# Non-invasive metabolomic profiling using near infrared spectroscopy

Thorir Hardarson

FERTILITETSCENTRUM, GÖTEBORG

# Potential conflict of interest

Fertilitetscentrum has collaborated with Molecular Biometrics for several years

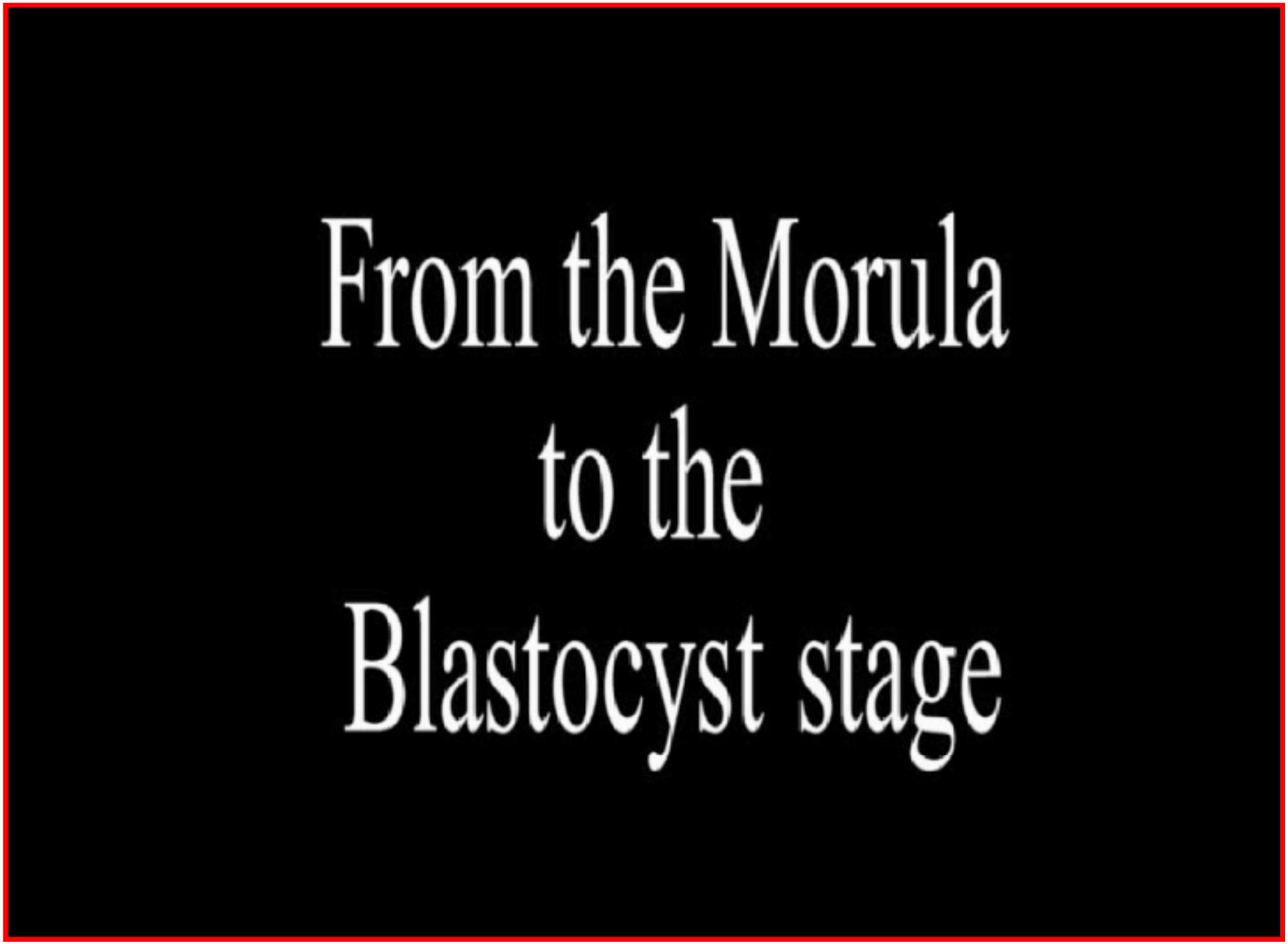
No commercial interest

# Content of the lecture

Background to metabolomics

Brief overview of retrospective studies

RCT at Fertilitetscentrum


From the Morula
to the
Blastocyst stage

# Selecting the most viable embryo

Morphology alone is not enough

Better techniques needed to select the optimal embryo

# PGS

# Prospective randomized controlled studies

Human Reproduction Vol.19, No.12 pp. 2849–2858, 2004
Advance Access publication October 7, 2004
doi:10.1093/humrep/deh536

## Comparison of blastocyst transfer with or witho preimplantation genetic diagnosis for aneuploid in couples with advanced maternal age: a prosp randomized controlled trial

Catherine Staessen[1,3], Peter Platteau[1], Elvire Van Assche[2], An Michiels[2], Tournaye[1], Michel Camus[1], Paul Devroey[1], Inge Liebaers[2] and André Va

[1]Centre for Reproductive Medicine and [2]Centre for Medical Genetics, University Hospital, Dutch-speaking (Vrije Universiteit Brussel), Laarbeeklaan 101, B-1090 Brussels, Belgium

[3]To whom correspondence should be addressed. E-mail: catherine.staessen@az.vub.ac.be

**BACKGROUND: It is generally accepted that the age-related increased aneuploidy rate is implantation and a higher abortion rate. Therefore, advanced maternal age (AMA) co group to assess the possible benefit of preimplantation genetic diagnosis for aneuploidy scr outcome after assisted reproductive technology (ART). METHODS: A prospective rando trial (RCT) was carried out comparing the outcome after blastocyst transfer combined w rescence *in situ* hybridization (FISH) for the chromosomes X, Y, 13, 16, 18, 21 and 22 in A years) with a control group without PGD-AS. From the 400 (200 for PGD-AS and 200 co allocated to the trial, an oocyte pick-up was performed effectively in 289 cycles (148 control cycles). RESULTS: Positive serum HCG rates per transfer and per cycle were the controls: 35.8% (19.6%) [%/per embryo transfer (per cycle)] and 32.2% (27.7%), respect fewer embryos were transferred in the PGD-AS group than in the control group ($P < 0$ rate (with fetal heart beat) was 17.1% in the PGD-AS group versus 11.5% in the contro $P = 0.09$). We observed a normal diploid status in 36.8% of the embryos. CONCLUSIONS arguments in favour of PGD-AS for improving clinical outcome per initiated cycle in p there are no restrictions in the number of embryos to be transferred.**

*Key words*: age/aneuploidy screening/FSH/preimplantation genetic diagnosis/RCT

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JULY 5, 2007 VOL. 357 NO. 1

## In Vitro Fertilization with Preimplantation Genetic Screening

Sebastiaan Mastenbroek, M.Sc., Moniek Twisk, M.D., Jannie van Echten-Arends, Ph.D., Birgit Sikkema-Raddatz, Ph.D., Johanna C. Korevaar, Ph.D., Harold R. Verhoeve, M.D., Niels E.A. Vogel, M.D., Eus G.J.M. Arts, Ph.D., Jan W.A. de Vries, Ph.D., Patrick M. Bossuyt, Ph.D., Charles H.C.M. Buys, Ph.D., Maas Jan Heineman, M.D., Ph.D., Sjoerd Repping, Ph.D., and Fulco van der Veen, M.D., Ph.D.

