

# Repeat Breeding

1. normal cyclic female
2. normal genitalia
3. mated in **three or more consecutive** estruses with fertile bull or inseminated artificially with fertile semen
4. fails to conceive

**Male factors:** High sperm abnormalities, Low sperm motility or Venereal diseases/infections

## Female factors

- Delayed ovulation - GnRH or hCG
- Anovulation - GnRH or hCG
- Fertilization failure - defective ovum - sexual rest for 2-3 months
- Luteal insufficiency - progesterone depot on 5<sup>th</sup>, 10<sup>th</sup> and 20<sup>th</sup> day after AI or 1000-1500 IU of hCG/ 50 $\mu$ g GnRH
- Endometritis
- Hydrosalpinx
- Pneumo-vagina: accumulation of air in the vagina - treated by **Caslick's operation**
- cyst

# FUNCTIONAL INFERTILITY

## Cystic Ovarian Degeneration (COD)

- more commonly in higher producing cattle
- more common during 2<sup>nd</sup> to 5<sup>th</sup> lactation (4 to 6 years of age)
- three type
  - **Follicular cyst/cystic degeneration of Graffian follicle**
  - **Luteal/Lutenized Cyst**
  - **Cystic corpus luteum**

## **Follicular cyst – Nymphomania – Sterility hump**

- presence of follicle or follicle like structures
- more than **25 mm in diameter if single follicular cyst**
- more than **17 mm in case of multiple follicular cysts**
- persist for more than 10 days
- **anovulatory** i.e. origin is from the follicle which fail to ovulate
- Show all signs of heat (exaggerated)
- **endometrium appears like ‘Swiss Cheese’** - marked hyperplasia
- **RX – LH, GnRH**

## **luteal cyst – Anestrus – Adrenal virilism**

- diameter of **corpus luteum more than 25 mm**
- persisting **for more than 10 days**
- **anovulatory i.e.** origin is from the follicle which fail to ovulate
- **RX – PGF<sub>2α</sub>**

Follicular cyst	Luteal Cyst
Thin, soft and fluctuating	Always hard
May be single or multiple	Always single
Animal is nymphomaniac (Buller)	Animal is anestrous
Incidence is higher	Incidence is lower.
Thickness of follicular wall is less than 3 mm	Thickness of luteal wall is more than 3 mm
Sterility hump	Adrenal virilism
P4 < 1ng/ml in serum is indicative of follicular cyst.	P4 concentration > 2ng/ml in milk and > 1ng/ml in serum
High estrogen in blood	Low estrogen in blood



**Sterility hump**

**Sterility hump** - Due to long standing cases of nymphomania, sinking of sacrosciatic ligaments and upward displacement of coccyx take place

### **Adrenal virilism -**

- masculine conformation
- will attempt to **mount other** cows
- **will not stand to be mounted** by other cows,
- the animal becomes very heavy and coarse.
- The neck of animal becomes thick and heavy

## **cystic CL – genetic inheritance**

- after ovulation - **ovulatory cyst**
- On rectal examination, CL appears similar to normal CL but the **cystic CL is slightly soft**
- Can maintain pregnancy if producing enough P4



## Segmental aplasia (White Heifer Disease)

- congenital defect of the reproductive tract
- segmental aplasia of the **Mullerian or Paramesonephric ducts**, especially an **imperforate hymen and associated with white coat color**
- Reported to occur in 10 % of Shorthorn breed
- Reported in Holstein, Jersey, Ayrshire breed
- Caused by **Single Recessive Sex Limited Gene** with linkage to the gene for **white coat color**
- **Tubular Genitalia**
  - **Absence** of either the cranial part of the vagina, cervix or the uterine body
  - **Uterus unicornis**
  - Cystic **dilation of uterine horn** due to aplastic body- large enough to simulate a 4 months pregnant uterus!
  - Uterine horn filled with yellowish to tan dark brown mucus
  - Persistence of **median wall** of the **paramesonephric duct- double cervix**

