Correlation between Day 2 and Day 3 Static Morphology on Pregnancy Outcome after Single Blastocyst Transfer

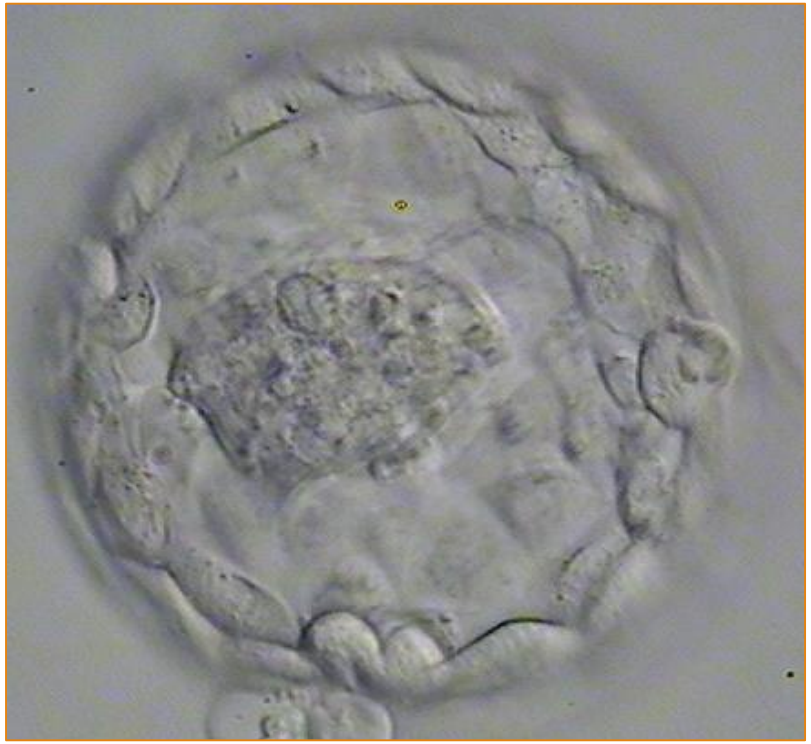
ALISON BARTOLUCCI, MS TS

CENTER FOR ADVANCED REPRODUCTIVE SERVICES

UNIVERSITY OF CONNECTICUT HEALTH CENTER

UCONN HEALTH

Does my past predict my future?



Does cleavage stage morphology predict pregnancy outcome when we transfer at the blastocyst stage?

We know morphology on the day of transfer can predict livebirth

Day 3 cell number, fragmentation and symmetry are predictive of livebirth from Day 3 transfers[1].

Blastocyst expansion, inner cell mass, and trophectoderm quality are predictive of livebirth from Day 5 transfers[1].

[1]Luke, B., M. B. Brown, J. E. Stern, S. K. Jindal, C. Racowsky and G. D. Ball (2014). "Using the Society for Assisted Reproductive Technology Clinic Outcome System morphological measures to predict live birth after assisted reproductive technology." Fertil Steril **102**(5): 1338-1344.

We know cleavage stage morphology can predict blastocyst development

- **Individual and cumulative scores from embryos on day 2 and day 3 have been shown to predict development to the blastocyst stage**
  - **Rijinders et al 1998; Racowsky et al 2000; Shapiro et al 2000; Fisch et al 2001; Langley et al 2001; Neuber et al 2003; Rienzi et al 2005; Braga et al 2014**

Is cleavage stage morphology still important once we have blastocysts ?

Are static morphology data from Day 2 and Day 3 important factors to consider when selecting blastocysts for transfer?

Is the cleavage stage morphology still predictive of ongoing pregnancy when controlling for the blastocyst quality?

Is it necessary to take the embryos out of the incubator on day 2 and/or day 3 if culturing to the blastocyst stage?

