



INTERNATIONAL FEDERATION OF FERTILITY SOCIETIES

Gamete Preparation & Embryo Culture

IFFS Embryology Workshop
TARTU, ESTONIA
21-23 September 2018

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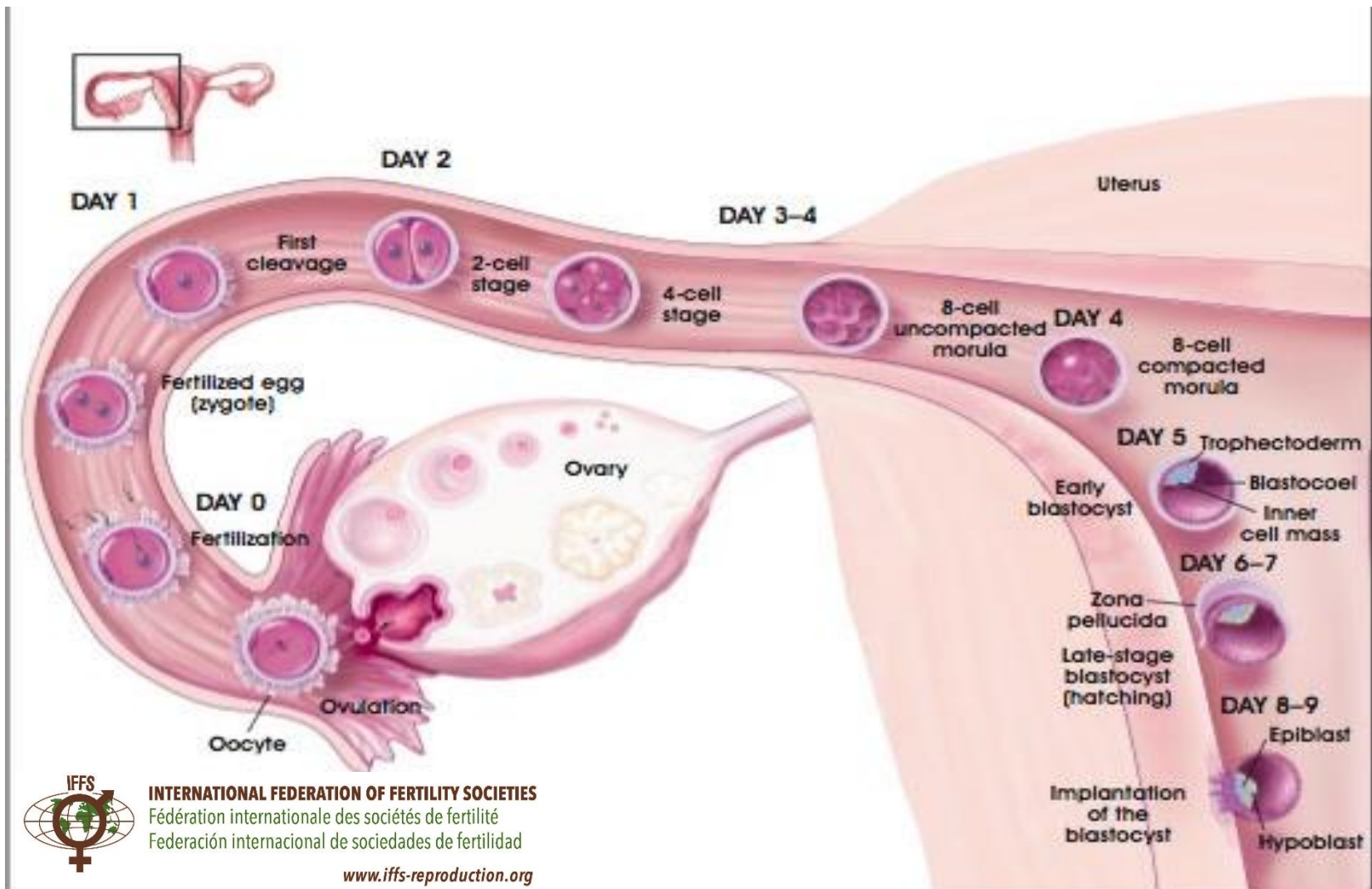


@IntFertilitySoc



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Why IVF is possible



Why IVF is possible....

- The preimplantation mammalian embryo is a free-floating entity
- This fact underlies the development of therapeutic IVF, and of all subsequent assisted conception procedures
- The success of IVF has relied on the ability to fertilise eggs and culture embryos *in vitro*
- The development of effective culture conditions has been a vital pre-requisite for the study of preimplantation embryogenesis, and for improvements in assisted conception treatment and outcomes



The beginnings of IVF.....



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Bob Edwards in his laboratory



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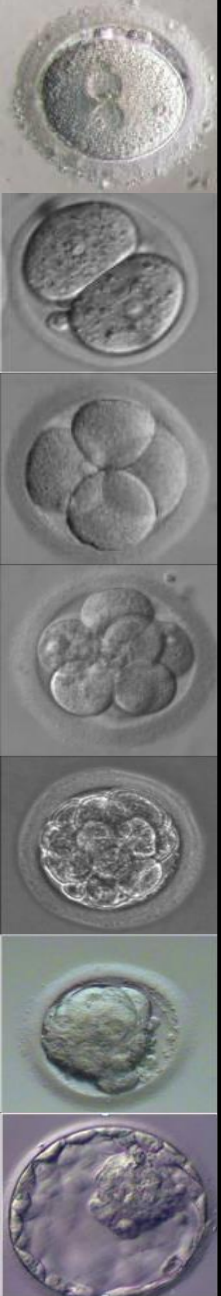
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Fertilisation in vitro



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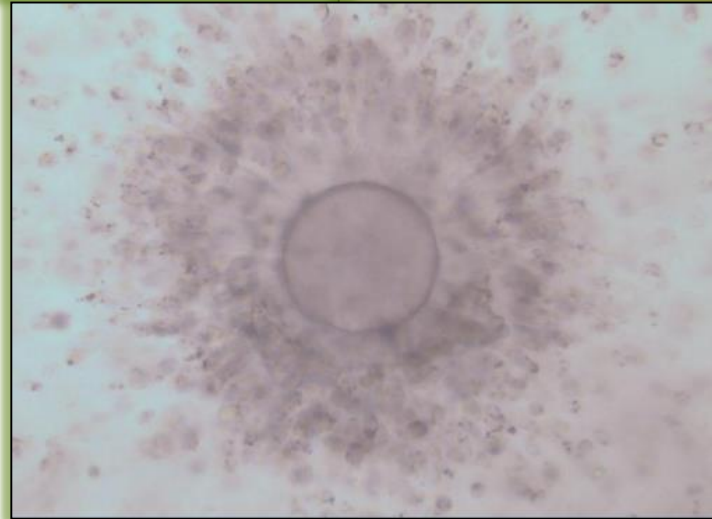


