Future perspectives for IVF laboratories

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Introduction

•The IVF laboratory is charged continuously to produce "better" oocytes and embryos and "better" procedures

So far, have we responded to these challenges

- **"Try something new"**

Have we learned something from such exercises?

- So far there appears to be no single "magic bullet" in the form of one specific "product" that is clearly superior to all others
- There seem to be a number of mutually interacting parameters

Introduction

Innovation → **The search for excellence – the best working system** ← Automation

→ Patients and IVF lab: Quality = successful outcome and affordable IVF

↑ Regulation/Legislation

Introduction

Infertility

- 10 - 15% of couples

→ Live style

→ Environment

Importance of ART

Introduction

ART

Clinicians – scientists – paramedical staff

Clinic – laboratory

The IVF laboratory is the "HART" of ART

Introduction

ART: situation

Success in ART:
The birth of a healthy baby (fresh and/or frozen)

Ovarian stimulation - Multiple pregnancies

The clinic:
IVM
Mild stimulation
Blastocyst transfer - SET

The IVF lab
SET – frozen embryos – sFRET
Freeze all - SFRET

AIM of the presentation

Three laboratory areas that warrant consideration and discussion

1. Optimizing embryo development in culture
2. Selecting the most viable/normal embryo for transfer
3. Optimizing cryopreservation procedures

AIM of the presentation

Future perspectives for IVF laboratories

- → **Knowing and understanding the past**
- → **Situate the present**
- → **Discuss future perspectives**

AIM of the presentation

Provide the IVF lab with the “best” technology available to:

- Produce “top”quality embryos
- Develop “top” methods to select “top” embryos
- Develop optimized cryopreservation procedures

  - → Efficiency (can it work)?
  - → Effectiveness (does it work)?
  - → Is it worth doing it?
  - → Compliant with EU directives, regulation and legislation?
  - → Technical challenges
  - → Costs?

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Optimizing embryo development in culture

Situation

- D2/3 ET < D5 ET?
- SET: Blastocyst transfer
  - → Better selection criteria
  - → Better synchronization between embryo and endometrium
- PGD – blastocyst transfer
- Consequence: in-vitro culture to the blastocyst stage
  - → Concern: long term culture in vitro and epigenetics
  - → Challenge: in-vitro cultured blastocysts = in-vivo cultured blastocyst
- Mimic the in-vivo environment!

Optimizing embryo development in culture

Past: the in-vitro culture environment and media (1)

- Suboptimal medium formulations and human serum supplementation in the eighties – ET D0, D1 and D2
- Grouped culture versus single embryo micro-drop culture
  - → No randomized trials
- Co-culture with feeder cell lines
  - → Kattal et al (2008) Role of co-culture in human IVF: a meta-analysis (Fertil Steril 90, 1O69 – 1076)
    - Systematic evidence-based review of randomized controlled trials

    Co-culture produces a significant improvement in blastomere number, implantation rates, and clinical and ongoing pregnancy rates

Optimizing embryo development in culture

Past: the in-vitro culture environment and media (2)

- Sequential culture = multiple step culture (Gardner et al, 1999)
  - → Back to nature principle and meeting the needs of the embryo
    - Complex
    - Commercially available
    - Expensive
    - Composition of the media not disclosed!
- Single step culture
  - → Let the embryo choose media formulations (Biggers et al, 2002)
    - Simple
    - Commercially available
    - Composition of the media not (always) disclosed!

Optimizing embryo development in culture

Future perspectives (1)

- One step versus multiple step culture?
  - → Sequential media use not absolute
  - → Do the randomized comparison
- The patient first
  - → Heterogeneity
  - → Evidence-based science and flexibility and creativity
    - The decision for the day of transfer should be made in the lab!!
  - → Epigenetics
  - → D5 ET in selected patients
- Novel incubators and Integrated cell culture observation
  - → Time lapse observations
  - → Stable physical conditions

Optimizing embryo development in culture

Future perspectives (2)

- In-vivo, gametes and embryos are exposed to the constricted "moist" environment of the female reproductive tract surrounded by oriented glycoproteins (mucins) (Smith G et al, 2008)
  - → Microfluidics (1)
    - Mimics more in-vivo environment
    - Technology utilizing characteristics of fluid movement in a micro- or nano-environment
    - Fluid movement is reliant on fluid density, viscosity, velocity and size/geometry of the microenvironment
    - Multiple streams of media through the same micro-channel

Optimizing embryo development in culture

→ Microfluidics (2)
- Cell proximity and spacing
- Stirred as opposed to still medium
- Adjust composition of media to nutritional requirements of embryos
- Minimal amounts of media
- "Lab on a chip" approach
- Infancy

Optimizing embryo development in culture

How can we maximize embryo development in-vitro? Conclusions
- Careful evaluation/controlled trials/evidence based medicine
- Costs involved? (for society/for the patients)
- Safety
- Plasticity
- Proficiency of staff
- Creativity and flexibility in the lab
- Continuous research
  - → Metabolism of the viable mammalian embryo: quietness?
  - → Developmental biology
  - → Molecular embryology
  - → Oviductal fluid dynamics and composition?

