Lecture 18: Embryo Development

Timing of Insemination

<table>
<thead>
<tr>
<th>Species</th>
<th>Time of Ovulation</th>
<th>Optimal Insemination Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>29 hr after start of estrus</td>
<td>End of estrus (12 hr after first seen in estrus)</td>
</tr>
<tr>
<td>Ewe</td>
<td>End of estrus</td>
<td>End of 1st day or start of 2nd day of estrus</td>
</tr>
<tr>
<td>Sow</td>
<td>End of estrus</td>
<td>End of 1st day or start of 2nd day of estrus</td>
</tr>
<tr>
<td>Mare</td>
<td>1-2 days before end of estrus</td>
<td>Every other day beginning day 3 of estrus</td>
</tr>
</tbody>
</table>

Errors in Fertilization

- **Polyspermy - polyandry**
  - Multiple sperm penetration
  - Invertebrates
    - Excess sperm eliminated because sperm centriole contributes to first embryonic cleavage spindle
  - Mammals
    - Sperm centriole not essential so development continues but fails early to midpregnancy due to multiploidy
    - Occurs most often in aged oocytes due to failure of zona block to polyspermy

- **Errors in Fertilization (cont.)**
  - **Polygyny**
    - Multiple maternal pronuclei + 1 paternal pronuclei
    - Artificially created only
      - Suppress extrusion of the PBII
  - **Androgenote**
    - Union of 2 paternal pronuclei
    - Artificially created only
      - From pronuclear exchange
  - **Gynogenote**
    - Union of 2 maternal pronuclei
    - Artificially created
      - Induced oocyte activation and suppression of PBII extrusion
  - **Parthenogenesis**
    - Activation of the oocyte without a sperm
    - Embryo is either haploid or gynogenesis occurs to form diploid
      - Platties - sperm activates but then gynogenesis occurs and sperm extruded from embryo

Errors in Fertilization (cont.)

Oocyte Development and Fertilization

LH Surge (0 hr)

Primary Oocyte

GV-Intact

MPF

Metaphase I

MV

Secondary Oocyte

Gyogenote

Union of 2 maternal pronuclei

Artificially created

Parthenogenesis

Activation of the oocyte without a sperm

Embryo is either haploid or gynogenesis occurs to form diploid

Platties - sperm activates but then gynogenesis occurs and sperm extruded from embryo
Zona Pellucida

Oocyte

Sperm Penetration of the Zona Pellucida and Fusion with the Oocyte (30 hr)

Embryo Development in the Bovine

4 cell (75 hr, day 3)
8 cell (90 hr, day 3)
16 cell (120 hr, day 4)
2 cell (62 hr, day 2)
Zygote (34 hr, day 1)
Hatched Blastocyst (day 5-11)
Trophoblast
Blastocyst (day 7-9)
Early Blastocyst (day 5-6)
32 cell Morula (day 5-6)
Expanded Blastocyst (day 8-10)
Tight Morula (day 6-7)

Fertilization to Cleavage

Zygote

Pronuclei
Polar Body
Blastomere

Imprinting

Sperm Pronucleus
Egg Pronucleus
Androgenote
Gynogenote

Fertilization to Cleavage

• Imprinting
• Maternal Gene Control
• Long Cell Cycle
Imprinting

Androgenote  
Gynogenote

No Inner Cell Mass  
Normal Placenta  
Fails during embryo development

Normal Fetus  
Small Placenta  
Fails Midpregnancy  
Normal Pregnancy

Maternal and Paternal Genomes Are Expressed Differently in the Embryo and Fetus

Gene Control of Development

Maternal Gene Control  
Embryonic Gene Control

Oocyte Growth  
LH Surge  
Fertilization  
Cleavage

• Transcription  
• Translation  
• No transcription  
• Translation  
• Translation  
• Post-Translation  
• Transcription  
• Translation  
• Post-Translation

Fertilization to Cleavage

Maternal Gene Control

Long Cell Cycle » Penetration to Cleavage

32 hour (Bovine)

Precompaction Cleavage

• Cell size decreases  
• Cell cycle  
• Embryonic gene control  
• Asynchrony of cell divisions  
• Movement into Uterus  
• Early pregnancy factor
Precompaction Cleavage

- Cell size decreases
- Cell cycle
- Asynchrony of cell divisions
- Embryonic gene control
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Precompaction Cleavage

- Cell size decreases
- Cell cycle
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Cell Cycle Lengths

1st Cell Cycle (zygote → 2 cell)
- $G_1$
- $S$
- $G_2 + M$
- Total = 32 hours

2nd Cell Cycle (2 cell → 4 cell)
- Short $G_1$ and $G_2$
- Total = 13 hours

Precompaction Cleavage

- Cell size decreases
- Cell cycle
- Asynchrony of cell divisions
- Embryonic gene control
- Movement into Uterus
- Early pregnancy factor

Asynchronous Cleavage - Inside Outside Theory

If a marked blastomere is placed into the interior of a 8-cell embryo, it and its progeny become part of the ICM.

