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Analysis of spare embryos following PGD/PGS

Follow-up data and how it can help guide your laboratory practices

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Overview

- Why perform follow-up
- FISH-based follow-up
- PCR-based follow-up
- Practical experience with an “underperforming” test
- Confirmatory FISH-reducing false positives

Why should your lab perform follow-up?

- Follow-up of untransferred embryos following PGD can help the lab:
 - Catch mistakes made during testing that could lead to a misdiagnosis
 - Learn about false-positive and false-negative results and how often they occur in the lab
 - Learn about mosaicism in early embryos and how it affects diagnosis, especially in aneuploidy screening
 - Manage underperforming lab tests and show areas that need improvement
 - Determine an internal error rate which should be available to patients at initial consultation

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



FISH-based follow-up testing

- Follow-up of untransferred embryos donated by couple to “research”
 - Our protocol is to choose at least one embryo that...
 - Has continued to progress following embryo biopsy
 - Had a FISH result on day-3
 - Preferentially choose embryos that had a single abnormality
 - Will spread “normal” embryos if they are donated for research to look at false-negative rate

FISH-based follow-up testing

- Steps
 - Remove zona pelucida using pronase
 - When zona is almost fully digested, the embryo is rinsed in 5% FBS/dPBS then placed on slide within etched area
 - Embryo is placed in 12.5 ul of Tween 20/HCl
 - Drops of Tween/HCl are added while visualizing blastocyst to dissociate the cells and lyse the cell membranes
 - Slide is allowed to air dry
 - Slide is fixed in Formalin followed by dehydration in sequential ethanol
 - Cells are located and mapped prior to addition of FISH probes
 - Follow-up embryos are always probed with the same mix as the original PGD testing

FISH-based follow-up testing

- Concordance and non-concordance
 - Our definition is as follows
 - Concordant embryo scores are those where the embryo score on day-3 matches the embryo score on day-5 following whole embryo spreading followed by FISH
 - Our definition allows for “different” abnormalities between day-3 and day-5 to be concordant
 - We also note “perfect” concordance in our data collection when the day-3 and day-5 scores match exactly

FISH-based follow-up testing

- Reporting
 - We prepare a report for each case that is followed-up that describes the testing performed, and details the results from day-3 and day-5
 - This report is placed in the chart with the original case
 - Our goal is to follow-up at least one embryo from each case biopsied at our center
 - All embryos that are followed-up are collected in a database that keeps track of concordance/non-concordance, embryos with no results and detailed data comparing each embryo

PCR-based follow-up testing

- Follow-up of untransferred embryos donated by couple to “research”
 - Our protocol is to follow-up as many embryos as possible, but only embryos that...
 - Have continued to progress following embryo biopsy
 - Had a PCR result on day-3

PCR-based follow-up testing

- Steps
 - Remove zona pelucida using pronase
 - Place whole embryo into PCR tube containing 2.5 ul of lysis mix (NaOH/DTT)
 - Cover with 2 drops of oil
 - Place in hotblock at 65°C for 10 minutes
 - Place samples on ice until all are collected
 - Add MDA master mix or PCR mix
 - Amplify DNA with same primers used in original testing

PCR-based follow-up testing

- Concordance and non-concordance
 - Our definition is as follows
 - Concordant embryo scores are those where the embryo score on day-3 matches the embryo score on day-5
- Reporting
 - We prepare a report for each case that describes the testing performed, and details the results from day-3 and day-5
 - This report is placed in the chart with the original case
 - Our goal is to follow-up at least one embryo from each case biopsied at our center
 - All embryos that are followed-up are collected in a database that keeps track of concordance/non-concordance, embryos with no results and detailed data comparing each embryo



Practical experience with an “underperforming” test

- Original test developed and validated on lymphocytes and blastomeres
- Probe set included
 - CEP X (Xp11.1-q11.1, DXZ1)
 - CEP Y (Yq12, DYZ1)
 - 21q subtelomere (D21S1575)
- Test offered to patients to determine gender of embryos at PGD as well as screen for aneuploidy of chromosome 21