ABSTRACT

## Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial

T. Hardarson[1,3], C. Hanson[2], K. Lundin[2], T. Hillensjö[1], L. Nilsson[2], J. Stevic[2], E. Reismer[1], K. Borg[1], M. Wikland[1] and C. Bergh[2]

[1]*Fertility Centre Scandinavia, Carlanderska Hospital, Box 5418, 402 29 Göteborg, Sweden;* [2]*Department of Reproductive Medicine, Sahlgrenska University Hospital, 413 45 Göteborg, Sweden*

[3]Correspondence address. E-mail: thorir.hardarson@fcivf.com

**BACKGROUND: Advanced maternal age (AMA) is an important parameter that negatively influences the clinical pregnancy rate in IVF, in particular owing to the increased embryo aneuploidy rate. It has thus been suggested that only transferring euploid embryos in this patient group would improve the pregnancy rate. The purpose of this study was to test whether employing preimplantation genetic screening (PGS) in AMA patients would increase the clinical pregnancy rate. METHODS: We conducted a two-center, randomized controlled trial (RCT) to analyze the outcome of embryo transfers in AMA patients (≥38 years of age) after PGS using FISH analysis for chromosomes X, Y, 13, 16, 18, 21 and 22. The PGS group was compared with a control group. The primary outcome measure was clinical pregnancy rate after 6–7 weeks of gestation per randomized patient. RESULTS: The study was terminated early as an interim analysis showed a very low conditional power of superiority for the primary outcome. Of the 320 patients calculated to be included in the study, 56 and 53 patients were randomized into the PGS and control groups, respectively. The clinical pregnancy rate in the PGS group was 8.9% (95% CI, 2.9–19.6%) compared with 24.5% (95% CI, 13.8–38.3%) in the control group, giving a difference of 15.6% (95% CI, 1.8–29.4%, $P = 0.039$). CONCLUSIONS: Although the study was terminated early, this RCT study provides evidence against the use of PGS for AMA patients when performing IVF. Trial registration number: ISRCTN38014610.**

*Keywords:* AMA; PGS; embryo biopsy; RCT; IVF

# New non-invasive methods

Time-lapse?

Genomics?

Proteomics?

Metabolomics?

# Non-invasive studies of human embryo viability

## *Pyruvate, lactate and glucose metabolism*

**Table I.** Pyruvate, lactate and glucose metabolism as a predictor of embryo development and viability—human studies.

| Study | Embryo stage examined | Altered metabolite associated with improved outcome | Technology used | Outcome |
|---|---|---|---|---|
| Hardy et al. 1989 | Day 2-4 | ↑ pyruvate uptake<br>No association with glucose uptake | Ultramicrofluorescence assay | Blastocyst development |
| | Day 5 | ↑ pyruvate uptake<br>↑ glucose uptake | Ultramicrofluorescence assay | Blastocyst development |
| Gott et al. 1990 | Day 2-4 | ↑ pyruvate uptake<br>↑ lactate production<br>No association with glucose uptake | Ultramicrofluorescence assay | Blastocyst development |
| | Day 5 | ↑ pyruvate uptake<br>↑ glucose uptake<br>↑ lactate production | Ultramicrofluorescence assay | Blastocyst development |
| Conaghan et al., 1993 | Day 2 – 3 | ↓ pyruvate uptake | Ultramicrofluorescence assay | Clinical pregnancy |
| Turner et al., 1994 | Day 2 | Intermediate pyruvate uptake | Ultramicrofluorescence assay | Clinical pregnancy |
| Gardner et al., 2001 | Day 4 | ↑ pyruvate uptake<br>↑ glucose uptake | Ultramicrofluorescence assay | Blastocyst development |
| Seli et al. | Day 2-3 | A trend toward<br>↑ pyruvate uptake<br>↑ glucose uptake | Proton NMR | Pregnancy and delivery |



From Botros *et al.* 2008

# Non-invasive studies of human embryo viability

## *Amino acid uptake and secretion*

**Table II.** Amino acid uptake and secretion by the embryo as a predictor of embryo development viability—human studies.

| Study | Embryo stage examined | | Altered metabolite associated with outcome | Technology used | Outcome |
|---|---|---|---|---|---|
| Houghton et al., 2002 | Day 2 – 3 | ↓<br>↓<br>↓ | amino acid turnover (sum of depletion and appearance)<br>glutamine, arginine, methionine uptake<br>alanine and asparagine release | HPLC | Blastocyst development |
| | 8 cell-Morula | ↓<br>↓<br>↓ | amino acid turnover (sum of depletion and appearance)<br>serine uptake<br>alanine and glycine release | HPLC | Blastocyst development |
| Brison et al., 2004 | Day 2 | ↓<br>↑ | glycine and leucine in culture media<br>asparagine levels in culture media | HPLC | Clinical pregnancy and live birth |
| Seli et al. 2008 | Day 3 | ↑ | glutamate levels in culture media | Proton NMR | Clinical pregnancy and live birth |

