



New technologies  
for implantation prediction

# Introduction

- The soaring success of (ART) has come with an increased rate of multiple pregnancies.
- To avoid obstetric complications, in many IVF centers.
- Single embryo transfer is one approach
- this strategy is still unacceptable for many couples

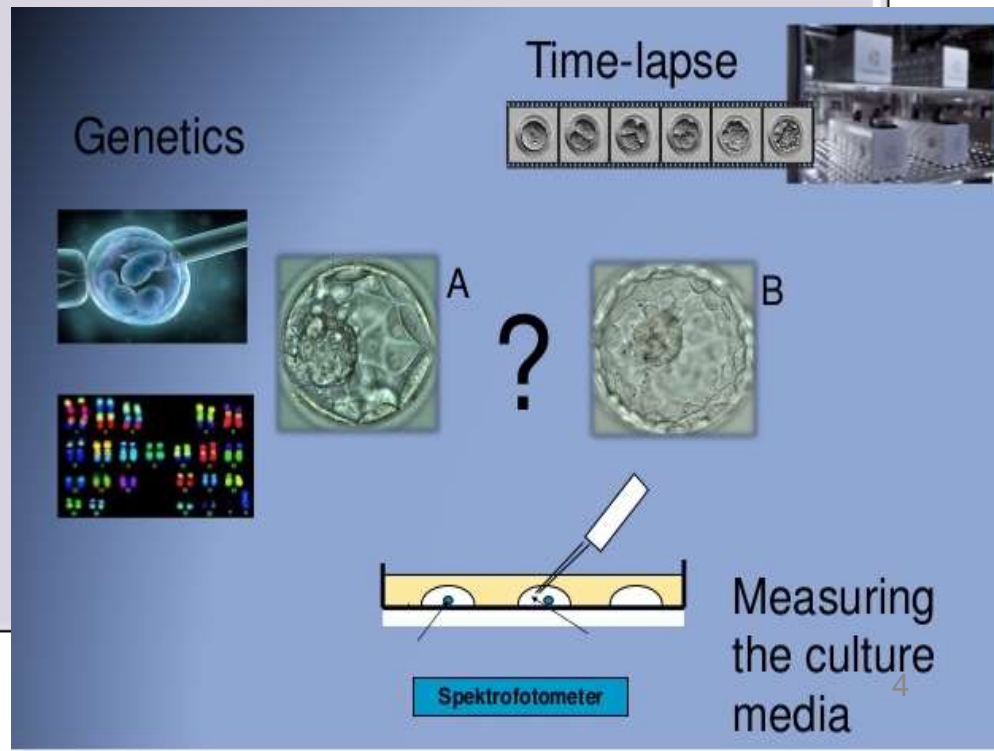


## **Improvement in implantation and pregnancy rates:**

- ✓ Improved embryo examination and selection
- ✓ Enhancement of ovarian stimulation
- ✓ Techniques of oocyte insemination
- ✓ Micromanipulation
- ✓ Embryo transfer
- ✓ Preimplantation genetic diagnosis
- ✓ Composition of culture media

# Embryo selection methods

- Static oocyte and embryo morphology
- Follicular fluid marker
- Cumulus cell marker
- PGS
- Time-laps, algorithms
- OMICS
- Non-coding RNA
- Biochemical markers



# Non-invasive methods of embryo evaluation

A close-up photograph of several glass test tubes and a pipette tip. One test tube in the foreground contains a vibrant blue liquid. The background is softly blurred, showing more laboratory equipment.

- Assess embryos without damage
- Selection of high quality embryos
- Reduces the number of transferred embryos
- Reduces multifetal pregnancies



# Morphological Assessment

- ✓ The Pronuclear-Stage Embryo
- ✓ Cleavage-stage Embryos
- ✓ Development to the Blastocyst Stage



Day 0 (Oocyte)



Day 0 (Sperm)



Day 0 (Insemination)



Day 0 (ICSI)



Day 1 (2 Pronuclei)



Day 2 (4 cells)



Day 3 (8 cells)



Day 4 (Morula)



Day 4 or 5



Day 5 or 6



Day 5 or 6



Day 6

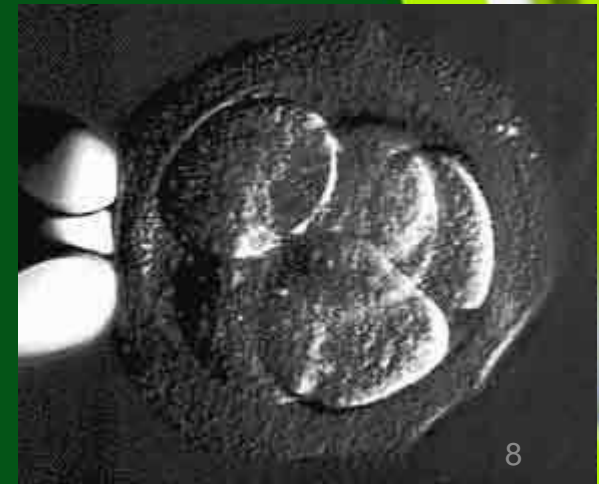
# Morphological Assessment

- Pronuclear size and symmetry;
- Size, number, equality and distribution of nucleoli
- Appearance of cytoplasm
- Polar body structure and placement
- Zona pellucida



# Thickness of zona pellucida:

- The embryos with an **regularly thick zona pellucida** have higher implantation rate than embryos with uniform thickness of zona pellucida. The embryos with thick zonae, greater than 15 microns have a smaller implantation rate than embryos with thinner zona.












