Laboratory Issues – Oocytes, Spermatozoa and Embryos

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ART Lab

1. Prepare sperm effectively
2. Retrieve eggs safely
3. Ensure correct technique used to fertilise the eggs
4. Maintain controlled lab environment
5. Maximise eggs potential – creating the best quality embryos
6. Select the “best” embryo for transfer
7. Freeze surplus embryos – maximum survival rates post thaw
8. Perform embryo transfers
9. Embryo biopsy (PGD/PGS)
Female Reproductive Potential

• Determined early in fetal life.
• During fetal development millions of primordial follicles are produced – development is paused in utero: **Primary oocyte**
  – At birth ~ 1 million
  – Puberty ~ 400,000
  – Menopause ~ 1,000
• Increased age associated with lower potential to achieve a pregnancy
Meiosis

Sexual reproduction relies on the fusion of paternal and maternal haploid gametes—the sperm and the oocyte, respectively—forming a new diploid organism.

Meiosis reduces the chromosome number of the parent cell so that four daughter cells are produced, each with one-half the chromosome number of the parent cell.
Gametogenesis

- **OOGENESIS**
  - X only + 23 chromosomes

- **SPERMATOGENESIS**
  - X or Y + 23 chromosomes
Folliculogenesis

The follicle contains a primary oocyte that is arrested in meiosis I. The oocyte continues meiosis and becomes a secondary oocyte, arrested in metaphase II.
Mature egg (MII)

- An egg cannot achieve fertilisation unless it reaches MII phase.
- The growth of human oocytes is a very slow process, taking many months to complete.
- The primordial oocyte undergoes a 100-fold increase in volume by the time it is mature, from 35 to 120 m in diameter, over a period of around 85 days.
- Egg released from graafian follicle at ovulation.
Aneuploidy

• Eggs and sperm- develop through meiosis
• Meiotic divisions contribute *solely* to the formation of haploid gametes. They consist of two successive divisions, without intervening DNA replication, meiosis I and II, which reduce the genetic content by half.
• In female- chromosomes frequently segregate incorrectly resulting in eggs with an *abnormal* number of chromosomes
• When fertilised – Aneuploid Embryos – fail to develop / miscarriage / genetic abnormalities
• As women age , errors in meiosis occur more frequently
Aneuploidy v Female Age

Figure 3: Percentage of day 5/6 embryos diagnosed as chromosomally “normal” following 24 chromosome screening.
Male Reproductive Potential

Spermatogenesis

• Differentiation primary spermatogonium into mature sperm is initiated at puberty
• Seminiferous tubules – sperm are formed and begin maturation process
• Requires the participation of several cell types, hormones, paracrine factors, genes and epigenetic regulators
Structure of the Spermatozoon

1. Head – containing condensed nucleus and acrosome (DNA)
2. Neck – containing centrioles
3. Middle piece – mitochondria
4. Tail – Flagellum
Semen Analysis
Production of Sperm sample

- Most common form of sperm retrieval is ejaculation – 2-3 days abstinence
- Time between collection to lab < 1 hr;
- Protected against temperature, shock, light
- Sterile container
- Container properly labeled
- Identity check performed
Semen Analysis - Implications in Fertility Treatments

Standard Parameters (WHO)

• Count \( \geq 15 \times 10^6 \) sperm/ml

• Motility \( \geq 40\% \)

• Morphology \( \geq 4\% \)

Antisperm Antibody Testing

\[ \begin{align*}
\text{IgA} & \leq 50\% \text{ binding} \\
\text{IgG} & \leq 50\% \text{ binding}
\end{align*} \]
Ejaculated sperm assessed for concentration and motility (initial and final)

Motile sperm to be separated from the non motile and abnormal sperm

Using a two part gradient system and centrifugation

Sperm then washed with culture media
Sub optimal parameters

• Oligospermia – low sperm counts
• Asthenospermia – low motility
• Teratospermia – reduced normal morphology
• Azoospermia – no sperm

❖ Impacts decision making process..........
Men who require Surgical retrieval include:

• Azoospermia
• Vasectomy
• CBAVD (CF Carriers)
• Spinal Cord injury
Seminiferous Tubules
Micro TESE – Procedure
Theatre/ Lab

