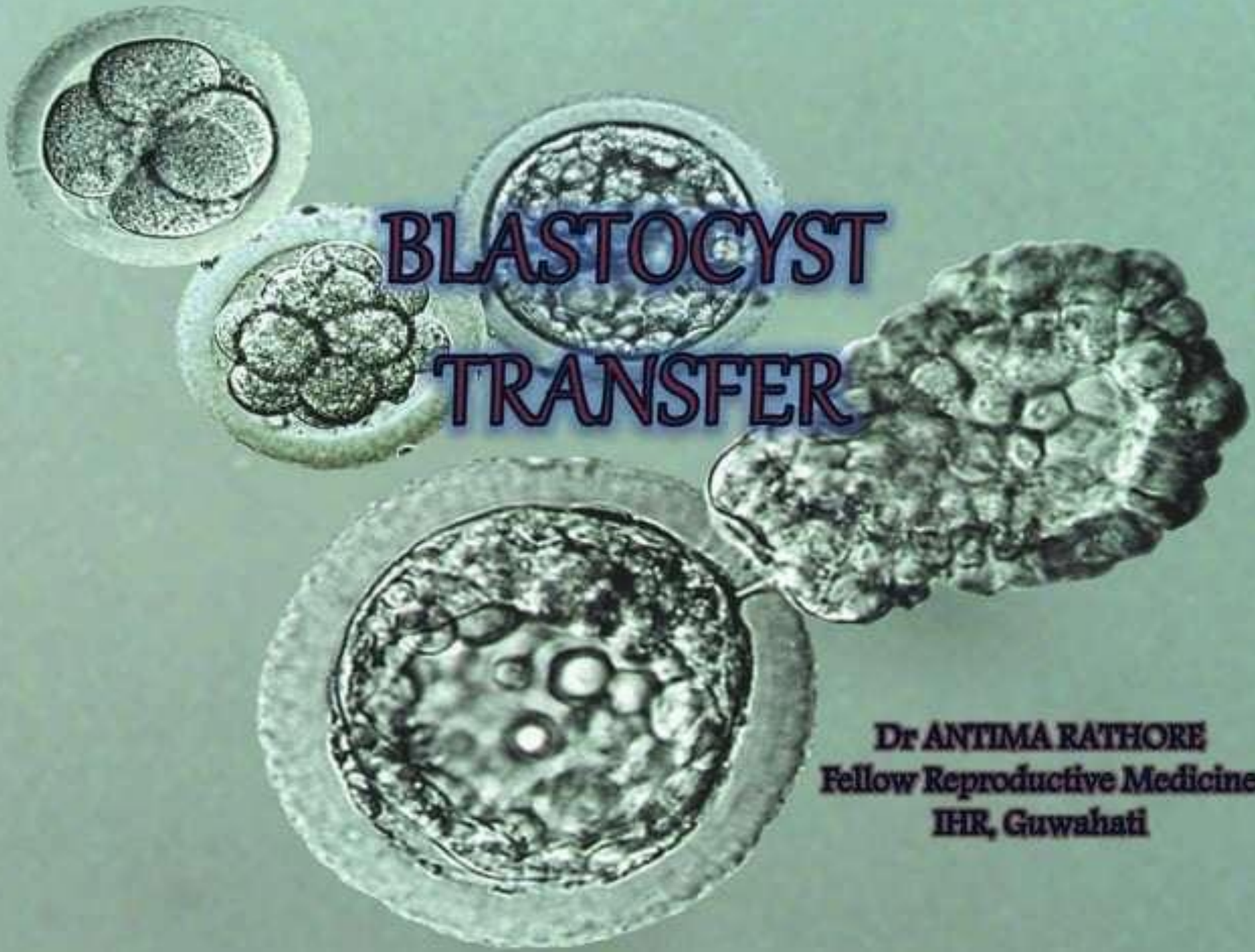


BLASTOCYST TRANSFER

Dr ANTIMA RATHORE
Fellow Reproductive Medicine
IHR, Guwahati

EMBRYO DEVELOPMENT

FROM EGG TO EMBRYO

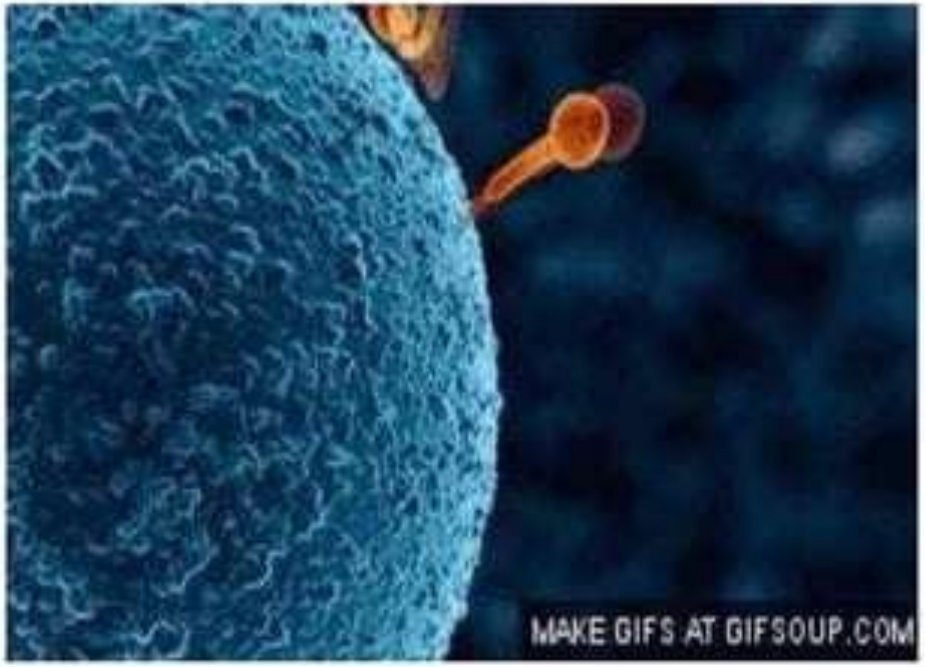
EMBRYO DEVELOPMENT

Fertilisation

Cleavage

Blastulation

Implantation



Embryo development Cont.

