

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

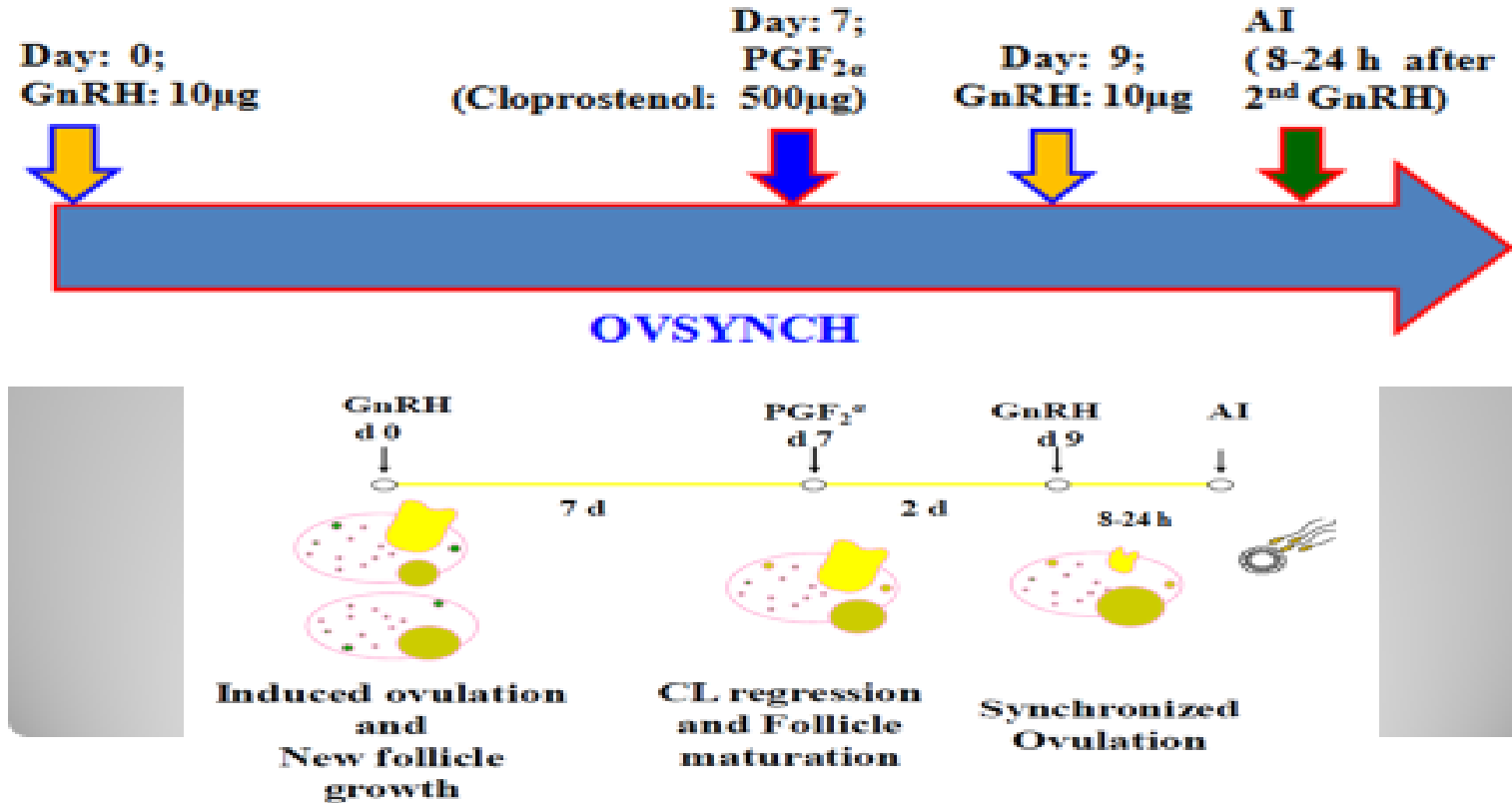
$PGF_{2\alpha}$

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

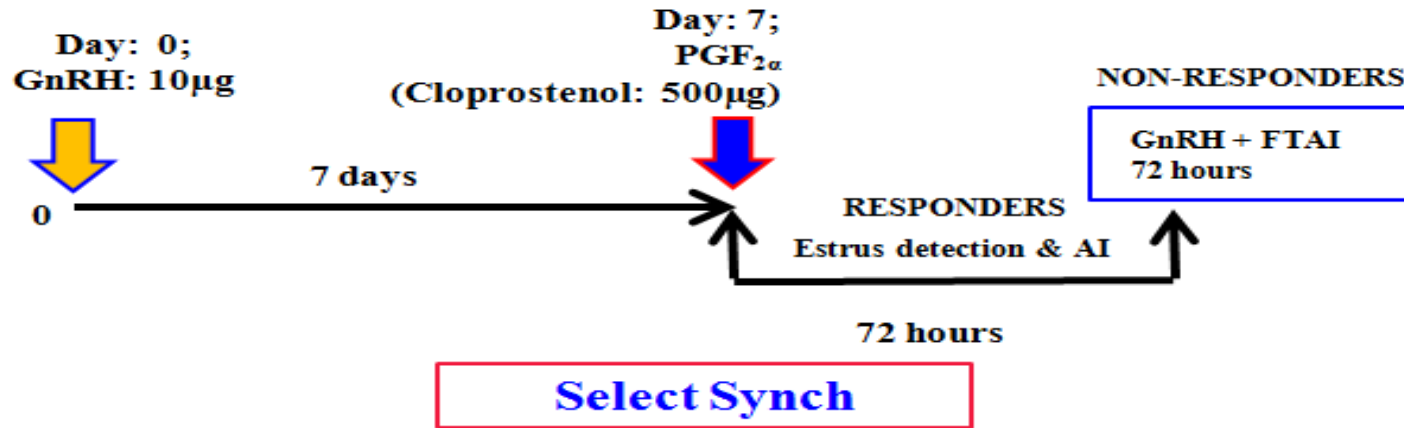
By extending the luteal phase

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

OVSYNVH – 0-7-9-11



SELECT SYNCH – 0-7 (AI – Heat detection)

