Embryoscoring

Mia Janssen

Stafvergadering 3 juni 2014

Importance of scoring

- 40-50% of fertilized oocytes grow to blastocysts and only 30-50% of the blastocysts implant:
  only 30% implantation rate /cycle, so we better choose the "good" one

- Worldwide a variety of embryo grading schemes:
  international consensus on embryo assessment : inter-clinic comparisons possible and validation of embryo morphology as an end-point in clinical trials and studies on new technologies and products in IVF (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, Istanbul 2010)

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Assessing oocytes

- Developmental competence of human embryos is directly influenced by the normality of nuclear and cytoplasmic maturation of the oocyte

  (The molecular and cellular anatomy of a cytoplasmic dysmorphism in the mature human oocyte: physiological implications for normal development ,Jonathan Van Blerkom 1992)
- Oocytes of different qualities, mostly due to desynchronization of nuclear and cytoplasmic maturation (Ebner et al., 2006)
- All anomalies should be divided into intracytoplasmic and extracytoplasmic dysmorphisms:
  - intracytoplasmic : incorporations, refractile bodies, dense central granulation, vacuoles, aggregation of sER
  - extracytoplasmic : first polar body morphology, perivitilline space size and granularity, discoloration, zona pellucida defects, shape anomalies

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sER, giant oocytes

Assessing fertilization

- A fertilized oocyte should have two pronucleï and two polar bodies
- Zygotes arising from IVF are observed ~1h behind those from ICSI
- Assessment is usually done 17 ± 1h post-insemination, 8% syngamy
- Proportion of zygotes in syngamy 24h post-insemination is a very sensitive key performance indicator and a post hoc indicator for oocyte maturity (Lawler et al., 2007)
- The pronucleï are of similar size, closely apposed and centrally located in the fertilized oocyte, have 4 -7 precursor bodies (NPBs)
- Pronuclear scoring takes into account the symmetry and alignment of the pronucleï, the number and position of NPBs (no static event)
- In animal studies, a lack of NPBs has been associated with imprinting errors in mouse and delayed embryonic genome activation (Svarcova et al., 2009)

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- Pronuclear score has a correlation with aneuploidy (Sadowy et al., 1998; Gianaroli et al., 2003; Edirisinghe et al., 2005) ; others found no positive predictive value (Salumets et al., 2001; James et al., 2006; Weitzman et al., 2010)
- Pronuclear score has in some papers a prognostic effect (Scott and Smith, 1998; Tesarik and Greco, 1999; Scott et al., 2000; Balaban et al., 2001; Nagy et al., 2003; Scott, 2003)

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Assessing early cleavage

- Time of first cleavage of the zygote predicts both embryo quality and implantation (Shoukir et al., 1997; Sakkas et al., 1998; Lundin et al., 2001; Salumets et al., 2003; Hammoud et al., 2008)
- Early-cleaving embryos cleave more evenly, which is strongly correlated with lower incidence of chromosomal errors (Hardarson et al., 2001)
- Cleavage earlier than 20h post-insemination has a poorer prognosis
- Cleavage directly into three or more cells, has been shown to be associated with chromosomal abnormality (Hardarson et al.? 2006)

Time lapse assessment?

Assessing cleavage-stage embryos (days 2 and 3)

- Most dysmorphisms : fragmentation, multinucleation, asymmetry… are associated with increased risk of post-meiotic abnormalities, such as mosaicism, monospermic polyploidy and haploidy
- Incidence of chromosome abnormalities increases from 50 to 60% in non-fragmented embryos to 70-90% in embryos with > 35% fragmentation; fragmentation was strongly correlated with mosaicism and other post-zygotic abnormalities, not with aneuploidy (Munné and Cohen, 1998; Magli et al., 2001; Munné et al., 2007)
- Chromosomal analysis of 1255 embryos demonstrated a highly significant relationship between maternal age and aneuploidy (Munné et al., 1995; Marquez et al., 2000) and between decreasing developmental competence (from normal to arrested) and an increase in post-meiotic abnormalities
- Too slow or too fast embryo cleavage rate has a negative impact on implantation rate (Edwards et al., 1980; Giorgetti et al., 1995;Ziebe et al., 1997; Van Royen et al., 1999)