- **Ovaries are normal and functional in white heifer diseases**
- **Estrus and Estrus Cycles**
  - **If ovulation occurs** in the ovary **adjacent to the abnormal horn**, regression of corpus luteum may not occur resulting into **permanent anestrus**
- **Fertility**
  - may conceive in unilaterally affected cases
  - **Sterile** - if the condition is **bilateral** and affects uterine horn, cervix and oviduct

## Puberty –

- age when **first estrus** is accompanied **with fertile ovulation**.
- **35-45%** of their mature body weight
- **breeding should be - 55%** of adult body weight.
- Before puberty majority - **anovulatory or silent heat** (about 3 months before the actual age of puberty).
- puberty should not be considered as sexual maturity.
- *hormone primarily associated with puberty – oestradiol and LH*
- *Before puberty – ovarian oestradiol - -ve feedback over hypothalamus – only pulsatile release of GnRH*  
*puberty – this negative feedback cease – increase GnRH and FSH and LH - estrus*

## **Age at Puberty:**

Sheep	: 7-10 months
Cattle	: 8-12 months
Buffalo	: 15-18 months
Horses	: 15-24 months
Doe	: 6-8 months
Sow	: 5-8 months
Bitch	: 6-12 months
Queen	: 5-12 months

## Regulation in seasonal long day breeders

- short days of winter - **pineal gland more active** - the inhibitory effect of the **pineal gland (melatonin)** – GnRH – anestrus
- long days – **pineal gland less active (melatonin)** – GnRH – estrus

## Seasonal short day breeders

- **Opposite of above**

# FOLLICULAR DYNAMICS

The process of **continual growth** of ovarian follicle leading to **preovulatory size** and its **regression or ovulation**, is known as follicular dynamics.

- Follicular development – **wave like pattern**
- **Recruitment – selection – dominance of follicle**
- **Dominant follicle** grow to full size other don't.
- Continuous process of **growth and atresia** - Throughout the estrous cycle, during pregnancy and other reproductive stages
- **Buffalo has predominance of 2 follicular waves** and **cattle has predominance of 3 follicular waves** during estrous cycle
- **Sow – no wave pattern but intermediate follicles**

# Ovulation

- Due to preovulatory LH surge
- key events
  - Elevated local blood flow (**hyperaemia**) due to - **prostaglandin E<sub>2</sub> and Vascular Endothelial Growth Factor (VEGF)**
  - **breakdown of the connective tissue** within the tunica albuginea and the basement membrane of the ovulatory follicle - **proteolytic enzymes (e.g., collagenase, plasmin)**
  - **contractions of the smooth muscle** within the theca externa layer of the follicle
  - **PGF<sub>2α</sub>** is involved in follicular rupture
  - **PGE<sub>2</sub>** in the remodelling of the follicular layers

## **OOGENESIS** - development and maturation of the ovum

- starts during the prenatal period.
- **At birth** - ovaries contain up to **150,000 primary or primordial** follicles - consists of an oocyte surrounded by a single layer of granulosa cells
- **Just after birth** - ovaries produce growing follicles, - **oocyte with two or more layers of granulosa cells**
- **Upto age of puberty** - **theca interna**
- **Later** - **Graffian or antral follicles** (**200 growing follicles** at puberty)