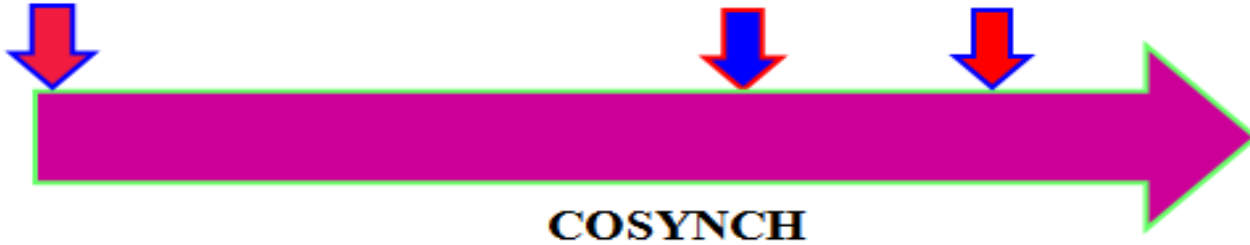


COSYNCH – 0-7-9

Day: 0;
GnRH: 10 μ g

Day: 7;
PGF_{2 α}
(Cloprostenol: 500 μ g)

Day: 9;
GnRH: 10 μ g + AI



Difference Between Ovsynch and Co-Synch Protocols:

Aspect	Ovsynch	Co-Synch
AI Timing	AI is performed 16-24 hours after the second GnRH injection.	AI is performed immediately with the second GnRH injection.
Sperm and Ovulation Timing	AI is timed closer to ovulation, allowing sperm and egg to meet at the optimal time.	Relies on sperm "waiting" for ovulation, as ovulation occurs ~24-30 hours after the second GnRH injection.
Convenience	Requires an extra trip to inseminate cows after the second GnRH injection.	Combines the second GnRH injection and AI into one visit, saving labor and time.
Labor Requirement	Slightly higher, as insemination happens separately.	Lower, as AI and the second GnRH injection are done at the same time.
Accuracy	More precise, as AI is closer to the actual time of ovulation.	Less precise, as AI occurs earlier, relying on sperm survival until ovulation.

INTRAVAGINAL DEVICES:

a. Progesterone releasing intravaginal device (**PRID**)

- progesterone - 1.55gm
- insert into vagina for 7-10 days
- inject $\text{PGF}_2\alpha$ one day before removal of device
- animal will come into heat within 2-4 days.

b. Controlled internal drug release device (**CIDR**)

progesterone - 1.38 grams

into the vagina for 7-12 days

inject $\text{PGF}_2\alpha$ at the time of removal of device

heat within 2-4 days.



EAR IMPLANTS

Synchromate-B: Implant contains **6 mg of norgestomet**

- Injection contains **5 mg estradiol valerate and 3 mg norgestomet**
- Injection is administered at the time of implant insertion.

Crestar: Implant contains **3 mg of norgestomet**

- Injection contains **5 mg estradiol valerate and 3 mg norgestomet**
- Injection is administered at the time of implant insertion.
- These implants are removed after **7-9 days**. **PG** is given at the time of implant removal afterwards animals come into heat between 24 to 72 hours.

Embryo Transfer Technology -

- increase the reproduction rate
- used to collection of an embryo from a **superior donor female** and its transfer to the uterus of a recipient female of the same species

History

- 1890 – **Rabbit** – offspring from embryo transfer
- 1949 - **Sheep and goat** – offspring from embryo transfer
- 1951 – **Cattle** - Birth of calf from embryo transfer
- 1982 – **Cattle** - Birth of calf from **IVF (Virgil)**
- 1983 - **Asian buffalo** - Birth of buffalo calf from embryo transfer
- 1989 – **Rabbit** - First live offspring from **sex-sorted sperm**
- 1996 – **Sheep** - lamb cloned (**Dolly**) from adult somatic cells
- 1988 – Buffalo - Production of the first buffalo calf following ET **in India**
- 1988 – Cattle - Production of first cow calf following embryo transfer **in India**

Superovulation

- induction of **multiple ovulations above the normal level**
- using **exogenous hormones**
- superovulatory response-**Three or more than three ovulations**
- In cattle and buffalo - superovulation protocol **during mid-luteal phase - best**
- Hormones used – **FSH and PMSG**
- **PMSG –**
 - Ad - longer biological half life
 - Disad - activity as both FSH and LH

Steps for ET

- **selection of donor - synchronization** of estrus between **donor and recipients – Superovulation – Breeding - EMBRYO COLLECTION AND TRANSFER**

Criteria for selection of donor:

- **Superior** individual and reproductive performance.
- **Regular cyclicity**.
- Absence of any deformity, pathological condition
- **younger donors are preferred.**