# Embryo Assessment In Pronuclear Stage

- high embryo score (>15)
- pregnancy 71%
- implantation 28%
- low embryo score
- pregnancy 8%
- implantation 2%

Time (h post insemin / ICSI)		Score
<b>DAY 1</b>	Equal size and symmetry of PN	10
18-19	Alignment between the PN and polar bodies	5
	Lack of heterogeneity and granularity in cytoplasm	10
	Presence of PN with both polarized or both not-polarized NPB	5
	A difference of less than 3 in the number of NPB in the PN	5
	Polar bodies are not displaced from each other	5

# Embryo Assessment In Cleavage Stage

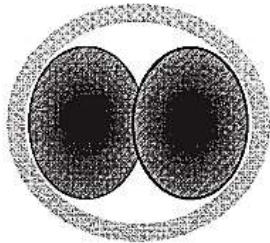


<b>A</b> <i>equal size blastomeres</i> 	<b>B</b> <i>unequal size blastomeres</i> 	<b>C</b> <i>defects of cytoplasm</i> 	
<b>1</b> <i>no fragmentation</i> 	<b>2</b> <i>fragmentation &lt;30%</i> 	<b>3</b> <i>fragmentation 30% – 50%</i> 	<b>4</b> <i>fragmentation &gt;50%</i> 

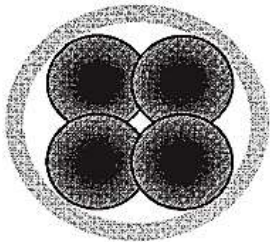
A - Symmetric blastomeres  
 B - Distinctly asymmetric blastomeres  
 C - Defects of cytoplasm

1 - No fragmentation  
 2 - Fragmentation less than 20%  
 3 - Fragmentation between 30-50%  
 4 - Fragmentation above 50%

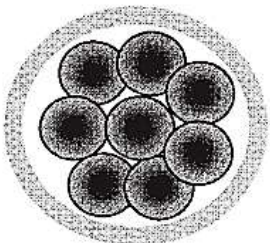
# Ideal features of the cleavage-stage embryo.



25 - 26h post insemination / injection  
embryo should be at the 2-cell stage with  
equal blastomeres and no fragmentation



42 - 44h post insemination / injection  
embryo should have 4 or more blastomeres  
and less than 20% fragmentation

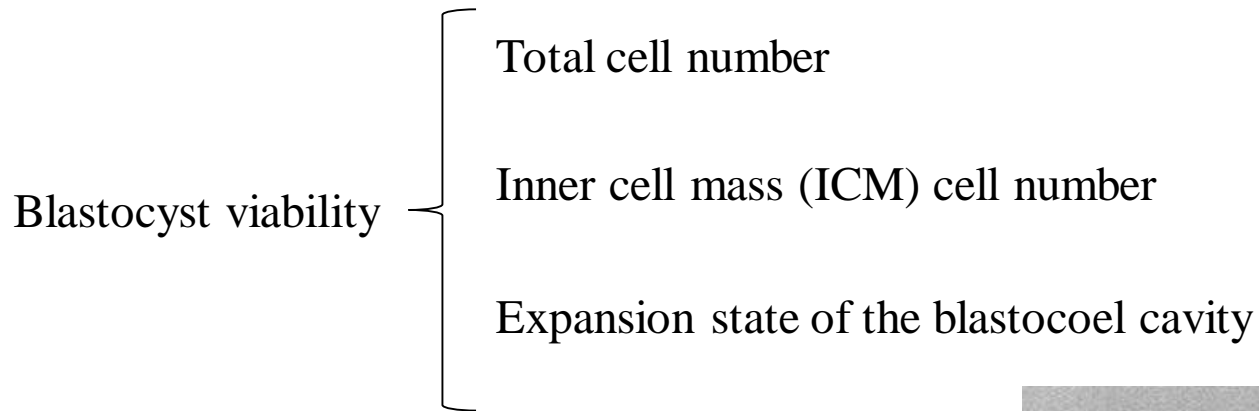


66 - 68h post insemination / injection  
embryo should have 8 or more blastomeres  
and less than 20% fragmentation

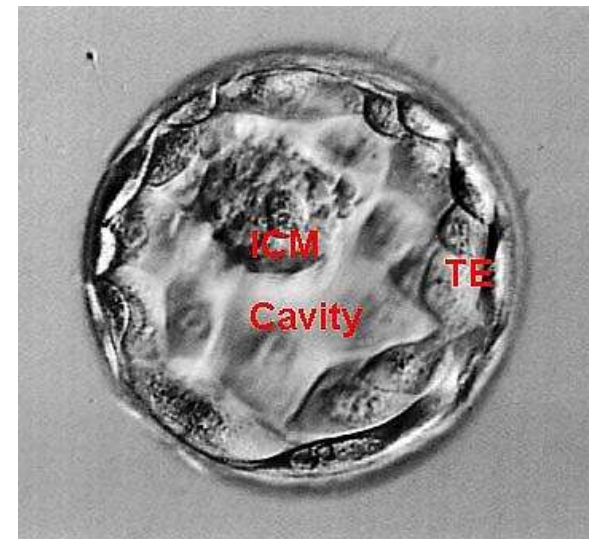
2-cell embryo	10
Nuclear membrane breakdown	5

Number of blastomeres $\geq 4$	10
Fragmentation of less than 20%	10
No multinucleated blastomeres	5

# Embryo assessment in blastocyste stage



Gardner showed that blastocysts of high quality led to the highest pregnancy and implantation rates.