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Selecting the most viable/normal embryo for transfer

Situation

Morphological aspects

- Not all top quality embryos implant
- Bad quality embryos do implant
- Patients have a cohort of top, good, fair and bad quality oocytes and embryos available (heterogeneity)

and …

Invasive tests of embryo viability (PGS – Genomics - transciptomics)

Selecting the most viable/normal embryo for transfer

Situation

- We need to establish a more rigorous selection process for defining the quality of individual embryos so that the one we choose for transfer is more likely to be viable
- A limiting factor is that these measurements ideally need to be non-invasive and not time consuming

(Sakkas, 2008)

Selecting the most viable/normal embryo for transfer

Past: selecting the most viable/normal embryo for transfer (1)

- Embryo Morphological characteristics (Static observations)
  - → Fragmentation
  - → Developmental speed
  - → Early cleavage
  - → Zygote score
  - → Blastocysts
- Pre-implantation genetic screening (PGS)
  - → Added value to morphology?
  - → Mosaicism
  - → Invasive technique - Technical flaws

Selecting the most viable/normal embryo for transfer

HOW TO LOOK AT EMBRYOS IN THE NEAR FUTURE ?

- **SELECTION OF OOCYTES AND SPERM**
- **TIME LAPSE EMBRYO OBSERVATIONS**
  - → Developmental Dynamics
  - → "Super High technology" microscopy
    - "Robotization"
    - "Special" incubators

Selecting the most viable/normal embryo for transfer

HOW TO LOOK AT EMBRYOS IN THE NEAR FUTURE ?

- **Non-invasive tests of embryo viability**

"Ideal" criteria for an embryo test (Sturmey R, 2008)
- Non invasive
- Sensitive
  - → Distinguish between individual embryos
- Simple
- Objective
- Robust
- Consistent
- Reliable
- Provide diagnostic information additional to embryo morphology

Selecting the most viable/normal embryo for transfer

HOW TO LOOK AT EMBRYOS IN THE NEAR FUTURE ?

Non-invasive tests of embryo viability

- **SECRETION OF FACTORS INDICATIVE OF VIABILITY**
  - → **SECRETOME**
  - → **PROTEINS**
  - → **METABOLIC**
    - **Metabolomic profiling**
    - **Metabolomic profiling using microfluidics**

Selecting the most viable/normal embryo for transfer: some thoughts on proteomics!

Sturmey, 2007

- No method capable of identifying and quantifying complex protein mixtures in a simple, single step
- 2D approaches have consistently identified same proteins
  - → Limited dynamic range – *getting better!*
- Relationship between analyte abundance and measured signal is complex
  - → Quantification difficult
- To date only a very few reports where full proteomic approaches have been applied to the early embryo

No complete 'proteome' yet analysed and without a suitable reference point this may never be achieved: infancy

Selecting the most viable/normal embryo for transfer: some thoughts on the secretome!

- Search for markers secreted into the culture environment
- Attractive idea
- sHLA-G one example

Caution

- → Need to ensure that protein/factor is produced in measurable quantities
- → Needs to be easily measured
- → Needs to relate to developmental potential!

Selecting the most viable/normal embryo for transfer: some thoughts on metabolomics!

- How does metabolomic profiling compare to morphological selection: more – less – equally predictive ?
- What would be the relationship between metabolomic profile and embryo morphology ?
- How does the metabolomic profile of individual embryos within one cycle presents: heterogeneous (as with embryo morphology) ?
- The metabolic profile will depend on the sequential medium used ?
- How does the metabolic profile of a day 3 embryo relate to its development towards day 5 ?
- What to measure: one metabolite (limited) versus a whole spectrum (what does it represent) ?
- Do we look for high metabolism or rather a more quiet metabolism ?
- Practical use ? (validation) Cost for the patient ?
- Evidence based medicine?

Selecting the most viable/normal embryo for transfer: conclusion

- Careful evaluation/controlled trials/evidence based medicine
- Costs? (for society/for the patients)
- Feasibility?
- Proficiency of staff
- Creativity and flexibility in the lab
- Continuous research
  - → Metabolism of the viable mammalian embryo: quietness?
  - → Developmental biology
  - → Molecular embryology
  - → Comprehensive study of proteins and their function

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Optimizing cryopreservation procedures

Situation

Cryopreservation importance:
- Ovarian stimulation – freeze all
- Tool to reduce multiple pregnancies
- Fertility preservation
  - → Severe diseases
  - → "Social" reasons

Dilemma:
What is the best for the patient slow controlled-rate freezing or vitrification?

