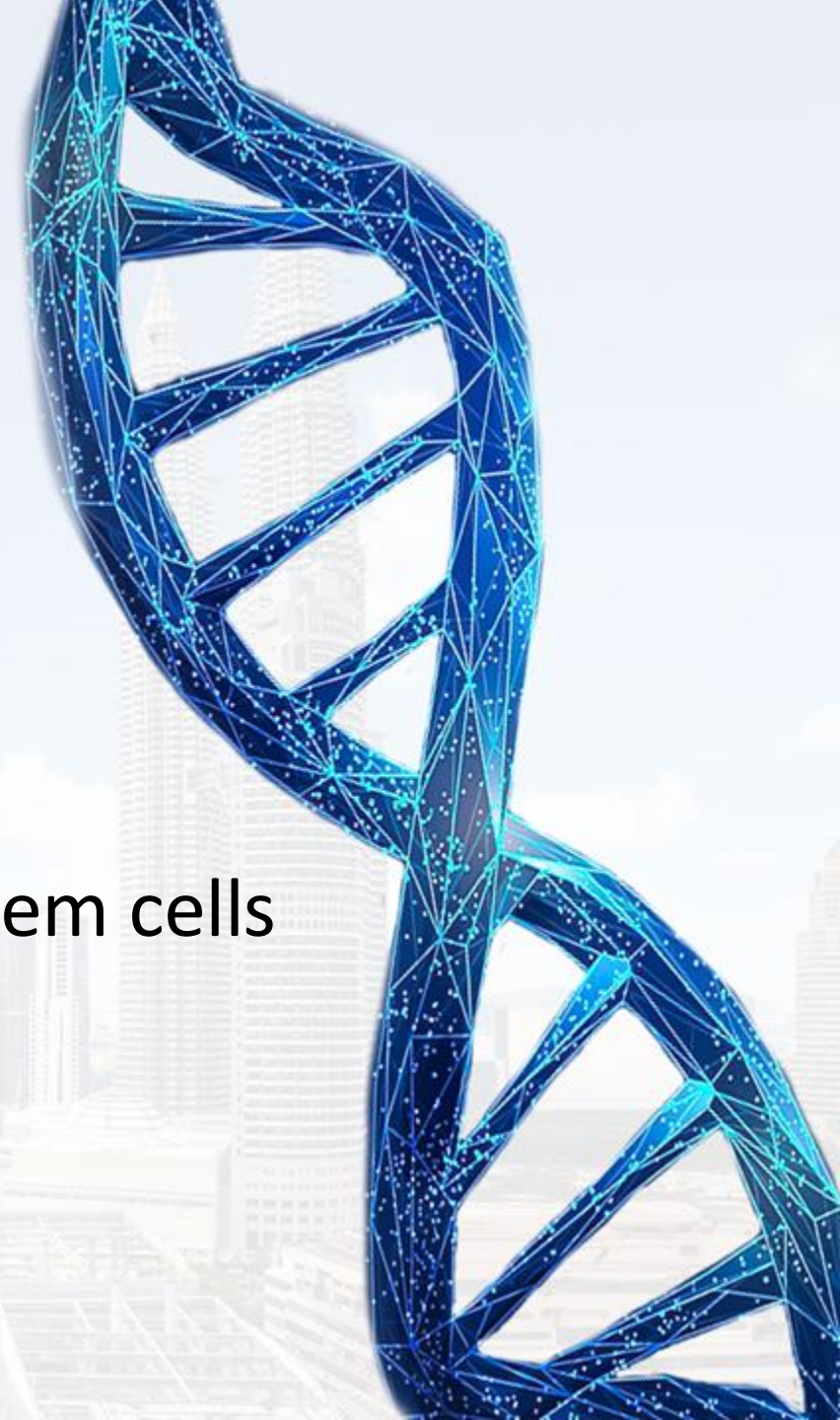
	SOMATIC GENE EDITING	VS.	GERMLINE GENE EDITING
EDIT	 Somatic therapies target genes in specific types of cells (blood cells, for example).		 Germline modifications are made so early in development that any change is copied into all of the new cells.
COPY	 The edited gene is contained only in the target cell type. No other types of cells are affected.		 The edited gene is copied in every cell, including sperm or eggs.
RISKS	Any changes, including potential off-target effects, are limited to the treated individual.		If the person has children, the edited gene is passed on to future generations.
NEXT GENERATION	 The edited gene is not passed down to future generations.		
CONSENSUS	 Somatic cell therapies have been researched and tested for more than 20 years and are highly regulated.		 Human germline editing is new. Heritability of germline changes presents new legal and societal considerations.

Gene Editing can be highly accurate

- For example: Xie etal. Genome Biology 2024
- The FokI catalytic domain can be fused to various DNA binding architectures to improve the precision of genome editing tools.
- Tested FokI-heterodimers fused with TALENs, FokI homodimers fused with RYdCas9, or FokI catalytic domains alone resulting in no significant off-target effects. These FokI genome editing systems exhibit undetectable off-target effects in mouse embryos
- Question the cost benefit?
- Edit the person with the genomic error or the embryo?

Postimplantation Embryos

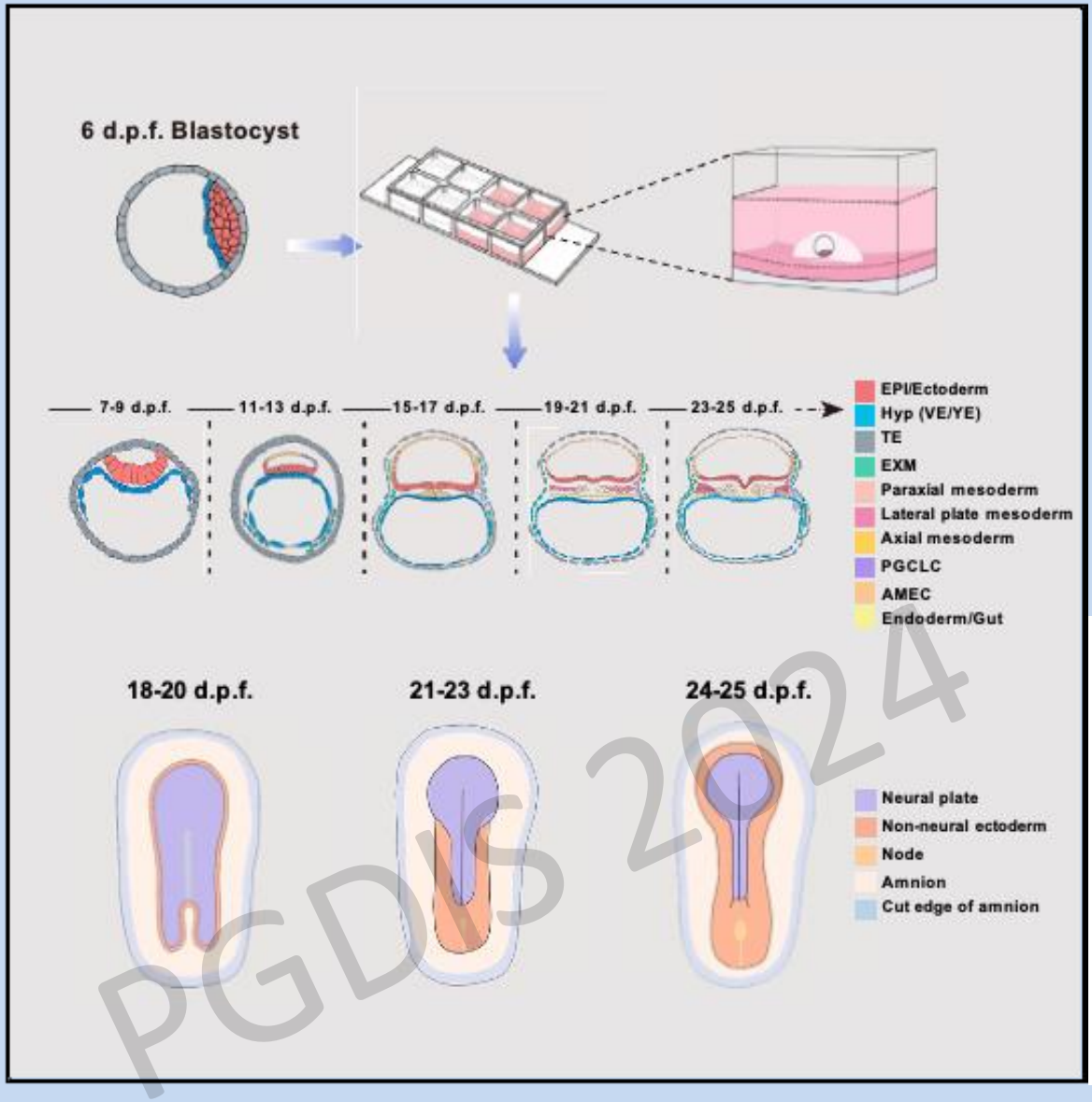
- Derived from IVF embryos
- Derived from pluripotent stem cells