- Donor must have **calved at least 60 days back.**

- Best time **to initiate** superovulatory treatment is **90-100 days post calving.**

- For **repeated** superovulation donors should be treated at least **60 days after 1st superovulatory treatment.**

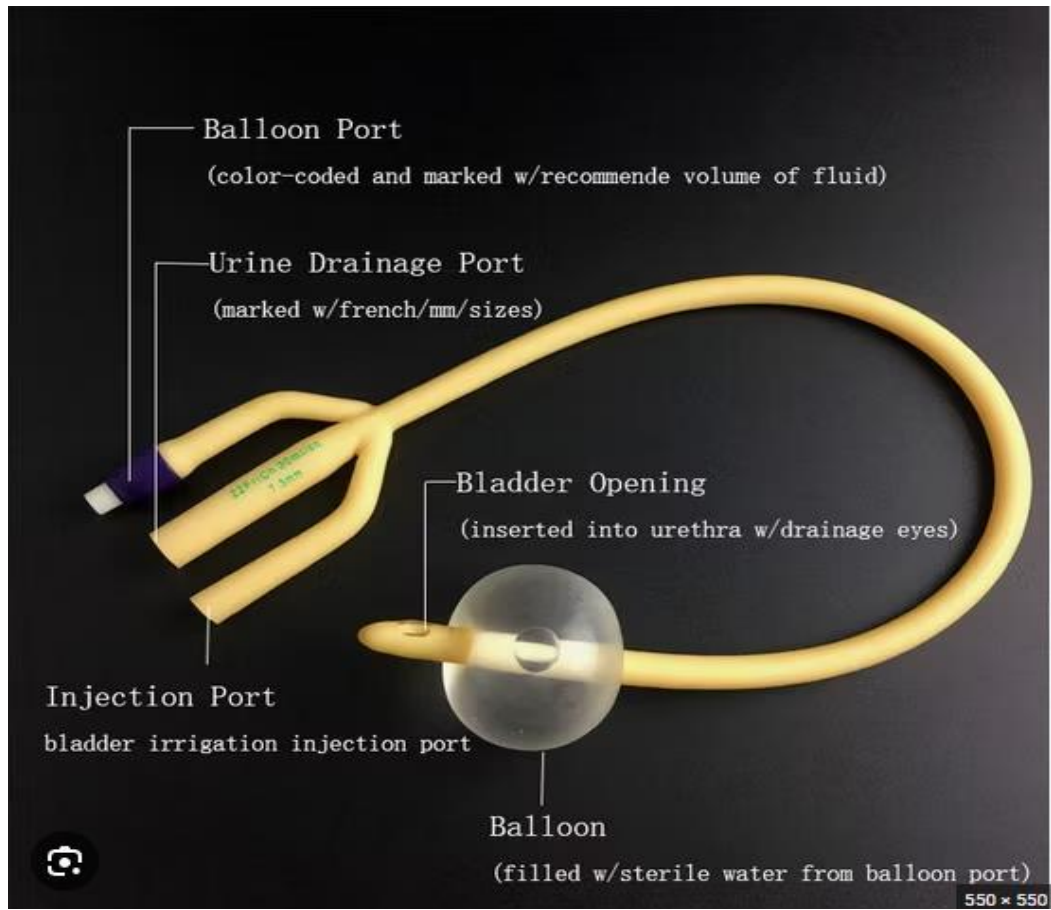
synchronization of estrus between **donor and recipients** for embryo transfer work.

BREEDING OF DONOR

- Heat detection
- **inseminated twice** at 10-12 hours interval using **twice dose** of semen
- Semen should be **deposited in both the horns**

EMBRYO COLLECTION

- **Non-surgical methods:** large animals
 - (A). Trans-cervical method - using 3-way Foley's catheter.
 - (B). Laparoscopic method -stab incision for Instrument
- **Surgical method:** small animals
 - mid-ventral incision under general/local anaesthesia
- **highest pregnancy rates** – embryo collection between the **eight cell (four cells in pigs) and blastocyst stage**



EMBRYO TRANSFER

Cattle and Buffalo: **trans-cervical** approach under epidural an

- recipients are per rectally examined for presence of corpus luteum
- deposit the embryo into the uterine horn to the corpus luteum side

Sheep and Goat:

- under local or ga through mid ventral laparotomy.

PREGNANCY DIAGNOSIS

Cyestiognosis - early and accurate diagnosis of pregnancy

Visual/ Managemental methods:

- A). Non return to estrus - **unreliable**
- B). Udder development – **low accuracy**

Clinical methods:

A). Trans-rectal palpation - **most widely used method**

- 42 days of gestation - safe and reliable
- **land mark** for rectal palpation
 - in cattle and buffaloes – **cervix**
 - mare - **ovaries**
- fetus is barely palpable during the **5½ – 6½ months** period due to **its location in abdominal cavity**
- **Fremitus - Palpation of middle uterine arteries**

B). Ultrasonography

- **Anechoic** : **liquids like follicular fluid, chorionic or amniotic fluid** **and** the images seen on screen appear **black**.
- **Hyperechoic**: **dense tissues such as fetal bones, bovine cervix etc.** and appear **as white** on screen.
- **Hypoechoic**: **soft tissues** and appear as **grey images** on dark screen

C). Radiography – **small animals (45days in bitch)**

D). Abdominal Palpation - in bovines during late gestation (7 months onward), in bitch

E). Laparoscopy

Laboratory methods:

A). Vaginal biopsy

- Histological assessment - number of layers of the stratified squamous epithelium of the vaginal mucosa
- **progesterone** - **decrease in the layers** of the stratum germinatum (vaginal epithelium cells: to 3 to 4 layers at 18-25 days of pregnancy)
- **estrogen** - **number of layers is high** at estrus

B). Immunological methods

Progesterone hormone assay - accuracy - low (75%)

Estrone sulphate – produced by feto-maternal axis or the conceptus

- in the milk of a cow at **105 days of gestation or later** is a **very reliable**

Cuboni test: To detect urinary estrogen

first developed by Cuboni (1934) and modified later (Galina, 1969).

