

Supplementary S1: Morphologic and Morphokinetic grading Criteria and scoring system

Supplementary S1A: Morphologic grading Criteria for embryos from all Arms (1 to 3) of the study:

Time Points of assessment: (as per Desai 2014 as seen in Goodman 2016)

- 16 – 18 HPI
- 42 HPI (d2)
- 66 HPI (d3)
- +- 90 HPI
- 114 HPI
- +- 138 HPI

Overall Evaluated morphologic parameters:

- oocyte Polar body presence?
- 2PN post-fertilization?
- cell number (Optimal?)
- Cell size & symmetry
- percent fragmentation.
- Multinucleation
- +- Blastocyst check – Optimal parameters:
 - Stage of development: Early blastocyst, Blastocyst, Expanded, Hatched/Hatching
 - Inner Cell Mass (ICM) grade: A,B, C
 - Trophectoderm (TE) grade: A, B, C

Breakdown of assessment:

Pre-fertilization	PB presence?			
16 - 18 HPI	2 PN?			
42 HPI (d2)	# Cells?	% Fragmentation?	Multinucleation?	Appropriate cell size / symmetry?
66 HPI (d3)	# Cells?	% Fragmentation?	Multinucleation?	Appropriate cell size / symmetry?
+- 90 HPI (morula)	Stage?	Inner Cell mass (ICM) grade?	Trophectoderm (TE) grade?	
114 HPI (exp blast)	Stage?	Inner Cell mass (ICM) grade?	Trophectoderm (TE) grade?	
+- 138 HPI (hatched blast)	Stage?	Inner Cell mass (ICM) grade?	Trophectoderm (TE) grade?	

Optimal Parameters:

- Day 0 check: oocyte parameters:
 - Examination of polar body presence
- Day 1 / fertilization check:
 - 2 Pronuclei present
- Day 2 check:
 - Four cells

- Minimal fragmentation (<10%)
 - No multinucleation
 - Symmetry at 4 cells
- Day 3 check:
 - Eight cells
 - Minimal fragmentation (<10%)
 - Symmetry at 8 cells
- Blastocyst check:
 - Stage of development: Early blastocyst, Blastocyst, Expanded, Hatched/Hatching
 - Inner Cell Mass (ICM) grade:
 - A (Good): Prominent, easily discernible, with many cells that are compacted and tightly adhered together
 - B (Fair): Easily discernible, with many cells that are loosely grouped together
 - C (Poor): Difficult to discern, with few cells
 - Trophectoderm (TE):
 - A (Good): Many cells forming a cohesive epithelium
 - B (Fair): Few cells forming a loose epithelium
 - C (Poor): Very few cells
- **Embryos graded on day 3 or day 5 (predetermined) and transferred according to preferential morphological grading (if in Arm 1 or 3)**

Supplementary S1B: Morphokinetic grading Criteria and scoring system

Morphokinetic Criteria recorded for ALL embryos in TLI (Arm 2&3):

(t0 = time of insemination)

- | | |
|--|---|
| - tPNf : time to pronuclear fading | - tBL : time to blastocyst |
| - t2 : time to 2 cells | - tEBL : time to expanded blastocyst |
| - t3 : time to 3 cells | - cc2 : duration of the 2 nd cell cycle (t3-t2) |
| - t4 : time to 4 cells | - cc3 : duration of the 3 rd cell cycle (t5-t3) |
| - t5 : time to 5 cells | - t4int : interval between 4 and 5 cells (t5-t4) |
| - t8 : time to 8 cells | - t5-t2 : interval between 2 cells and 5 cells |
| - t9 : time to 9 cells | - s1 : t2-tPNf |
| - tMor : time to morula | - s2 : t4-t3 |
| - tSB : time to start of blastulation | - s3 : t8-t5 |

Other qualitative Data:

- **Multinucleation (MU):**
 - more than one nuclei in a cell observed in 2–4-cell-stage embryos
 - recorded based on the number of cells affected and the number of nuclei per cell
- **Reverse Cleavage (RCLV):**
 - reabsorption of a blastomere after previous visual confirmation.
- **Direct uneven cleavage (DUC):**
 - evidence of one blastomere directly splitting to three
- **Irregular Division (ID)**
 - 1→3 Direct or rapid (>5h) cleavage from 1 to 3 cells, referred to as irregular division pattern
 - 2→5 Direct or rapid (>5h) cleavage from 1 to 3 cells, referred to as irregular division pattern

Morphokinetic Criteria, additional grading for Embryos from Arm 2 (and Arm 3 retrospectively):

HPI = Hours post-insemination

- **POSITIVE points:**
 - t5 = 45.8–57.0h HPI (+1)
 - s2 = 0-0.1h (+1)
 - s3 = 1.4-7h (+1)
 - tSB < 100 HPI (+1)
- **NEGATIVE points:**
 - cc2 < 5h (-1)
 - Multinucleation (-0.5)
 - Irregular division (-0.5)

- SCORE=_____

Embryo initially screened by morphology

THEN embryos with the highest morphokinetic score (MAX of 4 and MIN of -2.0) selected for transfer.