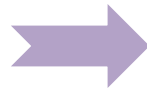




# Embryo assessment in blastocyste stage



Top-scoring blastocyst  
(Score of 3AA)

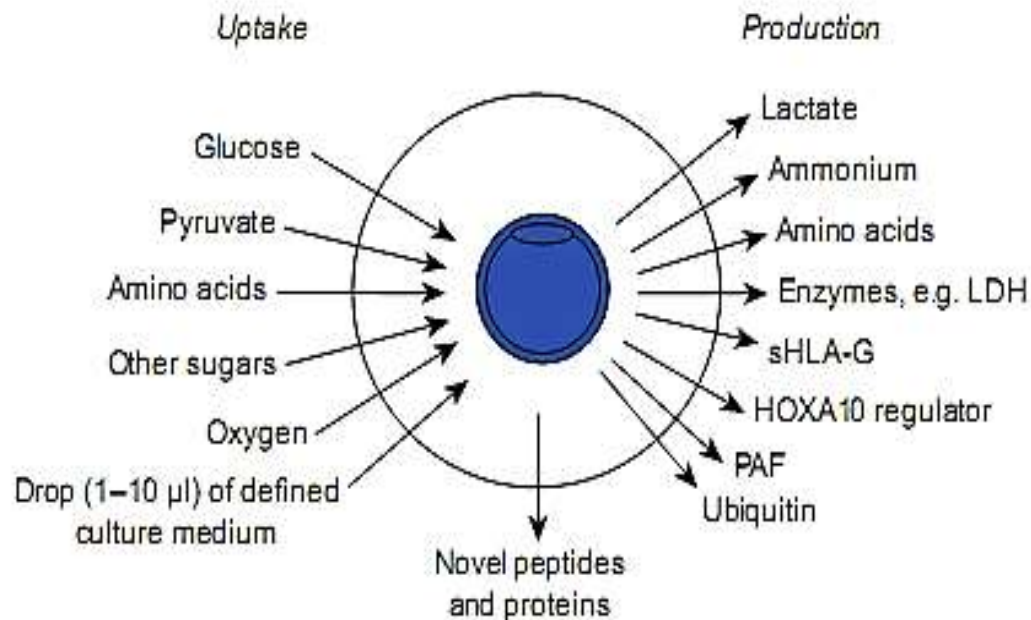


- ✓ full blastocoel cavity
- ✓ well-populated ICM
- ✓ tightly formed ICM
- ✓ a cohesive trophectoderm with many cells

