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Making sense of PGS: EBM, RCTs, mosaicism and reconciling the two sides

Disclosure information:
Anyone stating “nothing to declare” may be deluding themselves a little

List of disclosures:
Current funding (PhD) from Genesis Genetics (Cooper)
In 1993, in my sleep, I threatened to shoot Santiago Munne
A lot of good friends in the PGS world
… and you’ll hear a bit more about by other conflicts in a while

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Overview

• Aneuploidy and mosaicism
• Evidence based medicine in PGS
• Have we learned the right lessons from PGS?
• A way forward
• My real conflict of interest
• Conclusions
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Aneuploidy – the headlines

• The leading cause of mental retardation in humans
  • 1 in 700 children born with Down Syndrome

• The leading cause of pregnancy loss
  • ~1/3 of all first trimester losses are aneuploid
  • Trisomy 16, 21, 22, monosomy X

• A leading cause of obstetric complications
  • 5% of stillbirths (trisomy 21, 18, 13, 22, 9)
  • Intrauterine growth retardation (IUGR), Intrauterine death (IUD), high/low birth weight

• Can lead to imprinting syndromes through uniparental disomy
  • Prader Willi, Angelman

• A leading cause of infertility
  • XXY, XYY, XO
  • Declining quality of oocytes associated with age
  • Sperm aneuploidy and severe oligozoospermia

• A/The leading cause of (repeated) IVF failure

• So aneuploidy is bad and we should use all the tools at our disposal to eliminate it from IVF embryos?

• Or is it that simple?
  • MOSAICISM
What mosaicism is

• The existence of two or more chromosomally distinct cell populations in an embryo or foetus (includes the placenta)

• For the purposes of this presentation, I will focus mostly on whole chromosome aneuploid mosaics
How does mosaicism arise?

- Meiosis I non-disjunction
- Meiosis II non-disjunction
- Precocious sister chromatid/dyad separation
- Cell cycle control breakdown
- Reverse segregation
- Trisomy rescue
- Embryo correction
- Inter-chromosomal effect
- Weakened centromere cohesion
- Cohesin depletion
- Cohesion exhaustion
- Insufficient crossover maturation
- Altered recombination pattern
- Mitotic non-disjunction
- Chromosome loss
- Chromosome gain
- Anaphase lag
- Endoreduplication
- Chromothripsis
- Chaotic divisions
- Etc, etc, etc......
How mosaicism arises

Normal Meiosis

Post-zygotic error
Chromosome loss/gain
Mitotic non-disjunction

More likely to be <50% cells affected
How mosaicism arises

Meiotic Error

"Trisomy rescue"
Or "embryo correction"

More likely to be >50% cells affected
Mosaicism– in the “real world”
i.e. “in ongoing pregnancies”

• Most clinical outcomes similar to uniform aneuploidy
  • But can be milder

• Also, Intrauterine growth retardation (IUGR), Intrauterine death (IUD), Placental insufficiency, Uniparental disomy

• At least 30% of all trisomic conceptuses are mosaic
  • But most are confined to one germ layer (e.g. in the placenta)
  • 30% is probably an under-estimate
  • Very chromosome specific

• Mosaic trisomies can proceed uneventfully to term
  • If they are of post-zygotic origin
  • More than half post-zygotic trisomies are clinically uneventful
  • At least 2% of all normal pregnancies have post-zygotic trisomic cells
    • Almost certainly a underestimate
  • Meiotic mosaics usually result in a pathology (IUGR, IUD, neonatal death)
    • Most adverse clinical outcomes due to mosaicism are associated with errors of meiotic origin
What mosaicism is **not**

- Detection of normal and aneuploid cells in a 5-10 cell trophectoderm biopsy

- If you observe the above
  - Then you *almost certainly* have a mosaic embryo

- If you don’t observe the above
  - Then you *probably* have a mosaic embryo??

