european society of human reproduction & embryology

Organisation of the PGD laboratory and the need for accreditation

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UCL Centre for PGD

Summary

- Organisation of a PGD Centre
  - Different ways
  - The need for a successful IVF unit
  - Needs of the patient
  - Biopsy
  - Pretreatment workup
  - Clinical cycle
  - Misdiagnosis
- The need for accreditation
  - ISO
  - CPA

Setting up a PGD centre

- Two ways
- IVF centre and PGD centre in the same institute – preferred
- Transport PGD

Organisation of the PGD Centre

- Highly successful IVF unit
- Patients need genetic and specific PGD counselling
- Biopsy performed by trained embryologist
- Diagnosis performed by molecular biologist/cytogeneticist
- Accredited lab
- Patient information leaflets and consents
- Excellent communication between IVF centre and diagnosis lab
- Join the PGD Consortium

Successful IVF Unit

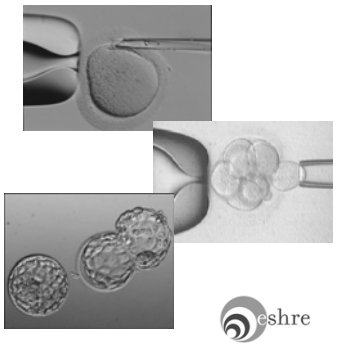
- No point doing PGD in an IVF unit with poor results
- Biopsy
- Selecting embryos on genetic and chromosomal status
- Morphology rarely taken into consideration

Evaluation of the patient

- Full report from genetics centre
- IVF and PGD specific information
- Standard IVF workup – confirm patients diagnosis
- Competent at embryo biopsy
- Suitable diagnostic workup
  - FISH
  - PCR

Biopsy

1. Polar Body
2. Cleavage Stage
3. Blastocyst



Pretreatment workup

- FISH
  - Sexing need to check for polymorphisms
  - Translocations protocols developed by cytogeneticist
  - For PGS – polymorphic sites
- PCR
  - Confirmation of mutation on proband and relatives
  - Suitable informative markers to detect contamination
  - Experienced molecular biologist

FISH

- Sexing for X linked disease
  - Ensure X and Y probes in first round
- Chromosome abnormalities
  - Suitable probes to detect all viable arrangements
  - 2 cells if only 1 informative probe
- PGS
  - 13, 14, 15, 16, 18, 21, 22, X and Y

FISH

- All probe combinations optimised on lymphocytes and patients DNA checked
- Metaphases (10) and interphase nuclei (100)
- Might need to also check on blastomeres
- Polymorphisms need to be known
- Who spreads the cells?
  - Different methods

PCR

- X-linked disease
  - Sexing only
  - Identification of mutation (specific diagnosis)
- Monogenic disorders
  - Monogenic
  - Triplet repeats
- HLA

PCR

- ICSI
- Tubing cells – who does it?
- Separate rooms for PCR and analysis
- Informative markers
- Contamination
- Allele dropout
- Workup including single cells
  - Heterozygous sample to check ADO (50 cells)
  - ADO <10%
  - Amplification efficiency > 90%
  - Assessing blanks

Clinical cycle

- Full consultation, information leaflets, relevant consents
- Need good number oocytes/embryos
- Patients must not have unprotected sex
- All cumulus cells removed (maternal contamination)
- ICSI for all molecular diagnosis (paternal contamination)
- Medium to support blastocyst growth
- Clear identification of biopsied cell and embryo number
- Ensure correct embryo transferred

Misdiagnosis

- Analysis of untransferred embryos
- Prenatal diagnosis
- Follow up of pregnant patients
- Follow up of babies born

- **The causes of misdiagnosis and adverse outcomes in PGD**
- Wilton, L, Thornhill, A, Traeger-Synodinos, J, Sermon, KD, Harper, JC
- Submitted to Human Reproduction

Possible causes of misdiagnosis

- **PCR**
  - Allele dropout
  - Contamination - sperm/cumulus/DNA/cells
  - Mosaicism
  - Transferring wrong embryo
  - Unprotected sex

- **FISH**
  - Contamination - cumulus
  - Mosaicism
  - Transferring wrong embryo
  - Unprotected sex

