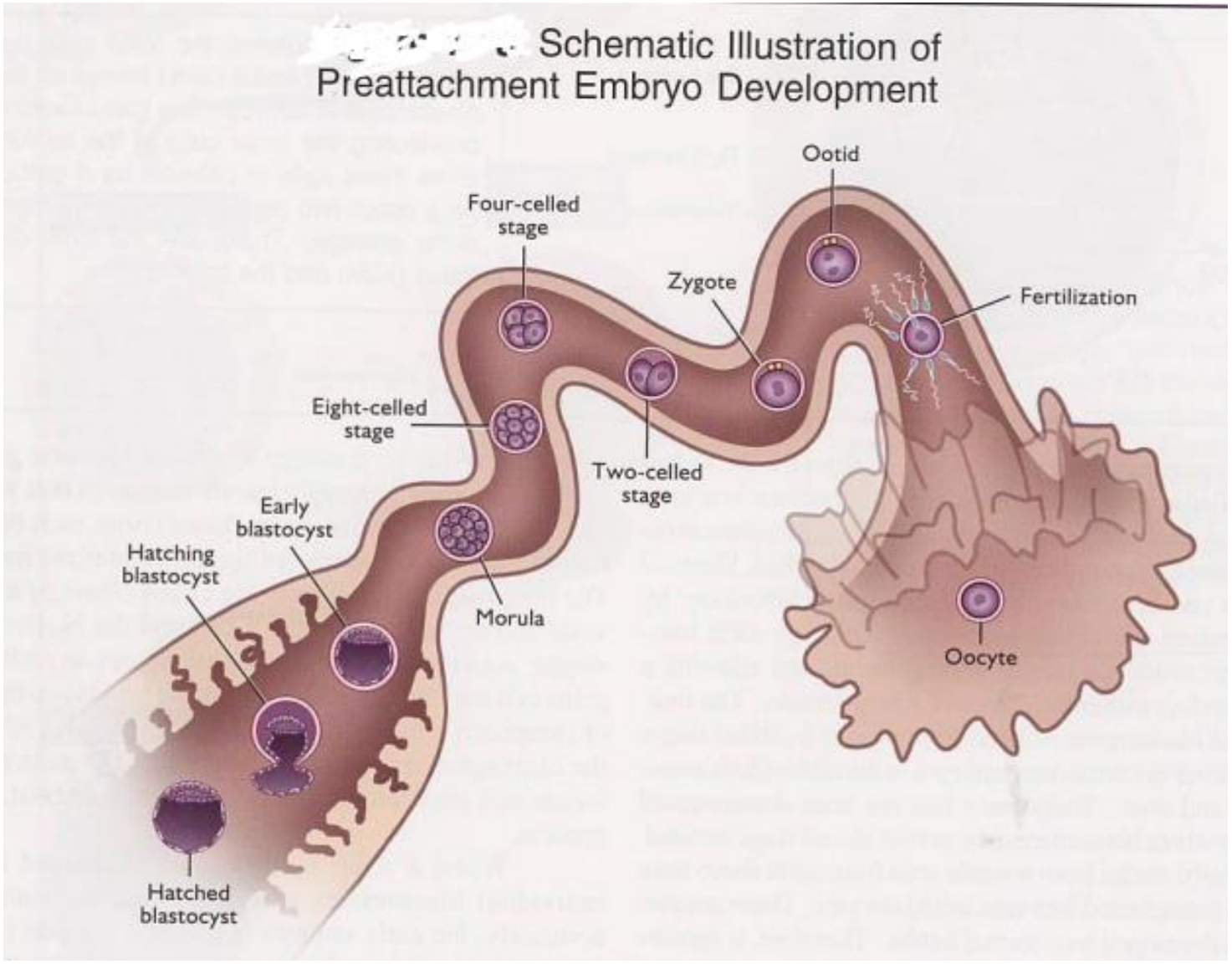
FERTILIZATION

Dr. Krishan Yadav
Assistant professor
Department of Veterinary Gynaecology and Obstetrics
MJFCVAS, Chomu


Schematic Illustration of
Preattachment Embryo Development
Ootid
Four-celled
stage
Zygote
Fertilization
Eight-celled
stage
Two-celled
stage
Early
blastocyst
Hatching
blastocyst
Morula
Oocyte
Hatched
blastocyst

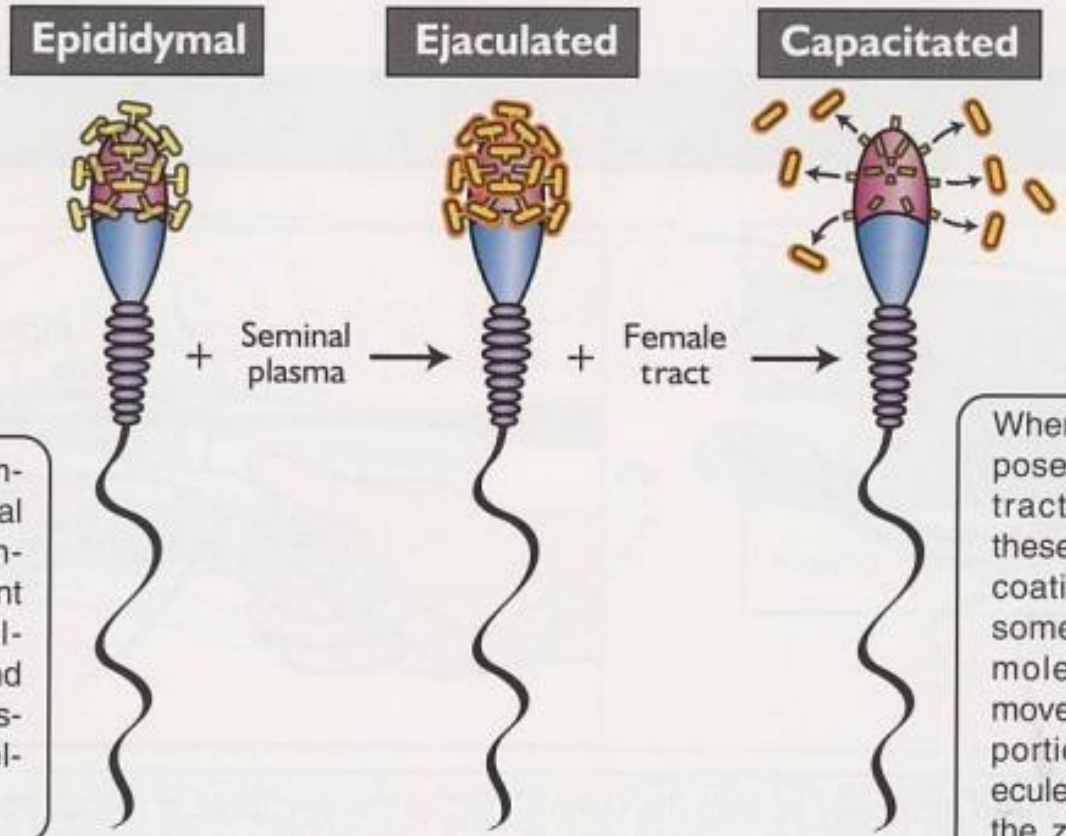
FERTILIZATION

For fertilization the ovum and spermatozoa should be mature. The ovum is at second meiotic stage at the time of fertilization and the maturation occurs after fertilization.