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- Uneven cleavage has a negative impact on pregnancy outcome (Giorgetti et al., 1995; Ziebe et al., 1997; Hardarson et al., 2001)
- Genetic analysis of blastomeres from uneven cleavage correlates with multinucleation and a higher degree of chromosomal aberration (Hardarson et al., 2001)
- Multinucleation is considered abnormal: occurence in up to 87% of cycles with 31-33% of the embryos affected (Jackson et al., 1998; Van Royen et al., 2003) ; 44% of patients have one or more embryo with multinucleation (Balakier and Cadesky, 1997)
- Multinucleation correlates with high degree of chromosomal aberration, higher degree of fragmentation and number of blastomeres on day 2 and 3, uneven cell size (Kligman et al., 1996; Hardarson et al., 2001; Van Royen et al., 2003)
- Transfer of multinucleated embryos leads to lower implantation, pregnancy and birth rates (Jackson et al., 1998; Pelinck et al., 1998; Hardarson et al., 2001; Van Royen et al., 2003)

- factors affecting multinucleation: culture media, improper temperature control during oocyte retrieval, stimulation high oestradiol levels ....(Winston et al., 1991; Van Blerkom, Pickering et al., 1990)

- Morphology cleavage score: cell number, equal blastomere size and number of mononucleated blastomeres on day 2 has a significant predictive value for implantation
- Study of 4042 individually cultured embryos: cell number on day 2 and the incidence of early cleavage were the most predictive parameters for good blastocyst quality (Guerif et al., 2007)

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Assessing morulae and blastocysts (day 4 – 6)

- compaction: allows examining the embryo after embryonic genome activation
- Examination of both cell types : TE reflects ability to attach and implant, ICM is crucial for the developmant of the fetus (Kovacic et al., 2004)
- Strong relationship between embryo cell number on day3 and blastocyst development
- Scoring of blastocysts by grading system of Gardner and Schoolcraft
- Significant linear trend in implantation rate related to the number of top-scoring blastocysts (Gardner et al., 2000) AA blastocyst transfer individually
- Blastocysts with a low score implant at a relatively high rate compared to cleavage-stage embryos

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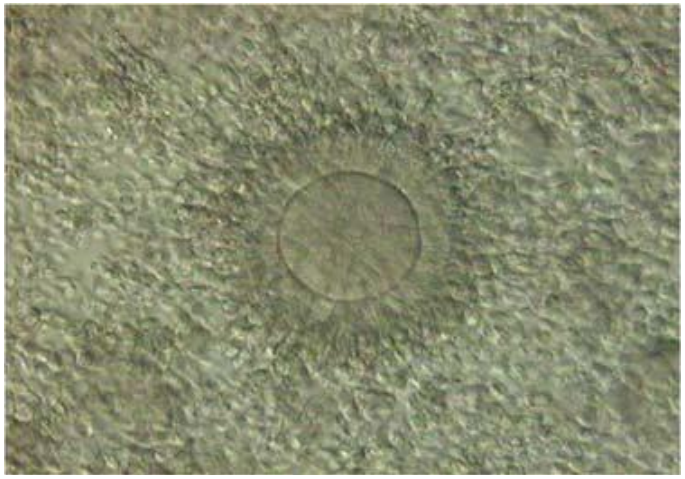
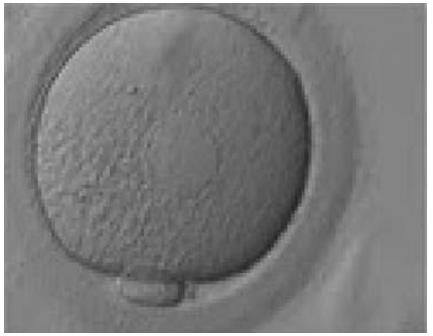
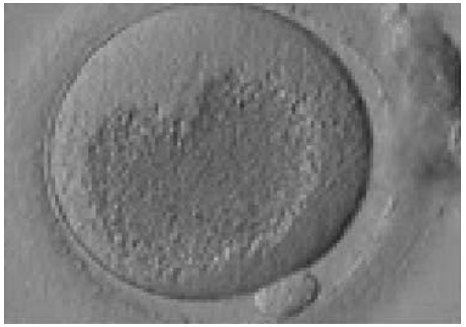
Embryoscoring in our Lab: comprehensible for all members in the team

