UNSW Embryology Course 2013

Human Gametogenesis and Fertilisation

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University of Sydney

August 2013
Gametogenesis & Fertilisation
Learning Objectives

- Meiosis
- Gametogenesis and aneuploidy
- Capacitation and hyperactivation
- Ovulation and oocyte maturation
- Fertilisation and polyspermy
- IUI, IVF, ICSI, PICSI and IMSI
- Assisted reproduction and society
Meiosis 1

Figure 1: MEIOSIS

Meiosis 1 (continued)

Figure 2: MEIOSIS

FIRST METAPHASE (Nuclear membrane breakdown and spindle formation)

FIRST ANAPHASE (Separation of homologous chromosomes)

FIRST TELOPHASE (Cytokinesis and nuclear membrane reformation)


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Meiosis 2

Figure 3: MEIOSIS

Why is meiosis a highly risky cell division?

Which aneuploidies result in subfertility or infertility?

Which aneuploidies increase with increased maternal age?
Aneuploidy: the scale of the problem

Incidence of aneuploidy during development

<table>
<thead>
<tr>
<th>Gestation (weeks)</th>
<th>Sperm</th>
<th>Oocytes</th>
<th>Pre-implantation embryos</th>
<th>Pre-clinical abortions</th>
<th>Spontaneous abortions</th>
<th>Stillbirths</th>
<th>Livebirths</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1-2%</td>
<td>~20%</td>
<td>~20%</td>
<td>?</td>
<td>35%</td>
<td>4%</td>
<td>0.3%</td>
</tr>
<tr>
<td>6-8</td>
<td></td>
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<tr>
<td>20</td>
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<tr>
<td>40</td>
<td></td>
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</tr>
</tbody>
</table>

Incidence of aneuploidy

- 1-2% Sperm
- ~20% Oocytes

Most common aneuploidies

- Various

Spermatozoa within the seminiferous tubule

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Eikonica
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Spermatogenesis

In what ways does meiosis differ during oogenesis?

What are the mechanisms underlying these differences?
Folliculogenesis/Oogenesis


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In Vitro Maturation

Prophase I → Metaphase I → Metaphase II

24-48 hours

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Actin-rich domain during PB formation in mouse oocyte:
Why is the timing of fertilisation regulated?

What are the mechanisms in the male and female that regulate fertilisation?
Graafian follicle

The follicle maintains the oocyte under meiotic arrest

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FSH
LH
FSH EGF?

Granulosa Cells

InsP3
Ca²⁺

Cumulus Cells

Oocyte

Gap junctions

FF-MAS

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Meiotic start and stop signals

Oogonia → Primordial oocyte → Preovulatory oocyte → GV → MI → MII

FF-MAS, Granulosa cells, LH

Meiotic start and stop signals


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The pathway to fertilisation

- Capacitation
- Hyperactivation
- Oocyte maturation
- Sperm-egg recognition
- The acrosome reaction
- Sperm-egg fusion
- Oocyte activation
- Pronuclei formation
- Syngamy

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Spermatozoa traversing ciliated epithelium

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Acrosome reacted spermatozoa in cumulus

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Sperm penetration of mature egg - Diagram

Sperm binding

Acrosome-intact

AR in Cumulus

Acrosome-reacting

Acrosome-reacted

Sperm-egg fusion

Sperm incorporation

CR=corona radiata

PVS=perivitelline space

ZP=zona pellucida


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Sperm penetration of the zona - TEM

Abnormal sperm
Reacted sperm
Sperm tails
Zona
Cytoplasm

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Spermatozoa penetrating the zona pellucida

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Sperm – egg membrane fusion - TEM

Midpiece
Fusogenic zone
Fusion
Ooplasm
Tail

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Spermatozoon binding to the oolemma

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Formation of pronuclei and syngamy


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How does the oocyte know it has been fertilised?

What is the mechanism by which the oocyte becomes activated?
Role of second messengers in fertilisation

Adapted from Developmental Biology, third edition by Scott F. Gilbert
Mature and pronuclear eggs – Nomarski optics

MII

1PN

2PN

3PN

August 2013 Gametogenesis & Fertilisation Courtesy Dr. D. Payne, Adelaide
Pronuclear ova - Diagram

Normal 2PN

Dispermy

Digyny

Parthenogenesis

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How does the oocyte prevent polyspermy?

