Genetic aspects and implications in IVF

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The interface between medically assisted reproduction and genetics:
Technical, social, ethical and legal issues

Eur J Hum Genet. 2006, 14:588

Risks of ICSI

- Genetic cause of fertility problem
- Use of immature sperm
- Micromanipulation

Genetic abnormalities in ICSI patients

- Increase of Y-chromosome microdeletions
- Increase of CF mutations
- Increase of chromosome abnormalities

Chromosome abnormalities in ICSI patients

- Oligospermia
  - Abnormalities: 2 - 9 %
  - Mainly structural abnormalities
- Azoospermia
  - Abnormalities: 2 - 9 %
  - Mainly sex chromosomal abnormalities

Chromosomal abnormalities transmitted by ICSI (I)

- 1995, In 't Veld et al:
  extremely high incidence (33%) of sex-chromosome abnormalities
- 1995, Liebaers et al:
  much lower (1%) but still higher incidence than in newborns (0.19%)
- 1998, Bonduelle et al:
  increased incidence of structural abnormalities

Chromosomal abnormalities transmitted by ICSI (II)

- Significantly increased number of de novo chromosome abnormalities (1.6 % vs. 0.56 %)
- About 3-fold increase of sex chromosome abnormalities
- Also increase of structural autosomal abnormalities

Bonduelle et al., Hum Reprod. 2002, 17:2600

Genetic counseling in case of normal karyotype

- Offer of amniocentesis
- Offer of ultrasound examination

General outcome of ICSI pregnancies

General outcome in terms of neonatal outcome, malformations and health seem to be comparable between ICSI and IVF.

Bonduelle et al., Hum Reprod. 2003, 18:342

What about TESE?

- Significantly higher aneuploidy rate in sperm derived from TESE (testicular sperm extraction) (Palermo et al., 2002)
- High incidence of mosaicism in embryos derived from TESE (Silber et al., 2003)

PGD and PGS

- PGD: ART used for genetic reasons
- PGS: Form of genetic screening (aneuploidy screening) used to improve ART results

Reasons for aneuploidy screening

- Advanced maternal age (AMA)
- Failed IVF
- Recurrent abortion
- Previous aneuploid conception
- Male infertility
- Selection of best embryo for SET

PGS for AMA: raging war or rearguard action?

- Staessen et al., 2004; Mastenbroek et al., 2007: no benefit of PGS for AMA
- Criticisms:
  - Vs Staessen et al.: two cells biopsied, more embryos transferred in control group
  - Vs Mastenbroek et al.: large proportion of embryos without diagnosis, group with limited experience
- In favour:
  - Only RCTs available
  - In depth analysis of comparative studies does not show benefit either

Way forward?

- ESHRE will set up large multi-centre RCT for PGS for AMA
- Other indications may be identified - but RCT's needed!
- PGS can have a role as PGD to avoid trisomic pregnancies

Aspects of PGS: Biopsy

- Polar body, cleavage stage, blastocyst
- Method of zona drilling (acid tyrode, laser)
- Number of cells biopsied

Biopsy

Biopsy of two cells in stead of one will:

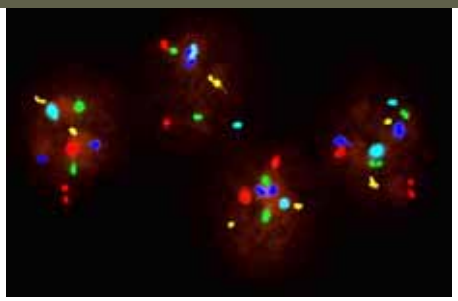
- Reduce false-negative rate and avoid more affected pregnancies
- Reduce embryos without diagnosis
- Increase false positive rate and exclude more normal embryos from transfer
- Not reduce implantation and pregnancy rates

Goossens et al., Hum Reprod, advance access

Which chromosomes?

- Increase implantation rate: 1, 5, 11 en 12
- Reduce abortion rate: 2, 7, 13, 15, 16, 18, 21, 22, X en Y
- Risk of live born trisomies: 13, 18 en 21

FISH result on blastomeres



Probes for chromosomes 13, 16, 18, 21 and 22: normal

FISH Efficiency

- Depends on probe
- Depends on number of probes applied
- Depends on biological material

FISH

Hybridisation efficiency

Lymfocytes	97 %
Amniocytes	94 %
Blastomeres	91%

Pehlivan et al., Reprod Biomed Online.;6:232

FISH

	amniocytes	blastomere(s)
Sensitivity	99.6	?
Specificity	99.9	?
Pos. pred. value	99.8	?
Neg. pred. value	99.8	?

Tepperberg et al., 2001

FISH

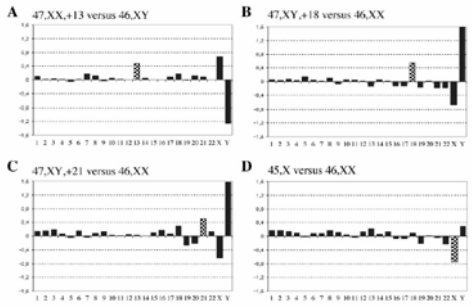
Increasing the number of FISH probes used will:

- Reduce false-negative rate and avoid more affected pregnancies
- Increase false positive rate and exclude more normal embryos from transfer

All chromosomes?

- Metaphase-CGH:
  - Work by Voullaire et al, Wells et al.
  - Too long for regular biopsy at day 3
- Array-CGH (aka molecular karyotyping)
  - Spits et al., 2006: elaboration of MDA
  - Le Caignec et al, 2006: proof of principle

Aneuploid single fibroblasts



Le Caignec et al., 2006, NAR

The influence of mosaicism

- Comparison of day 3 biopsy vs day 5 re-analysis in young women
- FISH for 10 chromosomes
- Total 2-cell biopsy: 121
- Normal: 43 (36 %)
- Aneuploid: 17 (14 %)
- Mosaic: 61 (50 %)
  - Abnormal/normal: 34 (28 %)
  - Abnormal/abnormal: 27 (22 %)

Baart et al.Hum Reprod, 2006, 21:223

Cost/effectiveness

- Costs: personnel, apparatus and probes etc.
- Effectiveness: more pregnancies?
- Effectiveness: less chromosomally abnormal children?

Embryo donation

Requires special attention, since it cannot be excluded that supernumerary embryos are at increased risk of carrying a known or unknown genetic defect due to parental infertility

Cryopreservation

- Cryopreserved oocytes exhibited serious disturbances of the microtubules immediately after thawing (Chen et al., 2003)
- Slightly higher incidence of chromosomally abnormal embryos after cryopreservation (Iwarsson et al., 1999)
- No apparent negative impact on children born (Wennerholm, 2000)

In vitro maturation of oocytes

- Risk for abnormalities due to imprinting errors
- Risk of chromosome abnormalities

Gamete retrieval prior to cancer treatment

- Risk of passing on mutated genes leading to cancer
- Possible hereditary nature of the cancer should be investigated
- PGD can be offered (eg BRCA1, FAP)

Conclusion

- Important interface between ART and medical genetics
- When in doubt: refer to clinical geneticist