SECTION 10

Ovulation & Early Embryo Development

FOLLICULOGENESIS

Types of follicles (Figure 6-1)

Primary (1°) follicles

Germ cell (ovum) surrounded by one layer of follicular (granulosa) cells

Formed during prenatal period

Highest number found

50 to 90 days fetal gilt

110 to 130 days fetal calf

Maximum number

About 75,000 in calf

Where do they all go?

An Example -- cow 20 years old

18 years productive life

18 ovulations/year = 324 ovulations in 18 years

Follicles that ovulate = about 0.5%
Follicles that become atretic = 99.5%

Secondary (2°) follicles

Ovum plus 2 or more layers of granulosa cells

Tertiary (3°) follicles

Ovum with many layers of granulosa cells plus an antrum

Graafian follicles (Figure 6-2)

Mature 3° follicle

Anatomy of Graafian follicle (Figure 6-2)

Layers (outside to inside)

- Theca externa
- Theca interna
- Basement membrane
- Granulosa cells
- Cumulus oophorus
- Corona radiata
- Antrum containing follicular fluid

Structure of follicular wall (Figure 6-3)

- Theca cells -- blood supply
Basement membrane

Granulosa cells -- No blood supply

**Oocyte and associated cells soon after ovulation**

**Anatomy (Figure 6-4)**

**Size**

.130 to .150 mm (130 to 150 µm) for bovine, ovine, porcine and equine species

**OOGENESIS**

**Introduction**

Formation and maturation of female gamete

Begins in prenatal period

Potential gamete in a 1° follicle is called an "oogonium"

Mitotic division of oogonia yield 1° oocytes

Ceases before birth

Number of potential oocytes is FIXED and decreasing at birth

Oocytes become arrested in meiotic prophase stage of diplotene (called dictyate oocytes)

Oocytes starting development before puberty become atretic

Maturation continues after puberty
During each cycle some oocytes start maturing--others remain in an arrested state

Process of oogenesis (Figure 6-5)

First step

Growth of oocyte
Enlargement of cytoplasm
Development of a Zona pellucida
Proliferation of follicular cells

Second step

Meiosis

$1^\circ$ oocyte (2N)--------- $2^\circ$ oocyte (1N) + 1st polar body
(most degenerate)

$2^\circ$ oocyte retains all cytoplasm and one-half of nuclear material

Completed just before ovulation in cow, sow, ewe and mare

Third Step

Begins immediately after meiosis I
Not completed unless fertilization takes place
During fertilization
Meiosis
2° oocyte (1N) + sperm (1N) ----------- zygote (2N) + 2nd PB

**OVULATION**

**Time of ovulation**

- Cow 10 to 12 hours after end of estrus
- Ewe 24 to 30 hours after onset of estrus
- Sow 35 to 45 hours after onset of estrus
- Mare 1 to 2 days before estrus ends

Ovulation occurs 24 to 45 hours after LH surge except in mare

Ovulation in mare occurs before peak in LH surge

**Process of ovulation**

- LH stimulates
  - Enzymes in follicular wall
  - Cytoplasmic and nuclear maturation of oocyte
- PGF2α and PGE2 involved in follicular rupture
- Enzymes cause follicular wall to weaken and thin Follicle ruptures, follicular fluid escapes with ovum

**Site of ovulation**

- Cow, sow, ewe -- any point on ovary except hilus
- Mare -- ovulation fossa
- Occurs at random between ovaries
Cellular events of ovulation

Layers oocyte must pass through:

Granulosa cells

Basement membrane

Theca interna

Theca externa

Tunica albuginea ovarii

Surface epithelium

After ovulation, theca and granulosa cells become luteal cells

Formation of corpus luteum

Corpus hemorrhagicum

Corpus luteum

Corpus albicans

Corpus luteum of pregnancy

TRANSPORT AND SURVIVAL OF GAMETES

SPERM TRANSPORT

Journey through male tract

Journey through female tract
Sperm distribution in female tract (Figure 6-6)

Sperm barriers and reservoirs (Figure 6-7)

Functions of cervix

- Barrier and reservoir
- Protection
- Energy requirements
- Filtration
- Capacitation

Functions of uterus

- Barrier and reservoir
- Myometrium
  - contractions
  - OT and E2β

Functions of oviduct

- Barrier and reservoir
- Peristalsis of musculature
  - Contractions of mesosalpinx
- Fluid movement by cilia
- Opening and closing of uterotubal junction
Time of sperm transport

Some spermatozoa reach oviduct within 15 to 30 min

Dead sperm also transported

Hormonal control of sperm transport

Ovarian steroids -- increase in E2ß & decrease in P4

Increase in PGF2α and PGE2

Increase in OT

Fertile life-span of spermatozoa

Species Fertile life (hours)

Bovine, porcine & ovine 24 to 48

Equine 72 to 120

OVUM TRANSPORT IN FEMALE TRACT

Reception of ova by fimbria

Transport in oviduct

Cilia

Smooth muscle

Mesosalpinx
Ampullary-isthmic junction (AIJ)

