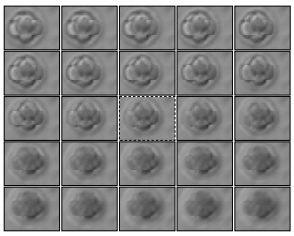
Multilevel morphometric imaging
Using
Computer assisted embryo analysis



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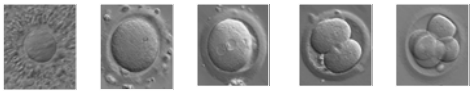
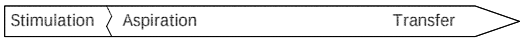
Key events from the mature oocyte to the early embryo reflecting embryo quality



A dynamic process



Embryo quality assessment

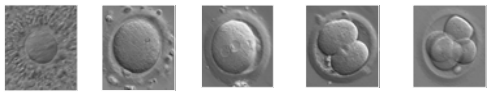
Embryo quality assessment without compromising the embryo

⇩

Non-invasive analysis

Minimal time in suboptimal environment

Light Microscopy

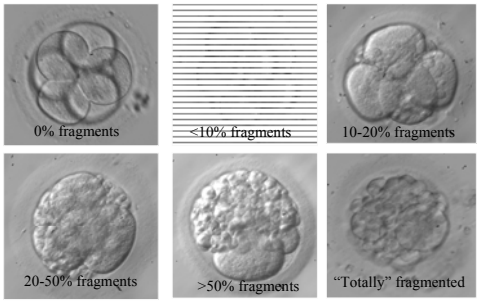


- **a non-invasive tool**
- **critical developmental stages can be detected**

Morphological parameters of embryo quality detectable by light microscopy

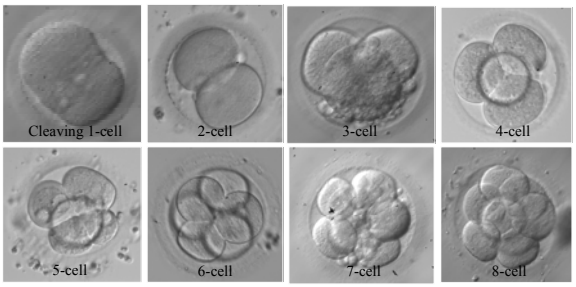
- **Fragmentation**
- **Number of blastomeres**
- **Blastomere size**
- **Nuclear status**

