CHAPTER-10

Basic Concepts of Animal Reproduction –

puberty, oestrus cycle, estrus length, gametogenesis (production of male and female gametes), ovulation, fertilization, embryogenesis, gestation and parturition

Objectives

- To understand the basic physiology of reproduction process in dairy animals
- To be acquainted with the reproductive cyclicity
- To recognize the process of sperm and oocyte formation, maturation, fertilization, pregnancy establishment and parturition

Introduction

Reproduction is one of the most important considerations determining the profitability of cattle production. Reproductive performance of a farm is reflected by the interval between two calving of a cow (Calving interval) and how many animals are culled for reproductive reason. These parameters are directly or indirectly influenced by certain parameters such as heat detection rate, conception rate and breeding period. Reproduction management, in broad sense, is manipulating all these contributory parameters to achieve a calving interval of 12-13 months with reproductive reasons contributing less than 8% in total culling of animals. Understanding the basics of animal reproduction would help in efficient reproduction management. In this chapter the basic physiology of animal reproduction is discussed.

Puberty

The term Puberty is defined as the achievement of the ability to reproduce. For the female, although the onset of sexual activity (in domestic animals) or first menstrual bleeding (in primates) is often used as the onset of puberty, When a male or female animal able to release gametes that have capacity to fertilize and characterized by showing the sign of estrus in females and by the presence of sperm **(**at least 50 millions of which >10% are motile**)** in the ejaculates in males has said to reached puberty.

At the onset of puberty, the circulating concentration of gonadotropins (GnRH) increases. Gonadotropin releasing hormone (GnRH) secreted from the hypothalamus. GnRH stimulates the anterior pituitary gland for the secretion of follicular stimulating hormone (FSH) and

Lutinizing hormone (LH). FSH and LH stimulate the gonad, which is ovary in female and testes in male for the growth and development of gamete (egg or sperm). After influence of FSH and LH on ovary and testes they start secreting hormone, estrogen in female and testosterone in male. Estrogen is responsible for the development of female sex organs and the sexual behaviour (e.g heat or estrus behaviour). Testosterone is responsible for the development of male secondary sex organs and secondary sexual characteristics. For the achievement of puberty and sexual maturity the secretion of these hormones in balance ratio, amplitude and the frequency of periodic impulses are necessary.

Age of puberty

The age of puberty is different in different animals. The following table is showing the age of puberty in different animals in normal conditions.

Animal species	Age of puberty (months)
Exotic Cattle	12 - 18
Zebu Cattle	18 - 24
Buffalo	24 - 30
Sheep	04 - 08
Goat	04 - 08
Mare (female horse)	12 - 24
Sow	06 - 10

The age of puberty is affected by many factors. Some important factors are as follows:

1. ***Hormonal****:* Estrogen and testosterone are also known as sex hormones. Disruption of the release of GnRH from the hypothalamus leads to affects in the release of FSH and LH and ultimately secretion of estrogen and testosterone from ovary and testis respectively. All these hormones are playing a very important role in female and male reproduction. Disruption or improper release of these hormones effects puberty.

2. ***Genetic Background*** –Breed of mother (dam), breed of bull (sire) that used in mating, within the breed, among different crossbred and pure breed have effect on age at puberty. Some of the good breed may reach at the age of puberty earlier than other breeds.

3. ***Nutrition*** – Ration offered to the growing animal plays an important role at the age of puberty. If growing animals are offered a balance ration as per the requirement regularly, the animal may reach puberty at proper time or may achieve puberty at a younger age. But the low quality feed or underfeeding causes slow in growth rate

that leads to delay in puberty. The onset of puberty is more closely related to body weight than age. So the attainment of a certain size is necessary for initiation of puberty in all species. For example, dairy cattle reach puberty when the body weight is 30-40% of the adult weight.

4. ***Environmental Factors*** – Temperature, humidity, rainfall, sunlight, etc. affects the fodder quality. So, animal may not get good quality fodder in all the season that hamper at the age of puberty. For example, in hot summer season the fibre content of forages increase. High fibre in the forage may not fulfills the nutrient requirement of growing animals and thus delayed in puberty. In hot summer season, animal have to maintain their body temperature by eliminating heat from their body. Animals release heat by radiation, convection, conduction, evaporation (sweating) and panting (Bird, Dog: increase respiration with protruded tongue).

