Forging a Bench to Clinical Pipeline in Human Germline Editing

Second International Summit on Human Genome Editing
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Hong Kong

Helen O'Neill MSc, PhD
Institute for Women's Health
University College London, UK


TIME
The Test-Tube Baby
Birth Watch in Britain


Evening News
Meet Louise, the world's first test-tube arrival
SUPERBABE


Women's Weekly
EXCLUSIVE
Meet the world's first test-tube baby
"I am the centre of a very special miracle" says Lesley Brown


THE WEEKLY NEWS
PARENTS TELL FRANK STORY
WORLD'S FIRST TEST-TUBE BABY
OUR MIRACLE CALLED LOUISE
SHOCK UPSET AFTER FIRST OPERATION


LE BÉBÉ DU SIÈCLE


Daily Mail
First test-tube baby is born and medical history made as a mother's dream comes true
IT'S A GIRL


woman


CHE RISCHI HA CORSO LOUISE?


Daily Mail
And here she is...
THE LOVELY LOUISE


parents
LES SEULES PHOTOS DU BÉBÉ-ÉPROUVETTE
LA VIRGINITÉ EN 1978
RENTRÉE DES CLASSES


stern
Exklusiv
Das Retorten-Baby


At 11.47 pm, on July 25th 1978, to every childless couple, hope was born.


News
ALL SET FOR BIG LAUNCH
Campaign ready to go
Happy birthday to you!
Louise invites 8,000 to celebrate at Thorpe Park


Now — Look at World's 1st test tube Baby Now
10!


OGGI
LA BAMBINA DELLA PROVETTA
LE VACANZE D'AMORE DI CAROLINE E JUNOT

Patrick Steptoe and Robert Edwards 1977

The New York Times SEPT. 15, 1974

The winner will be a brave new baby conceived in a test-tube and then planted in a womb.

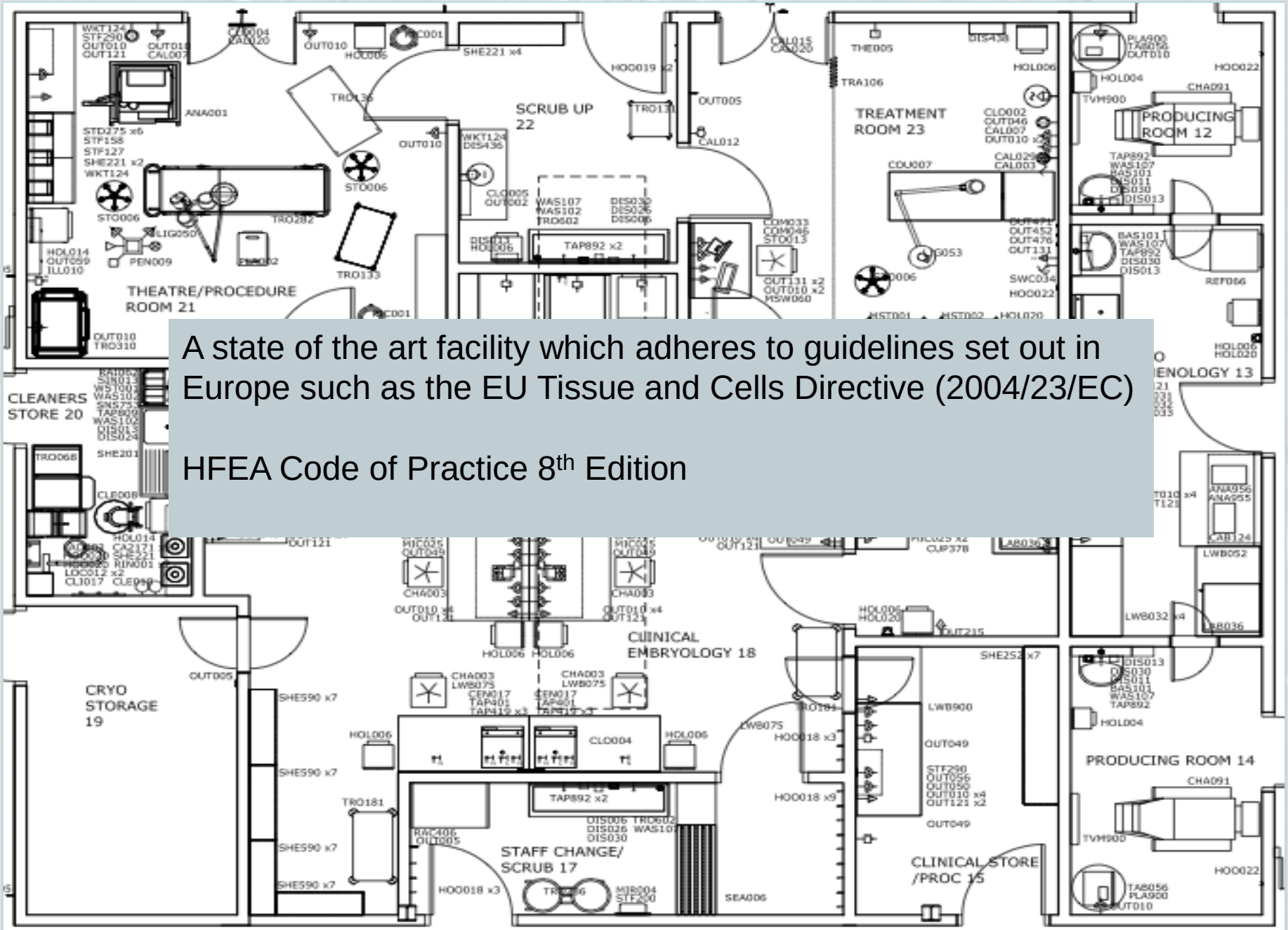
By DAVID RORVIK SEPT. 15, 1974

The winner will be a brave new baby conceived in a test-tube and then planted in a womb.

The Embryo Sweepstakes

Dr. James Watson, the Nobel biologist, told a Congressional subcommittee that a successful embryo transplant would soon occur. Watson foresaw "all sorts of bad scenarios" from the achievement. "All hell will break loose, politically and morally, all over the world," he concluded, a hit rashly, some felt.

