Lecture 19: Embryo Development
Animal Science 434
John Parrish

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 mid-pregnancy due to multiploidy
    - Occurs most often in aged oocytes due to failure of zona block to polyspermy

- **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**
  - **GVBD** (8 hr)
    - **MPF**
- **Metaphase I**
- **Metaphase II** (21 hr)
- **Secondary Oocyte**

- **Ovulation** (29 hr)
Zona Pellucida

Oocyte

Ca^{2+}

Perivitelline Space

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

Embryo Development in the Bovine

- **4 cell (76 hr, day 2)**
- **8 cell (90 hr, day 3)**
- **16 cell (120 hr, day 4)**
- **Hatched Blastocyst (day 9-11)**
- **32 cell Morula (day 5-6)**
- **Early Blastocyst (day 7-8)**
- **Tight Morula (day 6-7)**
- **Expanded Blastocyst (day 8-10)**
- **Blastocyst (day 7-9)**
- **Hatched Blastocyst (day 9-11)**
- **Zygote (34 hr, day 1)**

Fertilization to Cleavage

- **Zygote**
- **Pronuclei**
- **Polar Body**
- **Blastomere**
- **Zona Pellucida**
- **Perivitelline Space**

Fertilization to Cleavage

- **Imprinting**
- **Maternal Gene Control**
- **Long Cell Cycle**

Imprinting

- **Sperm Pronucleus**
- **Egg Pronucleus**

Imprinting

- **Androgenote**
- **Gynogenote**
Gene Control of Development

Maternal Gene Control

- Oocyte Growth
- LH Surge
- Fertilization
- Cleavage

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

Embryonic Gene Control

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
- Movement into Uterus
- Early pregnancy factor

**Cell Cycle Lengths**

1st Cell Cycle (zygote → 2 cell)
- G1
- S
- G2 + M
- Total = 32 hours

2nd Cell Cycle (2 cell → 4 cell)
- G1 (shortened)
- S
- G2 + M
- Total = 13 hours

**Precompaction Cleavage**

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

Maternal Gene Control

- Transcription
- Translation

LH Surge

Embryonic Gene Control

- Transcription
- Post-Translation

Fertilization

Cleavage

In vitro blocks to development often occur here!!!!!!

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

Embryo runs out of key factors coded for by maternal mRNA

Cell Cycle Length Increases

Pause in G1

32 hours

13 hours

15 hours

30 hours
**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

**Polarization (cont.)**

- Gap Junctions
- Tight Junctions
Cell Linage

- Polar Cells
- Non-polar Cells

- 2 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
- 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