Introduction to Andrology and Embryology

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What is Andrology and Embryology?

- ANDROLOGY: Study of medical conditions specific to males, particularly infertility and sexual dysfunction

- EMBRYOLOGY: Study of the formation and development of embryos from a fertilised egg cell to differentiation into tissues and organs


ANDROLOGY

Semen analysis - WHO Criteria (1999)

- Abstinence 2 – 5 days
- Concentration > 20 mill/ml
- Motility > 50% progressively motile
- Morphology > 30% normal (determined in-house)
- Antisperm antibodies < 20%

Classifications of sperm

- Normozoospermia: Production of sperm in normal numbers and motility

- Oligozoospermia: Low sperm count. Concentration < 20 Mill/ml

- Asthenozoospermia: Reduced sperm motility. Progressive motility < 50%

- Teratozoospermia: Reduced numbers of sperm with a normal appearance (morphology). Normal morphology < 30%

Oligoasthenoteratozoospermia

OATs: Reduced count, motility *and* normal forms
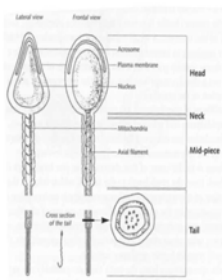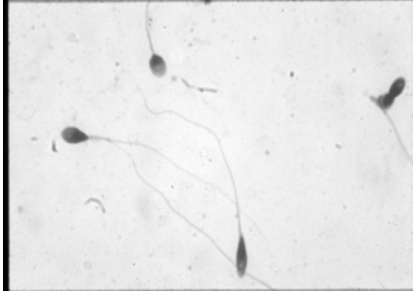
Sperm
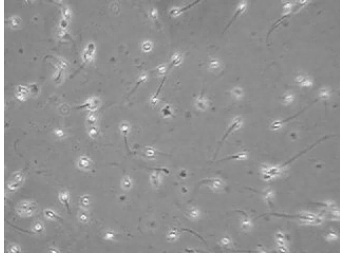
Sperm under phase contrast optics
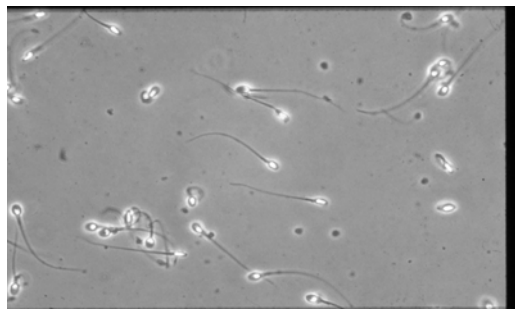
Normal mature sperm


- Head: 5um x 3um
  - Covered by acrosome cap
  - Contains densely packed nucleus
- Neck/Midpiece:
  - Contains mitochondria which supply energy for sperm movement
- Tail: 50um (0.05mm)
  - Made of protein fibres that contract on alternate sides giving wavelike movement that drives sperm forwards

Sperm after Diff-Quik staining

Further Classifications

- Aspermia: No ejaculate ie complete lack of semen
  - eg retrograde ejaculation

- Azoospermia: No spermatozoa in the ejaculate
  - obstructive: where sperm are created, but cannot mix with the rest of the ejaculatory fluid due to a physical obstruction (eg CBAVD)
  - non-obstructive: where there is a problem with spermatogenesis

Further classifications

- Cryptozoospermia: Apparent azoospermic sample but where sperm are found during analysis only after centrifugation

- Necrozoospermia: Samples with only dead sperm (not necessarily just immotile sperm)