If a marked blastomere is placed on the outside of a 8-cell embryo, it and its progeny become part of the trophoderm.
Asynchronous Cleavage Use
- Create embryos from different species
  - Placenta from one species
    - Host mother
  - Embryo from some other species
    - Donor mother

Precompaction Cleavage
- Cell size decreases
- Cell cycle
- Asynchrony of cell divisions
- Embryonic gene control
- Movement into Uterus
- Early pregnancy factor

Gene Control of Development

Transition from Maternal to Embryonic Gene Control

<table>
<thead>
<tr>
<th>Species</th>
<th>First begins</th>
<th>Development is dependent on</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>1 cell</td>
<td>2 cell</td>
</tr>
<tr>
<td>Rabbit</td>
<td>2 cell</td>
<td>8 cell</td>
</tr>
<tr>
<td>Pig</td>
<td>4 cell</td>
<td>8 cell</td>
</tr>
<tr>
<td>Cattle</td>
<td>4 cell</td>
<td>8-16 cell</td>
</tr>
<tr>
<td>Sheep</td>
<td>8 cell</td>
<td>16 cell</td>
</tr>
<tr>
<td>Human</td>
<td>4 cell</td>
<td>8 cell</td>
</tr>
</tbody>
</table>

Precompaction Cleavage
- Cell size decreases
- Cell cycle
- Asynchrony of cell divisions
- Embryonic gene control
- Movement into Uterus
- Early pregnancy factor

In vitro blocks to development often occur here!!!!!
Movement into the Uterus

- Occurs around day 4
- Cause:
  - Change in estrogen \(\rightarrow\) progesterone

Precompaction Cleavage

- Cell size decreases
- Cell cycle
- Asynchrony of cell divisions
- Embryonic gene control
- Movement into Uterus
- Early pregnancy factor

Early Pregnancy Factor

- Found at 24 - 72 hours after fertilization
  - Mice, hamster, sheep, cattle, swine, human
- Seen only in viable pregnancy
  - More recent experience in cattle may not agree with this
- Function
  - Sensitize the uterus to implantation
  - Basis for early pregnancy kit in cattle

Morula to Blastocyst

- Polarization
- Compaction

Polarization

- Polar Blastomeres
- Non-polar Blastomeres
- Microvilli
- Gap Junctions
- Tight Junctions
**Cell Linage**

- Polar Cells
  - 2 polar cells
- Non-polar Cells
  - 1 polar
  - 1 non-polar
  - 2 non-polar

**Compaction**
- Occurs at fixed time after fertilization
- Membranes are very close and begin to flatten, resulting in loss of the round cell outlines.
- Differentiation event
- Genome controlled and involves microtubules and microfilaments.

**Blastocyst Formation and Hatching**
- Blastocoel formation
- Hatching

**Blastocoel Formation**
- Morula
  - H₂O
  - Na⁺
  - Tight Junctions
  - Gap Junctions

**Blastocoel Formation**
- Early Blastocyst
  - Blastocoel
  - H₂O
  - Na⁺
  - Tight Junctions
  - Gap Junctions
### Blastocyst Formation and Hatching

- **Blastocoel formation**
  - not dependent on:
    - Cell number
    - Cell division
  - Embryonic genome expression required
- **Hatching**
  - Enzymatic digestion of zona
    - Plasminogen and plasminogen activator made by embryo
    - Softening of zona by uterine enzymes
  - Increase in size of blastocyst due to water pumping
  - Most important
  - Day 9 - 11 in cattle, 6 in swine, and day 7 - 8 in horses or sheep

### Formation of Twins

- **Dizygotic**
  - Not identical
  - Double ovulation
- **Monozygotic**
  - Identical
  - Several potential mechanisms

### Formation of Monozygotic Twins

- Siamese Twins