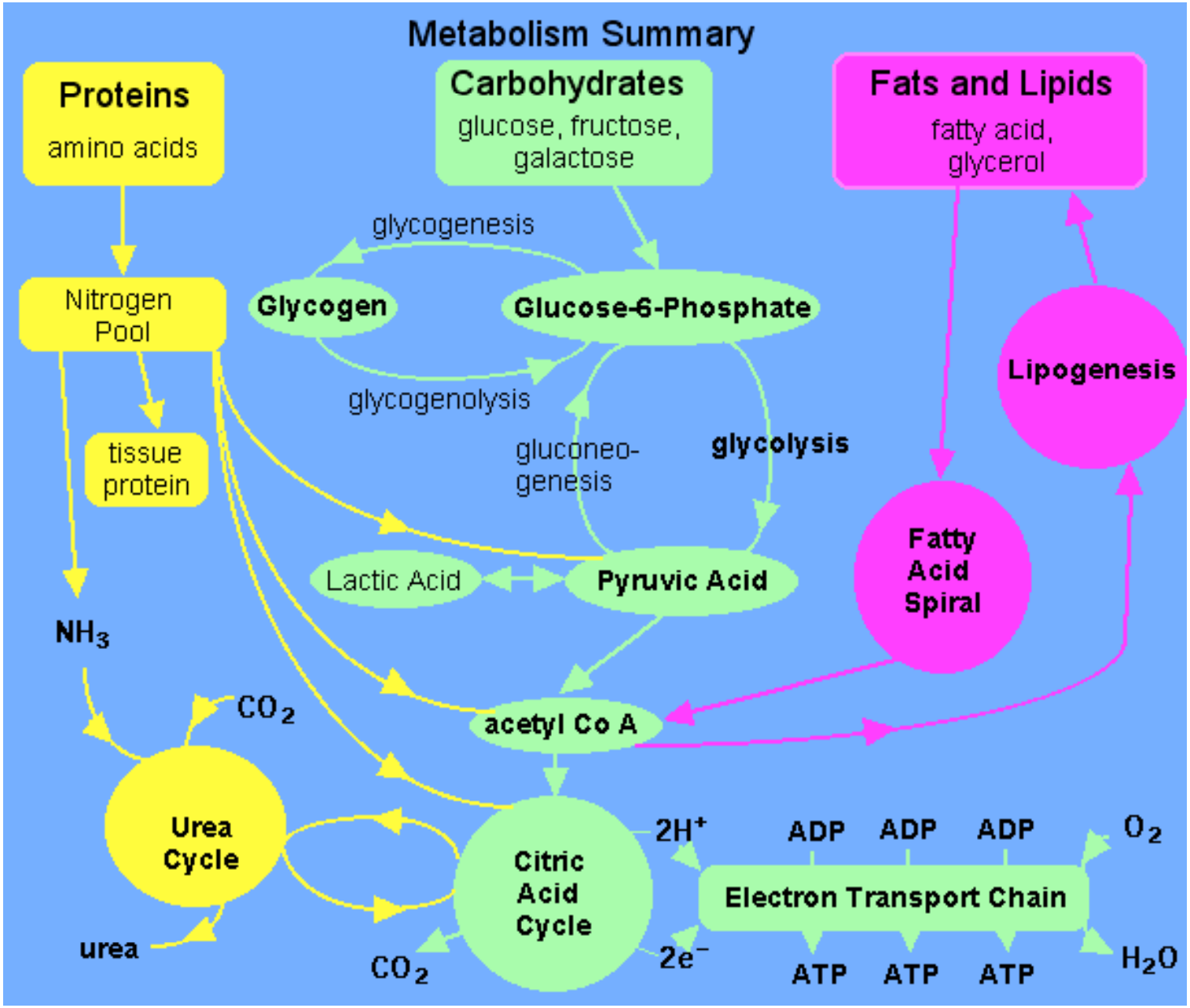


From Botros *et al.* 2008

# Metabolomics studies so far….

**Table IV.** Studies of non-invasive metabolomic profiling of embryo culture media to assess embryo viability in IVF.

| Study | Study design | *n* | Day of transfer | Number of embryos transferred | Analytical technique | Center | Findings |
|---|---|---|---|---|---|---|---|
| Seli *et al.* (2007) | Algorithm development | 36 | Day 3 | MET | Raman | YFC | A |
| Scott *et al.* (2008) | Blinded analysis | 41 | Day 3 and 5 | MET | Raman | RMANJ | B |
| Seli *et al.* (2007) | Algorithm development | 33 | Day 3 | MET | NIR | RMANJ | A |
| Seli *et al.* (2007) | Blinded analysis | 16 | Day 3 | MET | NIR | YFC | B |
| Seli *et al.* (2008b) | Algorithm development | 121 | Day 2 | SET | NIR | KLC | A, C |
| Seli *et al.* (2008b) | Blinded analysis | 60 | Day 2 | SET | NIR | KLC | B, D |
| Vergouw *et al.* (2008) | Algorithm development | 29 | Day 2 | SET | NIR | VUMC | A, C |
| Vergouw *et al.* (2008) Seli *et al.* (2008b) | Algorithm development | 304 | Day 3 | SET | NIR | VUMC | A, C |
| Hardarson *et al.* (2008) | Algorithm development | 137 | Day 5 | SET | NIR | FCG, SG | A, C, D |


Metabolism Summary
Proteins
amino acids
Carbohydrates
glucose, fructose, galactose
Fats and Lipids
fatty acid, glycerol
glycogenesis
Nitrogen Pool
Glycogen
Glucose-6-Phosphate
Lipogenesis
glycogenolysis
tissue protein
gluconeo-genesis
glycolysis
Fatty Acid Spiral
Lactic Acid
Pyruvic Acid
NH3
CO2
acetyl Co A
Urea Cycle
Citric Acid Cycle
2H+
ADP ADP ADP
O2
Electron Transport Chain
urea
CO2
2e−
ATP ATP ATP
H2O