- performed in the **mare for detection**
- **effective between 150 and 300 days of gestation of pregnancy**
- also predicts **fetal viability.**

PMSG(Pregnant Mare serum gonadotrophin)/eCG(Equine chorionic gonadotrophin)

- Endometrial cups - formed on day 35 and secrete eCG
- appear in blood on day 38-42
- continues to be secreted from day 40 to 140 days of pregnancy and disappear after day 150.
- **On farm testing:** National Research Centre on Equines (NRCE), Hisar has developed a kit named as *Pregmare Kit*
 - detects eCG in serum samples between day 35-125 post covering.

Bovine Pregnancy associated glycoproteins (bPAGs)

- Pregnancy specific proteins
- **pregnancy specific proteins (PSP or fetoprotein) and B**

BIOLOGIC TESTS

Aschien Zondek test / A-Z test

- identified **the presence of eCG** in serum of pregnant mare
- serum from mares is **injected to rat**
- SC route - 0.5 ml daily for 2 to 4 days or
- 5 ml intraperitoneally
- rats are killed (72 hr when injected intraperitoneally and 96 to 120 h later when injected subcutaneously)
- **positive test - presence of multiple corpora haemorrhagica on the ovaries and uterine edema.**
- **95 percent accurate** when performed between the **60 to 100 days** of pregnancy in the mare

Friedman Rabbit test

- Serum from test mare is injected (2 ml given i/v) to rabbits (14 to 20 weeks age)
- laparotomy performed 24 h later.
- positive test - presence of corpus haemorrhagica and uterine edema.

Toad test

- sperm cells are emitted by toads/frogs only when stimulated with female frogs or gonadotrophins.
- 1 ml of test serum from a mare is injected into the dorsal lymph sac of 2 male toads thrice at an interval of 1 hour.
- positive test - presence of the sperms in cloaca

PREGNANCY DIAGNOSIS IN BITCHES

- level of relaxin in the blood (after 26 days gestation)
 - **produced by the placenta**
 - ultrasound of the abdomen (**at 21-30 days gestation**)
 - best
 - palpation (at 30 days gestation)
 - radiographs (**after 45 days gestation**).
-
- **Progesterone concentration in the peripheral circulation of pregnant bitches is similar to non pregnant animals**

INFERTILITY

- Cystic ovarian degeneration
- Anestrous
- Silent estrous
- Delayed ovulation
- Anovulation
- Persistent CL

ANESTRUS – most common single cause of infertility in buffaloes

true anestrus

Long anestrus periods due to **cessation of cyclic activity** – ovaries without palpable follicle and CL

False anestrus - **physiological** (lactational, gestational) or **pathological** (due to pyometra and chronic metritis).

gestational heat - sometimes in 5-10% of cases the **pregnant animal also exhibits in first trimester of gestation estrus.**

SILENT ESTRUS: poor expression of heat symptoms

PREIMPLANTATION CHANGES

- **muscular activity and tonicity** of the uterus is **decreased**
- **Blood supply** to the uterine epithelium gets **increased**.

uterine milk - nutrition till **chorioallantoic placenta is established**

hormonal basis of implantation varies widely

- Progesterone plays a major role in determining the preimplantation changes in the **uterus but balance with estrogen is must**

Implantation

- attachment of **placental membranes with the endometrium** of uterus.
- **True implantation** - in **rodents and humans** - conceptus/blastocyst that penetrates the uterine mucosa by penetrating and phagocytizing the uterine luminal epithelium and “**buries**”