Growing monkey embryos from blastocyst to early organogenesis

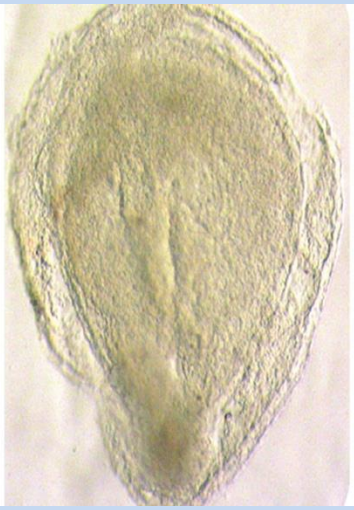
Graphical abstract

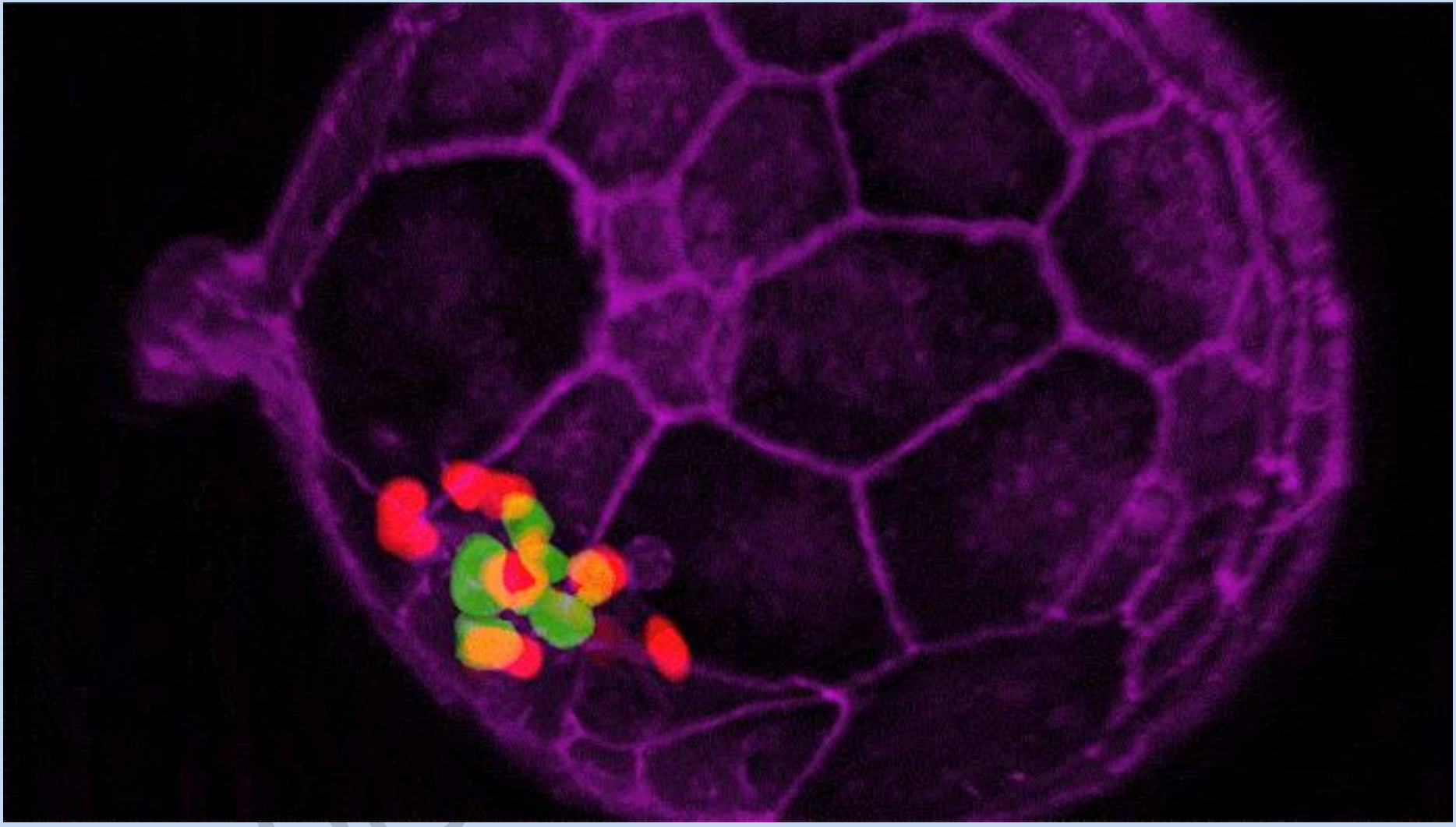
Gong etal. Cell 2023



Day 16-19 human embryo

Oxford Univ.