Estrous cycle

After puberty the female enters a period of reproductive cyclicity called as estrous cycle, which continues throughout most of her productive life except during pregnancy. Estrous cycle is the rhythmic sexual behaviour pattern and is the period between one estrus to subsequent estrus. Estrus is the period of sexual receptivity and commonly referred as heat. Based on the frequency of occurrence of estrous cycles, animals are divided into poly estrus (exhibit frequent, periodic estrous cycles over the year e.g. cow, sow), seasonally poly estrus (exhibit periodic estrus cycles only during a particular season e.g. sheep, mare) and monoestrus (exhibit only one estrus cycle per year (e.g. wild animals). Based on the ovarian activity, the estrus cycle is divided into follicular or estrogenic phase and luteal or progestational phase. The estrus cycle can be divided into four stages viz. pro estrus, estrus, metestrus and diestrus.

Proestrus is an ill-defined period, which is characterized by growth of graffian follicle and increased production of estrogen. This is the period during which the animal prepares herself for mating. The entire system is in a stage of development and excitement. Estrogen is rising at this time and is primarily responsible for the changes observed in animals.

Estrus is a well-defined period and assumes greater significance as proper detection of estrus is very much essential for successful AI. This period is characterized by sexual desire and begins with first acceptance and ends with last acceptance of the male. Generally it lasts for 12 - 24 hours. The willingness to mate is referred to as “standing estrus”. During this period the animal shows several behavioral changes due to changing hormonal profiles and this behaviour helps in deciding the stage of heat and time of insemination. Most commonly observed symptoms of estrus in cows are standing to be mounted, mounting on others, appearance of mucus from vulva, restlessness, increased phonation, drop in

milk yield, reduced feed intake, frequent urination, vulva edema etc. Not all these signs are exihibited by a singly cow and there is lot of individual variations in expression of heat. Similarly within the estrus period different behaviour are expressed at different times. For convenient, estrus period can be again divided into early heat (coming to heat), mid heat (standing heat) and late heat (going out of heat). During early heat, which lasts for around 8 hours, the animal stands, bellows, smell other cows and attempts to ride on other cows but will not stand to be mounted. Vulva is moist, red, slightly swollen and may have clear watery discharge. The mid heat, which lasts for approximately 18 hours, is characterized by standing to be mounted, frequent bellowing, highly excitedness and sometimes ride on other cows. There is clear, roapy mucus discharge from vulva. The second half of this period is the optimum time for breeding the animal. In the late heat, the animal will not stand to be ridden but attempts to mount, smells the other cows and may have mucus discharge from vulva.

Metestrus is the transitional period between ovulation and full development of corpus luteum and lasts for 3 - 5 days. In cows, capillary haemorhage occurs in the uterus and is excreted along with mucus. This phenomenon is called as post estrual or metestrual bleeding. There is no correlation between metestrual bleeding and conception.

Diestrus is the longest period of estrous cycle characterized by maturation of corpus luteum. During this period under the influence of progesterone, hypertrophy of endometrial gland and increased thickening of endometrium occurs for nourishing the embryo. The cervix is closed, vaginal mucus is scanty and vaginal mucus membrane is pale. Females is diestrus do not display sexual receptivity.

Oestrus cycle in dairy animals

Species	Length of estrous cycle	Proestrus	Estrus	Metestrus	Diestrus	Average length of estrus	Time of ovulation
Cow	21 days (Poly estrus)	3 - 4 days	12 - 24h	3 - 5 days	10- 14 days	18h	10 - 12h after the end of estrus
Buffalo	21 days (Poly estrus)	2 - 3 days	5-27h	3 - 5 days	10- 14 days	20h	33-40h after onset of estrus
Doe	21 days (Poly estrus)	2 - 3 days	24 - 48h	3 - 5 days	10- 14 days	30h	Near end of estrus