Site of Fertilization

Uterotubal junction (UTJ)

Transport time of ova in oviduct

72 to 90 hours in bovine, ovine, porcine and equine

Contraction of oviductal musculature

Direction/function

Delays progress of ovum at AIJ

Mixing of oviductal contents

Fertile life-span of ova

Species Fertile life (hours)

Bovine & ovine 16 to 24

Porcine & equine 6 to 10

Fate of unfertilized ovum

Fragments into cytoplasmic segments of unequal size

Disappears by phagocytosis

Transuterine migration

Where ova travel from one uterine horn through a common uterine body to opposite uterine horn
FERTILIZATION

Definition

Fusion of two cells to form one single cell (zygote)

Description of fertilization process

Position and state of ovum

Ovum in ampulla of oviduct

Cumulus cells

Present in sow, absent in cow, mare and ewe

Encounter of sperm with ovum

Capacitation

Fertilization process

Sperm form a loose, nonspecific attachment with zona pellucida

Sperm cells bind to receptors on zona pellucida

More than one sperm can bind to a single ovum

Bound sperm complete acrosome reaction in preparation for penetration of zona pellucida

Release of a variety of enzymes (i.e., hyalurondiase and corona penetrating enzyme)

Acrosome-reacted sperm can then penetrate zona pellucida
Accomplished by acrosin

Sperm head fuses with vitelline membrane

Cortical granules spill their contents into perivitelline space giving rise to zona reaction and vitelline block

Zona reaction guards against penetration of zona pellucida by other sperm

Penetration of vitelline membrane

Accomplished by phagocytosis

Entrance into cytoplasm

Tail separates from head

Syngamy

Union of pronuclei

Process of fertilization complete

Zygote has been formed

Duration of fertilization process

Varies from 12 to 24 hours

Polyspermy

Fertilization by more than one sperm cell

Results in zygote with polyploidy (>2n)

Incidence = 1 to 2%
Increases with aged ovum

Lethal condition

EMBRYONIC DEVELOPMENT

CLEAVAGE

Introduction

Zygote -- from syngamy to first division is completed

Embryo -- from 2-cell stage until differentiation is completed

Fetus -- from completion of differentiation to parturition

Conceptus -- whole product of conception at any stage of development including placenta

Definition

Process of cellular division without growth

Continues from first division to hatching of blastocyst

Nourishment for embryo during cleavage

Uterine secretions

Cytoplasm of egg

Process involved (Figure 7-1)

First cleavage yields a 2-cell embryo

Additional cleavages result in 4-, 8-, 16-, 32-cell, etc.
All divisions are mitotic

16- to 32-cell stage called MORULA

Fluid begins to collect in intercellular spaces and cavity forms (BLASTOCOELE) to give rise to a BLASTOCYST

Blastocyst contains two layers of cells

Trophoblast and inner cell mass

Rate of Cleavage

Days after ovulation

<table>
<thead>
<tr>
<th></th>
<th>Develop to 2-cell</th>
<th>Develop to 8-cell</th>
<th>reach uterus</th>
<th>Develop to Blastocyst</th>
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</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>1</td>
<td>3</td>
<td>3.5</td>
<td>7 to 12</td>
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<tr>
<td>Equine</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Ovine</td>
<td>1</td>
<td>2.5</td>
<td>3</td>
<td>6 to 7</td>
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<td>Porcine</td>
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<td>2</td>
<td>2</td>
<td>5 to 6</td>
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Blastocyst stage

Zona pellucida will hatch

Period of elongation
Metabolic rate increases

Twinning

Rate

Cattle

Beef = less than 1%

Dairy = 3.5%

Horses

Thoroughbreds = 1 to 2%

Draft-type = 3%

Humans

Natural rate 2%

From IVF >15%

Types

Monozygotic (Identical)

Originate from single fertilized ovum

More common in man and cattle

8 to 10% of twins in cattle are identical

30% of twins in humans are identical

Always same sex
Possible mechanisms

Separation of zygotic cells after first cleavage with each cell developing independently

Formation of two inner cell masses within blastocyst

Division of inner cell mass at time of hatching

After implantation, inner cell mass differentiates into two primitive streaks

Production of identical twins

Embryo splitting ("cloning")

Nuclear transfer

Can result in >2 identical offspring

Dizygotic (Fraternal)

Originate from a double ovulation in monotocous species

Two ova shed at same estrus, fertilized by two separate sperm

No more closely related than brothers and sisters

**Implantation**

**Definition**

When embryo becomes fixed in position and physical contact of placenta with maternal organism is established

Implantation in farm animals
Embryo remains in uterine cavity

Forms loose connection with uterine wall

by proteins call intagrin?