2PN

↓

4 CELL

↓

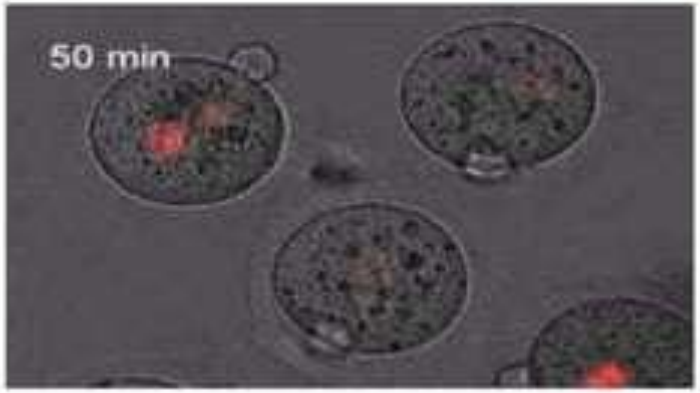
8 CELL

↓

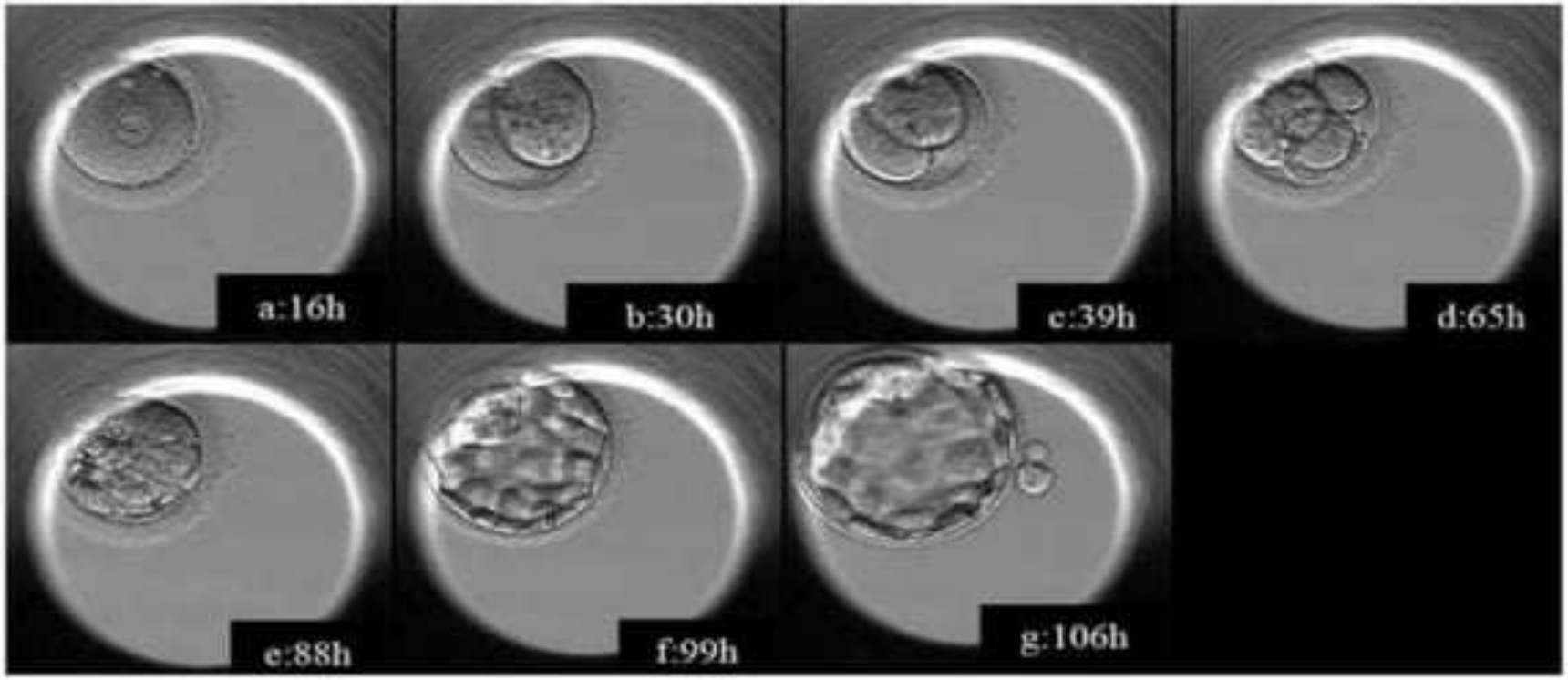
MORULA

↓

BLASTOCYST



EMBRYO DEVELOPMENT



Compacted

Stage I

Early Blastocyst

Stage II

BLASTOCYST

Stage III

Expanded Blastoyst

Stage IV

Hatching

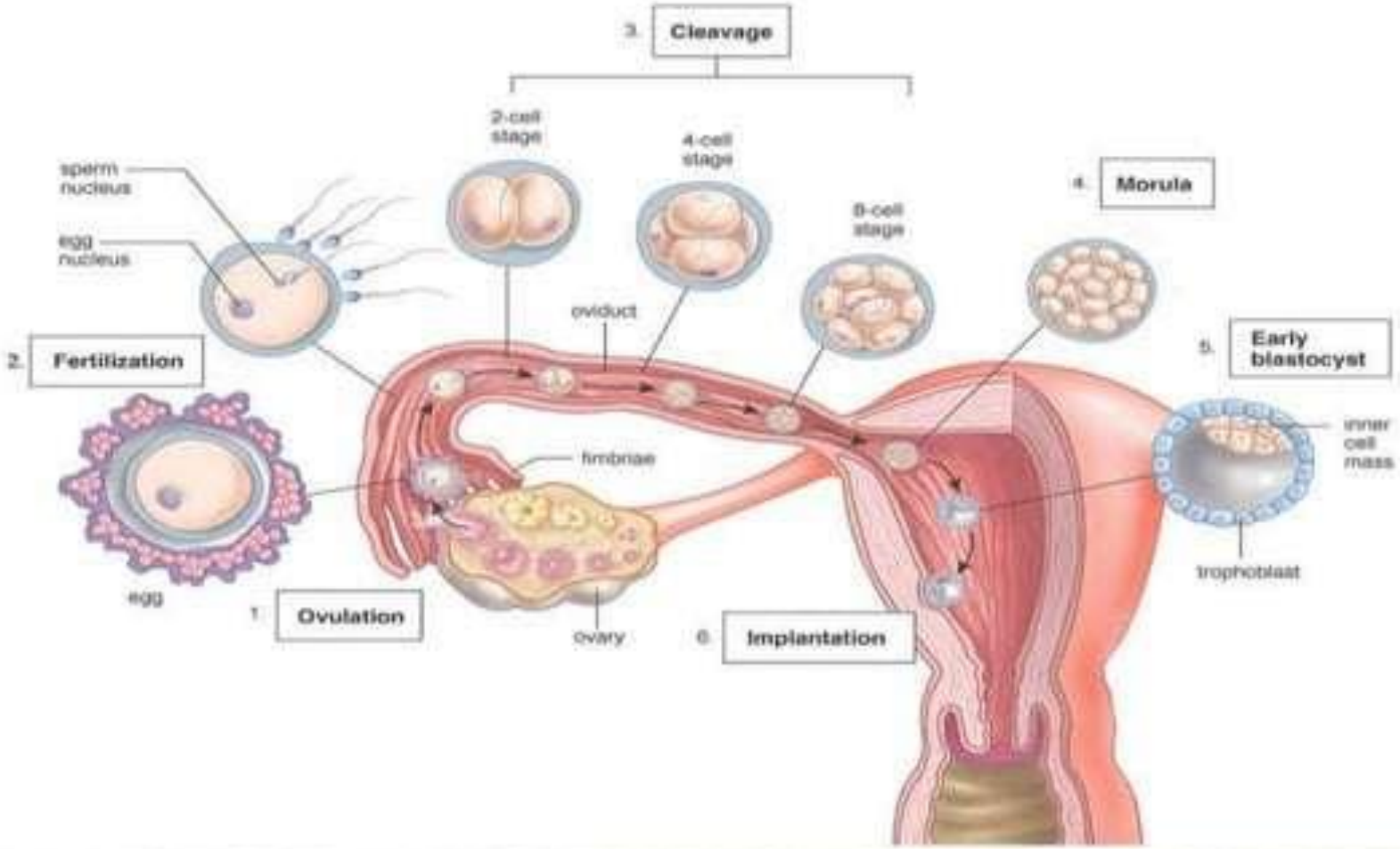
HATCHED

STAGES OF BLASTOCYST

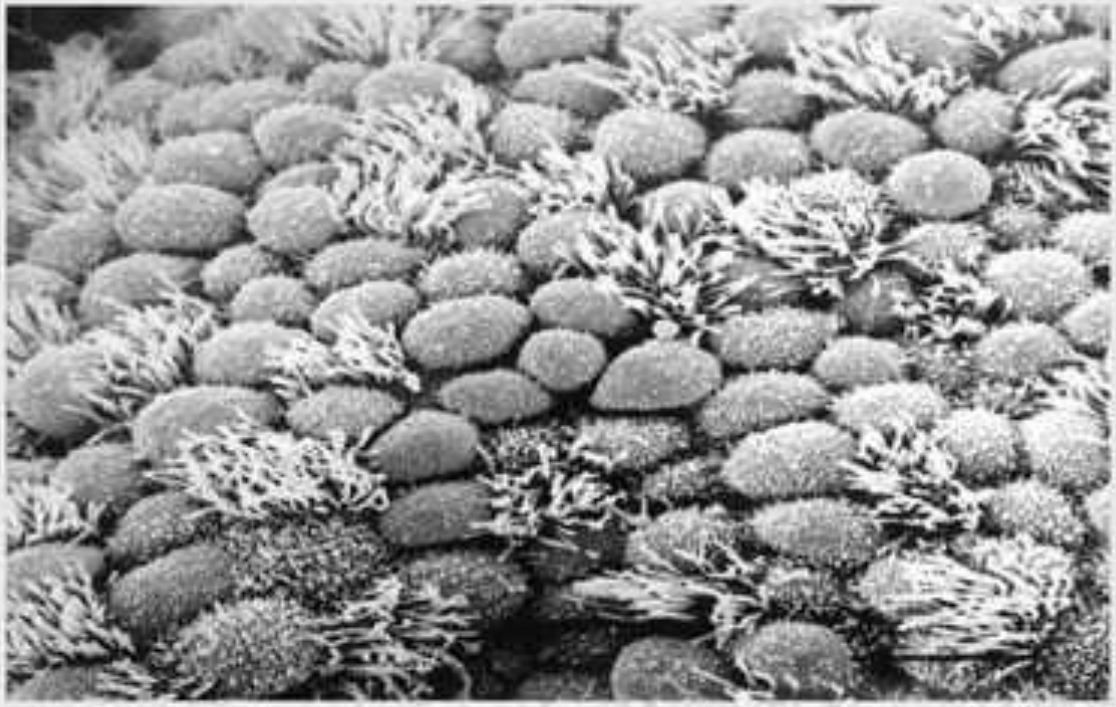
Stage V

& VI

EMBRYO DEVELOPMENT



IMPLANTATION



IMPLANTATION

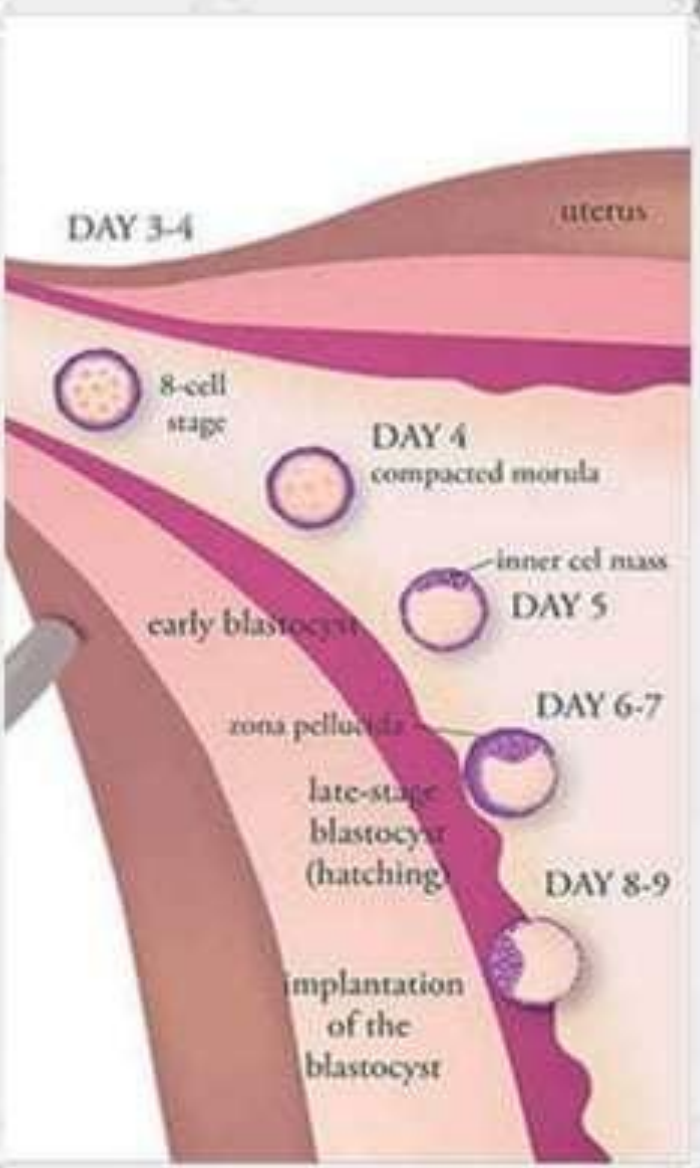
4th day Morula reaches the uterine cavity

5th day – Zona pellucida degeneration starts