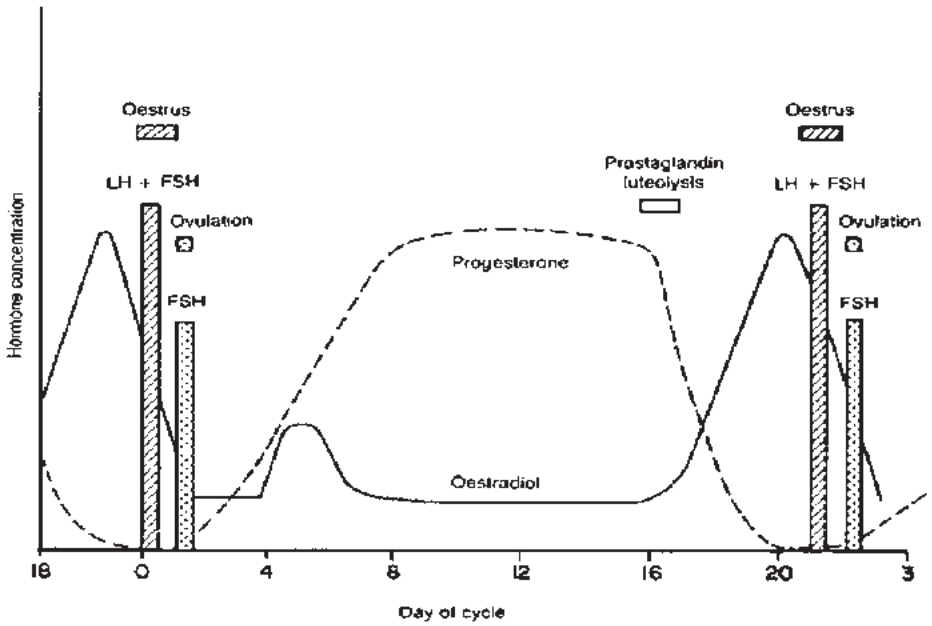


Fig: schematic diagram showing the level of oestrogen, progesterone and other related hormones in respect to the oestrous cycle.

Gametogenesis

The first phase in the sexual reproduction of an organism is gametogenesis. A process of formation of gametes from the germ cells in the testes and ovaries is known as gametogenesis. This process is termed as spermatogenesis in the male and oogenesis in the female. It is the fundamental biological process in both the sexes and the key event of gametogenesis is the reduction of the number of chromosomes (2n) to produce haploid (n) germ cells (sperm and oocytes). This haploid (n) chromosome number in the germ cell is achieved through meiosis. (For example, in goat, the chromosome number of somatic cells is 60 (2n), each sperm and each oocyte has only 30 (n) chromosomes). The formation of primary spermatocytes and primary oocytes, the spermatogenesis and oogenesis are similar. After this each primary spermatocyte divides meiotically and produces four spermatids, each destined to become a functional sperm. In female, on the other hand each primary oocytes divides meiotically and produces four cells. But the division of cytoplasm is unequal. Only one cell receive greater portion of cytoplasm and other three cells receive lesser cytoplasm which are known as polar body. The cell which received higher portion of cytoplasm and in bigger size give rise to functional oocytes also known as ovum. So, from each primary oocytes divides meiotically and produces only one functional oocytes (ovum) and the polar bodies were extruded from the cytoplasm (oolema).

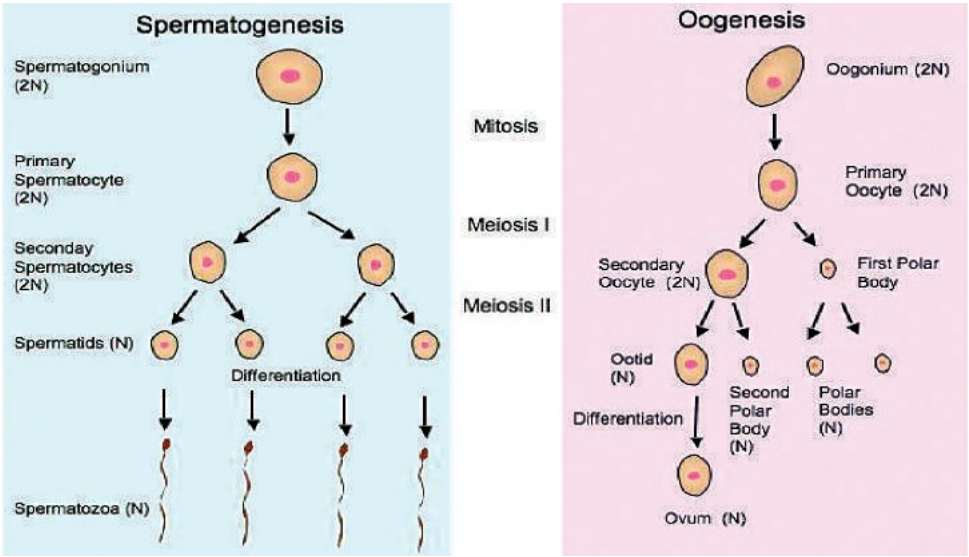


Fig: schematic diagram showing the process of spermatogenesis and oogenesis.

