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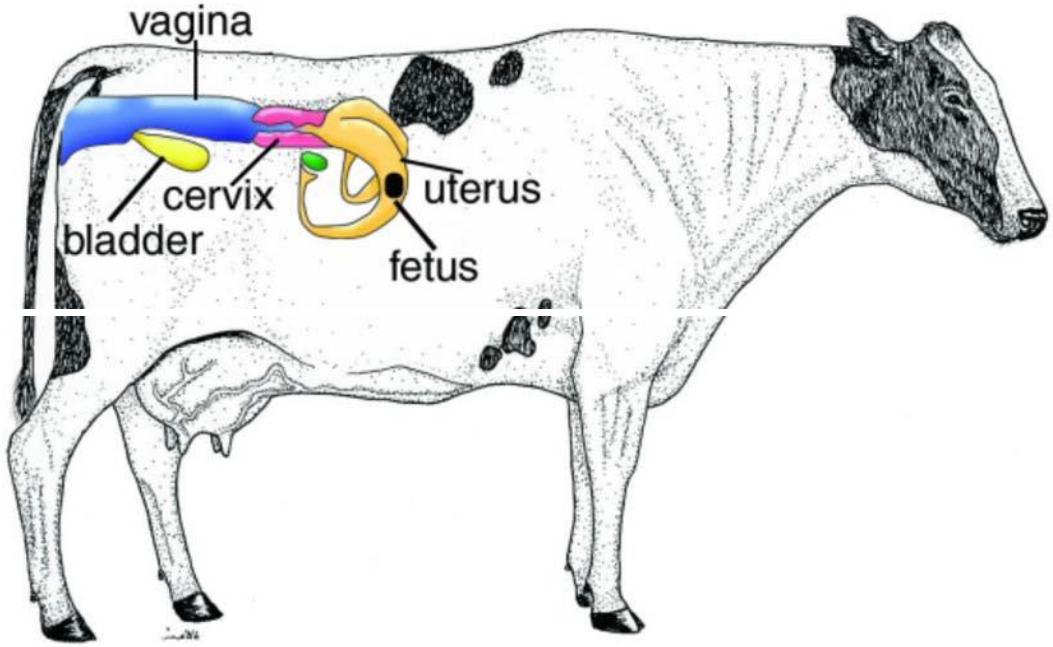
Animal Reproductive Physiology and Artificial Insemination



Reproductive Physiology and Artificial Insemination

Reproduction is the process by which animals produce offspring for continuing the species.
The process of reproduction begins with copulation, which is the mating of a male and female of the species.
Sperm cells from the male are deposited in the female reproductive tract and try to unite with an egg cell.

Fertilization is the union of **gametes** (the sperm and the ovum) to produce a **zygote** .

gametes are formed by **meiosis** . This means each contains only half the chromosomes of the body cells (**haploid**). the zygote contains full number of chromosomes (**diploid**). The zygote then starts to divide by **mitosis** to form a new animal with all its body cells containing chromosomes that are identical to those of the original zygote.

Fertility is the ability of animals to produce healthy offspring

fertility rate is the number of offspring born per mating.

Sterility or infertility is the inability animals to produce healthy offspring

Male reproductive system:

1- scrotum

Spermatogenesis need temperatures between 2 to 10 degrees Centigrade lower and then the body temperature to develop. This is the reason why the testes are located in a bag of skin called the **scrotal sacs** (or **scrotum**) that hangs below the body.

The function of scrotum is:

a- Thermal regulation of testes
b- Testes and epididymis protection

2- The Testes

The function of testes is:

1- production of the sperm

2- secretion of testosterone

Testes composed of:

a- seminiferous or **sperm producing tubules:**

A mass of coiled tubes in which the process of spermatogenesis was occurred.

b- (Leydig cell) : Cells lying between the seminiferous tubules called produce the male sex hormone **testosterone**.

c- Sertoli cell : special cell lining seminiferous tubules which nourish and protect sperm during spermatogenesis.

When the sperm are mature they accumulate in the **collecting ducts** and then pass to the **epididymis** before moving to the **sperm duct** or **vas deferens**.

3- Epididymis Storage site for maturation and nourishment of sperm.

4- Vas Deferens – Long tube connect epididymis to urethra .

The two sperm ducts join the urethra just below the bladder.

5- Urethra-passes through the penis and transports both sperm and urine.

6- **The penis:** The penis is found in protective covering (sheath) and is consists of connective tissue with numerous small blood spaces in it. These fill with blood during sexual excitement causing erection.

Dogs, bears, rodents have a special bone in the penis which helps maintain the erection. In some animals (e.g. the bull, ram and boar) the penis has an "S" shaped bend that allows it to fold up when not in use.

7- **glans penis** The end of the penis which is richly supplied nerves, that stimulated during copulation to induce ejaculation.

8- **Prepuce** is skin surrounding and protecting the head of the penis.

9- **Accessory Glands:** Three different glands may be involved in producing the secretions in which sperm are suspended, although the number and type of glands varies from species to species.

a- **Seminal vesicles** are absent in cats and dogs. When present they produce secretions that make up much of the volume of the semen, and transport and provide nutrients for the sperm such as fructose and vit c.

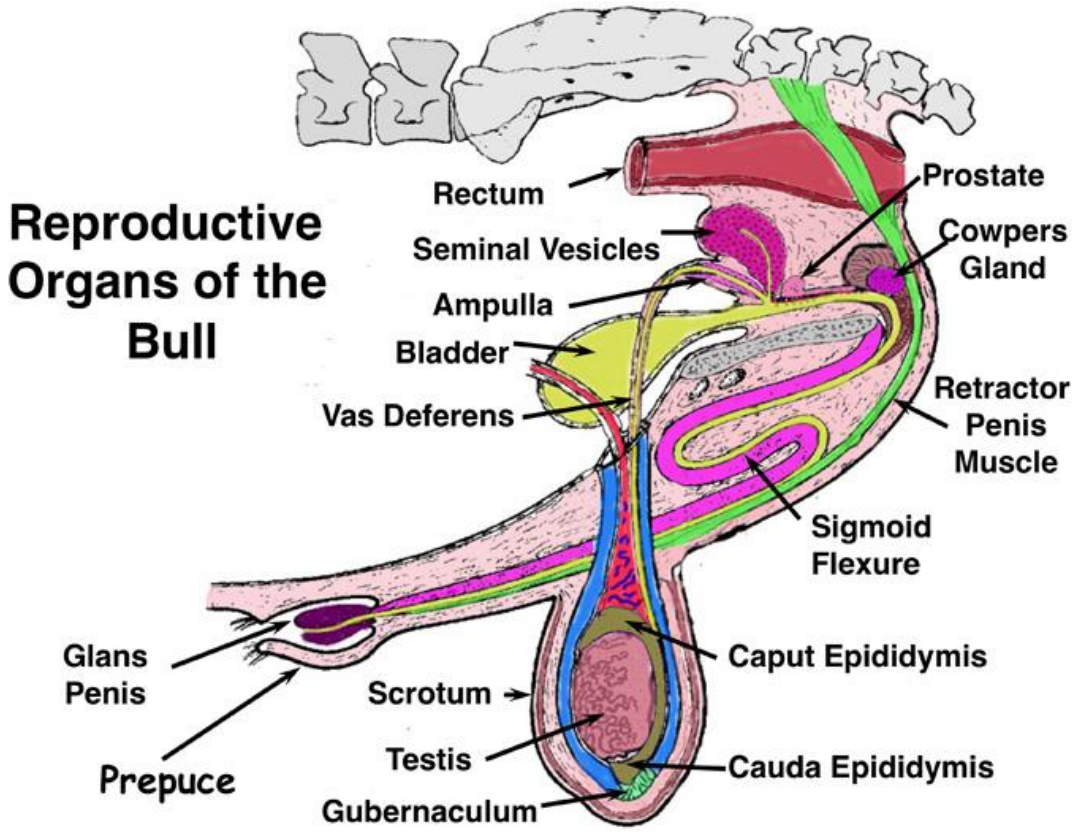
b- **prostate gland** is important in dogs and humans. It produces an alkaline secretion that neutralizes the acidity of the male urethra and female vagina.