Cumulative data I-IX: PCR misdiagnosis

Monogenics		
Myotonic dystrophy type 1	PND	TOP
SMA	Post	Born
ß-thalassemia	PND	TOP
ß-thalassemia	PND	TOP
Familial amyloid polyneuropathy	PND	Born
Cystic fibrosis	PND	Born
Cystic fibrosis (1 of twins)	Post	Born
CMT1A	PND	Born
CMT1A (twins)	PND	TOP of both twins
Fragile X	PND	Born
Sexing for X-linked disease		
46,XY in retinitis pigmentosa	PND	Born
46,XY in Duchenne muscular dystrophy twin	PND	TOP of one twin

Cumulative data I-IX: FISH misdiagnosis (1)

Sexing for X linked disease		
45,XO Haemophilia A	PND	TOP
46,XY Haemophilia A	Post	Born
46,XY Retinitis Pigmentosa (twins)	Post	Born
Translocations		
T13 after 45,XY,der(13;14)(q10;q10)	Mis	Mis
47,XX,+der(22)t(11;22)(q23.3;q11.2)	PND	TOP
46,XY,der(15)t(13;15)(q25.1;q26.3)pat	PND	TOP

Cumulative data I-IX: FISH misdiagnosis (2)

PGS		
T16 after 1st PB biopsy only	Mis	Mis
T16 after 1st PB biopsy only	Mis	Mis
47,XX,+16	Mis	Mis
47,??,+16	Mis	Mis
47,XX,+21	Post	Born
47,XXX	PND	Lost to follow-up
46,XY/47,XY,+18	PND	TOP
47,XY,+21	PND	TOP
Social Sexing		
Requested male but female foetus	PND	TOP

Key points for biopsy/diagnosis lab

- Counselling
- Appropriately trained staff
- ISO/accreditation
- Aware of misdiagnosis possibilities
- Quality control
- Records

The need for accreditation

- Excellent quality management
- Clear lines of communication
- Clear lines of reporting
- Continued professional development

References from International Organiziation for Standarization

- ISO 15189:2007
  - Medical Laboratories
    - Particular requirements for quality and competence
- ISO/IEC 17025:2005
  - General requirements for the competence of testing and calibration labs
- ISO 9001:2000
  - Quality Management systems
    - Requirements
- ISO 9000:2005
  - Quality Management systems
    - Fundamentals and vocabulary
- ISO 22870:2006
  - Point of care testing (POCT)
    - Requirements for Quality and competence

Laboratory accreditation in the UK

- **Clinical Pathology Accreditation (UK) Ltd**
- Clear route for diagnostic laboratories
- ISO 15189:2007
- Nothing specific for PGD

Accreditation for diagnostic lab

- ISO 15189
- CPA - The standards
  - A. Organisation and quality management system
  - B. Personnel
  - C. Premises and environment
  - D. Equipment, information systems & materials
  - E. Pre examination process
  - F. Examination process
  - G. Post examination phase
  - H. Evaluation and quality assurance

A. Organisation and quality management system

- quality manager
- quality manual
- quality policy
- quality management system
- document control
- record control
- sample control

B. Personnel

- professional direction
- staff orientation and induction
- job title and description
- contracts, terms and conditions
- line management and accountability
- education and continual professional development
- records of absence, accidents, occupational health, disciplinary action
- staff meetings and annual reviews

C. Premises and environment

- facilities for staff
- patients
- storage
- health and safety

D. Equipment, information systems & materials

- procurement of equipment
- management of data and information
- all equipment needs to be regularly maintained
  - service contracts
  - data of regular care, etc.
- consumables
  - need to log batch numbers
  - date of order/arrival/use/location
- primers and probes
  - same as consumables

E. Pre examination process

- information for users and patients
- specimen collection
- handling
- transportation
- reception
- storage
- referral to other labs

F. Examination process

- selection and validation of examination procedures
- clinical relevance, purpose of exam, specimen requirements and means of identification
- equipment and special supplies
- reagents, standard or calibrantes and internal control materials
- instructions for performance of the examination
  - limitations of the examinations
  - recording and calculation of results
- internal quality control procedures and criteria against which examination processes are judged, reporting reference limits
- responsibilities of personnel in authorising, reporting and monitoring reports
- hazards and safety precautions
- assuring the quality of examinations