Sperm production or spermatogenesis

Spermatogenesis is a complex process of producing sperm with half number of chromosomes (haploid) as somatic cells. Spermatogenesis takes place within the seminiferous tubules and eventually involves cell division and differentiation. Within the seminiferous tubule, germ cells progress first from the diploid to haploid state and then change the shape (metamorphosis) to become spermatozoa. The sertoli cells, support the germ cells, provide environment for germ cells to develop and secretes substances required for spermatogenesis. The entire process of spermatogenesis can be divided into three portions, spermatocytogenesis (proliferate phase), meiotic phase (production of haploid gamete) and spermiogenesis (differentiation phase or metamorphosis phase).

Spermatocytogenesis is the proliferative phase in which primitive germ cell are multiplied by a series of mitotic divisions. This phase begins with the mitotic division of spermatogonia. The spermatogonia are activated to divide and one or two divisions occur to maintain their population in a stem cell pool of the cells resulting from these mitotic divisions. Some spermatogonia stays in the “resting pool” while the remaining spermatogonia proliferate several times. Most of the type A spermatogonia divide to form intermediate spermatogonia, which then divide to form type B spermatogonia. From the type B spermatogonia primary spermatocytes are formed by mitotic division and this ends the process of spermatocytogenesis.

Meiotic Phase is the second phase of spermatogenesis, which involves primary and secondary spermatocytes. The primary spermatocytes undergo the first of two divisions that constitute meiosis. Division of the secondary spermatocytes completes meiosis and produces the haploid spermatids.

Spermiogenesis is characterized by the nuclear and cytoplasmic changes in the spermatid so that a spherical undifferentiated spermatid undergoes a remarkable transformation that results in fully differentiated, highly specialized spermatozoa. Spermiogenesis consists of four phases namely Golgi phase, cap phase, acrosomal phase and maturation phase.

Spermiation is the process by which the sperm cells embedded in the sertoti cells are released into the lumen of the seminiferous tubule for transport out of the testis.

Oogenesis or ovogenesis

The process of formation and maturation of the female gamete (the oocyte) is known as oogenesis. Oogenesis in mammals includes seven steps:

(a) Generation of primordial germ cells (PGC).

(b) Migration of PGCs to the prospective gonads.

(c) Colonization of the gonads by PGCs.

(d) Differentiation of PGCs to oogonia

(e) Proliferation of oogonia

(f) Initiation of meiotic prophase or prophase1.

The migrated germ cells in the ovary multiply and form primary oocyte. These are surrounded by a layer of follicular cells. The oocytes together with these follicular cells are known as primary follicles. The new born female calf has approximately 1,50,000 primary follicles in the ovary which decline to 75,000 by spontaneous degeneration in a 3 month old heifer. The cells lining the follicle multiply during estrous cycle and eventually a fluid filled cavity is formed around the oocyte. A layer of cells immediately surrounded the ovum (oocyte) which is connected by a bridge-like cellular growth known as the cumulus oophorus to the follicular cell layers.

At birth, all the oocytes from growing and dominant follicles are arrested at the diplotene stage of prophase 1. This dictyate stage is characterized by the enclosure of the chromosomes within the large nucleus, also known as Germinal Vesicle (GV). The oocytes remain in the arrested state until a few hours before ovulation. Ovulation is the process of release of oocyte from the dominant follicle known as grafian follicle. The infundibulum portion of the fallopian tube will pick up the released oocytes from ovary. The oocytes along with the oophorus cumulus cells move in the fallopian tube by ciliary movement and the contraction of the fallopian tube. When the oocytes come in contact with sperm, the oocytes stimulated from the arrested state. Then undergo the completion of meiosis division from diplotene stage of prophase1 and give rise to ovum and polar body. The polar body is extruded away from the cytoplasm.

