New Innovations and Technologies:

How and When in the Fertility Clinic?

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Overview

- Reproductive medicine is odd
- What is evidence based medicine?
- Which areas of reproductive medicine?
- The PGS story
  - Making sure we learn the right lessons
- The case for the defense and prosecution
- Some questions
- A way forward

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Reproductive Medicine is Odd

- One of the few occasions where
  - Patients have radical therapies with an intention other than benefiting their own health
  - Barely perceptible "good gardening" skills are so essential
  - So many different academic disciplines combine
    - Clinical medicine
    - Anatomy
    - Physiology
    - Endocrinology
    - Cell biology
    - Genetics
    - Biochemistry
    - Physics
- The only medical discipline where:
  - Physiologies of two individuals combine
    - Even if the two parties do not meet, (e.g. sperm donation)
  - For the sole purpose of producing a third
    - Fourth, fifth, sixth ….
- Some centres are better than others
  - "Good gardening" again
- So, at what stage do we consider the evidence-base good enough to introduce a new therapy
  - There are plenty
  - How is is different from other forms of medicine?

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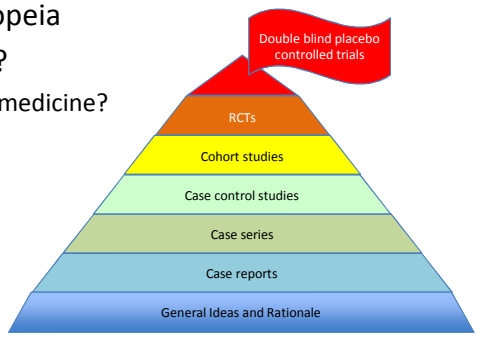
The definition of evidence based medicine (EBM)

- *An approach to medical practice intended to optimize decision making by emphasizing the use of evidence from* ***well designed and conducted research***

- It's up to us to decide what "well-designed" and "well-conducted" means

One view of EBM

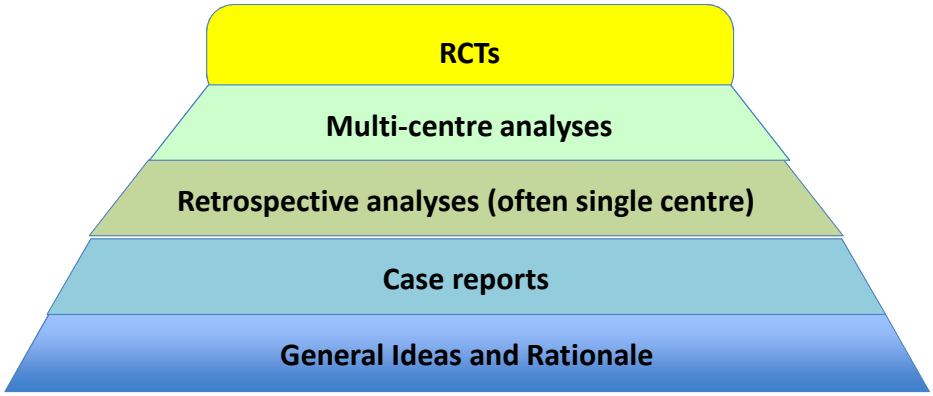
- Therapy should *only* be introduced into the clinic
  - After at least one favourable double blind randomised placebo controlled clinical trial
  - Until then, any treatment should only be part of a trial
  - And thus considered "experimental"
- Works for standard pharmacopeia
- Should it be the same for IVF?
  - Or other areas of reproductive medicine?



Problems with the standard pyramid in Reproductive Medicine

- Placebos are not usually relevant
- Skill of the operator (or lack of it) can negate any beneficial effect of the treatment
  - Any randomization can thus be rendered meaningless
  - More reliant on "good gardening"
- How "blind" is "blind" ?
  - Do people performing micromanipulation not know they're doing it?
- Results (e.g. retrospective) from single centres may be ***just as useful to the big picture*** as randomised trials
  - Meta-analyses may mask particularly bad (or good) practice by individual clinics

In reproductive medicine: The pyramid is more of a "hill"



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Areas we might want to consider

- ICSI
- Oocyte preservation
- Sperm DNA damage testing
- Metabolomic analysis
- Development of new culture media
- **PGS**