- Globozoospermia: Often referred to as 'round-head' defect. Sperm morphological defect where the acrosome is absent and sperm usually have small round heads
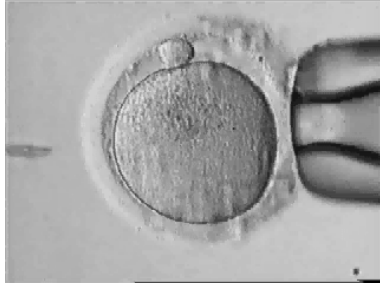
Globozoospermia
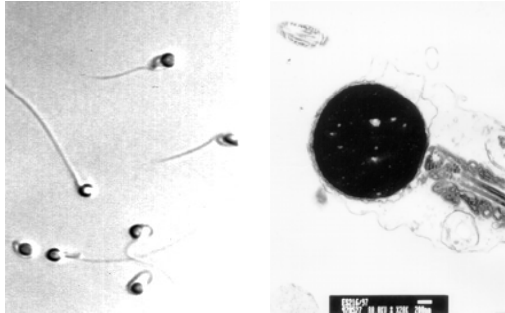
What can we do if the sperm is sub-optimal?

ICSI - indications

Intra Cytoplasmic Sperm Injection
1992: First baby born

- **sub-optimal semen analysis**
  - reduced conc, motility, morphology
  - globozoospermia etc
- **failed/low fertilisation at IVF**
- **non-ejaculated sperm**
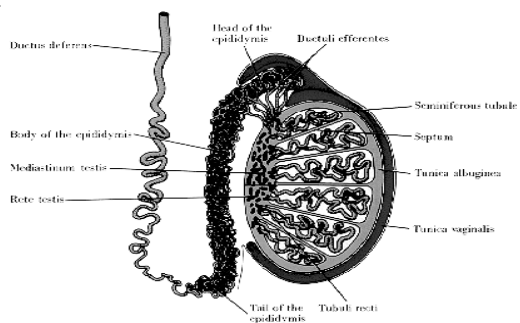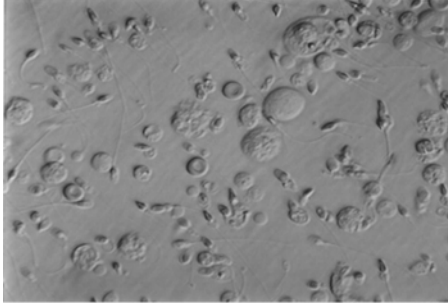- **frozen-thawed sperm - poor survival**

I.C.S.I.

Extraction of sperm from testicular tissue

- Where no sperm are produced in the ejaculate
- Surgical procedure to extract tissue direct from testes
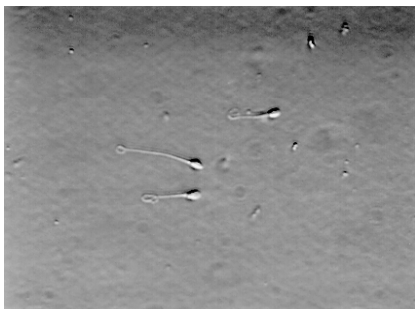- Samples can be frozen in advance of use

Male genital tract
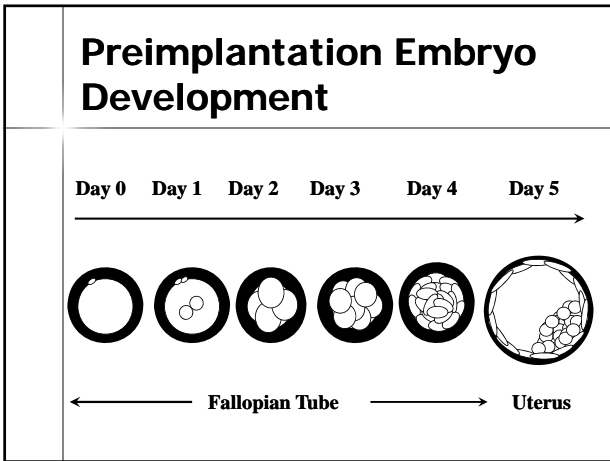
Testicular sperm aspiration (TESA)
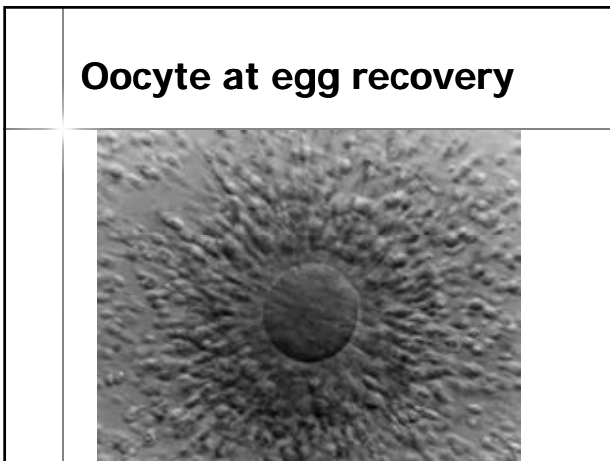