Ovulation

Ovulation is the process of releasing ova from the Graafian follicle. The process of ovulation is a gradual one. The oocytes were covered by the granulosa cells and formed primodial follicles. The granulosa cells grow and multiply around the oocytes which give rise to primary and growing follicles. The primodial and primary follicle does not contain any fluid inside the follicle. When the granulose cells in the follicles starts secreting fluid by the influence of FSH, give rise to antral follicle. As the follicular secretion increase the follicular size also increases. Continuous secretion of follicular fluid by granulose cells leads to acute bulging of the follicle with thinning of its wall at the point of bulging. The surge of LH causes contraction of smooth muscles of ovarian stroma leading to slow rupture of follicle and extrusion of ovum. In multiparous species more than one ova (oocytes) are released over a few hours during each cycle. However, in uniparous animals like cattle only one follicle matures and release during most of the cycles.

Ovulation occurs at the end or a little after the end of oestrus (desire) in all the animals. Based on the physiological mechanisms, ovulations are of two types. Spontaneously and induced ovulation. Species like Cat, rabbit, camel require mating stimulus for ovulation (ovulation fails in absence of mating stimulus) are known as induced ovulation. The cycle length in these animals differs depending on whether mating has taken place or not. In most of the farm animals' ovulation takes place during each cycle irrespective of whether coitus has taken place or not. Hence they are known as spontaneous ovulators. Even in these animals, stimulation of the genital tract has been found to hasten ovulation. The mating stimulus is important for timely ovulation. In all the species, oxytocin released from the posterior pituitary due to the stimulus of copulation.

Oocyte transport

When the oocytes (surrounded by cumulus oophorus cells) released from the graafian follicles (ovulation) pick up by the funnel like structure (infundibulum) of the oviduct (fallopian tube). The oocyte with the surrounding cumulus mass is swept into the oviduct by cilia present in the wall of oviduct. The oviductal contraction also helps in the movement of oocytes to the site of fertilization, i.e. the ampullary isthamus junction of oviduct.

Transport of spermatozoa

The spermatozoa are ejaculated as a mature cell. The bull, ram and man ejaculates on the face of the cervix and the cranial vagina. Millions of sperms or spermatozoa are ejaculated at the site of female reproductive tract. The ejaculated sperm have to move all the way of female reproductive tract to the site of fertilization. During mating or artificial insemination, oxytocin releases from the posterior pituitary by the reflex action in response to genital stimulation. This causes contraction of female genital tract. These musculature contractions

propel the sperms towards the oviduct. The fluid present in the uterus helps the sperms to swim. During the movement of spermatozoa in the tract, numbers of spermatozoa die due to phagocytosis and other barriers in female reproductive tract. Abnormal spermatozoa do not reach to the site of fertilization. During travelling of spermatozoa in reproductive tract, physiological and biochemical changes occurs in the spermatozoa, the process is known as capacitation. This change makes the spermatozoa able to fertilize the oocytes.

Capacitation: The spermatozoa must reside a minimum period in the female reproductive tract before gaining the ability to fertilize oocytes. It is speculated that during this time, glycoproteins from the sperm surface are removed, thus exposing receptor sites that can respond to oocyte signals and lead to acrosome reaction. This process allows the spermatozoa to bind to the ZP (zona pellucida), penetration the ZP and fertilize the oocyte. In another word, the spermatozoa gain the capacity to fertilize the oocyte and therefore, it is termed as capacitation.

Acrosome reaction: During fertilization, a sperm must first fuse with the plasma membrane and then penetrate the ovum to fertilize it. Fusing to the ovum usually causes little problem, whereas penetrating through the egg's hard shell can present more of a problem to the sperm. Therefore sperm cells go through a process known as the acrosome reaction which is the reaction that occurs in the acrosome of the sperm as it approaches the ovum. The *acrosome* is a cap-like structure over the anterior half of the sperm's head. As the sperm approaches the zona pellucida of the egg, which is necessary for initiating the acrosome reaction, the membrane surrounding the acrosome fuses with the plasma membrane of the oocyte, exposing the contents of the acrosome. The contents include surface antigens and numerous enzymes which are responsible for breaking through the egg's tough coating and allowing fertilization to occur.

Fertilization

The ovulated oocytes is surrounded by cumulus oophorus cells, zona pellucida and vitelline membrane. When the spermatozoa reach the oocytes, it has to cross all these barriers.

