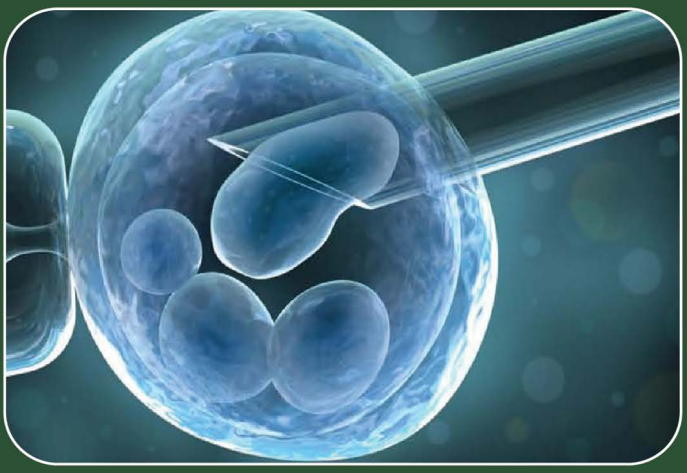

ISARCON 2017 Manual of Preimplantation Genetic Diagnosis & Screening, Vitrification Gametes, Embryo and Tissues

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Illuminating Fertility Treatments
Basics & Beyond

ISAR

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Status of PGS for Aneuploidy: Should We Do It and When?

4

Gad Lavy

BEFORE WE BEGIN

- Evidence-based medicine (EBM)
- Randomized controlled trials (RCT)

Evidence-Based Medicine

- Assist practitioners in making informed decisions regarding new interventions.
- Improve patient care
- Avoid unnecessary or unproven treatments (Healy 2006).[1]

EBM in Assisted Reproduction Technology (ART)

- Ovarian stimulation protocols: Well-established benefit
- Embryology:
  - Objective measure needed to assess new procedures
  - Difference between clinical and laboratory setting need to be explored and addressed.

Clinical Trials

- Meta-analysis
- Systematic review
- Prospective studies
  - Randomized controlled trial
  - Cohort study
- Retrospective studies
  - Case-control study
  - Cross-sectional study
- Case report and series.

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Randomized Controlled Trials

- Effect of drugs:
  - Study of streptomycin treatment for TB (Daniels and Hill 1952)[2]
- Surgical procedures:
  - Limitations imposed by complexity and need to consider skill and clinical judgment.
  - Value has been questioned
- Embryology:
  - Even more complex
  - Exact criteria have not been established.
- Removal of technological bias:
  - Quality of reporting lab (who will determine)
- Proper method of randomization:
  - Patients vs gametes/embryos
  - Order of aspiration, temp etc.
- Allocation concealment:
  - No method has been developed for embryology (Grimes and Schulz 2002)[3]
  - Fingerprinting of embryos with double transfer (Scott 2012)[4]
- Blinding (CONSORT)
  - Not as important in embryology but criteria need to be developed.
- Analysis and reporting of data
  - Subjects excluded or dropped
  - Poor data should be eliminated.

INTRODUCING NEW LABORATORY TECHNOLOGY AND EBM

- Hypothesis driven research
- Modeling in animal systems
- Testing in donated embryos
- Small clinical studies
- Large clinical trial
- Clinical application (Harper et al. 2012).[5]

EBM in Preimplantation Genetic Screening (PGS)

- Accepted
  - Overall low implantation with in vitro fertilization (IVF)
  - Many aneuploid embryos following IVF
  - Increase in aneuploidy with maternal age
  - High rate of mosaicism on day 3 embryos

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- Needs to be determined
  - Is PGS clinically effective and in which subgroups
  - Cleavage stage vs blast
  - Technology FISH vs CCS
  - Which CCS is best.

PGS in IVF

- Preimplantation genetic screening (PGS) with aneuploidy screening will improve results by selection of most competent embryo for transfer
- Possible indications:
  - Advanced maternal age
  - Repeated implantation failure
  - Recurrent pregnancy loss
  - Severe male factor
  - *Single embryo transfer*: Improve embryo selection.

Limitations of PGS

- *Cleavage stage*: PGS/FISH does not improve live birth
- *Blast Bx*:
  - Improve implantation rate but not number of babies born from a single cycle. Does not increase the number of euploid embryos.
  - May reduce number of viable babies
    Embryo damage possible
    - Biopsy
    - Vitrification/warming.

PREIMPLANTATION GENETIC SCREENING

- PGS 1.0
  - Cleavage stage (D-3) biopsy
  - FISH analysis (up to 12 chromosomes)
  - Blastocyst transfer
- PGS 2.0
  - Blastocyst (D5 or D6) biopsy
  - Comprehensive chromosome screening (CCS)
  - Vitrification vs fresh transfer.

PGS and IVF Success

- Does PGS improve clinical outcome
- PGS 1.0 vs PGS 2.0

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- PGS methodology, which is best
  - FISH
  - CCS
    - aCGH
    - SNP
    - qPCR
    - NGS

PGS 1.0

Cleavage Stage/FISH

- High rate of Mosaicism (50%) decreases accuracy
- Embryo damage due to low number of cells.

Fluorescent In Situ Hybridization (FISH)

- Original technology. Performed on cleavage stage embryos
- Limit in the number of chromosomes that can be analyzed (up to 12)
- High incidence of "no results".