- So is PGS useless because of the mosaicism issue?
Mosaicism in IVF embryos

• I would love to have the time to review all the literature
  • That’s for another day
  • And all the studies say something slightly different
  • One interpretation of existing data however is that most to all IVF embryos may have some form of mosaicism
It basically breaks down into 3

• Chaotic mosaicism
  • 5-10% of embryos?
    • Multiple abnormalities
    • No evidence that these can proceed to term
    • Surely we should eliminate by PGS

• Meiotic mosaicism
  • 2-20% in younger women, up to 50% older women??
    • Can lead to IUGR, IUD, UPD.....
    • Related to maternal age
    • Related to ovarian stimulation?
    • Rarely lead to unaffected conceptuses
    • Surely we should eliminate by PGS

• Post-zygotic mosaicism
  • May be up to the remainder of all embryos
    • These are trickier
    • Incidences may vary between clinics
    • If this is true then clinics need to improve their culture systems
  • Pathology depends on a number of factors
    • Proportion of cells affected
    • Germ layer(s) affected
    • Chromosome(s) involved
  • BUT BASIC RESEARCH IS LACKING
Is a bit of aneuploidy a good thing?

- Human cytотrophoblasts acquire aneuploidies as they differentiate to an invasive phenotype

- Cytotrophoblast – polar TE origin
  - Invades endometrium

- Is aneuploidy a normal part of cytотrophoblast differentiation?
  - Related to proliferation and invasive potential within the uterus?
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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

• But what does “well-designed” and “well-conducted” mean?
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 ( Add ons?)
  • How is is different from other forms of medicine?
One view of Evidence Based Medicine

• 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 many other areas of medicine
• Should it be the same for IVF?
  • Or other areas of reproductive medicine?
  • And should we apply this to PGS?
    • In the light of mosaicism
Problems with the standard pyramid in Reproductive Medicine

- Placebos are not usually relevant
- Skill of the operator (or lack of it) can negate any benefit 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
What is the evidence base for PGS?

• Not going to cover every last study
• Some randomised, non-randomised and single centre studies
PGD Aneuploidy vs. Morphology Pregnancy Rates: Meta-Analysis

Randomized Controlled Trials: PGS vs. Morphology

Pregnancy Rates

Does it work?

• Most (but not all) studies display some benefits of PGS
• BUT – attracted criticism because of study design
  • E.g. “intent to treat”
• We still await the results of the STAR trial
  • Illumina
• STILL AN ONGOING ARGUMENT
The case for the prosecution

• PGS is not properly validated

• *We must always* wait for the results of randomised trials (with intent to treat) before introducing a new technology because:

  • Clinics are motivated by the need to be seen to be innovating
    • And the money associated with charging patients for “the latest” therapy
    • Despite proper supporting evidence
    • “Add on” treatments are not good generally

  • Any treatment not validated 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 defence

• There is sufficient evidence justifying the continuation of PGS

• In reproductive medicine we cannot always wait for randomised trials 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 never have been introduced if subject to prior rigours of a randomised trial before being licenced
    - PGS
    - Karyomapping
    - ICSI
    - Some new variants on IVF culture media
  - When randomised trials are designed
    - Can take years
    - Poorly funded (unlike drug trials)
    - Benefits of the treatment may already be obvious without an RCT
    - Appetite to perform the trial may have waned

• The lessons of PGS tell us this
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

Empirical evidence against PGS

Empirical evidence supporting PGS
What I actually see

Empirical evidence pertaining to PGS. Be it positive or negative
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(Poor) Embryo Biopsy or FISH?
Mastenbroek et al. (2007)

Cleavage stage biopsy but no diagnosis (Sham) 6.0%

Cleavage stage biopsy and transfer of “normal” embryo (PGD by FISH) 16.8%

No embryo biopsy (Control) 14.7%

Some would argue this is evidence that it is cleavage stage biopsy (not FISH diagnosis) that is the problem
But is it biopsy in general, or just in the hands of some individuals performing it?
Learning the right lessons from PGS

• RCTs remain the gold standard but
  • Just because it is a randomized trial does not necessarily mean it is a good study
    • Especially if badly executed
  • Just because it is not a randomized trial does not necessarily mean it is not a good study
    • So should we reconsider, conceptually how we view EBM in reproductive medicine?

• DO NOT BE A JACOB OR A GIUSEPPE!!!
Is an open mind a good thing?

