10.50 – 11.20

The search for excellence in IVF laboratories: Towards "the best"
ESHRE Campus symposium
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Embryo and Blastocyst Morphology as a reflection of embryo viability

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Learning Objectives:

- **Why morphology for selection?**
- **Introduction to the concept of Sequential Embryo Selection (*SES*) by morphology**
- **Define timing for *SES***
- **Recap biological anomalies from day 1**
- **Introduce biological background to anomalies on Day 2/3**
- **Discuss the anomalies in the context of viability**

Introduction

Why Morphology?
Its All We Have!! (at present)
Can it work?
Yes if done correctly!
If done with biology in mind.
If done with strict QC/QA of performers
When do you do it?
Continually, carefully, *timed, according to "biology"*

Timing and Sequential Embryo Selection

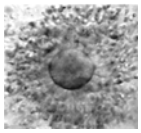
- **Gamete and embryo selection needs to be dynamic**
- **Embryos are not static and more than one point in an embryos development should be used to decide the embryo for transfer**
- **Using a sequential embryo selection technique (*SES*), a profile of the developing embryo can be drawn/profiled.**
- **If morphology is coupled to biology the profile can then help in selecting embryos with the maximum potential for implantation.**
- **The key element in developing a scoring system that can be used in a repeatable manner is timing; when are observations made.**

The Next Slide is the only one you need to remember and take home with you.
To gather data and build a data base, and to compare data (within your programme and between programmes), you need to be comparing like with like.

SCORING TIME POINTS

Time Point	hCG	ER	Insem.	D 1 Fert.	D 2 2-4 cell	D 3 6-8 cell	D5 Blast
Hours/ hCG	0	36-37	40	57-59	82-84	104-106	152-154
Hours/ Insem.	0	0	0	17-18	42-43	64-65	112-114

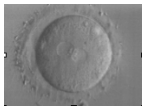
Most ER are 36-37 hours post hCG.
Maximum mature (M11) occurs 40-41 h post hCG
Time of maturation is important (Montag, 2008)
Stabilized PN's are from 16-18 hours post insem.
EC will start at 20 h post insem
Timing Timing Timing



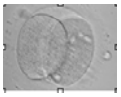
Gametes

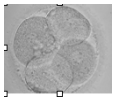
Abnormal gametes generally do not produce normal embryos

Early embryo parameters may be a window back to the gametes

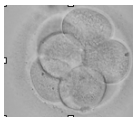


Day 1 and Day 2



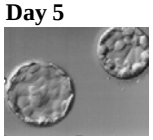


Day 3



Differentiation

Day 5

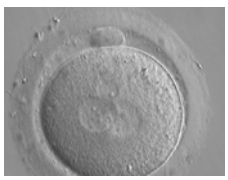


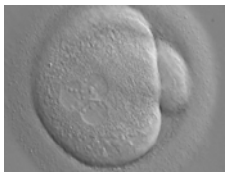
Later development reflects gene expression, differentiation, developmental controls

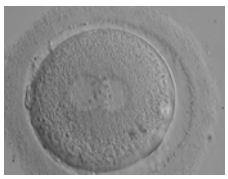
Day 1 Morphology

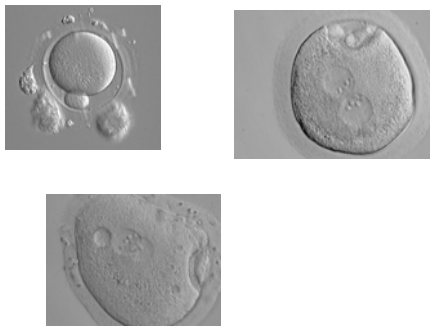
- **PN Score- Fertilized Oocyte Score**
- **Polarity**
- **Pre- vs. post- zygotic modifications for aneuploidy**
- **Earliest evaluation of the "unique embryo"**
- **Timing is crucial**
- **Ability to see in 3D is crucial**
- **Understanding the 3D nature of the nucleus, the spindle and how it moves is crucial**
- **Training/education/ knowledge is more important than a machine**