Fragmentation



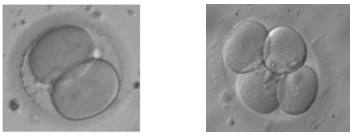
Number of blastomeres

reflecting the cleavage stage

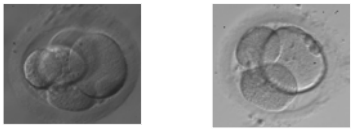


Blastomere size

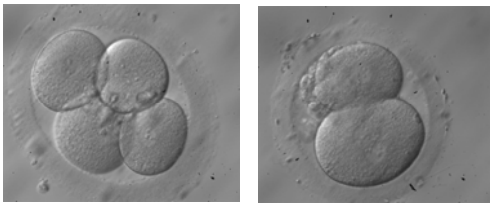
Reflecting different cleavage stages



Uneven sized blastomeres – reflecting decreased quality



Nuclear status



Traditional light microscopic embryo evaluation

Limitations

- **Subjectivity**
- **Time pressure**

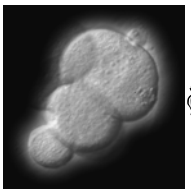
Assessment problems

- **Fragmentation**
- **Blastomere size**
- **Multinucleation**

Multilevel morphometric embryo imaging and analysis

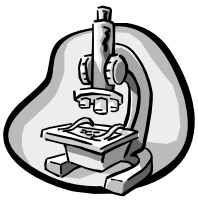
Using the computer-system

FertiMorph


The Fertility Clinic Rigshospitalet, Copenhagen, Denmark

Image House Medical Copenhagen, Denmark

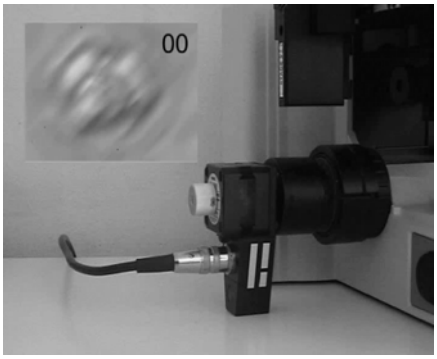



FertiMorph system

- **Multi-level imaging and analysis**
- **Integrating morphological information of the whole embryonic space**
- **Automatic calculations of morphometric information based on image sequences**

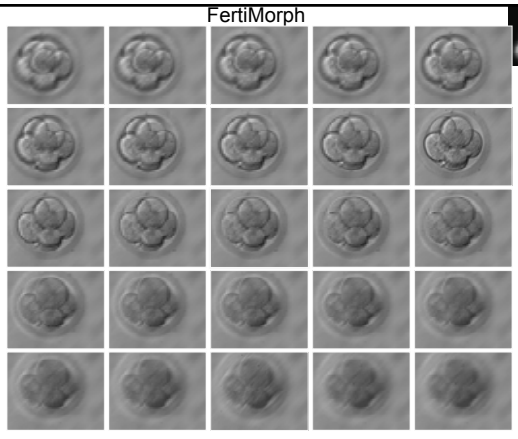
FertiMorph

Recording of digital image sequences



Hnida et al. (2003), Hum. Reprod. 19, 288-293

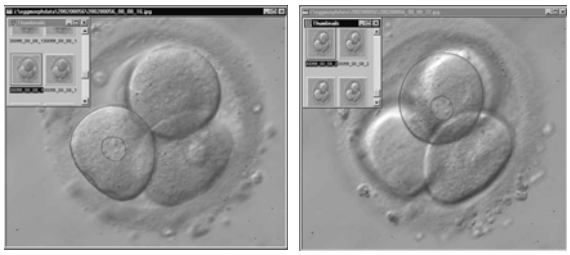
FertiMorph



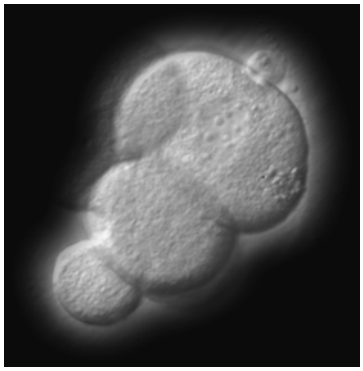
Hnida et al. (2003), Hum. Reprod. 19, 288-293

FertiMorph

Analysis of morphological structures



Hnida et al. (2003), Hum. Reprod. 19, 288-293

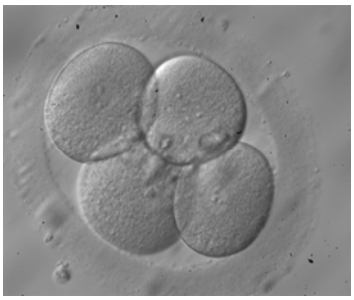


Morphometric measurements of a cohort of day 2 embryos

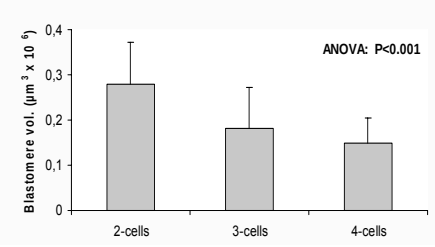
- **Assessing blastomere sizes at different embryonic cleavage stages**
- **Evaluating blastomere size as a biomarker of embryo quality focusing upon:**
  - **- embryo fragmentation**
  - **- multinuclearity**

- **Of each embryo image sequences were recorded on the morning of day 2 (48 hours after aspiration)**