c- **Cowper's glands** (bulbourethral glands) have various functions in different species. The secretions may lubricate, flush out urine or form a gelatinous plug that traps the semen in the female reproductive system after copulation .

Ejaculation discharges the semen from the erect penis. It is brought about by the contraction of the epididymis, vas deferens, prostate gland and urethra.

Semen

Semen is the ejaculated fluid which consists of 10% sperm and 90% fluid(from accessory gland) and as sperm pass down the ducts from testis to penis.



Female reproductive system

1- **Ovaries –** paired structures that produce eggs (ova) and the female hormones, estrogen , progesterone and relaxin.
2- **Oviducts (Fallopian tubes)** – paired tubes that transport the eggs from the ovaries to the uterus and sperm from uterus to the fertilization site in the upper third of oviduct.

Oviduct include:

a- **Infundibulum –** two funnel-like openings of the oviducts that pick up the eggs at ovulation and direct them to the body of the oviducts.
b- **Ampulla** upper part of oviduct where fertilization occur.
c- **Isthmus :** lower part of oviduct.

Broad ligaments : ligaments that support the female reproductive tract and arteries, veins, and nerves of the ovaries in the abdominal cavity.

3- **Uterus –** a major reproductive organ that consists of the uterine body and two uterine horns.

The embryo attaches to uterine body or uterine horn, depending on the species.
The uterus varies in shape between livestock species from long uterine
horns of the sow to relatively short uterine horns in the mare.

Functions of the uterus include:

• Passageway for sperm during copulation,
• Incubation and nourishment of the embryo during pregnancy, and
• Expulsion of the fetus during parturition by contractions.

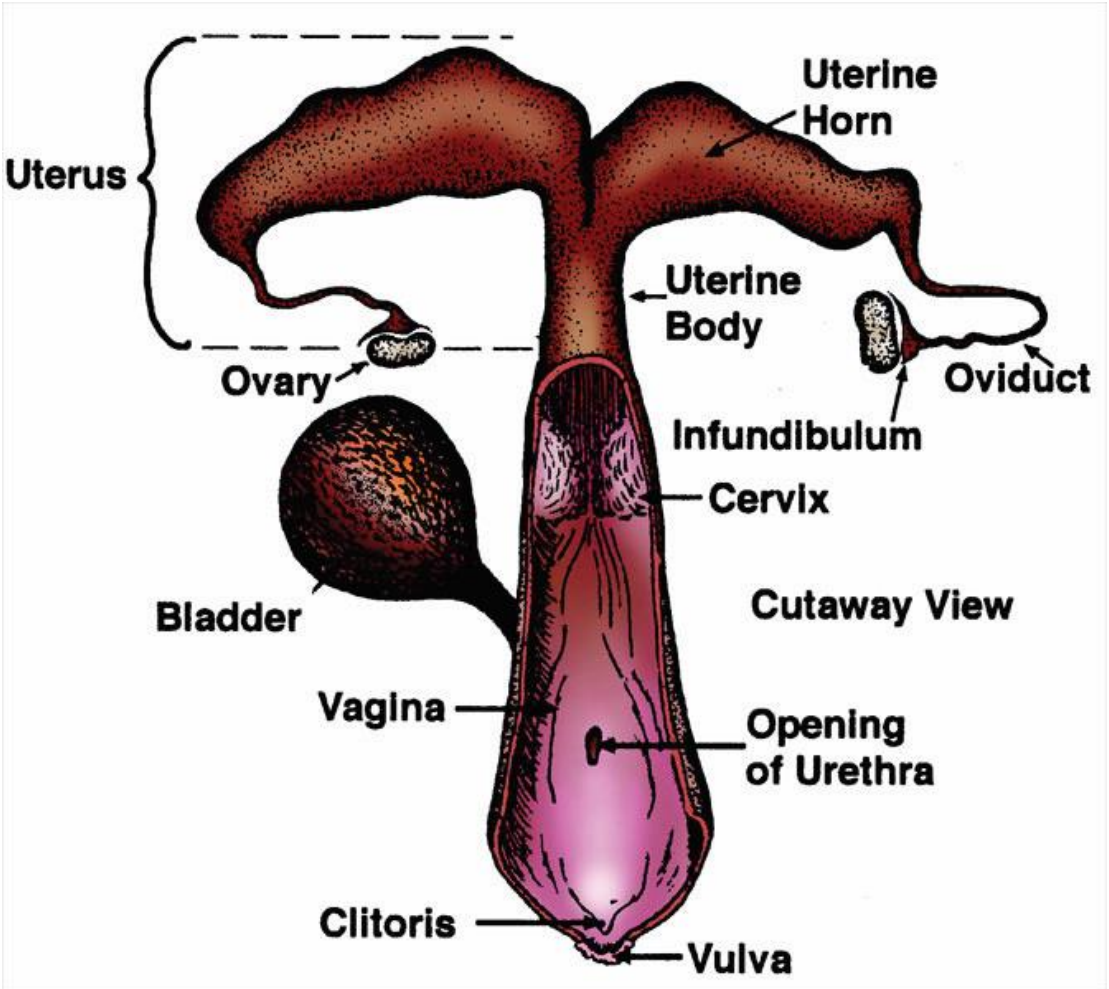
4- **Cervix** – a thick-walled mass of connective tissue with a small tube- like opening that joins the uterus to the vagina;

it serves as a passageway for semen during copulation and fetus during parturition. It also contains glands that secrete a waxy-like substance that seals off the uterus during pregnancy and between heat periods to protect against infection, disease, or foreign matter.