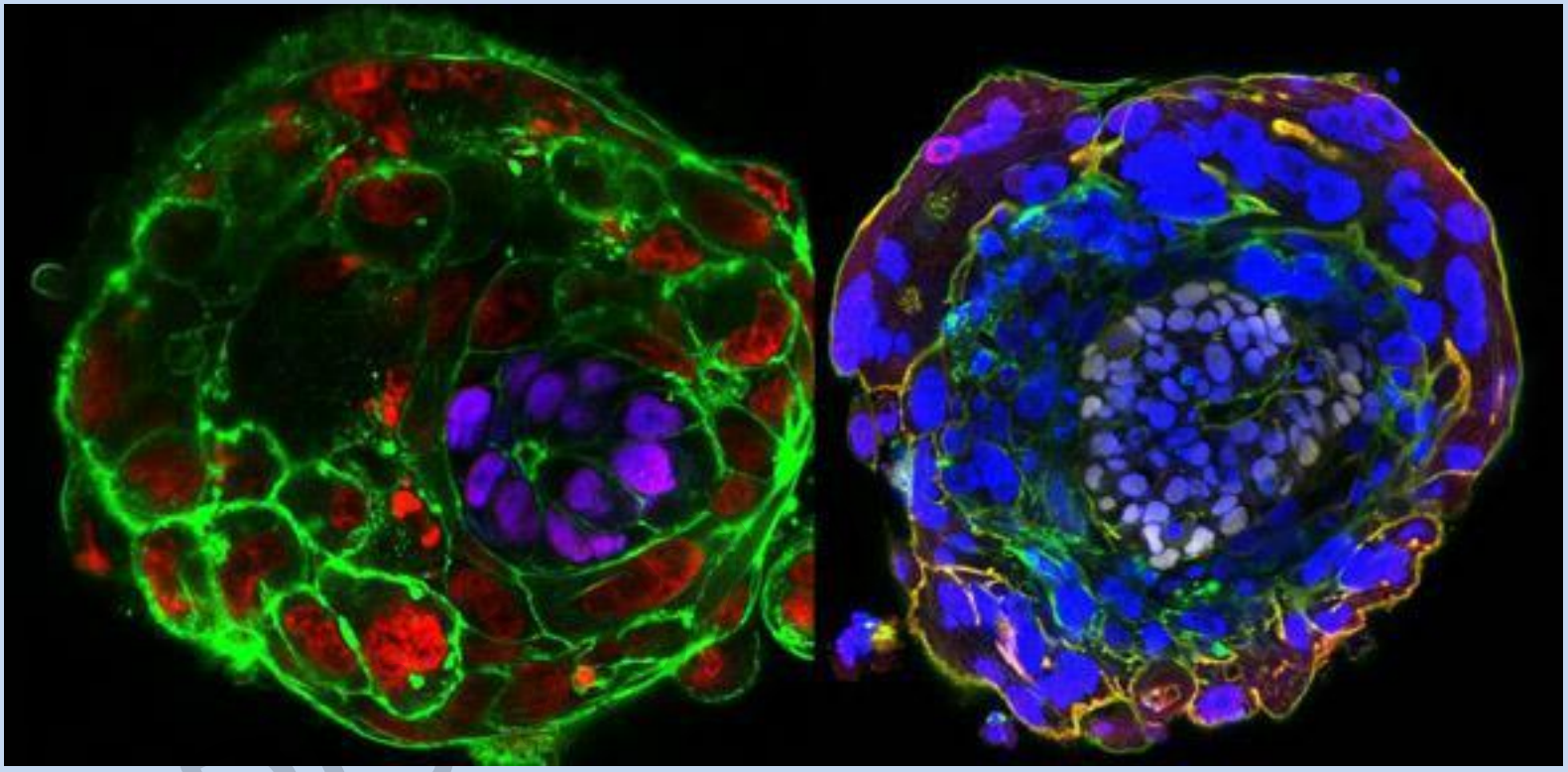




This is a 7-day-old embryo. If it developed further, the clusters in green would become cells that shape the body and the red/purple cells would form the placenta. Croft, Pelligrini, Brivanlou

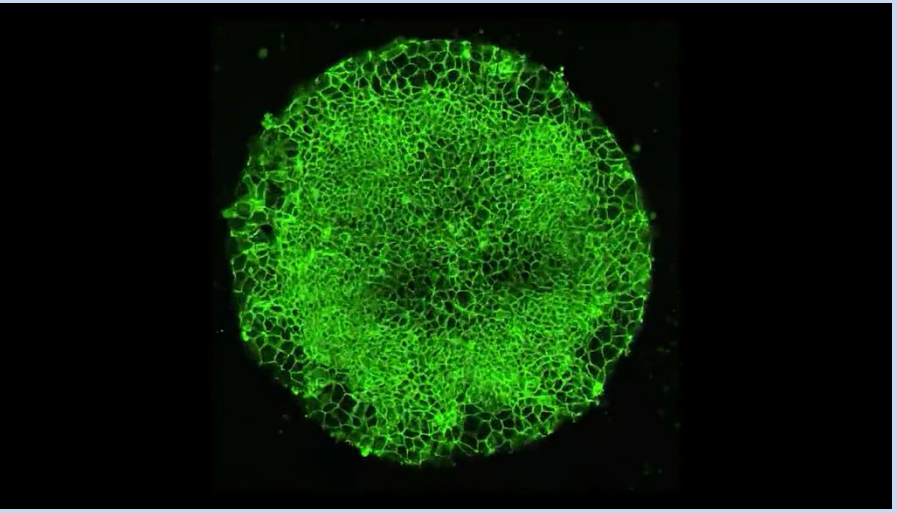
At day 10 of embryo development the pluripotent stem cells that will generate the future body self-organise to generate a cavity (the pro-amniotic cavity).

At day 11 of embryo development the pluripotent stem cells that will generate the future body self-organise to generate a cavity (the pro-amniotic cavity).

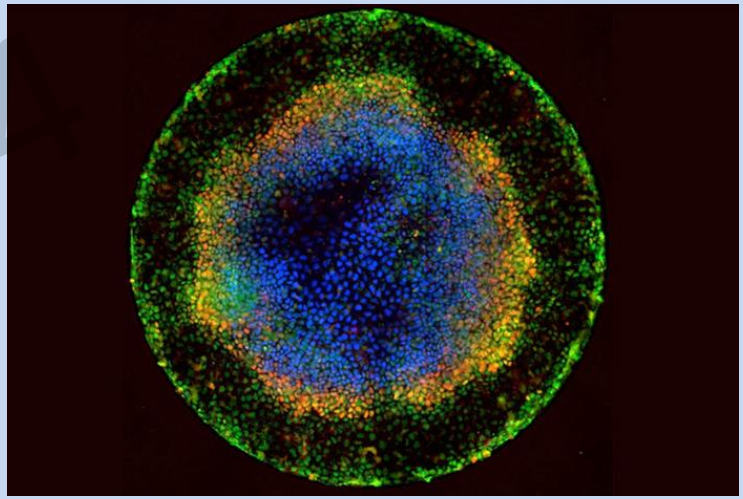


Shahbazi, MN et al. Self-organisation of the human embryo in the absence of maternal tissues (http://dx.doi.org/10.1038/ncb3347) . Nature Cell Biology; 4 May 2016;

