CULTURE AND EVALUATION OF BLASTOCYST MORPHOLOGY

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OVERVIEW - I

- **Blastocyst development**
  - Compaction
  - Cavitation
  - Differentiation
- **Blastocyst culture**
  - Effect of culture media
  - Effect of atmosphere

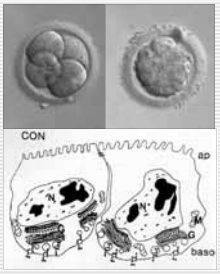
OVERVIEW - II

- **Blastocyst morphology evaluation**
  - Grading systems
  - Blastocyst quality and implantation
  - Blastocyst quality and monozygotic twins

BLASTOCYST DEVELOPMENT: Mechanism of compaction

Polarization of outer cells:

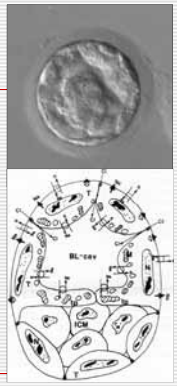
- Migration of nucleus and mitohondria to basal membrane.
- Tight junctions, desmosomes between cells.
- Na/K ATPase on basolateral membrane for ionic gradient.



(Watson, 1992)

Mechanism of cavitation

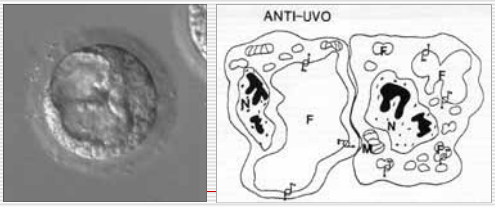
- Na/K ATPase ensures ionic gradient on basolateral membrane.
- Osmotic presure - water fills blastocoel.
- Tight junctions and desmosomes control leakage of fluid from blastocoel.
- Growth factors regulate the mechanism of cavitation.



(Watson, 1992)

Mechanism of abnormal cavitation

- Absence of tight junctions (no junctional proteins) between blastomeres.
- Abnormal polarization and organele distribution.
- Growth of vacuoles instead of blastocoel.



(Watson, 1992)

BLASTOCYST CULTURE: Embryo physiology

- Pre-compaction
  - Low biosyntetic rate
  - Low respiratory capacity
  - Pyruvate preffered nutrient
  - Maternal genome
  - Individual cells
- Post-compaction
  - High biosyntetic rate
  - High respiratory capacity
  - Glucose preffered nutrient
  - Embryonic genome
  - Transporting epithelium

Blastocyst Culture Media:

- Sequential Culture Media:

COOK IVF	Sydney IVF Cleavage Medium / Sydney IVF Blastocyst Medium
Vitrolife	G-1 v5 Plus / G2- v5 Plus
Origio – Medicult	BlastAssist System (Medium 1 / Medium 2) EmbryoAssist / BlastAssist ISM1 / ISM2 IVF Universal / ISM1 / BlastAssist
Fertipro	Ferticult / Ferticult G3
Irvine Scientific	ECM / Multiblast Medium
InVitro Care Inc.	IVC-One / IVC-Two / IVC-Three
Cooper Surgical - Sage	Quinn's Advantage Cleavage Medium / Quinn's Advantage Blastocyst Medium

- Mono Culture Medium:

IVFonline	Global (KSOMaa)

Comparison of blastocyst culture media by sibling oocyte study *(Maribor results)*

- Two sequential media from different companies

	Company A	Company B
Oocytes	**421**	**415**
Optimal day-2 embryos	**60.4%**	**57.4%**
Optimal day-3 embryos	**31.4%**	**44.4%***
Blastulation rate	**59.6%**	**55.6%**
Optimal blastocysts on day 5	**29.2%**	**17.7%****

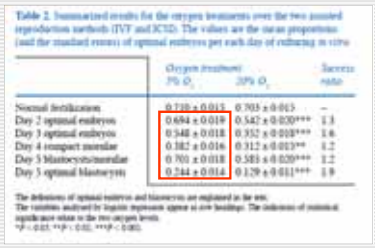
Effect of culture media

- Several types of commercially available media for prolonged embryo culture.
- Comparative studies:
  - Differences in dynamics of embryo development.
  - Differences in blastocyst quality.
  - No differences in pregnancy rates (cumulative ?).
- Lack of **robust multicentre randomized clinical trials** for comparing the influence of different culture media on human embryo development and clinical outcomes (*Biggers and Summers*, F&S, 2008; *Blake et al.*, Cochrane database, 2007).
  - Heterogenous patient populations, cycle preparations, laboratory techniques, embryo morphology evaluations, quality assurance…

Effect of culture atmosphere
(5% O_2 vs. 20% O_2)