Credit: Stephen Harbottle, Cambridge IVF



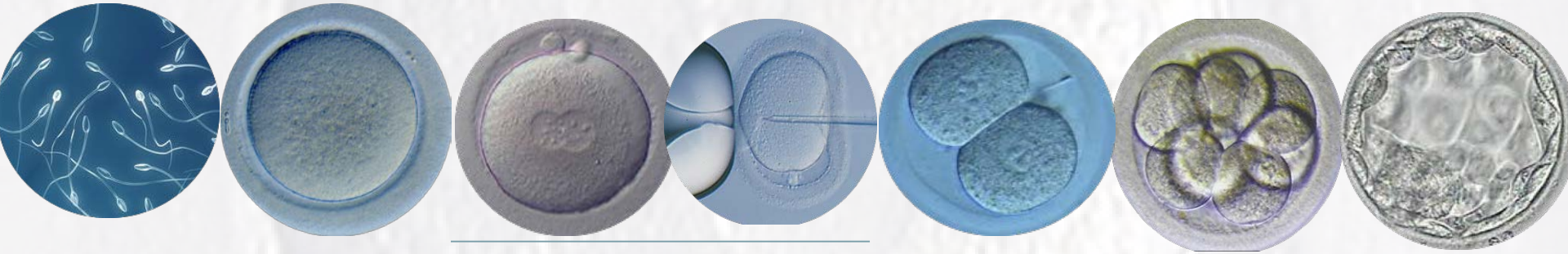
A state of the art facility which adheres to guidelines set out in Europe such as the EU Tissue and Cells Directive (2004/23/EC)

HFEA Code of Practice 8th Edition

Current Clinical Pathway –Assisted Reproduction

Day0

Day 1-5



Sperm Preparation

Oocyte Retrieval

FERTILIZATION

IVF

ICSI

Embryo Culture

Embryo Transfer

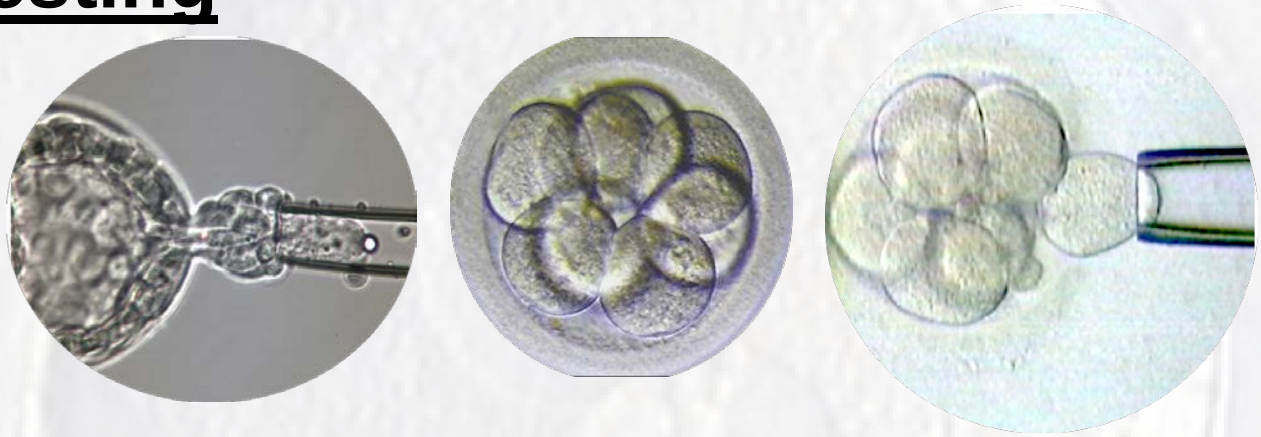
OR

VITRIFICATION

OR

TESTING

Preimplantation Genetic Screening, Diagnosis and Testing



- The Term “Genetic Screening” is intended for a procedure used to determine whether the level of genetic risk for a couple is acceptable
- New terminology PGT-A (aneuploidy)
  PGT-M (monogenic)
  PGT-S (structural)

Human Fertilisation & Embryology Authority

PGD conditions

This table shows all PGD conditions currently approved and awaiting consideration by the HFEA.
Please note that we are updating this database, and some approved conditions may not be displayed. If you are unsure if a condition has been approved or not, please contact pgd@hfea.gov.uk.