# Metabolomics

## WHY?

MB (James Posillico) – 4 years ago
New device – fast – promising

SET – spent media
Collaboration

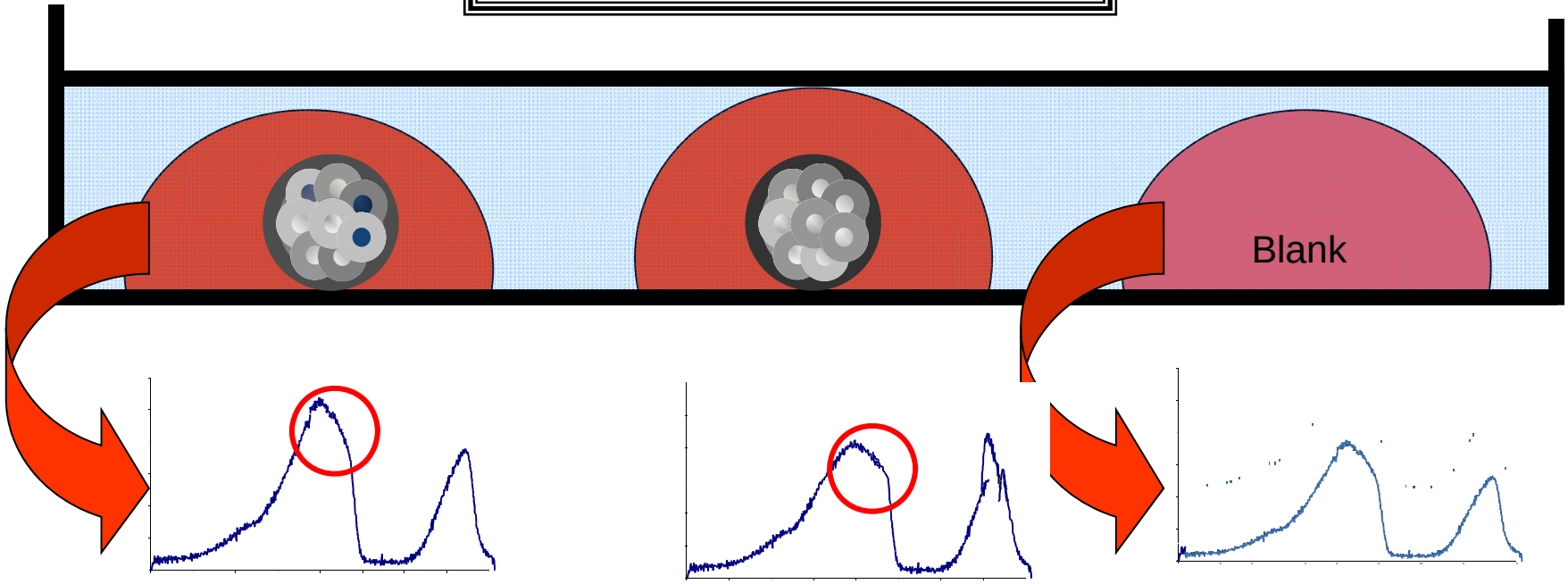
# Metabolomics
# ViaMetrics-E™ Methodology

- Clinically
  - How the embryo modifies its environment.
- Thesis
  - A viable embryo has a different metabolome than a non-viable embryo and this can be assessed by sampling the culture media.
- Biologically
  - Changes in concentrations of:

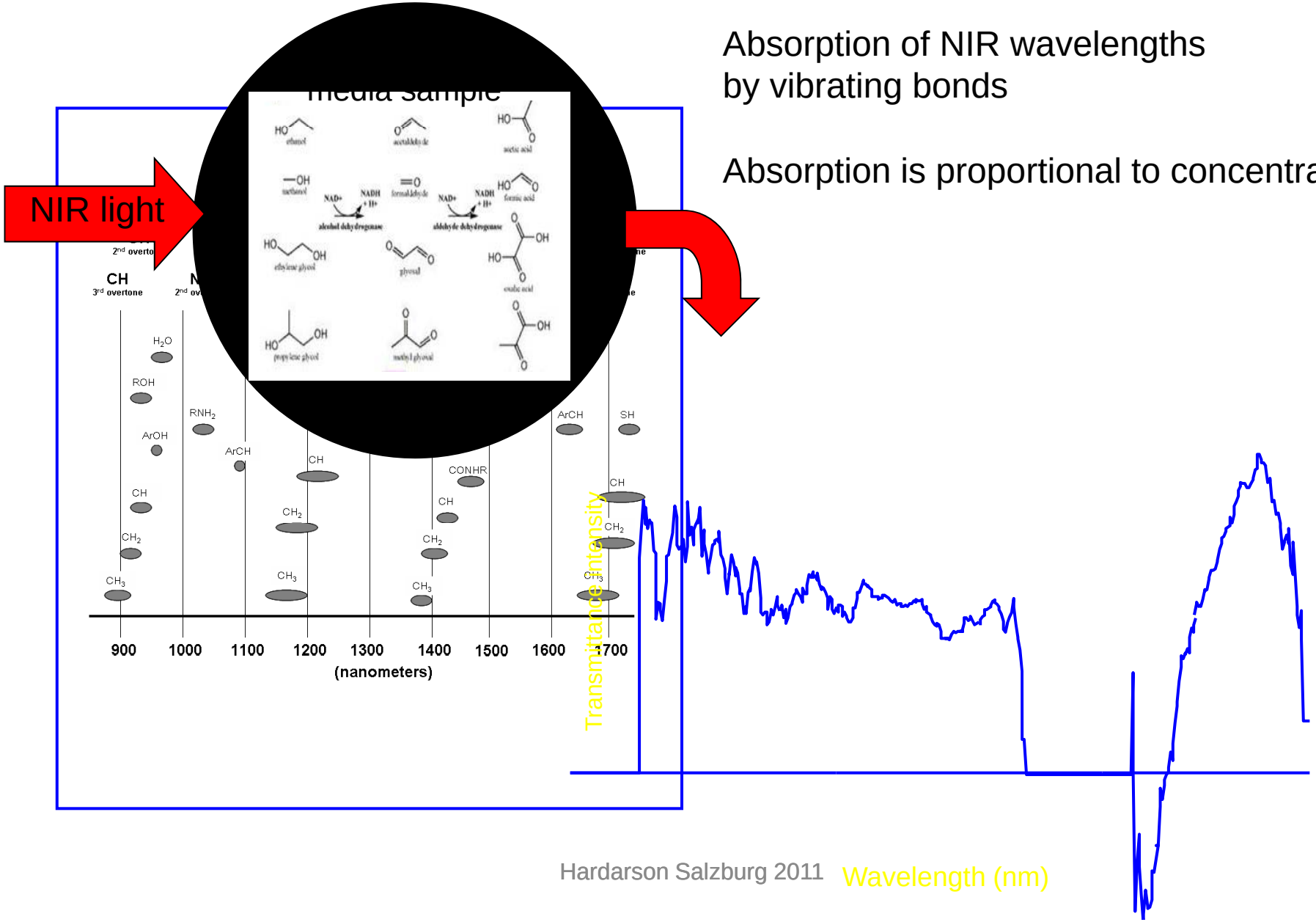
| Functional Groups | Constituents |
|---|---|
| •CH | •Albumin |
| •NH | •Lactate |
| •OH | •Pyruvate |
| •SH | •Glutamate |
| •C=C | •Glucose |