The spermatozoa attain maturity in the epididymis and for this the spermatozoa should remain in the epididymis for 10-15 days to attain the maturity. After ejaculation the female genital tract, the spermatozoa undergo capacitation.

Capacitation is a process by which some components of the spermatozoa are either removed or modified. The basic purpose of capacitation is to avoid premature acrosomal reaction which should occur at the time of fertilization. Acrosomal reaction is an indicator of completed capacitation. .

Figure 12-8. Conceptual Version of Mammalian Capacitation



The plasma membrane of epididymal spermatozoa contains a complement of surface molecules (proteins and carbohydrates) illustrated here as yellow T's.

When sperm are exposed to the female tract environment, these seminal plasma coatings, along with some of the surface molecules, are removed, thus exposing portions of the molecules that can bind to the zona pellucida of the oocyte.

The surface molecules in epididymal sperm become coated with seminal plasma proteins (orange halos) that mask portions of the membrane molecules.

The function of acrosomal reaction is that eggs are surrounded by glycoprotein coats through which sperm must pass before reaching egg plasma membrane.

Acrosome reacted sperm dissolve coat locally to produce a 'hole' through which the sperm swim.

Outer acrosomal membrane overlying plasma membrane destroyed (partially or totally) or become detached from main body of sperm.

The acrosomal reaction is a prerequisite for fusion between ova and spermatozoa plasma membrane and zona free ova cannot undergo fusion with non-acrosomal activated sperm even through attachment to the membrane surface occurs.

During this acrosomal reaction enzymes like hyaluronidase, corona penetrating enzymes (CPE) and acrosin are released from the acrosome.

Fertilization starts with penetration of spermatozoa. First the spermatozoa pass through the cumulus cells and the penetration is facilitated by the hyaluronidase enzyme in case of cattle, and arylsulfatase in sow.

Corona radiata penetration is facilitated by CPE After this, the spermatozoa reach the zona pellucida.

At the zona pellucida, it attaches with receptors. The receptors are species specific i.e the spermatozoa of same species attach with the ovum of same species.

Three glycoproteins, ZP1, ZP2 and ZP3 are synthesized by maturing oocytes. These are present in all mammals but with variation in these proteins, ZP3 functions as sperm receptor to which only sperm with an intact acrosome can bind.

Binding of sperm to sperm receptors occurs through an interaction with O-linked oligosaccharide on ZP3.

Then, the spermatozoa pass through the zona pellucida and get attached with vitelline membrane and the penetration is helped by acrosin enzyme. Once it attaches with the vitelline membrane zona reaction occurs.

During this reaction certain chemicals from the zona membrane are released and this causes reorganization of zona membrane and it prevents entry of other spermatozoas i.e cortical reaction or polyspermy block.

Cortical reaction or polyspermy block: Block to polyspermy is at zona pellucida in most mammals (e.g. sheep and swine) with a secondary physiologic block at vitelline membrane in rabbit (Vitelline block).

Initial polyspermy block is at sperm penetration of ovum when cortical granules are released into perivitelline space.

"Release of contents of cortical granules cause extensive reorganization of zona pellucida and/ or vitelline surface which causes hardening of zona pellucida and inactivation of ZPS receptors to prevent polyspermy, is termed as "cortical reaction/Polyspermy block".

The spermatozoa through phagocytosis digest the vitelline membrane and enter the cytoplasm of the ovum. Immediately after this vitelline block occurs.

Again, in this certain substances are released by the cortical granules of vitelline membrane and it prevents fertilization by other spermatozoa.

In this process, the tail of sperm is separated and get degenerated.. Subsequently, the cytoplasm of the ovum shrinks and it forms female pronucleus.

The head of spermatozoa forms male pronucleus. Both the pronuclei migrate towards the centre of ovum, come in contact with each other; their membrane are dissolved and the mixing of their contents takes place.

The fusion of male and female pronuclei is termed as Syngamy. After, this fusion the second polar body is released and fertilization process is completed.

CLEAVAGE

During this stage the zygote divide by several mitotic divisions (always).

The zygote or one celled stage is quite large having low nuclear to cytoplasm ratio.

To attain a ratio similar to somatic cells, cell divisions occur without an increase in cell mass. The process is referred to as cleavage.

By first mitotic division a two celled embryo is formed and each cell is known as blastomere.

Then, due to subsequent divisions a 4,8,16,32 and 64 celled embryo is formed. The 8-16 celled stage embryo is known as Morula.

Later, in the morula a fluid filled cavity called blastocoel is formed and this embryonic stage is known as Blastocyst.