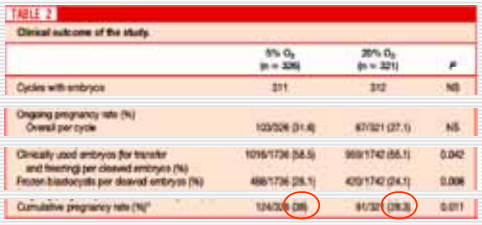
- Oxygen tension in the oviduct and uterus varies between 2 and 8%.
  - *(Fischer and Bavister 1993).*
- More reactive oxygen species (ROS) are generated in culture systems using high 20% oxygen.
  - *(Goto et al., 1993).*
- ROS affect embryo development.
  - *(Noda et al., 1994; Yang et al., 1998 ; Johnson and Nasr-Esfahani, 1994 ;Catt and Henman, 2000).*
- Contradictory conclusions from studies on O_2 effect on human embryos.
  - *(Dumoulin et al., 1995; 1999; Catt and Henman 2000; Bahceci et al., 2005; Waldenstrom et al., 2009).*

Effect of culture atmosphere
(5% O_2 vs. 20% O_2)
a prospective study on sibling oocytes



Kovačič and Vlaisavljević, RBMOnline, 2008

Effect of culture atmosphere
(5% O_2 vs. 20% O_2)
a prospective study on randomized ICSI cycles



Kovačič et al., F&S, in press

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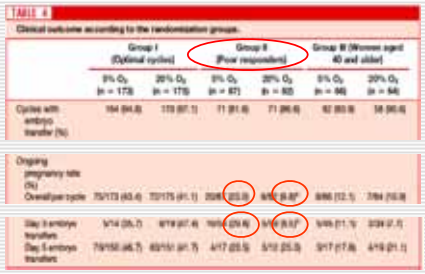


Kovačič et al., F&S, in press

Effect of culture atmosphere
(5% O_2 vs. 20% O_2)

- **Advantages of embryo culturing at reduced O_2:**
  - Better embryo development from day 2 to day 5.
  - Higher cumulative pregnancy rate.
  - Higher pregnancy rate in low responder cycles.

Blastocyst development in optimal vs. suboptimal cycles

Blastocysts in optimal cycles:

- Higher blastulation rate / lower transfer cancellation rate.
- Selection of 1 or 2 top quality blastocysts for transfer between optimal ones.

(Most of the reports in the literature)

Blastocysts in all cycles:

- Higher rate of total embryo arrest and cancellation of transfer.
- Selection of the best day-5 embryo for transfer between available ones.

(Vlaisavljevic et al., 2001; Kovačič et al., 2002)

BLASTOCYST MORPHOLOGY
Blastocyst 3 part scoring system

Gardner & Schoolcraft, 1999 **:**

Expansion and hatching status

1 Blastocoel cavity less than half the volume of the embryo

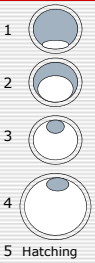
2 Blastocoel cavity more than half the volume of the embryo

3 Full blastocyst, cavity completely filling the embryo

4 Expanded blastocyst, cavity larger than the embryo, with thinning of the shell

5 Hatching out of the shell

6 Hatched out of the shell



Blastocyst 3 part scoring system

Gardner & Schoolcraft, 1999 **:**

Inner cell mass (ICM) score

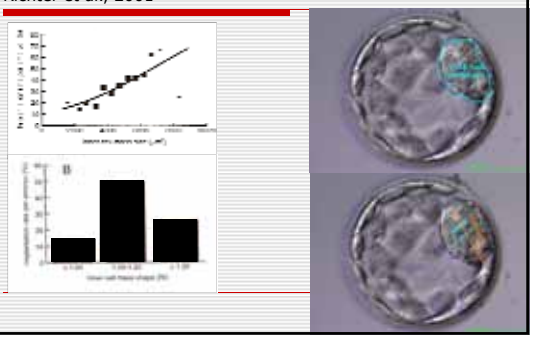
A Many cells, tightly packed
B Several cells, loosely grouped
C Very few cells

Trophectoderm (TE) score

A Many cells, forming a cohesive layer
B Few cells, forming a loose epithelium
C Very few large cells

Blastocyst evaluation by ICM morphometry

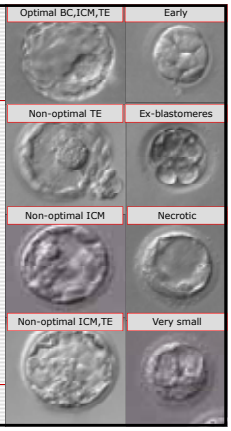
Richter et al., 2001



Blastocyst grading system

Kovačič et al., 2004:

- Definition of 8 morphology types of morulae and blastocysts, most frequently observed on day 5.
  - Blastocoel expansion,
  - ICM shape,
  - TE cohesivenes,
  - Amount of excluded blastomeres from blastocyst



Blastocyst grading system

Calculation the live birth rate per each blastocyst type.