Condition

OMIM #

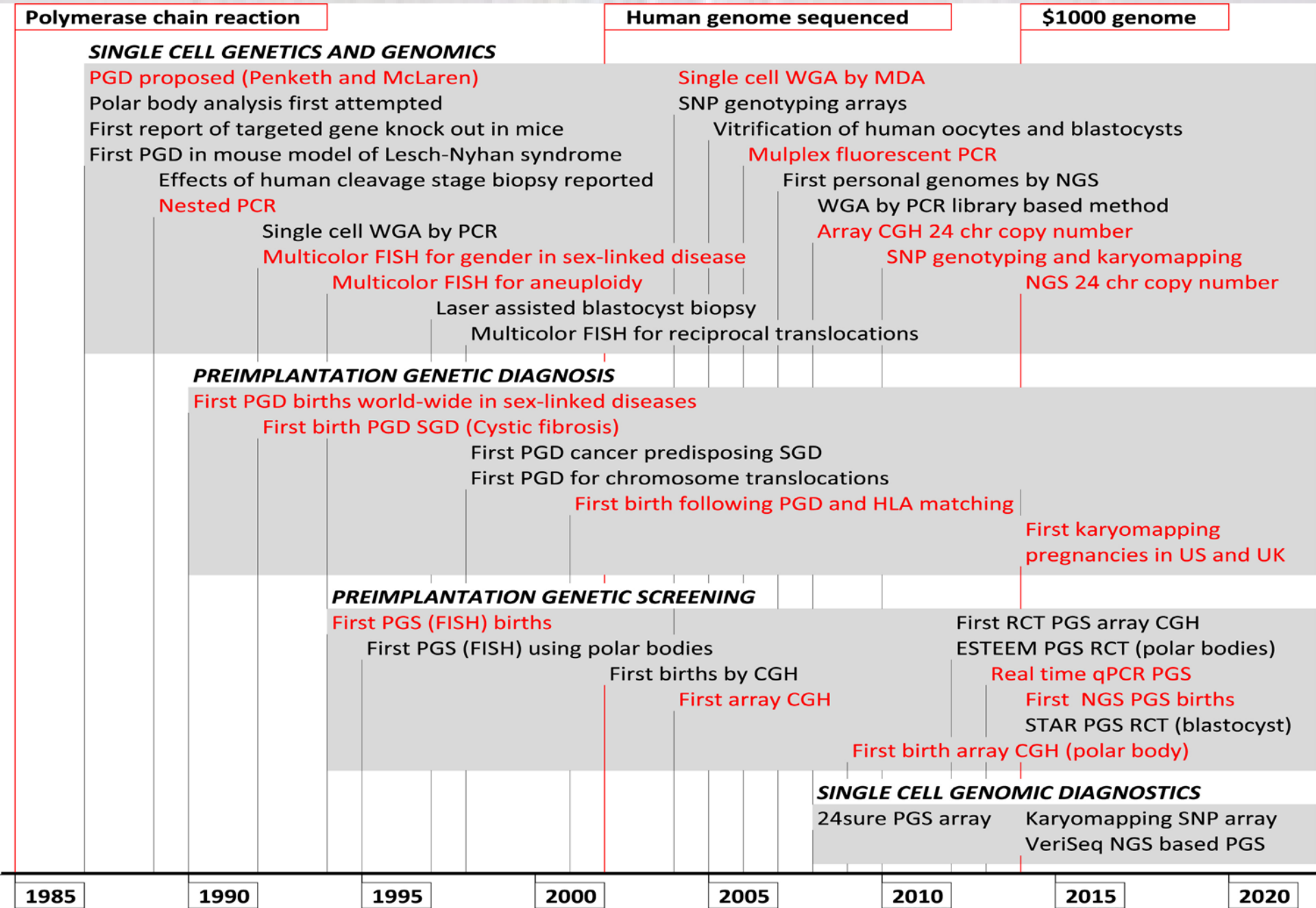
Status

All

Filter

Condition name	Status
Loeys-Dietz syndrome type 3	approved
3-Hydroxyisobutryl-CoA Hydrolase Deficiency (HIBCHD)	approved
46XY Sex Reversal 6 (SRXY6)	approved
Abetalipoproteinemia (also known as aconthocytosis, microsomal triglyceride transfer protein deficiency and Bassen-Kornweig syndrome)	approved
Achondrogenesis Type 1a	approved
Achondrogenesis Type 1b	approved
Achondroplasia (ACH)	approved
Acroleukopathy, Symmetric	awaiting approval
Acute Intermittent Porphyria (AIP)	approved
Acute Recurrent Autosomal Recessive Rhabdomyolysis	approved
Adenylosuccinate lyase deficiency (ADSLD)	approved
Adrenoleukodystrophy (Adrenomyeloneuropathy) (ALD)	approved
Adult-onset vitelliform macular dystrophy	approved
Adult-onset vitelliform macular dystrophy	approved
Adult-onset vitelliform macular dystrophy	approved
Agammaglobulinaemia (x-linked)	approved
Agammaglobulinemia and isolated hormone deficiency	approved
Aicardi Goutieres syndrome types 2, 3, 4, 5 and 6	approved
Aicardi-Goutieres Syndrome 1 (AGS1)	approved
Alagille Syndrome	approved

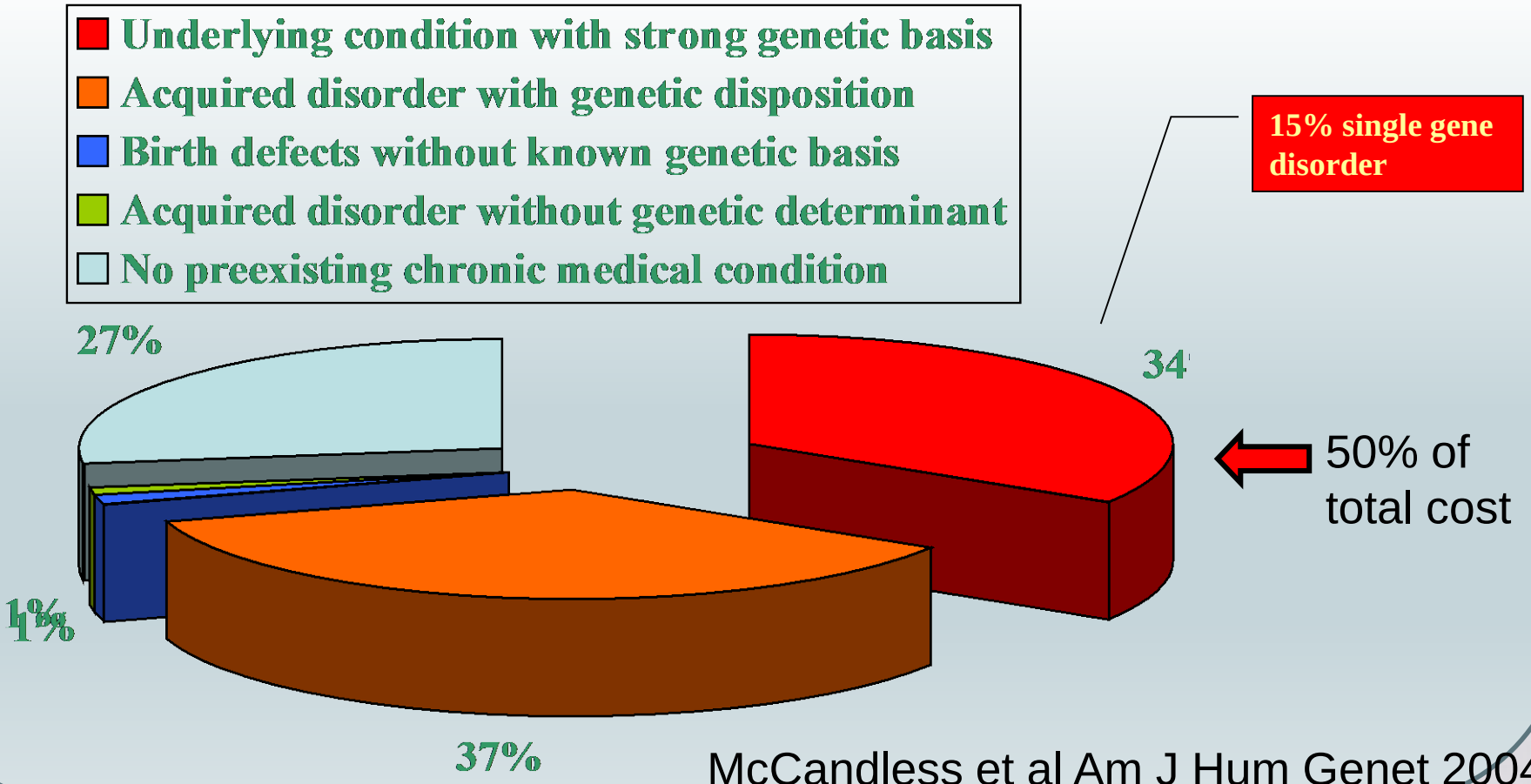
“Forty years of IVF” Niederberger et al 2018