Human Embryonic Models Derived from Pluripotent Stem Cells



Human Embryos Form Primitive Streak In Vitro

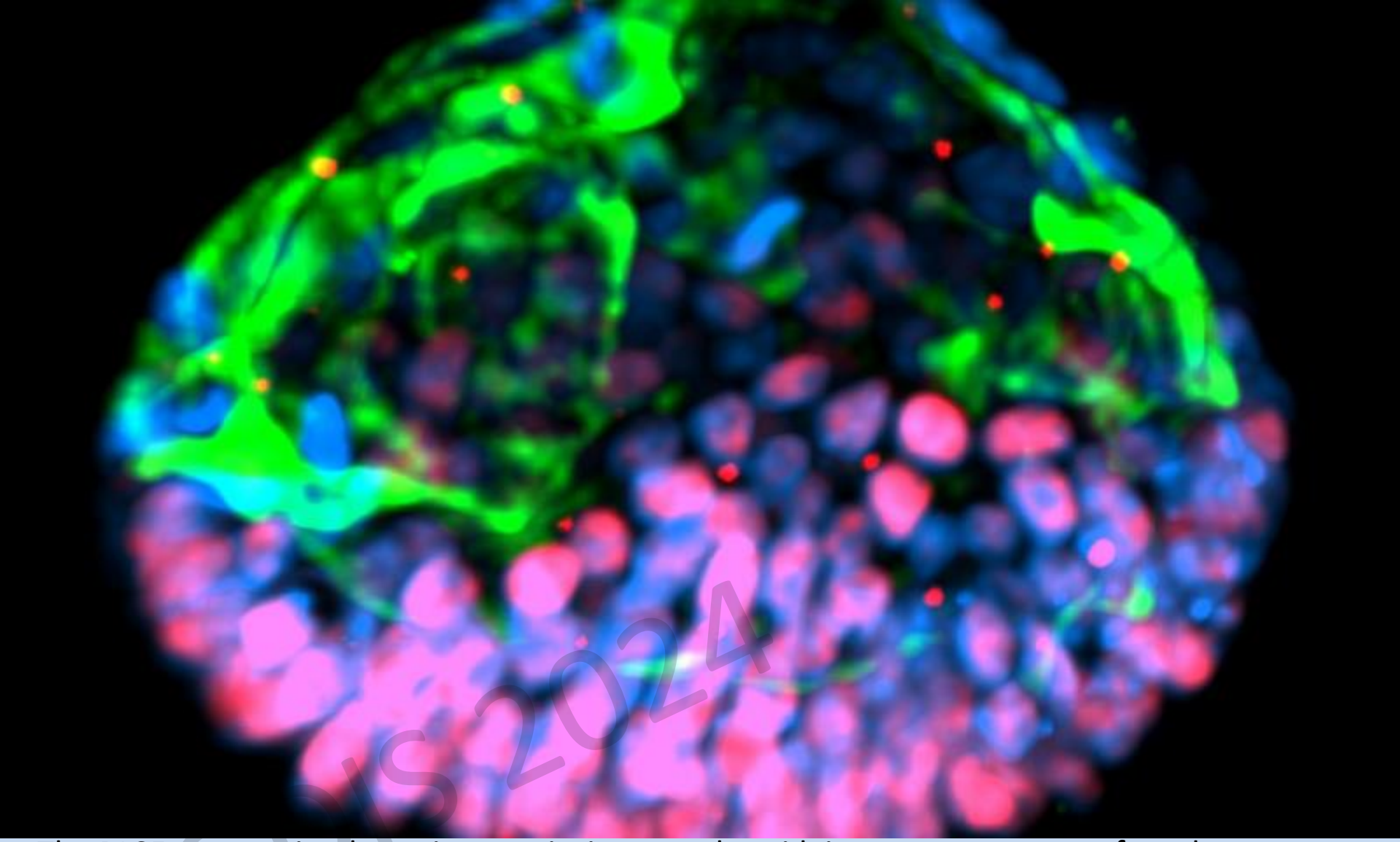


14 Day Human Embryoid – Ali Brivanlou

An Ethical Dilemma Looms

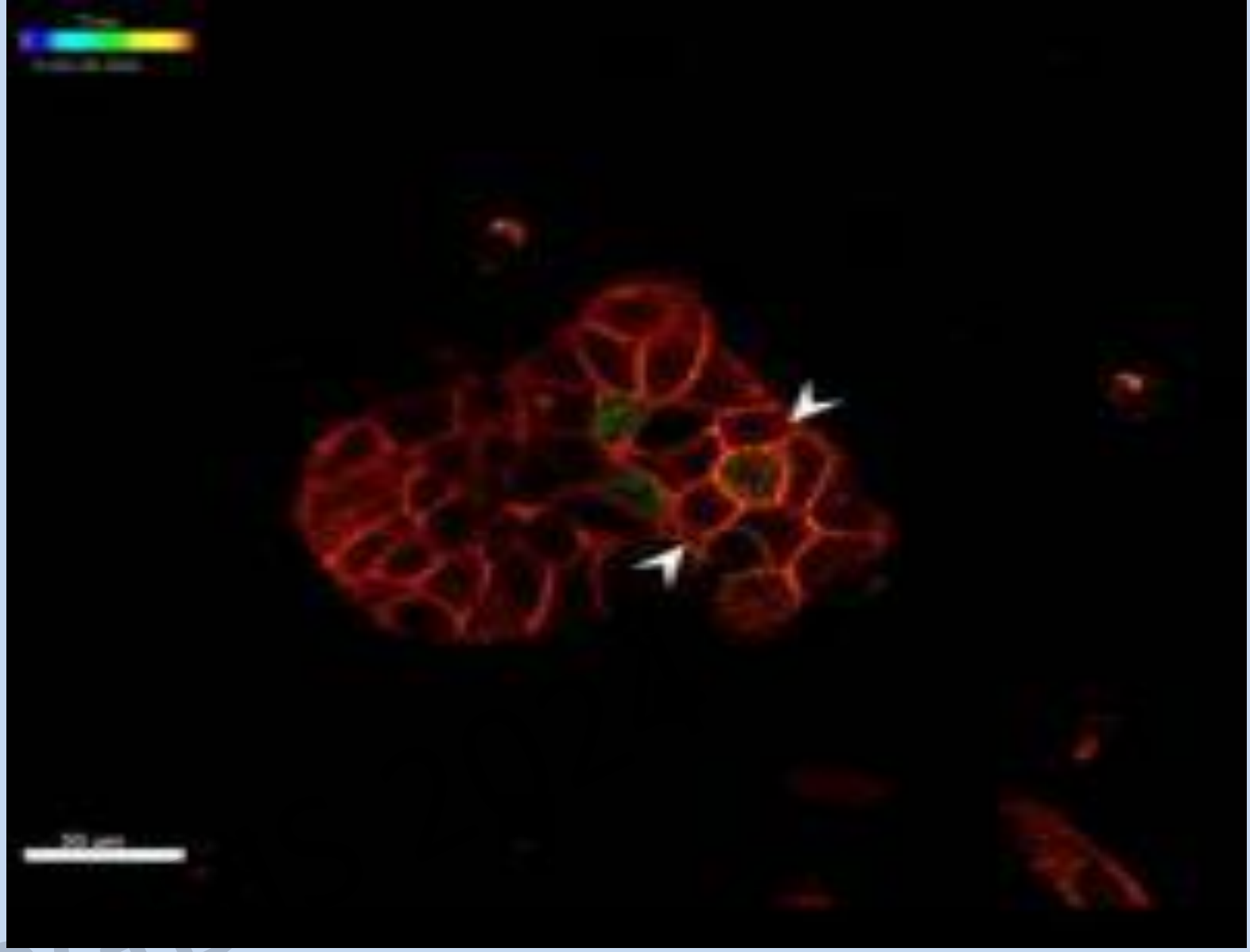
As biological research races forward, ethical quandaries are piling up. In a report published in the journal eLife, researchers at Harvard Medical School said it was time to ponder a startling new prospect: synthetic embryos.

John D. Aach and his colleagues explored the ethics of creating what they call “synthetic human entities with embryolike features” — Sheefs, for short. For now, the most advanced Sheefs are very simple assemblies of cells.



The PASE, or post-implantation amniotic sac embryoid, is a structure grown from human pluripotent stem cells that mimics many of the properties of the amniotic sac that forms soon after an embryo implants in the uterus wall.

Yue Shao et al, A pluripotent stem cell-based model for postimplantation human amniotic sac development, Nature Communications (2017).

