Evaluation of embryo morphology

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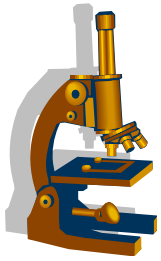
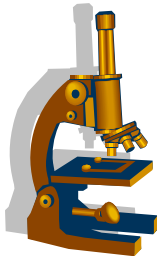
Evaluation of Embryo Morphology Why

- Evaluation of Embryo Morphology is an evaluation of **Embryo Quality**



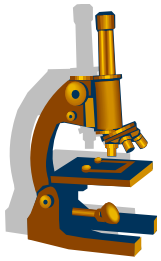
Evaluation of Embryo Morphology How

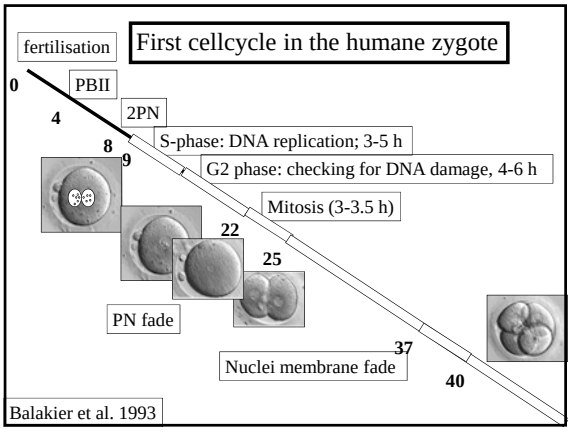
- Embryo evaluation is mostly done in light microscopy at 200 – 400 x magnification



Evaluation of Embryo Morphology When

- Embryos should be evaluated at **fixed time intervals**



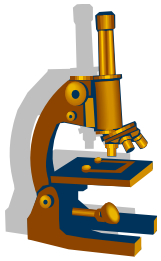


Evaluation of Embryo Morphology

- Developmental timing of the embryo is the **most important** parameter.

Evaluation of Embryo Morphology What

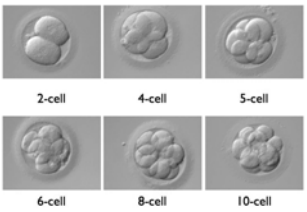
- Parameters indicating developmental timing
- Parameters indicating for chromosome failure or degeneration



Parameters indicating timing

- **Parameters:**

Number of blastomer at certain timepoints

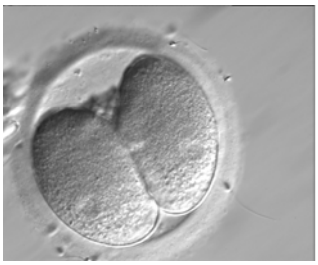


Parameters indicating timing

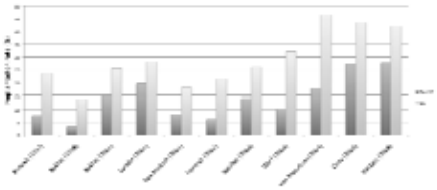
Parameter:

Presence of nuclei at certain timepoints

Number of blastomeres
Early cleavage



Early Cleavage



Early Cleavage
IVF vs ICSI

- **Lundin et al 2001 found that**
  EC after 25-27 in ICSI embryos was an independent predictor for live birth.

Early Cleavage Agonist vs Antagonist

- **Yan et al. 2009 found that:**

The Early Cleavage (EC) rate was significantly lower in the GnRH antagonist group compared to the GnRH agonist group.

In the GnRh agonist group the EC embryos resulted in significantly higher pregnancy(PR) rates than the late-cleaved embryos.

In the GnRH antagonist group there was no difference in PR between EC and late-cleaved embryos

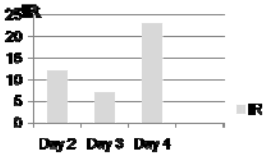
Developmental timing

- The timing of a cell cycle express the conditions of the cell.
- Number of blastomeres at certain time points express the timing of the cell cycles in the individual embryo.

Number of blastomeres and implantation

- **Ziebe et al. 1997 showed that**

Four cell embryos on day 2 had significant higher implantation rate compared to two and three cell embryos



Number of blastomeres and blastocyst formation

Alikani et al. 2000 describe that

the proportion of normal appearing blastocysts was significantly higher among embryos with 7-9 cell at day 3 compared to < 7 or > 9 cells.