The Burden of Genetic Conditions

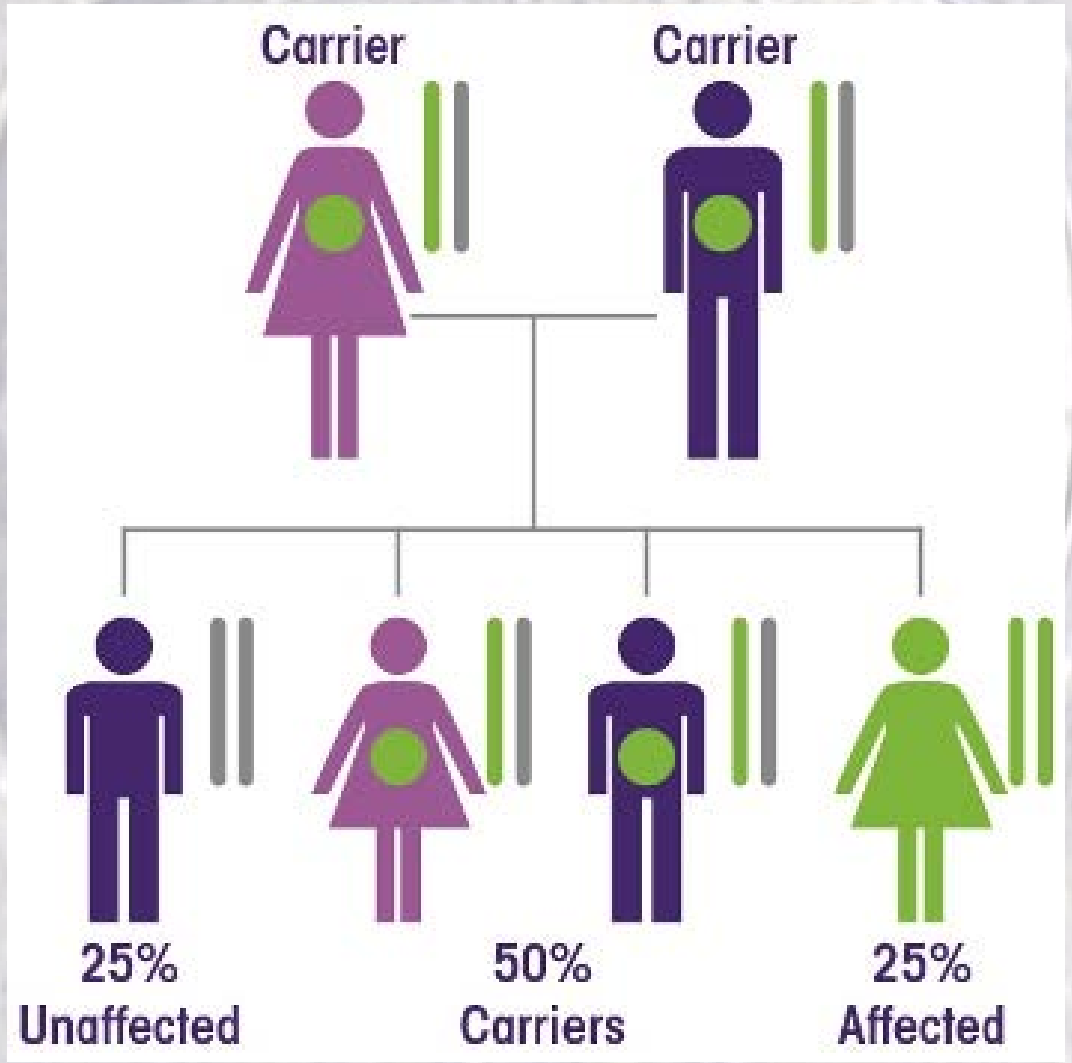
8,000 single gene disorders affecting 400 million people