# ViaMetrics-E™ Methodology

**Step 1: Single embryo culture**



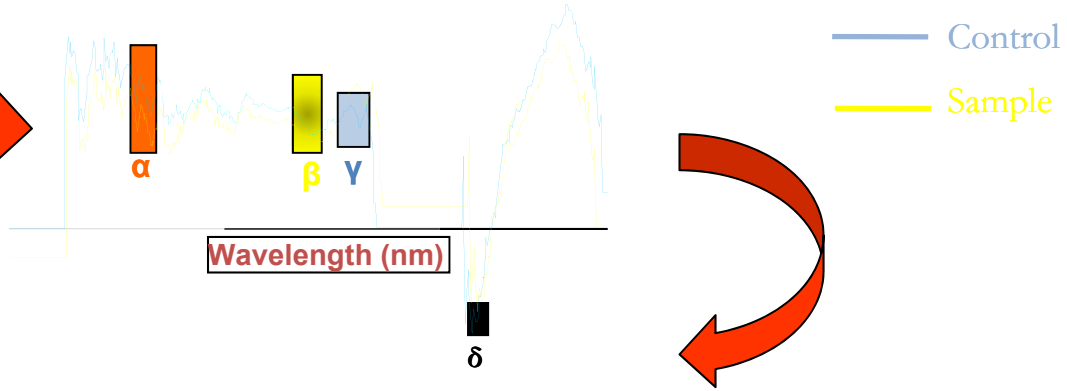

**Step 2: NIR Spectral analysis of media sample ratio against the blank**




Absorption of NIR wavelengths by vibrating bonds

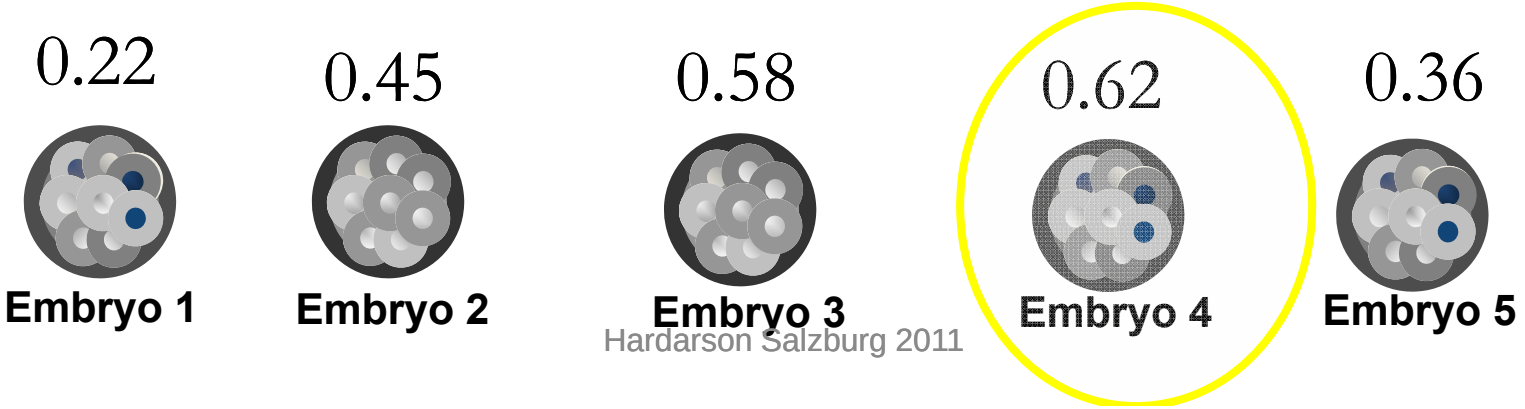
Absorption is proportional to concentration

## Step 3: Calculate score for each embryo




**ViaMetrics-E Score =**
**$\alpha(W\alpha) + \beta(W\beta) + \gamma(W\gamma) + \delta(W\delta)$**

## Step 4: Transfer embryo with highest Viability score



# We have previously shown…

ASRM 2007 – Hardarson *et al*. 2007 (*Fertil. Steril.* ***88****, S 307-308*)