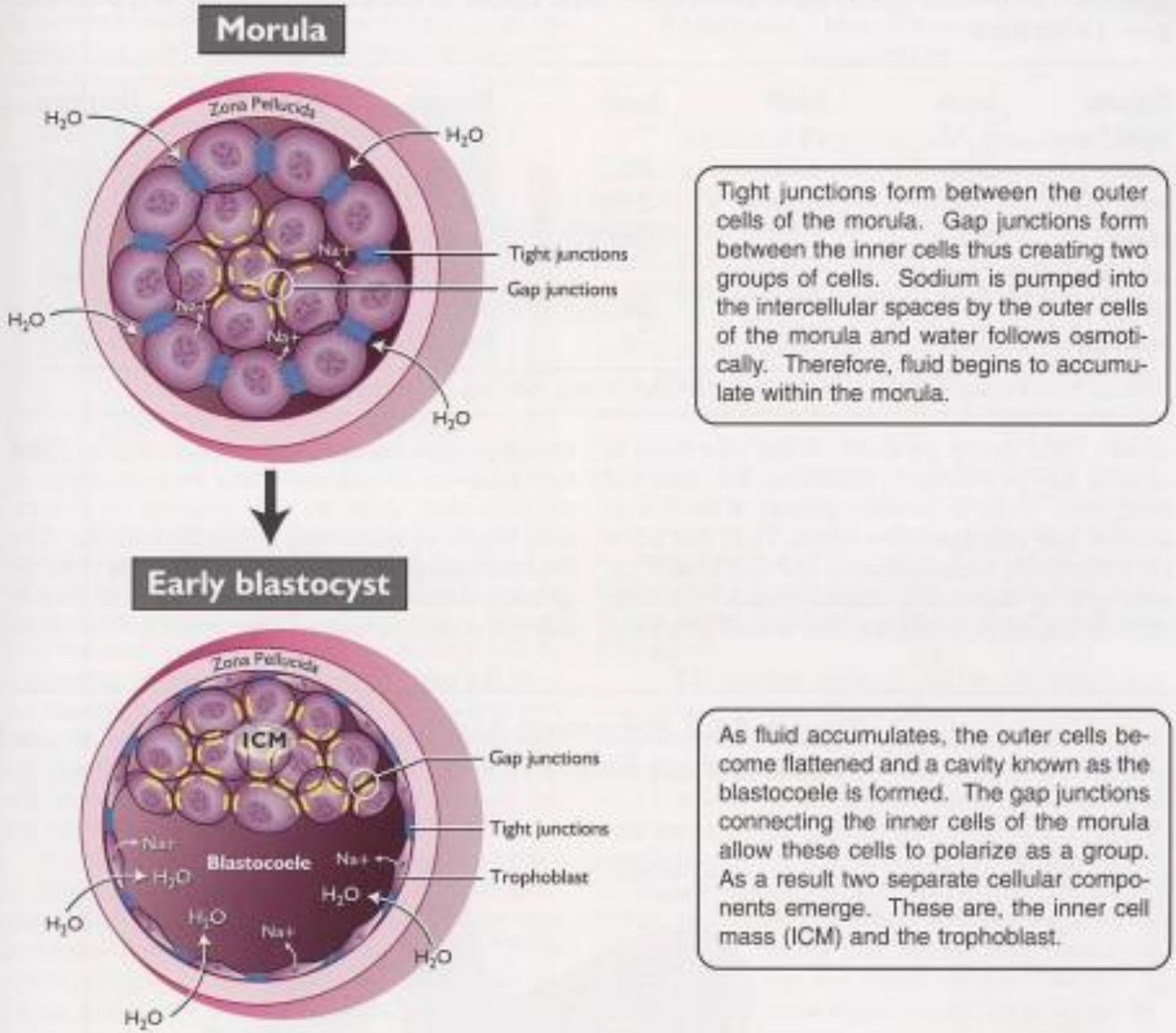
At this time blastomeres flatten on each other to form a round embryo and internal cellular components, and surface microvilli become asymmetrically positioned in a process termed polarization.

The combined processes of flattening of the blastomeres and polarization are referred to as compaction.

In sow, blastocyst is formed on day 5, in mare and sheep on day 6 and in cattle on day 7.

In cattle 16-32 celled embryo enters the uterus on day 3 or 4, days 5-8 in bitch and queen, whereas in sow a 4-8 celled stage embryo enters the uterus day In ewe (8 celled stage) it happens on day 3.

Figure 13-2. Transition of a Morula into an Early Blastocyst



Tight junctions form between the outer cells of the morula. Gap junctions form between the inner cells thus creating two groups of cells. Sodium is pumped into the intercellular spaces by the outer cells of the morula and water follows osmotically. Therefore, fluid begins to accumulate within the morula.

As fluid accumulates, the outer cells become flattened and a cavity known as the blastocoele is formed. The gap junctions connecting the inner cells of the morula allow these cells to polarize as a group. As a result two separate cellular components emerge. These are, the inner cell mass (ICM) and the trophoblast.

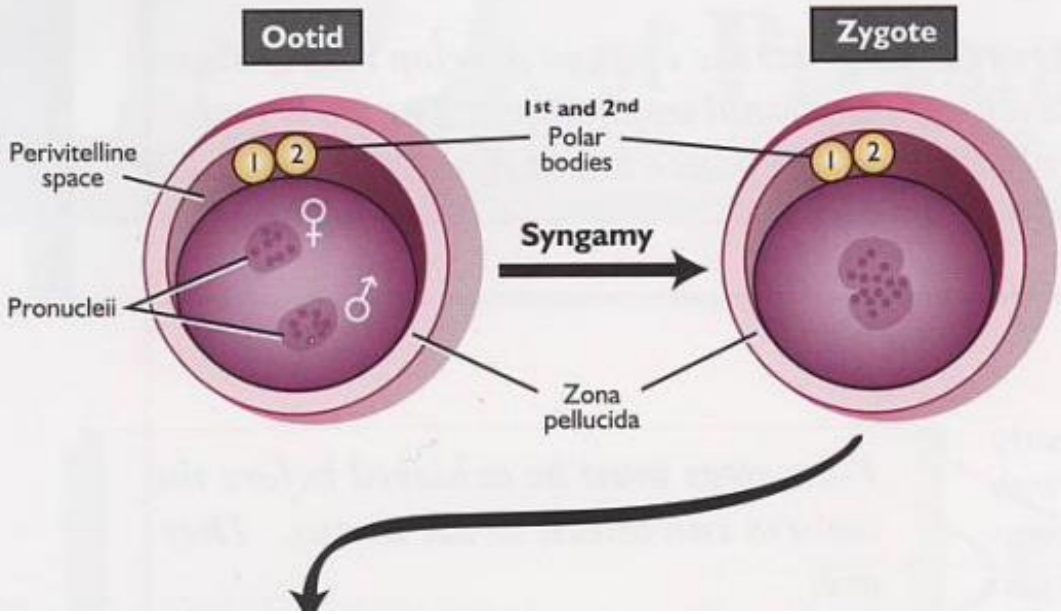
The tubal transport of fertilized eggs of mare probably takes 5-6 days, by which time they are at the blastocyst stage.

The egg is transported from the oviduct to the uterus irrespective of fertilization except in mare wherein the unfertilised egg remains in oviduct for several months.

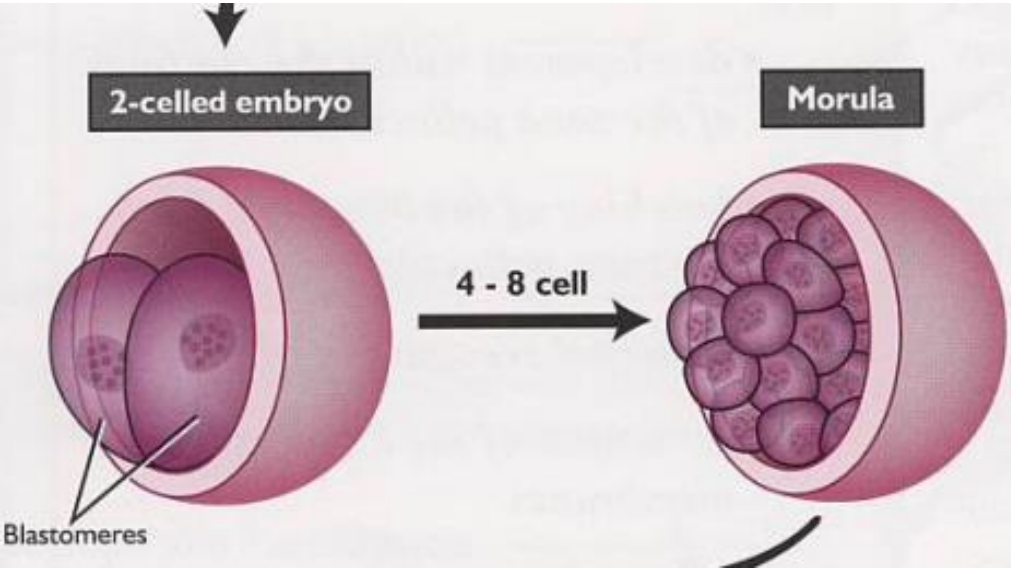
In the uterus the early zygote gets its nutrition from the uterine secretion known as Histotroph/Uterine milk.