As the sperm approaches the ovum, it has been prepared by capacitation so the acrosome now is capable of releasing enzymes to digest the bonds between the protecting cells of the cumulus oophorus and corona radiate. The major enzyme is hyalurnidase, but numerous other hydrolytic enzymes are present. The sperm penetrates the matrix of the outer cell mass, and as the sperm approaches the zona pellucida, enzymes of the inner acrosome membrane permit penetration. The sperm forms a channel through the zona and passes into penetration thus far. Chemical reactions between the product of the sperm and the zona pellucida cause a sealing to prevent penetration by other sperm. This is called the zona reaction. This thin membrane is the last barrier to penetration of the ovum. The sperm head lies flat against the vitelline membrane and fuses with it as the membrane

and vitellus engulf the sperm. There is an immediate sealing of the vitelline membrane called the vitelline block to prevent other sperm from penetrating.

Pronuclei formation

Penetration of the vitelline membrane is the stimulus for completion of the second metaphase, anaphase and telophase stage to expel the second polar body in to the perivitelline space and form the female pronucleus with 1N complement of chromosomes. The first polar body may or may not divide; therefore, two or three polar bodies may be present in the perivitelline space.The sperm head begins to enlarge once it penetrates the vitellus and gradually separates from the membranes and tail to form a rounded male pronucleus. The tail and membranes disintegrate.

Syngamy

Fusion of male and female pronuclei to create diploid zygote is called as synzamy. There is a loss of the nucleoli and nuclear membrane. The new nucleus and its surrounding cytoplasm are now considered as zygote, which is ready for mitotic cell division to form a new life.

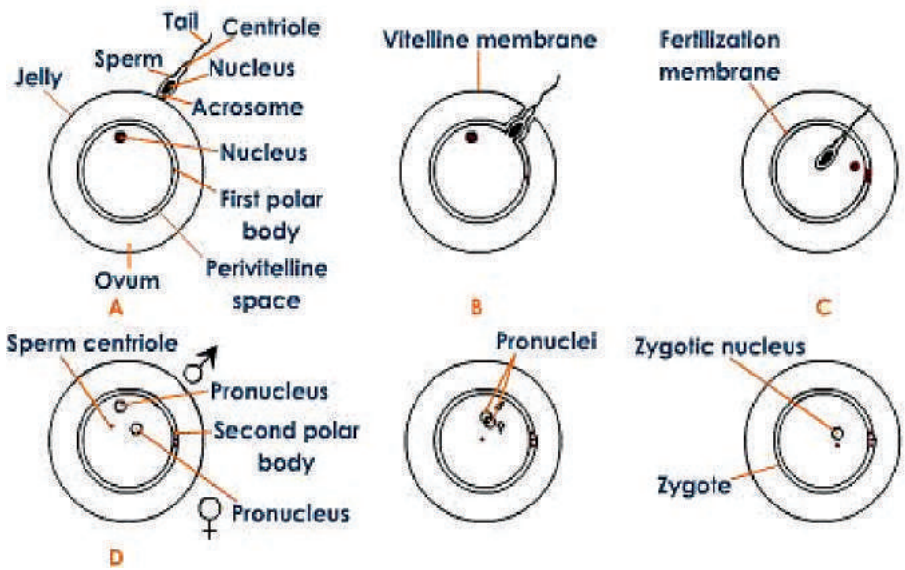
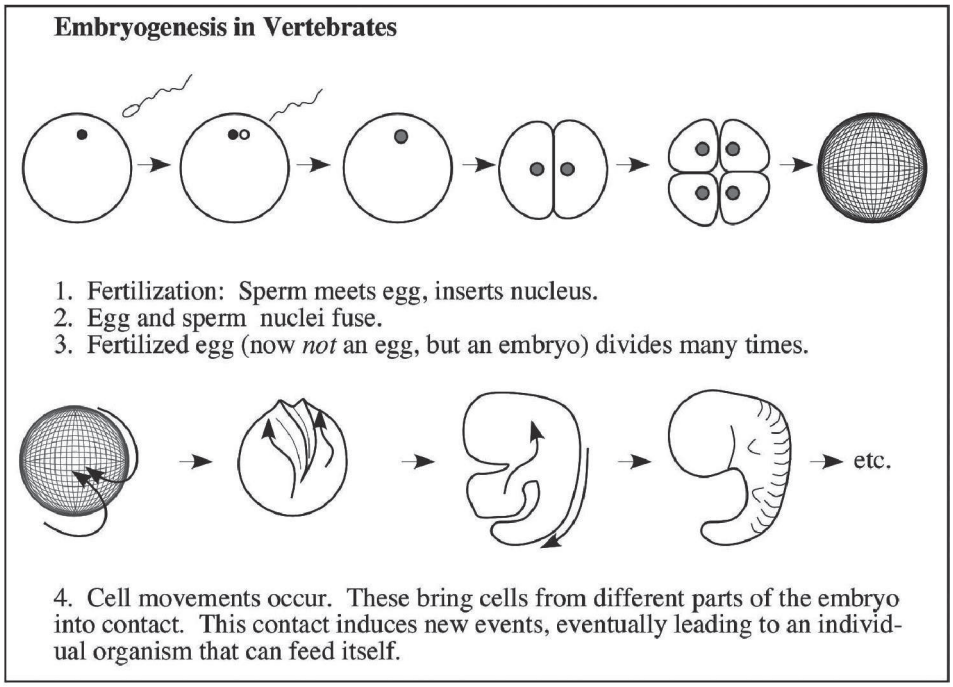