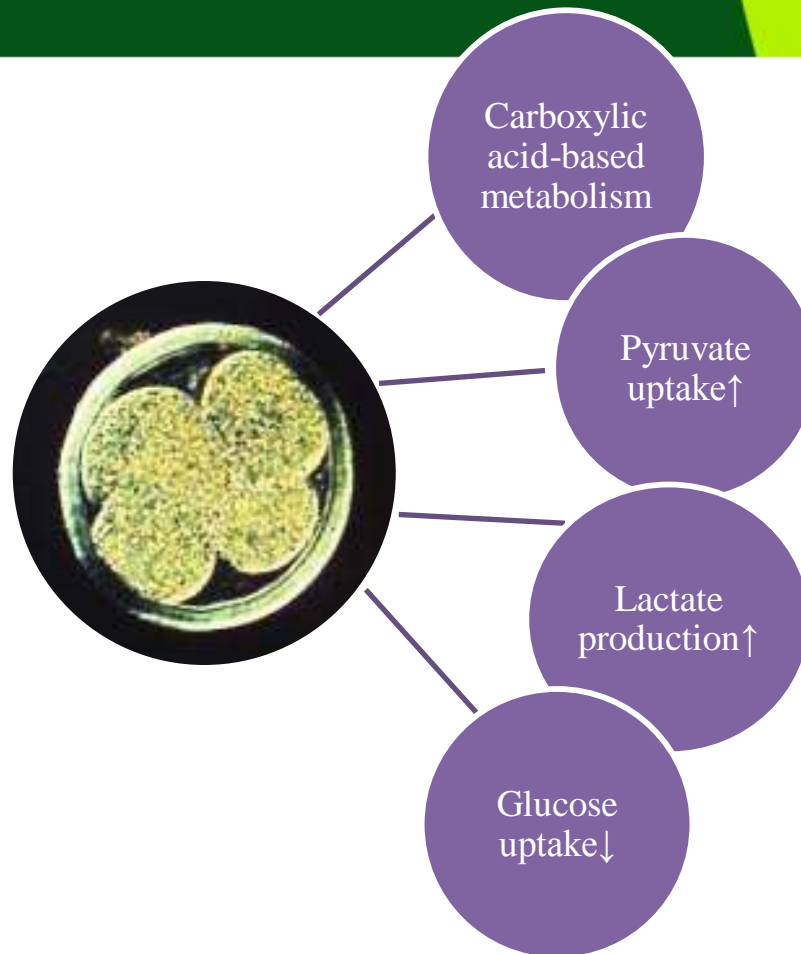


# Assessment of metabolism to select embryos

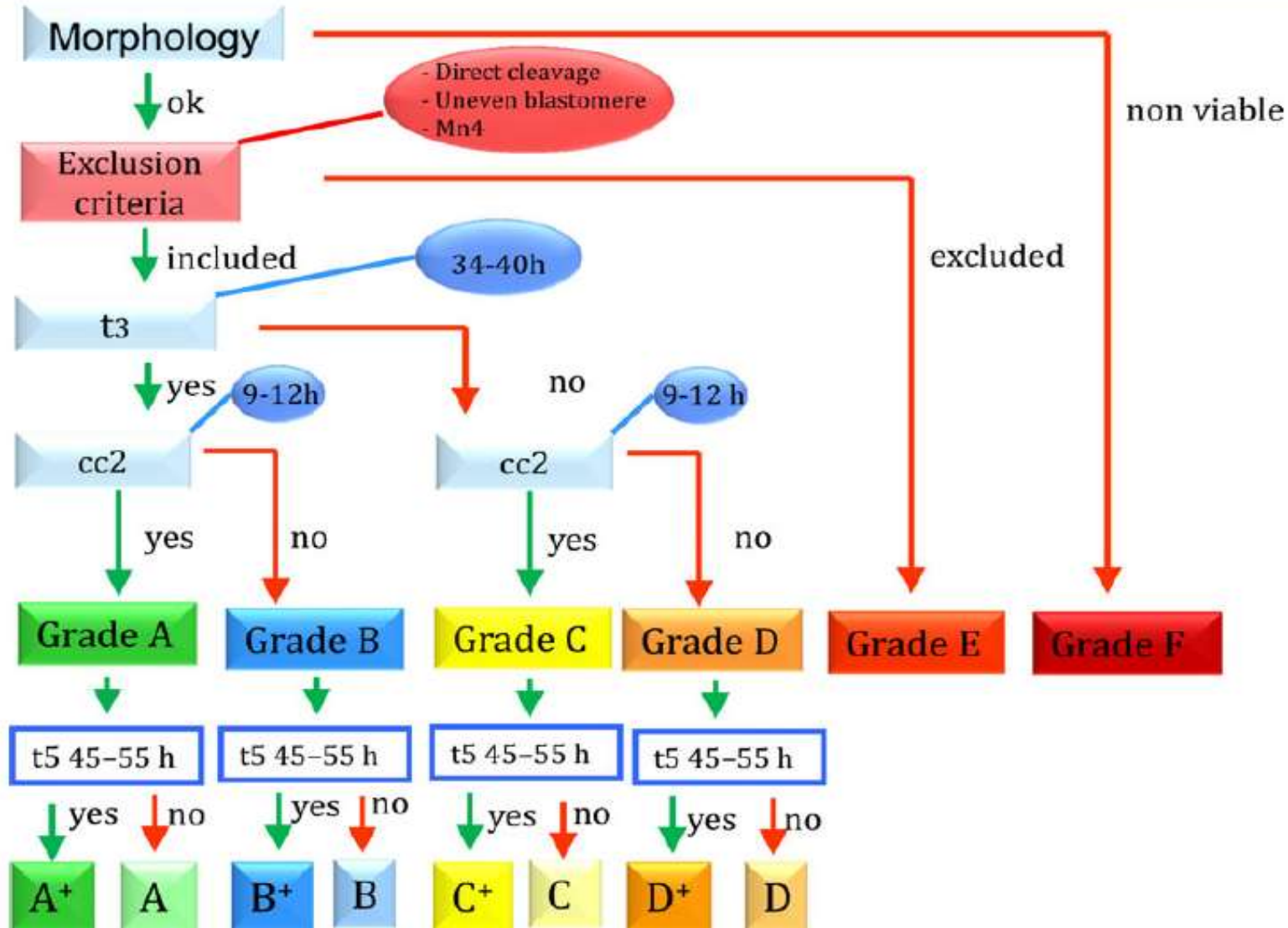
Many of the non-invasive approaches such as metabolomics, proteomics, secretomics, still remain as poorly applicable on a daily basis in the IVF laboratory.



Cleavage stage

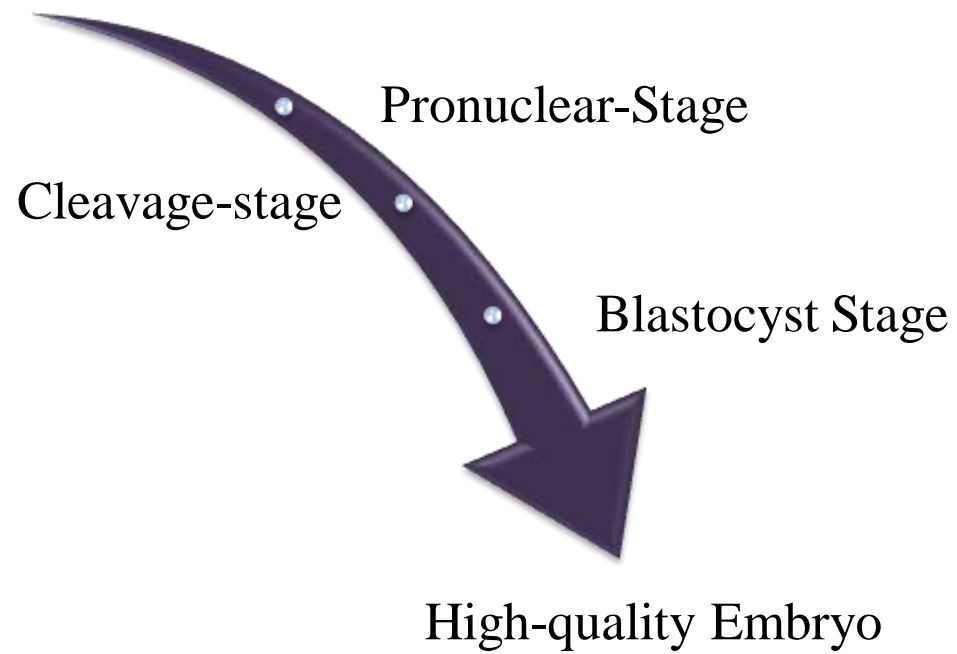


# Algorithm for embryo selection





## Morphology





# Another strategy: use a model that estimates the potential of transferred embryos

- qual
- Our best practices work well for embryonic transfer

## Algorithm to predict assisted reproductive technology pregnancy outcome reveals minimal embryo synergy

A mathematical model was developed to calculate the implantation probability for individual embryos based on the pregnancy outcome of in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cases with multiple embryos transferred. This model was used to calculate implantation probabilities of embryos of 31 morphological types using the outcome of 1,200 IVF/ICSI cases. The algorithm was validated by comparing the calculated pregnancy probability and multiple pregnancy probability with the actual outcome of 281 separate IVF/ICSI cases. Finally, an estimation of embryo synergy was calculated. (Fertil Steril® 2005;83: 782–4. ©2005 by American Society for Reproductive Medicine.)

The soaring success of assisted reproduction technology (ART) has come with an increased rate of multiple pregnancies. To avoid obstetric complications, many IVF centers have focused on developing strategies to reduce the rate of multiple births. Single embryo transfer is one approach that has been taken by many European ART programs (1–4); however, this strategy is still unacceptable for many couples.