IVF and Embryo Culture

- Culture medium
- Temperature & pH
- Gas phase



Egg collection



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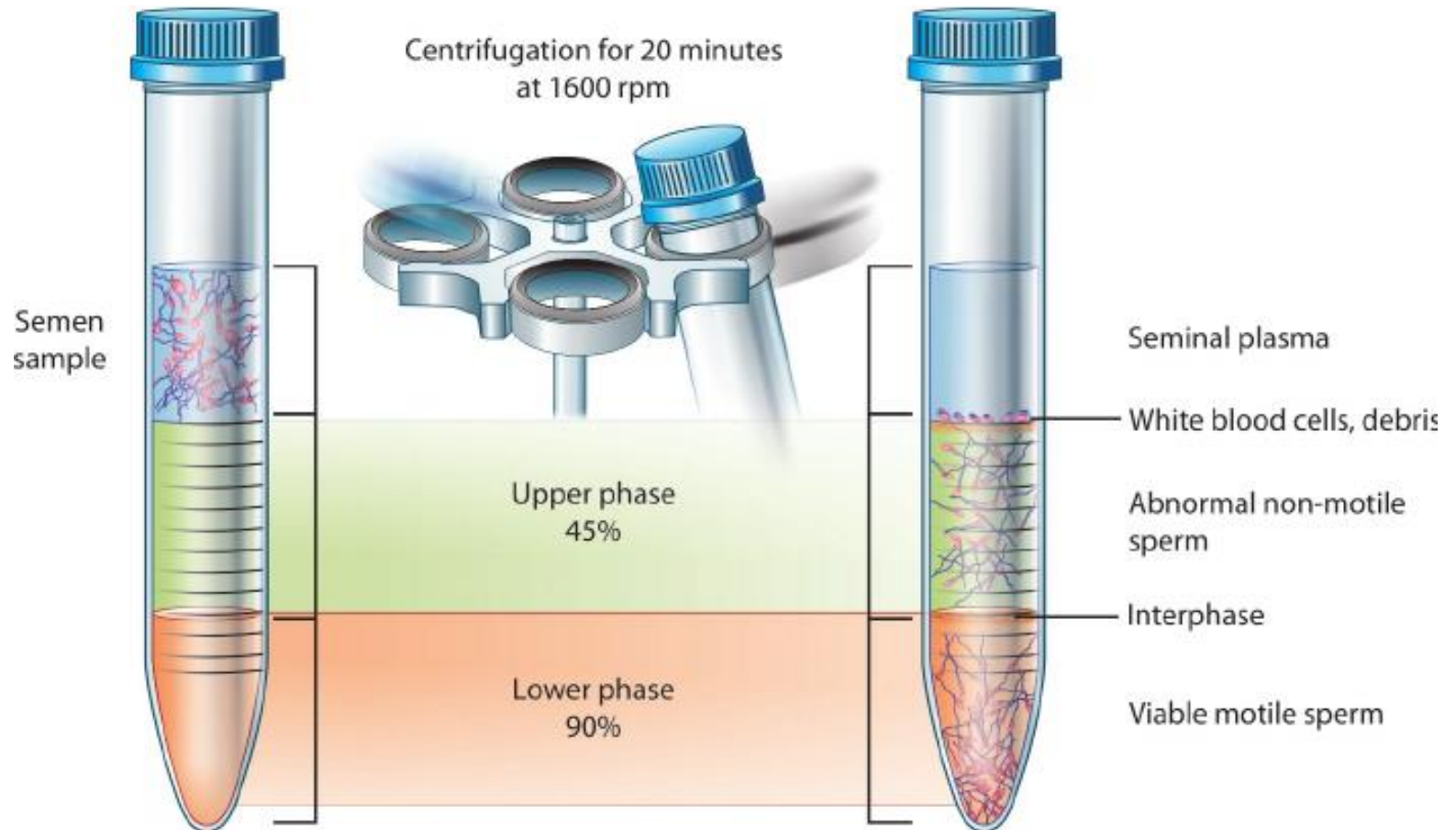
Semen preparation

- Semen analysis
 - Count (>15 million/ml)
 - Motility ($>40\%$ progressive)
 - Morphology ($>4\%$ normal forms)
- Preparation technique
 - Density gradient
 - Wash and swim-up
- Final preparation
 - IVF or ICSI