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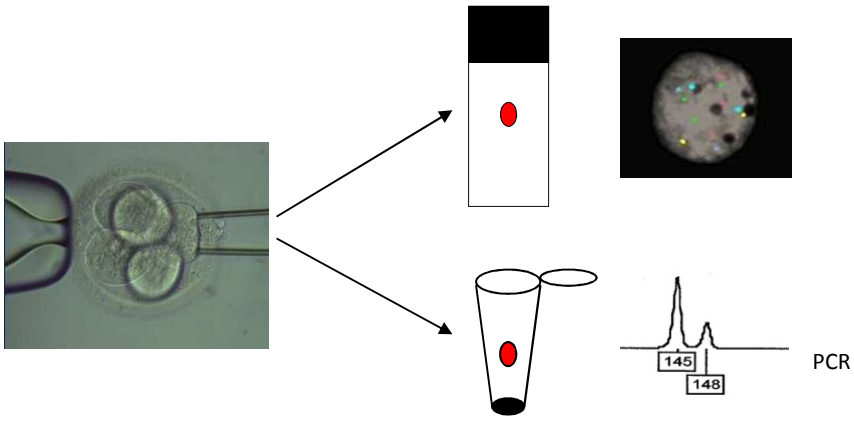
PGD on single cells

state of the ART c.2007

FISH

For PGS, chromosome translocations **and Sexing**

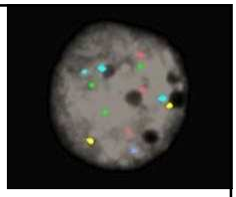


PCR

For single gene disorders and saviour siblings

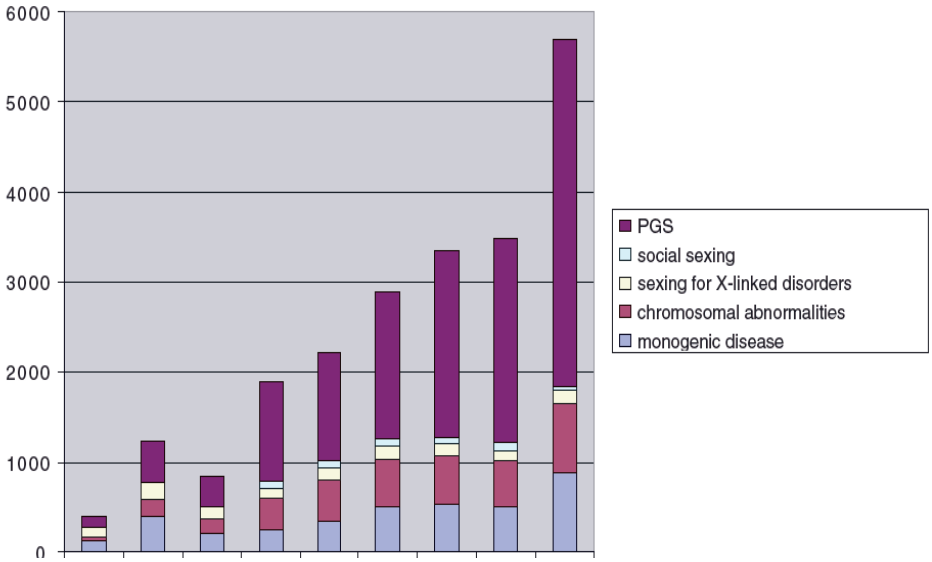
PGS

(AKA PGD-A)

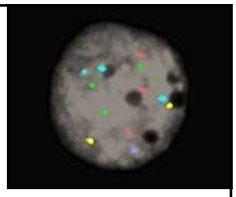


- Preimplantation Genetic **Screening**
- Munne et al 1993, 1994, 1995, 1996, 1997 …..
- Screening for chromosome abnormalities
  - **Non-targeted**
  - **Initially chromosome 13, 16, 18, 21, 22 (X and Y)**
- Referral categories
  - Advanced maternal age
  - Recurrent miscarriage
  - Recurrent implantation failure
  - Severe male factor infertility
- Rationale: transferring chromosomally normal embryos should
  - Improve IVF rates
  - Reduce miscarriage
  - Reduce the chances of affected live births and still birth

PGD circa 2007



Where it all went wrong



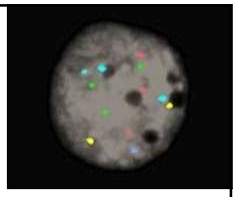
- Some retrospective analysis suggesting benefits in some clinics
- Most randomized trail data however suggesting ***no demonstrable clinical benefit***
- At least one study disagreed with the above
  - Mastenbroek et al 2007
  - Suggesting a *detrimental* effect of PGS

(Poor) Embryo Biopsy or FISH?