Picking up sperm from testicular tissue

Hypo-osmotic swelling (HOS) test

EMBRYOLOGY

Preimplantation Embryo Development

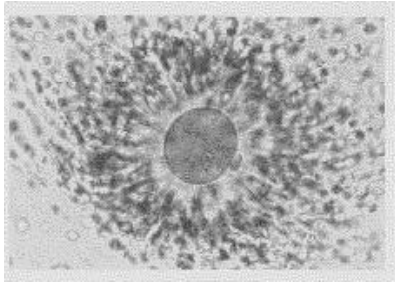| Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |

Fallopian Tube ⟶ Uterus

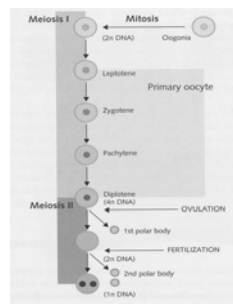## Oocyte at egg recovery

Mature Oocyte

Meiosis

- A two-part cell division process (I and II) which results in gametes with one-half the number of chromosomes of the parent cell
- Thus when sperm and egg come together the normal chromosome content of the parent is restored
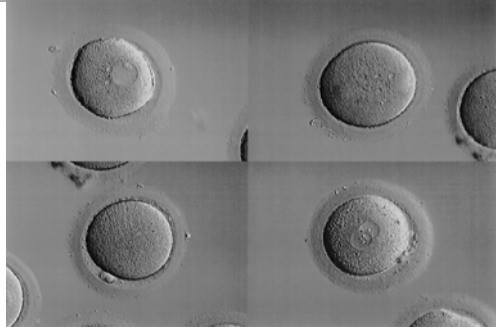
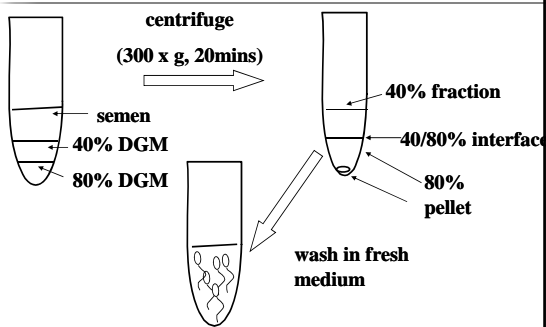# Classifications