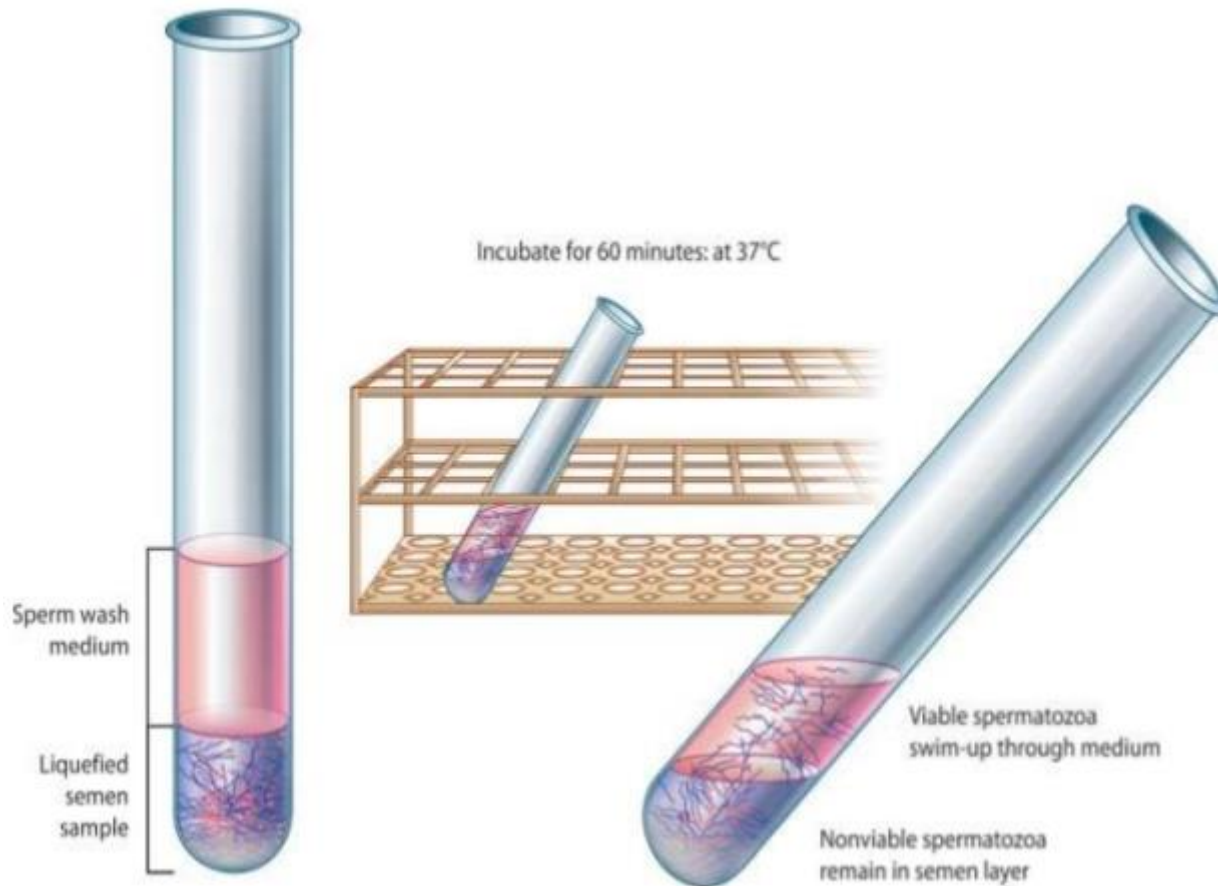
Semen preparation

Density gradient separation



Semen preparation

Wash and swim-up



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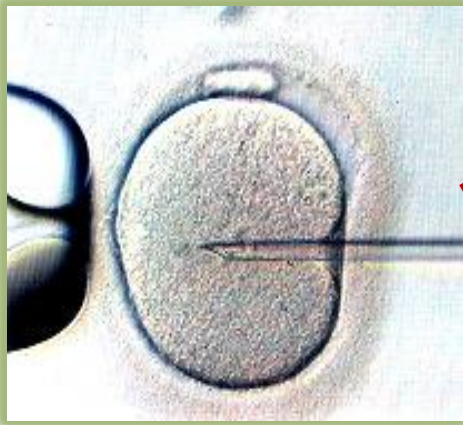
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Fertilisation *in vitro*

IVF



ICSI



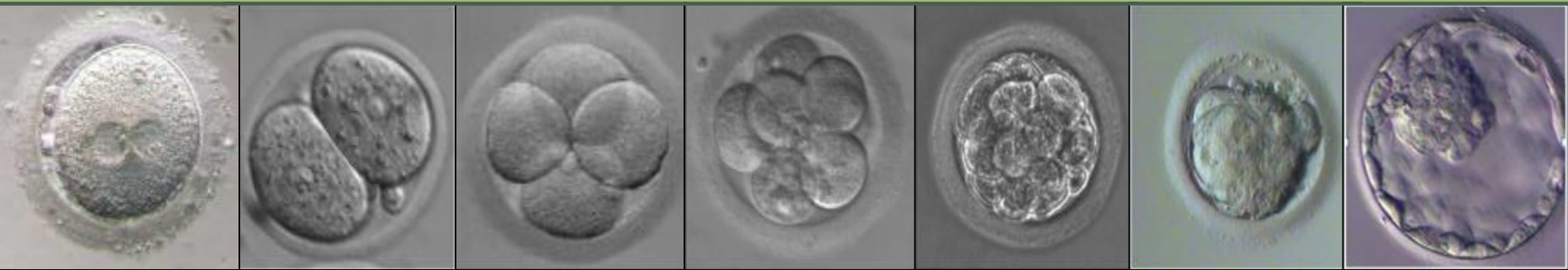
Fertilised egg



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Embryo Culture



2PN
Day 1

Cleavage stages
Days 2-3

Morula
Day 4

Compaction
Day 4

Blastocyst
Days 5/6



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Embryo Culture

- Culture medium
- Temperature & pH
- Gas phase



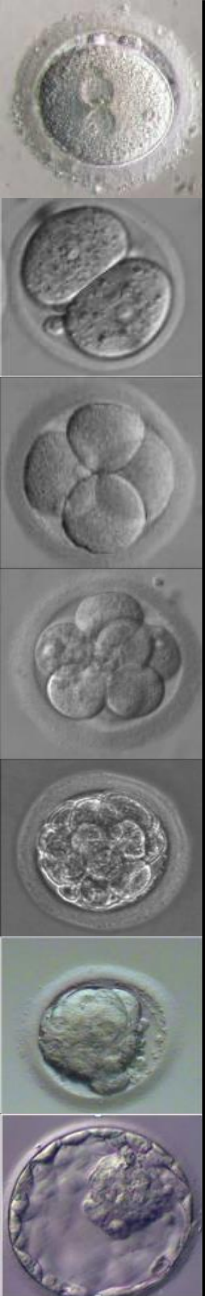


Culture medium

Key components

- Water
- Ions
- Energy source
- Buffer

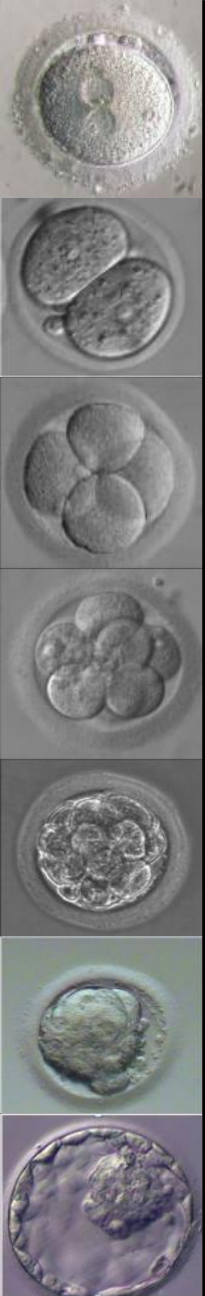




Importance of Buffers and pH

- Gene expression
- Metabolism
- Enzyme activity
- Maintenance of gap junctions
- Maintenance of the cytoskeleton
- Modulation of calcium levels
- Cell proliferation