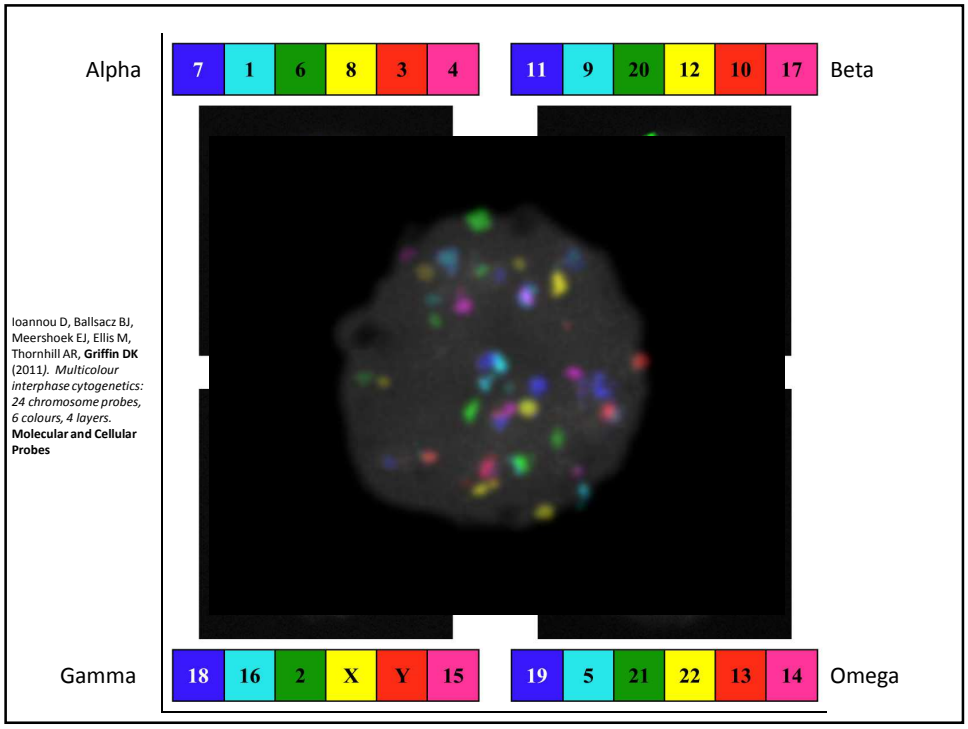
Mastenbroek et al. (2007)

	Live Births
Embryo biopsy but no diagnosis (Sham)	6.0%
Embryo biopsy and transfer of "normal" embryo (PGD)	16.8%
No embryo biopsy (Control)	14.7%

The problem with PGS



- **Cleavage stage biopsy (especially 2 cell) almost certainly the problem**
  - Negated any beneficial effect of chromosome screening
  - Particularly when performed sub-optimally
    - This probably varied between clinics
  - Some benefits of FISH screening but possibly limited
- There were probably some false positive results
  - Single cell not always representative of rest of embryo
  - Mosaicism
- 5-7 chromosome probes would have missed some abnormalities
  - False negatives
  - Solution to screen more chromosomes?
  - Well no
    - Too many false positives