6th day – blastocyst adheres to the endometrial epithelium.

7th day – T*rophoblast* differentiated into **Cytotrophblast** & **Syncytiotrophoblast**

8th day – blastocyst superficially embeds in the compact layer of the endometrium

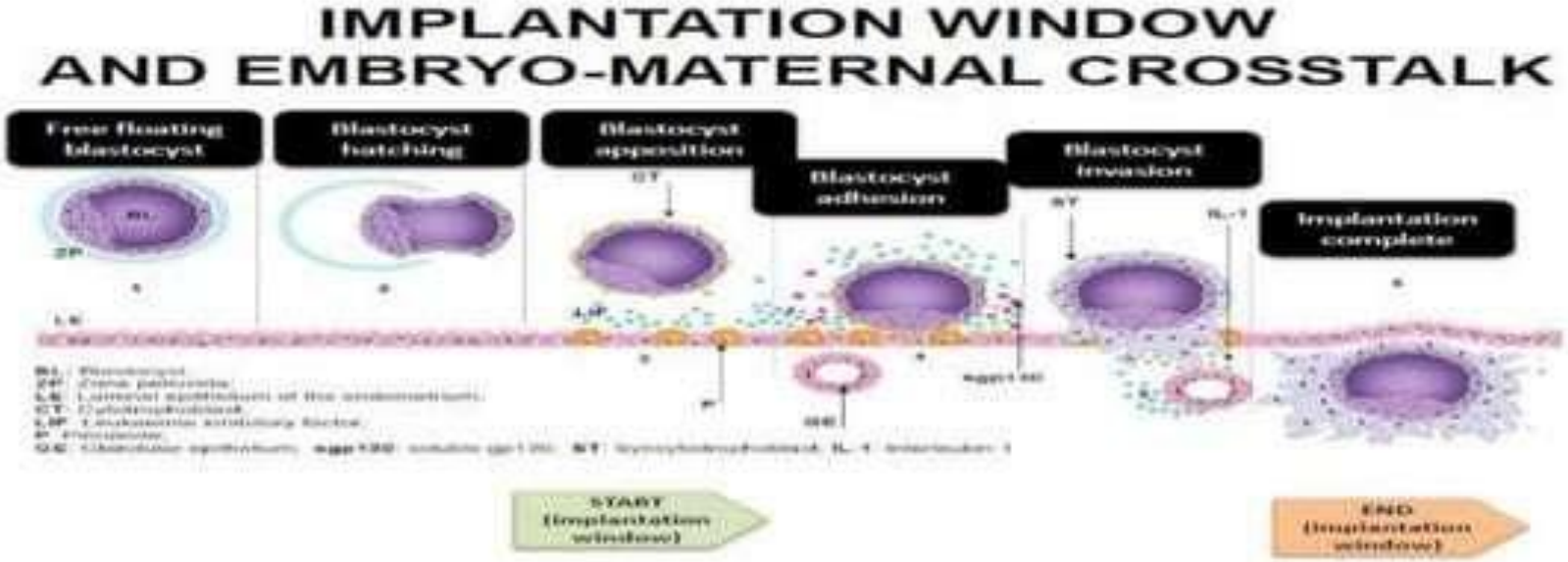


Embryo-Endometrium Communication

Day 5 – Free floating blastocyst in utero

Day 6 – Blastocyst hatching – **Start of Window**

Day 7 – Blastocyst apposition to endometrium at the beginning of the implantation window



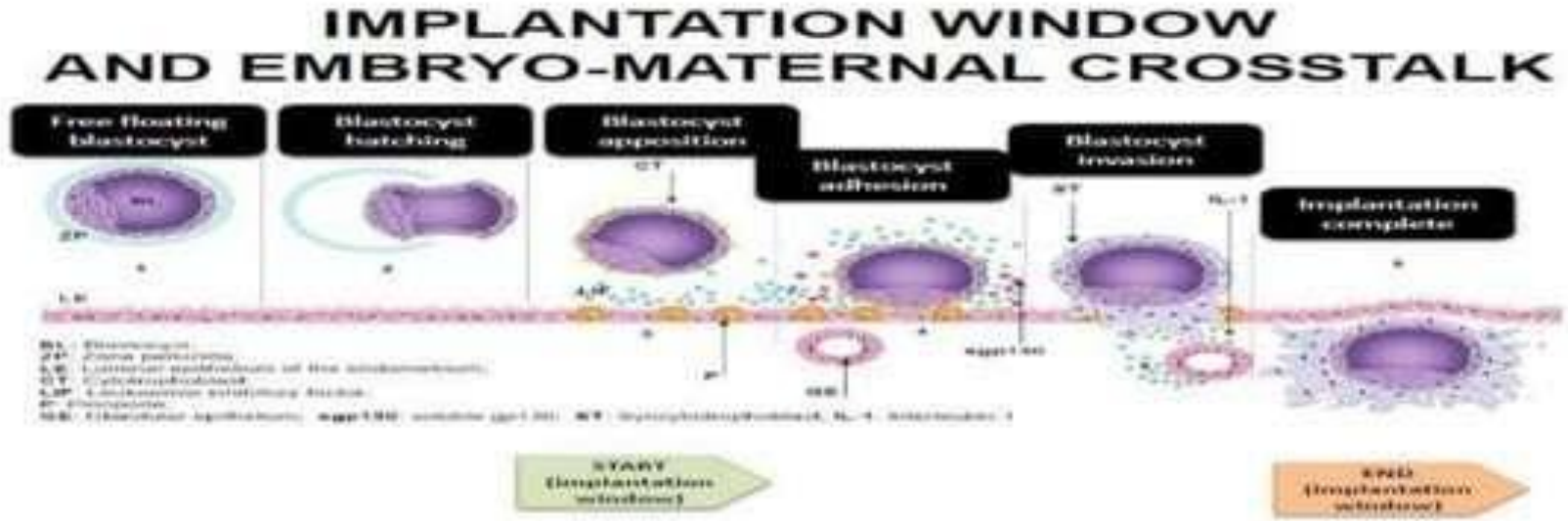
Justine S. Fitzgerald et al. Hum. Reprod. Update 2008;14:335-344