• If you and your partner are seeking fertility treatment
  • Do you not want a clinic that is 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?
From an ethical and legal perspective

- What are the implications of *not* implementing PGS?
  - The harm caused to a patient who has an aneuploid pregnancy
  - Assuming that they could, and would, have chosen to avoid this, had PGS been offered
- Other areas of reproductive medicine for which this applies?
General Ideas and Rationale

Case reports

Case series

Case control studies

Cohort studies

RCTs

Double blind placebo controlled trials

Multi-centre analyses

Retrospective analyses (often single centre)

Case reports

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General Ideas and Rationale
Have we learned the right lessons from PGS?

- We don’t do cleavage stage biopsy
  - Even if those labs that are good at it (some trials have shown a benefit)
- We don’t use FISH for PGS
  - Though some would argue it can be effective
- TE biopsy and CGH/NGS is now widely employed
  - Some would say that the Jacobs of this world have made the fortunes of the Giuseppes
- But let’s not be complacent
  - We need to learn more
  - We desperately need more research
  - And we need to look at the origin of the mosaic error
aCGH vs NGS
Did NGS improve things?

Does NGS just pick out a greater proportion of low level mosaics that would normally go to term?
Meiotic aneuploidy from Karyomapping

We could perform NGS and Karyomapping on the same sample TOMORROW
We could probably develop algorithms to derive Karyomapping data from the NGS
So what do I believe?

- Many/most/all human IVF embryos are mosaic
  - Some normal cells, some aneuploid, some chaotic
- BUT mosaicism is *by definition* not uniform
  - Meiotic, post-zygotic, chaotic
  - Many post-zygotic trisomies will proceed to term
    - Several studies provide evidence of this
- PGS is
  - Eliminating the chaotics – this *must* be a good thing
  - Eliminating the meiotics - this *must* be a good thing
  - Eliminating the post-zygotics - *this may or may not be a good thing*
- The likelihood of adverse outcomes increases with the number abnormal cells
  - The chances of detection increases the more cells are affected
  - Even post-zygotically PGS *should* be working
- BUT PGS is *less likely* to be effective in labs where
  - Post-zygotic aneuploidies are generally high
  - Pregnancy rates are low
  - Biopsy techniques are sub-optimal and damaging the embryo
  - If an RCT has a large number of these labs in it, then *it might not show a benefit of PGS*
PGS can work

• Alan Handyside’s talk this afternoon
• “One by one plus” programme
• “Freeze-all” strategy with PGS
• Impressive IVF success rates

• Alan Handyside, Michael Summers, Christian Ottolini, Sammy Neumann, Julija Gorodeckaja
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Personal opinion and the future

• PGS should continue
  • The signs are mostly good and we’ll only improve it by doing it

• NGS (Karyomapping) has great potential (mosaicism, meiotic origin)

• BUT there is much to improve
  • Karyomapping + NGS could be achieved tomorrow
  • Proper stratifying of referral categories
    • Who will (and will not) benefit?
  • More sophisticated analyses
  • Understanding mosaicism better
  • Improved External Quality Assessment (EQA)
  • New technologies
    • Blastocentesis, metabolomics, transcriptomics, time lapse
  • If we don’t continue to innovate we become a Giuseppe

• Good statistics (randomization) alone do not a good study make
  • We need all the “good gardening” as well
  • If we don’t consider the whole evidence base we become a Jacob

• Should we screen every IVF embryo for chromosome abnormalities?
A way forward for PGS

“Significant” abnormality
- Any meiotic abnormality
  - Segmental or whole chromosome
- Trisomy 21, 18, 13
- Any monosomy or deletion
- Uniparental disomy
- Any chaotics

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

No detected chromosome abnormality

Do not transfer

Refer to genetic counsellor
- Consider
  - Level of abnormality
  - Chromosome involved
  - Prospects for re-biopsy and analysis
  - Availability of other embryos
  - Low level errors that might benefit implantation

Consider for transfer

There are bound to be false positives and negatives however these can ultimately be minimized by understanding mosaicism better
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Answers the world needs to know