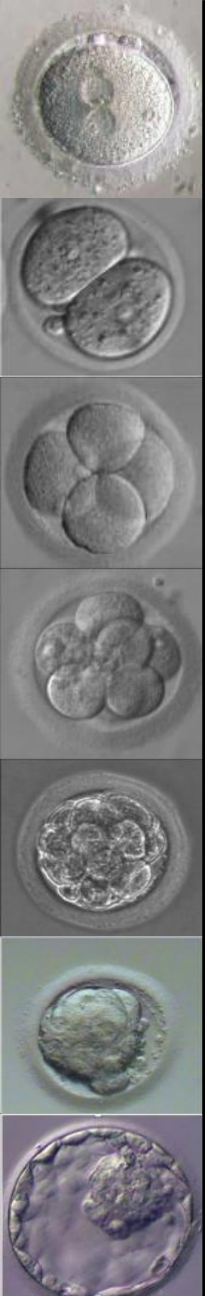




Buffers

- In air (egg collection, ICSI)
 - HEPES
 - MOPS
- Culture in 5-6% CO₂
 - Sodium bicarbonate

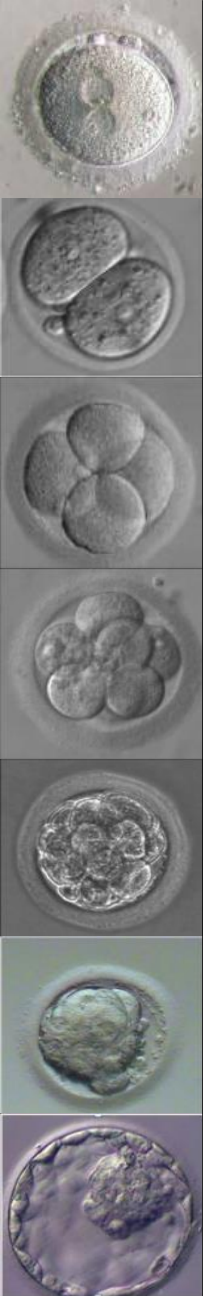




IVF and Embryo Culture

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Incubators



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Chest incubator

Designed for long-term culture

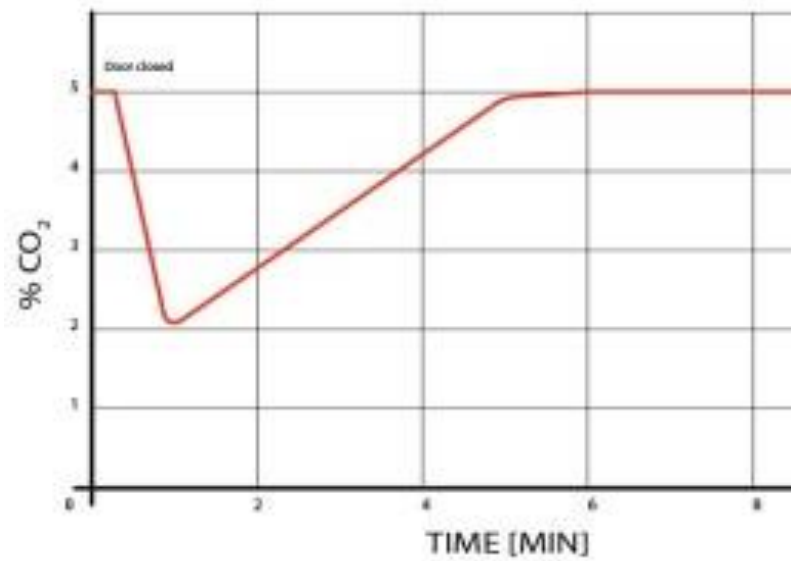
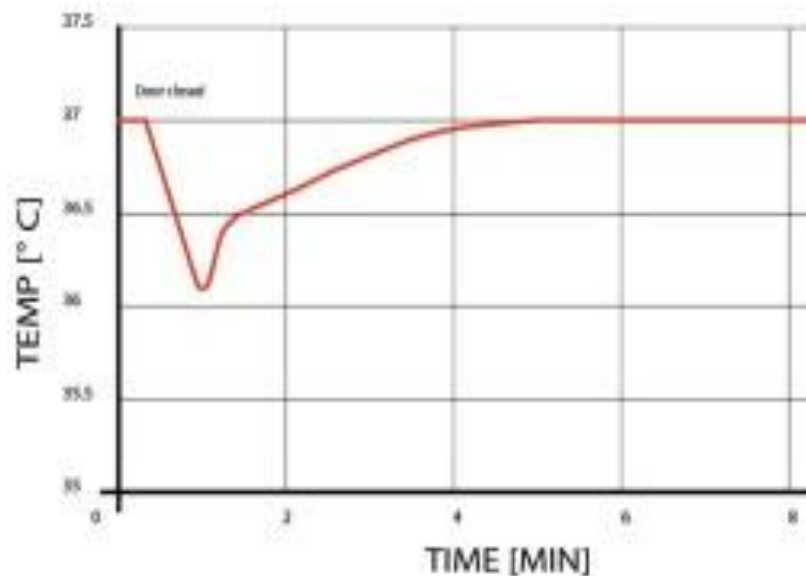


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Chest Incubator

Temperature and CO₂ recovery times after 15 second door opening x 1



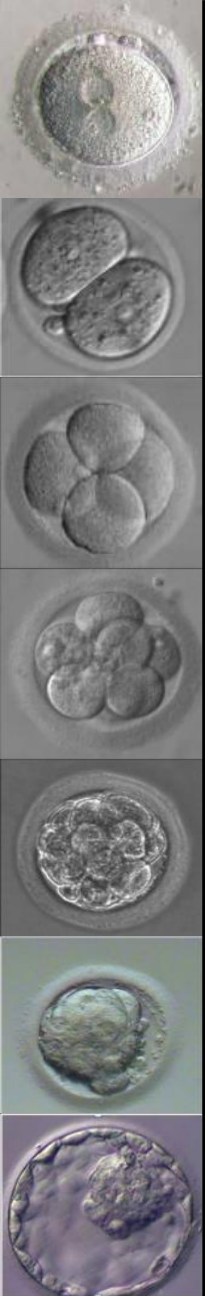
Innovation: 6 inner doors



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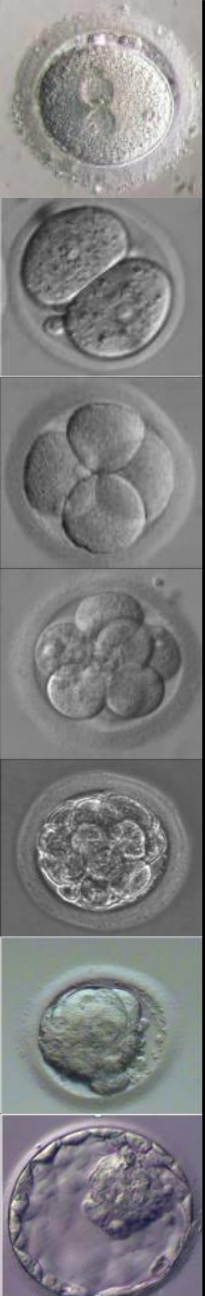
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Bench-top incubator