RCT for Cleavage Stage PGS/FISH

- Mastenbroek et al. (2011):[6] FISH
  - Large multicenter study (408 patients; 836 cycles)
  - Implantation lower in the PGS group (23% vs 35%)
- Treff et al. (2011):[7] Safety of biopsy
  - Cleavage stage biopsy decreased implantation
  - Blastocyst biopsy does not decrease implantation
- Scott et al. (2012):[8] Embryo fingerprinting CCS
  - Implantation rates aneuploid (4%) vs normal (41%).

PGS/FISH

Systematic Reviews

- Mastenbroek et al. (2011):[9] Nine RCT included
- PGS has no benefit in increasing pregnancy success
- PGS reduces implantation rates.

Cleavage Stage PGS (PGS 1.0)

Lessons Learned

- PGS 1.0 was adapted very quickly worldwide and supported by many non-RTCs

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- It was eventfully proven to be detrimental and to reduce pregnancy success
- PGS 2.0 has replaced PGS 1.0 and is increasing in popularity
- PGS 1.0 caused significant harm to thousands of patients due to reduction in pregnancy success. We should not allow this to happen again with PGS 2.0 (Gleicher et al. 2014).[10]

Blastocyst Biopsy Advantages

- Improvement in culture conditions
- Improvement in biopsy technique (no damage 54% vs 51% implantation rate) as compared to cleavage stage (implantation rate 30% vs 50%)
- Improvement in cryopreservation (vitrification)
- 5–10 cells removed from trophectoderm. Reduces error rate.

Blastocyst Biopsy Disadvantages

- Reduces chances of embryo transfer
  - 50% of zygotes survive to D-3
  - 25% to D-5
- Higher risk of monozygotic twining
- Higher risk of male offspring
- Risk of epigenetic modifications and adverse neonatal outcome (Prac comm 2013).

Blastocyst Bx limitations

- Mitotic nondysjunction (post fertilization) leads to mosaicism and possible errors
  - Cleave stage 50%
  - Blastocyst 3–5% (Brezina et al. 2015)[11]
  - Self-correction is possible and thus even those embryos diagnosed with mosaicism
  - Proportion of moscaicism required for normal birth is unknown
  - False positive/negative difficult to determine as abnormal embryos are discarded.

PGS 2.0

- PGS 2.0 compared to morphological criteria alone improves clinical implantation in good prognosis patients
- The findings are independent of CCS method used

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- When the same number of embryos were transferred in both control and PGS group the implantation, OGP and delivery rates were higher with PGS
- SET (PGS tested) = DET (untested) (Dahdouh et al. 2015).[12]

PGS CLINICAL USE

What is the Evidence

- Couples at risk of aneuploidy
  - Age
  - Multiple IVF failures
  - Previous aneuploid pregnancy
  - Recurrent pregnancy loss (RPL)
- Couple interested in SET.

Note: The primary population that has been targeted for PGS are coupes undergoing IVF without evidence of genetic abnormalities and thought to be at a high risk of having an aneuploid embryo.

Elective Single Embryo Transfer (eSET) EarlY Studies

- Increases the success per transfer but not the cumulative pregnancy rates
- Need to account for all embryos (fresh/frozen) to determine benefit
- RCT eSET/PGS vs eSET/no PGS + SET/PGS FET
- Success was the same (Thurin et al. NEJM 2004).

eSET recent studies

- High rate of multiple with DET without PGS
- High success with SET/PGS
- Forman et al. F&S 2013[13]
- Forman et al. AJOBG 2014[14]

Forman Studies

- These studies are often quoted as proof as to the benefit of PGS in clinical practice.
- Careful review of the study shows some basic flaws that may invalidate the conclusion.

Recurrent IVF implantation Failure (RIF)

- Higher rate of aneuploidy observed in these patients
- RCT of couple with RIF
  - Two studies with day 3 biopsy and blastocyst transfer

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  - No difference is implantation rates compared to controls who did not have PGS
- No data to support PGS for RIF.

Advanced Maternal Age (AMA)

- RCT for effect of PGS no success with AMA
  - All used FISH
  - Reduction in success in the PGS group (26% vs 13–23%) (Mastenbroek et al. Hum Rep 2011).[6]
  - Increase in success with D-3 FISH and blastocyst transer vs blastocyst transfer/No PGS (Rubio et al. F&S 2013)[15]
- Available evidence does not support PGS for AMA.

Recurrent Pregnancy Loss

- Most sAbs are associated with chromosomal abnormality. Higher in RPL
- Studies
  - No RCT using blast culture and CCS
  - Observational studies show improvement in outcome compared to controls. However, pregnancy success without intervention is high.
- PGS may reduce risk of sAb in RPL patients
- No evidence that PGS increases take home baby rate in women with RPL.

CONCLUSION

- PGS is performed to identify de novo aneuploidy in couples presumed to be chromosomally normal to allow selection of those embryos with a normal karyotype.
- PGS with cleavage stage embryos and FISH decreases chances of live birth.
- Blast biopsy with CCS is the preferred method.
- Major clinic utility is to reduce multiples without reducing delivery rates allowing SET with high success.
- However, consider no PGS with SET followed by SET/FET if not successful, is as success is the same.
- The value of PGS to select best embryos for transfer in couples with no known chromosomal or genetic abnormality.
- There is no high quality evidence that PGS improves cumulative live birth rates in these couples, including those with AMA, RIF and RPL.

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- Among those with RPL, PGS will reduce the risk of subsequent loss but not increase the chances of having a live born baby.

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