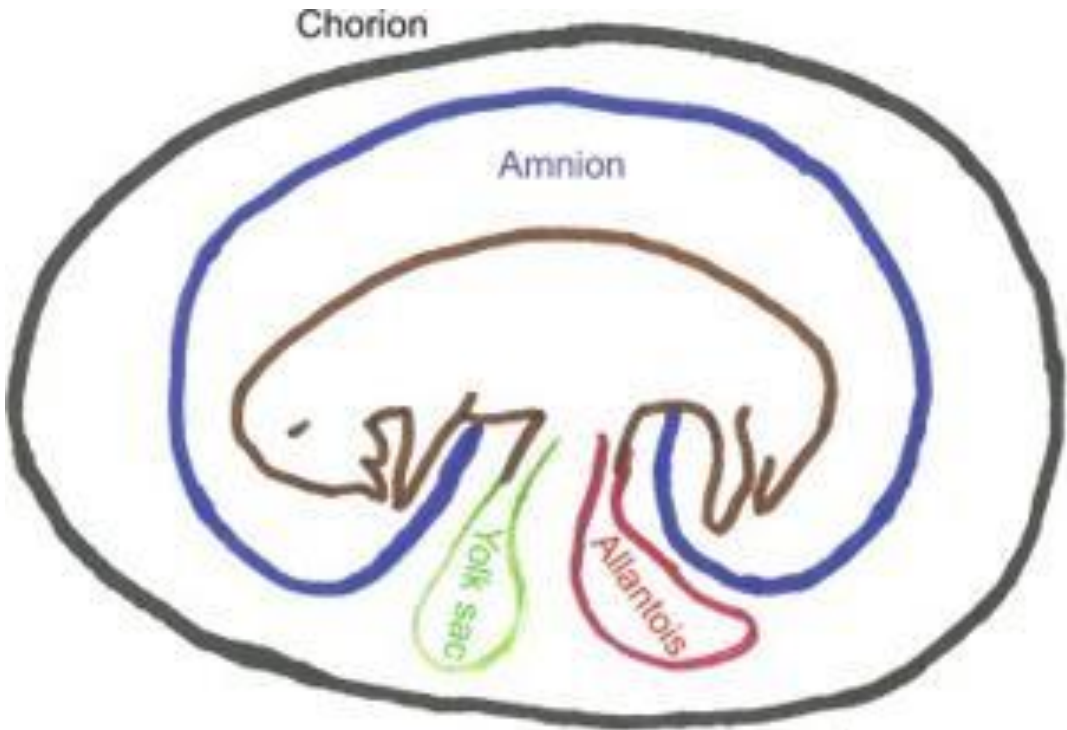
- In **large animals the attachment is superficial and non-invasive -conceptus** never disappears from the luminal compartment.
- **Ruminants - caruncular and intercaruncular areas of the uterine endometrium and trophoblast (cotyledon) of conceptus**
- **Papillae** - finger-like villi from trophoblasts - transitory attachment in **cow and ewe**
- **binucleate giant cells** - from uninucleate cells of the trophoblast - **cow and sheep** - characterized Attachment
- **multinucleate cells or a syncytium** - **binucleate giant cells** fuse with **uterine surface epithelial cells**
- **In sow - diffused microvilli.** trophoblastic surface in these areas becomes modified to form specialized absorptive structures (**areolae**)
- Mare - day 35 - **endometrial cups** - chorionic girdle cell + uterine endometrium and **microcotyledon**

Maternal Recognition of pregnancy

- Critical period of signalling **by the developing conceptus** to prevent luteolysis in order to sustain the pregnancy
- Conceptus (embryo and extra embryonic membranes)

Species	MRP	Day of definite attachment
Sow	12	18
Ewe	12-13	16
Cow	16-17	18-22
Mare	14-16	36-38

- **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**)
- **embryo partition** - two cellular populations
 - **inner cell mass** will give rise to the **body of the embryo**
 - **trophoblastic cells** will become **the fetal component of the placenta**
- **Zona hatching** - free-floating embryo



EXTRAEMBRYONIC MEMBRANES – protection, nutrition, collection of waste

- conceptus undergoes massive growth
- Germ disc – ectoderm mesoderm and Endoderm
- 1. **yolk sac** - regresses
 - persists for 4-6 weeks **in horse** before it becomes a **remnant in the fetal membrane**
- 2. **Chorion** – Trophoblast + Mesoderm
- 3. **amnion – amniotic fluid** - protect from mechanical injuries
 - double walled sac that completely surrounds the fetus except at the umbilical ring.
 - **Amniotic fluid** - early to mid gestation, from the **amniotic epithelium and from fetal urine** (stop later on),
 - later on - **mucoid** amniotic fluid is then the secretions of the **nasopharynx and saliva** of the fetus
 - **AMNIOTIC PLAQUES**

4. allantoic sac - liquid waste from the embryo

- Allantoic fluid – colour - Clear, watery and amber, Contains albumin, fructose and urea
- **Hippomanes** - irregular shaped masses in this fluid

PLACENTA - apposition of fetal membranes to the endometrium to permit physiological exchange between the fetus and the mother

primitive placenta - yolk sac or amniotic chorion

Amniotic Fluid

Species	Amount (in ml)
<i>Cow</i>	2000 – 8000
<i>Mare</i>	3000 – 7000
<i>Goat</i>	400 – 1200
<i>Ewe</i>	350 – 750
<i>Sow</i>	40 – 200
<i>Dog and Cat</i>	8 - 30

Allantoic fluid

Species	Amount (in ml)
<i>Cow</i>	4000–15,000
<i>Mare</i>	8000–18,000
<i>Sheep and goat</i>	500–1500
<i>Sow</i>	100–200
<i>Dog</i>	10–50
<i>Cat</i>	3–15

Embryo - an organism in the early stages of development (**has not acquired an anatomical form that is readily recognizable**)

Fetus - potential offspring that is still within the uterus, but is generally **recognizable**.

Conceptus - product of conception.

- The **embryo** during the early embryonic stage.
- The **embryo and extraembryonic membranes** during the pre implantation stage
- The **fetus and placenta** during the post-attachment phase.

Placentation

- union of **fetal membranes with the endometrium**.
- It protect the fetus from the external physical injury.
- It stores the fetal waste products in the allantoic fluid.
- It synthesise many enzymes and hormones which helps in maintenance of pregnancy.
- It provide nutrition to the fetus from the dam.

Deciduate or conjoined or placenta vera : **bitch, cat, rodents and primates**.

attachment of trophoblast within the endometrium is **deep and firm**.