Oocyte Scoring



we don't score the oocytes, just make notations if special disorders are observed in cumulus-oocyte complex, in zona pellucida, in perivitelline space, polar body, cytoplasm (sER disks) and large vacuoles





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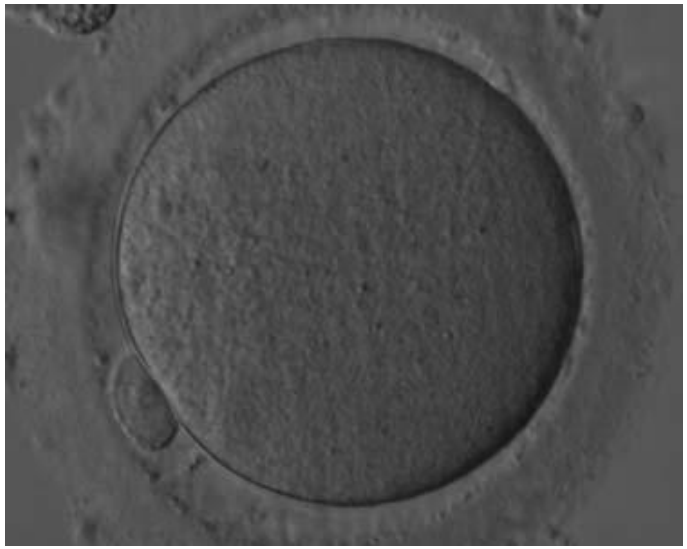

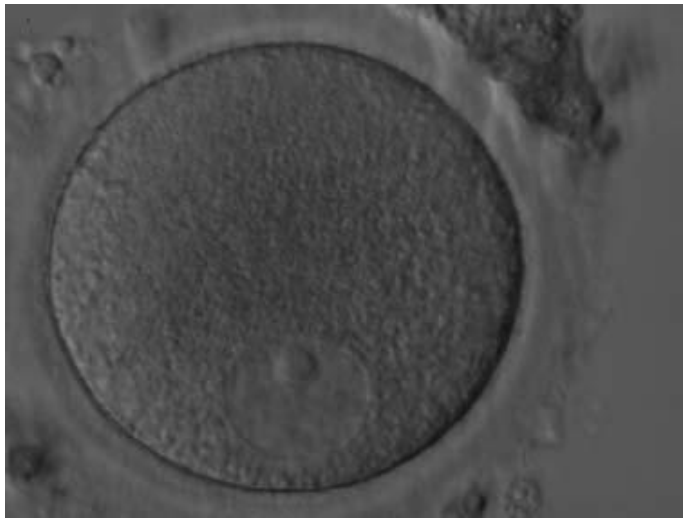

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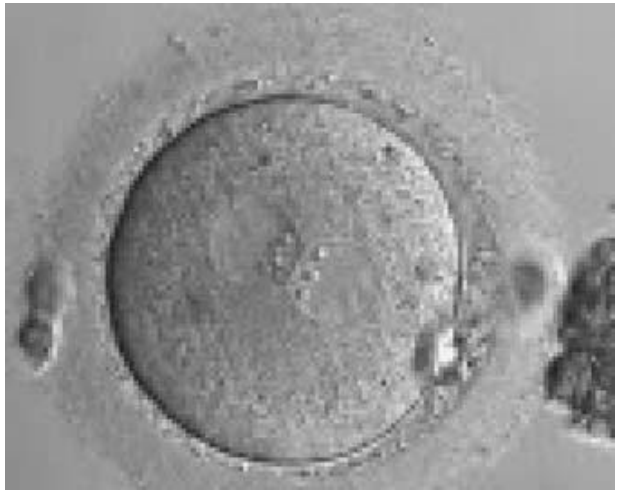
Fertilization check: 2 polar bodies, centrally located, even sized, with even number and size of NPBs