The blastocyst is contained in the zona pellucida and due to further divisions it elongates due to which zona pellucida ruptures and the blastocyst comes out. This is known as zona hatching and it occurs in uterus 4 to 8 days post ovulation.

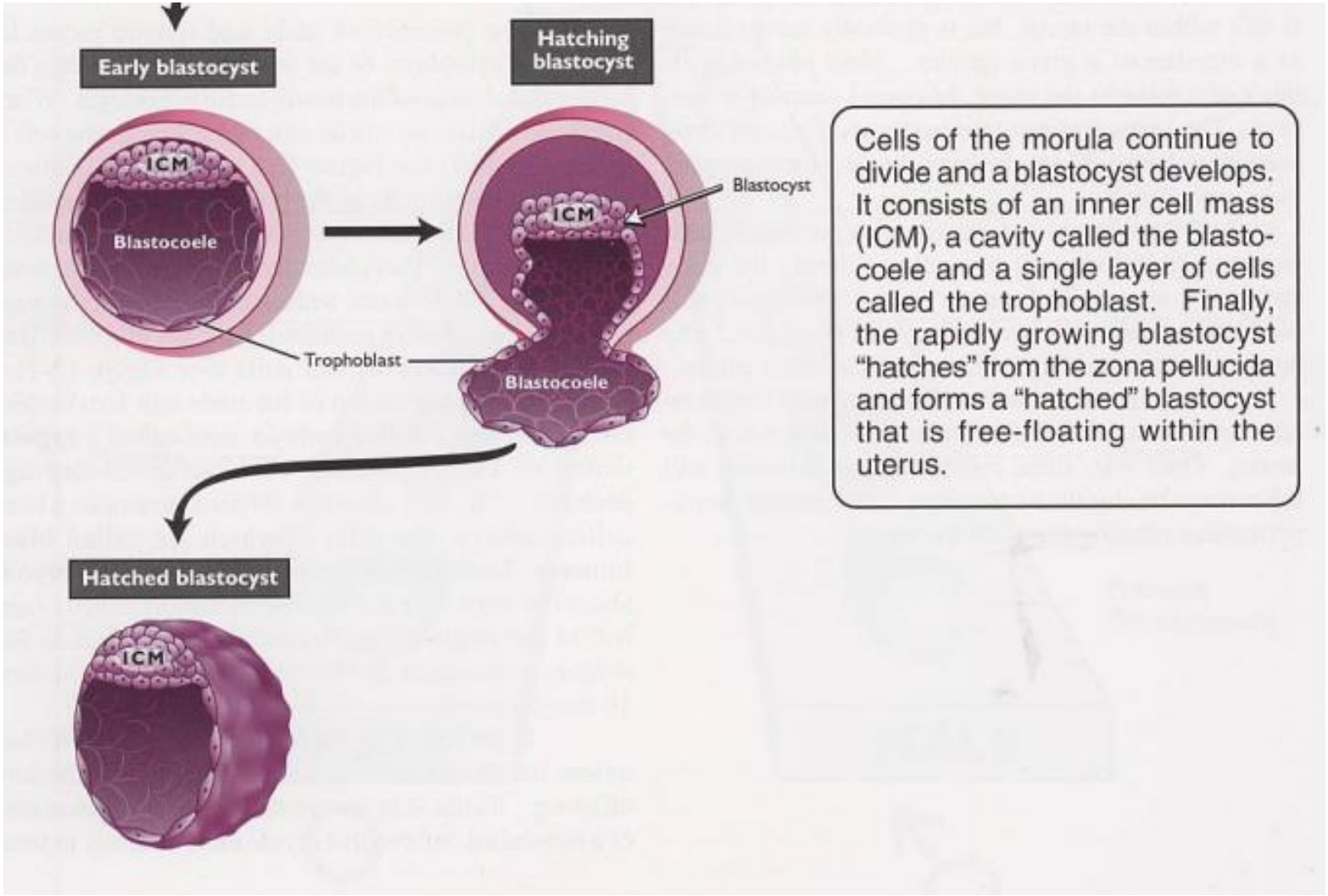
Figure 13-1. Preattachment Development of the Embryo



In the ootid, male and female pronuclei along with the first and second polar bodies are present. Fusion of the male and female pronuclei into a single diploid nucleus constitutes syngamy. Shortly thereafter, the zygote undergoes cleavage (mitotic divisions) and gives rise to daughter cells called blastomeres.



Cleavage divisions continue. A four-celled embryo gives rise to an eight-celled embryo. After the eight-celled stage, a ball of cells is formed and this embryonic stage is referred to as a morula.



Cells of the morula continue to divide and a blastocyst develops. It consists of an inner cell mass (ICM), a cavity called the blastocoele and a single layer of cells called the trophoblast. Finally, the rapidly growing blastocyst "hatches" from the zona pellucida and forms a "hatched" blastocyst that is free-floating within the uterus.

Afterwards, the elongation is very fast and blastocyst starts differentiating into extra embryonic-membrane from which the placenta develops. Also, there is formation of germ cell layer in the blastocyst.

The germ cell layers are ectoderm, mesoderm and the endoderm.

From the ectoderm the central nervous system and external organs like hair, eye, ear, nail and sweat glands develop.

From the mesoderm all the internal organs except digestive system develop.

From the endoderm, the digestive system, liver, lung and pancreas develop.

The organogenesis occurs during 2^{nd} to 6^{th} weeks and on day 21 the heart beat starts in the embryo.

The development of fetus in fetus can be divided in to three periods:

1. Ovum period: The period from fertilization till implantation or attachment.

2. Embryonic period: It is the period from implantation till differentiation or organogenesis.

In Cattle, it is the period from 15 to 45 days

In Sheep, it is the period from 11 to 34 days

In Mare, it is the period from 12 to 55 days

3. Fetal period: It is the period from differentiation till parturition.

In Cattle, it is the period from day 45 till parturition

In Sheep, it is the period from day 34 till parturition

In Mare, it is the period from day 55 till parturition

Stem cells: Pluripotent and totipotent cells with infinite regeneration capacities like embryonic cells, bone marrow cells and germ cells.