- At the time of expulsion of placenta, **a part of endometrium is lost** and there is **bleeding**

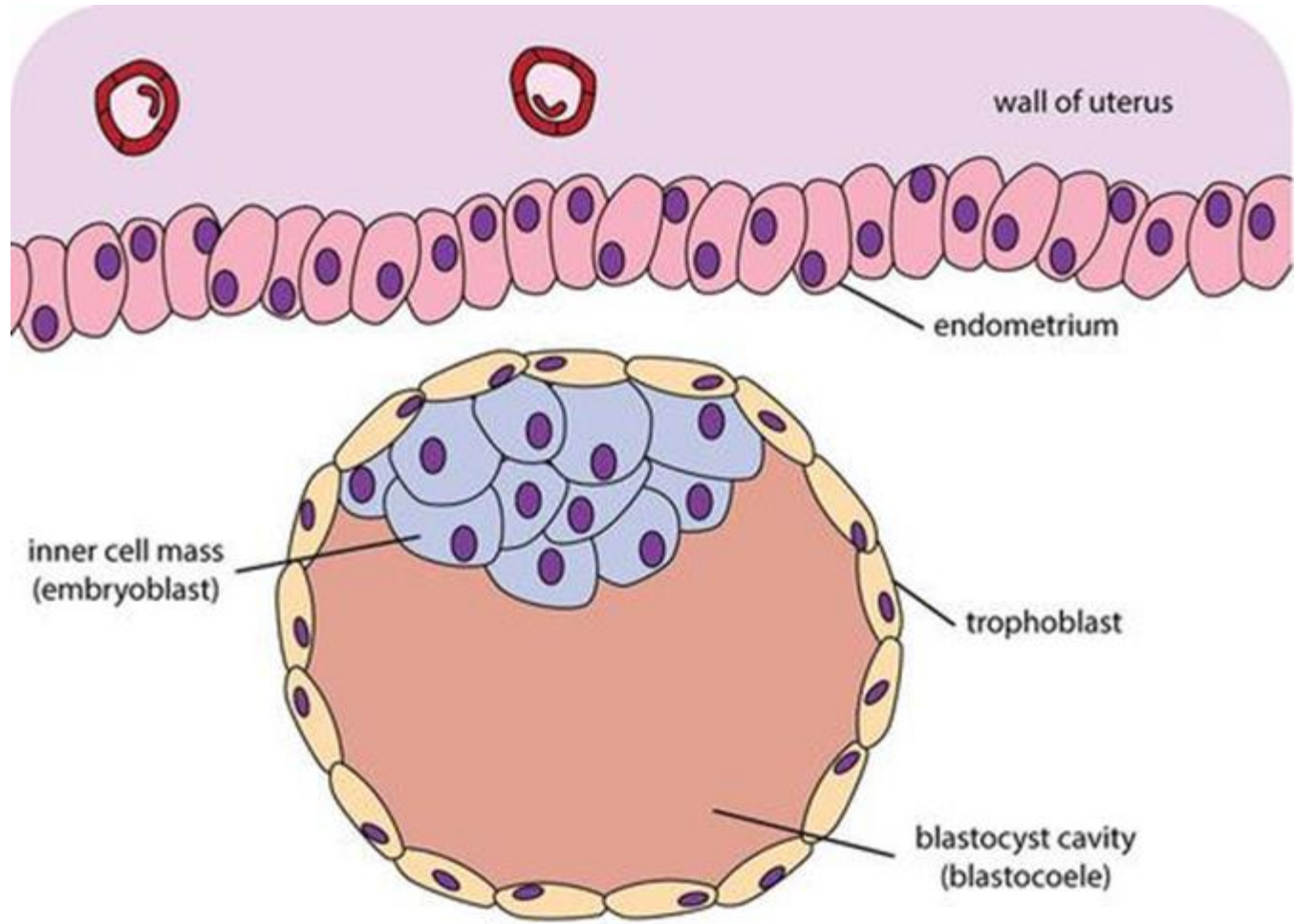
Non-Deciduate: **ruminants, equines and swine**

attachment of trophoblast is **superficial**

- **no part of endometrium** is lost at the time of expulsion of placenta and **no bleeding** takes place

Histologically/ separation between fetal and maternal blood

- **Epitheliochorial: in mare, sow, and camel** - all layers
Chorion of the foetus is attached with the **endometrium of uterus**.
- **Syndesmochorial (Ruminants)** – 5 layers
All tissues of the previous type are present with the exception of the **maternal epithelium**.
- **Endotheliochorial: in bitch and cat.** – 4layers
The chorion of fetus is attached with **endothelium of blood vessels of uterus**
- **Haemochorial - in human being and monkeys.** - 3 fetal layers
The chorion of fetus directly contacts with **blood of uterus**. Blood is lost at the time of separation of placenta in these species.



Six tissue layers that separate the maternal and fetal blood in

1. Uterine vascular endothelium
2. Uterine stroma/Connective tissue.
3. Uterine epithelium

4. Fetal trophoblast (Allanto-chorionic epithelium)
5. Fetal stroma/Connective Tissue
6. Fetal capillary endothelium

1. Diffuse Placenta (Horse, Pig)

•**Type:** Epitheliochorial

•**Description:**

- The fetal chorion comes into contact with the uterine epithelium (outermost maternal layer).
- **All layers** of maternal and fetal tissues (epithelium, connective tissue, endothelium) **remain intact**.

•**Function:**

- Minimal invasion ensures limited exchange of materials between the mother and fetus.

•**Example Animals:** Horse, pig.