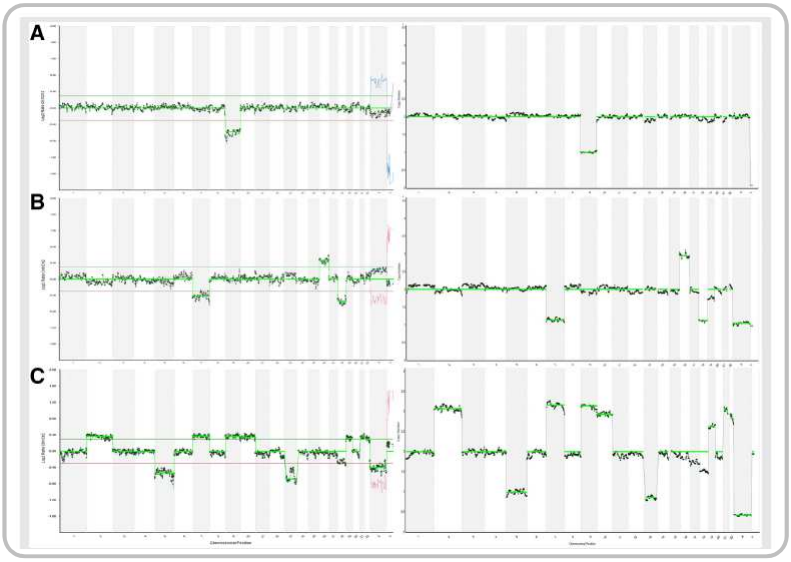


Ioannou D, Ballsacz BJ, Meershoek EJ, Ellis M, Thornhill AR, **Griffin DK** (2011). *Multicolour interphase cytogenetics: 24 chromosome probes, 6 colours, 4 layers.* **Molecular and Cellular Probes**

The PGS Renaissance

- Switch to trophectoderm biopsy
  - One randomized trail even suggested FISH could be effective
  - Rubio and colleagues 2014
- Adoption of whole karyotype screening
  - Array CGH
  - NGS

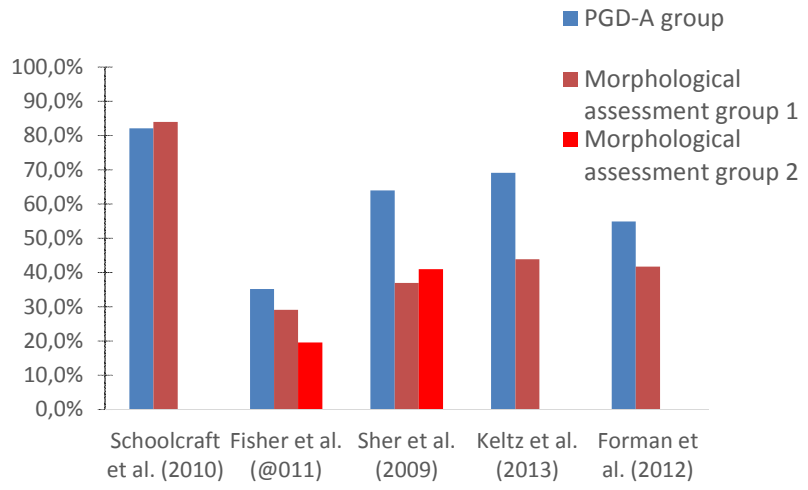
Array CGH NGS



Does it work?

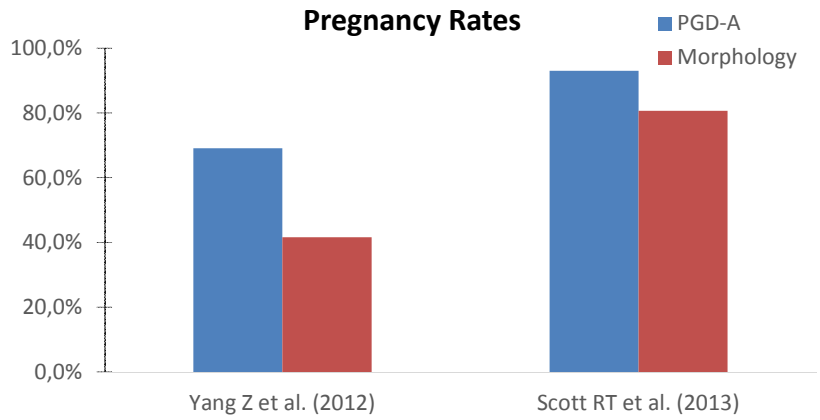
- Both Meta-analyses and RCTs have been performed

PGD Aneuploidy versus Morphology – Pregnancy Rates: Meta-Analysis



Lee, E. et al., 2015

RCT: PGD Aneuploidy Testing versus Morphology



Lee, E. et al., 2015

Does it work?