Prior studies

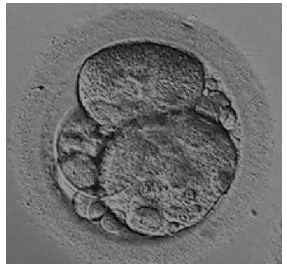
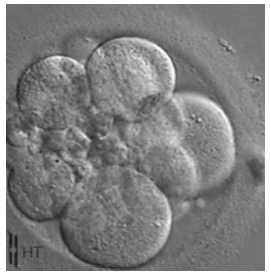
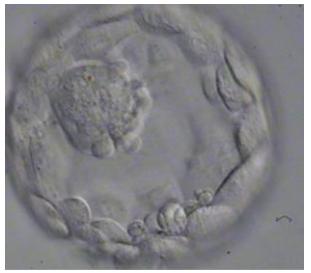
Racowsky et al 2003 – slightly significant correlation between day 3 cell number and pregnancy outcome after transfer of 194 expanding and fully expanded blasts.

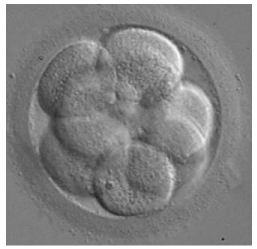
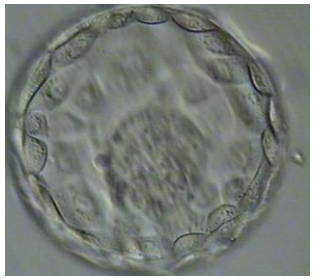
Guerif et al 2007– implantation and livebirth after blastocyst transfer not influenced by early cleavage, Day 2 cell number, PN score or fragmentation.

Guerif et al 2010 – no significant correlation between PN morphology, early cleavage or day 2 morphology and pregnancy outcome after adjusting for blastocyst quality in 407 single blast transfers.

Objective of this study

Determine significance of Day 2 and Day 3 static morphology on pregnancy outcome after transfer at the blastocyst stage using a large multi-clinic database.

Materials and methods

Analysis of de-identified data from 52 clinics within the US and Canada provided by eIVF ®.

8352 single blastocyst transfers occurring between January 1 2010 – September 30 2016.

Primary outcome measure = Ongoing pregnancy, defined as positive fetal heart tones beyond 12 weeks gestation.

Inclusion and exclusion criteria

INCLUSION CRITERIA

Patients 18-40

Single embryo transfer

Day 5 Transfer

EXCLUSION CRITERIA

Donor oocyte

Gestational carrier

Thawed oocytes

Preimplantation genetic testing (PGT)