5,747 children's admissions to a pediatric hospitals

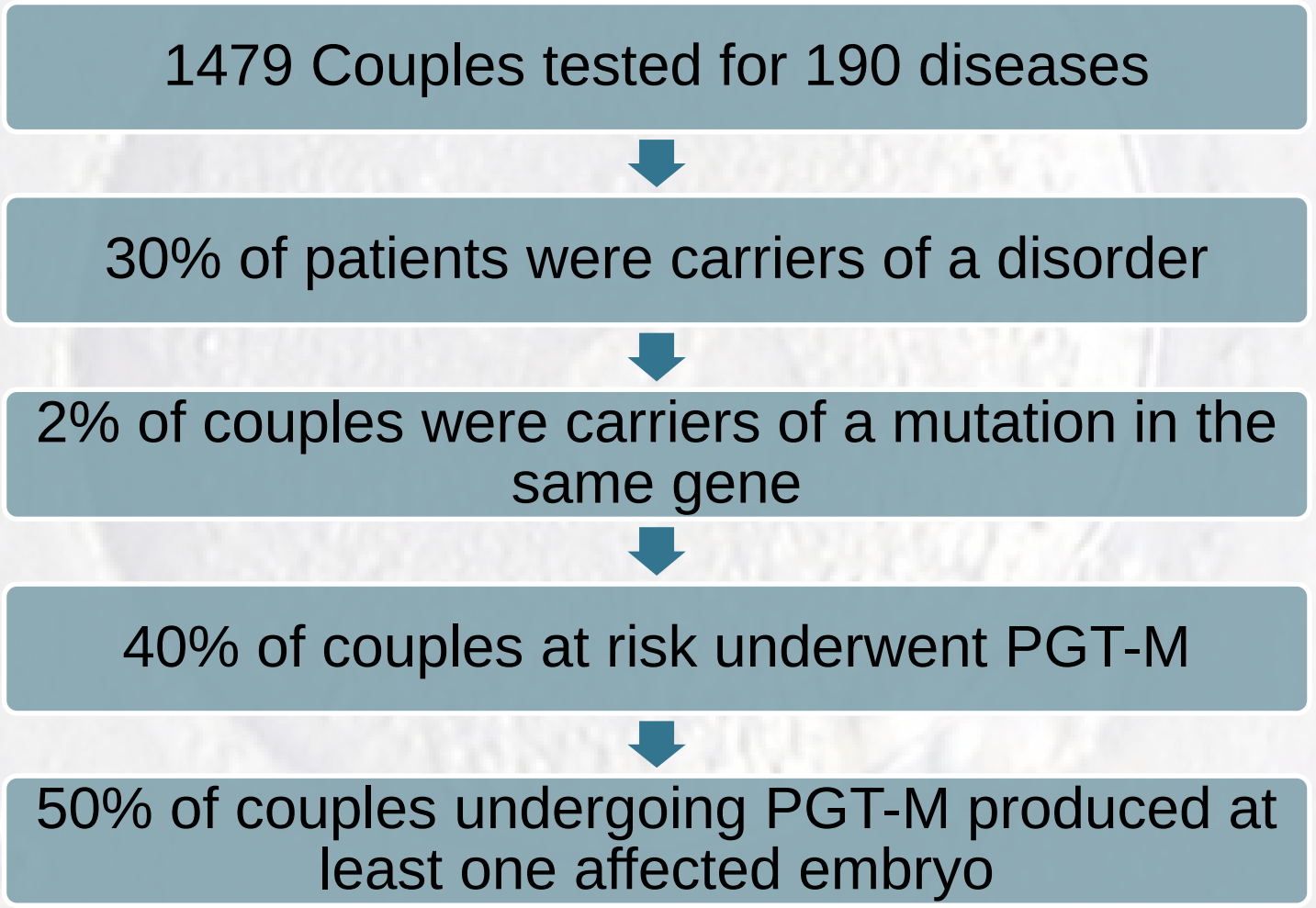


McCandless et al Am J Hum Genet 2004

Preconception Carrier Screening



Expanded Carrier Screening: example in IVF



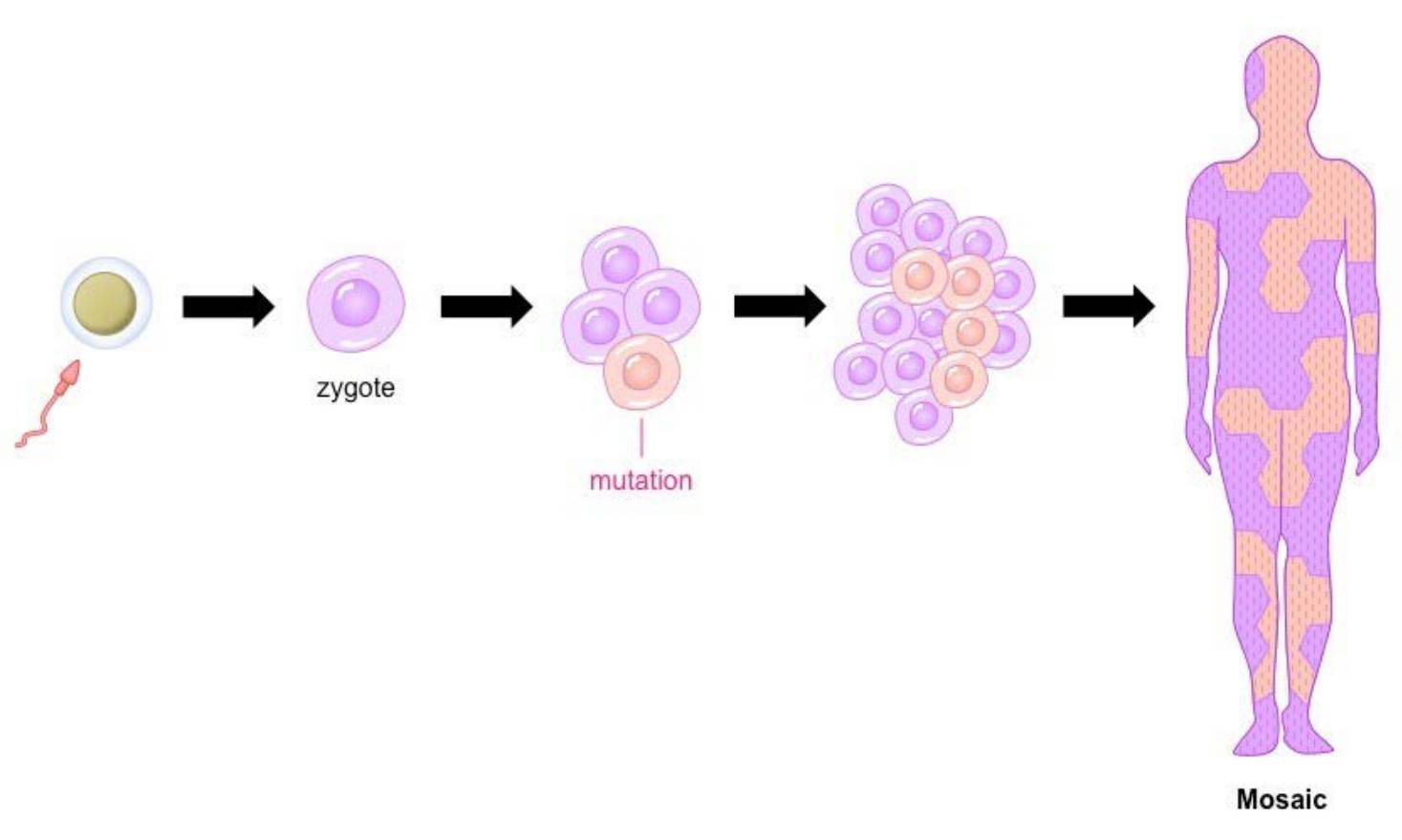
Kumar et al ASRM 2014,2015

How to Develop a Proper approach (ECS)

Disorders selected for inclusion should meet several of the following consensus-determined criteria:

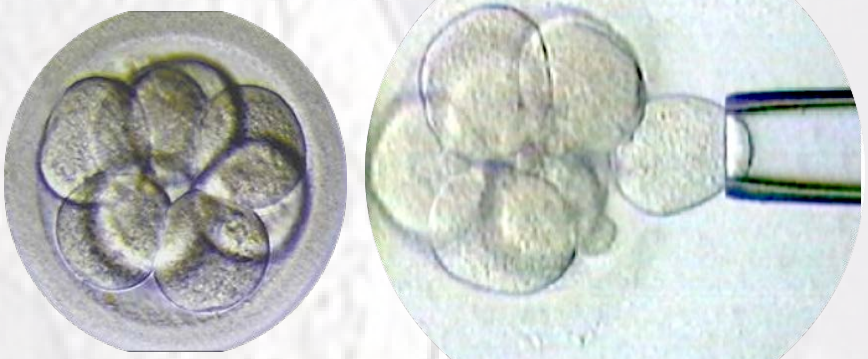
- Carrier frequency 1:100 or greater for AR (1/40,000 prevalence)
- Have a well defined phenotype
- Have a detrimental effect on the quality of life
- Cause cognitive of physical impairment
- Require surgical or medical intervention
- Early onset of disease in life
- Adult onset diseases not to be included
- Autosomal Dominant conditions- not recommended for carrier screening

Mosaicism- should we transfer mosaic embryos?