Uterine glands in sow provide nutrition to embryo/fetus during entire gestation.

Polyspermy

It refers to fertilization of ova by more than one spermatozoa. It is due to ageing of the gametes. Due to this polyploidy occur.

The zygote may survive for some time but early embryonic death occurs.

Physiologic polyspermy is common in birds and reptiles.

the incidence of polyspermy in most mammalian species is only 1 to 2%.

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.

Ageing of gametes

It occurs if the animal is not inseminated at proper time. If insemination is too early then spermatozoa become older or aged. If inseminated too late then ageing of ovum occur. Due to ageing polyspermy may occur.

Parthenogenesis

It is the development of individual from egg without fertilization. It is common in invertebrates, If the egg is subjected to electric stimulation or hyaluronidase treatment it may develop up to blastocyst stage. In mammals due to ageing the ovum may develop up to second polar body stage.

Twinning

It is natural and common in sheep and goat. In mare about 60% of females double ovulations take place. If twinning is there, then in majority of cases either one fetus dies or both get aborted. Rarely both are born alive.

It can be diagnosed by per-rectal examination or ultrasound and the one of the embryonic vesicle/fetus is destroyed manually.

In cattle it is rare and can occur 0.5-2% of cases.

It is highly undesirable in mono-ovulatory animals and may lead to dystocia and retention of placenta.

Twins can be:

a) Dizygotic: These develop from two separate fertilised eggs ovulated during the same estrous cycle. These are relatively common and may be of same or opposite sex.

b) Monozygotic: These develop from single fertilised egg. These are always of same sex, same blood group and are phenotypically and genetically similar.

Twinning may occur due to separation of blastomeres and up to 16 cells stage is blastomere is totipotent means capable of developing into an independent living being or it may develop due to formation of two inner cell masses in the zygote.

As compared to dizygotic, monozygotic are less common because the chances of abortion are more in monozygotis.

With twin pregnancy in cattle, 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. Therefore, around 95% of heifers born co-twin with a male are sterile freemartins.

Freemartinism is a distinct form of intersexuality that arises as a result of vascular anastomosis of the adjacent chorioallantoic sacs of heterozygous fetus.

The vascular anastomosis occurs as early as 30 days of gestation. If there is death of male twin of a heterozygous pair after this time with the other being carried to term.

Therefore, it is possible for a single born freemartin to occur. The external genitalia of freemartin heifer may appear normal; the internal genitalia are grossly abnormal.

The gonads are typically vestigial but in minority of cases have undergone masculinization and the gonads resemble testes (parenchyma contains recognizable tubules and interstitial tissue).

Structures developed from paramesonephric duct are absent or hypoplastic. Development of mesonephric ducts is related to the degree of masculinization of gonads. Vestigial gonads of freemartins are devoid of follicle and oocytes.

The newly born freemartin can be recognized by prominent clitoris with an obvious tuft of hairs at the ventral commissure of vulva, although these signs are not always reliable.

In adult female the length of vagina is about 30 cm compared with 8-10 cm in freemartin. In calves of-4 weeks of age the vagina is normally 13-15 cm in length compared with 5-6 cm in a free martin. The most accurate method of diagnosis is demonstration of sex chromosome chimerism in cultured lymphocytes.

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 and by day 12, the zygotes become stabilized and are equally distributed between both the uterine horns (in sow & bitch).

In mare, the zygote migrates about 10-14 times daily between 12-14 days. By day 25 it become stabilized and usually found in the uterine horn which is opposite to the corpus luteum.

In cattle, the migration does not occur. But, in sheep if there are twins then zygote migrate from one to another horn and in case of single zygote no intrauterine migration occurs.

Implantation

It is the attachment of embryo with the endometrium of uterus. The trophoblast of embryo attaches with uterus by secreting proteolytic enzymes. In large animals the attachment is superficial and non-invasive. In sow, attachment starts on day 12 completed by 18 and attachment is by diffused microvilli.

Maternal Recognition of pregnancy

After fertilization certain signals are produced by the zygote which prevents regression of CL. So, the critical period during which the signals are passed to the dam or female is known as Maternal Recognition of Pregnancy (MRP).

In other words, it is defined as "Critical period of signalling by the developing conceptus to prevent luteclysis in order to sustain the pregnancy".

In different species different substances are produced by the developing embryo which helps in maintenance of pregnancy. Initially, the conceptus of domestic farm species must physically cover a large portion of maternal endometrium to regulate release of PGF2α to prevent luteolysis.

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

In sheep, between days 12-21 a protein called Interferon-τ /OTP-τ (Ovine Trophoblastic Protein Tau, Mol. weight 18-20 kDa) is produced which again prevent the synthesis of PGF2α.

OTP- tau secreted by embryo prevents the rise in endometrial oestrogen-α receptors that precedes the rise in endometrial oxytocin receptors.

The consequence is that OTP-τ indirectly suppresses the expression of endometrial receptors.

Oxytocin of luteal and possibly of central origin can thus no longer bind to the uterus, and this result in a reduction in the pulsatile secretion of PGF2α.

In sow, by the day 12, the embryos are more or less becomes evenly distributed and this process is known intra-uterine spacing.

The estrogen production by conceptus plays a vital role in the maternal recognition of pregnancy and the extension of the life span of CL.

The administration of exogenous estrogens parentrally in non-pregnant sows prolongs the life span of the CLs (i.e luteotrophic) and extends the inter-oestrous interval.

After the initial secretion of estrogen at day 11, a second sustained release of estrogen by the conceptus is necessary between day 14 and 18 for luteal persistence beyond day 25.

These estrogens do not prevent the synthesis of PGF2α but cause its sequestration into uterine lumen and thus prevent its entry in uterine vasculature (inhibition of endocrine action).

Sow conceptus also produces interferons (Type I and II) at the time of elongation. Other important factor for maintenance of pregnancy in sow is the number of foetuses present in the uterus.

To maintain pregnancy in sow, there should be at least two foetuses in each uterine horn.