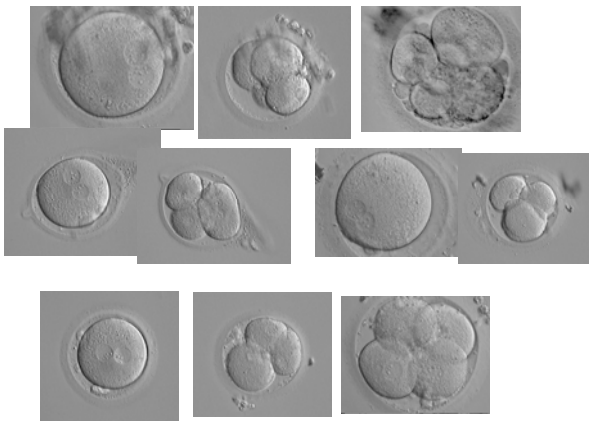
Abnormal PN Morphology





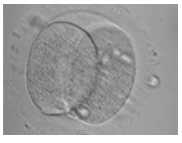




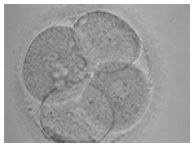


Day 2 Morphology/ Scoring

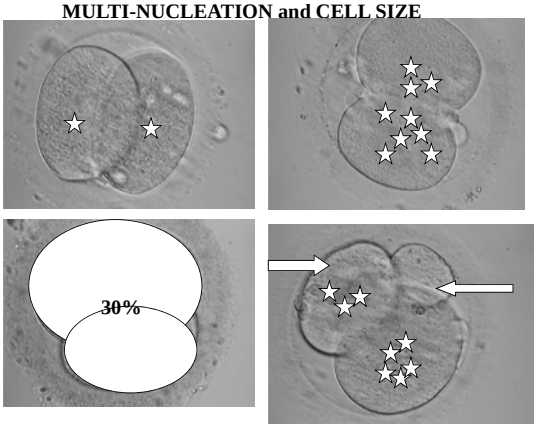
- **Cell number**
- **Blastomere relative size**
- **Status of nucleation**
- **Fragmentation**



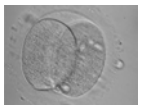
- **Timing is important**
- **Embryos are very dynamic on Day 2**



MULTI-NUCLEATION and CELL SIZE



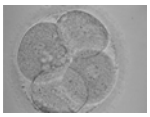
Mono and Multinucleation



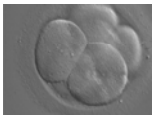
Mononucleated 2-cell

Multinucleated 2-cell

Multinucleated 2-cell

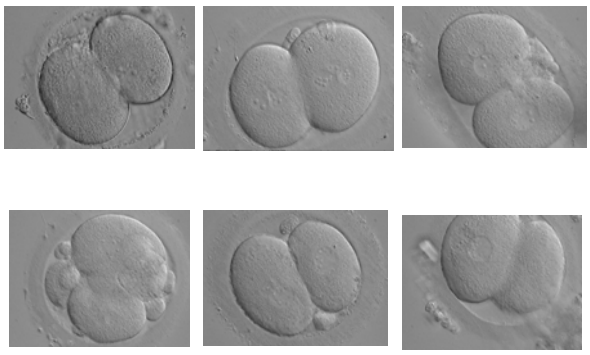


Mononucleated 4-cell



Multinucleated 4-cell

2-CELL MultiNucleated and Fragmented Nuclei

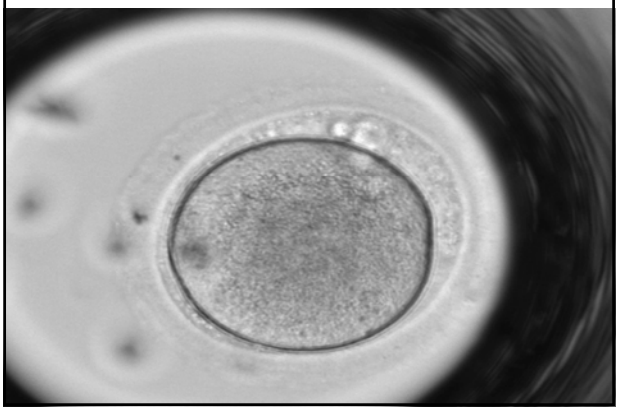


What are the origins of MN?
What happens with MN?