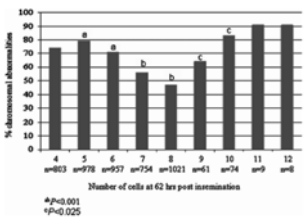
Number of blastomeres and Aneuploidy

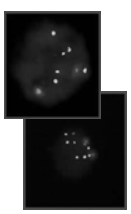
- **Magli et al 2001, 2007 found that:**

The incidence of aneuploidy was highest among embryos that were 3-4 or 5-6 cells day 3 compared to embryos that were 7-8 cells.

The incidence of aneuploidy was also higher in embryos with more than 9 cells day 3.

Number of blastomeres and Aneuploidy





"These observations suggest that timing is a crucial factor in determining embryonic development. In agreement with previous observations, any deviation from the expected cleavage stage has dramatic effects on implantation."

M.C. Magli, Italy

Number of blastomeres and early pregnancy loss

- **Hourvitz et al. 2006, found that**

five or less blastomeres in the best embryo transferred at day 3 were predictive for early pregnancy loss.

Presence of nuclei

- **Palmstierna et al. demonstrated in 1998 that**

visible mononucleated blastomeres are a strong predictor of pregnancy in IVF treatment.

- **Saldeen et al. 2005 confirmed that**

the presence of a single nucleus in each of the blastomeres in a 4 cell embryo day 2 is predictive for implantation.

Presence of nuclei

- **Hnida et al 2007 found that**.

2 cell embryos with no visible nuclei day 2 (44 h after fertilization) were closer to the next cell cycle than 2 cell embryos with visible nuclei.

Presence of nuclei

- **Lemmen, Agerholm and Ziebe (2008) has demonstrated that**

Synchrony in re-appearance of nuclei after first cell division is significantly associated with pregnancy success.

Time-lapse

Nuclei size

- **Agerholm et al. 2008 found that:**

Nuclei with loss of chromosomes are smaller than nuclei with gain of chromosomes (p< 0.01, 95 % CI -2.92 – (-0.68)).

Bi or multinucleation

- **Jackson et al. 1998, Pelinck et al. 1998 and Van Royen et al. 1999 states that**

The presence of two or more nuclei in at least one blastomere is correlated to low implantation rates.

Binukleation and chromosome status

- **Agerholm et al. 2008 found that :**

Nuclei from binucleated blastomeres are more often chromosomal abnormal than nuclei from mononucleated embryos.

(P < 0.01)

Binucleation and blastomer size

- **Agerholm et al. 2008 also found that:**

Binucleated cells > than mononucleated cells from mononucleated embryos

(p = 0.048, 95% CI 0.60 - 7.1)

Binucleated cells > end mononucleated cells from binucleated embryos

(p = 0.03, 95% CI 0.59 - 9.22)

Binucleation and Chromosome status

- **Ziebe et al 2003 found:**

a significantly increased rate of chromosomal abnormality for embryos containing unevenly sized blastomeres

- **Hardarson et al 2001 also indicate that:**

Embryos with unevenly sized blastomeres correlate with increased rates of multinucleation

Fragmentation and implantation

- **Gioretti et al. 1995 and Van Royen et al. 1999 have found that**

High amounts of fragmentation correlates negatively with implantation and pregnancy

- **Ziebe et al 2003 found that**

Degree of Fragmentation correlates with chromosome abnormality day 3 but not day 2.

Blastomer size and chromosome status

- **Hardarson et al. 2001, Ziebe et al. 2003, Agerholm et al 2008 found that**

Embryos with uneven sized blastomeres had a significantly higher proportion of blastomeres with abnormal chromosome complement

Embryo appearance and Chromosome status

- **Ziebe et al 2003 found**

No correlation between localization of the fragment and chromosome abnormality in the embryo.

No correlation between cytoplasm appearance and chromosome abnormality in the embryo.

Evaluation of Embryo Morphology

Can you use all these information's?

Can you go home and use the time points in your own lab ?

Can you go home and use the morphology ?

?