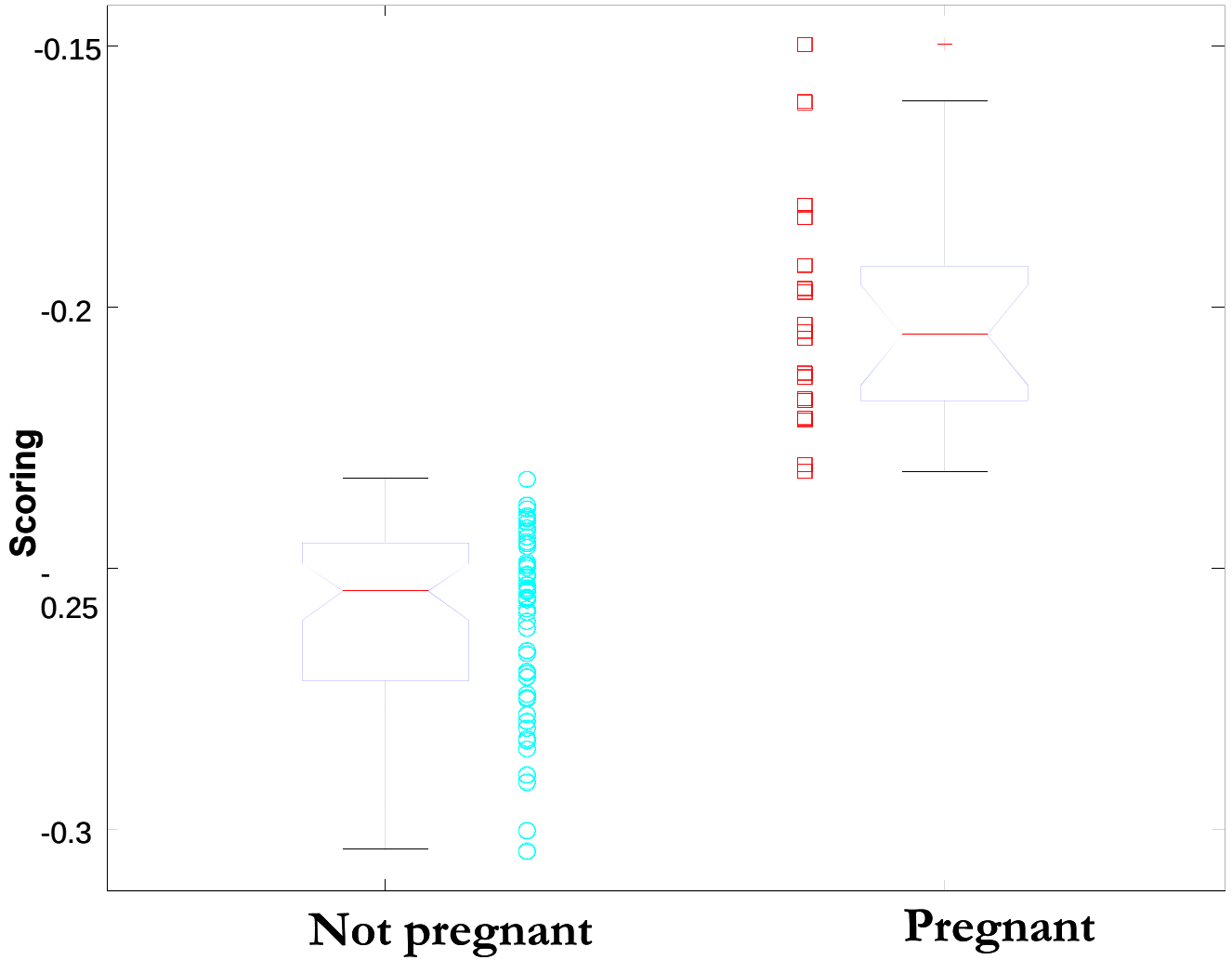
NIR predicts outcome of day 2 embryos

ASRM 2008 - Hardarson *et al*. 2007 (*Fertil. Steril.* ***90****, S 77*)

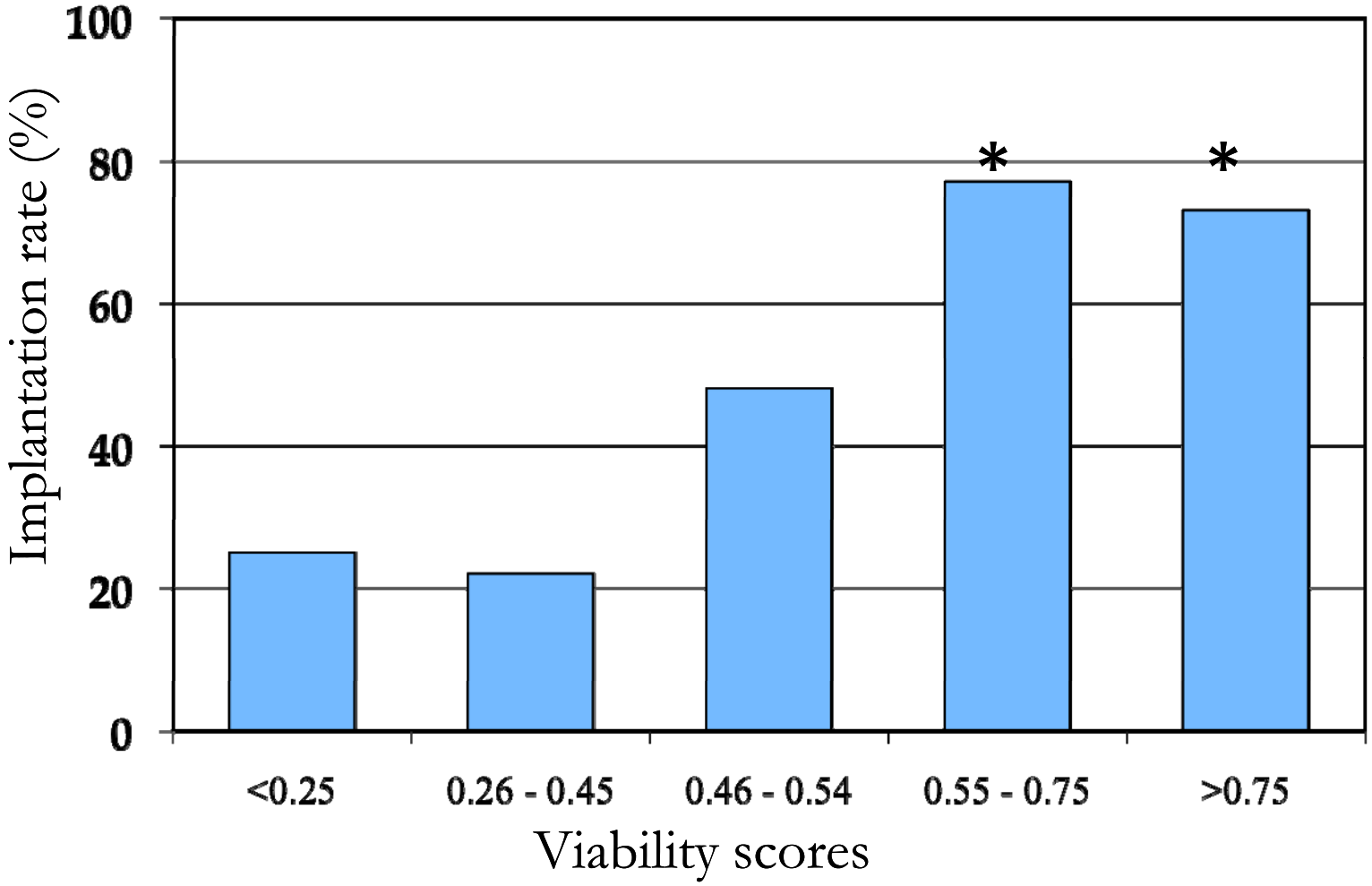
NIR predicts the outcome of day 5 embryos

