State of the art on

Practical aspects of non invasive selection of gametes, embryos and blastocysts in a modern IVF laboratory

Main conclusions

I- Andrology

DNA fragmentation

- Is higher in infertile compared to fertile patients
- To implement DNA fragmentation test each laboratory should establish first a) which test to use, and b) the cut-off index of normality
- No indication for routine use in male evaluation
- Unclear correlation with embryo morphological quality

ICSI

- Is an efficient non-physiological technique that cannot be made more physiological

IMSI

- There is no evidence supporting the advantage of its use
- More attention should be paid to sperm morphology (sperm selection at at least 40X objective)

II- Before expression of the embryonic genome

Oocyte morphology:

- The presence of blood clots in cumulus cells predicts poor quality oocytes
- Several characteristics of dysmorphism are associated with a decrease in oocyte viability – it is the decision of the laboratory how to use them
- Insemination of giant oocytes should never be performed
- Insemination of oocytes with aggregation of smooth endoplasmic reticulum should be avoided

Pronuclear morphology

- Scoring of PN number must be done
- PN location and morphology change with time ➔ time of observation, time-lapse

Early cleavage

- It is predictive of embryo implantation
- It occurs earlier in ICSI than in IVF
- ➔ time of observation, time-lapse, dependent on culture media?

III- After expression of the embryonic genome

Cleavage

- Many (static) data available
- New (continuous) information is coming from time-lapse studies
- Clinical relevance should be validated by RCTs
- Developmental kinetics dependent on culture media?

Morula stage

- Only a few data available
- D4 at least as good as D3 and Day 4 similar to Day 5 transfer

Blastocysts

- Scoring human blastocysts is difficult
- Definition of an optimal blastocyst considers both ICM and TE (Istanbul Consensus Meeting)
- Some variants have an unknown / doubtful significance (strings, cellular or extracellular structures in PVS or in the blastocoele)

IV- Cryopreservation and Polarization

Cryopreservation

- Oocyte / embryo cryopreservation: there is a correlation between pre-cryo morphology and post-cryo results
- Intact embryos after thawing/warming (100% survival) perform better
- Vitrification performs better, at least for oocytes and blastocysts
- To decide the application of slow-freezing or vitrification; several aspects needs to be taken into consideration, including costs and practical aspects

Polarization

- The study of birefringence is relevant for research purposes
- The birefringence associated with the meiotic spindle is an unclear marker of oocyte quality, however it is an important research tool
- The birefringence associated with the ZP is a good marker of oocyte quality
- Relevance for embryo stage selection not known

V- <u>Metabolomics</u>

Glucose

- Metabolic studies valuable for defining improved culture media and as a selection tool
- Glycolytic activity is stable through blastocyst development
- Blastocysts leading to pregnancy have a higher glucose-uptake
- Measurements of glycolytic activity is cumbersome and time consuming, with low predictive power

Amino acids

- Difference in AA turnover can be seen for blastocyst vs. non blastocyst producing embryos, for gender, for maternal ages
- “Quiet” metabolism „better“ than high metabolism
- DNA damage levels in blastocysts correlate to high AA turnover, but not to embryo morphology
- Technical issues remain, clinical trials needed

Oxygen consumption

- Oocyte oxygen consumption differs: per patient, within each patient, per female age, with environmental conditions
- Embryo oxygen consumption correlates with embryo quality and time of cleavage
- Valuable for assessing metabolism
- Predictive power for earlier stages uncertain

- Technical issues remain regarding the probe measuring oxygen

Near infrared spectroscopy

- Initial studies showed correlation of a „metabolic pattern“ to embryo viability and
- Higher viability score correlated (retrospectively) to implantation
- Transferred to an algorithm, used for prediction in a randomised controlled trial
- Randomised trial; improved results for NIR group on day 2, but better results for controls on day 5 (no difference overall)
- Variability issues amongst instruments