Evaluation of Embryo Morphology Why

Definitions

- We must use the same definitions for the different parameters

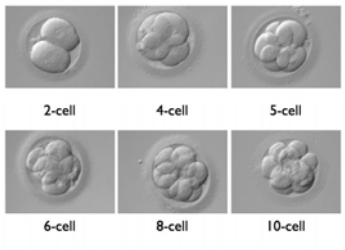
Number of blastomeres When

Standardization:

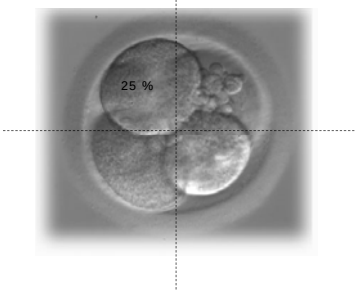
- We have to be sure that we are evaluating at the same development

Number of blastomeres

- When

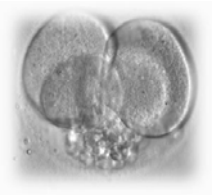


Fragmentation volume



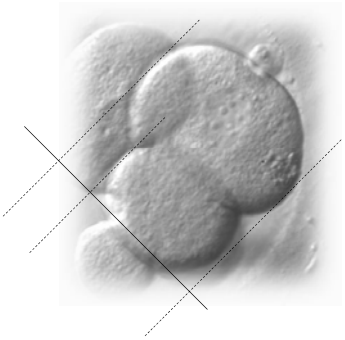
Localization of the fragments

Local



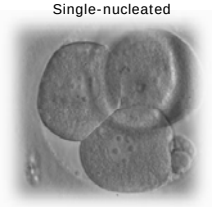
Dispersed

Blastomere size

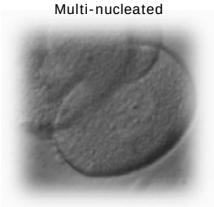


Bi or Multi-nucleated blastomeres

Single-nucleated

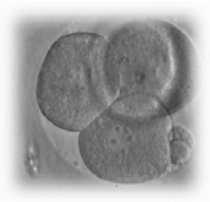


Multi-nucleated



Presence of nuclei

- **When**



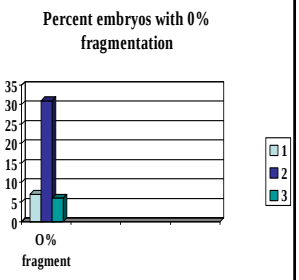
Evaluation of Embryo Morphology

Standardization

- Same Media same distribution
- Different Media different distribution
- Same stimulation protocol same distribution
- Different stimulation protocol different distribution
- Same temperature same distribution …….ect.

Standardization

Three clinics with same embryo scoring system but different media



Evaluation of Embryo Morphology

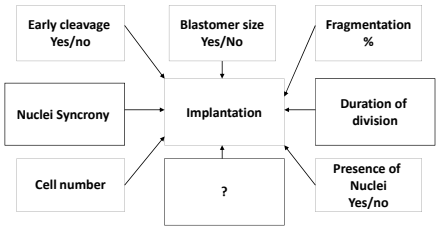
- Remember to drop unpredictable parameters !

Evaluation of embryo morphology

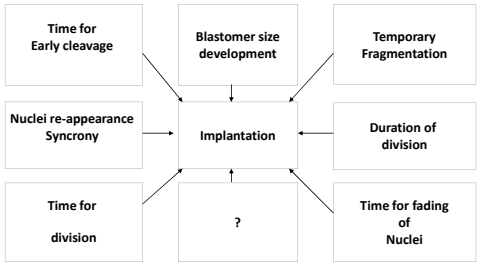
We are doing good and we have found a system that enable us to select embryos that in 75 % of the cases are overall chromosomally normal (7 chromosomes)

Ziebe et al. 2003.

Evaluation of Embryo Morphology Static evaluation



Evaluation of Embryo Morphology Time-Lapse



Disclosure

- Beside being labdirector at the Fertility Clinic in Brædstrup I work as clinical coordinator for the company Unisense FertiliTech which have invented an instrument that can do time-lapse evaluation of embryos in a clinical setting.
- I do however also have a time-lapse equipment from Nikon, so the following slides are a mix from both systems.

Evaluation of embryo norphology

Can we learn something from Time-lapse

Film

Take home message

- Timing in the development is crucial
- Syncrony is important
- Uneven blastomer size is not good and could be a sign for binucleation
- Binucleation is not good but could be dependent on the development
- Fragmentation is temporary
- Presence of nuclei is important but when is crucial

Take home message

- FIND your own timing for your parameters in your lab !.....and that goes for Time-lapse and NIR evaluation too !!