# Viability score correlated to implantation rate

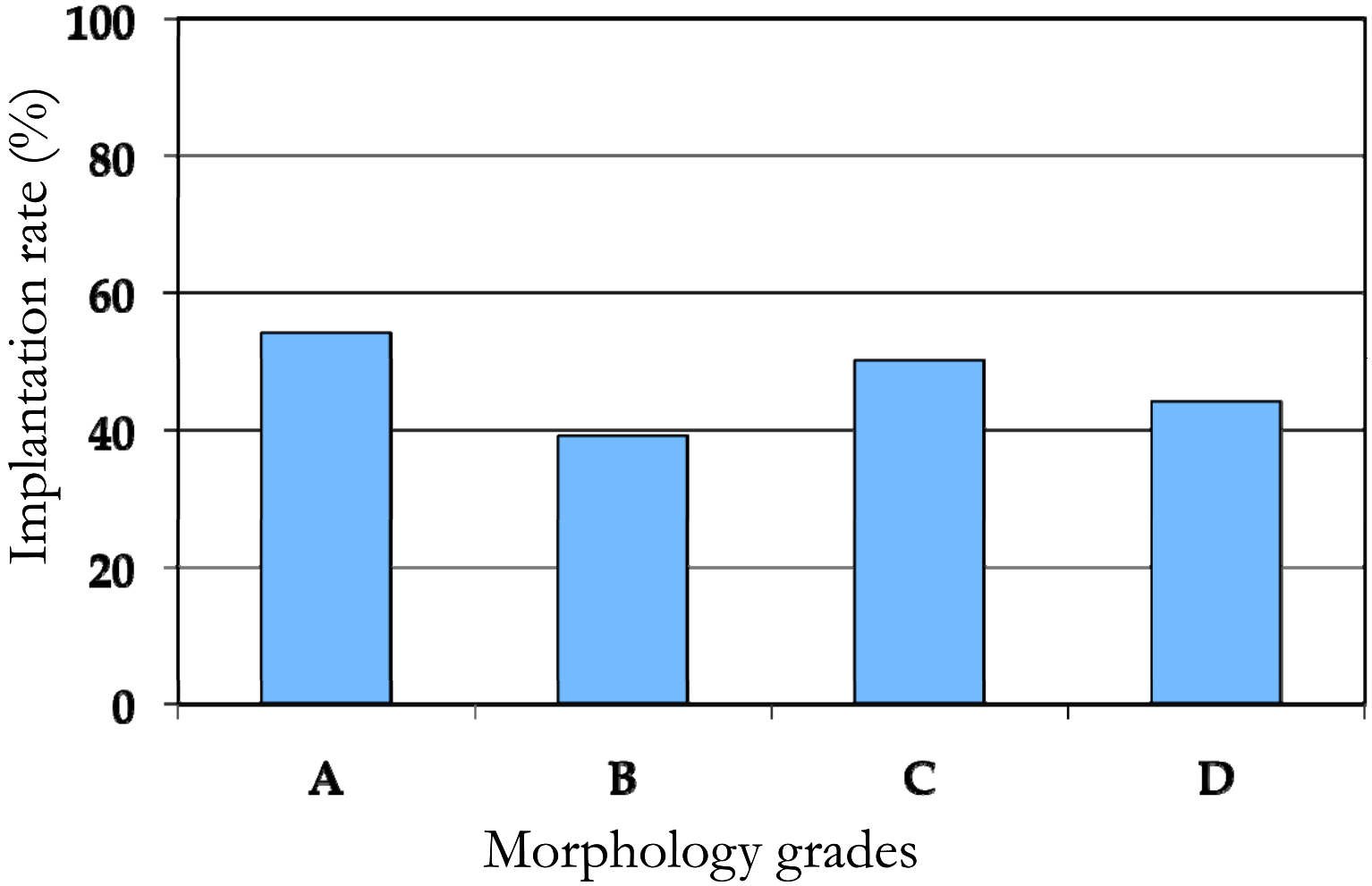
## D*ay 2 transfers, (n= 75)*



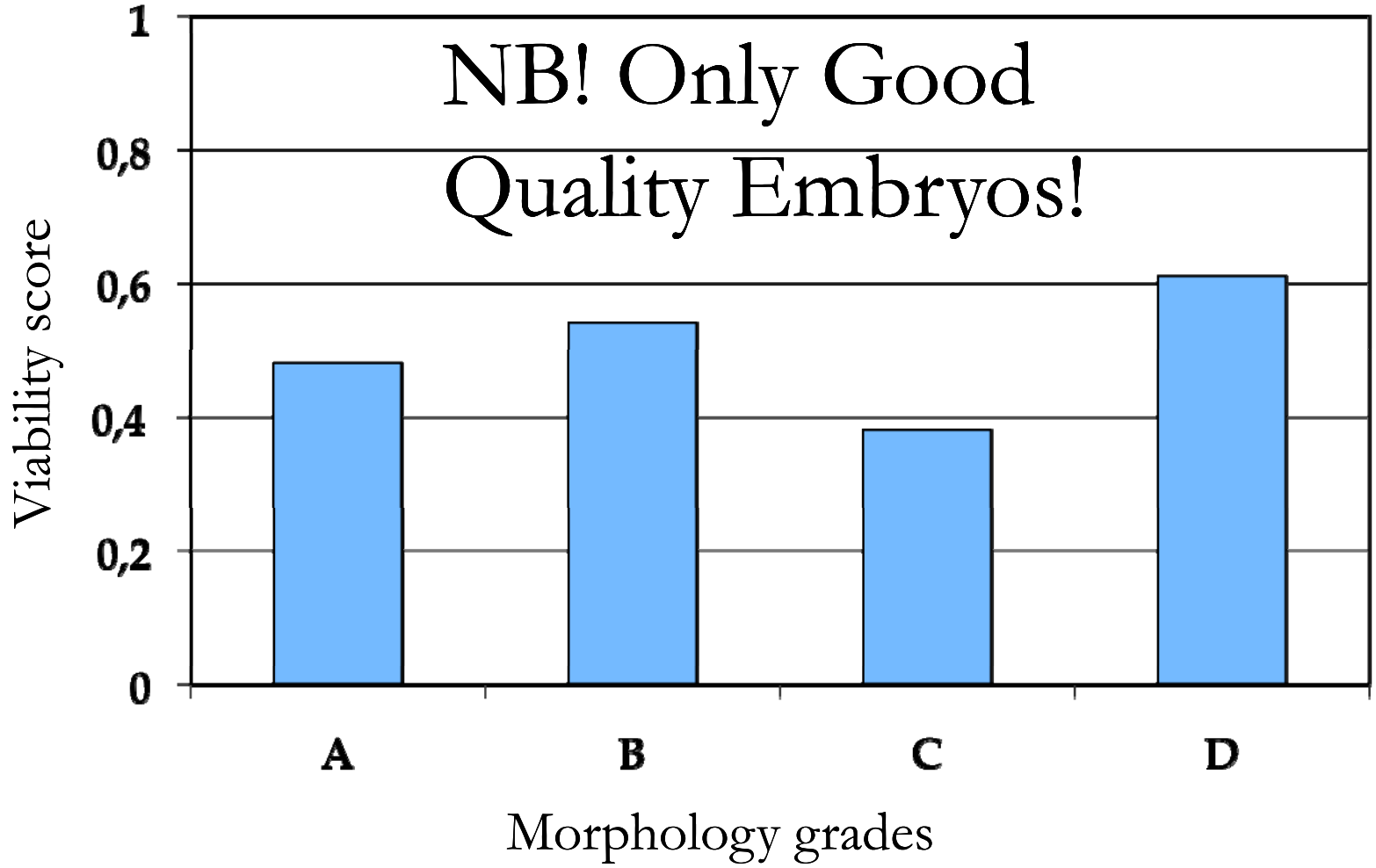
# Viability score correlated to *implantation rate* for sET (n=137) on Day 5