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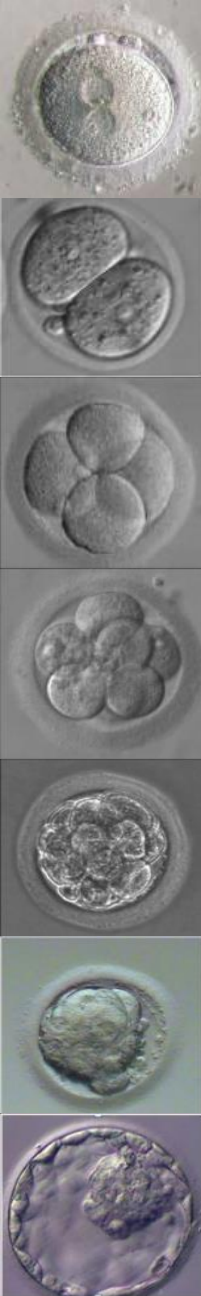
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IVF and Embryo Culture

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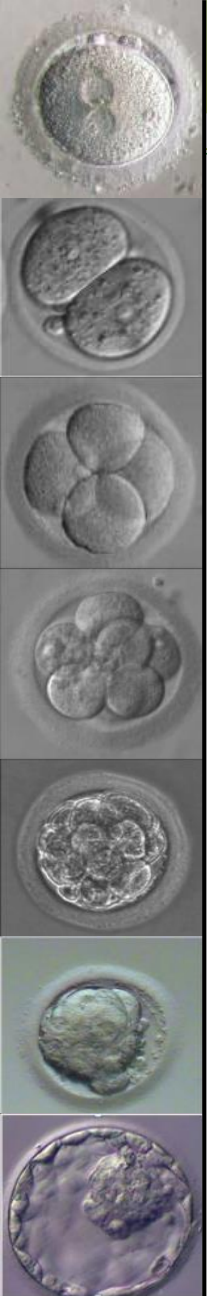




Gas phase

- Embryo culture may be in atmospheric (20%) or reduced (5%) oxygen
- High oxygen has adverse effects on development in several animal species
- Some dispute remains over possible benefits of low oxygen in human embryo culture



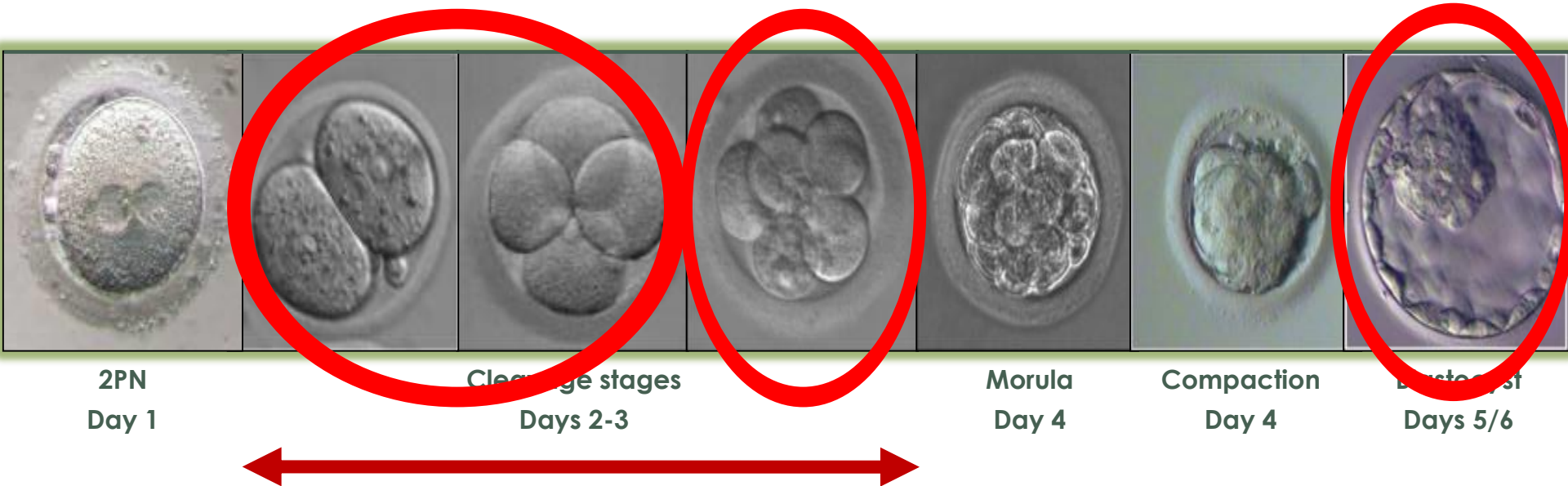


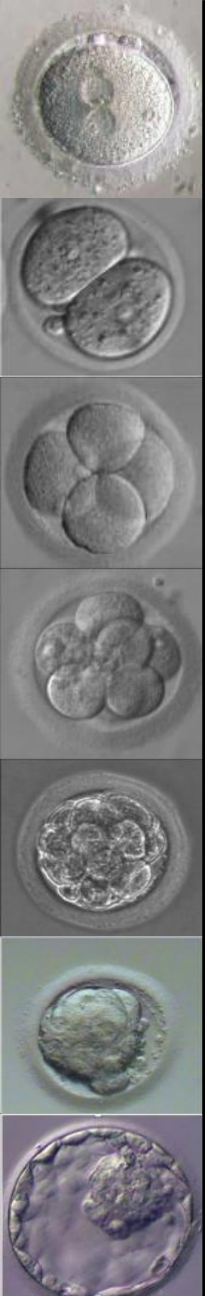
Oxidative stress

- REDOX state of cells
- Gene expression
- Metabolism
- Differentiation



When to do embryo transfer?