## DEVELOPMENTAL EVENTS

Multiplication by mitosis  
Migration to genital ridge

**BIRTH**-rabbit, ferret, mink, vole, hamster

Final interphase  
DNA synthesis  
Meiotic prophase begins

**BIRTH**-Most mammals

Growth of oocyte and follicle

**PUBERTY**

Follicular maturation

**OVULATION**-dog, fox

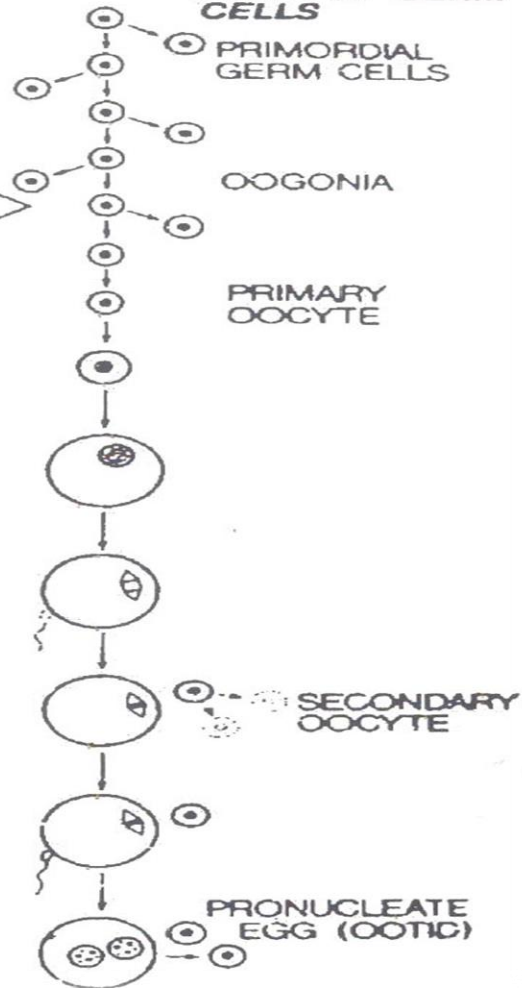
First meiotic division begins  
Sperm penetration- dog, fox

First polar body emitted (may divide)

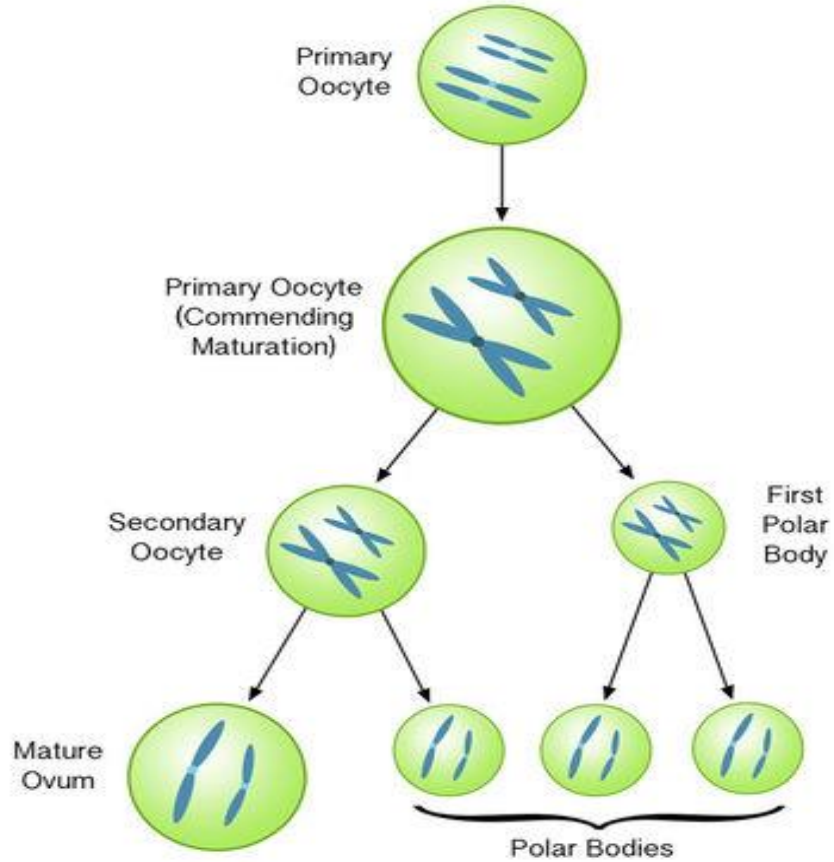
**OVULATION**-Most mammals

Sperm penetration- Most mammals  
Second meiotic division,  
fertilization, and emission  
of second polar body

## STATE OF GERM CELLS



# OOGENESIS



## **prenatal period**

embryonic gut cells developed into--- primary germ cells/oogonia --migrate to cortex of embryonic gonads

**oogonium (2N)** -- formed at day 25-35 of gestation  
undergo mitosis and produce **primary oocytes (2N)**.

The primary oocyte divide by **meiosis** but the division is arrested **at prophase stage** immediately after birth