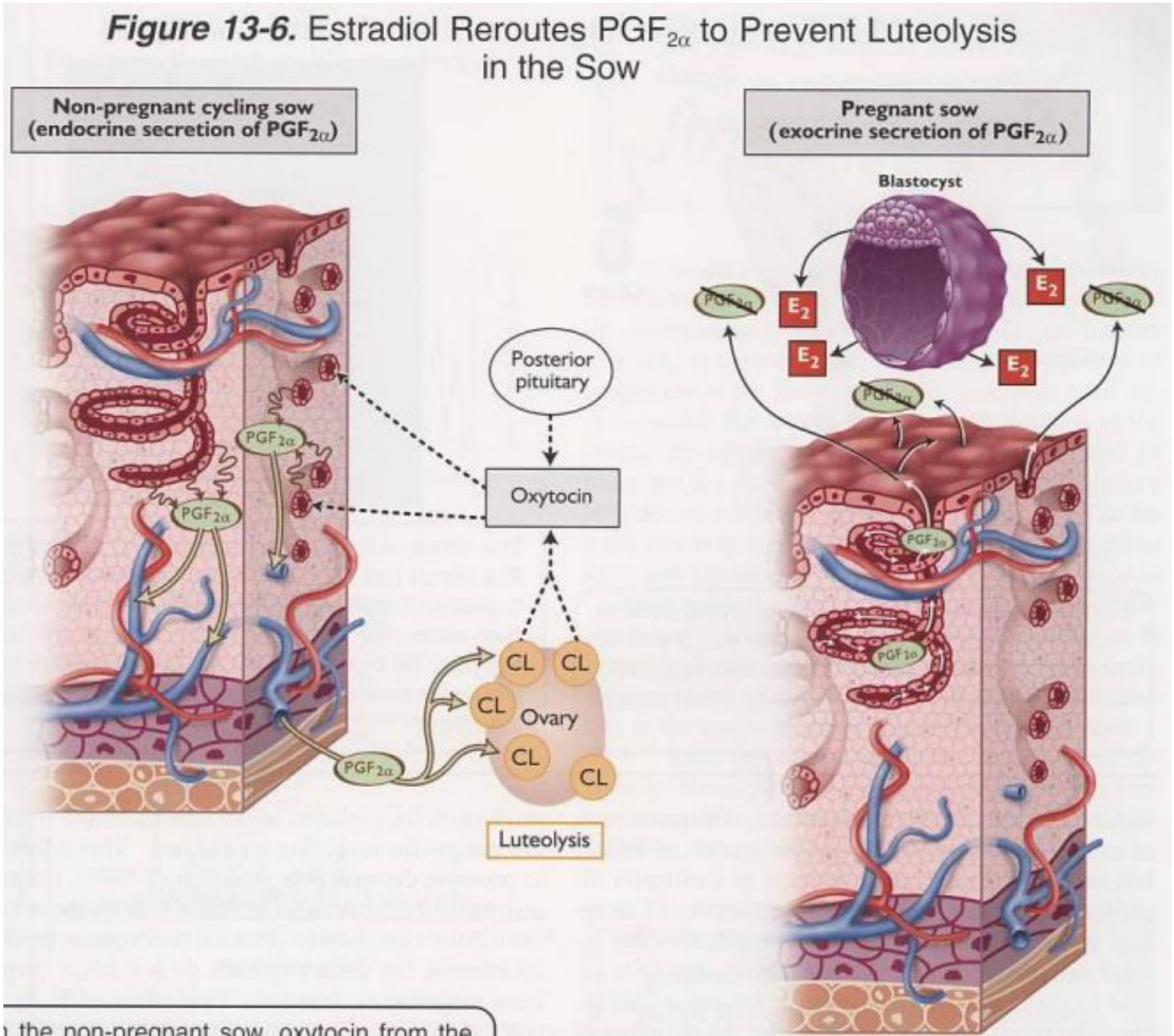
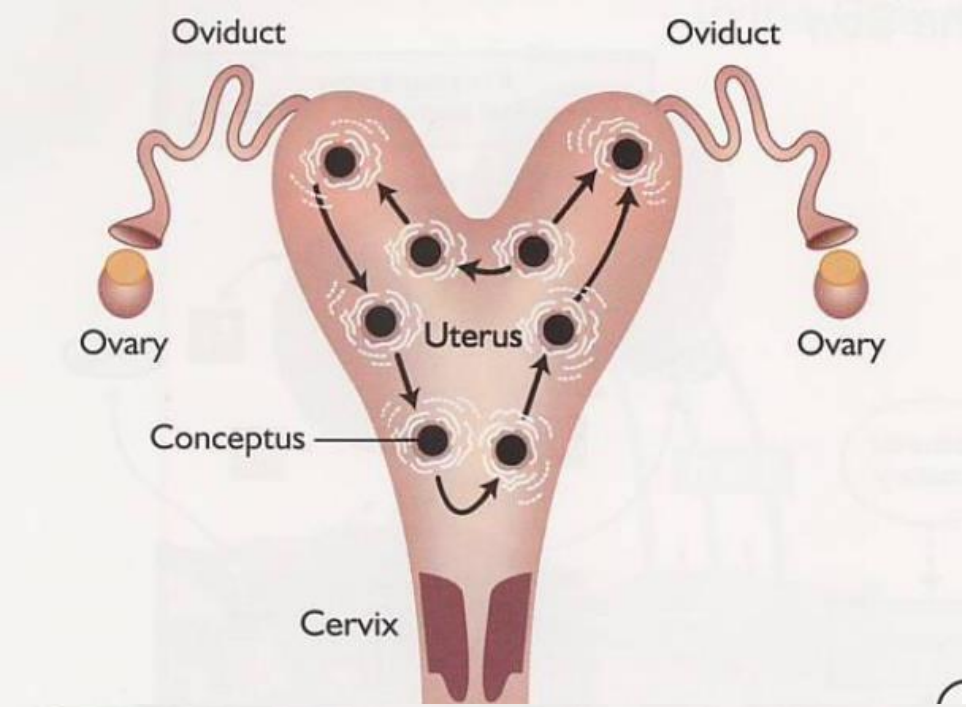


Figure 13-6. Estradiol Reroutes PGF2α to Prevent Luteolysis in the Sow
Non-pregnant cycling sow (endocrine secretion of PGF2α)
Pregnant sow (exocrine secretion of PGF2α)
Blastocyst
E2
E2
E2
E2
PGF2α
PGF2α
PGF2α
PGF2α
PGF2α
PGF2α
Posterior pituitary
Oxytocin
CL
CL
CL
Ovary
CL
CL
Luteolysis