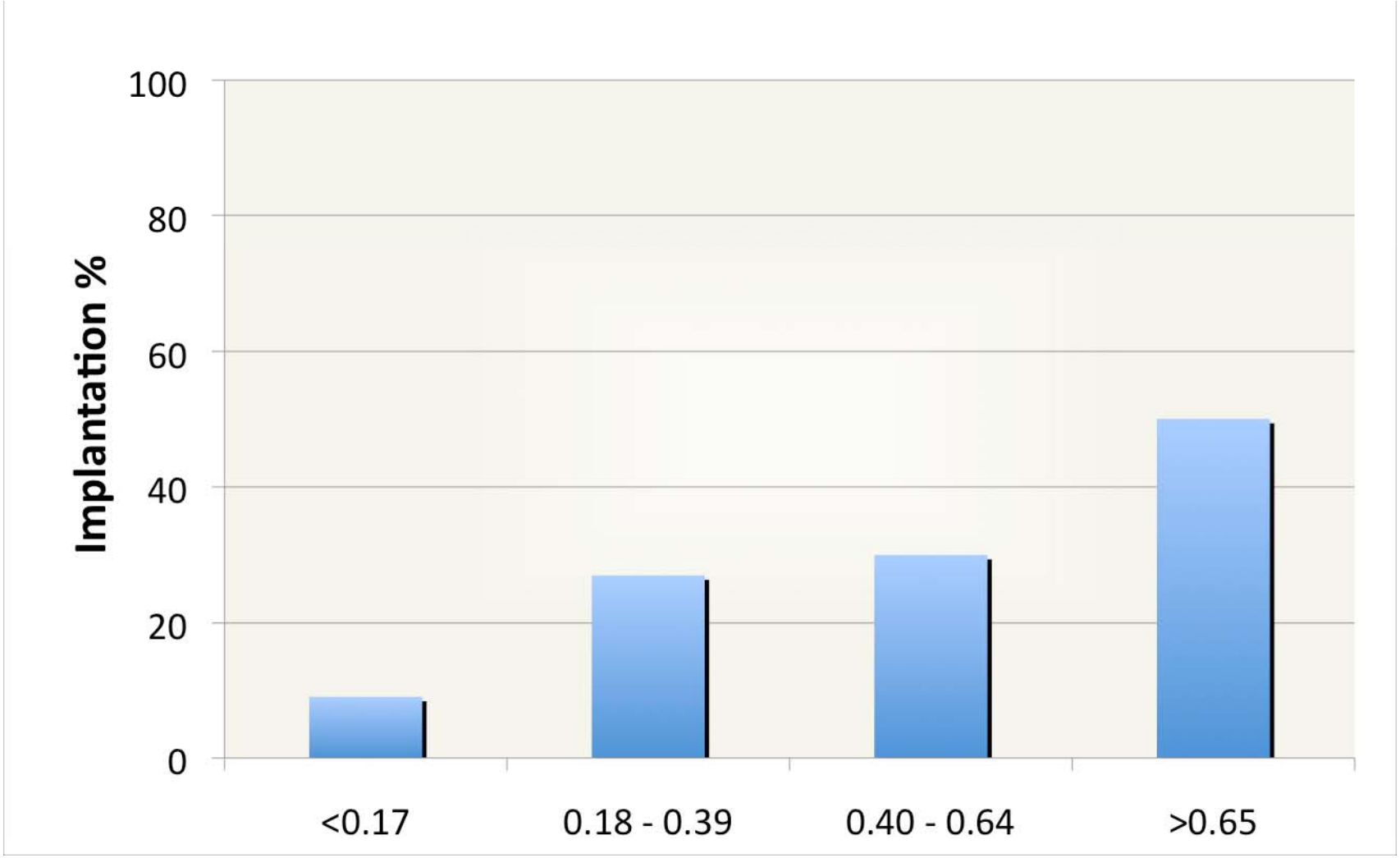
# Morphology correlated to *implantation rate* for sET (n=137) on Day 5



## Morphology correlated to *viability score* for sET (n=137) on Day 5



# Viability score correlated to implantation rate



## SHADY GROVE CLINIC, USA

*47 SET spent media collected and analysed (SAGE )*

The algorithm trained using the SGC data

The trained algorithm used to blindly predict outcome at FC

## FERTILITETSCENTRUM GOTHENBURG, SWEDEN

*42 SET spent media collected and analysed (Vitrolife CCM)*

*Reproductive BioMedicine Online*

NIR spectroscopy and embryo selection

# Cross-validation and predictive value of near-infrared spectroscopy algorithms for day-5 blastocyst transfer

[1]Aisling Ahlström, BSc., [1]Matts Wikland, M.D., PhD., [1]Lena Rogberg, BSc., [2]Jeannette Siques Barnett, MSc., [2]Michael Tucker, PhD., and [1]Thorir Hardarson, PhD.
[1]Fertilitetscentrum, Carlanderska Hospital and **Göteborg Universitet**, Sweden.
[2]FC, Shady Grove Fertility Center RSC, 15001 Shady Grove Road, Rockville, USA.
Correspondence address: Aisling Ahlstrom, Fertilitetscentrum, Carlanderska Hospital, Box 5418, SE-402 29, Gothenburg, Sweden. Email: aish.ahlstrom@fcivf.com



doi: 10.1016/j.rbmo.2011.01.009

# Conclusions

These pilot studies indicate that viability score improves prediction of implantation compared with morphology

To show that the viability score truly is an independant predictor of clinical precnancy a randomized controlled trial is needed


Hardarson Salzburg 2011

# NIR - RCT

*At Fertility Centre Gothenburg, Sweden*

**Aim:** To study if NIR spectroscopy of spent culture media combined with morphology better predict an embryo's potential than embryo morphology alone.

**Primary end-point:** Ongoing pregnancy rate after SET.

**Inculusion critera:** Patients seeking treatment at the clinic having two or more GQE

**Duration**: Estimated 2 years with 752 patients randomized.

## Interim analysis performed

January 2011

# Interim analysis

*287 patients randomized*

Conditions:

< 3% in favor of the NIR study group = stop the study

➢3% but less than 10% in favor of the NIR group => continue, but add more patients

➢10% or more in favor of the NIR group => continue as planned

The study was stopped

In total, 327 patients randomized, results being analyzed and will be published

# Preliminary results

Data will be provided at course (unpublished data)

# Future for metabolomics


Thank you for
your attention!