**At birth - ovaries contain primary oocyte**

**From birth to puberty - primary oocyte remains dormant and is known as Dictyate oocyte**

# Puberty

- gonadotrophins (FSH, LH) initiate the **first meiotic division**
- **primary oocyte (2N) -- secondary oocyte (N) and a polar body**. The polar body is retained in the perivitelline space (i.e between zona pellucida and vitelline membrane).
- Second meiotic division - - - arrested at **metaphase stage** (till fertilization)
- **At Fertilisation – entry of spermatozoa - zygote and second polar body**
- **In farm animals true ovum does not exist**

# Ovulation

- *bitch----- at primary oocyte stage*
- *in most of mammals----- at secondary oocyte stage.*

Species	Time of ovulation	Time of AI/Service
Cattle buffalo	10-12 hour <b>after end</b> of estrus	10-12 hour after onset of estrus(mid to end of estrus)
Mare	1-2 day before the end of estrus	2-4 day after onset of estrus
Sheep	24-30 hour after onset of estrus(towards the end of estrus)	12-24 hour after onset of estrus
Goat	24-40 hours after onset of estrus (towards end of estrus)	24-36 hours after onset of estrus
Sow	30-40 hours after onset of estrus(towards the end of estrus)	24-36 hours after onset of estrus

**Site of fertilization** - ampullary isthmic junction

**Transport of spermatozoa**

- **Rapid transport** - oxytocin of female and  $\text{PGF}_{2\alpha}$  present in the semen
- **Colonization – reservoir - cervix**
- **Slow release and transport** - entry to the oviduct

**Barriers**

- cervix acts as the largest barrier (**Bypass in mare and AI**)
- Uterotubal junction
  
- spermatozoa are transported to both the oviducts but **slightly more towards the ovulating site**

### Fertile life of ovum and sperm (in female genital tract):

Species	Sperm	Ovum
Cattle	30-48 hrs	20-24 hrs
Horse	72-120 hrs	6-8 hrs
Sheep	30-48 hrs	16-24 hrs
Swine	24-72 hrs	8-10 hrs

### Gestation length in different species:

- Bitch: 2 months  $\pm$  2 days
- Sow: 4 months  $\pm$  4 days
- Sheep 5 months  $\pm$  5 days
- Cow 9 months  $\pm$  9 days
- Buffalo: 10 months  $\pm$  10 days
- Mare: 11 months  $\pm$  11 days
- Camel: 13 months  $\pm$  13 days
- Elephant: 24 months  $\pm$  24 days

**Abortion:** The expulsion of **dead embryo or fetus** that has reached **recognizable size** (non viable fetus irrespective of gestation length)

