Clinical Experience with Metaphase and Array CGH

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Introduction

- High incidence of chromosome abnormalities seen as most significant factor in the limiting of pregnancy rates in humans.
- Current pregnancy and implantation rates in IVF indicate traditional embryo selection methods, such as morphology, are insufficient.
- Additional information on oocyte and embryo competence, such as the chromosome complement, may act as an effective prognosticator.

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Preimplantation Genetic Screening

RATIONALE

- Greater than 50% first trimester pregnancy losses display degree of aneuploidy. The incidence of aneuploidy can be presumed to be even higher in embryos meaning a high rate of aneuploidy even in morphologically normal embryos.

STRATEGY

- Identification and transfer of only euploid embryos.

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Preimplantation Genetic Screening

DILEMAS

- What are we looking for and how much information do we really want?
- Where and when do we want to look for it?
- Is this information representative of what the embryo has the potential to be and do?
- Is this information improving embryo selection and increasing live birth rates?
- Which patients will benefit most?

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Is this information improving embryo selection and increasing live birth rates?

Axioms for increasing ART success rates

- Must increase pregnancy rate sufficiently to compensate for the procedure.
- Must be skilled in procedures ie. biopsy, extended culture, cryopreservation etc.
- Must be skilled in diagnostic accuracy.

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PGS at CARE Fertility, Nottingham

2003-2007

- **PGS via blastomere biopsy + FISH c'somes 13, 15, 16, 18, 21, 22, X & Y.**
  - Experienced and competent embryology and diagnostic team.
  - Successful SGD PGD program.
  - Poor pregnancy results.

2007

- **PGS via polar body or blastomere biopsy and metaphase CGH**
  - Clinical trial with pb biopsy cohort and blastomere biopsy cohort.
  - Very strict inclusion criteria.
  - Experienced and competent embryology and diagnostic team.
  - Required vitrification of blastocysts and significant wait for results.
  - Acceptable pregnancy results.

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Clinical trial – PB biopsy cohort

No. patients treated	18
No. patients with blastocysts frozen	15
No. blastocysts frozen	94
No. blastocysts from euploid pb	22 (24%)
No. blastocysts from aneuploid pb	46 (50%)
No. blastocysts from inconclusive pb	24 (26%)
No. failed tests	2
No. patients with euploid blastocysts for ET	8 (53%)
No. patients no euploid blastocysts for ET	7* (47%)

* Mixture of aneuploid and inconclusive

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Clinical trial – PB biopsy cohort

8 patients underwent ET of blastocysts with euploid pb results

- 14 blastocysts thawed
- 12 surviving
- 4 x SET, 4 x 2ET
- 4 x +ve βHCG
- 6 x FHS
- Clinical pregnancy rate 50%
- Implantation rate 50%

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Clinical trial – blastomere biopsy cohort

No. patients treated	9
No. patients with blastocysts frozen	8
No. blastocysts frozen	27
No. euploid blastocysts	5 (18%)
No. aneuploid blastocysts	18 (67%)
No. blastocysts with inconclusive results	4 (15%)
No. failed tests	0
No. patients with euploid blastocysts for ET	3 (38%)
No. patients no euploid blastocysts for ET	5* (62%)

* Mixture of aneuploid and inconclusive

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Clinical trial – blastomere biopsy cohort

3 patients had euploid embryos for transfer

- 5 blastocysts thawed
- 4 surviving
- 1 failed thaw
- 2 patients had 2ET
- 1 x +ve βHCG
- 0 FHS

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Reassessing the PGS Service

DILEMAS

- **What are we looking for and how much information do we really want?**
  - CGH allows analysis of whole chromosome complement, giving more complete diagnosis than FISH. However, still need to look at how much information we need on each c'some. What does resolution of test need to be to be able to make accurate diagnosis of whole c'some aneuploidy without revealing data we do not know the clinical significance of?