5- **Vagina** – reproductive structure that serves as the receptacle for the penis during copulation and the birth canal at parturition; it also serves as a passageway for expelling urine, as the urethra joins the bladder to the vagina prior to the opening at the vulva.

6- **Vulva –** the external portion of the female reproductive tract that serves to protect the internal system from infection, to initially receive the penis at copulation, and to act as a passageway for urine.

7- **Clitoris –** a sensory erectile organ just inside the vulva.



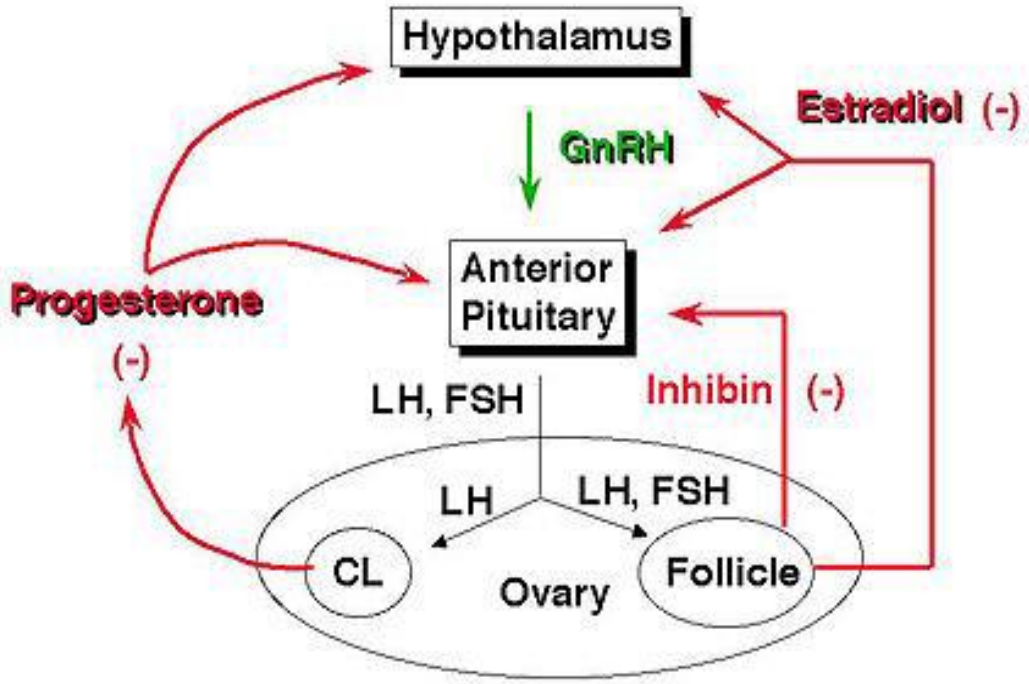
Endocrine glands and Reproduction

Endocrine glands are glands that secrete their products, hormones, directly into the blood without a duct. The major glands of the endocrine system include the pineal gland, pituitary gland, pancreas, ovaries, testes, thyroid gland, parathyroid gland, hypothalamus and adrenal glands.

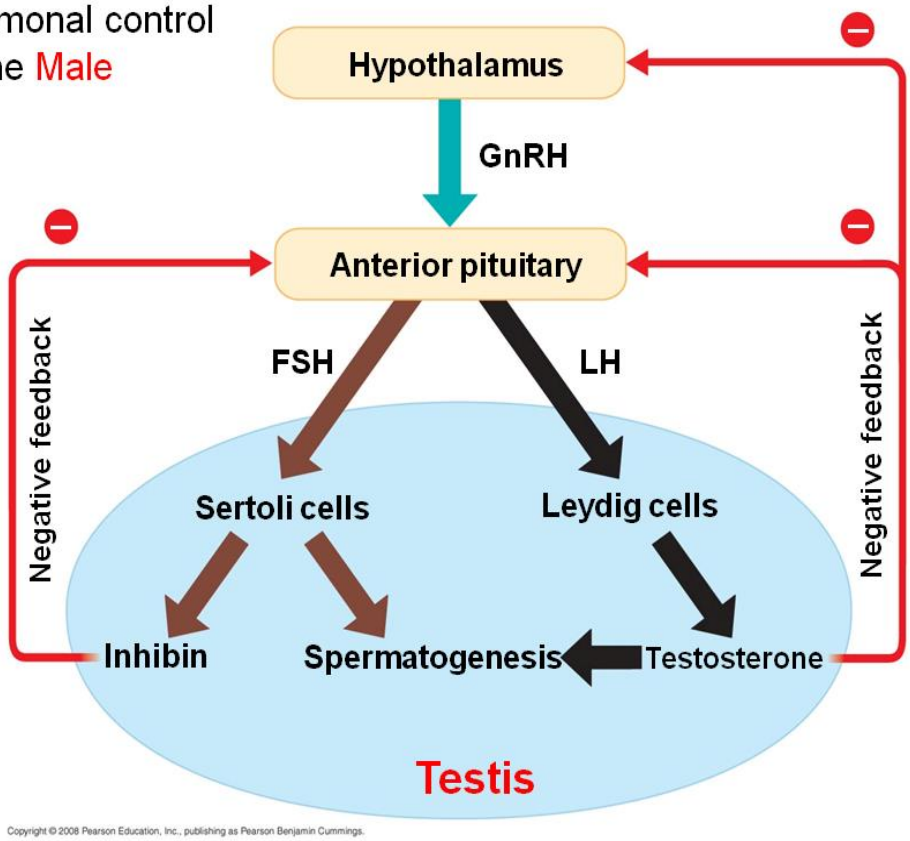
Hormones are chemical mediators produced by the endocrine glands that regulate the function of target organs in another part of the body.

Organs that produce reproductive Hormones are:

- **Pineal gland (photoperiod)** in seasonally reproductive animals such us sheep and mare.
- **Hypothalamus**
- **Pituitary gland**
- **Ovaries**
- **Testes**
- **Placenta**



Hormonal control in the Male



Functions of reproductive hormones in male and female:

- **Puberty**
- **Sperm and ova production and ovulation.**
- **Development and Maintenance of Accessory Sex Glands**
- **Secondary Sex Characteristics**
- **Libido**
- **lactation**
- **parturition**