**Still birth:** Delivery of **dead fetus at full term**. It is common in swine and bitch (Polytocus species).

**Premature Birth:** Delivery of **live fetus** before full term.

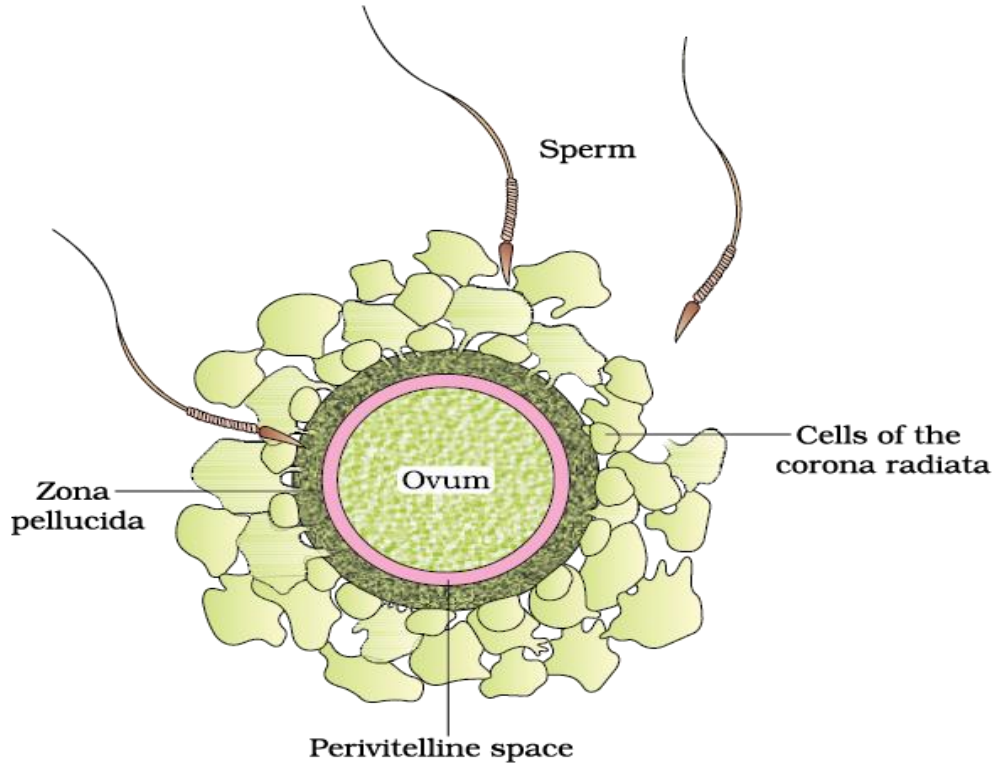


- **Capacitation** - some components of the spermatozoa are either removed or modified to avoid premature acrosomal reaction (- in the female genital tract )
- **Acrosomal reaction - indicator of completed capacitation.**
- ova cannot undergo fusion with non-acrosomal activated sperm
- enzymes from the acrosome - hyaluronidase, corona penetrating enzymes (CPE) and acrosin

### **Layer wise penetration**

- cumulus cells -- Hyaluronidase (arylsulfatase in sow)
- Corona radiata --- corona penetrating enzymes (CPE)
- zona pellucida --- acrosin and species specific receptors **Zona reaction**
- Sperm attached with vitelline membrane
- vitelline membrane --- phagocytosis **Vitelline Block**
- Entry of sperm into cytoplasm

# FERTILIZATION



- ✓ **cortical reaction** - certain chemicals **from the zona membrane and contents of cortical granules** causes **reorganization/ hardening of zona membrane** and it prevents entry of other spermatozoas i.e or **polyspermy block**.
  
- ✓ **Vitelline block** - in this certain substances are released by the cortical granules of vitelline membrane and it prevents fertilization by other spermatozoa.
  
- tail of sperm is separated and get degenerated.
- cytoplasm of the ovum shrinks and it forms **female pronucleus**.
- The head of spermatozoa forms **male pronucleus**.
- **The fusion of male and female pronuclei - Syngamy.**
- second polar body is released and fertilization process is completed.

# CLEAVAGE

- zygote - mitotic divisions
- zygote or one celled stage - low nuclear to cytoplasm ratio.
- cell divisions - without increase in cell mass.
- Cells - **blastomere**
- **Morula** - 8-16 celled stage embryo.
- **Blastocyst** – in morula a fluid filled cavity (**blastocoel**)
- **Polarization** – internal **cellular components** and surface microvilli become **asymmetrically positioned**
- **Compaction** - flattening of the blastomeres and stick together.
- **Histotroph/Uterine milk** - early zygote gets its nutrition from the uterine secretion. (**but in sow – for entire gestation**)

## **Blastocyst formation**

**Sow**

on day 5

**Cattle**

on day 7

**Mare**

on day 6

**Bitch**

**Sheep**

## **Uterus entry**

4-8 celled on day 2

16-32 celled on day 3 or 4

blastocyst stage – 5 or 6d

days 5-8

8 celled on day 3

- **Prostaglandins of F series** - inhibit transport of embryo into the uterus
- **prostaglandins of the E series** - accelerate their delivery to the uterus.