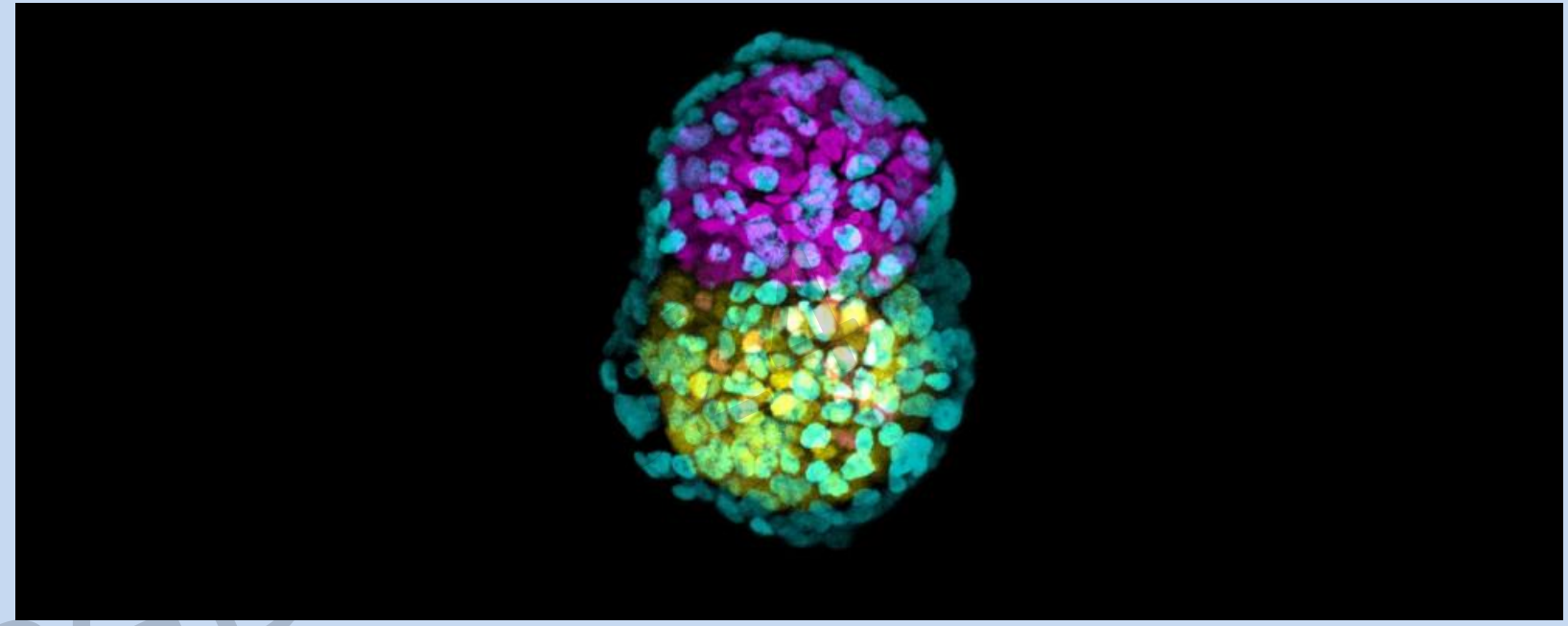


Still from a recording of live pluripotent cells, in which the cells with higher levels of Myc (green) cause the death of their neighbors (white arrowheads). This system works through the elimination of cells that begin to differentiate prematurely, in a process mediated through 'cell competition' based on the expression levels of the gene Myc. CREDIT CNIC

Artificial Mouse Embryo Made in a Laboratory

The embryo, grown in a dish from several types of stem cells, went through gastrulation, a significant stage in development.