Blastomere size

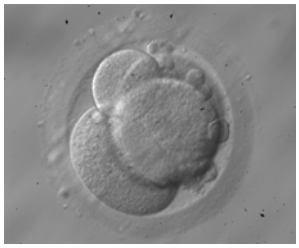
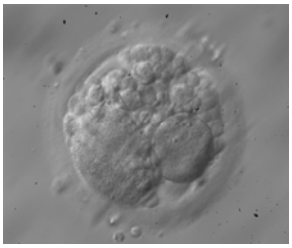


Blastomere Size at Different Cleavage Stages

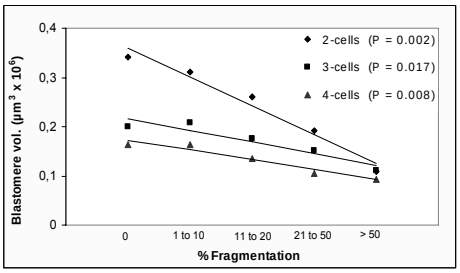


Hnida et al. (2003), Hum. Reprod. 19, 288-293

Fragmentation

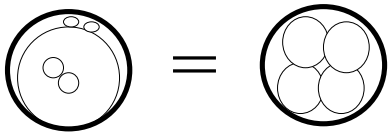



Blastomere Size and Fragmentation



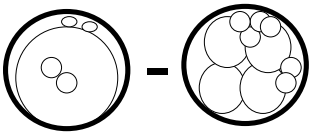
Hnida et al. (2003), Hum. Reprod. 19, 288-293

Total Cytoplasmic Volume



Hnida et al. (2004), J. Assist. Reprod. Genet., 21, 335-400

Total Cytoplasmic Volume



Hnida et al. (2004), J. Assist. Reprod. Genet., 21, 335-400

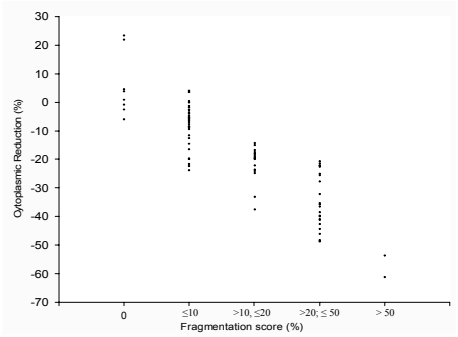
Cytoplasmic reduction

- **To analyse the total blastomere volume in relation to the volume of the zygote**
- **To use the cytoplasmic reduction to quantify the degree of fragmentation in the single embryo**
- **To compare this new method with the traditional light microscopic evaluation of fragmentation**

Hnida et al. (2004), J. Assist. Reprod. Genet., 21, 335-400

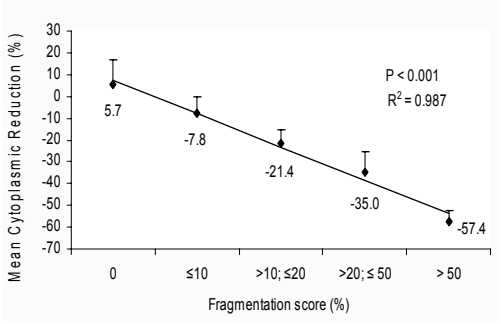
- **Of each embryo image sequences were recorded on the morning of day 1 (zygote stage) and day 2 (early embryo)**

Cytoplasmic Reduction and Fragmentation Score



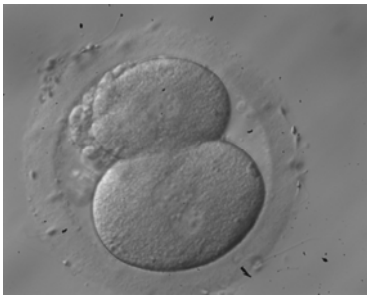
Hnida et al. (2004), J. Assist. Reprod. Genet., 21, 335-400