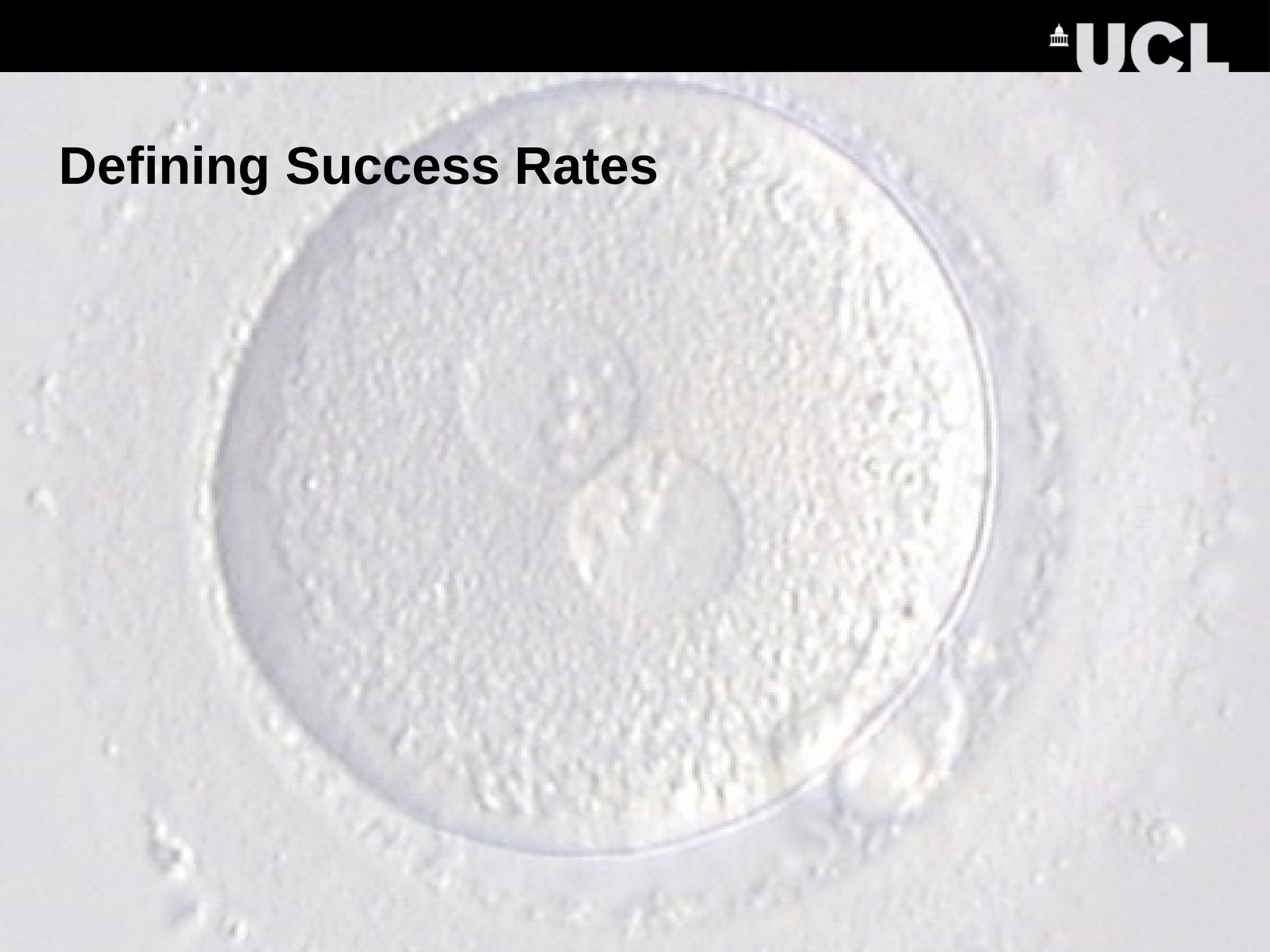
What is an acceptable level of mosaicism for considering transfer?

An evidence-based scoring system for prioritizing mosaic aneuploid embryos following preimplantation genetic screening Romana Grati et al 2018



- IVF
- Embryo transfer
- Embryo biopsy
- Intracytoplasmic sperm injection ICSI
- Preimplantation genetic testing PGT
- Assisted hatching
- Gamete intrafallopian transfer GIFT
- Zygote intrafallopian transfer
- Gamete and embryo cryopreservation