Embryo-Endometrium Communication

Day 8 – Blastocyst adhesion

Day 9 – Blastocyst Invasion

Day 10 – Implantation complete – **End of Window**



Justine N. Fitzgerald et al. Hum. Reprod. Update 2008;14:335-344

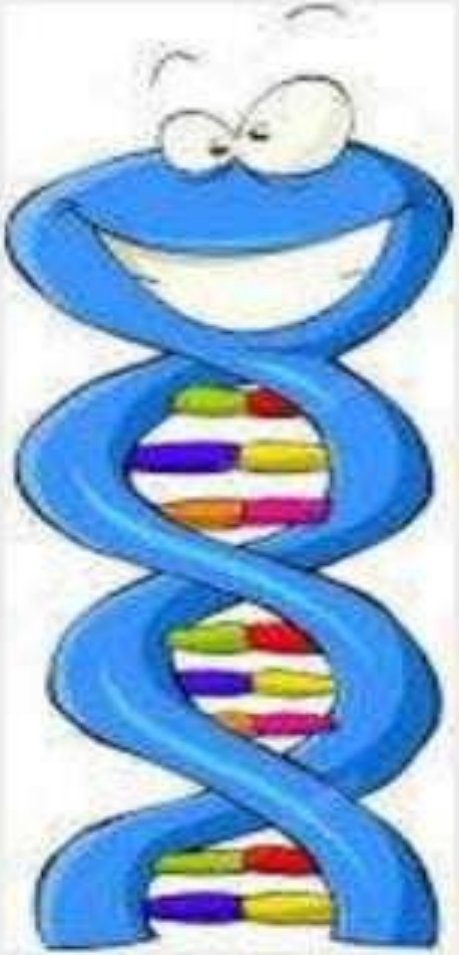
Genomic Activation

GENOMIC ACTIVATION

- Maternal to Zygotic Genomic Transition
- **Maternal gene down-regulation** far outweighs **embryonic gene up-regulation**
- **4-8 cell embryo**

GENOMIC ACTIVATION

- Maternal mRNA degradation
- Embryonic gene transcription activation
- Epigenetic changes (Phosphorylation and acetylation of hystones)



GENOMIC ACTIVATION

- Expression of house keeping gene
- Result – Totipotent to pluripotent (cellular diferentiation)
- EGA failure leads to **embryo arrest** and eventual **implantation failure**

transfer

HISTORY

The first ever IVF birth

England ; July 25, 1978


Evening News

Meet Louise, the world's first test-tube arrival

SUPERBABE

Patrick Steptoe and Robert Edwards

- BLASTOCYST TANSFER

Steptoe PC. Edwards RG. Birth after the reimplantation of a human embryo. Lancet. 1978 ; 12:2:366

HISTORY

1998 - First pregnancies following embryo culture in sequential media and transfer at the blastocyst stage of development

David Gardnar

Jones GM, Trounson AO, Gardner DK, Kausche A, Lolatgis N, Wood C. Evolution of a culture protocol for successful blastocyst development and pregnancy. Hum Reprod. 1998 ;13,169,77

ADVANTAGES

- More physiological – Synchronisation between Embryo and Uterine Endometrium
- Allow best embryo transfer
- High Implantation Potential (50–63%) as compared to Cleavage stage embryo (10–30%)
- Single Embryo Tansfer

ADVANTAGES

- Higher live-birth rate following fresh transfer in
- Good prognosis patient
- Repeated miscarriages or IVF failures

- PGS – EUPLOID EMBRYO TRANSFER – Single Embryo Transfer – Decrease Twinning

ADVANTAGES

- Reduced Uterine contraction at the time of implantation

- Allow time for Preimplantation Genetic Diagnosis (PGD)/ Preimplantation Genetic Screening (PGS)
  [not much relevant in the era of Frozen Embryo Transfer]

- Due to the larger diameter of blastocysts the rate of ectopic pregnancy might be decreased after blastocyst transfer 60% to 65% (Shoolcraft 2001)

DISADVANTAGES

- Cycle Cancellation
- Practical Laboratory-related Issues
- High Rate of Multiple Pregnancies
- Monozygotic Twinning
- Cryopreservation
- Neonatal and Long-Term Outcome Issues

CLEAVAGE STAGE

ADVANTAGES

- Uterus is best incubator
- Large number of embryos available for transfer
- Ease of culture

TRANSFER

DISADVANTAGES

- Difficult to select the best one
- End up freezing nonviable one
- Embryo exposed to hostile uterine environment for longer time

Cycle Cancellation

Number of surviving embryos diminishes with the length of time in vitro, extending culture to the blastocyst stage

↓

Reduce the number of viable embryos available for cryopreservation and subsequent thaw transfer

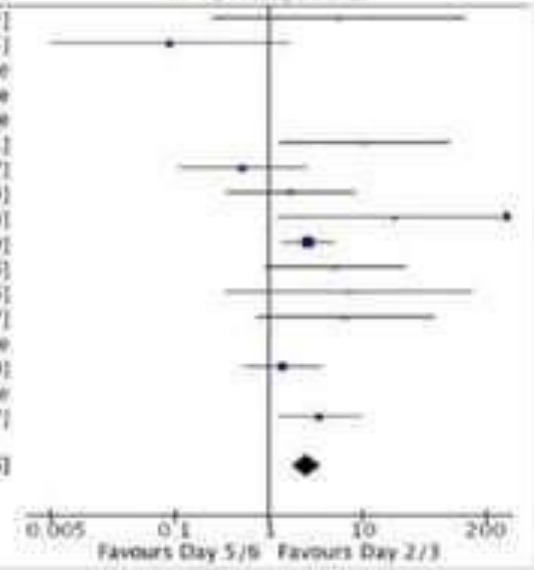
- Under standard IVF culture conditions, only about 25 to 60% of human embryos progress to the blastocyst stage after 5 days of culture

Cycle Cancellation

It has been shown that having even 8 zygotes on day-1 has about 11-13% chance of no blastocyst growth at day 5

Study or Subgroup	Day 5/6 Events	Total	Day 2/3 Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Gardner 1998	2	45	0	47	1.1%	5.46 [0.25, 116.92]
Aziminekoo 2015	0	57	5	61	12.5%	0.09 [0.00, 1.65]
Bungum 2003	0	61	0	57		Not estimable
Coskun 2000	0	100	0	101		Not estimable
Devreker 2000	0	11	0	12		Not estimable
Emiliani 2003	10	99	1	94	2.2%	10.45 [1.31, 83.31]
Frattarelli 2003	3	29	5	28	10.8%	0.52 [0.11, 2.47]
Hreinsson 2004	4	64	1	80	5.9%	1.71 [0.37, 7.94]
Karaki 2002	9	80	0	82	1.0%	21.92 [1.25, 383.14]
Kolibianakis 2004	36	226	16	224	31.4%	2.58 [1.39, 4.80]
Levitas 2004	6	23	2	31	3.0%	5.12 [0.93, 28.26]
Levron 2002	1	46	0	44	1.1%	7.16 [0.36, 142.76]
Mena 1998	6	58	1	58	2.1%	6.58 [0.77, 56.47]
Papanikolaou 2005	0	80	0	84		Not estimable
Papanikolaou 2006	11	175	8	176	17.8%	1.41 [0.55, 3.59]
Rienzi 2002	0	50	0	48		Not estimable
Van der Auwera 2002	18	70	6	66	10.9%	3.46 [1.28, 9.37]
Total (95% CI)		**1274**		**1303**	**100.0%**	**2.50 [1.76, 3.55]**
Total events	108		47			

Heterogeneity: $Chi^2 = 17.22$, $df = 11$ ($P = 0.10$); $I^2 = 36\%$
Test for overall effect: $Z = 5.11$ ($P < 0.00001$)



Failure to transfer embryos for cleavage stage and blastocysts.

Glujovsky, Cleavage-stage versus blastocyst transfer, Fertil Steril 2016.