Reproductive hormones and their origin and function

Gland	Hormones	Function
hypothalamus	Gonadotrophin-Releasing Hormone (GnRH)	Causes release of FSH and LH from anterior pituitary
Anterior Pituitary	FSH (follicle stimulating hormone)	1. Initiates follicular growth (oogenesis) 2. Influence spermatogenesis
	LH (luteinizing hormone)	1. Stimulates growth of tissues in gonads which secrete hormones 2. Acts with FSH to cause ovulation. 3. Causes corpus luteum formation
	Prolactin	1. Ovary: maintains functional capacity of the corpus luteum 2. Mammary gland: stimulates formation of milk in alveoli 3. Causes broodiness in birds
Posterior Pituitary	oxytocin	1. Uterus: stimulates contraction (birth, avian egg laying) 2. Mammary gland: initiates let-down of milk into ducts and cisterns
Ovary (Follicle)	Estrogen	1. Stimulates growth of accessory reproductive organs and secondary sex characteristics 2. Induces estrus (heat) 3. Mammary gland: stimulates development of the duct system
Ovary (Corpus Luteum) Placenta	progesterone	1. Inhibits release of FSH & LH 2. Prepares uterus for implantation 3. Maintains pregnancy 4. Mammary gland: develops alveolar system

Gland	Hormones	Function
Ovary (Corpus Luteum)	relaxin	Relaxes pubic bones and cervix (birth)
Testes	testosterone	1-Stimulates sexual desire 2. Stimulates growth of accessory reproductive organs and secondary sex characteristics
Uterus	Prostaglandin F2 α	Causes regression of corpus luteum in sheep, cattle and swine Contaction of uterus during parturition

Spermatogenesis

Spermatogenesis is the process of producing the male gametes(sperm).

Males start producing sperm when they reach puberty, which is vary among animals. Sperm are produced in large quantities (~200 million in human and 2-5 billion/ml in goat and sheep) to maximize the likelihood of sperm reaching the egg. Sperm are continually produced as males need to be ready to use the small window of fertility of the female.

Sperm production occurs in the testes of the male, specifically in the **seminiferous tubules**.
Sertoli cells is important in supporting and protection of the developing sperm,

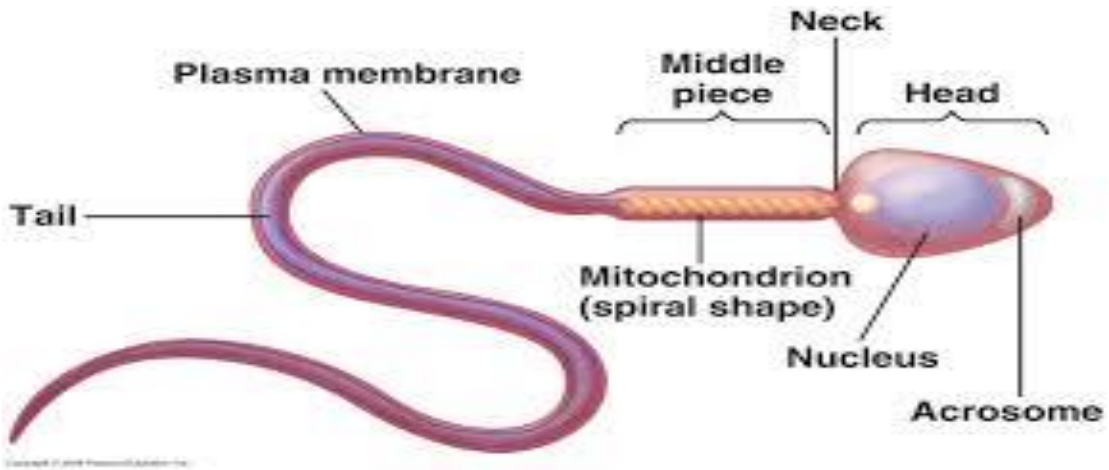
Spermatogonia are the initial pool of diploid cell that divide by mitosis to give two identical cells. One of these cells will be used to replenish the pool of spermatogonia – these cells are A1 spermatogonia. This replenishment of spermatogonia means that males are fertile throughout their adult life. The other cell – type B spermatogonium – will eventually form mature sperm.
Type B spermatogonia replicate by mitosis several times to form identical diploid cells linked by cytoplasm bridges, these cells are now known as primary spermatocytes. Primary spermatocytes then undergo meiosis.

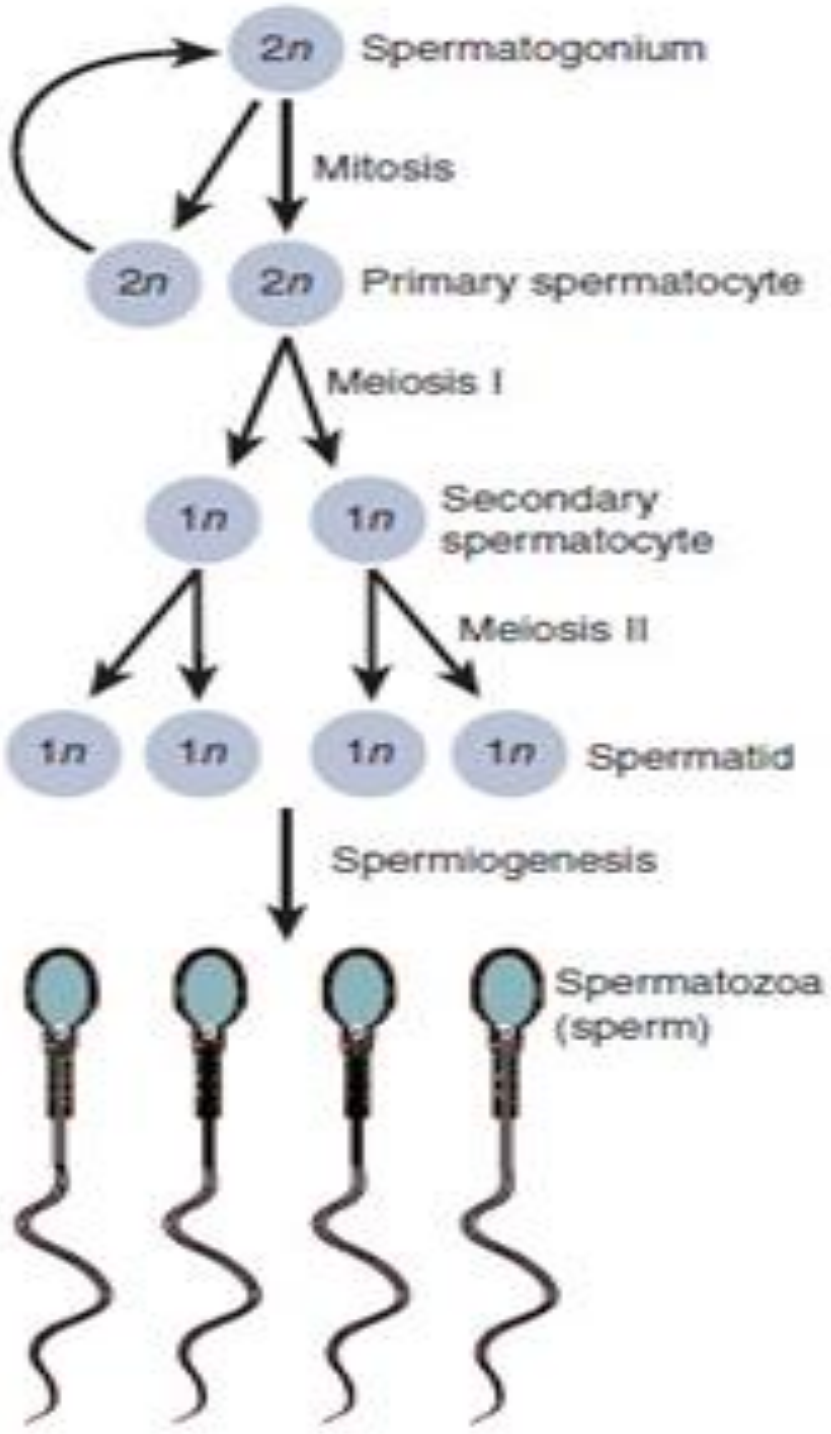
- **Meiosis I** produces two haploid cells known as secondary spermatocytes
- **Meiosis II** produces four haploid cells known as Spermatids