Defining Success Rates

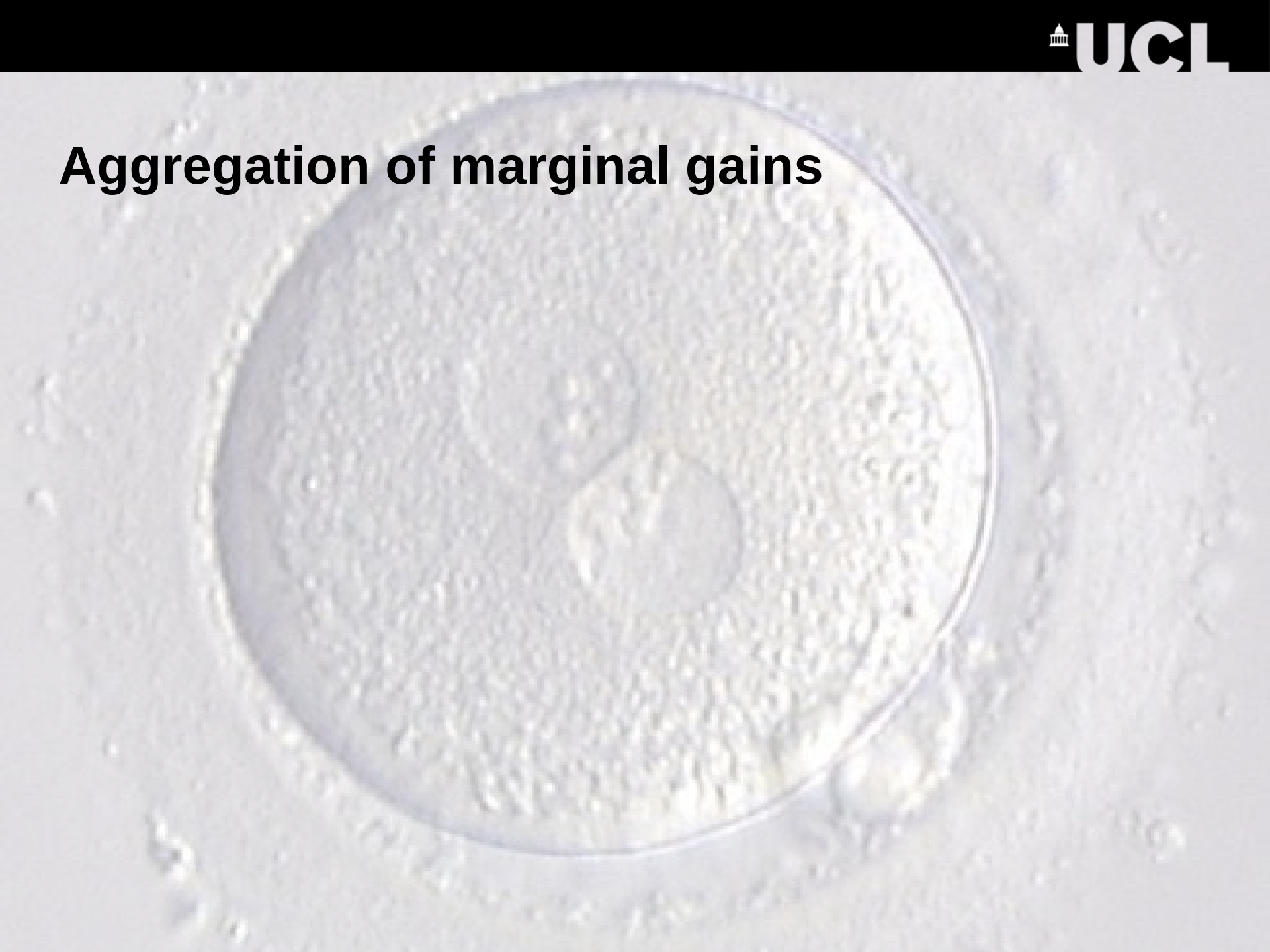


Evidence based standards for clinical use

1. Hypothesis driven research
2. Animal modelling
3. Testing in donated gamete/embryo material
4. Pre-clinical investigation
5. Larger clinical trials (EBM??)
6. Routine Randomised control trials

Are we using poor quality embryos for research?

Aggregation of marginal gains



Testing various timepoints

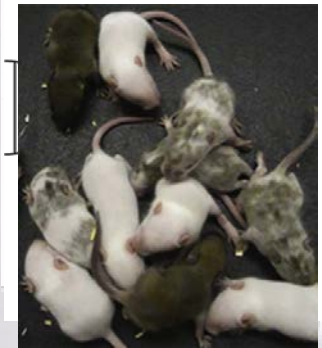
- https://www.nature.com/articles/s41536-018-0050-7
- “Maternally deposited RNAs and proteins could cause a delay in the manifestation of mutant phenotypes potentially masking roles these genes have in early developmental processes. We were unable to evaluate the contribution of maternally deposited proteins to early development as antibodies were not commercially available. As an

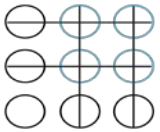
Prediction of editing outcomes

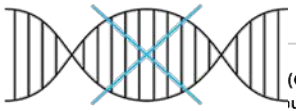
- Our finding that editing precision is site-specific and can be predicted has important implications. For practical reasons, knowing what editing outcome is likely to occur at a given site has obvious advantages and maximizes the chance of having a desired sequence alteration, both for clinical and research applications.

Re-evaluating published mosaicism

	A	B	C	D	E	F	G	H	I
1	**Paper**	**Year**	**Gene**	**Type of mutation**	**Mode of delivery**	**Protein or mRNA (or other)**	**Endonuclease that was used**	**Guide used**	**Mosaicism**
2	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4166609/	2014	Tyrosinase gene (Tyr)	mono- and bi-allelic null mutations	Pronuclei and cytoplasmic injection	RNAs	Cas9	**Tyr4a** - PAM (GGT) TACGTGGATAGCCGGTATTG **Ty44b** - PAM (GGA) TAGCCGGTATTGTCTCTGAG	**Pronulei, biallelic** - 33 pups. 1 albino, 3 mosaics and 29 fully pigmented. **Pronuclei, monoallelic** - 28, 3 albino, 19 mosaic and 6 fully pigmented. **Cytoplasom monoallelic** - 12, 6 albino, 4 mosic and 2 fully pigmented.
3	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5450330/	2017	Phosphoprotein 1 (Spp1) and Tyrosinase gene (Tyr)	knock-in	Pronucleus Injection	Ribonucleoprotein (RNP)	Cas9	**Spp1** - ATGGACTGAGGTCAAAGTCT (AGG) PAM **Tyr** - GGGTGGATGACCGTGAGTCC (TGG) PAM	17/46 Spp1 and 11/39 Try knock-in
4	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5314402/#S1	2017	Notchless (Nle) and Sox2	knock-in	Pronuclei and cytoplasmic injection	RNA	Cas9 and Cas9 nickase	**Sox2** - caccGTGCCCCTGTCGCACATGTGA **Nle1** - caccGTGGGGACGCAGGATGGCGG **Nle2** - caccCCCCACGTGGAGGAGAACCG **Nle3** - caccTGGTGGTAGGTGTACGCGCA	**Sox 2 Cas9 pronuclus** = 75, 40 KI , 8 WT, 46 mosaic. **Cytoplasom** = 59, 25 WT, 6 WT, 27 mosaic. **Nle Cas9 1 pronuclus** = 50, 17 KI, 9 WT, 8 mosaic. **Nle Cas9 1 cytoplasom** = 53, 21 KI, 10 WT , 15 mosaic. **Nle Nickase 2&3 pronucleus** = 63, 9 KI, 9 WT, 24 mosaic
5	http://www.biotechniques.com/BiotechniquesJournal/2015/October/Highly-efficient-	2015	lipopolysaccharid eresponsive vesicle trafficking, beach and anchor containing (Lrba) gene	Knockout	Pronucleus Injection	RNA	Cas9	CCTTTCTCGTGAACTAGTCA (TGG)	targeting efficiency for mouse zygotes was 32%