Surgically retrieved sperm

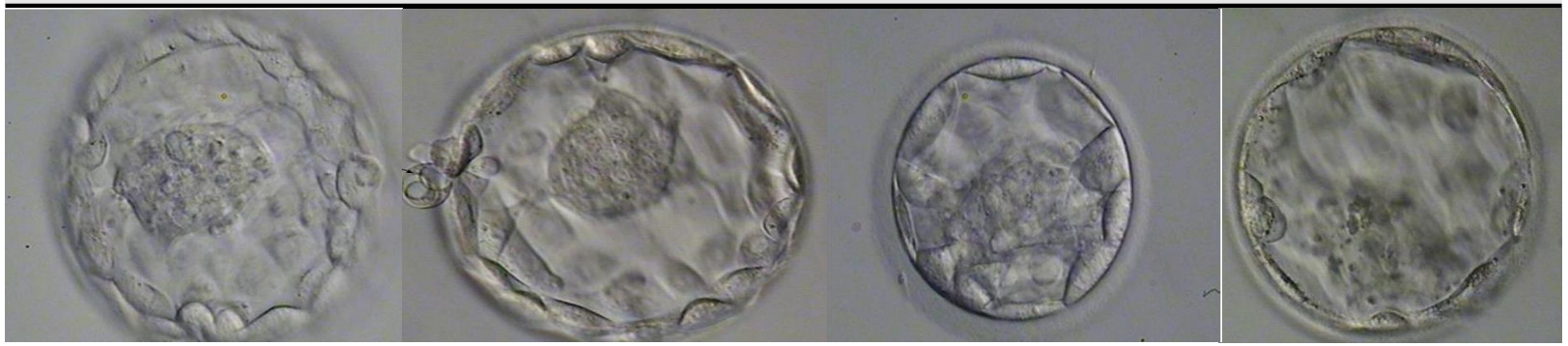
Cleavage stage morphology categories

SART[1] grading parameter	Data analysis groups: Day 2	Data analysis groups: Day 3
Cell number	<4, 4, >4	<8, 8, >8
% Fragmentation	0-10%, 11-25%, >25%	0-10%, 11-25%, >25%
Symmetry	perfect, moderately asymmetric, severely asymmetric	perfect, moderately asymmetric, severely asymmetric

[1] Racowsky, C., M. Vernon, J. Mayer, G. D. Ball, B. Behr, K. O. Pomeroy, D. Wininger, W. Gibbons, J. Conaghan and J. E. Stern (2010). "Standardization of grading embryo morphology." Fertility and Sterility **94**(3): 1152-1153. 0

Blastocyst morphology categories

SART grading parameter	Data analysis groups	Gardner score[1] equivalent
Degree of expansion	Early, expanding, expanded, hatching or hatched	1-2, 3, 4, 5-6
Inner cell mass	Good, fair, poor	A,B,C
Trophectoderm	Good, fair, poor	A,B,C



[1]Gardner, D. K. and W. B. Schoolcraft (1999). "Culture and transfer of human blastocysts." Curr Opin Obstet Gynecol **11**(3): 307-311.

Data analysis

Performed a univariate analysis to identify significant relationships between patient and cycle variables and pregnancy outcome in this dataset.

Analyzed day 2, day 3 and day 5 morphology categories in univariate analysis.

Performed multivariate regression and controlled for significant patient and cycle variables. Odds ratios (OR) and 95% confidence intervals (CI) generated for each factor.

SPSS (IBM, Armonk, NY) used for all analyses with $\alpha=0.05$

UNIVARIATE ANALYSIS

Patient and Cycle Variables

Variable	Category	Ongoing Pregnancy OR (95% CI)	P-value
Age	<35	1	
	35-37	0.67 (0.60 – 0.74)	<0.001
	38-40	0.44 (0.37 – 0.52)	<0.001
AMH	>3.5	1	
	1.5-3.5	0.96 (0.83 – 1.12)	0.599
	1.0-1.49	0.85 (0.67 – 1.07)	0.159
	0.5-0.99	0.59 (0.46 – 0.75)	<0.001
BMI	18.5-24.99	1	
	<18.5	1.02 (0.78 - 1.33)	0.887
	25-29.99	0.93 (0.71 – 1.22)	0.608
	>29.99	0.69 (0.53 – 0.92)	0.012
Embryos Frozen	None frozen	1	
	1 or more frozen	2.32 (2.08 – 2.59)	<0.001

Blastocyst morphology

Variable	Category	Ongoing Pregnancy OR (95% CI)	P-value
Day 5 Expansion (n=6900)	Fully exp. Blast	1	
	Early blast	0.389 (0.333 – 0.456)	<0.001
	Expanding blast	0.687 (0.598-0.789)	<0.001
	Hatching/hatched	1.008 (0.885-1.147)	0.907
Day 5 ICM (n=8351)	Good	1	
	Fair	0.674 (0.605-0.752)	<0.001
	Poor	0.436 (0.315 – 0.604)	<0.001
Day 5 TE (n= 8351)	Good	1	
	Fair	0.669 (0.604-0.742)	<0.001
	Poor	0.398 (0.314 – 0.505)	<0.001

Day 2 morphology

Variable	Category	Ongoing Pregnancy OR (95% CI)	P-value
Day 2 Cell Number (n=4450)	4 cells	1	
	< 4 cells	0.935 (0.773 – 1.132)	0.491
	> 4 cells	0.907 (0.745 – 1.105)	0.334
Day 2 Fragmentation (n=1278)	0-10%	1	
	11-25%	0.947 (0.646 – 1.387)	0.779
	> 25%	0.762 (0.214 – 2.715)	0.675
Day 2 Symmetry (n=1011)	Perfect	1	
	Moderate	0.783 (0.605 – 1.1013)	0.063
	Severe	0.794 (0.347 – 1.817)	0.585