The cytoplasmic bridges break down and the spermatids are released into the lumen of the seminiferous tubule – a process called **spermiation**.

The spermatids undergo spermiogenesis (remodeling and differentiation into mature spermatozoa) as they travel along the seminiferous tubules until they reach the epididymis.
From the seminiferous tubule they travel to the rete testis, which acts to "concentrate" the sperm by removing excess fluid, before moving to the epididymis where the sperm is stored and undergoes the final stages of maturation.

Spermatogenesis takes approximately **70 days**, therefore in order for sperm production to be continuous and not intermittent, multiple spermatogenic processes are occurring simultaneously within the same seminiferous tubule, with new groups of spermatogonia arising every 16 days (spermatogenic cycle). Each of these populations of spermatogenic cells will be at different stages of spermatogenesis.
Note that once sperm leave the male body and enter the female reproductive tract, the conditions there cause the sperm to undergo **capacitation**, which is the removal of cholesterol and glycoproteins from the head of the sperm cell to allow it to bind to the zona pellucida of the egg cell.





Spermatogenesis

Oogenesis

Oogenesis is the process of producing the female gametes(ova).

Oogenesis differs from spermatogenesis in that it begins in the fetus prior to birth. Primordial germ cells (which originate in the yolk sac of the embryo) move to colonize in the cortex of the primordial gonad and replicate by mitosis to peak at approximately 7 million by mid-gestation (~20 weeks). Cell death occurs after this peak to leave 2 million cells which begin meiosis I before birth and are known as **primary oocytes**. Therefore, female is born with approximately 2 million primary oocytes arrested in meiosis.

The primary oocytes are arranged in the gonads in clusters surrounded by flattened epithelial cells called follicular cells and these form **primordial follicles**. The primary oocytes are arrested in prophase stage of meiosis I.

During childhood, further **atresia** (cell death) occurs, leaving ~40,000 eggs at puberty.

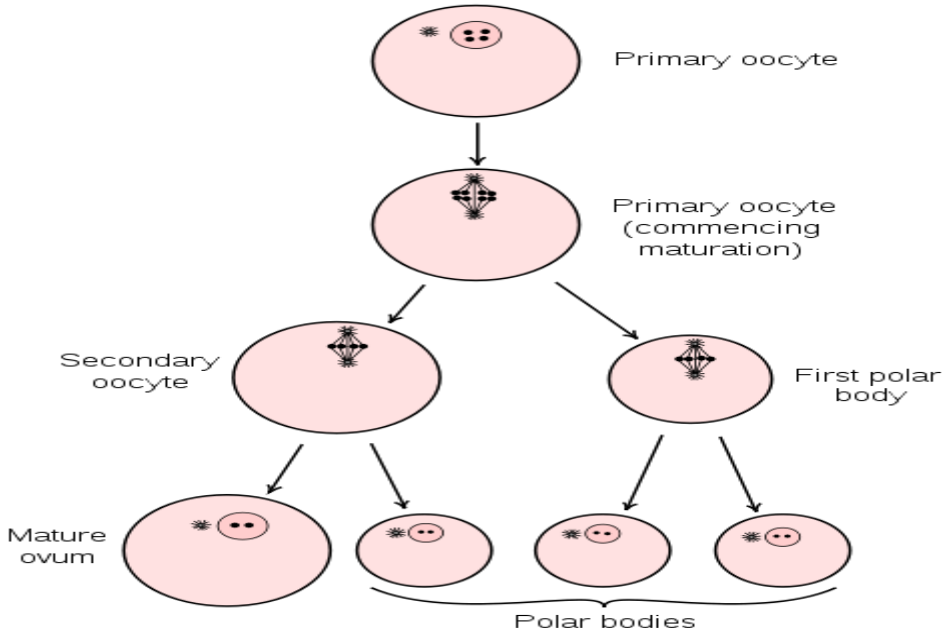
Once puberty begins, a number of primary oocytes (15-20) begin to mature each month, although only one of these reaches full maturation to become an oocyte.

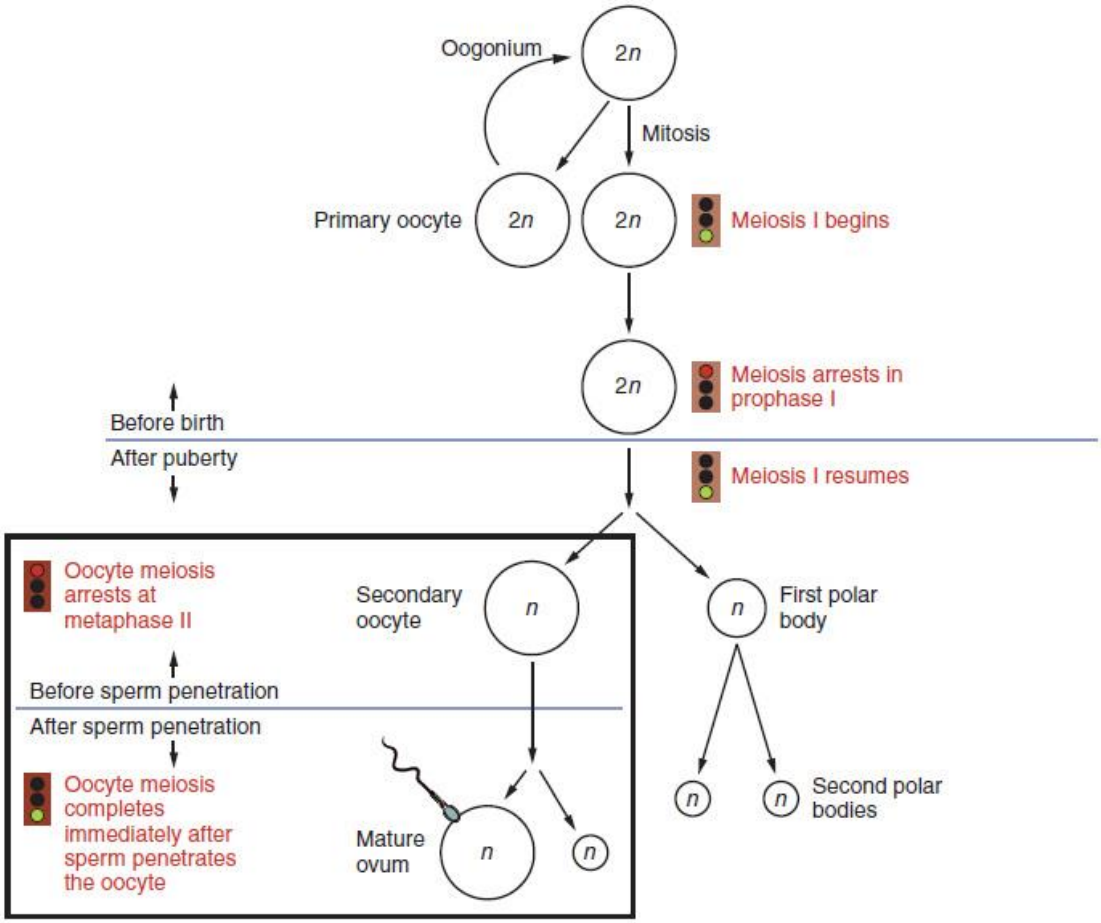
The primary oocyte completes its first meiotic division producing a Secondary oocyte and the first polar body. The Secondary Oocyte enters Meiosis II and is arrested at metaphase II. The secondary oocyte is diploid.