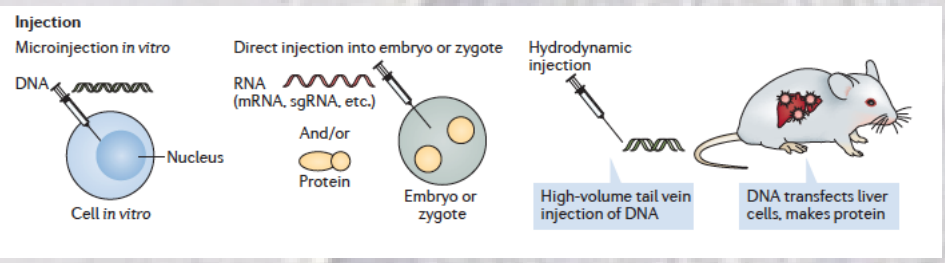


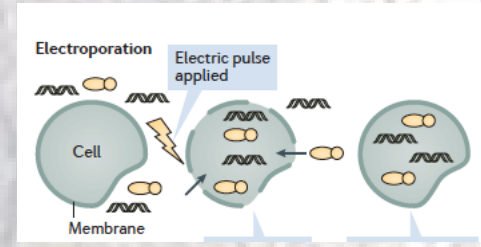


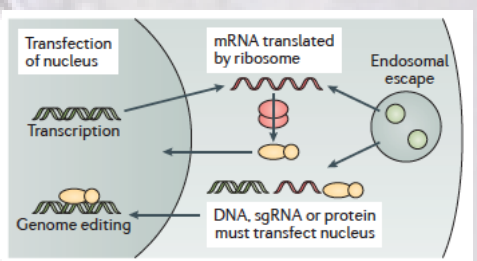
DESKGEN

O'Neill et al unpublished

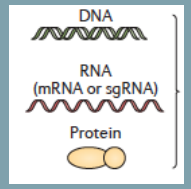
Dissecting the technology

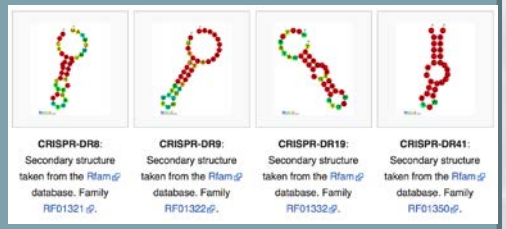






- 1. Method of delivery.
- 2. Mode of Delivery
- 3. Endonuclease activity
- 4. Guide design
- 5. Method of repair (non-homologous end joining (NHEJ) or homology directed repair (HDR))





Consistency is Critical

- Comparison of guide design tools. Reference genomes
- Vs difficulty of consistency with embryos and assessing natural variations.

Are we already editing the germline?

- Pat hunt research Bisphenols
- Long term outcomes IVF- delivery
- IVF- disease prevalence/birthweight

3 Registries in Western Australia on births, births after assisted conception, and major birth defects in infants born between 1993 and 1997

ICSI	26/301	(8.6%)
IVF	75/837	(9.0%)
Natural	168/4000	(4.2%)

Infants conceived with use of ICSI or IVF have **twice as high a risk of major birth defects** as naturally conceived infants

Imprinting disorders and ART procedures

Syndromes	Odds Ratio (95% CI)
Beckwith-Wiedemann (maternal origin)	5.8 (3.1-11.1)
Angelman (maternal origin)	4.7 (2.6-8.5)
Prader-Willi (paternal origin)	2.2 (1.6-3.0)
Silver-Russell (paternal origin)	11.3 (4.5-28.5)

Estimated Frequency: **ART conception:** 11/ 10,000 births

General population: 2/ 10,000 births

- Relative Risk is low

Possible causes:

-ART procedures cause aberrant maternal and paternal imprints

-Aberrant imprints on gamete DNA are the cause of male and female infertility

Public perception matters

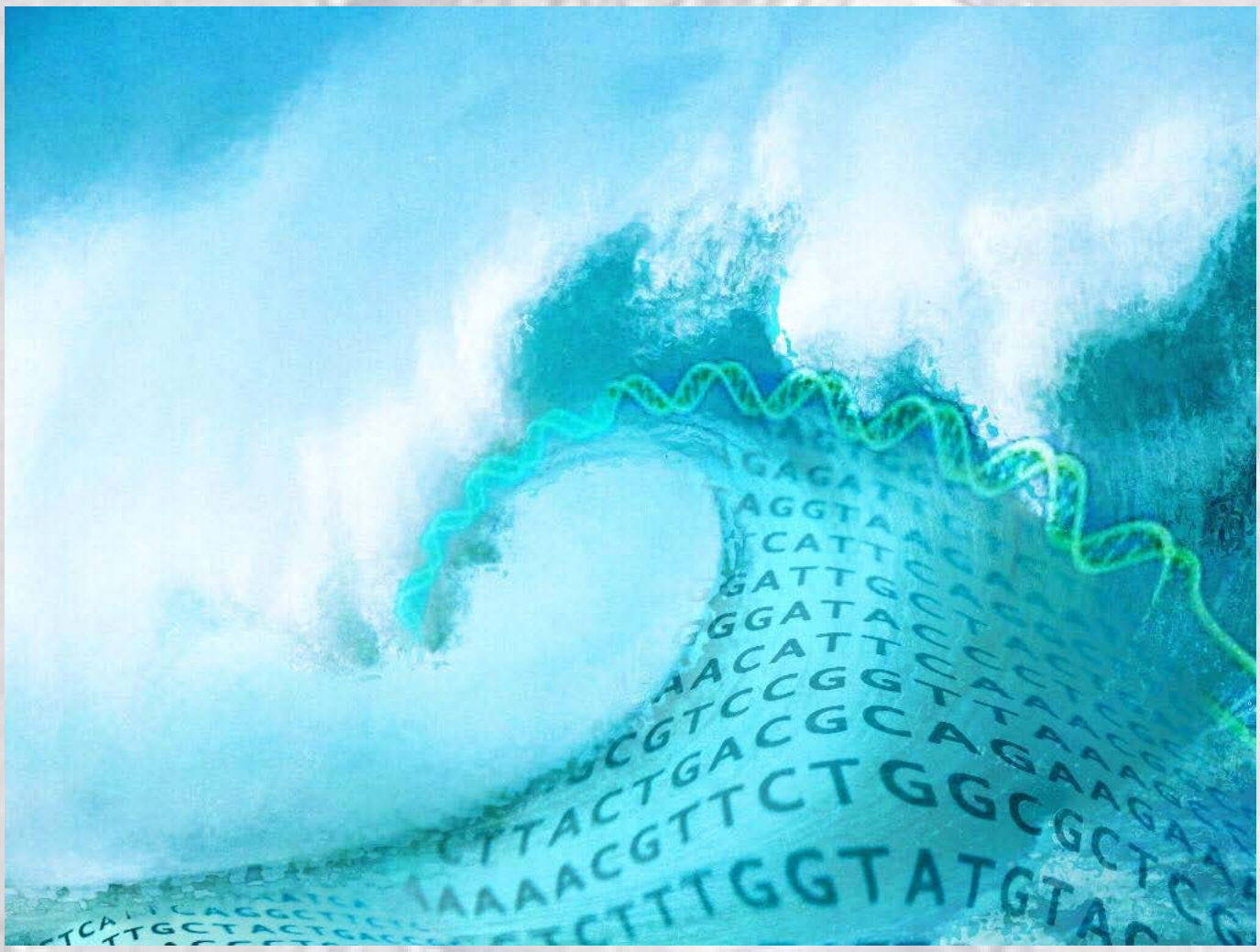
- Carrier screening data from Plantinga et al., 2016
- NSL Genome editing
- Mitochondrial replacement therapy legislation


1
2
3
4

Is all ART artificial?

- Artificial ovulation (induction of ovulation)
- Artificial insemination
- Artificial fertilisation (ICSI)
- Artificial culture for growing of embryos
- Artificial conception?

- Artificial gametogenesis


GAGAT
AGGTA
CATT
GATTG
GGATA
AACATT
CGTCCGGT
TTACTGACGC
AAAACGTTCTGGC
TTTGGTATGTA

Easy Right?