17±1 hour post insemination

Score: symmetrical – non symmetrical - abnormal



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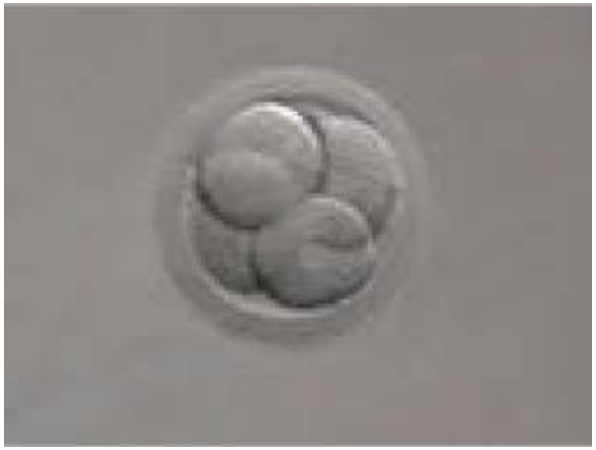
Cleavage-stage

-Day 2: 44±1 hour post insemination



-Day 3: 68±1 hour post insemination

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Scoring:

-Assessment of cell number : n

-Even or uneven sized : 1 or 2

-Fragmentation : <45µm-<40µm

<10% 1

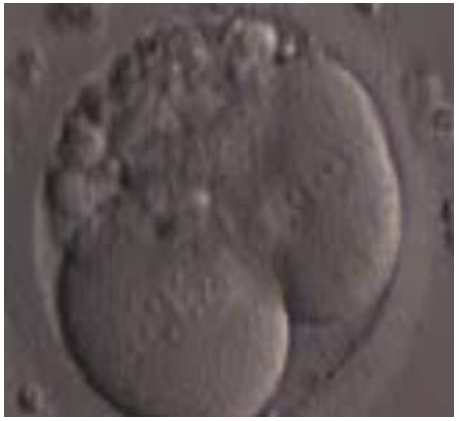
10-25% 2

>25% 3

- Notation of multinucleation

Day 2: 4.1.1

Day 3: 8.1.1



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Day 4 assessment

92±1 hout post insemination

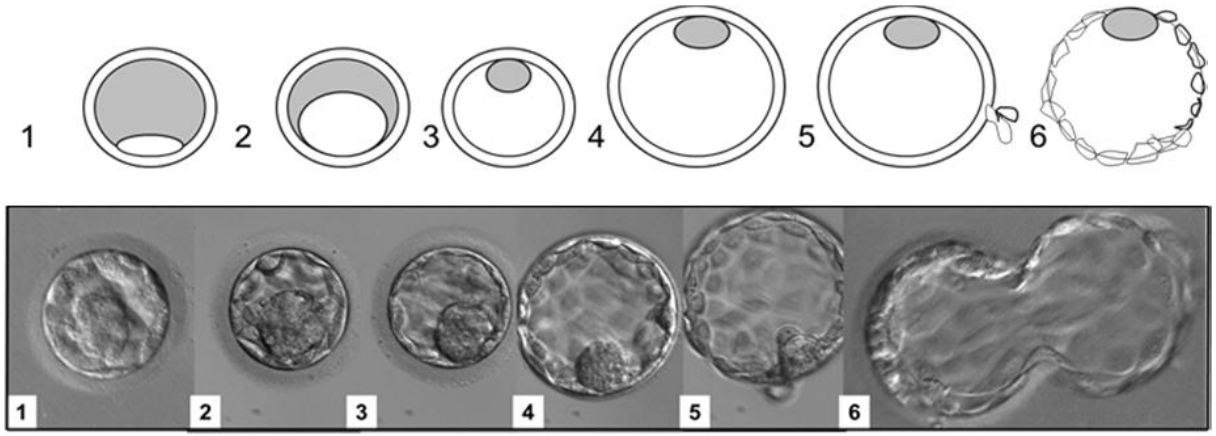
MOR1 : evidence of compaction that involves all the embryo volume