CHOCRANE REVIEW 2016

Cycle Cancellation

- Psychological implication

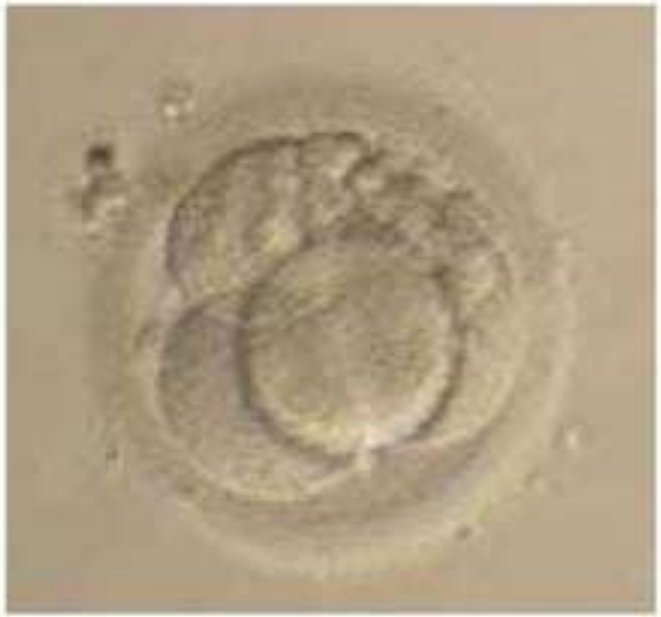
a. Patient

b. Clinician

- Financial implication

Practical Laboratory-related Issues

- Some evidence suggests – the **numbers** of blastomeres and the degree of fragmentation observed on day 3 are associated with the potential for blastocyst formation



- However, these associations do not necessarily correlate with blastocyst viability

Practical Laboratory-related Issues

- Ability to produce blastocysts varies widely among patients

↓

0% to almost 100%

- Lack of established markers for predicting blastocyst development

↓

Therefore, increases risk of having no embryos to transfer despite observations of adequate development in vitro on day2–3

Practical Laboratory-related Issues

Recent focus is on identifying clinical factors associated with blastocyst development and pregnancy

- Patient Age
- Parity
- Antral Follicle Count
- Fertilization Technique
- Number And Quality Of Embryos

Practical Laboratory-related Issues

- Substandard laboratory environment, fluctuations in temperature or pH of culture conditions can severely affect blastulation

- Proper maintenance of humidity, osmolality of culture media and optimal O2 concentrations are all imperative to prevent embryonic block

Multiple Pregnancy Rates

- 53 % (double blastocyst transfer)
- Solution – Elective Single Blastocyst

Multiple Pregnancy Rates

		Single Embryo Transfer	Double blastocysts transfer
Multiple Pregnancy Rate		1-2 %	25-44%
Live Birth Rate	Self	63- 65 %	61-63 %
	Donor	63 %	74%

ASRM ,2013

Monozygotic Twinning

- Incidence 3–5%
- Risk increased in the range of 2- to 3-fold following blastocyst transfer compared with cleavage-stage transfer

- Associated with

a. Female age <35 years
b. Assisted hatching
c. ICSI

PERINATAL OUTCOMES

Table 1 Unadjusted outcome rates for singleton births from IVF/ICSI in Canada, 2001–2009, after ET on Day 3 or Day 5/6.

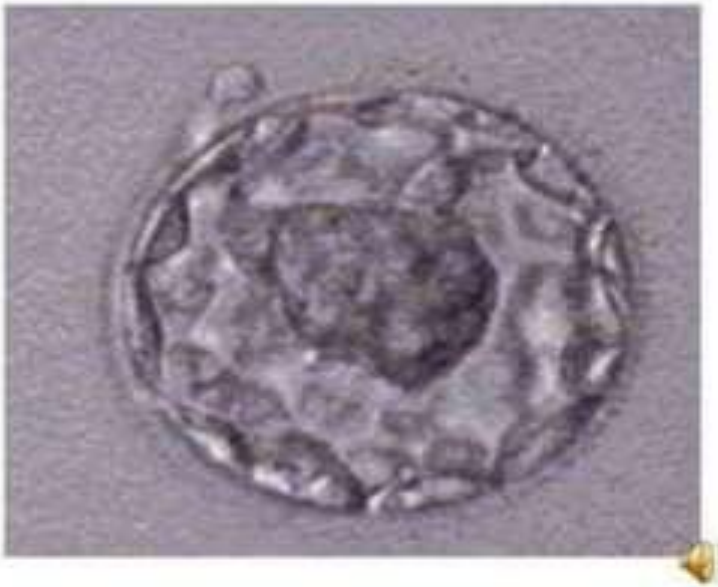
Outcome	Day 3		Day 5/6		P value
	Instances/deliveries with data	Percentage	Instances/deliveries with data	Percentage	
Preterm birth (<37 weeks)	1335/9442	**14.1**	548/3194	**17.2**	**<0.001**
Very preterm birth (<32 weeks)	251/9442	2.7	95/3194	3	0.34
Low birthweight (<2500 g)	897/9109	9.8	274/2985	9.2	0.28
Very low birthweight (<1500 g)	155/9109	1.7	50/2985	1.7	0.92
Congenital anomalies	215/9506	2.3	78/3206	2.4	0.58
Stillbirth	98/9506	1	33/3206	1	0.99
Neonatal death	39/9506	0.4	13/3206	0.4	0.97

Bold indicates the main findings.

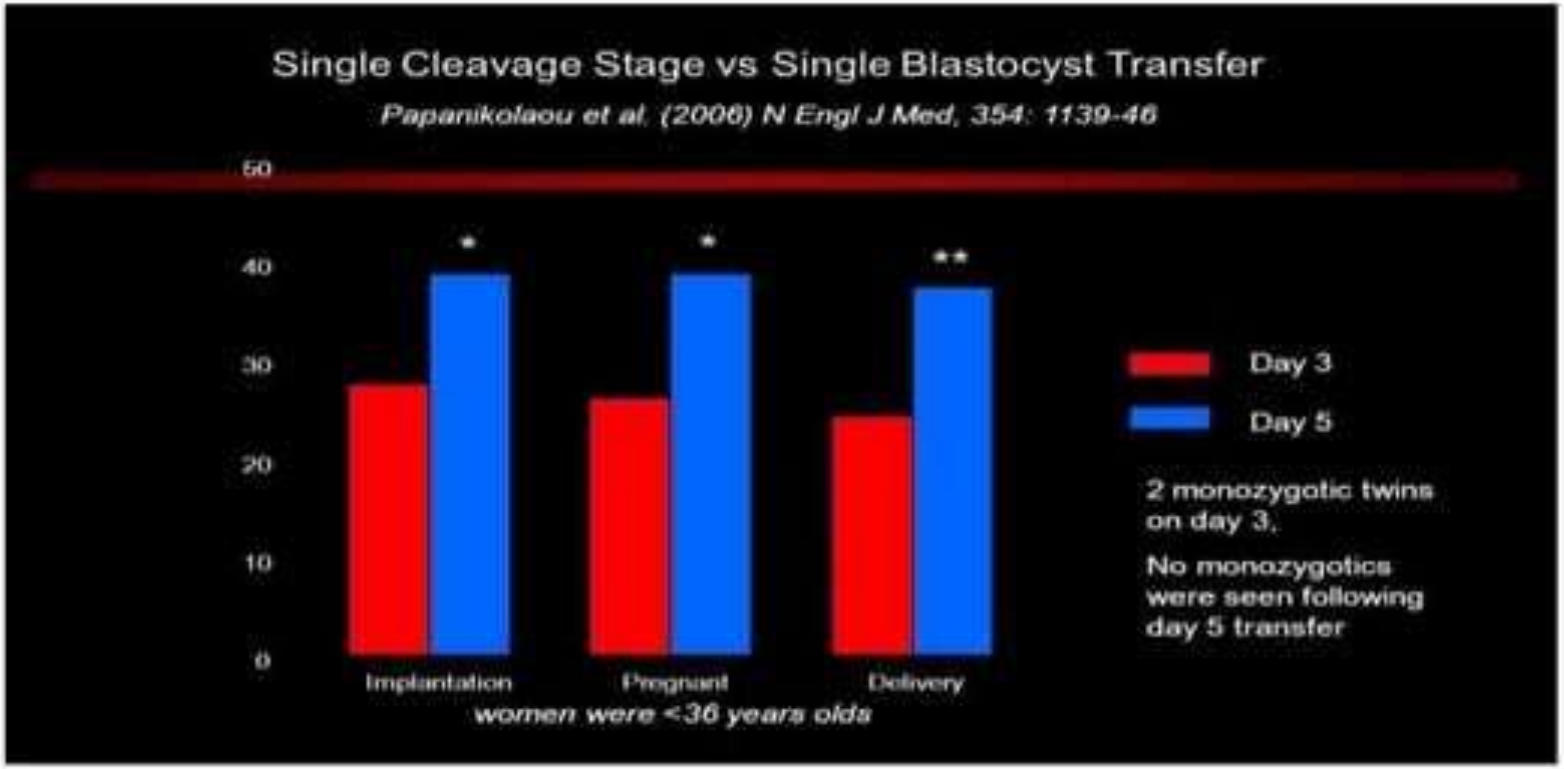
Day 3 vs Day 5 Transfer

Cleavage stage

Blastocyst stage



Day 3 vs. Blsatocyst



Cleavage vs. Blastocyst Transfer

	Cleavage stage transfer	Blastocyst transfer
Live birth rates (Fresh Cycle)	30.3-37.2%	39.1-43.2%
Cumulative pregnancy rate (FET) only 1 study	71.9%	53.2%
Cumulative pregnancy rate (Fresh)	~	~
Miscarriage rate	~	~