Figure showing the process of fertilization (penetration of sperm, pronuclear formation, fusion and zygote formation)

Embryogenesis

The new cell divides to form two blastomeres, which are smaller daughter cells. Cells divide from 2 to 4 cell and then 8, 16 so on without loss in mass. The zygote continues its cell division and forms a ball of cells called the morula, which transforms into a hollow

sphere of cells in the zona pellucida. The fluid is formed within these cells and the embryo is term as blastocyst. Further embryonic development results in hatching from the zona pellucida. The whole process is shown below in the form of a diagram.



Gestation

Gestation or pregnancy period begins with the fertilization of the ovum by the sperm and terminates with the birth of a young one. The period of gestation varies considerably among species of farm animals. The average gestation period of cattle is 282 days, buffaloes 310 days, and sheep and goat 151 days. Generally, male calves are carried few days longer than female calves.

Table: Gestation period of different domestic animals

Animal species	Gestation period (days)
Sahiwal cow	282
Holstein cow	282
Buffalo	310
Sheep	144-151
Goat	146-152
Horse	340

Parturition

Parturition is the physiological mechanism that enables the uterus to expel fetus after a certain period of development and nourishment. The process is called **"foaling"** in mares, **"calving"** in cows and buffaloes, **"lambing"** in ewes, and **"kidding"** in goats.

The mother (dam) shows a number of signs of approaching parturition, which are usually divided into following four stages.

(a) ***Preliminary stage:*** This stage may last for hours or even days. The ligaments in the pelvic region and around the tail head begin to relax and sink a few days before calving. The dam exhibits difficulty in movement. The external genitalia are swollen, enlarged, and flabby, and strings of mucous are seen. The mammary glands are enlarged and become tender. A clear, waxy fluid oozes out of the teats when pressure is applied.

(b) ***Dilation of the cervix:*** This stage merges with preliminary stage. Uneasiness increases and the animal may lie down and get up frequently. Frequency of micturition (urination) increases. At this time the cervix is fully dilated, and the next stage follows without any appreciable break in the sequence of events.

(c) ***Expulsion of the fetus:*** This stage begins with the fetus entering the dilated cervix and the birth canal. During this time, uterine contractions occur at about two-minute intervals and the amnion is not ruptured. There is a rush of fluid from the uterus when the water bag ruptures, and the animal appears relieved. Normally, the forefeet, with the muzzle lying behind and over them, appear at the vulva. The young one is expelled from the fetal membranes by a forceful and painful effort by the mother.

(d) ***Expulsion of membranes:*** The expulsion of the fetal membranes is commonly known as the delivery of the afterbirth. The placenta is expelled by action of uterine contraction within half an hour to eight hours after parturition.

Activities

Obtain ovaries from slaughter houses and study the structures

Observe estrus in animals

Review Questions

1. Write the process of sperm formation.
2. How oocyte is ovulated from the ovaries?
3. During which period of the estrous cycle the animal shows signs of heat?
4. Name two species in which the ovulation does not take place spontaneously?