ETHIOPIAN VETERINARY ASSOCIATION (EVA)

Bovine Reproduction

A continuous Professional Development (CPD) Module

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Preface

Bovine reproduction with optimal fertility is the major factor determining the bovine farm efficiency and profitability. The dairy cows have been selected for high performance; milk productivity has steadily increased due to genetic improvement combined with better nutrition and management. On the other hand, selection of animals for high milk production has also changed the metabolic adaptation and reproductive physiology of animals, leading to decreased reproductive efficiency. Delayed puberty, sexual maturity, extended calving to conception interval, reduced calving rate, long days open and poor conception rate continue to remain as major constraints in considerable population of animals leading to reduce lifetime production. This existing situation calls for a thorough understanding of the basics and advanced concepts of anatomy, function and pathology of reproduction so that they can be applied on animals to improve the overall reproductive efficiency. Therefore, in this module, we attempted to provide the trainee with basic and advance concepts of bovine reproduction.

In this module, updating knowledge in the area of bovine reproduction including the anatomy and physiology of reproductive organs, breeding soundness evaluation (BSE), estrous cycle, estrous detection methods, physiology of fertilization, pregnancy diagnosis, diseases/accident of gestation, and reproductive technology (artificial insemination, synchronization, MOET, OPU, IVF, semen sexing) are covered. We strongly believe that the modules will be immensely useful for the frontline veterinarians those providing veterinary services (public and/or private), artificial insemination technicians, and dairy farm managers and paraprofessional in updating their knowledge on bovine reproduction.

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COURSE INTRODUCTION

1.1 Module Title	Bovine Reproduction
1.2 Module Code	TBA
1.3 Credit Points	TBA
1.4 Target group	This CPD module is organized for frontline veterinarians those providing veterinary services (public and/or private), artificial insemination technicians, DVM students, BVSc students and paraprofessional.
2. Course Format	
2.1 Description	There is a clear gap in inseminating bovine, pregnancy diagnosis, dystocia management, and adopting reproductive technologies. This module deals with functional anatomy of male and female genital organs, artificial insemination, Breeding soundness evaluation, semen evaluation. In addition, the course discourse pregnancy diagnosis, heat detection, gestation accidents and management of dystocia. Moreover, reproductive technologies will also be covered.
2.2 Duration (69 hrs)	TBA (To Be Assigned)
2.3 Objectives	At the completion of this module the trainees should able to: - Have in-depth knowledge, skill and attitude about bovine reproduction - Apply breeding soundness examination principles in bulls certification - Detect estrus and inseminate animals - Palpate corpus luetum and follicle - Confirm pregnancy in cows - Perform laboratory for semen analysis - Manage, plan and control reproduction in cow
2.4 Learning outcomes	- Trainees could confirm pregnancy at different stage of pregnancy - The trainees become efficient inseminator - Success in pregnancy rate in cows/heifers will be improved - Dystocia will be corrected and managed properly.
2.5 Contents	
Chapter 1 (5 hrs)	Anatomy and Physiology of Reproductive System
Chapter 2 (3 hrs)	Breeding Soundness Evaluation (BSE) and Breeding Systems
Chapter 3 (5 hrs)	Neuroendocrine Control of Reproduction
Chapter 4 (6 hrs)	Estrous Cycle
Chapter 5 (25 hrs)	Physiology of Fertilization and Pregnancy Diagnosis
Chapter 6 (10 hrs)	Parturition and Postpartum Complications

Chapter 7 (12 hrs)	Reproductive Technology
Chapter 8 (4 hrs)	Infertility
2.6 Learning Approach	Lecture, discussion, brain storm, Demonstration, hands on touch
2.7 Measurement of learning	Oral questions, question and answer, Written test, assignment, practical etc

