Emerging technologies in PGD

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Challenges of PGD

1. Sample sufficient genetic material from oocytes or early embryos without adversely affecting their viability and development
2. Perform rapid and reliable genetic diagnosis on single or very few cells

Embryo biopsy

- Three stages: polar body, cleavage, blastocyst
- Cleavage stage biopsy still most widely used approach
  - ~90% of cycles reported to ESHRE PGD Consortium
- Techniques have changed little in past 20 years
- All methods of embryo biopsy:
  - Are labour intensive
  - Require a high degree of skill
  - Can take months for even experienced staff to learn
  - Take a significant proportion of time available to perform genetic test

Lasers and optical tweezers

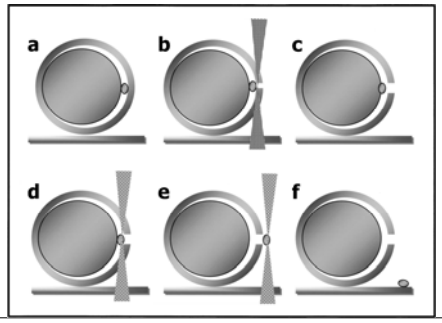
- Lasers now commonly used to open zona pellucida
- Optical tweezers used for
  - Cell sorting (Ashkin et al., 1987)
  - Sperm manipulation (Clement-Sengewald et al., 1996)

Polar body biopsy with optical tweezers

Clement-Sengewald et al.,2002

- Cutting laser
  - Breach zona pellucida
- Optical tweezers
  - Trap polar body
  - Drag polar body through zona pellucida
  - Polar body placed on polyethylene naphthalene membrane
- Laser pressure catapulting
  - Propel polar body into lid of PCR tube

Clement-Sengewald et al.,2002



Optical tweezers for PB biopsy

- Lasers are tangential to oocyte
- Micromanipulation not required
  - No micromanipulators
  - No glass pipettes
  - Minimal staff training
  - Rapid (~ 40 secs)
- Human contact minimised
  - Reduce contamination risk in PCR based cases

Laser tweezers for clinical embryo biopsy

- Can laser tweezers trap/manipulate blastomeres?
- Not tested clinically
  - Unknown impact on embryo development
- Approach holds promise but further research needed

Non-invasive genetic analysis?

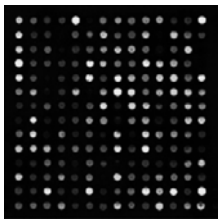
- Ideal approach would be to avoid cellular sampling of embryos
- 4D confocal fluorescence microscopy
  - Observation of individual bivalent chromosomes (Schuh and Ellenberg 2007)
- 3D tomography of single cells
  - Identification of cellular organelles (Choi et al., 2007)
- STED nanoscopy
  - High resolution imaging of interior of living cells (Hein et al., 2008)

Advances in genetic testing

- Many advances in single cell genetic testing since the advent of PGD
  - Expansion of available FISH probes and fluorochromes
  - mf-PCR
  - Rapid tests
  - Etc, etc
- Metaphase CGH
  - Full molecular karyotyping
  - Demonstrated value of analysing every chromosome
  - Identified existence of partial aneuploidy in embryos
  - Time-consuming, labour intensive
  - Requires embryo cryopreservation if done on cleavage stage embryos

Array-CGH

- Same principle as metaphase CGH
  - Template is solid support
  - Spotted with known short sequences of DNA
  - Sequences specific to different chromosomal regions
  - Chromosomal loss or gain identified by relative fluorescence ratio
  - Rapid analysis, easily automated



- Successfully applied to single cells

Potential future technologies

- "Lab on a chip"
  - Microfluidic PCR (see Zhang and Xing, 2007)
  - Small chips
    - Fast reaction times
    - Rapid heating and cooling times
  - Small reaction volumes
    - Use less reagents
  - Used to amplify multiple genes from single bacteria (Ottesen et al., 2006)
  - Can be coupled to analysis and information chips
    - Closed system
    - Minimise sample handling