IVF and Embryo Culture

Culture medium

- Temperature & pH
- Gas phase



Sequential or single medium?

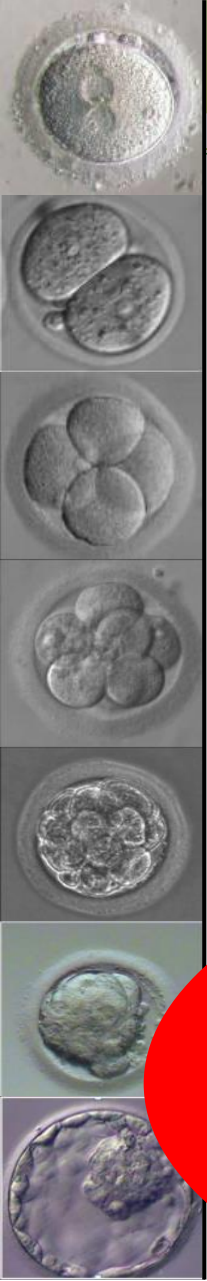


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Blastocyst Transfer





Prerequisites for successful blastocyst culture

- Low oxygen
 - 5% O₂
- Better incubators
 - Universal use of Micro incubators
- Sequential media
 - Cleavage medium (lactate/pyruvate, D1-D3)
 - Blastocyst medium (glucose) D3-D6





Culture Media: Sequential or Single-Step?

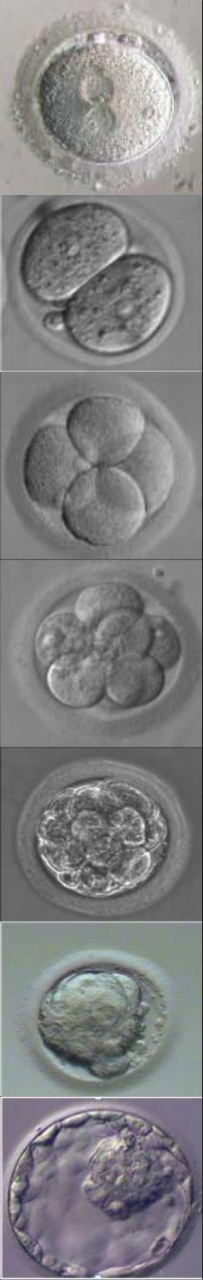
Arguments

Sequential media

- *In vivo* data in the human have shown different concentrations of pyruvate, lactate and glucose in the oviduct and uterus that change throughout the menstrual cycle

Single step medium

- There are no direct data from the human
- Embryo metabolism is not strictly determined by the environment
- *In vitro*, embryos may be able to adapt to a constant environment by altering their metabolism, provided constituents of culture media fall within tolerable ranges



Culture Media: Sequential or Single-Step?

Carbohydrate metabolism

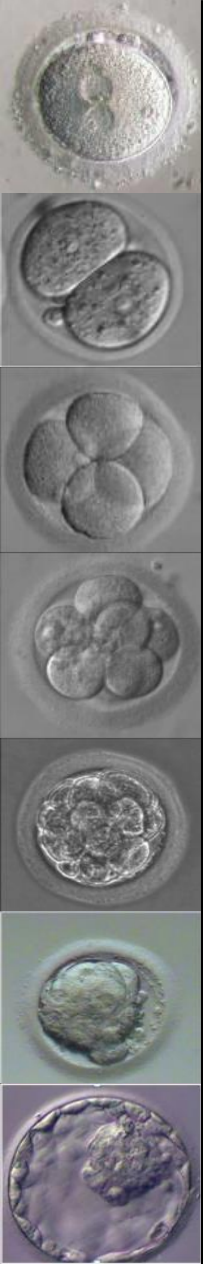
Sequential media

- Mouse and human embryos use pyruvate as the primary energy source, changing to glucose after the 8-cell stage
- During early cleavage, glucose should be reduced to 0.5mmol/l or omitted altogether

Single step medium

- Glucose is present at a significant concentration at any phase of the cycle in the human oviduct and uterus
- Glucose is important for ribose synthesis and therefore its complete omission is likely to be detrimental





Culture Media: Sequential or Single-Step?

Glutamine metabolism

Sequential media

- Accumulation of ammonia in culture medium as a result of deamination of glutamine has been shown to induce exencephaly in mice
- Human embryos show impaired development in the presence of high concentrations of ammonia in culture medium

Single medium

- Incidence of exencephaly was not confirmed, either in mice or other animal models, in the presence of similar concentrations of glutamine
- Glutamine can be substituted in culture medium by stable dipeptides of glutamine which do not produce ammonia





Culture Media: Sequential or Single-Step?

Amino acids

Sequential media

- Essential amino acids appear to inhibit development, at least up to the 4-cell stage (day 2)
- Non-essential amino acids stimulate cleavage to the 8-cell stage (day 3) and facilitate blastocyst formation and hatching (day 5/6)

Single Step medium

- Human embryos make no distinction between essential and non-essential amino acids

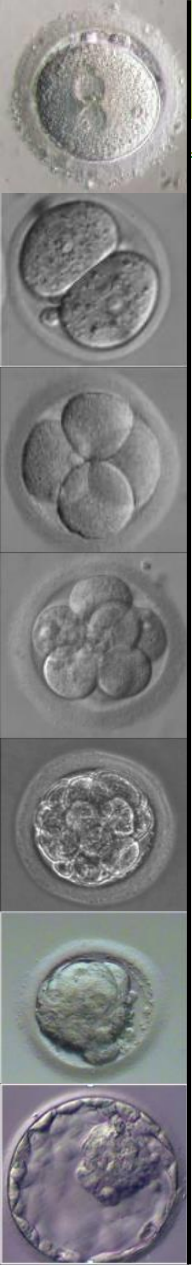




So where does this leave us?

- Many different culture media are available commercially
- The exact components of none of these are known
- All *claim* to enhance culture conditions by supplementation with essential and non-essential amino acids, vitamins and even growth factors and cytokines.....
- There is no absolute evidence in support of the need for sequential culture media





Culture Media: Sequential or Single-Step?