MOR2 : compaction involves the majority of the volume of the embryo

MOR3 : disproportionate compaction involving less then the half of the embryo,with 2 or 3 cells remaining as discrete blastomeres

Day 5 : blastocyst stage

116±1 hour post insemination



The expansion grade scale ranges from 1 (least expanded) to 6 (completely hatched).

Grade 1: the fluid-filled cavity takes up less than half the space of the embryo.

Grade 2: the fluid-filled cavity takes up more than half the space of the embryo.

Grade 3: the blastocyst cavity has expanded into the entire volume of the embryo, pressing the trophectoderm cells up tightly against the inside of the zona.

Grade 4: Expanded blastocyst, where the blastocyst has increased beyond the original volume of the embryo and caused the zona pellucida "shell" to become super thin.

Grade 5: Embryo has breached the zona and is hatching out of its shell

Grade 6: Embryo is completely hatched.

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So the embryo is given a number grade (1-6), followed by a letter grade for the inner cell mass and then the trophectoderm (A,B or C).

For the inner cell mass:

A: Many cells, tightly packed

B: several cells, loosely packed

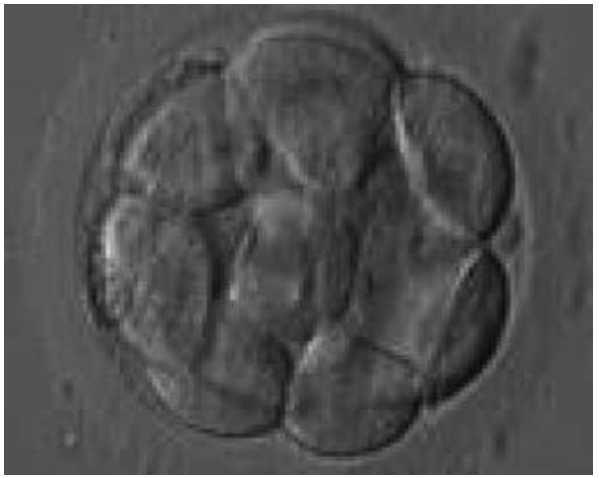
C: very few cells

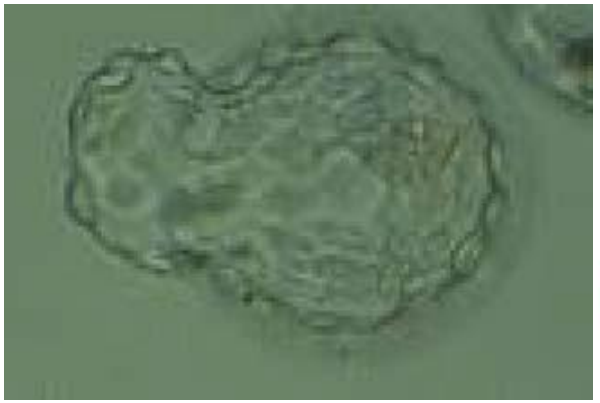
The trophectoderm grading goes like this:

A: many cells, forming a cohesive layer

B: Few cells, forming a loose layer

C: Very few large cells.





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Our results jan 2014 - apr2014

- 47 embryo's transferred with best score (44 cycli): 16 pregn/42 known 38%
- 205 embryo's ET with good score (151 cycli): 47 pregn/143 known 33%
- 13 embryo's ET with bad score (11 cycli): 1 preg/11 known 9%

Thank you.

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