ABBREVIATIONS

ACTH	Adrenocorticotropic Hormone
AI	Artificial Insemination
BNC	Binucleate Cell
bPAG1	Bovine Pregnancy Associated Glycoprotein 1
BTB	Blood Testis Barrier
CIDR	Control Internal Drug Release
CL	Corpus Luteum
eCG	Equine Chorionic Gonadotropin
EIA	Electro Immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
FSH	Follicular Stimulation Hormones
GnRH	Gonadotropin Releasing Hormones
IBR	Infectious Bronchorenitis
ICM	Inner Cell Mass
IVF	Invitro Fertilization
LH	Luteinizing Hormones
MGA	Melegestrol Acetate
MOET	Multiple Ovulation and Embryo Transfer
MRP	Maternal Recognition of Pregnancy
N2	Nitrogen
NSPC	Number of Service Per Conception
OPU	Ovum Pick-Up
P4	Progesterone
PPP	Presentation, Position and Posture
PSP	Pregnant Specific Protein
RFM	Retained Fetal Membrane
RIA	Radio Immunoassay

1. ANATOMY AND PHYSIOLOGY OF REPRODUCTIVE SYSTEM

Upon completion of this chapter, trainees should be able to:

- Explain the anatomical features of bull and cow reproductive system
- Describe the implication of gonads in reproduction
- Have in-depth knowledge of anatomy and physiology of female animals
- Clearly refine how reproductive management can be practiced with the reproductive system.

1.1 Male

Understanding of bull's reproductive system will help producer better understand breeding soundness examinations, reproductive problems and breeding impairments. The reproductive tract of bull consists of testis in scrotum, accessory organs (gland and ductus) and penis.. These organs work in concert for formation, maturation and transport of spermatozoa. The secondary sex organs are the epididymis, vas deferens and penis. The three accessory sex glands include the seminal vesicles, prostate and bulbourethral gland (Cowper's gland).

The reproductive organs and hormone of the male animal may be considered to fulfil the following major functions;

- Firstly, the production of spermatozoa
- Maintenance of spermatogenesis
- Secondly maturation, storage and transport of spermatozoa
- The production of masculine behavior (libido & aggression)
- Finally, the deposition of semen within the female genital tract via the penis.

Scrotum

It is a two lobe sac; consists of skin, tunica dartos, scrotal fascia, parietal vaginal tunic. It is highly populated with sweat glands with little fat and endowed with large numbers of thermosenstive nerves which governs sweat glands and respiratory rate of animals. The scrotum provides a favorable environment for the production and maturation of spermatozoa, enhance movability and a protective sac for testis.

T**estis**

Testes are formed at the level of ribs in retroperitoneal location and after descending it is located outside the body cavity in the scrotum (temperature essential for normal sperm formation: 4-5 °C degrees below body temperature). It is composed of seminiferous tubules separated by fibrous septa extending from the tunica albuginea into about 250 lobules. The tubules in each septum join to form a larger straight tubule that lead into rete testes. The seminiferous tubules have a thin basement membrane, a central lumen & 2 types of lining cells (spermatogenic and sertoli cells) and the interstitial tissue between the seminiferous tubules is composed of Leydig cells (LH dependent and responsible for testosterone production). Sertoli (sustentacular**)** cells is FSH dependent and responsible for blood testis barrier (BTB) and produce androgen binding protein. Testis is responsible for sperm and hormone production.

Epididymis

Convoluted tubule connects to testis by a series of efferent ductus measuring 5.5-6 m in length. Surrounded by smooth muscle enhancing rythemic contraction of epididymis for sperm transport. It has 3 parts: head (caput), body (Corpus) and tail (Cauda). It has the following functions:

- Sperm transport, Maturation of sperm, Storage of sperm
- Sperm concentration, Absorption of testicular fluid & degenerated sperms

Surgical removal of the tail of the epididymis (epididectomy) used as a means of sterilization for teaser (Gomer) bulls for estrus detection. Epididectomized bulls will still service cows in the usual manner, but will not deposit sperm in the female reproductive tract.