Another strategy is to use a model that estimates the potential of transferred embryos. In several works (5–9), the qualitative estimation of embryo potentials were suggested. The disadvantage of these studies is that the qualitative approach works well only with the best embryos and does not take into account patients with a poor embryo

intracytoplasmic sperm injection (IVF/ICSI) (1,200 cases). During the second phase, the validity of the mathematical model was tested on 281 new IVF/ICSI cases. This model was also used to calculate the probability of multiple pregnancy and embryo synergy. Pregnancy was diagnosed by a positive serum  $\beta$ -human chorionic gonadotropin (hCG) test 16 days after embryo transfer. Multiple pregnancy was determined by the number of gestational sacs identified during ultrasound exam.

Embryos were assigned a “type” based on the number of cells in the embryo and embryo grade (11). We calculated the embryo implantation probability ( $p$ ) for embryos of more than 30 different morphological “types.” The numerical variant of the least-squares method was used to calculate  $p$  for embryos of all types. The resulting values of



## The Kidscore™ D5 algorithm as an additional tool to morphological assessment and PGT-A in embryo selection: a time-lapse study

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# Kid score

- 912 embryos from 270 patients
- October 2016 and June 2018
- embryos were cultured for up to five or six days in an Embryoscope® time-lapse incubator and evaluated based on the KIDscore™ D5 algorithm (KS5).
- Biopsies for PGT-A screening were performed in 778 (85.31%) embryos. •

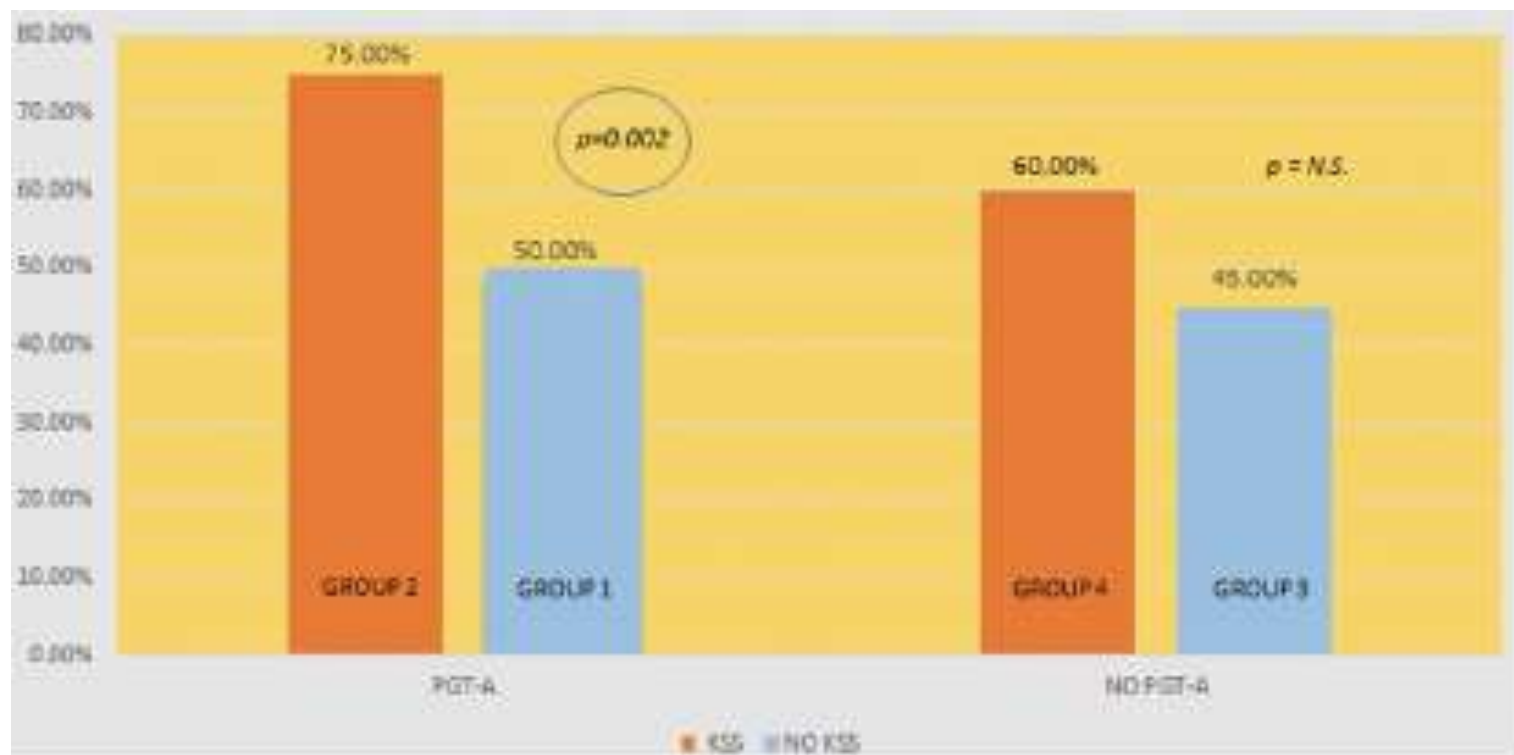


<b>Table 1. Clinical results</b>				
<b>(n=135)</b>	<b>PGT-A</b>			
	<b>KS5 (Group 2)</b>	<b>No KS5 (Group 1)</b>	<b>TOTAL</b>	<b>p-value</b>
Transferred embryos	48	86	135	
Patient mean age	29.27	31.83	30.55	
Total positive $\beta$ -hCG	36	43		
Positive $\beta$ -hCG (%)	<b>75.0%</b>	<b>50.0%</b>		<b>0.002</b>
Gestational sac w/heartbeat	32	42		
Ongoing Pregnancy	<b>66.7%</b>	<b>48.8%</b>		<b>0.037</b>
<b>(n = 50)</b>	<b>No PGT-A</b>			
	<b>KS5 (Group 4)</b>	<b>No KS5 (Group 3)</b>	<b>TOTAL</b>	<b>p-value</b>
Transferred embryos	10	40	50	
Patient mean age	26.7	30.22	28.46	
Total positive $\beta$ -hCG	6	18		
Positive $\beta$ -hCG (%)	60.0%	45.0%		<b>0.396</b>
Gestational sac w/heartbeat	2	12		
Ongoing Pregnancy	20.0%	30.0%		<b>0.529</b>