Sequential

Jan – June 2014

- 652 cycles
- Age 35.7 yrs
- No 2PN/cycle: 6.5
- IR 24.9%
- 42% eSET
- **44.12% utilisation***

Single Step

Jan - June 2015

- 784 cycles
- Age 35.5 yrs
- No. 2PN/cycle: 6.5
- IR 24.4%
- 49% eSET
- **48.92% utilisation***



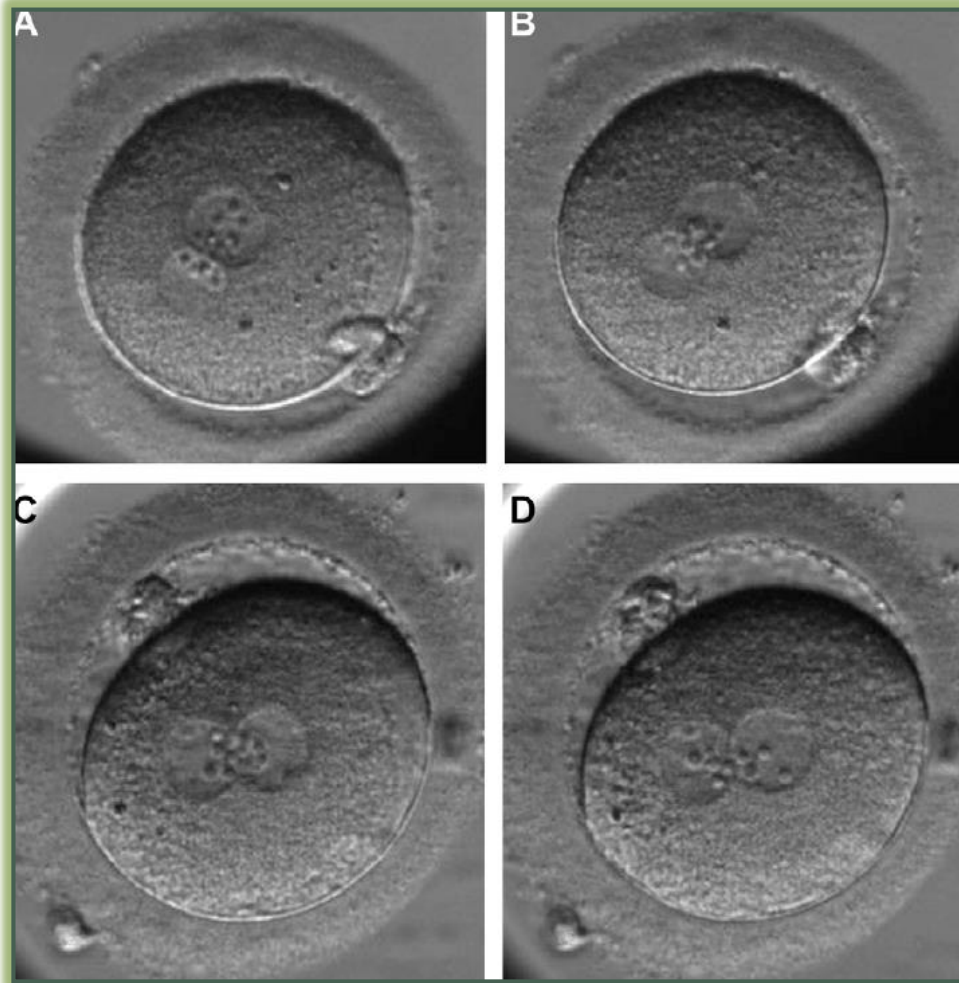


Embryo selection

- Identify embryos most likely to implant
- Criteria for scoring embryos at different stages of development

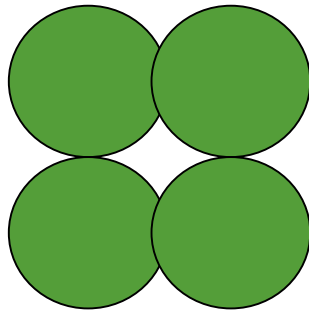


Pronucleate stage (Day 1)

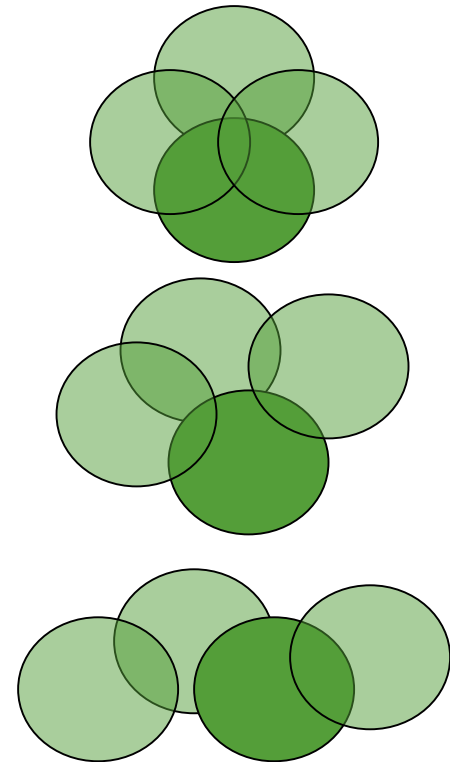


Cleavage stage (Day 2-3)