Univariate analysis shows day 2 parameters not predictive of pregnancy outcome after blastocyst transfer

Day 3 morphology

Variable	Category	Ongoing Pregnancy OR (95% CI)	P-value
Day 3 Cell Number (n= 7640)	8 cells	1	
	< 8 cells	0.658 (0.577 – 0.751)	<0.001
	> 8 cells	1.225 (1.094 – 1.372)	<0.001
Day 3 Fragmentation (n= 3113)	0-10%	1	
	11-25%	0.815 (0.653 – 1.017)	0.070
	> 25%	0.747 (0.329 – 1.696)	0.486
Day 3 Symmetry (n= 2197)	Perfect	1	
	Moderate	0.860 (0.723 -1.024)	0.090
	Severe	0.571 (0.333-0.979)	0.041

MULTIVARIATE REGRESSION ANALYSIS

Multivariate regression

Variable	Category	Ongoing Pregnancy OR (95% CI)	P-value
Day 5 Expansion	Fully exp. Blast	1	
	Early blast	0.651 (0.514 – 0.824)	<0.001
	Expanding blast	0.816 (0.695 – 0.957)	0.013
	Hatching/hatched	0.987 (0.853 – 1.143)	0.861
Day 5 TE	Good	1	
	Fair	0.763 (0.673 – 0.866)	<0.001
	Poor	0.563 (0.425 – 0.745)	<0.001

Controlling for the significant patient and cycle variables – age, BMI, AMH and embryos frozen

Multivariate regression

Variable	Category	Ongoing Pregnancy OR (95% CI)	P-value
Day 3 Cell Number	8 cells	1	
	< 8 cells	0.771 (0.217 -2.747)	0.689
	> 8 cells	0.840 (0.347 – 2.036)	0.699

Controlling for Day 5 morphology

Multivariate regression results

MORPHOLOGY ASSOCIATED WITH ONGOING PREGNANCY

Day 5 Expansion

Day 5 Trophectoderm Score

MORPHOLOGY NOT ASSOCIATED WITH ONGOING PREGNANCY

Day 2 Morphology

Day 3 Morphology

Day 5 ICM

Study limitations

Retrospective design

Variation from large number of clinics

- **Stimulation and lab protocols**
- **Timing of observations**

Limited morphology data

No multi-nucleation data

Media and incubator type not indicated

Conclusions

What do these results suggest?

Static morphology from Day 2 and Day 3 are not predictive of pregnancy outcome after transfer at the blastocyst stage.

The influence of blastocyst morphology on pregnancy outcome is due to the parameters on Day 5, not that of earlier morphology scores.

It may not be necessary to take the embryos out of the incubator on day 2 and/or day 3 if culturing to the blastocyst stage.

Does this suggest cleavage stage morphology is irrelevant?

- These data show that static “snapshots” from Day 2 and Day 3 are not associated with ongoing pregnancy after blastocyst transfer.

- Time-lapse microscopy has revealed that embryo cleavage is dynamic and time sensitive.

- Proper prospective trials may show that early morphometric and morphokinetic events observed along a continuous timeline can be predictive of pregnancy even after controlling for blastocyst quality.

But in the absence of time-lapse?

While there may be no benefit to performing static morphology observations at the cleavage stage, there may be a benefit to not performing them.

Uninterrupted culture from day 1 to day 5 may result in improved blastocyst development and quality[1]

- **Less handling**
- **Reduced exposure to fluctuations in temperature and ph.**

Prospective trial needed

[1]Zhang, J. Q., X. L. Li, Y. Peng, X. Guo, B. C. Heng and G. Q. Tong (2010). "Reduction in exposure of human embryos outside the incubator enhances embryo quality and blastulation rate." Reprod Biomed Online **20**(4): 510-515.

Thank you