Time of implantation

(days after ovulation)

<table>
<thead>
<tr>
<th>Specie</th>
<th>Beginning</th>
<th>Complete</th>
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</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>28 to 32</td>
<td>40 to 45</td>
</tr>
<tr>
<td>Ovine</td>
<td>14 to 16</td>
<td>28 to 35</td>
</tr>
<tr>
<td>Porcine</td>
<td>12 to 13</td>
<td>25 to 26</td>
</tr>
<tr>
<td>Equine</td>
<td>35 to 40</td>
<td>95 to 105</td>
</tr>
</tbody>
</table>

DIFFERENTIATION

Definition

Time when cells are forming specific organs in body of embryo

Formation of germ layers (Figure 7-2)

Endoderm or entoderm (inner most layer)

Organs formed

Digestive system, liver, lungs, pancreas,
thyroid and most other glands

Mesoderm (middle layer)

Organs formed

Circulatory system, skeletal system, muscle, reproductive system, kidneys and urinary ducts

Ectoderm (outer layer)

Organs formed

Central nervous system, sense organs, mammary glands, sweat glands, skin, hair and hooves

Formation of extraembryonic membranes (Fig. 7-3)

Amnion

Inner-most membrane

Folds around embryo

Forms amnionic cavity

Contains fluids that suspends embryo, protects it and permits free growth

Yolk sac

Present during early gestation

Contains source of nutrients for early embryo

Regresses by time of implantation
**Allantois**

Outpouching of hindgut of embryo

Connects to embryonic bladder

Allantoic cavity contains fluids high in waste products

**Chorion**

Outer-most layer

Allantois enlarges and fuses with chorion to form ALLANTO-CHORION

Allanto-chorion attaches to endometrium during process of implantation

**Formation of organs**

**Nervous system**

Brain and spinal cord

First to start Last to complete

**Circulatory system**

Embryonic heartbeat detected by day 16, 20, 22 and 24 in sow, ewe, cow and mare, respectively

**Digestive system**

**Reproductive system**

Sexual differentiation (days of gestation)
Bovine day 45

Ovine day 35

Porcine day 30

Equine day 45

Dual duct system develops

Mullerian duct (genetic female)

Develops into oviduct, uterus, cervix & vagina

Wolffian duct (genetic male)

Develops into epididymis and vas deferens

Embryonic gonads

First appear as genital ridges

Genetic male

Primordial germ cells form gonocytes which differentiate into spermatogonia

Genetic female

Primordial germ cells form gonocytes which differentiate into oogonia

Period of differentiation
<table>
<thead>
<tr>
<th>Specie</th>
<th>Time (days)</th>
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<tbody>
<tr>
<td>Sow</td>
<td>7 to 28</td>
</tr>
<tr>
<td>Cow</td>
<td>13 to 45</td>
</tr>
<tr>
<td>Ewe</td>
<td>11 to 45</td>
</tr>
<tr>
<td>Equine</td>
<td>13 to 50</td>
</tr>
</tbody>
</table>

Problems during differentiation

- Freemartin
- Hermaphrodite

**MATERNAL RECOGNITION OF PREGNANCY**

**Importance**

Presence of embryos in uterus must be signaled before implantation begins to prevent regression of CL

**Mechanism**

Bovine & ovine (Figure 7-4)

Conceptus secretes a protein (Trophoblastic Protein-1, TP-1; bovine, bTP-1; ovine, oTP-1) which binds to its receptor in endometrium
Stimulates release of Endometrial Prostaglandin Synthetase Inhibitor (EPSI)

EPSI acts with arachidonic acid and Prostaglandin Synthetase to prevent formation and secretion of PGF2α.

Porcine

Blastocysts synthesize estrogens during elongation phase.

Estrogen responsible for maternal recognition of pregnancy.

Exogenous estrogens injected into unmated pigs between days 11 and 15 of estrous cycle induces pseudopregnancy in which CL are maintained (luteotropic effect) for over 100 days.

Equine

Details of mechanism are not fully understood.

Mobility of embryo in uterus may be involved.

Recent research indicates mechanism may be similar to that of pig.

Time of maternal recognition of pregnancy

**Species Days**

Bovine 16 to 17

Ovine 12 to 13

Porcine 11 to 12

Equine 14 to 16
EMBRYONIC MORTALITY

Embryonic mortality denotes death of fertilized ova and embryos up to end of implantation

Regarded as a normal process to eliminate unfit genotypes in large litters of swine and multiple pregnancies in cattle, sheep and horses
termed "embryo wastage"

<table>
<thead>
<tr>
<th>Specie</th>
<th>Days of Gestation</th>
<th>Embryos Lost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>8 to 16</td>
<td>25 to 40</td>
</tr>
<tr>
<td>Ovine</td>
<td>9 to 15</td>
<td>25 to 40</td>
</tr>
<tr>
<td>Porcine</td>
<td>8 to 16</td>
<td>25 to 40</td>
</tr>
<tr>
<td>Equine</td>
<td>30 to 36</td>
<td>30 to 40</td>
</tr>
</tbody>
</table>

Possible causes

P4 deficiency

Inbreeding

Increasing maternal age
Overfeeding

High environmental temperature

Multiple embryos

Overcrowding *in utero*

Chromosomal aberrations

Lactation

Defective semen

Immunologic incompatibility

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