Optimizing cryopreservation procedures

Past: cryopreservation procedures (1)

- Slow controlled rate freezing
  - → Equilibrium freezing with DMSO (Trounson et al, 1983)
    - For advanced cleavage stage embryos only?
  - → Quasi-equilibrium freezing with PG-S (Lassalle et al, 1985)
    - For early cleavage stage embryos of good-quality and zygotes only?
  - → Quasi-equilibrium freezing with Glyc-S (Cohen et al 1985)
    - For blastocysts only
- Ultra rapid freezing
  - → Ultra-rapid freezing a new low cost and efficient procedure for the cryopreservation of human embryos (Trounson et al, 1987)?

Optimizing cryopreservation procedures

Future perspectives

Vitrification of oocytes and embryos: the next breakthrough in IVF?

Optimizing cryopreservation procedures

Stated advantages of vitrification:

Vitrification is a new, simple, low cost, safe and efficient procedure for the cryopreservation of oocytes, embryos and blastocysts (*Kuwayama et al (2005, 2007), Al - Hasani et al (2007), Mukaida et al (2007), Liebermann et al (2007)*

No ice crystals – Flexibility

Questions:

- Is vitrification an efficient procedure (can it work)?
  - Recent published data of the vitrification of human embryos and blastocysts indicate that vitrification "apparently" works and produces even "better" results than conventional freezing

Optimizing cryopreservation procedures

Questions:

- Is vitrification a simple procedure?
- Is vitrification a low cost procedure?
- Is vitrification a safe procedure?

Optimizing cryopreservation procedures:

Vitrification: future aspects

→ There is a need for more robust and safe protocols

- Very low CPA (< 1M)
- Injection of non permeating sugars
- Special devices to obtain super high cooling rates

- Molecular cryobiology
- Nanotechnology

Thoughts on vitrification

Vitrification as clear as a glass?

→ Vitrification can work

→ Safety issues unanswered

→ Technical challenges

- Are we really vitrifying?

→ Costs

Optimizing cryopreservation procedures: conclusions

Vitrification: the next breakthrough in ART?

→ Vitrification has a future

→ No randomized controlled trials (children)

→ No conclusive data on health of children

→ The overall success rate (extensive literature survey) (FHB/embryo warmed) is 5-10% for oocytes, 11.2% for human zygotes, 17.2% for D3 embryos and 18.3% for blastocysts, and these results are lower than their unfrozen counterparts (Blake et al, 2007)

→ Effectiveness?

→ Vitrification is still a research procedure?

The fourth element

Three laboratory areas that warrant consideration and discussion

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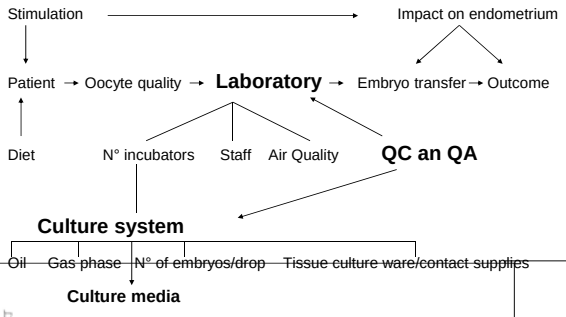
3. Optimizing cryopreservation procedures

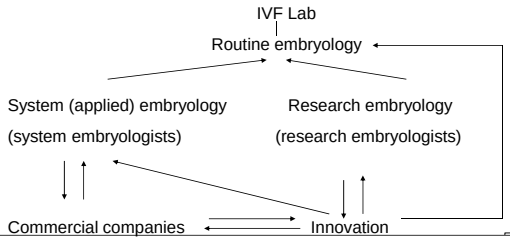
4. Holistic approach of IVF (Gardner, 2008)

Holistic approach of IVF



Holistic approach of IVF

Future perspectives for human IVF laboratories



Holistic approach of IVF: some thoughts

QC/QA – directives – regulation

Benefits

→ Safety issues

→ Traceability

Thoughts

→ Costs

→ Quality garanteed? ART success rates garanteed?

→ Creativity and flexibility!

Inform correctly regulators and discuss with them!

General conclusions

Future perspectives for human IVF laboratories

→ A plea for an evidence-based approach – prospective randomized controlled trials

→ Affordable IVF

→ Competent, trained and certified staff

→ Research to maintain and increase efficiency and effectiveness

→ Relation with commercial companies

→ Let us speak the same embryology language worldwide!!