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Evaluation of Results:
Follow-up and Misdiagnosis

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Overview

- Errors and Misdiagnosis
  - Definition, Causes/Solutions, How much of a problem is it???
- Follow-up of untransferred embryos
  - Why??, How??, Outcome
- Follow-up of DNA from live-born children
  - Why??, How??, Reporting, Cost/payment, Prenatal testing
- Minimizing errors in the lab
  - Linked markers, more stringent human controls, control sets, EQA

Errors and Misdiagnosis

- Definitions:
  - **Adverse Misdiagnosis**: a misdiagnosis that results in a severe adverse event for the patient
    - Birth of a child affected with the tested mutation when the embryo was diagnosed as unaffected during PGD
    - Termination of a fetus affected with the tested mutation when the embryo was diagnosed as unaffected during PGD

Errors and Misdiagnosis

- Definitions:
  - **Benign Misdiagnosis**: a misdiagnosis that does not result in a severe adverse event for the patient
    - Birth of an unaffected carrier child when the transferred embryo was thought to be free of the tested mutation
    - Follow-up of untransferred embryos reveals a misdiagnosis but no pregnancy during cycle

Errors and Misdiagnosis

- Most Likely Causes/Solutions-Human Errors:

Error	Solution
Unprotected sex during IVF Cycle	More stringent teaching of patients
Sample mix-up during embryo biopsy and PGD testing	More robust systems including witnessing steps
Misinterpreted report	Better training of clinical staff
Use of wrong reagents, primers or probes	More robust lab SOPs and witnessing steps, employee competency testing
Improper segregation analysis	Better training of staff and oversight by more experienced staff

Errors and Misdiagnosis

- Most Likely Causes/Solutions-Intrinsic Errors:

Error	Solution
Haploid cell removed during biopsy	Removal of two cells during biopsy and/or addition of linked markers
Chromosomal mosaicism	Removal of two cells during biopsy and/or addition of linked markers
Allele drop out (ADO)	Removal of two cells during biopsy and/or addition of linked markers
Trisomic rescue/Uniparental disomy	Removal of two cells during biopsy and/or addition of linked markers

Errors and Misdiagnosis

– Most Likely Causes/Solutions-Extrinsic Errors:

Error	Solution
Probe or primer failure	More robust pre-clinical validation and/or use of WGA to allow repeat sample testing
Contamination by parental cells or DNA	Removal of cumulus cells/ICSI to reduce the risk and/or use of linked markers to assess contamination
Contamination by lab staff or carry-over contamination	More stringent control of environment and/or the use of linked markers to assess contamination

Errors and Misdiagnosis

– How much of a problem is it?

  – Not much data available

  – Most misdiagnosis are probably never realized due to failure to achieve pregnancy and live birth following a misdiagnosis and absence of evidence of misdiagnosis (carrier versus unaffected baby born but never tested in follow-up)

Errors and Misdiagnosis

– How much of a problem is it?

  – Actual reports to the ESHRE PGD Data Collection are very low

    – 24 reported misdiagnosis in 15, 158 cycles = 0.16%

  – Each PGD lab should have an idea of an in-house error rate which can be reported to patients at their consult

    – Usually reported to be approximately

      – 1% for PCR-based tests

      – 5% for FISH-based tests

Follow-up of untransferred embryos

- Why???
  - May help the lab determine that an error was made that could lead to an adverse misdiagnosis if the patient achieves pregnancy
  - Can help identify errors in initial diagnosis that can be investigated and lead to protocol or program changes to eliminate or reduce errors
  - Helps each lab determine their own misdiagnosis rate which should be available to patients at initial counseling

Follow-up of untransferred embryos

- Donating embryos to research
  - IRB approved program to systematically collect embryos for future research and our follow-up program
  - Specific consent that spells out the options to the patient for untransferred embryos
    - Disposal
    - Research
    - Donate to another couple (only normal embryos)
  - Genetic counselor speaks to each patient about untransferred embryos and coordinates receipt of embryos into research program, including follow-up

Follow-up of untransferred embryos

- How??
  - Rebiopsy of untransferred embryos followed by single cell analysis of any/all cells remaining
  - Whole embryo analysis following digestion of zona using multiplex PCR
  - Whole embryo analysis following digestion of the zona using whole genome amplification (WGA) and multiple analysis steps

Follow-up of DNA from live-born babies

- Why???
  - May help the lab determine that an error was made that could lead to an adverse misdiagnosis if the patient achieves pregnancy
  - Can help identify errors in initial diagnosis that can be investigated and lead to protocol or program changes to eliminate or reduce errors
  - Helps each lab determine their own misdiagnosis rate that should be available to patients at initial counseling

Follow-up of DNA from live-born babies

- How??
  - Collection of cord blood at delivery followed by DNA extraction and analysis using the original single cell protocol
  - Collection of cheek swab material followed by DNA extraction and analysis using the original single cell protocol
- Reporting
  - Must make it clear to the patient what will happen with the sample and how results will be handled since this is outside of the normal testing pattern of the PGD laboratory

Follow-up of DNA from live-born babies

- Cost/Payment
  - Must make it clear to the patient what, if anything, test will cost and how it will be billed
- Offer of free prenatal testing
  - Because we are an infertility and genetics clinic with shared resources, we offer all monogenic disease PGD patients a free prenatal test (amnio or CVS) to help us get as much follow-up data as possible

Minimizing errors in the lab

- Linked markers
  - Controls for and helps assess ADO
  - Controls for and helps assess contamination
- Protocol checking and witnessing
  - Will help reduce human errors of wrong reagents, miscalculations, etc.
- Double checking of embryo numbers/sample numbers
  - Controls human errors such as sample switches and incorrect embryo making it to transfer

Minimizing errors in the lab

- Control sets
  - Helps assess the assay on the day of biopsy and should ideally include the following samples:
    - Single cell controls (heterozygous or homozygous affected)
    - Normal, affected, and parental DNA samples
    - No DNA reagent negative control
    - Negative wash controls (one per biopsied cell)
  - Report and result checking
    - Helps control human errors such as result mix-ups and misinterpretation of the results
  - External quality assessment (EQA)
    - Covered by Sandi Deans

Thank you…

- Local organizing committee
- ESHRE
- Genetics & IVF Institute
  - PGD lab
  - Embryology lab
  - Clinical genetics staff and genetic counselors