Morphologic type of blastocyst	*Transferred blastocysts*	*Transferred blastocysts with known outcome*	*Implantations (%)*[a]	*Miscarriages (%)*	*Births (%)*[b]
Optimal					
B1	**766**	**706**	**366 (51.8)**	**47 (12.8)**	**319 (45.2)**[c]
Suboptimal					
B2	**71**	**61**	**22 (36.1)**	**2 (9.1)**	**20 (32.8)**
B3	**178**	**145**	**44 (30.3)**	**5 (11.4)**	**39 (26.9)**
B4	**111**	**87**	**25 (28.7)**	**5 (20)**	**20 (23)**
B5	**73**	**62**	**16 (25.8)**	**5 (31.3)**	**11 (17.7)**
B6	**87**	**72**	**17 (23.6)**	**5 (29.4)**	**12 (16.7)**
B7	**26**	**26**	**3 (11.5)**	**1 (33.3)**	**2 (7.7)**
B8	**84**	**82**	**6 (7.3)**	**5 (83.3)**	**1 (1.2)**
Total	**1396**	**1241 (88.9)**	**527 (41.3)**	**75 (14.2)**	**424 (34.2)**

a P<0.0001; b P<0.0001

Kovačič et al., RBM Online, 2004

Blastocyst grading system

Grading the blastocyst types by their implantation ability.

Kovačič et al., RBM Online, 2004

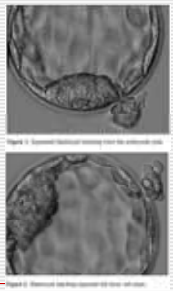
Some morphologic characteristics of blastocysts with impact on implantation

- **Location of herniation**

Table 1. Implantative behaviour of blastocysts hatching at different spots around the zona pellucida

	Study group hatching from ICM	Mixed group	Control group hatching from TE
n	29	26	83
Clinical PR	21 (72.4)	16 (61.5)	27 (55.9)
MFR	5 (23.8)	5 (~9.2)	4 (14.8)
IR	28/[illegible] (66.7)[A,B]	21/52 (40.4)[A]	31/[illegible] (40.8)[B]

[A] p=0.009; [B] p=0.0[illegible]



Ebner et al., JTGGA, 2004

Other morphologic characteristics of blastocysts

- **Cytoplasmic strings** *(Scott, 2000)*
- **Vacuoles** *(Ebner et al., 2005)*
- **Included blastomeres**

Prolonged embryo culture to day 6 ?

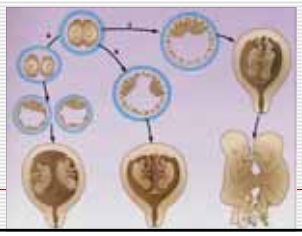
- **23.9%** of compact day-5 embryos reach the stage of expanded blastocyst with round ICM on day 6 ***(our results)***.
- Higher implantation ability of day-5 vs. day-6 blastocysts:
  - **37.4% vs. 20.6%**
    - *(Shapiro et al., F&S, 2001).*
  - **22.1% vs. 3.6%**
    - *(Barrenetxea et al, F&S,2005).*
  - **35.2% vs. 21.2%** in fresh transfers
    37.4% vs. 37.7% (D6 in frozen cycles)
    - *(Shapiro et al., F&S, 2008)*
- Better implantation if day-6 blastocysts are frozen and transferrred in one of the next fresh cycles.

Morphological characteristics of frozen / thawed blastocysts

- **Effect of freezing on morphology:**
  - Collapsed blastocoel
  - Necrosis
  - Damaged cells
- **Selection criteria for suitability for transfer:**
  - Reexpansion
  - ICM normality

Blastocyst and monozygotic twins

- **5.0%** *Behr et al.,2000.*
- **3.9%** *da Costa et al.,2001.*
- **3.4%** *Cornell programme.*



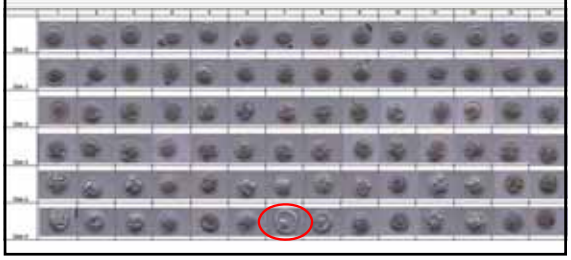
Can we predict monozygotic twins from blastocyst morphology?

- Embryos which resulted monozygotic twins
  - **1.9% MZT** per pregnancy in SBTs *(our results)*

  - **?** per double blastocyst transfer

How to evaluate day-5 embryos ?

- **Short culture**
  - precise sequential embryo evaluation required (each day at the same time).
- **Prolonged culture**
  - sequential evaluation is recommended but selection only on day-5 is enough for clinical use.



CONCLUSIONS: How to evaluate day-5 embryos ?

- Evaluate day-5 embryos after 152 hours post hCG under the microscope at at least 200x magnification.
- Turn the embryos during evaluation.
- Pay regard that ICM morphology is the most predictable parameter for the live birth.
- Ascertain that the structure within the blastocyst is really an ICM (not a blastomere).
- Take into account that blastocyst can be collapsed during the observation.
- Suboptimal blastocysts or morulae should be evaluated by taking into account the dynamics of development, before the final selection decision is done.
- Allow >10 cell-embryos or compact embryos on day 5 to be cultured for an additional day.