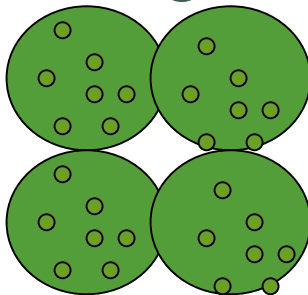
No. of blastomeres



Blastomere symmetry

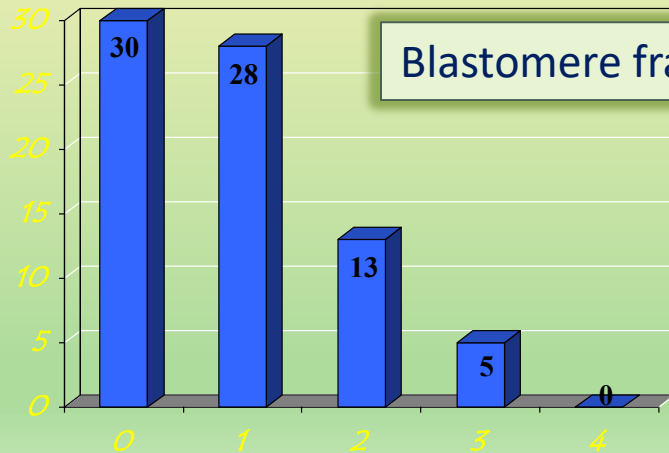


Extent of fragmentation

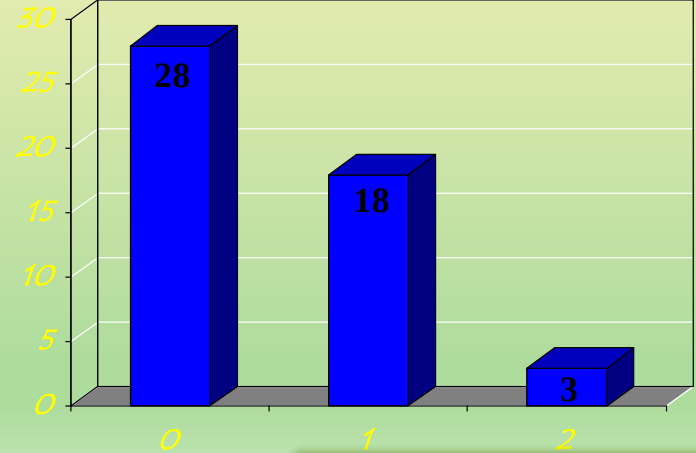


Cleavage stage morphology and implantation rate

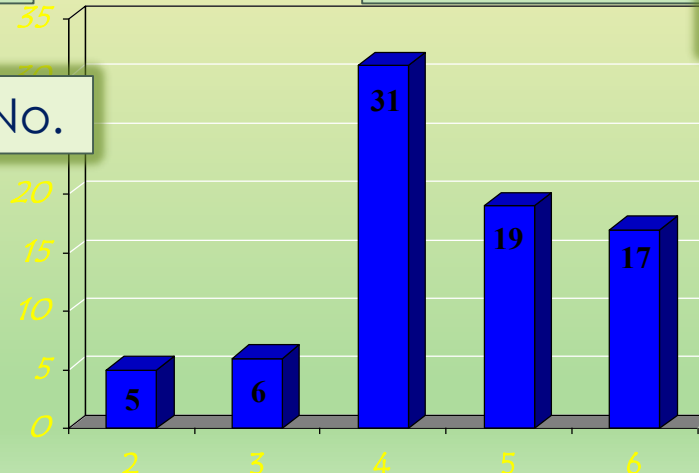
Blastomere fragmentation



Blastomere symmetry



Blastomere No.

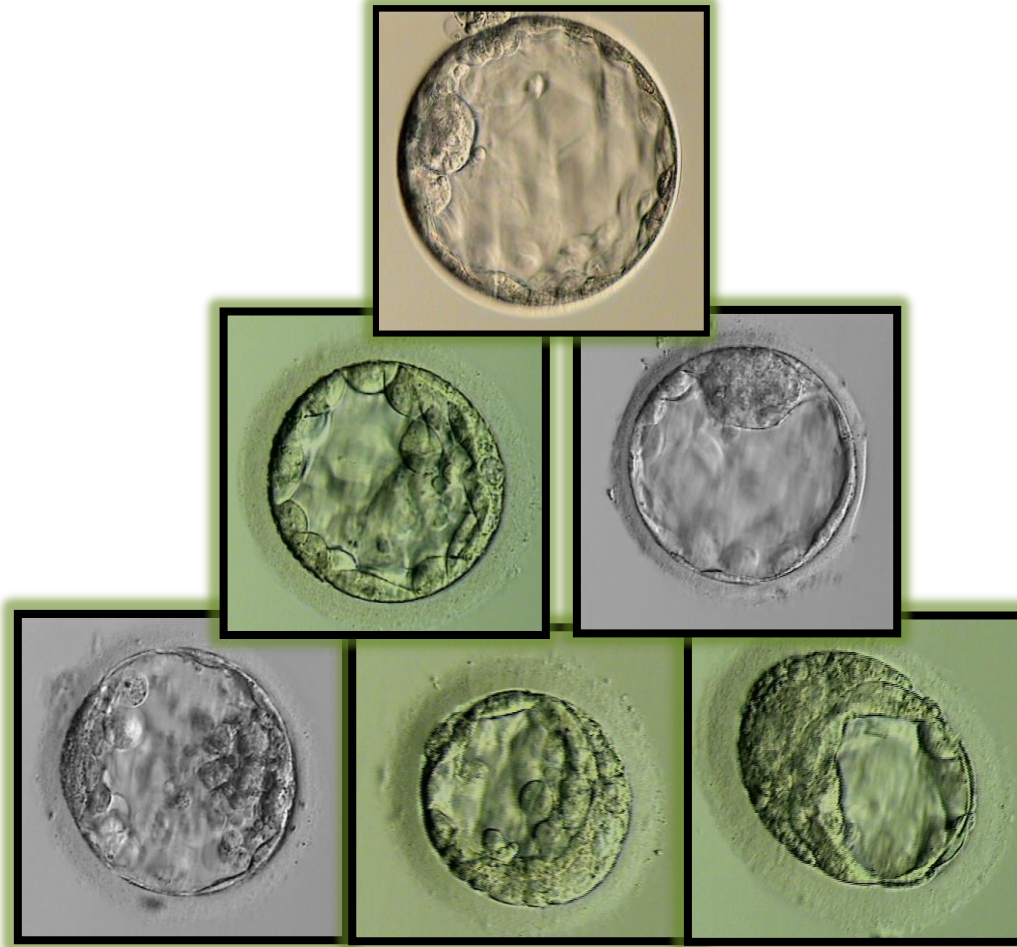


All $p < 0.001$



Blastocyst Stage (Day 5)

Best
↓
Worst



Time lapse monitoring for embryo selection

Eeva



Embryoscope



Time lapse imaging and morphokinetics

- Detailed analysis of specific parameters
 - Pronuclear breakdown
 - Time of cleavage divisions
 - Interval between cleavage divisions
- Derivation of *centre-specific* algorithms

Time lapse imaging and morphokinetics

- May assist in “deselection” of embryos
- Focus of much attention and hype
- Reality not living up to promise

Embryo selection



- Reducing multiple pregnancies whilst maintaining pregnancy rates remains a challenge
- Current methods for embryo selection remain unsophisticated
- Day 5 transfer is *one approach* for embryo selection
- Better methods for selection of embryos on Day 1, Day 2 or Day 3 should still be sought



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