• A cell by cell comparison of a large cohort of embryos
  • To establish overall levels of mosaicism in different germ layers
  • Some of this may be estimated from existing NGS data
  • Meiotic vs post-zygotic errors
• What if any approaches can be used to improve levels of aneuploidy?
  • Improved culture (post zygotic)
  • Improved ovarian stimulation/patient management (meiotic)
  • Comparison with model systems (e.g. mouse, pig, cattle)
• More trials
  • Stratified patient groups (e.g. AMA vs RIF vs RPL vs Oligospermia ....)
  • A “neutral” trial
• Are patterns of confined mosaicism laid down in the embryo or as a result of differential survival?
• If most embryos have some form of (mosaic) aneuploidy, is this normal?
• Which aneuploid mosaics are not likely to have adverse outcomes?
• Are IVF embryos representative of in-vivo fertilized embryos?
  • Hassold and colleagues suggest Meiosis I errors are far more common in liveborn (non-IVF) individuals
  • Handyside et al 2012 suggest that MI and MII errors are equally likely
    • Of course looked at IVF embryos
  • Is chromatid separation an IVF-induced phenomenon?
    • Increased MII rate, PSSC, reverse segregation
• Nuclear organization
  • Spatio-temporal organization of chromatin in 4 dimensions
  • Relationship to gene expression
• Non-invasive detection of chromosome error
  • Metabolomics
  • Time lapse
Here is my real conflict of interest

• I’m not a clinician
• I don’t run a diagnostic lab
• I don’t have shares in any companies
• But I am a researcher
  • And I run a research lab
  • My career and personal well being (including being invited to lovely conferences like this one) depend on myself and others performing good research

• **It has never been more important to perform basic research**
  • First on model systems
  • But moving to human embryos

• The companies, governments, research councils and charities **must, must, must** get their heads together more
  • Lobby for a greater slice of the funding pie

• There is a prize
  • A greater understanding of aneuploidy an mosaicism generally
    • Probably the biggest genetic hazard facing mankind
  • If we can get PGS right then, in future **every** IVF cycle may involve some variant of it
Polar TE

Mid TE

Mural TE

56.2%
(27/48)

47.4%
(37/78)

30.0%
(12/40)

aCGH

Tiffany Stankewich Tyl Taylor
2 embryos only

So only proof of principle at this stage

Tyl Taylor
Is there an animal model?

- Yes!
  - Cattle

- For food production reasons cattle IVF is commonplace

- Ovum pick up (OPU) similar to egg collection in humans

- Some companies now pioneering “coasting” protocols
  - Similar in principle to mild stimulation protocols
    - “A period of no gonadotrophin administration after initial stimulation and before inducing ovulation”
  - Opportunity to compare same animals (or close relatives) with same protocols
Cattle Karyomapping to optimise food production and delivery of superior genetics: The first liveborn calves

Turner KJ¹, Silvestri G¹, Smith C², Dobson G², Black DH², Handyside AH¹,³, Sinclair KD⁴, Griffin DK¹

- 77 embryos successfully generated SNP chip results
- 37 Karyomapped to date, analysed for
  - Chromosome copy number
  - Parent of origin
  - Number of cross-over events
- ~50% have a chromosome abnormality
  - Including 10% (4 embryos) no paternal chromosomes
  - Apparently parthenogenetically activated

- 50 embryo transfers
  - 18 led to pregnancies
  - 9 ongoing,
  - 4 aborted,
  - 5 born
  - 1 soon after birth of post-natal infection
- NB aneuploidy results not yet forming part of selection strategy
- More transfers and pregnancies imminent
- We continue to improve protocols
  - In most recent run 2/2

![Embryos by Number of Abnormalities](image)
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• We need to 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

• We need to consider the role of meiotic vs. mitotic errors and we need to do it now
  • Selectively screen out meiotic and chaotic but not necessarily all post-zygotic errors

• Stratify the patient groups
  • Who will and will not benefit from PGS?
    • AMA vs RPL vs RIF vs Oligospermia

• Introduce a proper EQA scheme

• WE NEED NOT TO BE A JACOB NOR A GIUSEPPE – LISTEN AND LEARN FROM EACH OTHERS’ ARGUMENTS

• Consider appropriate “staged” introduction protocols for new innovations
  • Karyomapping for aneuploidy, Blastocentesis, 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

• WE NEED MORE RESEARCH
• Especial thanks to Prof Sally Sheldon (Kent Law School)
• Showed the work of Tyl Taylor, Kara Turner, Giuseppe Silvestri
• The “One by One” team at the Bridge Centre London. Alan Handyside, Michael Summers, Christian Ottolini
• The real Jacob and Giuseppe

• www.kent.ac.uk/cisor