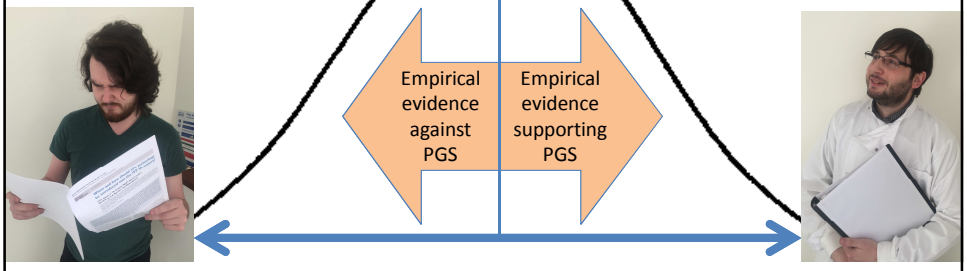
- We still await the results of the STAR trial
- STILL AN ONGOING ARGUMENT
  - How to best explain it?

Jacob and Giuseppe: Two straw men

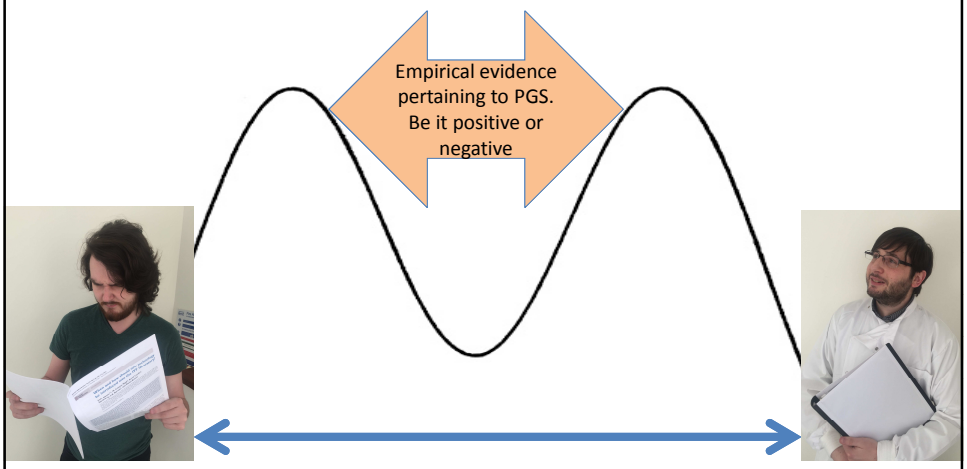
Jacob is a medical statistician who is very against PGS in all its forms. He gets very angry when he reads any evidence that supports PGS and will always find an excuse to criticize it. He hides behind the banner of "evidence-based medicine" advocating that more and more complex analyses need to be done before PGS is ever put into clinical practice. In his own publications he will be selective about evidence that supports his point of view and has made a career out of trashing PGS.

Giuseppe is a clinician who will always advocate PGS. He is motivated in part by good press for his IVF unit and generating income to keep it open. He hides behind the mantra of "I will always do what I think is best for my patients" advocating that PGS is effective, whatever the evidence. In his own publications he will selectively trash any evidence that suggests PGS is ineffective and has made a career out of treating patients using PGS, always publishing his findings that show it in a positive light.

What I would like to see

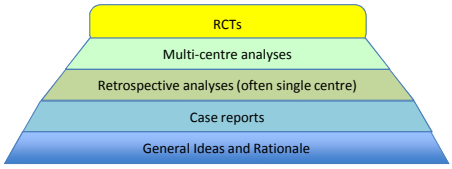


What I observe



Learning the right lessons from PGS

- RCTs remain the gold standard but
  - Just because it's a randomized trial does not necessarily mean it's a good study
    - Especially if badly executed
  - Just because it's not a randomized trial does not necessarily mean it's not a good study
    - Remember the hill