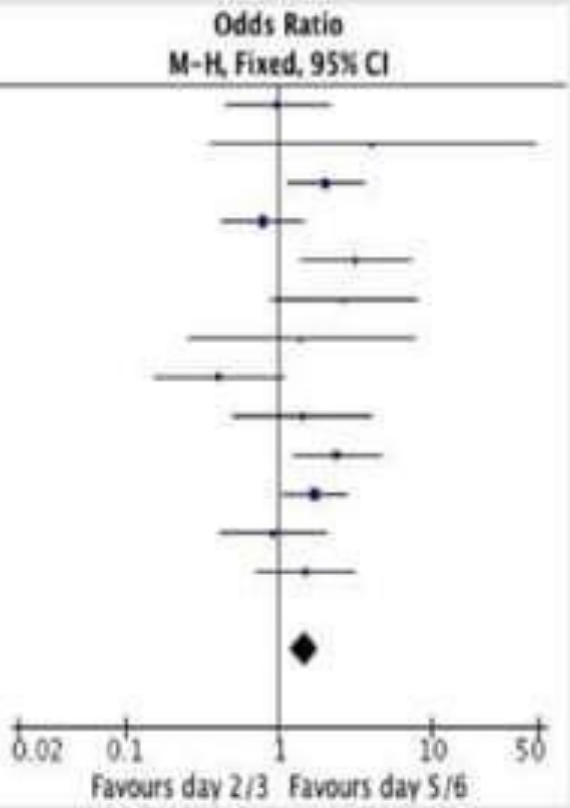
CHOCRANE 2016

The Chochrane Review 2016

Study or Subgroup	Day 5/6 Events	Total	Day 2/3 Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Brugnon 2010	22	55	21	52	8.8%	0.98 [0.45, 2.13]
Devreker 2000	3	11	1	12	0.5%	4.13 [0.36, 47.30]
Elgindy 2011	52	100	35	100	11.4%	2.01 [1.14, 3.55]
Emiliani 2003	33	82	41	89	16.0%	0.79 [0.43, 1.45]
Fernandez-Shaw 2015	25	60	11	60	4.4%	3.18 [1.39, 7.31]
Frattarelli 2003	15	29	8	28	2.7%	2.68 [0.89, 8.02]
Levitas 2004	3	23	3	31	1.5%	1.40 [0.26, 7.66]
Levron 2002	8	46	15	44	8.6%	0.41 [0.15, 1.09]
Livingstone 2002	14	30	11	29	4.1%	1.43 [0.51, 4.04]
Papanikolaou 2005	38	80	23	84	8.0%	2.40 [1.25, 4.60]
Papanikolaou 2006	56	175	38	176	17.5%	1.71 [1.06, 2.76]
Rienzi 2002	24	50	24	48	8.7%	0.92 [0.42, 2.04]
Van der Auwera 2002	24	70	17	66	7.8%	1.50 [0.72, 3.15]
Total (95% CI)		**811**		**819**	**100.0%**	**1.48 [1.20, 1.82]**
Total events	317		248			

Heterogeneity: $Chi^2 = 21.83$, df = 12 ($P = 0.04$); $I^2 = 45\%$

Test for overall effect: $Z = 3.68$ ($P = 0.0002$)

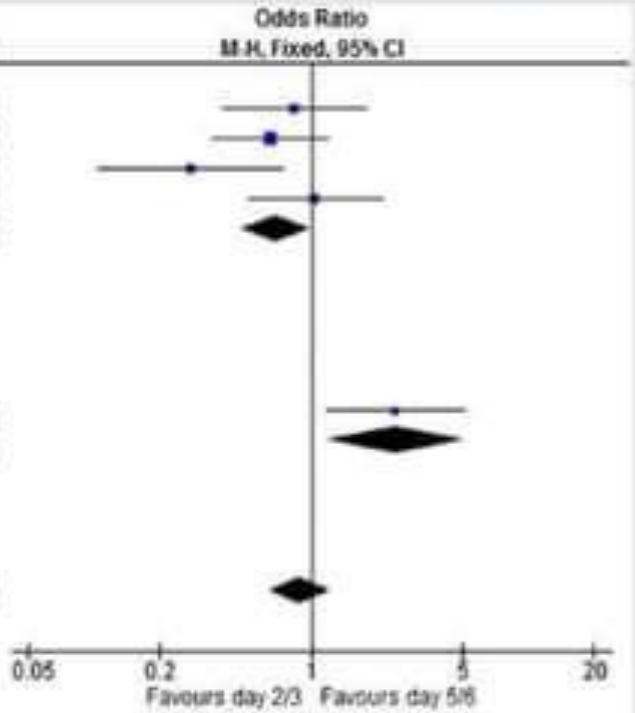


Live-birth rate in fresh cleavage-stage transfers and fresh blastocyst transfers.

Glujovsky. Cleavage-stage versus blastocyst transfer. Fertil Steril 2016.

The Chochrane Review 2016

Study or Subgroup	Day 5/6 Events	Total	Day 2/3 Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
2.5.1 slow freezing						
Brugnon 2010	24	55	25	52	18.2%	0.84 [0.39, 1.79]
Emiliani 2003	43	82	56	89	32.0%	0.65 [0.35, 1.20]
Rienzi 2002	31	50	41	48	19.9%	0.28 [0.10, 0.75]
Van der Auwera 2002	24	70	22	66	18.6%	1.04 [0.51, 2.12]
Subtotal (95% CI)		**257**		**255**	**88.7%**	**0.69 [0.48, 0.99]**
Total events	122		144			
Heterogeneity: Chi² = 4.85, df = 3 (P = 0.18); I² = 38%						
Test for overall effect: Z = 2.03 (P = 0.04)						
2.5.2 vitrification						
Fernandez-Shaw 2015 (1)	33	60	20	60	11.3%	2.44 [1.17, 5.12]
Subtotal (95% CI)		**60**		**60**	**11.3%**	**2.44 [1.17, 5.12]**
Total events	33		20			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.37 (P = 0.02)						
Total (95% CI)		**317**		**315**	**100.0%**	**0.89 [0.64, 1.22]**
Total events	155		164			
Heterogeneity: Chi² = 13.76, df = 4 (P = 0.008); I² = 71%						
Test for overall effect: Z = 0.75 (P = 0.46)						
Test for subgroup differences: Chi² = 9.12, df = 1 (P = 0.003), I² = 89.0%						



Footnotes

(1) Both cumulative pregnancy and live birth rates given, same numbers except for one voluntary termination in blastocyst group due to anomaly after VET

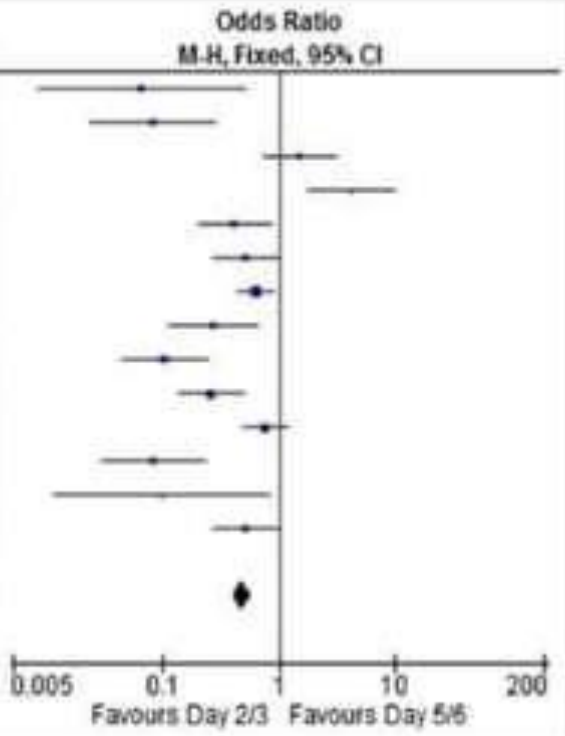
Cumulative pregnancy rate for fresh and frozen cleavage-stage transfers and blastocyst transfers grouped by freezing techniques.