The oocyte enlarges due to an increase (x 50) in cytoplasmic volume. The oocyte is now 100-150 µm.

Ovulation occurs at this stage once the first meiotic division has been completed and secondary oocyte formed within the dominant follicle. The exception to this is in the case of the Bitch where ovulation occurs after the Primary Oocyte arrested in prophase I.

The secondary oocyte completes meiosis II once fertilization of the secondary oocyte by the sperm has occurred.


Primary oocyte
Primary oocyte (commencing maturation)
Secondary oocyte
First polar body
Mature ovum
Polar bodies


Oogonium
2n
Mitosis
Primary oocyte
2n
2n
Meiosis I begins
2n
Meiosis arrests in prophase I
Before birth
After puberty
Meiosis I resumes
Oocyte meiosis arrests at metaphase II
Secondary oocyte
n
n
First polar body
Before sperm penetration
After sperm penetration
Oocyte meiosis completes immediately after sperm penetrates the oocyte
Mature ovum
n
n
n
n
Second polar bodies

Folliculogenesis and ovulation

The primary oocytes undergo 3 stages:

- Pre-antral
- Antral
- Preovulatory

Pre-antral Stage

The primary oocyte grows dramatically whilst still being arrested in meiosis I. The follicular cells grow and proliferate to form a stratified cuboidal epithelium. These cells are now known as granulosa cells and secrete glycoproteins to form the zona pellucida around the primary oocyte. Surrounding connective tissue cells also differentiates to become the **theca folliculi**, a specialized layer of surrounding cells that is responsive to LH and can secrete estrogen under its influence.

Antral Stage

Fluid filled spaces form between granulosa cells, these eventually combine together to form a central fluid filled space called the antrum. The follicles are now called **secondary follicles**. In each estrous cycle one of these secondary follicles becomes dominant and develops further under the influence of FSH, LH and estrogen.

Pre-Ovulatory Stage

The LH surge induces this stage and meiosis I is now complete. Two haploid cells are formed within the follicle, but they are of unequal size. One of the daughter cells receives far less cytoplasm than the other and forms the **first polar body**, which will not go on to form an ovum. The other haploid cell is known as the secondary oocyte. Both daughter cells then undergo meiosis II, the first polar body will replicated to give two polar

bodies but the secondary oocyte arrests in metaphase of meiosis II, 3 hours prior to ovulation.

Ovulation

The follicle has grown in size and is now mature – it is called a **Graafian follicle** which is a blister-like mass on the surface of the ovary that contains a developing ovum and produces and stores estrogen.
The LH surge increases collagenase activity so that the follicular wall is weakened, this combined with muscular contractions of the ovarian wall result in the ovum being released from the ovary and being taken up into the fallopian tube via the fimbriae (finger-like projections of the fallopian tube).

Corpus hemorrhagicum – a small hemorrhage or blood-clotted area that develops at the site of a ruptured follicle and lasts 2 – 3 days.

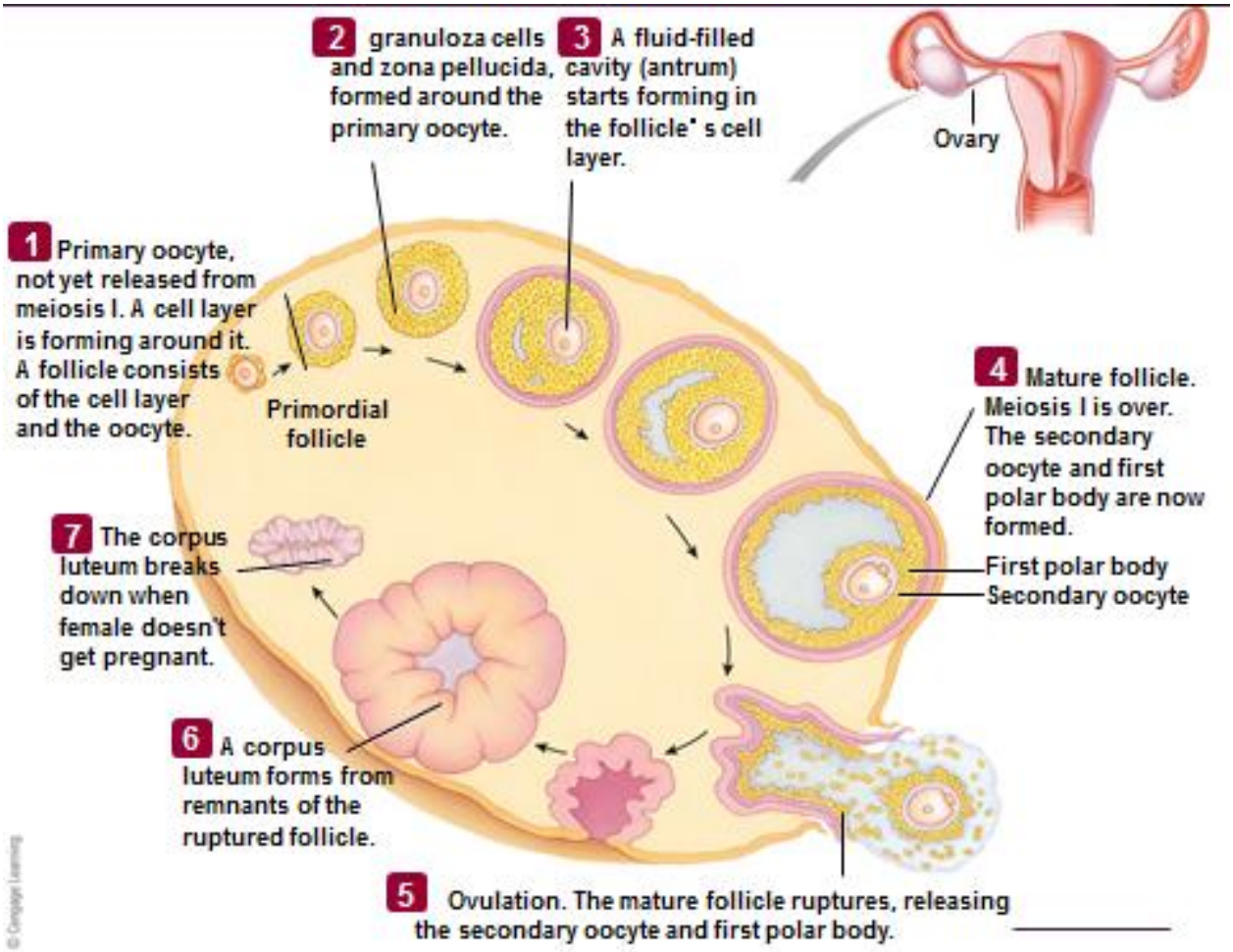
Corpus luteum – a yellow body of cells that develops in place of the corpus hemorrhagicum and produces progesterone.