Practical experience with an “underperforming” test

- Follow-up of untransferred embryos

| | “Original” Test | Percentage |
|---------------------|------------------------|-------------------|
| Concordant | 7 | 50.0% |
| Discordant | 7 | 50.0% |
| Undetermined | 0 | |
| Total Spread | 14 | 100% |

test

2 cells from embryo on follow-up (day-5)

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Practical experience with an “underperforming” test

- “New” test developed and validated on lymphocytes and blastomeres
- Probe set included
 - CEP X (Xp11.1-q11.1, DXZ1)
 - CEP Y (Yq12, DYZ1)
 - LSI21 (D21S259, D21S341, D21S342)
- Test offered to patients to determine gender of embryos at PGD as well as screen for aneuploidy of chromosome 21

Practical experience with an “underperforming” test

- Follow-up of untransferred embryos

| | “New” Test | Percentage |
|---------------------|-------------------|-------------------|
| Concordant | 9 | 60.0% |
| Discordant | 6 | 40.0% |
| Undetermined | 3 | |
| Total Spread | 18 | 100% |

Practical experience with an “underperforming” test

- Original test was clearly “underperforming” with a large number of false-positive results
- The issue was clearly a split-signal problem
- Technologists suspected that the true result should have been “normal” but could not, with confidence, score the cells as “normal” for chromosome 21.
- Validated a new test with a different chromosome 21 probe
- Encountered the same problem clinically-test has been discontinued

Confirmatory FISH

- Any nucleus that contains a single aneuploidy (monosomy or trisomy) will be hybridized with a special second round mix which includes a different probe for the chromosome in question to either confirm or overturn the original score

Confirmatory FISH

- As a way to combat false-positive results, we are developing tests to “confirm” results of FISH-based tests before the final report is issued
- This will allow us to confirm single aneuploidies seen by FISH with a second probe that localizes to a different region of the chromosome prior to embryo transfer
- Should cut down on false-positive results and allow transfer of more “normal” embryos

Confirmatory FISH

- 8-probe Aneuploidy Screening
 - Vysis PB kit followed by home-brew X, Y, 15
 - Any probe in the first round can be “confirmed” in the second round
 - i.e. if we see monosomy 16 in the first round on a particular nucleus, the second round mix will be X, Y, 15, 16*
 - Each nucleus on a slide (we spread as many as 8 on a slide) can be probed with a different second round mix
 - i.e. position A was scored as monosomy 16 and will be probed with X, Y, 15, 16*, while position E was scored as trisomy 21 and will be probed with X, Y, 15, 21* at the same time

Confirmatory FISH

- 5-probe Aneuploidy Screening
 - Vysis PGT kit
 - Does not use a second hybridization routinely
 - Will add a second round hybridization so that any probe in the first round can be “confirmed” in the second round
 - i.e. if we see monosomy 18 in the first round on a particular nucleus, the second round mix will be 18*
 - Each nucleus on a slide (we spread as many as 8 on a slide) can be probed with a different second round mix
 - i.e. position A was scored as monosomy 18 and will be probed with 18*, while position E was scored as trisomy 21 and will be probed with 21* at the same time

Confirmatory FISH

- First worked out probe mixes and washing conditions using lymphocytes
- Continued development of conditions on day-3 blastomeres from embryos donated for research
 - Each blastomere located and scored for first round mix
 - First round probes stripped using our validated probe strip protocol
 - Second round hybridization with mix of X, Y, 15 and 1 of “confirmation” probes as listed previously
 - Embryos were of unknown aneuploidy status so we only scored for signal intensity of confirmation probe and other included probes

Confirmatory FISH

- Current status
 - Began offering confirmatory FISH in 2009 clinically
 - Continued follow-up of untransferred embryos that underwent confirmatory FISH analysis on day-5 or 6
 - All data from follow-up of these embryos is kept in a dedicated database to assess the results
 - Original cell score
 - Confirmatory cell score
 - Follow-up embryo score

Confirmatory FISH

- Current Status and the future
 - Currently used for 8-probe and 5-probe FISH panels
 - Has increased our concordance calls from 2009 compared to 2008 by approximately 10 % for both panels
 - We continue to database findings including cells that undergo CF and follow-up

Thank you...

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