**Table 2.** Embryos are matched with their respective KS5 scores (1 to 6). Number of euploid, transferred, and implanted embryos, and gestational sacs in each group are shown

<i>Score KS5</i>	<i>Embryos</i>	<i>Euploid</i>	<i>Transfer</i>	<i>Positive β-hCG</i>	<i>Implantation rate</i>	<i>p-value</i>	<i>Gestational sac w/heart beat</i>	<i>Ongoing pregnancy rate</i>	<i>p- value</i>	<i>Euploidy rate</i>	<i>p-value</i>
<b>1</b>	269	130	51	25	<b>49.0%</b>	<b>0.045</b>	24	<b>47.1%</b>	<b>0.105</b>	<b>48.3%</b>	<b>0.006</b>
<b>2</b>	4	2	0	0	0.00%		0	0.00%		50.0%	
<b>3</b>	211	111	31	18	<b>58.1%</b>	<b>NS</b>	18	<b>47.4%</b>	<b>NS</b>	<b>52.6%</b>	<b>0.042</b>
<b>4</b>	5	3	3	2	66.7%		2	66.7%		60.0%	
<b>5</b>	115	74	29	17	<b>58.6%</b>	<b>NS</b>	15	<b>51.7%</b>	<b>NS</b>	<b>64.3%</b>	<b>0.042</b>
<b>6</b>	223	138	25	20	<b>80.0%</b>	<b>0.045</b>	18	<b>72.0%</b>	<b>0.105</b>	<b>61.9%</b>	<b>0.006</b>



**Figure 1.** Implantation rate (with and without PGT-A) vs. embryo selection (with and without KS5 selection).





- Our highly encouraging results showed the advantages offered by time-lapse technology in assisted reproduction. We firmly believe that predictive algorithms should be used as an accessory tool to traditional morphological assessment and PGT-A and incorporated into the protocols of assisted reproduction laboratories. An easy-to-implement method, predictive algorithms use basic events in embryo development observed by an embryologist as input to produce a score with which the best embryos can be selected for transfer.

RESEARCH


Open Access

Individualized embryo selection strategy developed by stacking machine learning model for better in vitro fertilization outcomes: an application study



# Baseline characteristics of the variables included in the data sets

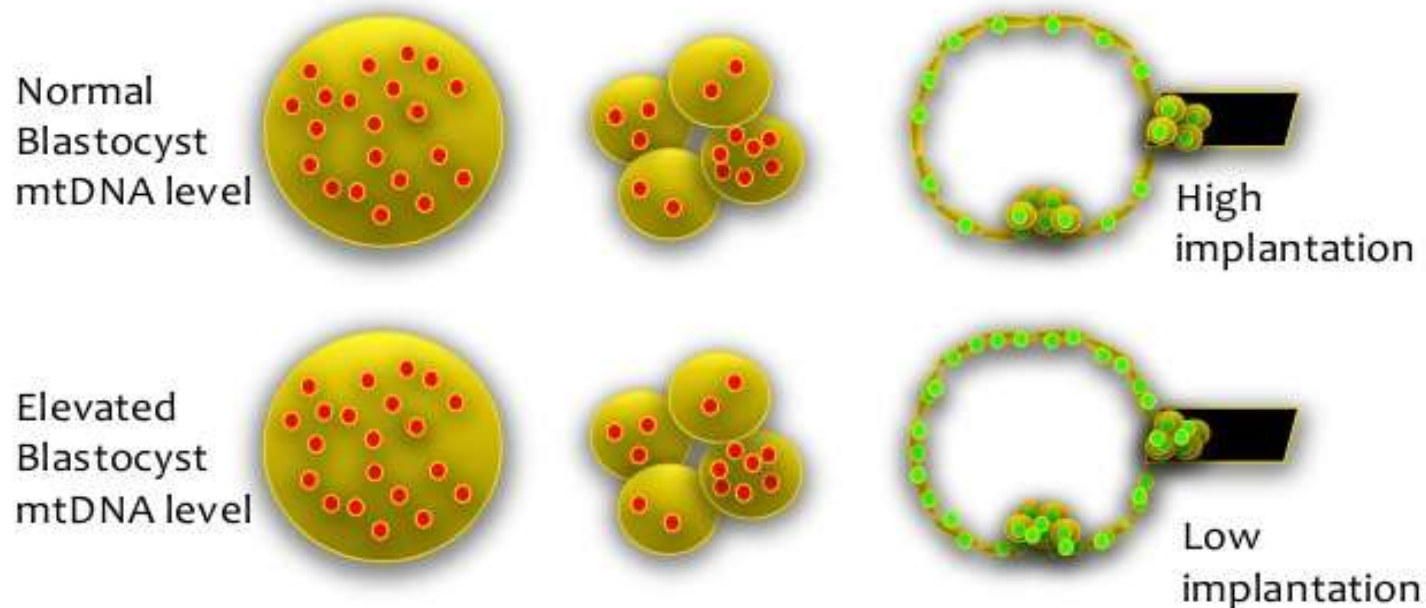
- Age\*, y
- Attempt times of IVF\*
- Antral follicle count\*
- Follicle stimulating hormone\*, IU/L
- Luteinizing hormone\*, IU/L
- E2 per mature oocyte
- E2 on HCG day\*, pmol/L
- Endometrial Thickness\*, mm
- MetaphaseII (M II)\*
- 2pronucleus(PN)\*
- Oocyte Number\*
- 2PN/MII\*
- **Male factor**
- Frozen Sperm Oligospermia
- Asthenospermia
- Azoospermia
- **Female Factor**
- Endometriosis
- Ovulation Disorder
- Unknown