Corpus albicans – a white body of connective tissue that is the result of the degeneration and re-absorption of luteal tissue.

Fertilization

The secondary oocyte will only complete meiosis II on fertilization, giving off a third polar body once meiosis II is completed and a fertilized egg. If fertilization never occurs, the oocyte degenerates 24 hours after ovulation, remaining arrested in meiosis II.

If the egg is fertilized however, the peristaltic movements of the fallopian tube move the egg to the uterus where it can implant into the posterior uterine wall.



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Estrous cycle

Estrous (heat): is the period during which the female will accept the male for mating.

Estrous cycle: The estrous cycle is measured as the time between two subsequent estrous periods. The estrous cycle begins with the ovulation of a follicle and ends with the ovulation of the next follicle.

Animals classified according to the frequency of estrous cycle into:

1- Poly estrous animals: Estrous cycles occur regularly throughout the year e.g. cow and sow and rodents
2- Seasonally poly estrous animals:
   a- Long day breeders: mare
   b- Short day breeders :sheep and goat.
3- Mono estrous animals: one or two estrous cycle per a year e.g. dog , fox ,wolves and bears.

Signs of Estrus:

1. Bellowing and aggressive behavior.
2. Swelling and reddening of the vulva.
3. Females will mount each other
4. clear mucus discharge from vulva.
5. sometimes simple bleeding.

Stages of the Estrous Cycle

1- **Proestrus** : follicle development and estrogen increases.
2- **Estrus**: sexual receptivity, ovulation occur in all animals except cow ovulation occur during metestrous.
3- **Metestrus**: initial development of corpus luteum and progesterone increase
4- **Diestrus**: mature phase of corpus luteum

Follicular phase = (proestrous+ estrous) =20% of estrous cycle

Luteal phase=(metestrous+ diestrous)= 80% of estrous cycle

Follicular phase mean that animals under influence of ovarian follicle.

Luteal phase mean that animals under influence of corpus luteum.

Anestrous : along period of sexual rest. The reproductive system is quiescent. The causes of anestrous are:

1- Pregnancy
2- Lactation
3- Non breeding season(in seasonally reproductive animals)
4- Diseases such as luteal cyst.
5- Nutritional deficiency
6- Stress factor.

	Cow	Ewe	Mare
Estrous cycle (day)	21	17	21
ovulation	12 hr. after end of estrous	Near end of estrous	5 day after onset of estrous
Proestrus (day)	3-4	2-3	2-3
Estrus (hr)	12-18	24-36	4-8
Metestrus (day)	3-4	2-3	2-3
Diestrus (day)	10-14	10-12	10-12

Puberty

puberty is the stage in which an animal becomes physiologically capable of sexual reproduction , that mean the production of gametes (sperm and ova) began in this stage accompanied with libido and ejaculation in male and estrous cycle in female.

Sexual maturity: the stage in which an animal become capable to fertilization , pregnancy and normal parturition that mean it reach full reproductive efficiency.

	Puberty(month)	Maturity(month)
cow	7-9	18
bull	9	24-30
ewe	5-10	12
ram	5-7	18-20
mare	12-24	36
stallion	10-14	24

Factors affecting puberty

1- Breeds
2- Species of animals : short lived animals matured earlier.
3- Sex: bulls and rams matured later than female due to delayed access to appropriate weight.
4- Environmental factors such as light and temperature.
5- Nutrition
6- Weight : weight gain accelerate maturity.
7- Health status: diseases delayed puberty
8- Genetic factors

Implantation

It is the process by which the Blastocyst penetrates the superficial layer of the endometrium of the posterior wall of uterus. It began at 6^{th} day and completed at $11\text{-}12^{th}$ day after fertilization.

Fertilization occur in the upper third part of oviduct.

After fertilization the zygote began to divide forming 2 ,then 4 the 8 then 16 cells and when reach 32 cells is called morula which formed in the third day of fertilization and reach uterus in fourth day to develop into blastocyst.

Placental or fetal membrane:

1- Yolk Sack
   - In birds to nourish embryo.
   - In mammal atrophies but source of blood cells and primordial germ cells.

2- Amnion
   - Non-vascular, fluid filled.
   - Fluid produced by fetus.
   - Protective cushion.

3- Allantois
   - Contain blood vessels and Brings blood vessels to chorion

- Fuses with chorion so called allantochorion or chorioallantois

4- Chorion

- Outermost membrane.
- Attachment to mother.