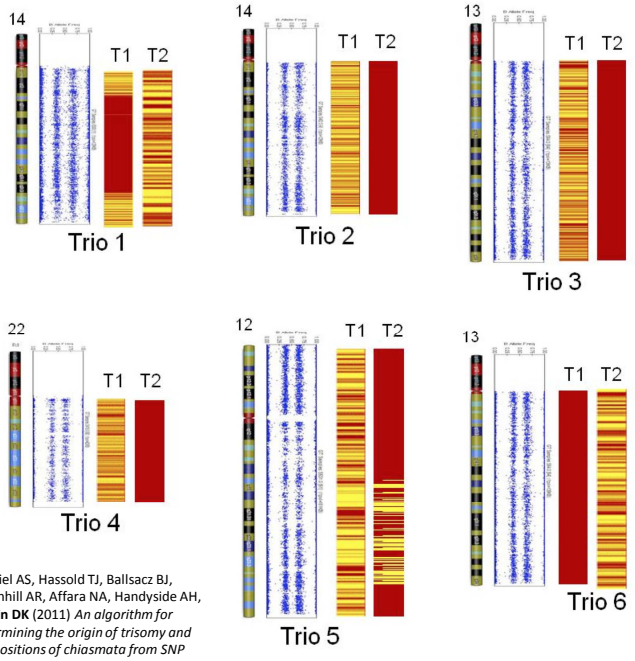
- **NEITHER A JACOB NOR GIUSEPPE BE!**

Learning the right lessons from PGS

- Don't do cleavage stage biopsy
  - Even if you know you're good at it
- The benefits of FISH are limited
  - But not negligible
- TE biopsy and CGH/NGS is better
  - But let's not be complacent

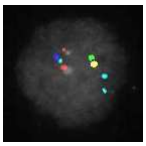
Consider the mechanisms of moscaicism

- It is incontrovertible that a significant proportion of embryos are mosaic
- Mosaicism can either arise
  - From a meiotic aneuploidy in which some cells became normal
  - From a normal conceptus that acquired some aneuploidy post-zygotically
- An mosaic embryo with a meiotic aneuploidy will either
  - not implant
  - lead to a miscarriage
  - lead to obstetric complications (e.g. IUGR)
  - lead to an affected child
  - often display uniparental disomy in the "normal" cells
- An embryo with multiple chromosome abnormalities will not develop
- **We should not be transferring these embryos**
- Equally, some/many/most post-zygotic mosaic trisomies will be **normal**
  - We need to get better at detecting these
  - And ask whether they lead to normal live births
    - Or have a reduced chance of implantation (e.g. deletions and monosomies)

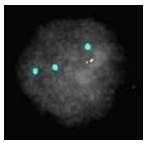


Gabriel AS, Hassold TJ, Ballsacz BJ, Thornhill AR, Affara NA, Handyside AH, **Griffin DK** (2011) *An algorithm for determining the origin of trisomy and the positions of chiasmata from SNP genotype data* **Chromosome Research** 19:155-63

“Karyomapping case” for PGS

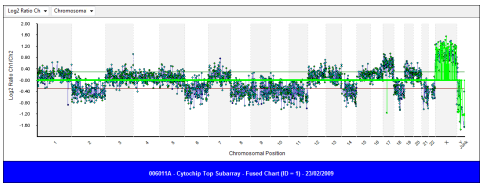


13, 16, 18, 21, 22
Monosomy 18, 21, 22

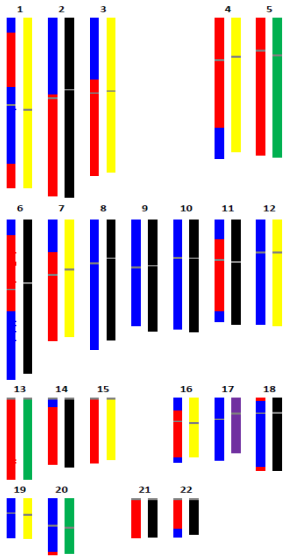


X, Y, 21
Trisomy X
Monosomy 21

Array CGH



Karyomap

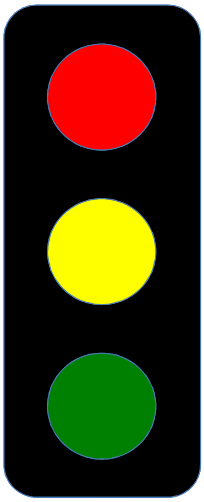


What might a future strategy look like?