Science Today Jul 25, 2018

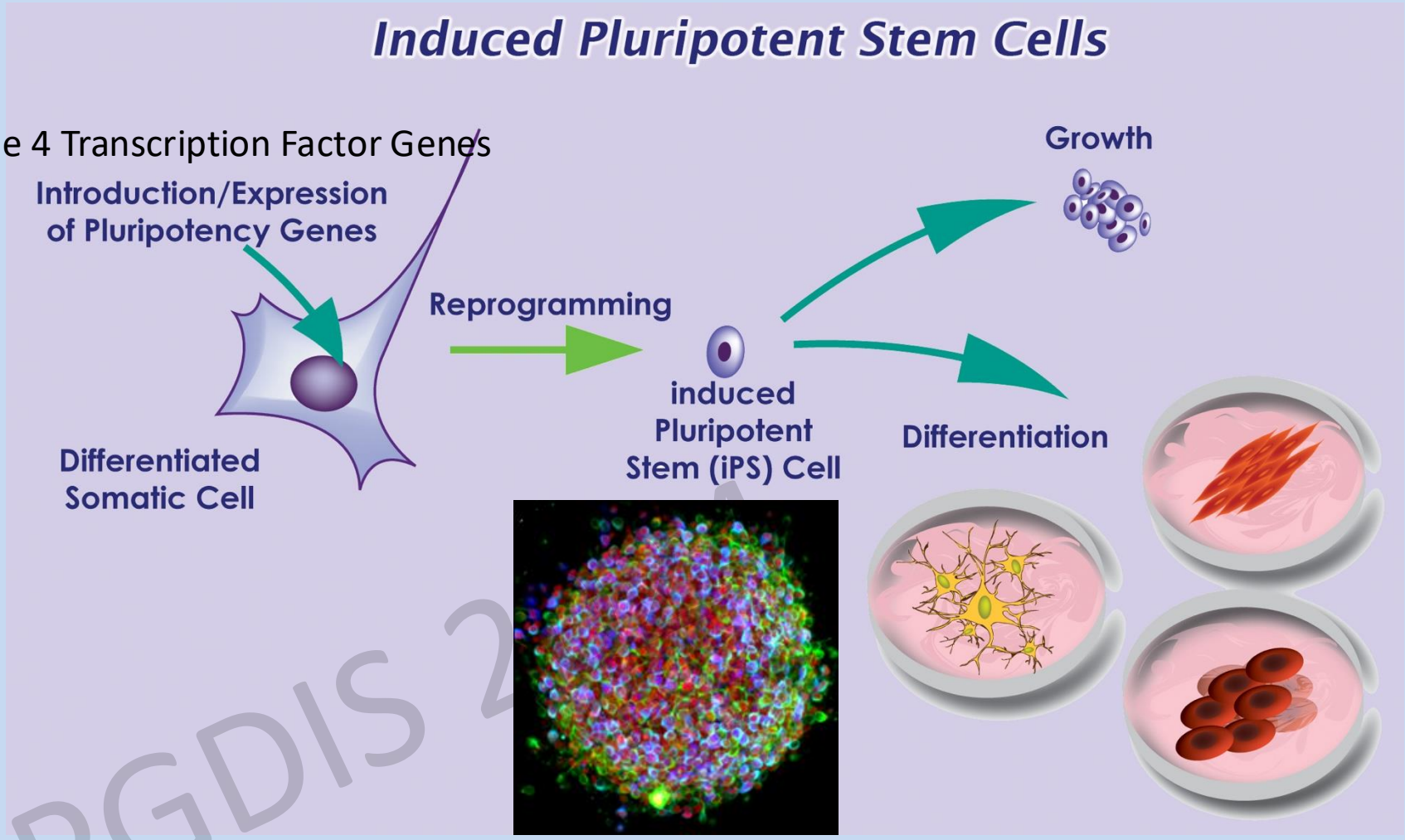


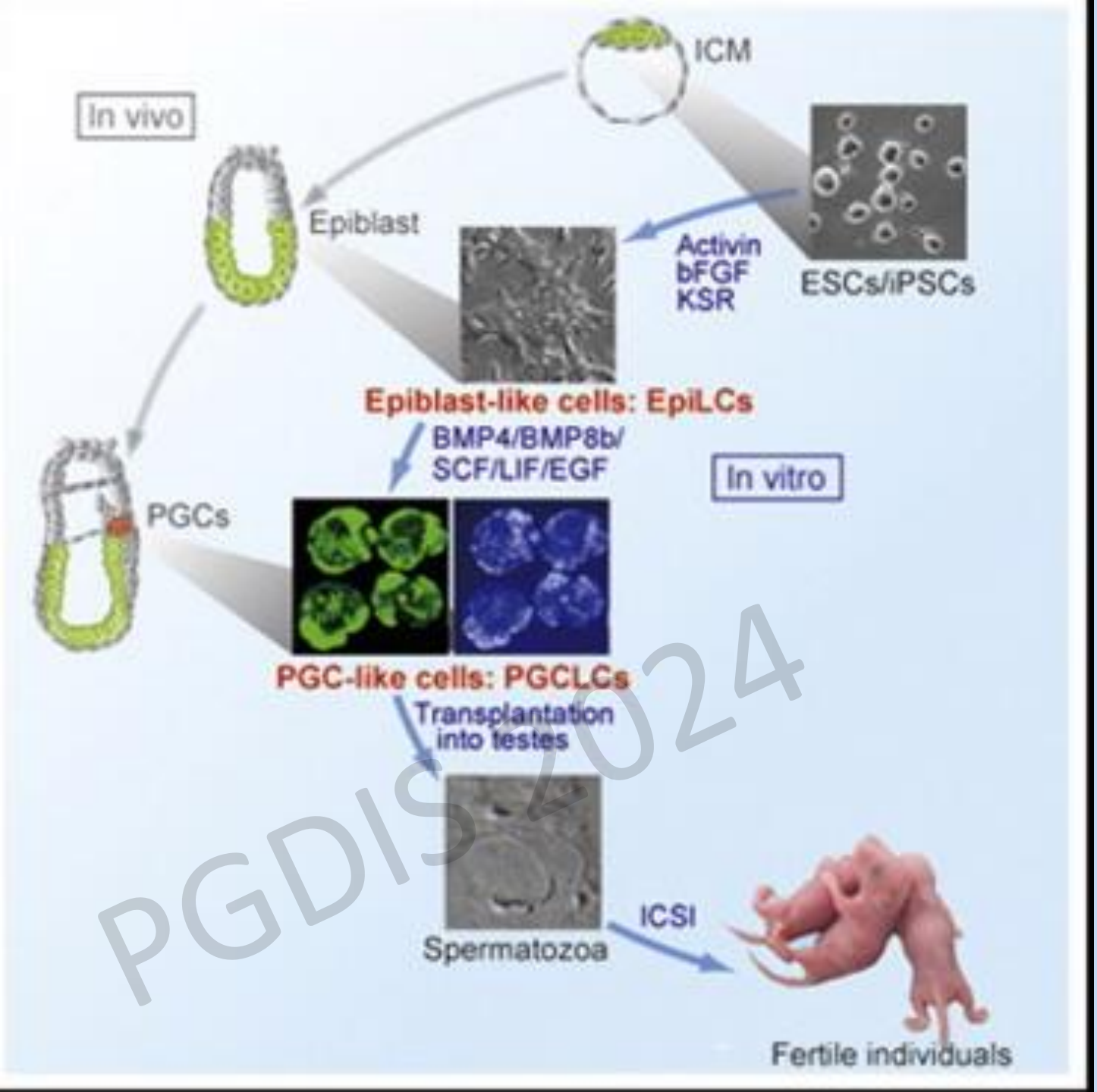
Use of Pluripotent Stem Cells for Correction of Sterility

- Development of sperm from iPSCs
- Function when transplanted

- Oocytes from iPSCs
- Developmental potential

Making the Equivalent of Embryonic Stem Cells from Blood, Skin etc





Development of Viable Sperm from Pluripotential Stem Cells

Hayashi etal. 2011

Therapy for Azoospermia?