G. Post examination phase

- reporting results
- the report
- the telephoned report
- the amended report
- clinical advice and interpretation

H. Evaluation and quality assurance

- evaluation and improvement processes
- assessment of user satisfaction and complaints
- internal audit of quality management system
- internal audit of examination processes
- external quality assessment
- status of preventive, corrective and improvement actions

Quality assurance

- Staff need adequate training
- Ongoing assessment
- Explicit protocols

External quality assessment (EQA) FISH

- Working with
  - Cytogenetics European Quality Assessment scheme (CEQA)
- Ros Hastings, Alan Thornhill, Sjoerd Repping, Joyce Harper
  - Online registration closed
  - Stage 1 - Send in a case which gets assessed
  - Stage 2 – Test on various on line cases

EQA PCR

- Working with
  - UK National External Quality Assessment Scheme (UKNEQAS) for Molecular Genetics
- Zandra Deans, Sioban SenGupta, Martine De Rycke Francesco Fiorentino, Gary Harton, Céline Moutou, Pamela Renwick, David Robinson, Jan Traeger-Synodinos
  - Pilot study EQA for CF
  - Stage 1
    - DNA from 'parental cell lines' sent to labs
    - Labs test DNA and return feasibility report
  - Stage 2
    - Tubed single cells from 'offspring cell lines' sent to labs
    - Labs test and return diagnostic report

Pregnancy and baby follow up

- Retrospective study – Alison Lashwood
- Postal questionnaire for parents
- Part 1 – centre; complete basic details
- Part 2 – patients; reproductive history, pregnancy, birth
- Part 3 – patients; growth and development
- Part 4 – patients; history of the patients
- Prospective study?

Limitations of PGD

- Patients have to go through IVF
- Cost
- All embryos may be affected
- Making diagnosis from 1-2 cells
- Have been misdiagnosis
- Success rate lower than IVF

Guidelines on PGD

ESHRE PGD Consortium Best Practice Guidelines for Clinical Preimplantation Genetic Diagnosis (PGD) and Preimplantation Genetic screening (PGS) Human Reproduction, 20 (1):35-48.

The Preimplantation Genetic Diagnosis International Society (PGDIS) Guidelines for good practice in PGD: programme requirements and laboratory quality assurance, *Vol 16. No 1. 2008 134-147 Reproductive BioMedicine Online*

Consortium working groups

- Diagnostic laboratory accreditation: Chair Katerina Vesela
- Misdiagnosis: Chair Joanne Traeger-Synodinos
- Aneuploidy screening: Chair Sjoerd Repping
- Database: Chair Celine Moutou

Consortium training

- Biopsy and FISH workshop: Prague, 2006
- Basic genetics for ART practitioners:
  - Brussels, 2006
  - London, 2007
  - Athens, 2008
- Biopsy trouble shooting day: Lyon 2007

Interface between ART and Genetics

- Joint project between ESHRE and ESHG
- Document and guidelines on interface between ART and genetics:
  - Editorial: Kääriäinen, H., (2006), Eur. J. Hum. Genet, 14, 505
  - Document: Soini et al., (2006), Eur. J. Hum. Genet, 588-645
  - Recommendations: Eur. J. Hum. Genet, 2006, 14, 509-511
- On PGD, genetic screening in gamete donors, etc…

What makes a good PGD centre?

COMMUNICATION

Excellent IVF Platform

Excellent Diagnostics Laboratory

Integration of Services

Rigorous Quality Control/Quality Assurance

Commitment to Follow-up

Comprehensive Ethical Review

TRANSPORT PGD

Data VII – size of centres

	1 to 10 cycles	11 to 100 cycles	>100 cycles
N° of centres	6	30	3
N° of cycles	31	1327	2001
N° of pregnancies	5	296	491
% pregnancies	16 %	22 %	25 %

Consortium web site – www.eshre.com

- PGD consortium members and the treatments they offer
- Summary of our activities
- Recommended reading
- Publications of the consortium
- Guidelines
- Newsletters
- Training and education
- Cumulative tables of results
- PGD mail
- Patient support group
- Statutes
- How to become a member

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