Glujovsky. Cleavage-stage versus blastocyst transfer. Fertil Steril 2016.

The Chochrane Review 2016

Study or Subgroup	Day 5/6 Events	Total	Day 2/3 Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Brugnon 2010	42	55	51	52	3.4%	0.06 [0.01, 0.50]
Bungum 2003	36	61	54	57	6.3%	0.08 [0.02, 0.28]
Fernandez-Shaw 2015	39	60	33	60	3.2%	1.52 [0.73, 3.17]
Gardner 1998	29	45	14	47	1.3%	4.27 [1.78, 10.24]
Hreinsson 2004	15	64	34	80	6.3%	0.41 [0.20, 0.86]
Karaki 2002	22	80	35	82	6.9%	0.51 [0.26, 0.98]
Kolibianakis 2004	114	226	145	234	19.3%	0.62 [0.43, 0.91]
Levron 2002	12	46	25	44	5.2%	0.27 [0.11, 0.65]
Motta 1998	15	58	45	58	9.1%	0.10 [0.04, 0.24]
Pantos 2004	16	81	79	162	11.8%	0.26 [0.14, 0.48]
Papanikolaou 2006	115	175	126	176	11.8%	0.76 [0.48, 1.20]
Rienzi 2002	18	50	42	48	7.5%	0.09 [0.03, 0.23]
Ten 2011	20	28	26	27	2.1%	0.10 [0.01, 0.83]
Van der Auwera 2002	26	70	35	66	6.2%	0.52 [0.26, 1.04]
Total (95% CI)		**1099**		**1193**	**100.0%**	**0.48 [0.40, 0.57]**
Total events	519		744			

Heterogeneity: Chi² = 82.95, df = 13 (P < 0.00001); I² = 84%
Test for overall effect: Z = 8.25 (P < 0.00001)



Embryo freezing rate for cleavage stage and blastocysts

Glujovsky: Cleavage-stage versus blastocyst transfer. Fertil Steril 2016

LIMITATIONS OF THE COCHRANE REVIEW

- Numbers of RCT – small
- Studies included were more than 10 years old
- Blastocyst transfer yields a better clinical pregnancy as well as live birth rate, if one considers pregnancy rate per transfer attempt

Blastocyst culture and transfer in clinical-assisted reproduction: a committee opinion

The Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology

American Society for Reproductive Medicine, Birmingham, Alabama

The purposes of this Practice Committee Opinion, which replaces the 2006 ASRM Practice Committee Opinion of the same name (Fertil Steril 2006;90:S174–7), are first, to review the literature regarding the clinical application of blastocyst transfer and second to identify the potential risks and laboratory issues related to use of this technology. This document does not apply to patients undergoing blastocyst culture and transfer for preimplantation genetic testing/screening. (Fertil Steril® 2013;99:667–72. ©2013 by American Society for Reproductive Medicine.)

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CONCLUSIONS

- Evidence supports blastocyst transfer in "good prognosis" patients. Consideration is warranted to transfer of a single embryo given the high risk of multiples in this patient population.

- Blastocyst or cleavage-stage embryos can be used for unselected or poor prognosis patients as the pregnancy/livebirth rates are not significantly different; however, in these populations there is a higher risk of embryos not progressing to blastocyst stage resulting in fewer/no embryos available for transfer

SEQUENTIAL TRANSFER

- Both cleavage stage embryo(s) and blastocyst(s) are sequentially transferred in the same cycle

- Advantages
  - Blastocyst transfer along with the insurance against potential cancellation by having a cleavage stage transfer as well

- Disadvantage
  - Cost
  - Patient inconvenience
  - Increase in multiple pregnancy rates
  - Possible chance of harming the transferred embryos during the second transfer

Day 5 vs. Day 6 Blastocyst Transfer

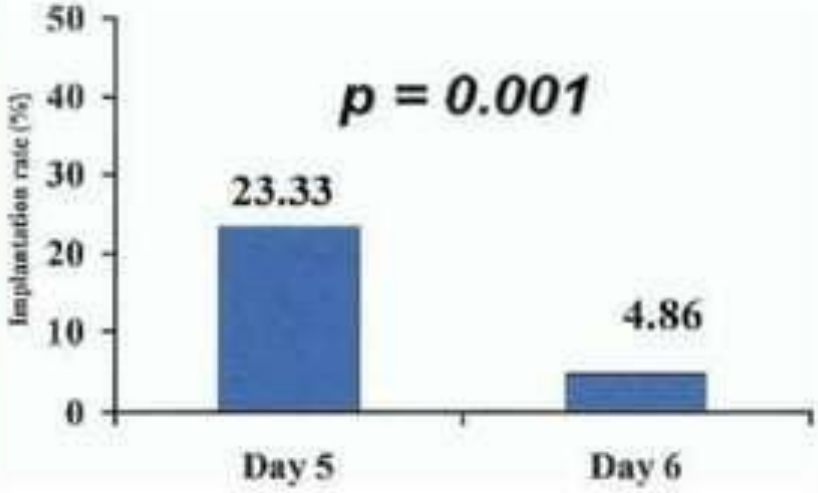
Fresh IVF cycles

Higher implantation and pregnancy rates with the transfer of blastocysts developing on Day 5 compared with those developing on Day 6

(Khorram et al., 2000; Shapiro et al., 2001; Barrenetxea et al.,2005)

FIGURE 1

Comparison of implantation rates between patients transferred on day 5 and day 6.



Barrenetxea. Blastocyst culture after failed transfers. Fertil Steril 2005.

Day 5 vs. Day 6 Blastocyst Transfer

Frozen-thawed Blastocyst Transfers

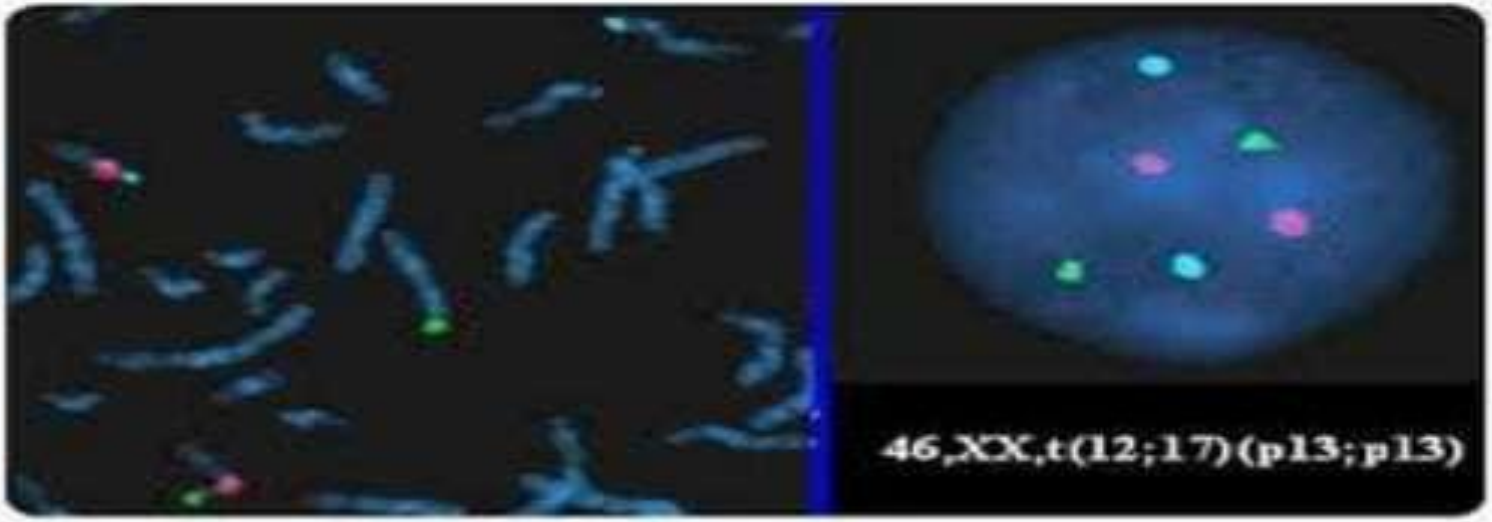
- Conflicting results -

  whether the rate of blastocyst formation prior to cryopreservation affects treatment outcome

