Results of the Virtual Academy of Genetics (VAoGEN) questionnaire on “Mosaicism in PGS”

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Disclosure information: Nothing to declare
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James Surowiecki 2004

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The Surveys
Anti-Mullerian hormone (AMH) and antral follicular count (AFC)
Minimising the risk of infection and bleeding at trans-vaginal ultrasound guided ovum pick-up
Vitrification, GnRH trigger and differed ET
Mechanical Infertility
Oocyte Donation
Reproductive Immunology Practice in IVF
Embryo Culture and Catheter Loading
Egg Collection and Embryo Transfer Techniques
PCOS – Definition, Diagnosis and Treatment
Poor Responders
The use of GnRH agonist in IVF protocols
Metformin Use In IVF Patients
Frozen-Thawed Embryo Transfer
Updated survey on the use of progesterone for luteal phase support in stimulated IVF cycles
Progesterone Support In IVF
IVF Worldwide surveys - publications


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ONLINE EDUCATIONAL PROGRAM

History of PGD and PGS

- A Brief History of Preimplantation Genetic Diagnosis and Preimplantation Genetic Screening
  Jason Franasiak, USA
  Richard T. Scott, Jr, USA | Read More

PGD and PGS: State-of-the-Art

- Development and current best practice for translocations
  Francesco Fiorentino, Italy | Lecture | BIO

- Single gene diagnosis and aneuploidy detection using karyomapping
  Tony Gordon, UK | Lecture | BIO
A Statement on the use of Preimplantation Genetic Screening (PGS) of chromosomes for IVF patients

On September 26th and 27th 2015, under the auspices of The Virtual Academy of Genetics, Cogen held its 1st meeting on Controversies in Preconception, Preimplantation and Prenatal Genetic Diagnosis.

This meeting gathered together Key Opinion Leaders from around the world to inform, discuss and consider many of the questions of our time in relation to genetics and the place of the new technologies in driving the future of medical practice in the field of human reproduction.

The Undersigned have issued the Statement below and welcome debate and comment in this forum.

CONSENSUS STATEMENT ON PGS

For all practitioners of IVF there is the clinical imperative to achieve the highest chance of a live birth per single attempt, reducing the time to delivery for each patient; to reduce the incidence of miscarriage; reduce the number of miscarriage pregnancies; decrease the number of non-viable embryo transfers (unnecessary IVF transfer cycles); eliminate the freezing of embryos that are chromosomally abnormal; to diagnose patients with no chance to deliver with IVF, and, given the high incidence of embryo aneuploidy in all IVF cycles, to minimise the chance of transferring an aneuploid embryo. The alternative to using PGS is to transfer embryos one by one, and studies have shown this can result in up to 50% patient drop out following a miscarriage.

To this aim, scientific endeavour in many laboratories and clinics worldwide has focused upon the development of methods to detect and screen out chromosome anomalies from human embryos using technologies to identify whole chromosome copy number. All IVF cycles have a significant risk of aneuploid embryos, from an average of around 30% in young female patients and also egg donors, up to 90% in women in their early 40s. Methods such as array CGH, qPCR and Next Generation Sequencing (NGS) are delivering high-fidelity information allowing for accurate assessment of chromosome copy number of embryos.

A wide-ranging expert critical evaluation of the new pre-implantation genetic screening (PGS) technologies, taking into consideration clinical data from the application of full
COGEN Position Statement on Chromosomal Mosaicism Detected in Preimplantation Blastocyst Biopsies

BACKGROUND

The occurrence of aneuploidy (an incomplete or abnormal chromosome copy number) in human embryos is a natural phenomenon in embryos conceived either in vivo or following the use of in vitro fertilization (IVF) technology; this has been understood since the early 1980s. Alterations from the euploid (complete) chromosome copy number can occur at any age, but the incidence of aneuploidy increases with maternal age (>50% risk in women over 40 years old and approximately 50% risk in women age <35). Aneuploidy is the largest single cause of miscarriage, and thus the ability to evaluate chromosome copy number prior to embryo transfer has been a clinical imperative of the IVF community for many years. In recent years, screening methods known as preimplantation genetic diagnosis for aneuploidy (PGD-A) or preimplantation genetic screening (PGS) have been developed to accurately assess the copy number of all 24 chromosomes from a single or multiple cell(s) biopsied from human preimplantation embryos.