- 
- Sperm Retrieval
  - Ejaculation
  - MESA
  - TESA
  - PESA
  - Stimulation Protocol
  - Agonist Protocol\*
  - Antagonist Protocol
  - Endometrial Type
  - A\*
  - B

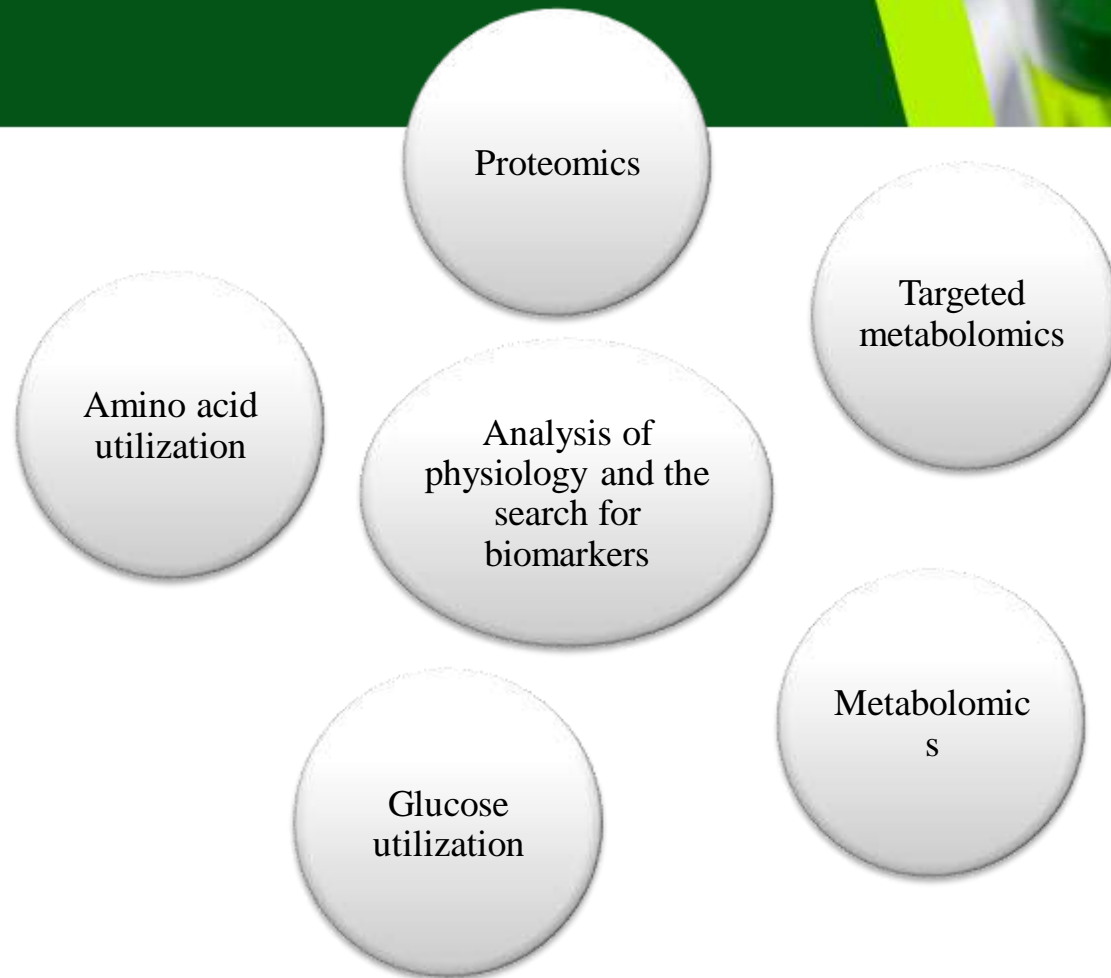
- C\*
- Infertility
- Primary
- Secondary\*
- Fertilization Method
- IVF
- ICSI\*
- Embryo Features
- Number of Blastomere\*
- Fragmentd\*
- Equalitye\*

## Mitochondrial DNA levels as a marker of embryo viability in IVF

- Egg mitochondria.
- Embryonic mitochondria (replicated after genome activation)