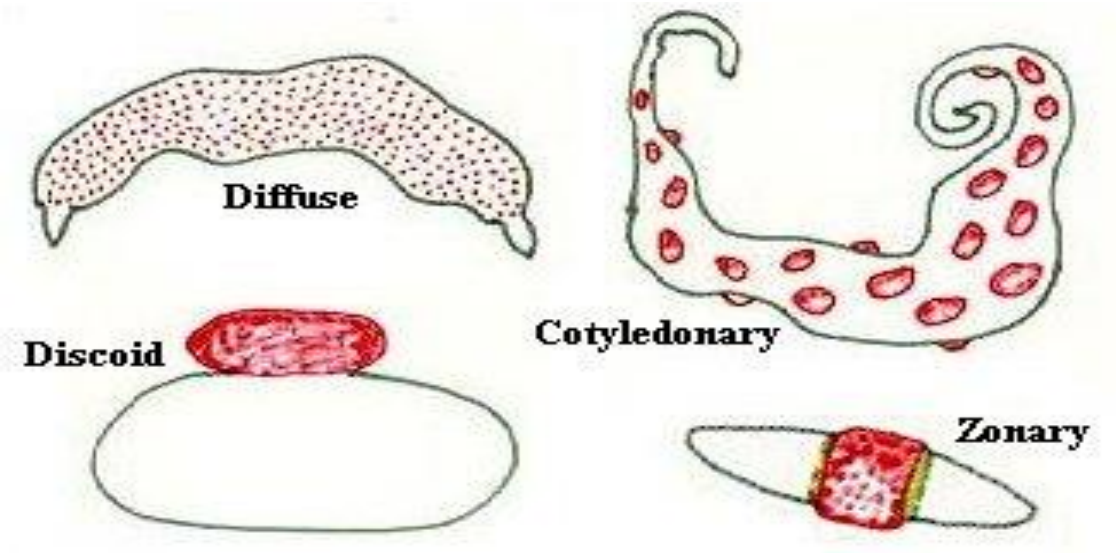
Classification of placenta according to shape and Contact Points:

1- **Diffuse**: Almost the entire surface of the allantochorion is involved in formation of the placenta. Seen in horses and pigs.

2- **Cotyledonary**: Multiple, discrete areas of attachment called cotyledons are formed by interaction of patches of allantochorion with endometrium. The fetal portions of this type of placenta are called cotyledons, the maternal contact sites (caruncles), and the cotyledon-caruncle complex a placentome. This type of placentation is observed in ruminants which is about 80-120 .

3- **Zonary**: The placenta takes the form of a complete or incomplete band of tissue surrounding the fetus. Seen in carnivores like dogs and cats, bears, and elephants.

4- **Discoid**: A single placenta is formed and is discoid in shape. Seen in primates and rodents.



Function of placenta:

1- Regulates the exchange of oxygen and CO2
2- Provide nutrients to the fetus
3- Waste materials away from the fetus
4- Antibodies between the fetus and mother.
5- Endocrine function(secretion of hormones).
6- Protect and cushions the fetus

Pregnancy diagnosis in cow

1- Economic Importance.
2- Detect pregnant animals for better feeding and management.
3- Early treatment of non-pregnant animals
4- To reduce calving interval
5- Increase milk production

Rout s of pregnancy diagnosis:

1- Breeding history
2- Cessation of estrous: 3-6% of pregnant cows may exhibit estrous during first trimester.
3- Development of udder : from 4-5 months in heifer and 7-8 months in pluriparous and older animals.
4- Change in temperament.
5- Relaxation of pelvic ligament.
6- Abdominal ballottement of fetus from 6 months onwards in lower right flank.
7- Fetal heart auscultation 6-7 months onwards (right flank).
8- Vaginal examination with vaginal speculum we see thick mucus discharge (pregnancy mucous seal) at external opening of cervix which is closed and the vagina dry and pale.
9- Laboratory method: the blood or urine progesterone level is used as indicator in this method, the test is conducted 21-24 days after insemination, but is give false result in following:
   a- Diestrous phase with corpus lutium actively produce progesterone.
   b- Embryonic death after 24 days of gestation.

      Recently available tests detect so called early conception factor (ECF) or pregnancy-associated glycoprotein in blood samples. They are reported to

detect the pregnancy-associated glycoprotein within 48 hours of conception.

Because of the high incidence of embryonic mortality this test should be treated only as an indication of conception. Pregnancy should be confirmed later by rectal or ultrasound.

10- Ultrasound method.

11- Rectal palpation: most practical, immediate method 35 (experienced vet).

In 55-60 days of gestation avoid rupture of amniotic vesicle and early embryonic death. The cervix used as a guide for detecting other structure of female reproductive system.

Fetal membrane slip by thumb and finger at 120 days(4 months) and the placentomes are large enough to palpate through uterine wall. After 90 days the fetus can be palpated except in 6-7 months become too deep in abdominal cavity due to increase in weight. After 7 months the fetus can be palpated due to increase in size and weight.

Gestation period in some animals

animal	**(day)**
cow	280
Mare and donkey	330-360
Sheep and goat	150
Buffalo	320
Dog and cat	60
camel	360

Artificial insemination:

Artificial insemination is the process by which semen can be obtained from male and then diluted and kept refrigerated (at 5 ° C) or frozen (at -79 ° C or -196 ° C) until it is placed in female during estrous.

Advantages of artificial insemination:

1- Prevents injuries to animals that can occur from natural breeding
2- Allows farmers to keep cows indoors and out of harsh climatic conditions
3- Avoids the spread of sexually transmitted infections
4- Increases bull efficiency—one ejaculation can be used on multiple cows
5- Promotes high quality genetics that allow farmers to get higher yields with fewer inputs.
6- Achieves a more uniform calf crop by having a shorter calving season
7- Is usually cheaper than natural breeding
8- Removes the risks of having to keep a bull on the farm

Rout of semen collection:

1- artificial vagina (AV): require the training of males, which is often time-consuming and could last for few weeks but the the semen collected is relatively clean and it is similar to the natural ejaculate .

2- electro-ejaculation(EE). The EE allows the collection of semen from males that are untrained and unable to mount females but give less sperm concentration.
3- vaginal collection vial (VCV) a glass vial inserted into the vagina of the female shortly before mating which also does not require the preparation of males.

Extender(diluent): are materials used to dilute semen so that it can be used to inseminate large number of female. The sperm concentration of bull semen is very high and when extended with appropriate extender, one single collection can be used to 1000 cows.

Properties of good diluent:

1- same osmotic pressure to the seminal plasma.
2- buffering capacity.
3- protect sperm from cold shock.
4- nutrient for sperm metabolism.
5- controlling microbial contamination.
6- protect sperm against freezing-thawing damages and preserve sperm viability without more reduction in fertility.

Common composition of diluents:

1- **Tris**
2- **Citric acid**
3- **Fructose**
4- **Alpha amylase**
5- **Glycerol**
6- **Lactose**
7- **Distilled water**
8- **Egg yolk**
9- **Penicillin and streptomycin**

Two ways of semen preservation

1. cooled semen-can use for 2-3 days

2. Frozen semen (at -196 °C) with liquid nitrogen can use for more than 100 years

Puberty

is manifested by the male through exhibition of libido and production of spermatozoa while the female exhibits estrous and ovulation.

Maturity: reproductive organs of male and female are fully grown or developed and animals can reproduce efficiently.

Factors affect puberty:

1- Species
2- Sex
3- Breed
4- Nutrition
5- Environmental factors
6- Diseases