Blastocyst

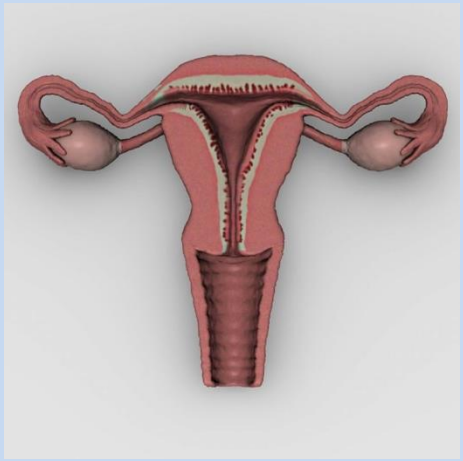
8-cell

4-cell

2-cell

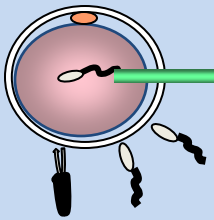
Embryos

Transfer to patient

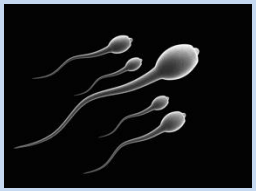


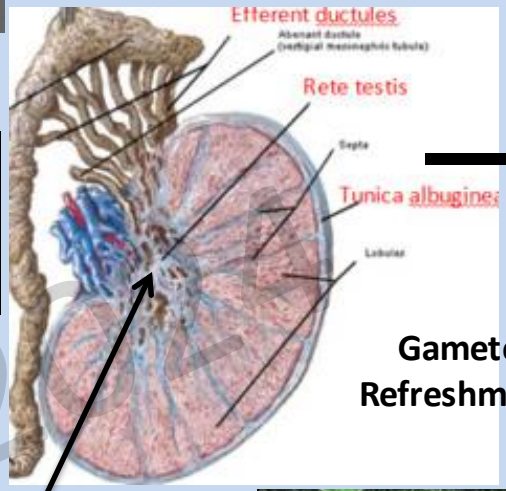
Steroid replacement therapy

ICSI

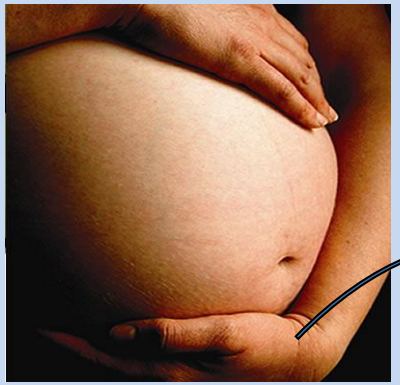


IVF

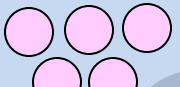


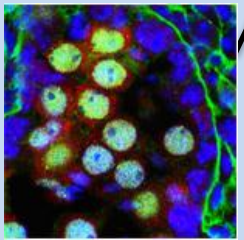


Gamete Refreshment



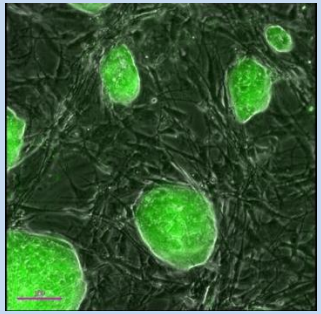
MII eggs



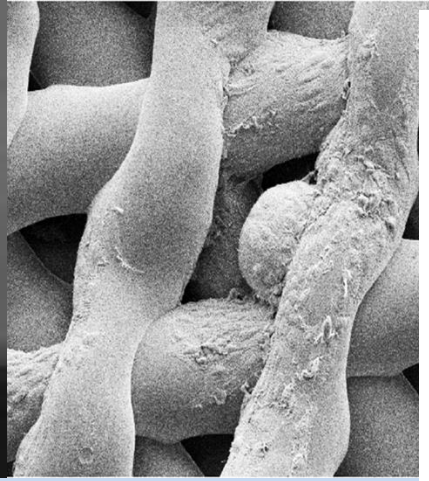


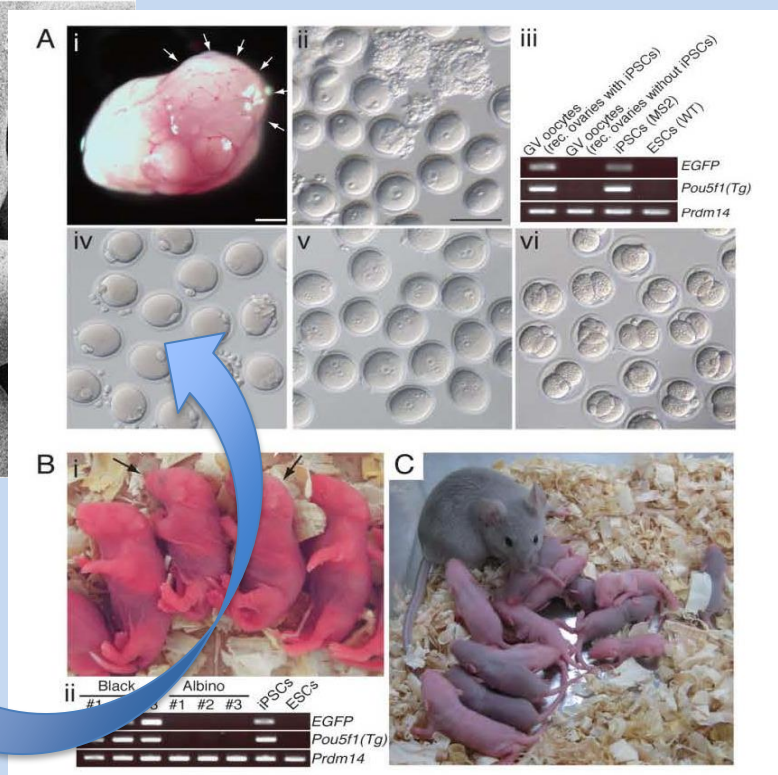
PGCS

iPSCs

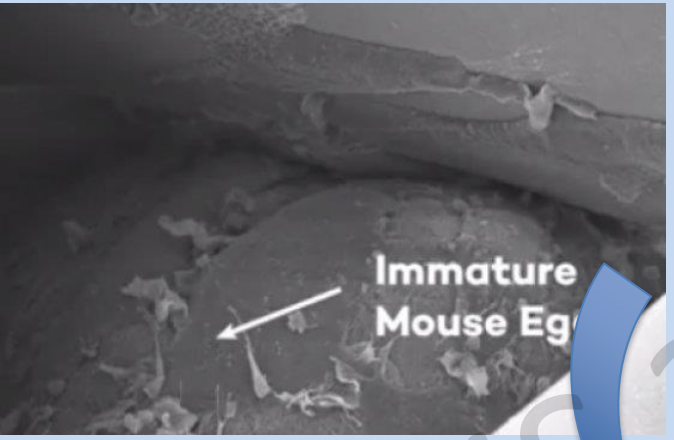


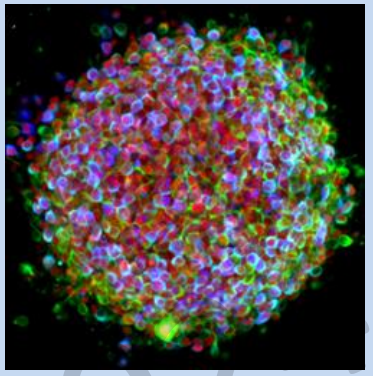
A tissue paper (green) supports the growth of an ovarian follicle (purple) in this SEM image ADAM JAKUS/NORTHWESTERN UNIVERSITY