However, since 1983 and the introduction of chorionic villus sampling (CVS), a discrepancy between the chromosomal makeup of the placenta and the fetus has been documented in approximately 1-2% of ongoing pregnancies; this is known as “confined placental mosaicism” (CPM). Since trophoderm biopsy is now widely viewed as the preferred method of chromosome analysis, this phenomenon is often observed in PGS of blastocysts. In the case of mosaicism, the blastocyst biopsy may have a single chromosome abnormality (pseudodiploidy) in one cell line while the other cell line is diploid; if one of the cell lines then undergoes further differentiation to a trophoderm cell, CPM will result. In such cases, PGD or PGS will miss the abnormality, and the presence of mosaicism may be an important factor in the decision to transfer the embryo.
Embryonic mosaic aneuploidy: the presence of cell lineages with different chromosomal constitution

Detection of mosaicism at blastocyst stage with the use of high-resolution next-generation sequencing

Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts
Mosaicism in Preimplantation Genetic Screening (PGS): what is your opinion?

The purpose:
• To evaluate the extent of mosaicism in PGS clinical practice
• To gain insight on the views and practices of the ART community regarding this issue

This survey was suggested and composed by:

Ariel Weissman  Yuval Yaron  Simon Fishel
December 1 through December 31, 2016

RESEARCH & EDUCATION

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Survey on Mosaicism in Preimplantation Genetic Screening (PGS): what is your opinion?

It is now apparent that mosaicism exists in a material number of embryos as part of the human conception, and some PGS technologies are now able to detect its presence. This survey is designed to evaluate the extent of mosaicism in PGS clinical practice and to gain insight on the views and practices of the ART community regarding this issue. Your opinion is important whether you perform PGS or not.

Background

Aneuploidy is the leading cause of implantation failure, miscarriage and congenital abnormalities in humans. It is for this reason that preimplantation genetic screening (PGS) was introduced into clinical practice. By...
The Surveys: Quality Assurance

- Minimization of duplicate reports from a clinical unit
- Minimization of possible false data

A software that assesses the consistency of three parameters
Comparison with existing data of units registered to the IVF-Worldwide
- Name of the unit
- Country
- E-mail address
≥2 match with archived data on the website

- Data is analyzed by cycle and not by center
### Geographic distribution of survey respondents

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At what stage of development are the majority embryos biopsied in your center?

- Polar body biopsy (PB1 and PB2): 0%
- Polar body biopsy (PB1 only): 0%
- Blastomere biopsy (1 cell): 7.9%
- Blastomere biopsy (>1 cell): 2.1%
- Trophoblast biopsy (day 5 only): 8.7%
- Trophoblast biopsy (day 5 or 6 depending blastocyst stage): 90.1%
5) At what stage of development are the majority embryo biopsies being performed in your clinic?

PGS Survey - September 20, 2015 - November 17, 2015
In trophectoderm biopsy, what is the optimal number of cells that you aim to obtain?

- Less than 5: 27.7%
- 5-7: 58.1%
- 8-10: 6.6%
- More than 10: 7.6%
GOOD PRACTICE RECOMMENDATIONS
“For reliable detection of biological mosaicism, a minimum of 5 cells (but, importantly, <10) should be biopsied, with as little cell damage as possible.”

PGDIS Position Statement on Chromosome Mosaicism and Preimplantation Aneuploidy Testing at the Blastocyst Stage

“For reliable detection of mosaicism, ideally 5 cells should be biopsied, with as little cell damage as possible.”

Trophectoderm biopsies with the highest relative DNA content are associated with lower live-birth rates after single euploid embryo transfer

Neal et al., Fertil Steril 2017
7) Which method of genetic testing is predominantly used in your clinic for determination of embryo ploidy status?