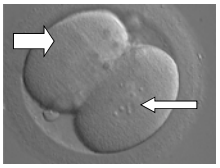
- **Fragmented Nuclei vs. Multi nucleation (Circay et al.,**
- **Karyokinesis without Cytokinesis (>1 cell)**
- **Duplication of the MTOC**

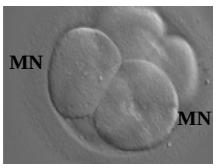
- **May act like 3PNs and explode**
- **Result in fragmented nuclei and aneuploidy**
- ***Meriano et al. 2004/ Hardarson et al. 2001/ Moriwaki et al. 2004/Pickering et al. 1995/ Scott et al, 2007/ Kligman et al. 1996, Sorimachi et al., 1998***

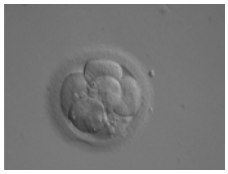
Exploding MN Cell



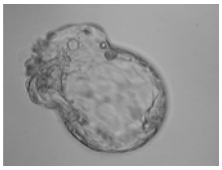
Complex Abnormal







Good Grade 8-cell



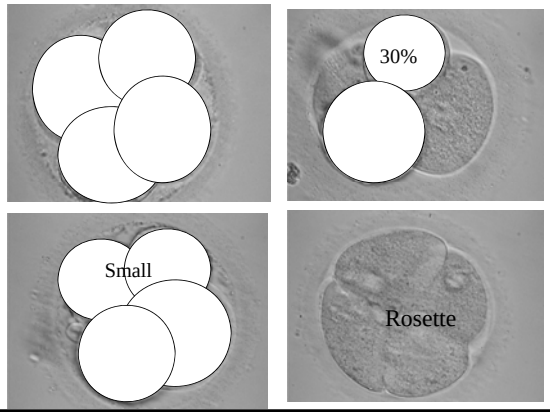
Good Blastocyst (D6)

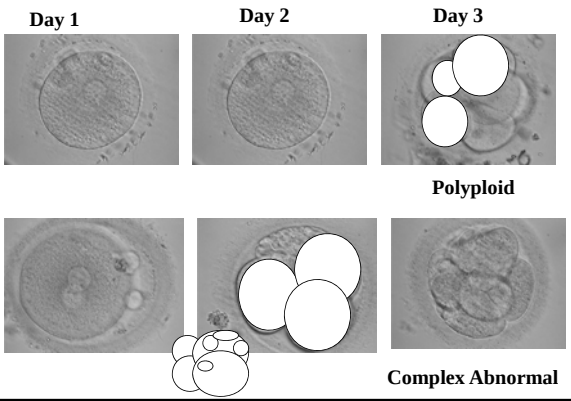
XXXY
1x 16
1x 21
3x 22
0x 15

Day 2 Cell Size
Why is it important?

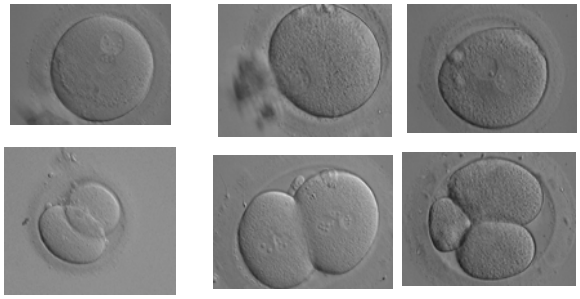
- **Polarity**
- **Distribution of cell components**
- **Embryo axes**
- **The meiotic and mitotic spindle**
- ***Hardarson et al., 2001; Van Royen et al., 2001; Hnida et al., 2004 Antczak , 1997 Roux et al.1995, Gardner R, 2066, Ciray et al, 2005***