Cytoplasmic Reduction and Fragmentation Score

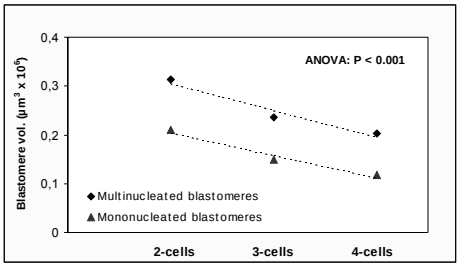


Hnida et al. (2004), J. Assist. Reprod. Genet., 21, 335-400

Nuclear status

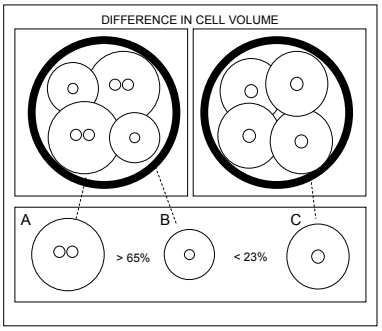


Blastomere Size and Multinuclearity



Hnida et al. (2003), Hum. Reprod. 19, 288-293

Blastomere Size and Multinuclearity



Hnida et al. (2003), Hum. Reprod. 19, 288-293

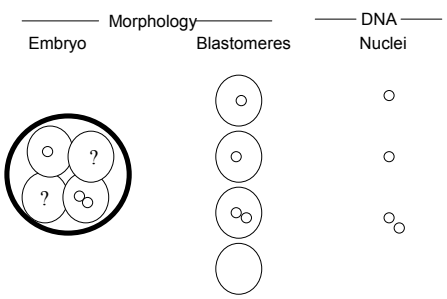
Morphological detection of nuclear structures

Hnida et al. (2005), Hum. Reprod.20, 665-671

Background

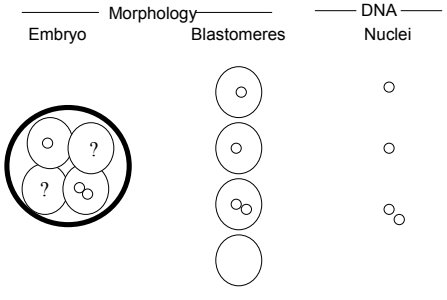
- **Nuclear structures are not always easy to identify by light microscopy**
- **Good methods to identify nuclear status are of great importance**

Method: Study III



Hnida et al. (2005), Hum. Reprod.20, 665-671

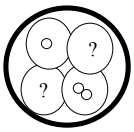
Results: Study III



Total	**94%**	**100%**
<10 % fragmentation	100%	
10-20 % fragmentation	86%	

Hnida et al. (2005), Hum. Reprod.20, 665-671

Morphological detection of nuclear status o day 2 embryos



- **Traditional analysis**
- **Computer-assisted analysis**
- **Validation by DNA staining techniques**

27 % of the embryos were classified incorrectly by the traditional evaluation method

4 % of the embryos were classified incorrectly by the computer-assisted analysis

Conclusions of the biological findings obtained by using The FertiMorph-system

- **Blastomere volume at the 4-cell stage is half the volume compared to the 2-cell stage**
- **Blastomere size may function as a biomarker of embryo quality at least in regard to**
  - **Fragmentation**
  - **Multinuclearity**
- **Cytoplasmic reduction reflects the degree of fragmentation in the single embryo and might be a more precise and standardised method to quantify fragmentation**

Key technological approaches of computer-assisted, multilevel embryo analysis

- **Based on image sequences information of the whole embryonic space can be included in the morphometric analysis**
- **Using the FertiMorph-system detailed and objective measurements of at least oocyte, blastomere and nuclear sizes can be performed**
- **Computer-assisted detection of nuclear status is more precise compared with traditional analysis**
- **The time limitation of the traditional analysis can be overcome**

Improvements in embryo evaluation via computer-based systems offers:

- **More critical/detailed information can be collected during assessment**
- **Embryos spend minimal time outside the incubator**
- **Better standardisation**
- **Better documentation**
- **Quality control of scoring procedures**

Final conclusion

- A combination of light microscopic analysis with computer-assisted morphometric measurements may result in a more precise and detailed embryo morphology evaluation compared to the traditional embryo assessment

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- Implementation of more standardised procedures in embryo scoring systems may contribute to improve embryo selection and thus the clinical results