• Micro-TESE average time it takes for a single procedure varies from 3-4 hours in theatre
• The tissue processing time is approx 1.5 hours in the laboratory
• Time searching for sperm can be 3-4 hours with multiple embryologists searching
• When sperm found – cryopreservation or used on day egg collection
Oocyte Retrieval

Follicle numbers do not equal egg numbers
Day 0
Oocyte Retrieval

- Follicles are aspirated
- Oocytes are isolated from follicular aspirates
- Oocytes placed in culture media drops
- Oocyte maturity assessed based on appearance of surrounding cells
Oocytes - EPU
Day 0 – Egg Collection Day

Insemination Technique

- Depends on sperm quality or patient history (previous IVF cycles)
  - Standard Insemination (IVF)
  - Microinjection (ICSI)
Day 0
IVF or ICSI?

**IVF**
Add sperm to culture drops containing eggs

**Advantages**
Non invasive

**Disadvantages**
A low incidence (<1%) of fertilisation failure

**ICSI**
Injecting a single sperm into each mature egg

**Advantages**
Essential for couple with male factor infertility
Lower risk of fertilisation failure

**Disadvantages**
Invasive
Eggs require denuding
ICSI Dish Prep & procedure
ICSI – Microscope
Microinjection (ICSI)

A single sperm is immobilized and drawn into a fine pipette for injection into the egg.

The egg is held steady using a “holding” pipette.

The polar body is positioned at 12 o’clock to minimize disruption of DNA in meiotic spindle

The sperm is expelled into the cytoplasm of the egg and the pipette is withdrawn from the egg.
Requirements for Fertilisation

- Capacitation of sperm
- Acrosome reaction
  - Sperm penetration the zona pellucida
  - Binding and fusion to the Oocyte membrane
Day 1: Fertilisation Check

1 x PN - abnormal

3 x PN - abnormal

Multiple PN, fragmenting

Degenerate
Fertilisation (day 1)

**Normally** Fertilised egg (2 Pronuclei Visible; 2\textsuperscript{nd} PB extruded)

**Unfertilised**

**Abnormal** (>2 PN)

eg.

- Standard Insemination more than 1 sperm has entered
- ICSI – Second polar body hasn’t been extruded by egg as above
SYNGAMY

• Follows fertilisation and migration of the female and male pronuclei towards each other.
  – Duration 6-10 hours after pronuclei formation
  – Migration of pronuclei dependent on centrioles inherited from sperm
Day 2 Embryos

- Cleavage Stage – 2-6 CELLS
- Mitotic division of the zygote
- Formation of blastomeres
  - Cleavage begins ~26 hrs after fertilisation
  - Blastomeres are totipotent
Embryo Arrest

• IVF embryos can permanently arrest in mitosis at the 2- to 4-cell cleavage stage showing no indication of apoptosis

Reasons for arrest:
• Most arrested embryos (70%) display gross chromosomal anomalies
• Inadequate egg maturation
• Failure to activate the embryonic genome
Embryo Fragmentation

- During cell division fragments of cytoplasm break off.
  - Programmed cell death
  - Cytoskeletal and spindle defects
- Extensive fragmentation have decreased blastulation rate, >15%
  fragmentation blastocyst rate declines
Day 3 Embryo cohort
• During compaction the blastomeres flatten against each other so that the boundaries between the blastomeres can no longer be distinguished.
Day 4 - Morula

8-16 cell stage embryo undergoes compaction to yield a morula

Final stage before the formation of blastocoel cavity
How does the cavitation occur?

• An osmotically driven movement of water into the embryo which forms the fluid filled cavity, called the Blastocoel.
• The movement of other ions (chloride, bicarbonate, Na+, K+) begin this energy dependant movement.
• As the fluid increases into the cavity, it then separates the cells into two parts.
Day 5 - Blastocyst

Inner Cell Mass

Trophectoderm
Blastocyst Cell Types

**TROPHECTODERM (TE)**
The outer single layer of epithelial cells adjacent to the zona pellucida of the blastocyst which will eventuate into the placenta.

**INNER CELL MASS (ICM)**
An massed area of cells inside the trophectoderm and collected together at one side of the cavity, which develops into the baby.
Day 5 - 6
Embryo development
Day 5 Embryo Transfer

Advantages

• Embryo Selection
• Reduction in number of embryos for ET resulting in reduction in multiple gestations
Guidelines – Which embryo is best?
Why Blastocyst ET?