4-cell Blastomer Morphology

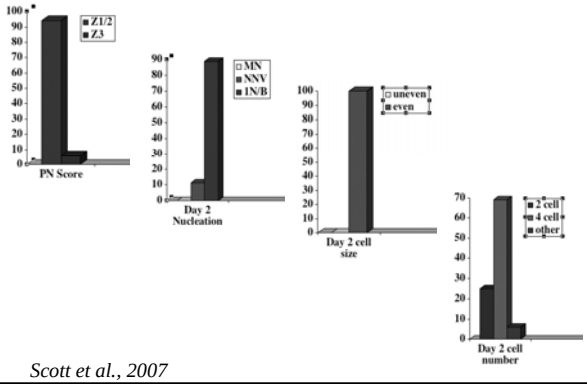




Day 1 and Day 2 Correlations

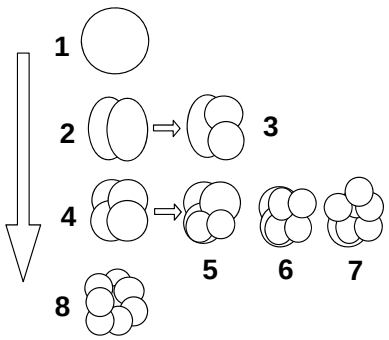


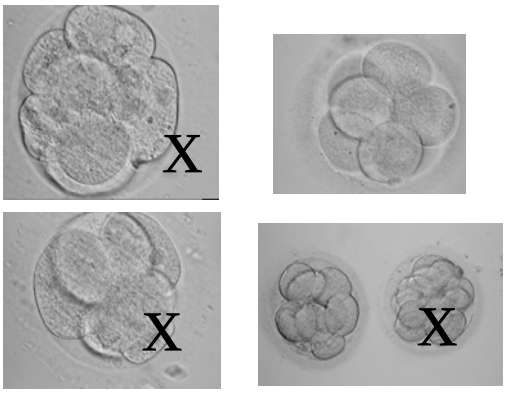
Deliveries according to early morphometrics