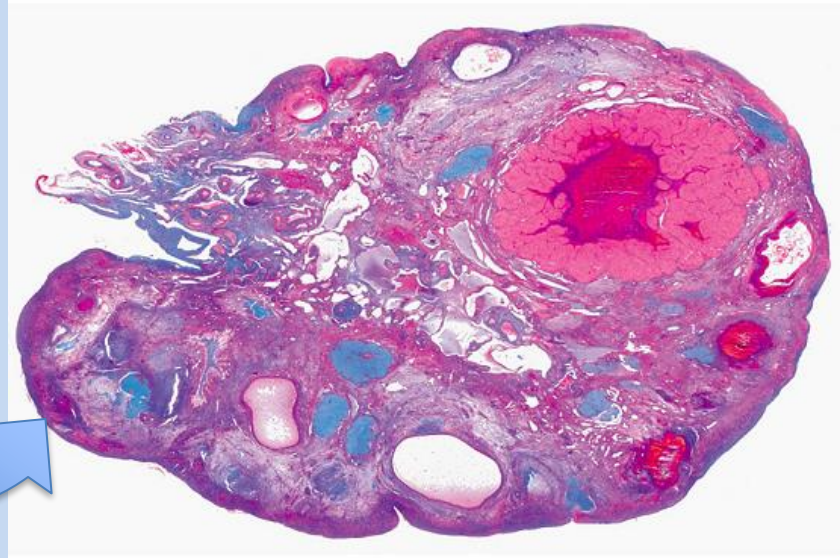


3D Printing Ovary





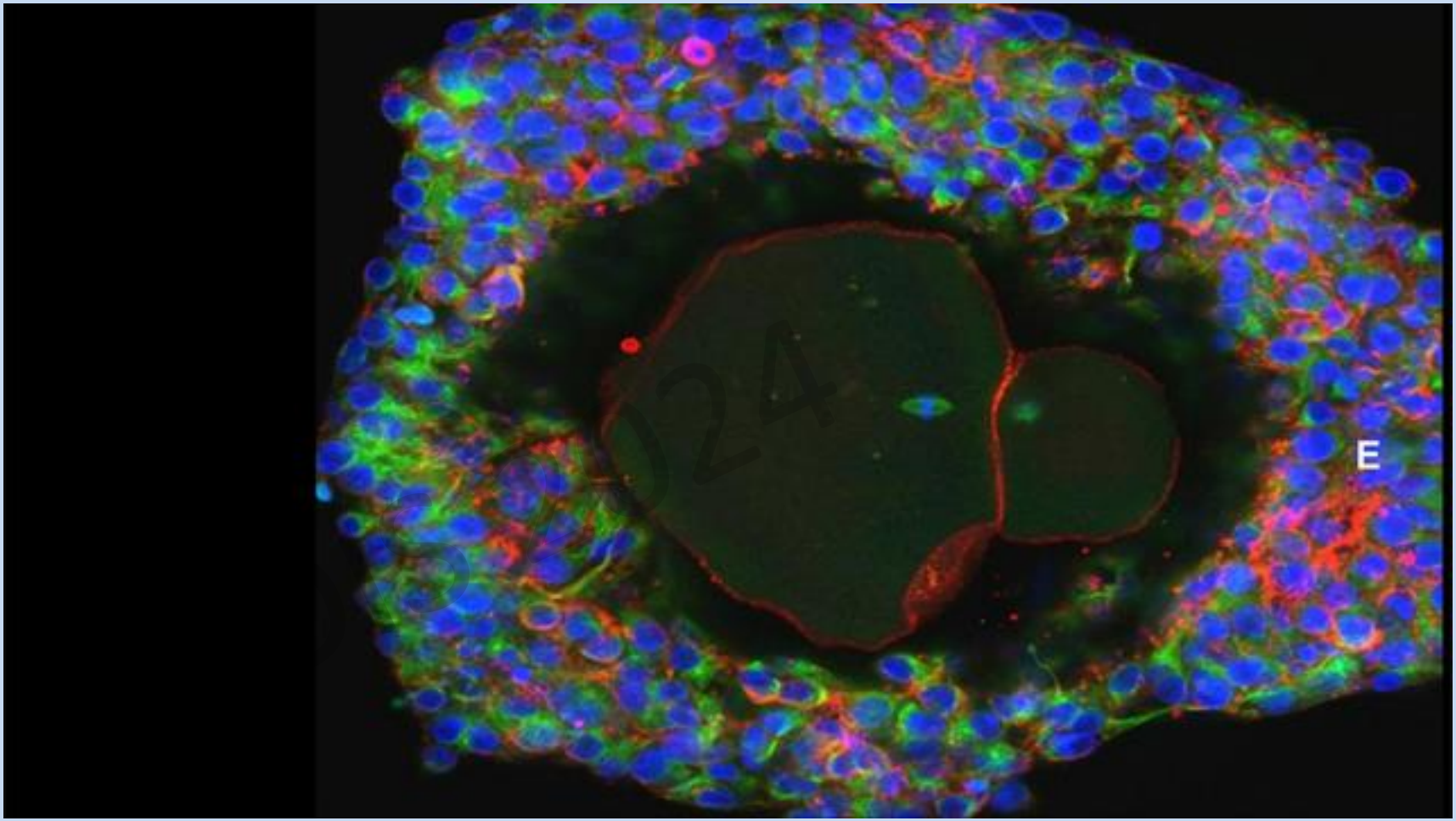
iPSC

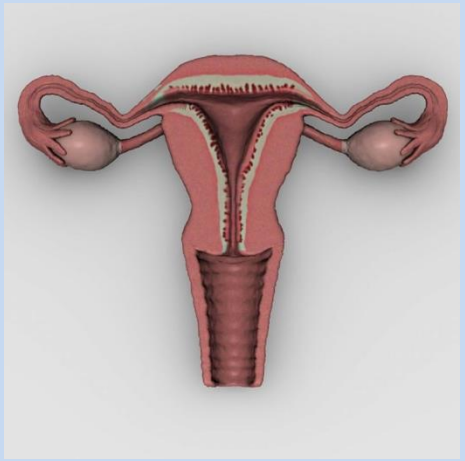


Hayashi etal. Science 2012
Hayashi & Saitou Nature Protoc 2013
Hikabe etal. Nature 2016 (HayashiLab)

Laronda etal Nature Comm 2017
Woodruff Lab

For the first time, scientists have nurtured human eggs from an immature state to the stage where they would be ready to be fertilized by sperm. The maturation was done entirely in the lab, researchers reported last month (January 30 2018) in Molecular Human Reproduction.





8-cell

4-cell

2-cell

Blastocyst

Embryos

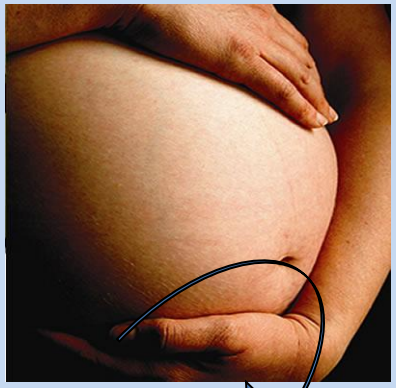
Transfer to recipient

Steroid replacement therapy

ICSI

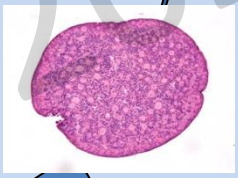
Husband

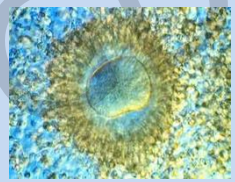
IVF



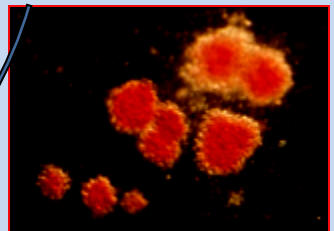
MII eggs

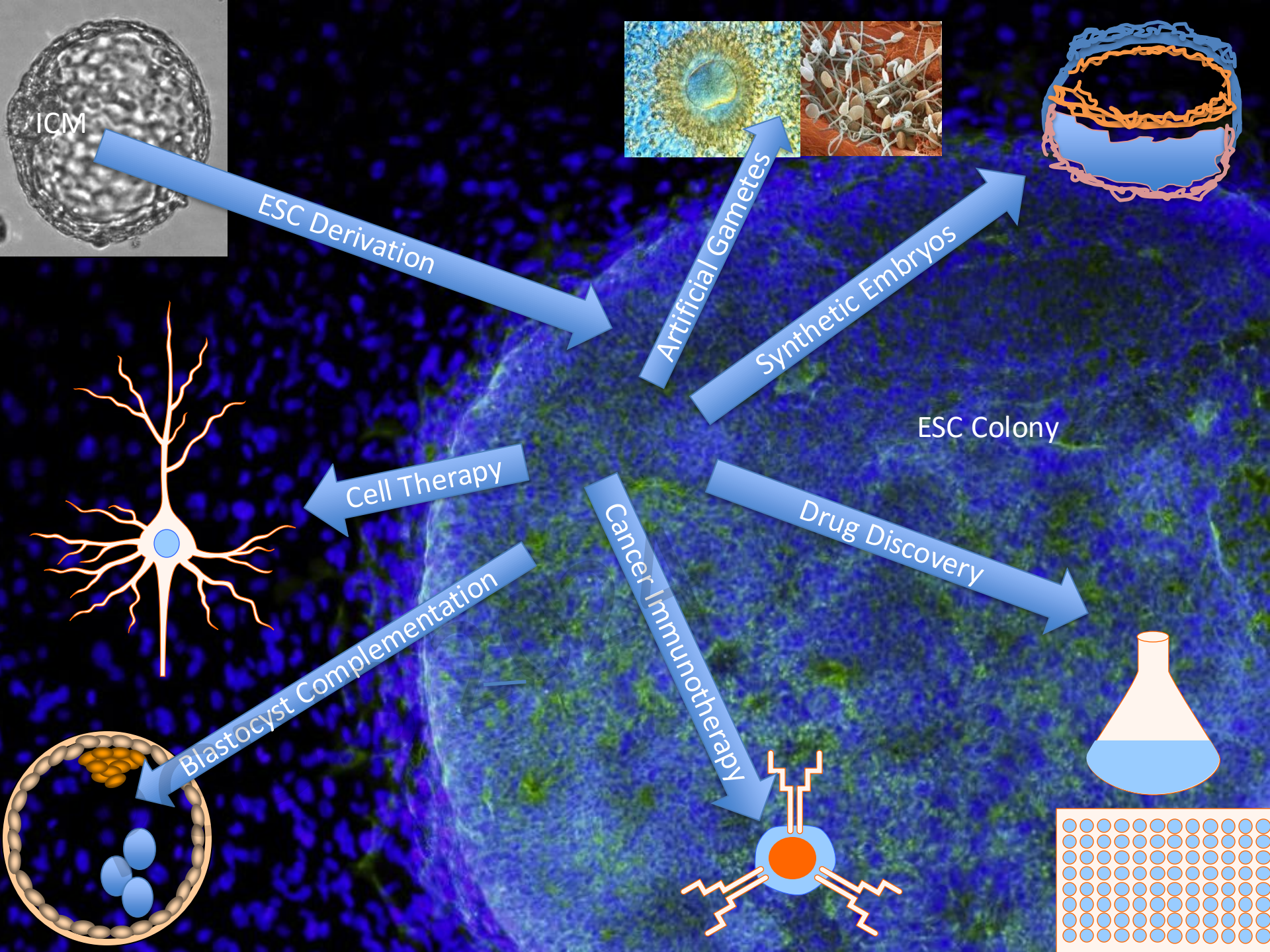
Gamete Refreshment





iPSCs




ICM
ESC Derivation
Artificial Gametes
Synthetic Embryos
ESC Colony
Cell Therapy
Drug Discovery
Cancer Immunotherapy
Blastocyst Complementation

The right to safe reproduction

- Simple superovulation
- Use ICSI only for poor sperm
- Simple culture systems and lab incubator
- Vitrify excess embryos
- Don’t try PGT-A
- Single embryo transfers

THANK YOU

PGT and BEYOND...