Different stages of maturity during meiosis

- Germinal Vesicle:
  - Name from enlarged nucleus
  - In prophase of 1st meiotic division
- Metaphase I:
  - Arrested at this stage in meiosis I
  - No polar body
- Metaphase II:
  - Mature egg able to be fertilised
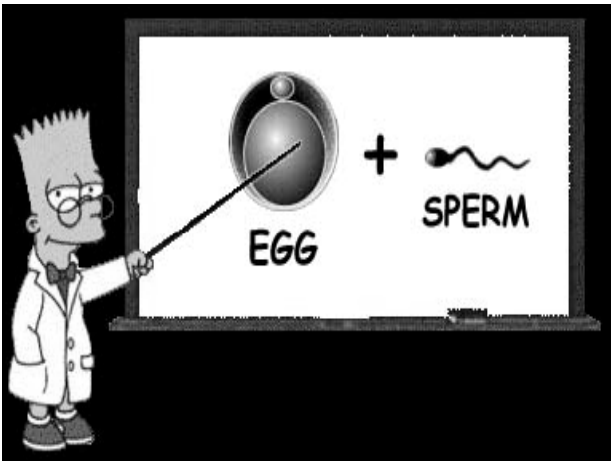 - Has a single polar body

Oocyte maturation

Density gradient centrifugation

centrifuge

(300 x g, 20mins)

semen

40% DGM

80% DGM

40% fraction

40/80% interface
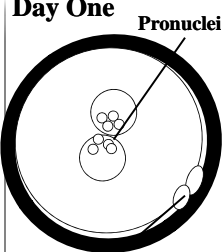
80% pellet

wash in fresh medium

Insemination

Conventional IVF or ICSI
- Oocyte retrieval at 35 hrs post hcg
- Insemination / injection 4 – 6 hrs post oocyte retrieval
  – CIVF: 100 – 250 K motile sperm per ml
  – ICSI: 1 sperm / egg
- Incubated overnight

Day 1 - Pronucleate Embryo

Day One

Pronuclei

Polar bodies

Embryo grading

Which embryos to transfer?

- Law permits transfer of 2 embryos or exceptionally 3
- Pronucleate morphology
- Early cleaving embryos
- Morphological appearance of cleaved embryos
- Progression to blastocyst

Grading of Pronucleate Embryos
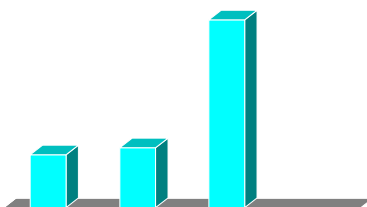
Z1

Z2

Z3

Z3

Z4

Z4
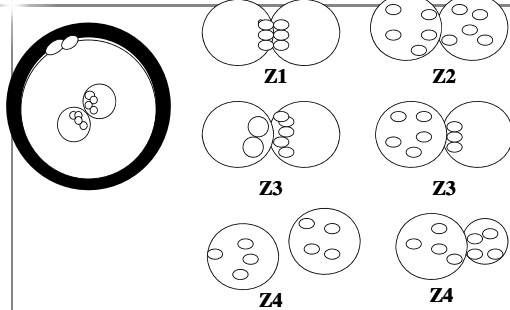
Pronucleate Morphology

- Better embryo morphology
- Higher pregnancy rates
- Reduced embryonic arrest
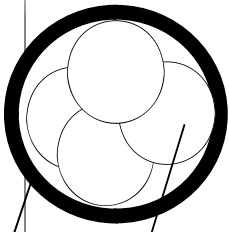- Higher implantation rates
- Higher multiple pregnancy rates

  – Scott et al. 2000, 2007

CPR in Relation to Number Early Cleaving Embryos

-Shoukir et al 1997
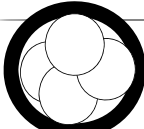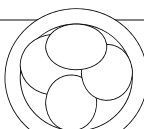-Wharf et al 2004

Day Two Embryo

Zona Pellucida   **Blastomeres**

Usually 2 – 4 cell
Selection possible
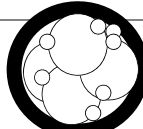
Cleavage Stage Embryo Grading
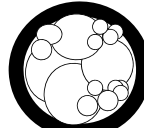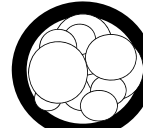
Grade A   **Grade B**   **Grade C**