PGS Survey - September 20, 2015 - November 17, 2015

- Array CGH: 59%
- Real-time quantitative PCR: 5%
- SNP microarray: 7%
- Next generation sequencing: 16%
- FISH: 9%
- Other: 2%
- I don't know: 3%
- Total: 100%
Do you think next generation sequencing is required to reliably detect mosaicism?
NGS and embryonic mosaicism

NGS has brought embryonic mosaicism to the spotlight

aCGH is less suitable for establishing the exact level of low-grade mosaicism compared with NGS

Munne et al., Fertil Steril 2016
Sermon et al., Mol Hum Reprod 2016
Where is genetic testing being performed in the majority of cases in your center?

- In-house genetic laboratory: 34.5%
- Centralized/referral laboratory: 65.5%
This highlights the need for uniformity and standardization in reporting practices.
Do you receive information on mosaicism from the laboratory?
Comparing in house genetic laboratory to centralized/referral laboratory

- Yes – any degree of mosaicism is reported as 'mosaic' for the chromosome(s) involved: 34.4% (in-house) vs. 17.8% (centralized/referral)
- Yes – any degree of mosaicism is reported as 'mosaic' and the % mosaicism is provided for the chromosome(s) involved: 18.1% (in-house) vs. 12.8% (centralized/referral)
- Yes – the sample is reported as 'mosaic' for the chromosome(s) involved but only above a certain mosaicism threshold: 0.9% (in-house) vs. 4.7% (centralized/referral)
- Yes – the % mosaicism for the chromosome(s) involved is reported but only above a certain mosaicism threshold: 18.8% (in-house) vs. 8.3% (centralized/referral)
- No – samples are reported as euploid or aneuploidy only: 33.2% (in-house) vs. 31.2% (centralized/referral)
- Other: 8.7% (in-house) vs. 11.1% (centralized/referral)
What is the percentage of mosaic embryos that you encounter in your practice?

- Less than 5%: 21.6%
- 6%-10%: 24.4%
- 11%-20%: 11.7%
- More than 20%: 7.9%
- I don't know: 34.5%

≥10% 46%
Do you ever re-biopsy mosaic embryos?

- Yes: 19.3%
- No: 63.9%
- Only under special clinical circumstances: 16.7%
At what level of mosaicism would you consider the embryo as abnormal?
Should mosaic embryos be stored for potential therapeutic use or discarded?

- Performing PGS: 72.4%
- Not performing PGS: 68.8%

- Should be stored for potential therapeutic use: 12.8% (Performing), 7.3% (Not performing)
- Should be discarded: 14.8% (Performing), 24% (Not performing)
- Should be stored for potential therapeutic use following individual counseling: 72.4% (Performing), 68.8% (Not performing)
Should mosaic embryos be transferred?

- Yes, if no euploid embryos are available for transfer: 45.8%
- No, mosaic embryos should never be transferred: 14.9%
- Yes, but only if mosaicism degree is below a certain threshold: 31.1%
- Yes, but it depends on the specific chromosome involved: 33.5%

Legend:
- Performing PGS
- Not performing PGS
What kind of mosaic embryos would you consider transferring?

- Only monosomic: Performing PGS (2.2%) and Not performing PGS (8.3%)
- Both monosomic and trisomic: Performing PGS (2.8%) and Not performing PGS (3.1%)
- All mosaic embryos below a certain threshold (see question 12): Performing PGS (26.5%) and Not performing PGS (11.5%)
- I would never consider transferring mosaic embryos: Performing PGS (8.2%) and Not performing PGS (31.3%)
- It would depend upon the specific chromosome involved: Performing PGS (60.3%) and Not performing PGS (45.8%)
TRANSFERRING MOSAIC EMBRYOS IN ABSENCE OF ALTERNATIVES

If non-mosaic euploid embryos are not available
No option for undergoing another IVF cycle
A mosaic embryo may be considered for transfer.

In such circumstances, the following constitutes good practice:

Embryos with lower levels (20-40%) of mosaic aneuploidy are preferential to those with higher levels (40-70%)

In the case of complex mosaicism (mosaicism observed across multiple chromosomes), transfer is not recommended
RECOMMENDATIONS FOR PRIORITIZING MOSAIC EMBRYOS FOR TRANSFER

If a decision is made to transfer a non-complex, low-level mosaic embryo, one can prioritize selection based on the specific chromosome involved.