Cotyledonary Placenta (Cow, Sheep)

•**Type:** **Synepitheliochorial**

•**Description:**

- Fetal villi attach at specific points called **cotyledons** (specialized regions).
- **Binucleate cells (BNCs)** and **trophoblast cells** fuse with maternal cells to enhance nutrient and gas exchange.

•**Function:**

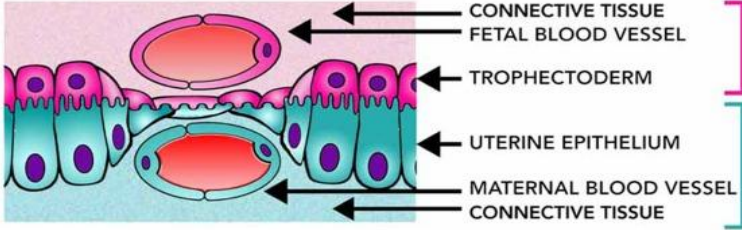
- More efficient exchange than diffuse placentas due to localized attachments.

•**Example Animals:** Cow, sheep.

- **Zonary Placenta (Cat, Dog)**
- **Type:** Endotheliochorial
- **Description:**
 - Fetal chorion invades through maternal epithelium and connective tissue, reaching the endothelium of maternal blood vessels.
 - Fewer maternal layers separate fetal tissues from maternal blood, increasing exchange efficiency.
- **Function:**
 - Allows better nutrient and oxygen transfer than diffuse or synepitheliochorial types.
- **Example Animals:** Cat, dog.

- **Discoid Placenta (Human, Mouse)**
- **Type:** Hemochorial
- **Description:**
 - Fetal chorion comes into direct contact with maternal blood (maternal endothelium is completely breached).
 - The most invasive placenta, **with no maternal layers** remaining between fetal chorion and maternal blood.
- **Function:**
 - Highly efficient nutrient and gas exchange, critical for species with high fetal demands.
- **Example Animals:** Humans, mice.
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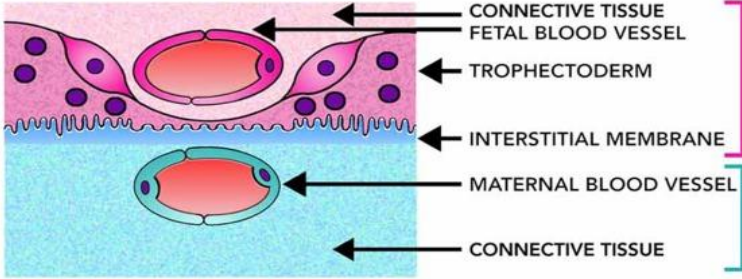
EPITHELIOCHORIAL



CONNECTIVE TISSUE
FETAL BLOOD VESSEL
TROPHECTODERM
UTERINE EPITHELIUM
MATERNAL BLOOD VESSEL
CONNECTIVE TISSUE

FETAL TISSUE
MATERNAL TISSUE

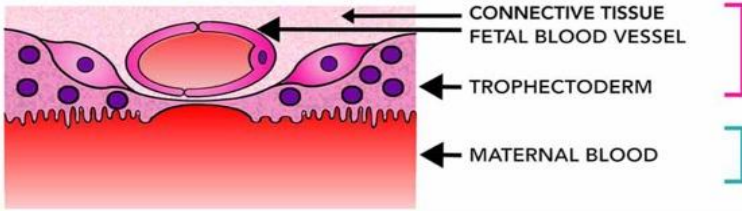
ENDOTHELIOCHORIAL



CONNECTIVE TISSUE
FETAL BLOOD VESSEL
TROPHECTODERM
INTERSTITIAL MEMBRANE
MATERNAL BLOOD VESSEL
CONNECTIVE TISSUE

FETAL TISSUE
MATERNAL TISSUE

HEMOCHORIAL



CONNECTIVE TISSUE
FETAL BLOOD VESSEL
TROPHECTODERM
MATERNAL BLOOD

FETAL TISSUE
MATERNAL TISSUE

Based on SITES OF CHORIONIC ATTACHMENT

Diffused: mare (endometrial cups), sow and camel

Chorionic villi are present throughout the chorion and all the villi attached with endometrium. The villi are absent in the area just adjacent to the cervix.

Cotyledonary: cattle, buffalo, sheep and goat.

Placentomes - Cotyledons (fetus part) + caruncles (maternal parts).

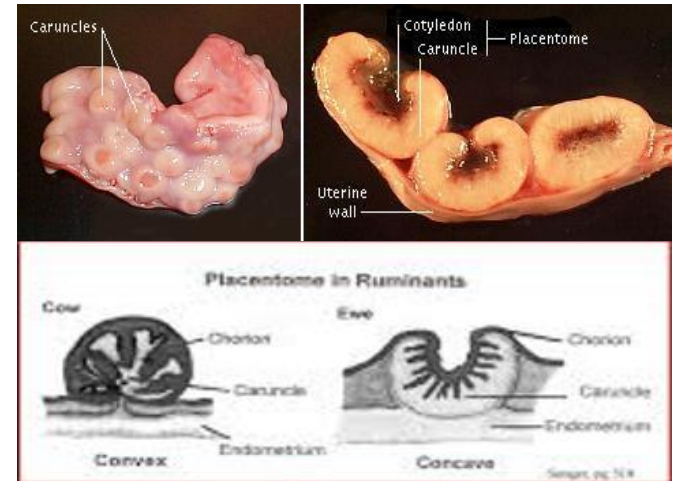
Placentomes are **convex in cattle and concave in sheep**

Zonary: bitch and cat (carnivores)

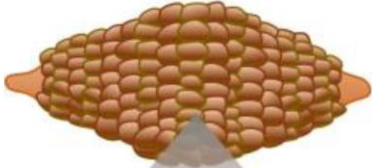
The villi are present in zones in a diameter of 1-3 inches.