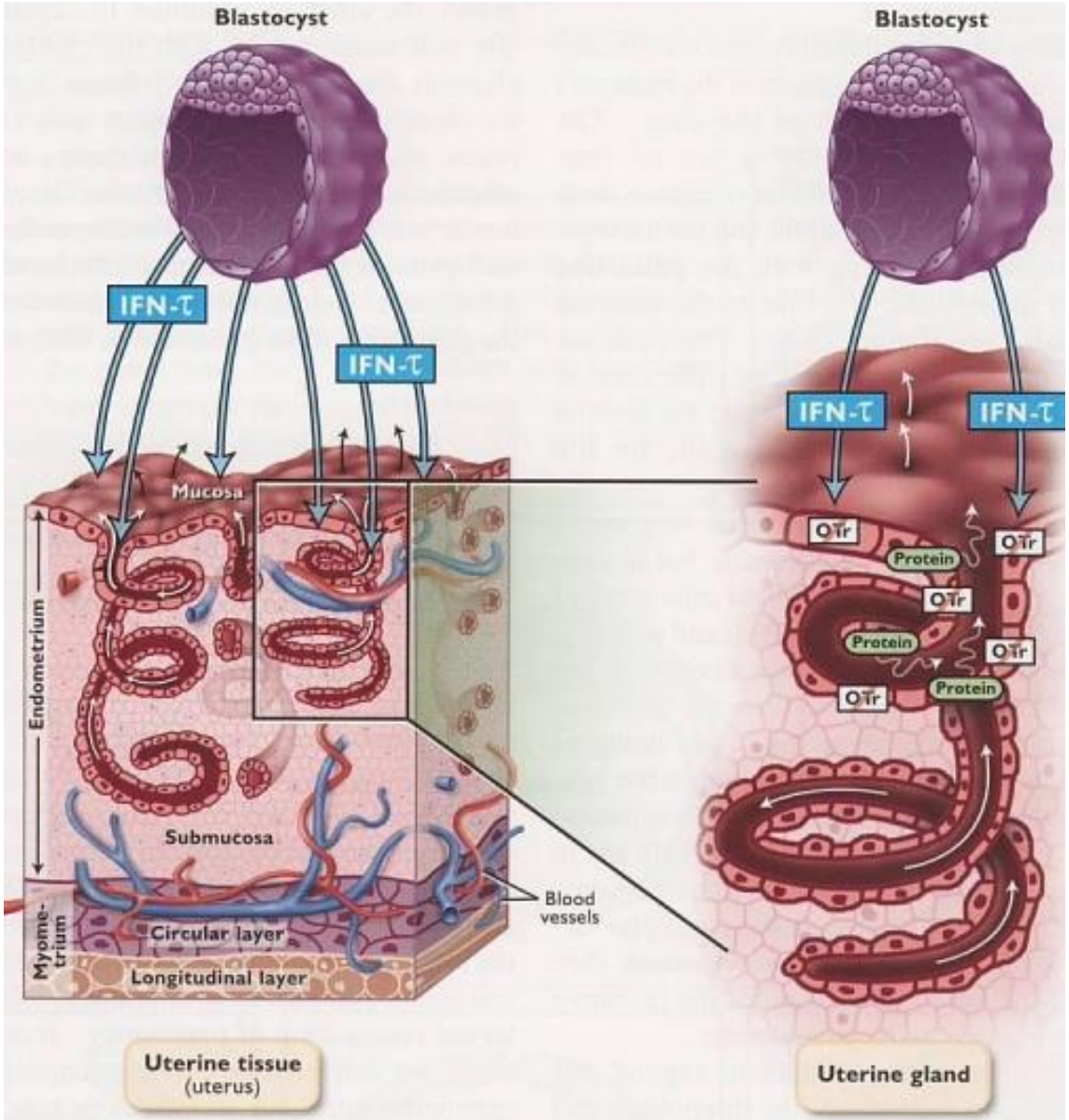
In mare, embryo migrates 12-14 times daily between days 10-14 which prevents the synthesis of PGF2α.



Each black sphere represents a "stopping spot" in which the conceptus will spend between 5 and 20 minutes. The migration of the conceptus probably distributes pregnancy factors (white lines) over a wide surface of the endometrium.

In cows, between days 16-17 bovine Trophoblastic Protein (bTP-1, Mol. weight 24kDa) produced by the embryo which prevents the synthesis of PGF2α from the endometrium of uterus. bTp- 1 shows immunological cross-reactivity with OTP-1 as well as having a high amino acid sequence homology with both oTP-1 and interferon-α (Interferon-α), it also possesses antiviral activity.

Bovine Trophoblastic Protein is also known as bovine interferon-τ (bIFN-τ). This exerts its antiluteolytic effect by modifying oxytocin receptors) thereby inhibiting the synthesis from Arachidonic acid and subsequent release of PGF2α.


Blastocyst
Blastocyst
IFN-τ
IFN-τ
IFN-τ
IFN-τ
Mucosa
Endometrium
Submucosa
Myome-trium
Circular layer
Longitudinal layer
Blood vessels
OTr
Protein
OTr
Protein
OTr
Protein
OTr
Uterine tissue
(uterus)
Uterine gland

Placental attachment.in ruminants is characterised by appearance of binucleated cells arising from uninucleated cells.of the trophoblast. Binucleated cells appear on the day 17 and are present throughout the gestation. These cells migrate and fuse with the underlying uterine surface epithelium to form multinucleated cells or a synctium. These synctium may be involved in immunologic protection of the conceptus and the transfer of the placental lactogen synthesized by the binucleated cells into the maternal vascular circulation.

Placenta

After attachment of fetus within uterus, it gets nutrition from the dam. For this specialized parts of fetus come in contact with uterus. The placenta is formed by the union of trophoblast with the endometrium. The main functions of the placenta are:

a). It protect the fetus from the external physical injury.

b). It stores the fetal waste products in the allantoic fluid.

c). It synthesise many enzymes and hormones which helps in maintenance of pregnancy.

d). It provide nutrition to the fetus from the dam.

In general placenta can be:

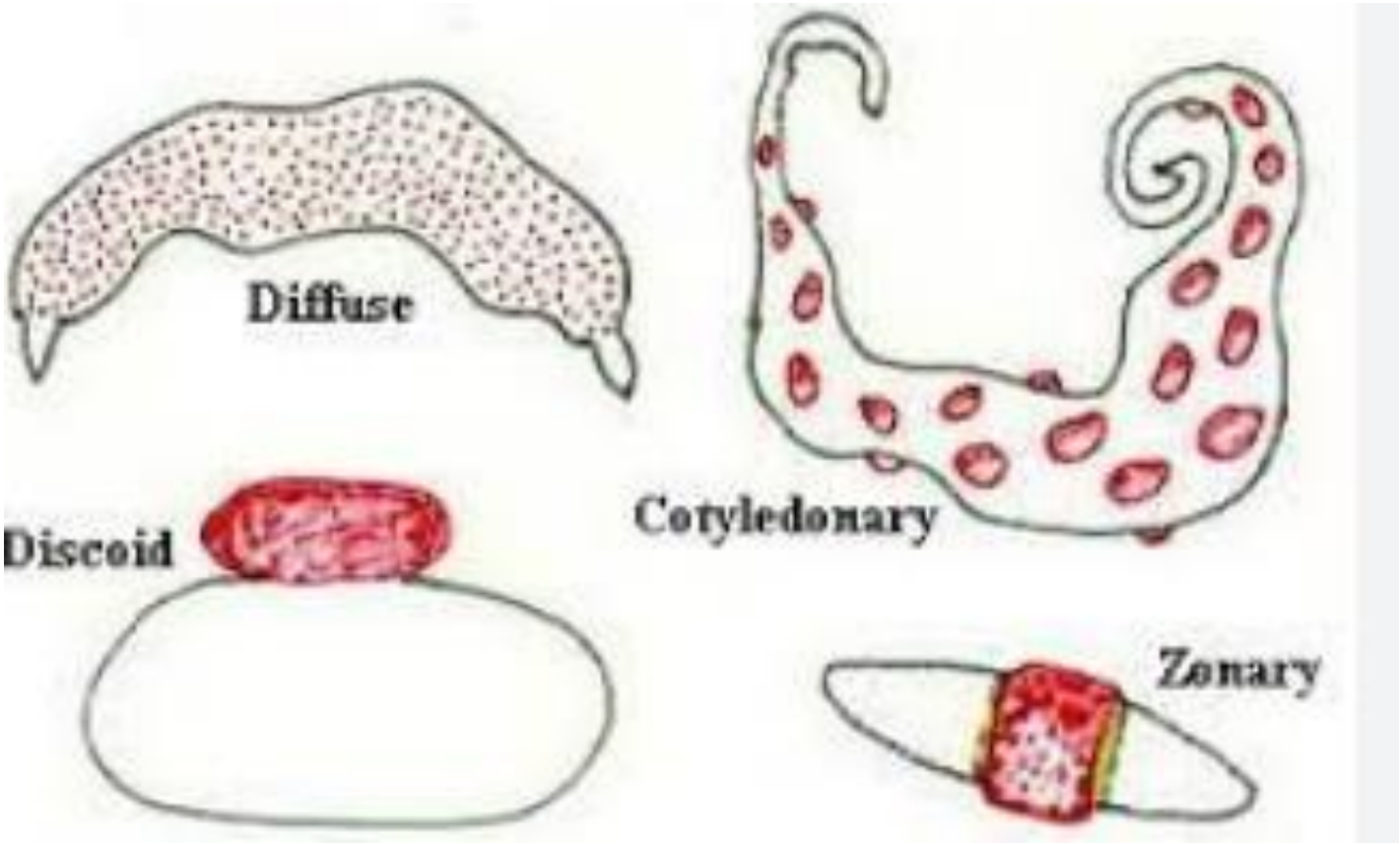
1. Deciduate: The attachment of trophoblast within the endometrium is deep and firm) At the time of expulsion of placenta, a part of endometrium is lost and there is bleeding. It is seen in bitch, cat, rodents and primates.

2. Non-Deciduate: It is observed in most of the domestic animals. The attachment of trophoblast is superficial, no part of endometrium is lost at the time of expulsion of placenta and no bleeding takes place.

On the basis of distribution of chorionic villi, the placenta can be:

1) Diffused: It is found in mare, sow and camel. Chorionic villi are present throughout the chorion and all the villi get attached with endometrium. The villi are absent in the area just adjacent to the cervix. This area is known as cervical star In Sow, the villi are present in a radiating manner and the circulating areas containing villi are known as Areolae. The areole are present opposite to uterine glands for absorption of nutrients. Uterine glands are devoid of any attachment.

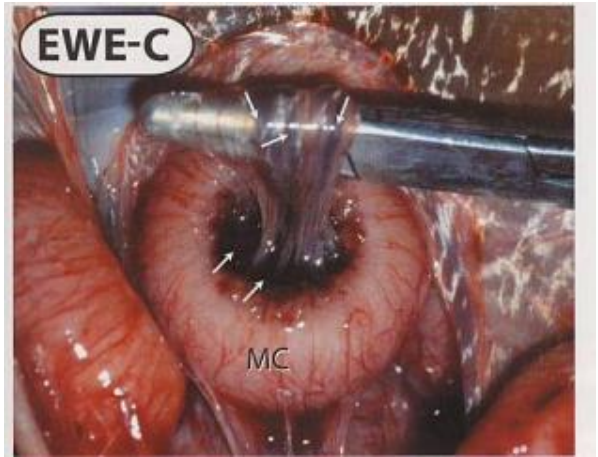
*In mare and sow, at the site of contact between allantochorion and endometrium, there is formation of multibranched interdigitations which are known as microcotyledonary placenta.

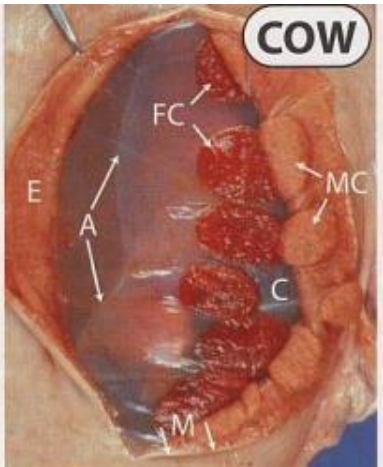

Diffuse
Cotyledonary
Discoid
Zonary

2) Cotyledonary/Multiple placenta/Placenta multiplex: It is seen in cattle, buffalo, sheep and goat. Initially, villi are present throughout the surface of chorion and later villi are restricted to opposite to caruncles and rest of the villi get degenerated.

These villi form cotyledons. These villi form cotyledons which get attached with caruncles and the union results into placentomes.

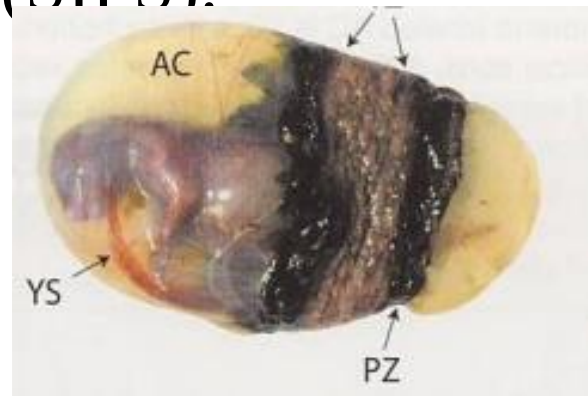
Placentomes are convex in cattle and concave in sheep.





3) Zonary: It is found in bitch and cat. The villi are present in zones in a diameter of 1-3 inches.

*In bitches, chronic haemorrhagic discharge after whelping due to failure of sloughing placental masses is called Sub Involution of Placental sites (SIPS).



4) Discoidal: it is found in human beings and monkey. The villi are present in an oval disc like structure.

Histologically, the placenta can be:

1). Haemochorial: It is observed in human being and monkeys. The chorion of fetus directly contacts with blood of uterus. Blood is lost at the time of separation of placenta in these species.

2). Endotheliochorial: It is observed in bitch and cat. The chorion of fetus is attached with endothelium of blood vessels of uterus.

3). Epitheliochorial: It is found in mare, sow, and camel. Chorion of the foetus is attached with the endometrium of uterus.

4). Synepitheliochorial/Syndesmochorial: It is found in cattle, buffalo, sheep and goat i.e in ruminants. Placental attachment in ruminars is characterised by appearance of binucleated cells arising from uninucleated cells of the trophoblast. These cells migrate and fuse with the underlying uterine surface epithelium to form multinucleated cells or a synctium.

Thank you