(Marek et al., 2001; Behr et al., 2002; Liebermann and Tucker, 2006; Richter et al., 2006; Levens et al., 2008; Shapiro et al., 2008)

Aneuploidy Rate

- Blastocyst -34 %
- Day 2/3 – 70-75%



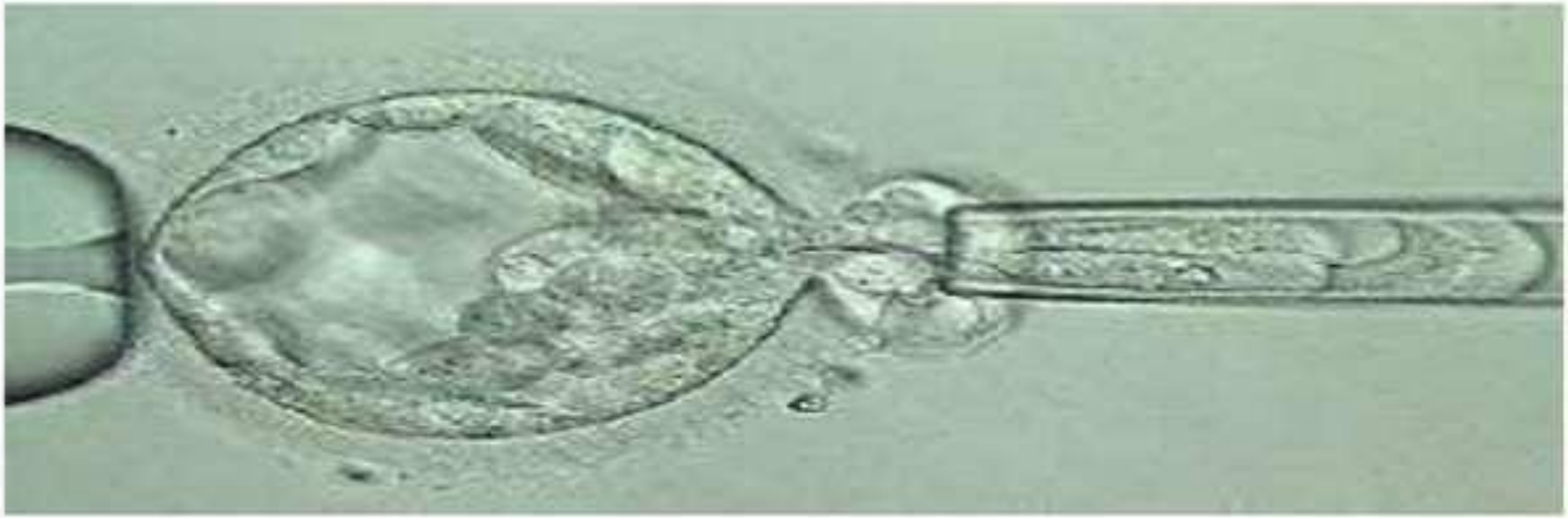
(Sandalinas *et al.* 2000)

Trophoectoderm Biopsy

Mosaicism

Blastomere – around 60 %

Blastocyst – 5-6%



Assisted hatching

- Marginal improvement in clinical pregnancy rate
- Association with monozygotic twinning has been suspected
- ? Selected cases

To Blast Or Not To Blast

Whenever possible – Blastocyst Transfer

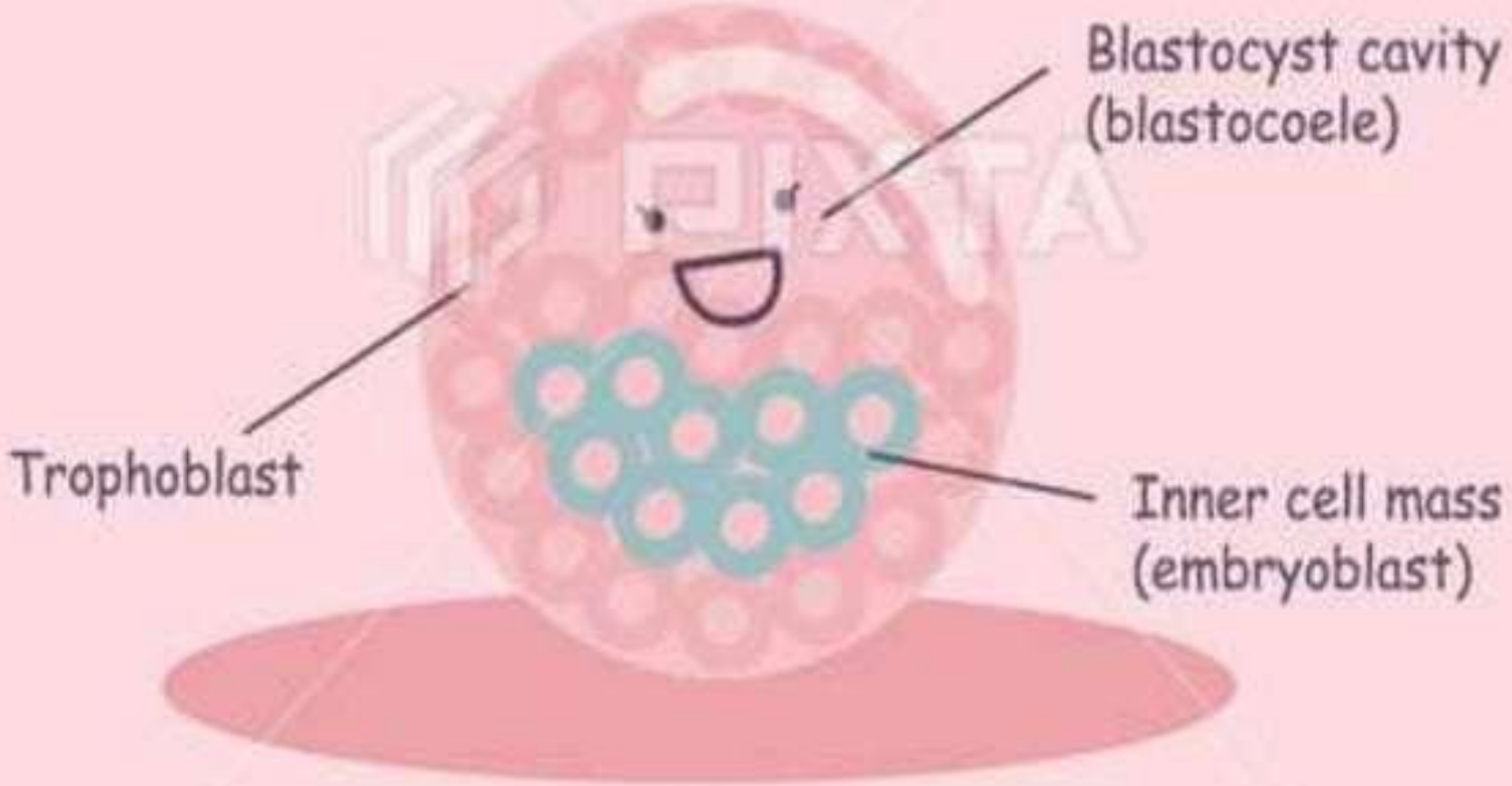
Suspicion of cycle cancellation

<5-6 8 cell stage embryo available / Poor quality 8 cell embryo

↓

8 cell stage transfer

Thank You


Blastocyst cavity
(blastocoele)
Trophoblast
Inner cell mass
(embryoblast)

The limits to the number of Embryos to Transfer ASRM Guidelines, 2017

Euploid Embryo	Single embryo
< 35 yrs	Preferably single embryo
35-37 yrs	Strongly recommend single embryo
37-40 yrs	Not more than 3 cleavage stage Or Two blastocysts
41-42 yrs	Not more than 4 cleavage stage Or 3 blastocysts
> 43 yrs	Insufficient data
Co existing medical condition	Single embryo

- Ideal goal

Singleton pregnanacy

who do not meet criteria for a favorable prognosis may have an additional embryo transferred according to individual circumstances.

donor cycle – age of donor

- Alpha/Eshre Consensus Meeting Istanbul 2010 Timing by consensus for each of the scoring points was reached and related to postinsemination (PI)
- – 2pn 16-18 h PI 18 hrs PI
- – EC 24 h +/- 1 hour PI
- –Day 2 check 44 h +/- 1 hour PI
- – Day 3 check 68 h +/- 1 hour PI
- – Day 4 check 92 h +/- 2 hours PI
- – Day 5 check 116 +/- 2 hours PI

Day of Division	Type of Twin
Till Day 3	DADC
between day 3–7	MCDA
7–14 days	MCMA
> 14 days	SIAMESE TWINS