Discoidal: human and monkey.

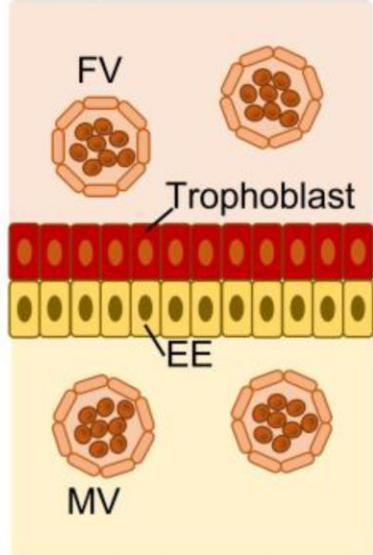
The villi are present in an oval disc like structure.



Diffuse placenta
(Horse, Pig)



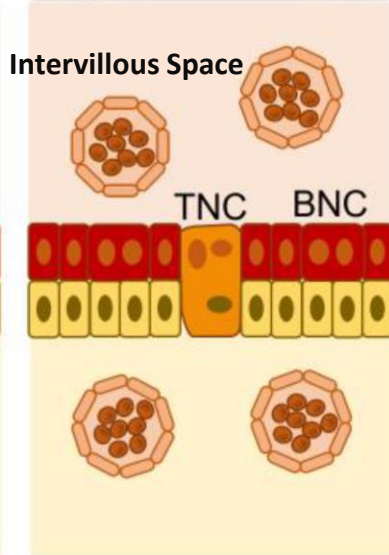
Epitheliochorial



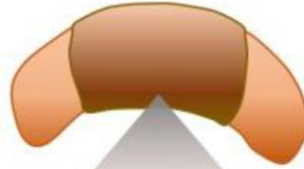
Cotyledonary placenta
(Cow, Sheep)



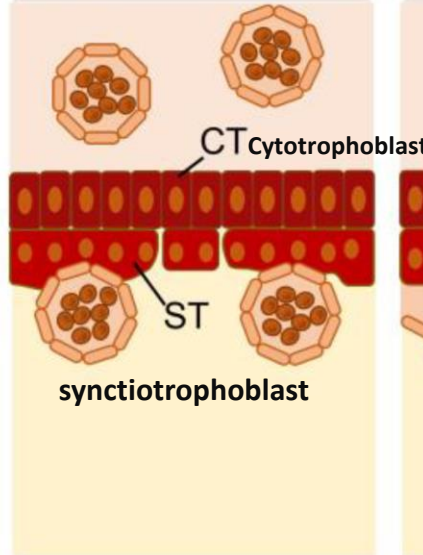
Synepitheliochorial



Zonary placenta
(Cat, Dog)



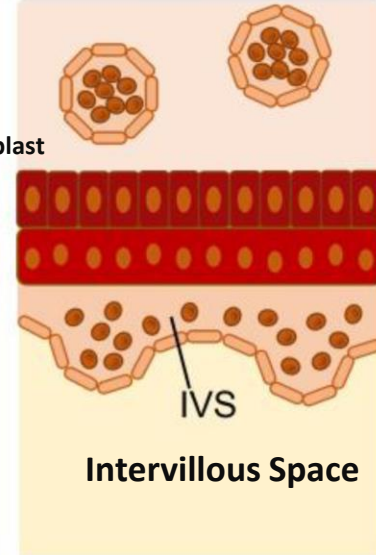
Endotheliochorial



Discoid placenta
(Human, Mouse)



Hemochorial



Chorionic villi (Fetal tissue)

Endometrium (Maternal tissue)

- **FV (Fetal Vessel):** Carries fetal blood.
- **EE (Endometrial Epithelium):** Outermost maternal tissue layer.
- **TNC/BNC (Trophoblast Giant Cells/Binucleate Cells):** Specialized cells in synepitheliochorial placentas for fusion and better exchange.
- **IVS (Intervillous Space):** Space in the maternal tissue filled with maternal blood for exchange in hemochorial placentas.
- **CT/ST (Cytotrophoblast/Syncytiotrophoblast):** Layers of trophoblasts involved in maternal-fetal interface.

Species	Classification of Chorioallantoic Placentas		
	Chorio Villous Pattern	Maternal-Fetal Barrier	Loss of Maternal Tissue at Birth
Pig	Diffuse	Epitheliochorial	None (nondeciduate)
Mare	Diffuse and Microcotyledonary	Epitheliochorial	None (nondeciduate)
Sheep, goat, cow, water buffalo	Cotyledonary	Epitheliochorial	None (nondeciduate)
Dog, cat	Zonary	Endotheliochorial	Moderate (deciduate)
Man, monkey	Discoid	Hemochorial	Extensive (deciduate)

Type of Placenta	Maternal Layers intact			Examples
	Endometrial Epithelium	Connective Tissue	Uterine Endothelium	
Epitheliochorial	✓	✓	✓	Equine Swine
Synepitheliochorial	✓	✓	✓	Ruminants
Endotheliochorial	×	×	✓	Canine Feline
Hemochorial	×	×	×	Humans Rodents