“Significant” abnormality
Any meiotic abnormality
Segmental or whole chromosome
Trisomy 21, 18, 13
Any monsomy?
Any deletion?
Uniparental disomy

Abnormality possibly compatible with normal live birth
Post-zygotic trisomies and segmental duplications

No detected chromosome abnormality



Do not transfer

Refer to counsellor
Consider
Level of abnormality
Prospects for re-analysis
Availability of other embryos

Consider for transfer

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The case for the prosecution

- We *must always* wait for the results of randomised trials because:

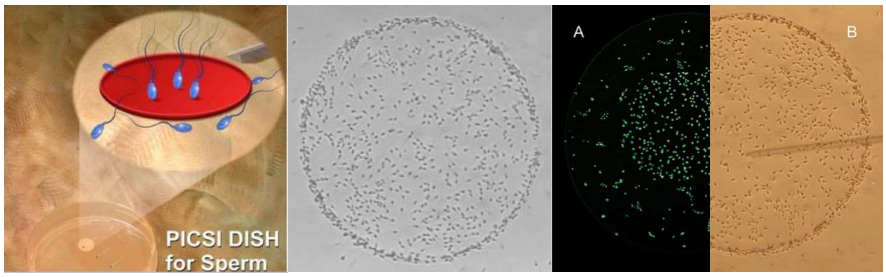
- Clinics are motivated by the need to be *seen* to be innovating
  - And the money associated with charging patients for "the latest" therapy
  - Regardless of convincing evidence supporting their efficacy
- Any treatment not validation by RCTs should only be part of a trial
  - It is unethical and unfair to patients to subject patients to such treatments unless the case is proved (with an RCT)
  - "How do you sleep at night" unless you believe this?
- The lessons of PGS tell us this

The case for the defense

- We *cannot always* wait for randomised trials ahead of implementation because:

- Clinics (especially private clinics) depend, for their survival and the employment of their staff, on their ability to innovate quickly
- Innovation is good, clinics that do not innovate typically have a low success rates
- The following would **not** likely have ever been introduced if subject to prior rigours of a randomised trial before being licenced
  - PGS
  - ICSI
  - Some new variants on IVF culture media
- When randomised trails are designed
  - Can take years
  - Underfunded (unlike drug trials)
  - Benefits of the treatment may already be apparent without randomization
  - Appetite to perform the trial may have waned
- The lessons of PGS tell us this

HAB SELECT



HABSelect Hyaluronic Acid Binding Sperm Selection
Dr David Miller : PI

- Hyaluronic acid
  - Natural substance found on/within cumulus-oopherus complex that sperm encounter when they reach the egg

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From an ethico-legal perspective

- What are the implications of *not* implementing PGS ?
  - The harm caused to a patient who has an adverse outcome (e.g. trisomic conceptus)
  - Assuming that they could, and would, have chosen to avoid this, had PGS been offered
- Are there other areas of reproductive medicine for which this applies?

Is an open mind a good thing?

- If you and your partner are seeking fertility treatment
  - Do you not want a clinic that is unequivocally dedicated to making that treatment work?
    - The best "gardeners"
  - Not one that has an open mind when part of a trial?
  - Do you not want to know the results of that clinic on whether a particular treatment works in their hands?
    - Not the results of an RCT?