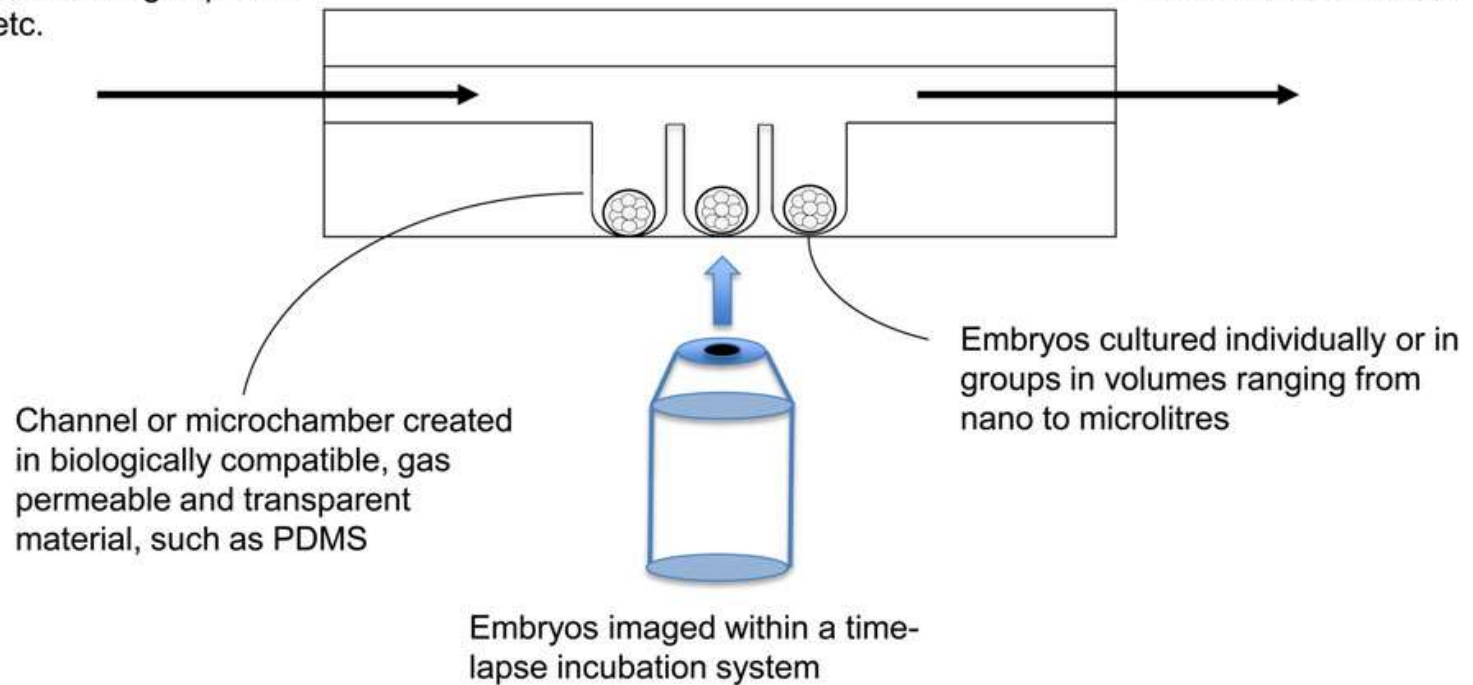


Medium Introduced:

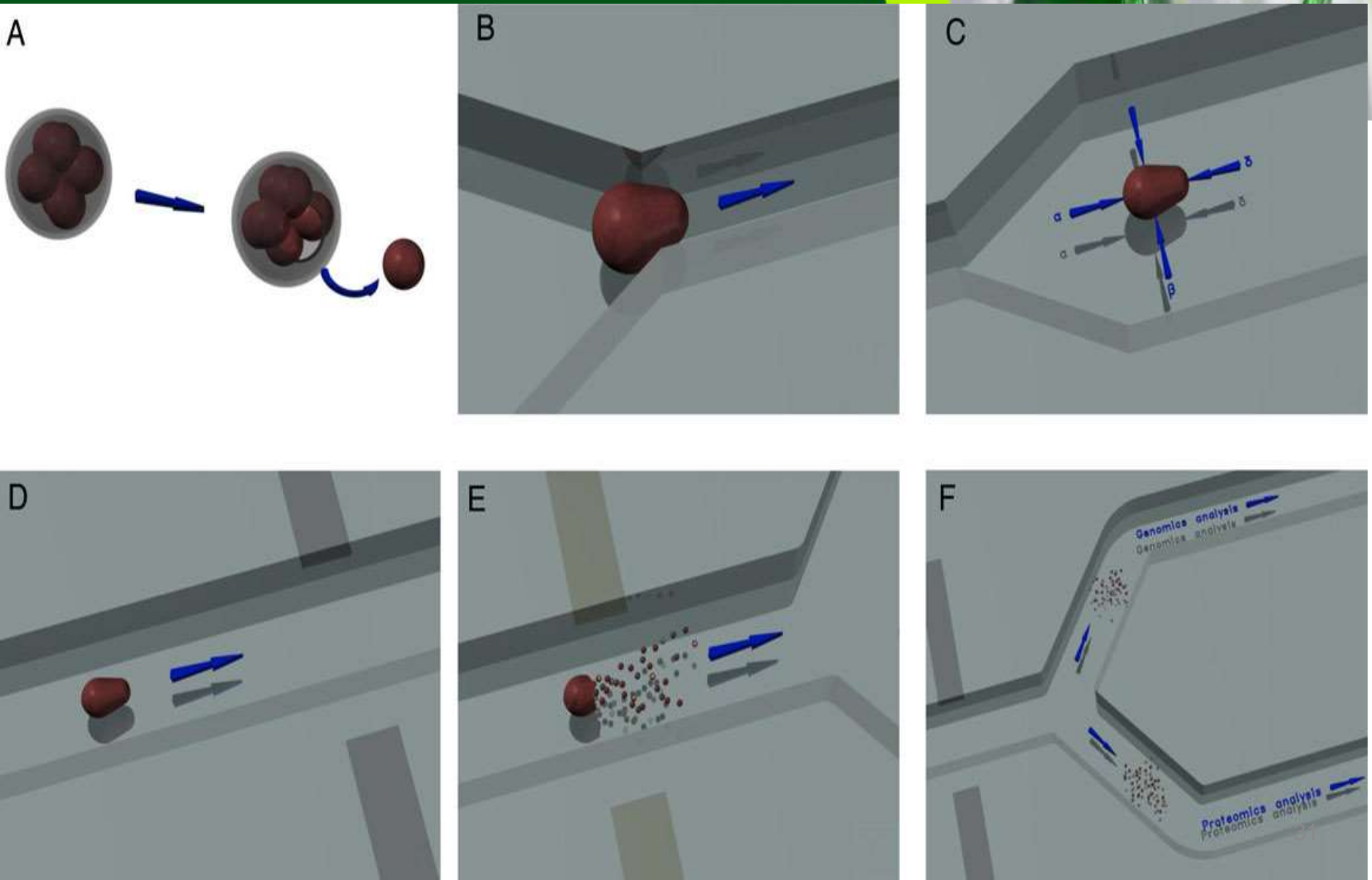
Changing metabolite pool,  
introduction of stage specific  
factors etc.

Medium Expelled:

Analysis of  
metabolites/biomarkers



# Concept microfluidic device for biomechanical analysis of a preimplantation embryo





**THANK  
YOU**

**DOCTORS AND NURSES**