- **zona hatching** – rupture of zona pellucida and blastocyst comes out. This occurs in uterus **4 to 8 days post ovulation**.
- formation of germ cell - **ectoderm, mesoderm and the endoderm**
- **ectoderm** – CNS, external organs like hair, eye, ear, nail and sweat glands
- **mesoderm** - all the internal organs except digestive system develop.
- **Endoderm** - the digestive system, liver, lung and pancreas develop.
- **organogenesis** - **2<sup>nd</sup> to 6<sup>th</sup> weeks** and on **day 21 the heart beat** starts in the embryo.

## development of fetus - three periods

- **Ovum period:** from fertilization till implantation
- **Embryonic period:** from implantation till organogenesis.
  - cattle - from 15 to 45 days
  - Sheep - from 11 to 34 days
  - Mare - from 12 to 55 days
- **Fetal period:** from differentiation till parturition.
  - In Cattle - from day 45 till parturition
  - In Sheep - from day 34 till parturition
  - In Mare - from day 55 till parturition

## Polyspermy

- **fertilization of ova by more than one spermatozoa.**
- due to ageing of the gametes.
- The zygote may survive for some time but **early embryonic death occurs.**
- **Physiological polyspermy** is common in birds and reptiles
- The **Pig** appears **most susceptible to polyspermy**, especially as a result of **delayed mating or insemination** up to 15% of the eggs are penetrated by more than one spermatozoa.

## Parthenogenesis

- development of **individual from egg without fertilization.**
- common in invertebrates.
- In **mammals due to ageing** the ovum may develop **up to second polar body** stage.



**Twinning** - common in **sheep and goat**.

- In mare about 30% of females - double ovulations take place. If twinning is there, then in majority of cases either one fetus dies or both get aborted.
- Twins can be:
- **Dizygotic:** develop from **two separate fertilised eggs ovulated during the same estrous cycle**.
- **Monozygotic:** develop **from single fertilised egg**.
  - These are always of same sex, same blood group and are phenotypically and genetically similar.
- up to **16 cells stage is blastomere is totipotent** - each cell - capable of developing into an independent living being.

## freemartin –

- twin pregnancy in cattle (dizygotic) – male and female
- **Humoral theory - fusion of chorioallantois** of adjacent conceptus results in a **common blood circulation** and **transfusion of fetal androgens**/Mullerin inhibiting substance/factors from male to female side.
- **Cellular theory** - During the migration of primordial germ cells, **germ cells from the male fetus pass** to the female fetus in early pregnancy resulting in XY cells in the genital ridge and cause free martinism.
  
- **external genitalia** of free martin heifer may or may not be appear **normal**
- **internal genitalia** are grossly **abnormal** - devoid of follicle and oocytes.
- diagnosis - demonstration of **sex chromosome chimerism** in cultured lymphocytes. (most accurate method)

## Intrauterine migration of embryos

- In **polytocus animals** (sow & bitch) and in **mare**, it is common.
- It is **essential for survival of fetus**.
- After entry into the uterus the free zygote moves from one uterine horn to the second and there is **mixing of zygotes** of both the uterine horns
- **in sow & bitch - day 12 - the zygotes become stabilized** and are equally distributed between both the uterine horns
- **In mare**, the zygote migrates about **12-14 times daily** between 10-14 days. **By day 25 it become stabilized** and usually found **in the uterine horn which is opposite to the corpus luteum**.
- **in sheep** if there are twins then zygote migrate from one to another horn and in case of **single zygote no intrauterine migration occurs**.
- **Pig** – one fetus in each horn is must
- **Sheep** - protein called **Interferon-  $\tau$ /OTP- $\tau$  (Ovine Trophoblastic Protein Tau)** - prevent the synthesis of  $\text{PGF}_{2\alpha}$ . /**expression of oxytocin**

## **Estrus synchronization**

- to bring a group of females into estrus at pre determined time

### **By shortening of luteal phase**

- luteolytic agents that are prostaglandin  $F_{2\alpha}$  or its analogues and estrogen which result in estrus and ovulation within 2-3 days

#### **Cattle & Buffalo**

##### **PGF<sub>2α</sub>**

- Single Injection, if CL is present – AI after 2 days
- Two injections at 11 to 12 days apart - AI after 2 days

### **By extending the luteal phase**

- administration of a progesterone therefore
- CL regresses after withdrawal of progesterone