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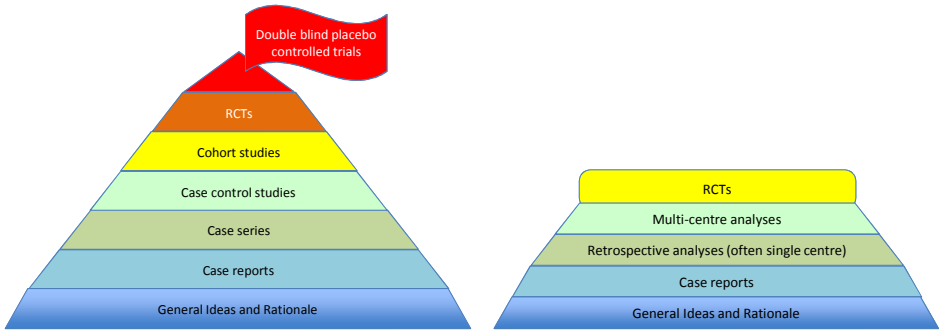
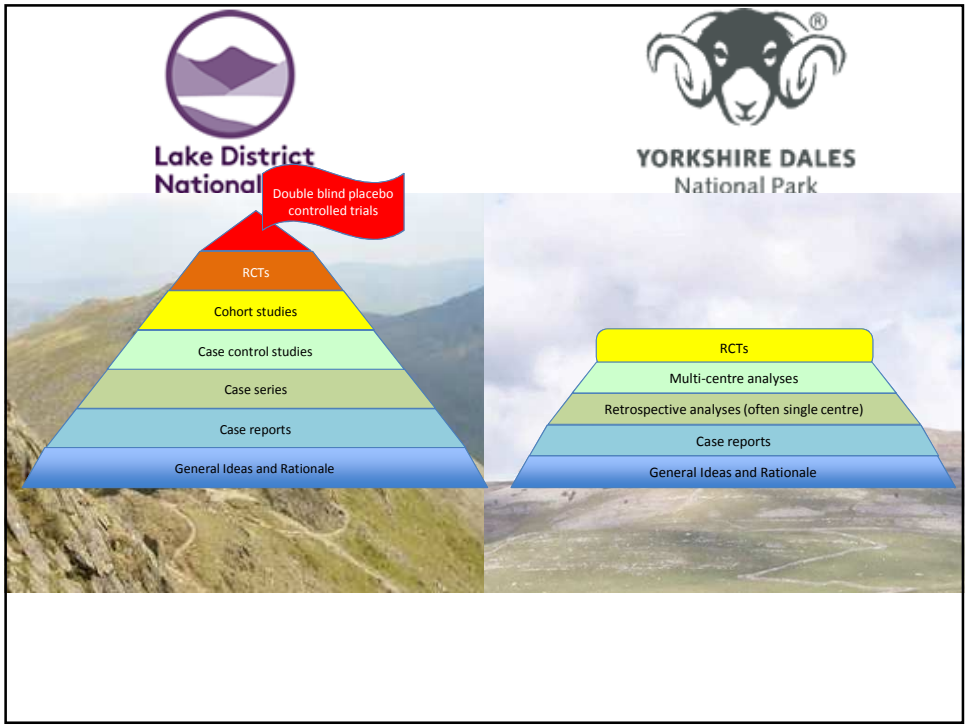
A way forward

- Understand mosaicism better – THE BASIC QUESTION OF THE LEVEL OF ANEUPLOIDY IN EACH GERM LAYER IN HUMAN BLASTOCYSTS **HAS NOT BEEN SATISFACTORILY ANSWERED**
  - Human embryos
  - Model systems e.g. cattle
    - More easily manipulated
- Consider the role of meiotic vs. post-zygotic errors
  - Selectively screen out meiotic but not necessarily all post-zygotic errors
- Stratify the patient groups
  - Who will and will not benefit?
  - For PGS
    - AMA vs RPL vs RIF
- Introduce a proper EQA scheme
- **WE NEED NOT TO BE A JACOB NOR A GIUSEPPE**
- Consider appropriate "staged" introduction protocols for new innovations
  - Blastocentesis
  - Karyomapping for aneuploidy
  - Time lapse
- We need to appreciate the similarities and differences in evidence based medicine between our field and others
  - Good statistics (randomization) alone do not a good study make
    - We need all the "good gardening" as well
  - Let patients know where on the "hill" the evidence base is




Lake District
National Park
YORKSHIRE DALES
National Park
go4awalk


Lake District
National
YORKSHIRE DALES
National Park
Double blind placebo controlled trials
RCTs
Cohort studies
Case control studies
Case series
Case reports
General Ideas and Rationale
RCTs
Multi-centre analyses
Retrospective analyses (often single centre)
Case reports
General Ideas and Rationale

CENTRE FOR INTERDISCIPLINARY STUDIES OF REPRODUCTION

Especial thanks to Prof Sally Sheldon
Kent Law School

University of Kent