Grade D   **Fragmented**

Embryo Grading

	A	B	C	D
A	34.2% 608/1778	28.5% 69/242	33.8% 138/408	17.2% 5/29
B		22.5% 9/40	17.8% 18/101	12.5% 1/8
C			31.5% 112/356	17.0% 15/88
D				12.2% 5/41

For elective transfer of 2 embryos in patients < 38yrs since 1995

Day Three Embryo
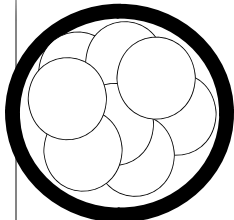
Usually 6 – 8 cell
Further selection possible

2007 – Patients with 2 ET

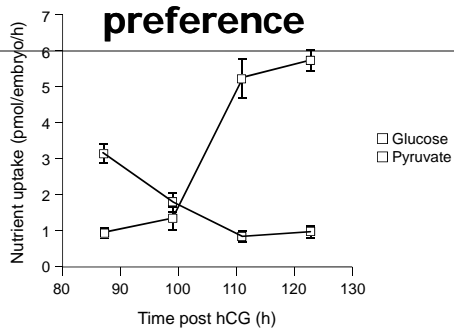| Day ET	Day 2	Day 3
No. Transfers	217	424
No. Clinical Pregnancies	57 (26.3%)	173 (40.8%)

Oxford IVF Results
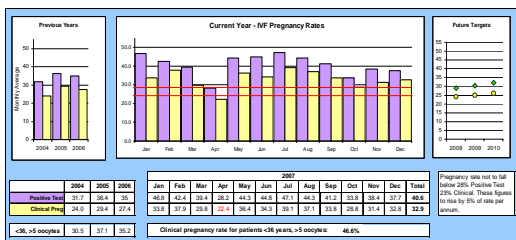
Changes from Day 2 to Day 3 of development

## Number 2PN Embryos Progressing Through Successive Stages
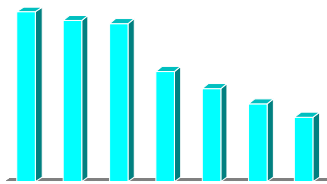
- Woodward et al. 1994

Embryo metabolite preference

Nutrient uptake (pmol/embryo/h) vs Time post hCG (h)

Legend: □ Glucose, □ Pyruvate
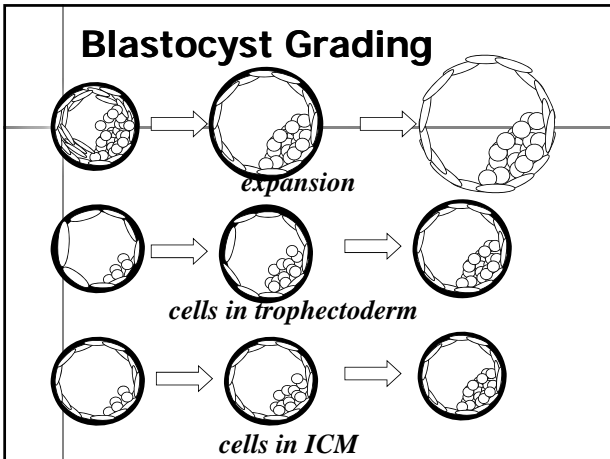
Sequential Media

- Reflects changing embryonic environment
- Composition alters with changing metabolic needs of the developing embryo
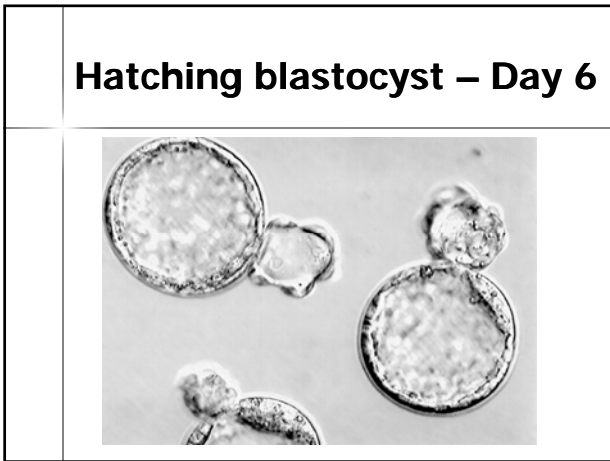- Contains amino acids, carbohydrates and vitamins