How might polyspermy occur unwittingly in vitro?
Fertilisation events - Diagram

A: sperm-egg fusion
B: second maturation division
C: cortical reaction
D: zona reaction
E: association of male & female pronuclei

a: Sperm aster forms after sperm incorporation


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Cortical reaction at fertilisation - TEM

Zona

CG

Ooplasm

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Block to polyspermy – nature's way - TEM

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Zona morphology following fertilisation

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Sperm binds egg receptor

G protein activated

Membrane potential changed

IP$_3$ produced

phospholipase C activated (PLC)

diacylglycerol produced (DAG)

protein kinase C activated (PK-C)

Ca$^{2+}$ released from ER

cortical reaction

Na$^+$ ion influx

activation of egg

increase in intracellular pH

Na$^+$ / H$^+$ exchanger activated

Adapted from Developmental Biology, third edition by Scott F. Gilbert

IP$_3$ as a second messenger in fertilization

FAST BLOCK OF POLYSPERMY

SLOW BLOCK OF POLYSPERMY

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Ovulation induction and Intrauterine insemination

- Patent fallopian tubes
- Ovulatory menstrual cycles
- Success rate = 10% - 15%
- Increases up to 4 attempts
- Total motile sperm count ≥ 10 million
In vitro fertilisation (IVF)

Success rate = 25% - 30%
What if the sperm concentration or sperm motility is too poor for IUI or IVF?
Intra-Cytoplasmic Sperm Injection (ICSI)

- Only one sperm required per egg
- Success rate = 25% - 30%
What if there are no sperm being ejaculated?

- Retrograde ejaculation?
- Genital tract obstruction?
- Very low sperm production?
Surgical Sperm Recovery

Testicular sperm extraction

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PICSII


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Acridine Orange Fluorescence

- Fixation with methanol and glacial acetic acid (9:1)
- Staining with Acridine Orange solution for 10 minutes
- Metachromatic shift in fluorescence
  - Green (native, double-stranded DNA)
  - Red (single stranded DNA breaks)
HA selection of sperm


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DNA integrity of HA selected spermatozoa

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green sperm in semen</td>
<td>54.9 ± 2.0</td>
</tr>
<tr>
<td>Green sperm outside HA spot</td>
<td>56.1 ± 1.9</td>
</tr>
<tr>
<td>Green sperm inside HA spot</td>
<td>99.1 ± 0.2</td>
</tr>
<tr>
<td>Red sperm in semen</td>
<td>45.0 ± 1.9</td>
</tr>
<tr>
<td>Red sperm outside HA spot</td>
<td>43.9 ± 1.9</td>
</tr>
<tr>
<td>Red sperm inside HA spot</td>
<td>0.9 ± 1.9</td>
</tr>
</tbody>
</table>

Mean ICSI procedure duration (s)

PICSI = 450.0 \pm 30.5
SSlow = 284.1 \pm 10.1
p < 0.001
Intracytoplasmic Morphologically Selected Sperm Injection (IMSI)


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RCT of ICSI vs IMSI


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<table>
<thead>
<tr>
<th>Outcome</th>
<th>ICSI</th>
<th>IMSI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of ICSI procedure (min)</td>
<td>13.55 ± 5.43</td>
<td>20.54 ± 9.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2-pronuclei fertilization rate (%)</td>
<td>80.97 ± 15.06</td>
<td>81.60 ± 10.65</td>
<td>NS</td>
</tr>
<tr>
<td>Embryos with 4 blastomeres on day 2 post fertilization (%)</td>
<td>34.70 ± 21.88</td>
<td>30.43 ± 16.23</td>
<td>NS</td>
</tr>
<tr>
<td>Embryos with 8 blastomeres on day 3 post fertilization (%)</td>
<td>31.65 ± 17.21</td>
<td>33.61 ± 16.34</td>
<td>NS</td>
</tr>
<tr>
<td>Grade 1 and 2 embryos on transfer day (%)</td>
<td>4.84 (63.95)</td>
<td>5.01 (66.44)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean no. of embryos transferred&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.76 ± 0.46</td>
<td>2.72 ± 0.48</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy per initiated cycle (%)</td>
<td>36/81 (44.4)</td>
<td>47/87 (54.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Live birth rate per initiated cycle (%)</td>
<td>31/81 (38.3)</td>
<td>38/87 (43.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>42/215 (19.5)</td>
<td>66/228 (28.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Multiple pregnancy rate (%)</td>
<td>6/36 (16.7)</td>
<td>16/47 (34.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SD or n (%).
ICSI = intracytoplasmic sperm injection; IMSI = intracytoplasmic morphologically selected sperm injection; NS = not significant.

<sup>a</sup>Six patients (three in each group) who did not undergo embryo transfer due to total fertilization failure (one in IMSI) or pending ovarian hyperstimulation syndrome (two in IMSI, three in conventional ICSI groups) are excluded from this analysis.
ART, Ethics & Society

- Mitochondrial disease and ooplasmic transfer
- Haploidosiation of somatic cells to derive gametes
- Microarray CGH and sperm selection