- **Where and when do we want to look for it?**

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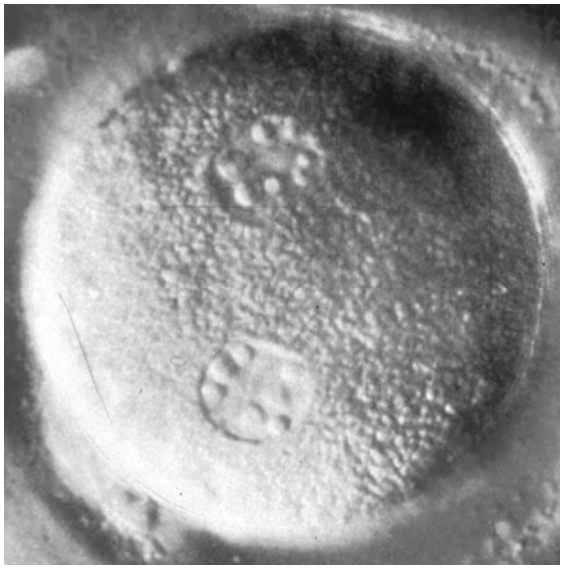
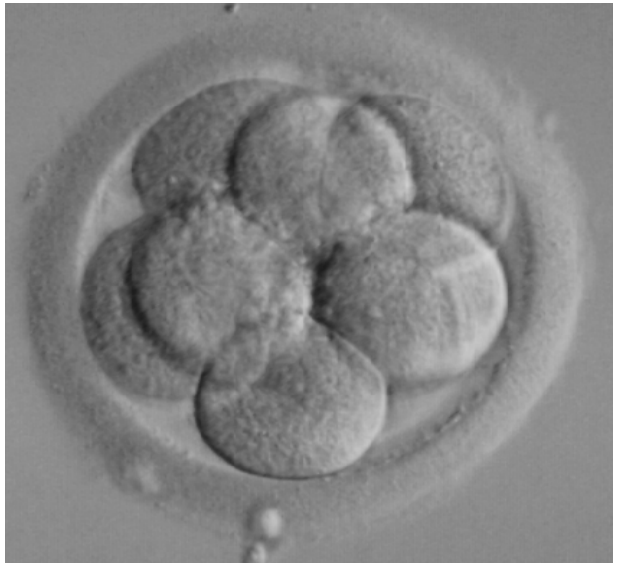
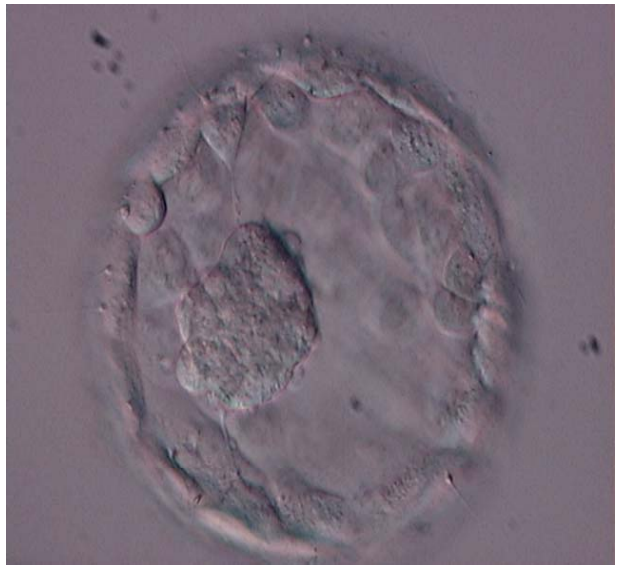
What to biopsy?



POLAR BODY



BLASTOMERE



TROPHECTODERM

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PGS at CARE Fertility, Nottingham

2008

- **Introduction of clinical array CGH service**
  - Experienced and competent embryology team with successful SGD PGD service
  - Skilled diagnostic team
  - 24-48hr results turnaround
  - No need for vitrification of embryos
  - Allows day 3 or day 5 ET

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Array CGH data

No. patients treated	27
No. polar bodies biopsied	235
No. polar bodies tested	194
No. euploid polar bodies	54 (28%)
No. aneuploid polar bodies	99 (51%)
No. failed tests	41 (21%)

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Patient demographic

- Age range 29-45yrs (mean age 40yrs)
- TTC 1-11yrs (mean 5yrs)
- 10 x immune issues identified
- 68 cycles (range 0-13)
- 8 x never conceived
- 46 pregnancy losses (range 1-9)
- Success rate estimated <5%

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Array CGH data

- 17/27 patients had euploid results.
  - 1 patient had 2PN freeze due to OHSS risk
  - 1 patient had oocytes vitrified after pb biopy
- 14 patients had embryo transfer of euploid embryos only
- 2 patients +ve βHCG
- 14% clinical pregnancy rate

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Preimplantation Genetic Screening

DILEMAS

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- **Is this information improving embryo selection and increasing pregnancy rates?**
- **Which patients will benefit most?**

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Is this information representative of what the embryo has the potential to be and do?

Polar body

- Can an aneuploid oocyte result in a euploid embryo?

Blastomere

- Mosaicism.

Trophectoderm

- Comparisons with ICM.
- Confined placental mosaicism.

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Is this information improving embryo selection and increasing pregnancy rates?

- Clinical trial results acceptable.
  - Implantation rate of 50% is much higher than conventional treatment and equivalent to SGD PGD results.
  - Results per cycle started will be poor due to high % of patients not reaching ET.
- Clinical pregnancy rate in array CGH patient population is increased beyond expected success rates in this demographic.
  - Still a poor prognosis group due to multiple indications for testing – not a magic bullet!

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Which patients will benefit most?

- Should we move beyond traditional indications to aid embryo selection for all patients and help moves to single embryo transfer?
  - HFEA dropping restrictions from next version of code of practice and have already done so for CARE Nottingham.

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Conclusions

- To our knowledge, this is the first clinical application of array CGH and polar body analysis.
- Metaphase CGH has been used clinically since 2001 but necessitated freezing of embryos while waiting for results
- CGH offers prospect of more complete diagnosis in terms of chromosome complement.
- As a molecular technique it negates the need for chromosome spreading and many common artefacts of traditional cytogenetic techniques.
- Array CGH allows for total automation of hybridization and analysis, reducing labour intensity and subjectivity.

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Conclusions

- Majority of embryo aneuploidies have been identified as originating from the oocyte, specifically female meiosis I.
- Analysis of the polar body as opposed to the blastomere is potentially a less invasive and less detrimental procedure.
- Negates diagnostic concerns regarding mosaicism.
- Will not identify mitotic errors or contribution of sperm.

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Conclusions

- Changing HFEA guidance in the UK allows wider application of such techniques.
- May aid moves towards single embryo transfer and help multiple birth minimisation.

- **We need to understand more about very early embryonic development and chromosome lineage to be sure we are looking for the right thing in the right place at the right time …**

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Thanks for listening

Thanks to

All the staff at CARE Fertility, Nottingham, and Bluegnome Ltd who were involved in development and validation and the introduction of array CGH as a clinical treatment.

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