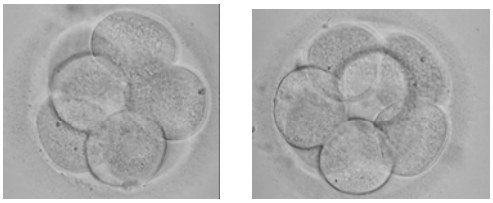
Scott et al., 2007

Relative cell sizes during mitotic divisions





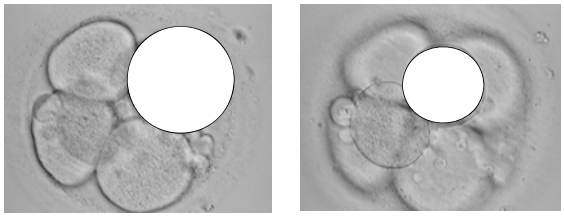
eSET Day 3 with *SES*



Z1 6/7
4 even
1n/b
Pregnant

Z2 6/7
4 even
1n/b
Delivered

eSET Day 3, SES Rules not followed



Z1 5/7
4 cell, 1 blastomer smaller
2 1n/b
2 NNV
Not Pregnant

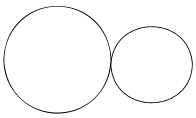
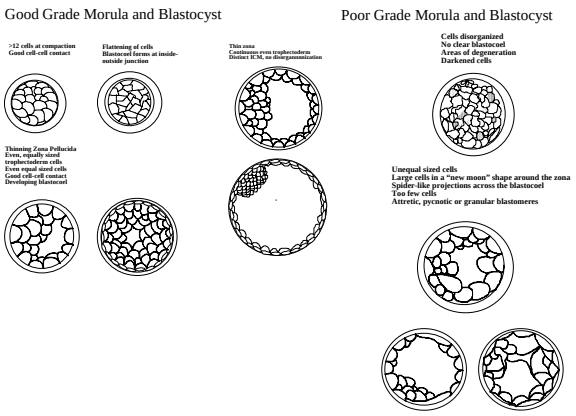
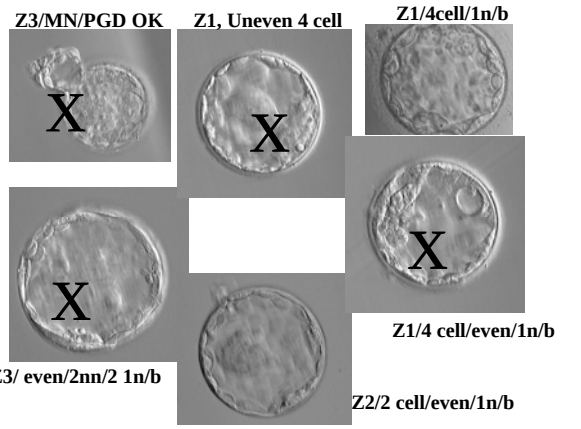
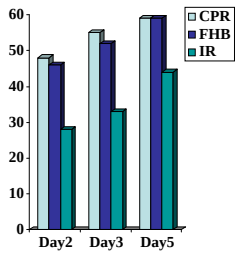


Figure 5





SES and Day of ET



- **CPR and FHB rates NS between the days of ET**
- **IR significant.**
- **This is due to poor vs. high prognosis patients**
- **However, Day 2 ET with selected embryos is as good as Day 3 or 5**
- **Mean # in ET 2.1 in all groups for all ages**

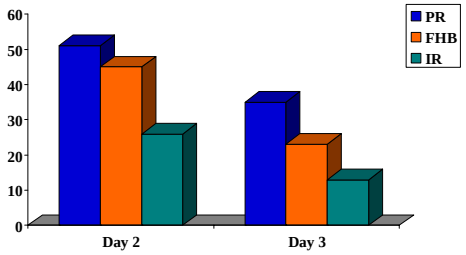
Day 2 ET Criteria
Based on previous cycles

- 3rd cycle without conception
- Previous cycle with <50% cleavage D2-D3
- Severe male factor with <50% fertilization
- Previous failed Tesa/Pesa cycle
- Previous failed PGS cycle

Prospective Randomized Trial
Day 2 vs. Day 3

- 3 or greater cycle, <38, no severe male factor or female pathology
- Intended 120 in each arm
- Interim analysis at 60 in each arm, because of a clinical outcome bias we were seeing

Data from PRT



Embryos Scored and Selected Sequentially

- Day 1 PN scoring
- Day 2
  - Cell number
  - Multinucleation
  - Cell size
- Day 3
  - Cell number
  - Fragmentation
- Day 5
  - Expansion
  - ICM
  - Trophectoderm

⇨ Need >3 Z1/Z2 in <35 >3 in >35 or repeat failure. If not = Day 2 ET

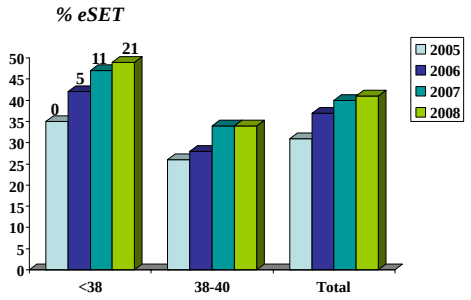
⇨ Need >3 4-cell/even/ 1 n/b in <35, that also pass D 1 >4 in >35 or repeat failure. If not = Day 2 ET

⇨ Need >3 8-cell/even from D1 + D2 gate in <35, >4 in >35 or repeat failure. If not = Day 3 ET

⇨ D5 ET when all the above is met on each day

Scott et al. 2007

Introduction and use of *SES*
Impact on Delivery Rates



2008 = Delivery and ongoing