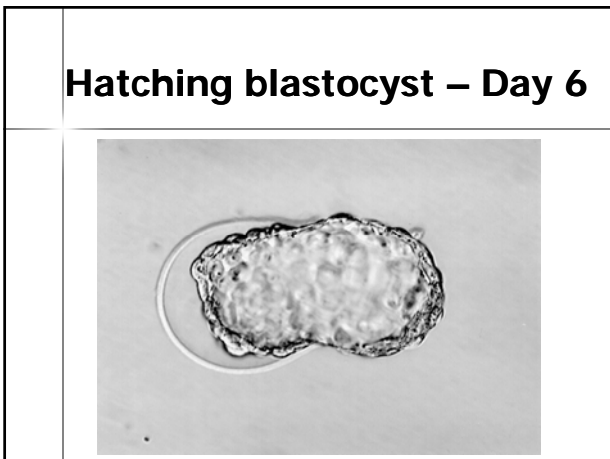## Day Five Blastocyst

ICM

Trophectoderm
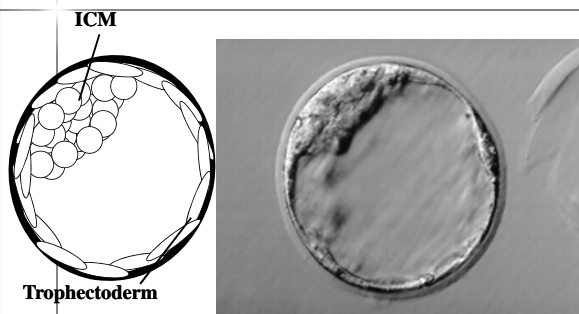
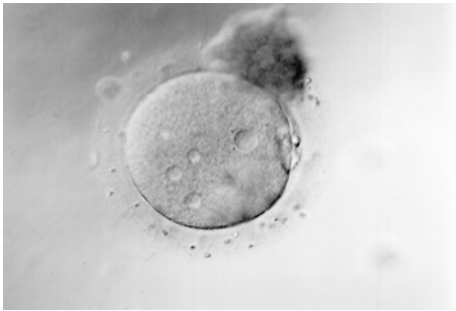## Blastocyst transfer - potential advantages

- Synchronisation of embryos with endometrium

- Improved embryo selection

- Increased implantation rate

- Decreased multiple pregnancy rate by moving towards single embryo transfer

Blastocyst Grading

expansion

cells in trophectoderm

cells in ICM

Hatching blastocyst – Day 6

Hatching blastocyst – Day 6

What can go wrong?

Abnormal oocyte development

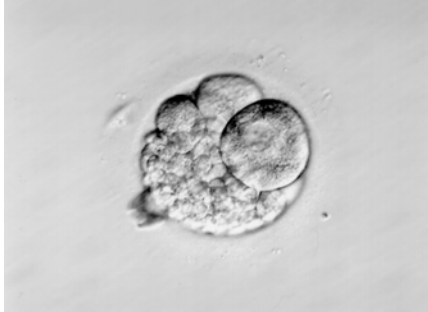**Abnormal oocyte development**

Normally fertilised

Uni-Pronucleate Embryo

3 Pronucleate Embryo

Abnormal development

Summary

- Accurate diagnostic assessment of sperm samples is essential for appropriate treatment
- Important to know and understand what is normal/abnormal for both sperm and embryos before and after treatment
- Important to be aware of abnormality rates, especially after IVF to troubleshoot and optimise your own system