- Embryos mosaic for trisomies capable of live born viability (chromosomes 13, 18, 21, 22) are of lowest priority.
- Embryos mosaic for trisomies associated with uniparental disomy (chromosomes 14, 15) are low priority.
- Embryos mosaic for trisomies associated with intrauterine growth retardation (chromosomes 2, 7, 16) are low priority.
- Mosaicism involving chromosomes 1, 3, 4, 5, 6, 8, 9, 10, 11, 12, 17, 19, 20, have not been associated with the aforementioned adverse outcomes; only adverse outcomes have been observed when mosaicism is present in the fetus.
- Mosaic monosomies seem to implant with a similar incidence to mosaic trisomies. They may contain trisomic cell lines, and should be considered to have similar risk as their counterpart trisomies.
How would you follow a pregnancy following transfer of mosaic embryos?

53-59%
If ongoing pregnancy is established after transfer of a mosaic embryo, the developing fetus should be retested for aneuploidy by prenatal diagnosis.

Amniocentesis is recommended over CVS as the mosaicism persists in the trophoblast.

Reproductive outcomes of mosaic embryo transfers should be conveyed to the PGS laboratory performing the original diagnosis.

And where possible a follow-up on mosaic data should be obtained from the live birth.
Which of the following statements best describes your views regarding mosaicism in preimplantation embryos?

- Mosaicism is a serious problem that puts in doubt the entire concept of PGS. Until the issue is resolved, the use of PGS should be avoided.
  - Performing PGS: 2.4%
  - Not performing PGS: 58.3%

- Mosaicism is a problem but with proper counseling the practice of PGS should continue.
  - Performing PGS: 15.8%
  - Not performing PGS: 18.8%

- Mosaicism, while challenging, provides an opportunity to further discriminate between embryos during PGS.
  - Performing PGS: 9.7%
  - Not performing PGS: 0%

- Mosaicism may be an issue, but has always existed in IVF. More study is needed, but PGS should continue with appropriate safeguards and counseling.
  - Performing PGS: 66.4%
  - Not performing PGS: 22.9%

- The concern over mosaicism in human embryos is exaggerated by over-sensitive technology.
  - Performing PGS: 5.7%
  - Not performing PGS: 0%
What types of studies would be most valuable to better understand mosaicism?

- Better characterization of detection limits and technical requirements of PGS technologies for mosaicism detection: 38.3% (Performing PGS), 34.4% (Not performing PGS)
- Retrospective studies using NGS of samples previously tested with other PGS methods to establish the 'true' incidence of embryo mosaicism: 33% (Performing PGS), 7.3% (Not performing PGS)
- Retrospective studies using NGS of embryos previously tested euploid with other methods to establish the clinical impact of mosaicism: 35.8% (Performing PGS), 12.5% (Not performing PGS)
- Prospective studies about the impact of mosaicism on clinical outcomes: 55% (Performing PGS), 60.4% (Not performing PGS)
- Distribution of mosaicism throughout embryos donated for research (e.g. multiple biopsies for each embryo) and between TE biopsy and ICM: 52.3% (Performing PGS), 62.5% (Not performing PGS)
- Other: 7.6% (Performing PGS), 0% (Not performing PGS)
Survey on chromosomal mosaicism detected during preimplantation genetic screening: Conclusions

Embryonic mosaic aneuploidy is common in preimplantation embryos

New techniques used for CCS, particularly NGS, can now detect its presence with high accuracy

More studies are needed before definite conclusions can be drawn regarding:
• detection
• clinical significance
• management
of embryonic mosaicism in the context of PGS

Standardization of reporting of mosaicism in PGS is urgently required
The results support the COGEN Position Statement on Chromosomal Mosaicism Detected in Preimplantation Blastocyst Biopsies 2nd COGEN Meeting in Barcelona 2016
Chromosomal mosaicism detected during preimplantation genetic screening: results of a worldwide Web-based survey

Ariel Weissman, M.D., Gon Shoham, B.Sc., B.Med.Sc., Zeev Shoham, M.D., Simon Fishel, Ph.D., Milton Leong, M.D., and Yuval Yaron

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Thank you

http://www.lodmosaic.org/home.html