Clinically:
• To reduce multiple-pregnancy rates whilst maintaining pregnancy rates
• Higher twinning rates with blasts (33% vrs 16.5%) after equal number of embryos transferred (Scwärzler et al, 2004)

Embryonic factors:
• 1. Better (self-)selection
• 2. Higher implantation potential
• 3. Limitations of d2/3 (time lapse)
• 4. Better cryopreservation results
• 5. PGS
Day 3 ET v D5: Tough questions!

- Will we “lose” embryos by culturing them to the blastocyst stage?
- Can any given day 2/3 embryo become a viable blast in vivo but not in vitro?
- Can an embryo of poor quality on d2/3 become a good quality blastocyst?
- Is poor/no blastocyst development evidence of poor development potential or a consequence of (poor) in vitro culture conditions?
- Health of children born?
Factors Affecting Choice ET Day

- Laws/Regulations
- Culture Conditions
- Patient/Doctor Requests
- # Embryos available
- Clinic policies (ESET)
Trend -% Blastocyst transfers

Source: HFEA report: Fertility treatment in 2011 and 2014
Diagnosis of a genetic disorder or chromosomal abnormality in early human embryos, before a clinical pregnancy has been established. Euploid (Unaffected) embryos are only transferred to the uterus.
Multiple Events in ART

- Many significant variables within ART framework
- External factors cannot be controlled
- The degree of variability during internal processes should be minimised
Reducing environmental stress imposed upon gametes and embryos in the IVF laboratory is crucial in optimizing culture conditions and development.
Laboratory Set up
Managing culture conditions for the oocytes and embryos

- Culture Media
- $\text{CO}_2$ (5.0 - 6.0%)
- Temperature ($37^\circ\text{C}$)
- VOC
- Non-Embryo Toxic: MEA
Monitoring Temperature

• Increase:
  – Decrease in cleavage rates and blastocyst formation

• Decrease:
  – Again leading to a decrease in the development potential of the embryo
  – Damage of meiotic spindle can lead to aneuploid embryos
Lab Environment

- **Daily Quality Control (QC)**
- Temperature: Digital thermometers/thermocouples
- Gas
- Low level lighting
- Air quality (HEPA)
- VOC measurements
- Minimal walking with dishes
- Minimise Incubator openings

- 3 monthly Quality Control

- 6-12 monthly servicing and calibration of all equipment
Data Loggers
Data Loggers- Report
IVF Lab QC : Consumables

• MEA Tested products - external
• Culture media & oil -(storage & cold chain) : Data loggers - Pre- defined tolerances
• In house Testing – Batch/Lot numbers
  ➢ High grade Plastic ware
  ➢ Gloves – sperm survival tests
  ➢ EPU needles
  ➢ ET catheters
ESHRE recommends KPI’s are part of the QMS
What KPI’s are relevant in ART?

- Pregnancy rate per EPU
- Pregnancy rate per embryo transfer
- Cumulative pregnancy rate (fresh + frozen embryos)
- Define pregnancy rate – Live birth rate
- Implantation rate
- Time to achieve pregnancy
- Reduction in multiple pregnancy – healthy singleton babies
- OHSS minimised
- Controversial – no consensus
What KPI’s should we be monitoring in ART lab?

1. Oocyte maturity
2. IVF Normal fertilisation rates
3. ICSI Normal Fertilisation rates
4. ICSI Degeneration rate
5. % patients with failed fertilisation
6. Embryo survival rates – post vitrification
7. % cancelled cycles post EPU
8. Embryo utilisation rate
9. The list is not complete..............................
Overview of ICSI .......
Blastocyst Hatching

Blastocyst hatching is the process whereby the expanded blastocyst breaks through and escapes from the Zona Pellucida (ZP).
Implantation
Challenges faced ........

• Patients fail to develop follicles – cycle cancelled
• No eggs at egg collection
• Eggs collected are immature
• Discordant egg and follicle number
• Man fails to produce semen sample
• Azoospermic semen sample
• Eggs fail to fertilise
• Eggs fertilise abnormally
• Eggs may fertilise but not develop
• Eggs may develop to day 3 ........then do not progress
• Embryos are poor quality

• And many more things…
PERFECTING A PROCESS