Vas deferens/ ductus deferens

It starts at the epididymal tail & its terminal section enlarges to form an ampullary portion that joins the duct of the seminal vesicle to form the ejaculatory duct which passes through the prostate to open in the prostatic urethra. Readily separable from the rest of the spermatic cord (vasectomy) (for castration). Bulls may also be sterilized by a vasectomy at this region. It has a tick muscular wall; at its terminal end it gets wider thickened, and this portion is furnished with branched tubular glands and forms ampulla. Its functions:

- Transports of sperm by peristaltic movement usually during courtship and pre-coital stimulation
- Nutrient for stored sperm
- Sperm stayed alive motile and fertile for 3 days then loose its fertilizing capacity
- Absorb dead sperm

Urethra

Two vas deferens eventually unite into a single tube, the urethra. It is a common passageway for semen from the reproductive tract and urine from the urinary tract.

Accessory Glands

Vesicular Gland

It is a paired glandular tissue lied dorsocrainail to pelevic urethra and it is lateral to the terminal portion vas deferens. Compact and lobulated in bull and ram, firm consistency. Seminal vesicle and vas deferens have a common ejaculatory orifice into urethra. It secretes:

- Vesicular secretion is significant portion of the ejaculate (ex, 50% in bull:, sorbotol, inositol) which act as a vehicle to sperm activity
- Fructose for energy to sperm, citric acid as buffer to sperm
- K+ and Na+ to control the equilibrium of osmotic pressure
- Flavin which give yellow coloration to normal ejaculate in few bulls

Prostate Gland

A pyramidal-shaped, firm elastic gland that lies b/n bladder & pelvic urethra. It has a fibromuscular capsule, extends into the substance of the gland separating the external part into lobules (Pars Externa). The inner disseminated portion of the gland is made up of periurethral glands & is not lobulated (Pars Enterna). In older intact male animals, the prostate may become enlarged and interfere urination and surgical intervention is difficult for the inner disseminated portion. Functions:

- Produces alkaline that gives semen its characteristic odor.
- Secrete large amount of minerals that regulate the buffering system of seminal plasma
- Secrete amino acids for sperm nutrition
- It is alkaline in reaction to neutralize the acidic sperm coming from the cauda epididymis

Cowper's glands:

Oval, paired located dorsal to the urethra at its pelvic end close to ischial arch and covered completely with bulbocavernosus muscle. In bulls almost hidden by bulbospongiosus muscle. Each has a s single excretory duct at the posterior of pelvic urethra. Functions:

- Its secretion flushes the urethra free of urine i.e. for cleaning & neutralize

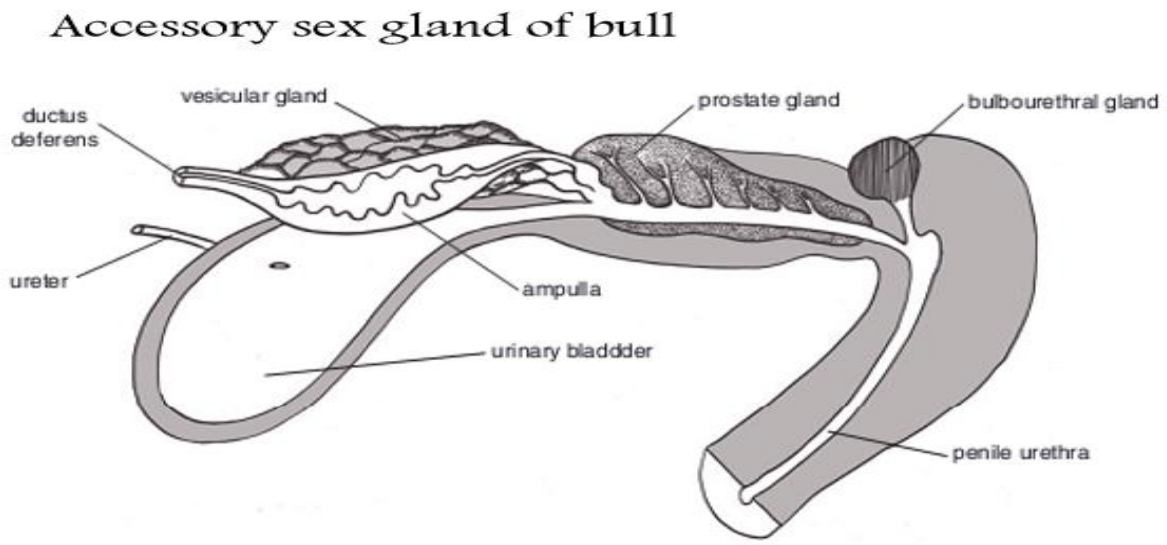
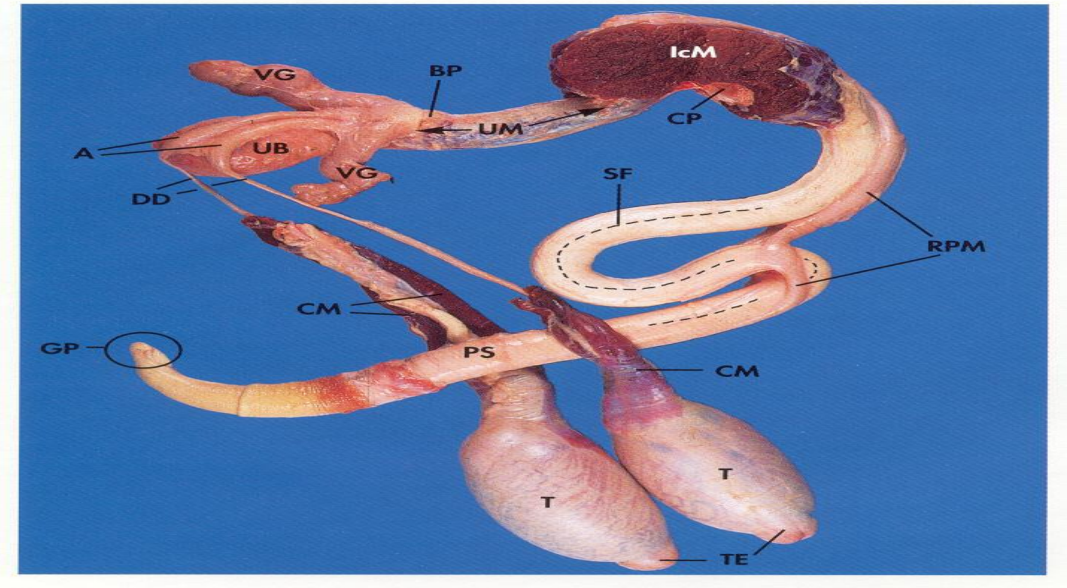


Figure 1.1 Accessory sex gland of bull (Frandson et al., 7th ed.)

Penis

Male copulatory organ consists of base, shaft, glans penis. Glans penis is highly populated with sensory nerves. Bull has fibroelastic type of penis. Two retractor penis muscles attached the penis at the end of **S** shaped sigmoid flexure. The structure in penis are: Corpus spongiosum, Corpus Cavernosum and Tunica albuginea. Improper function of retractor muscles may let penis and sheath lining protrude at all times then exposing to infection.



NB; A=Ampulla; VG=vesicular gland; BP=body of prostate; GP= Glans penis; ICM=Ischiocavernosum muscle; CP=Crus penis ; UM-urethralis muscle; RPM=Retractor Penile Muscle; DD=Ductus deferens

Figure 1.2 Reproductive tract of bull

1.2 Female

Understanding the anatomy and physiology of female reproductive system is fundamental to good cattle management especially when using artificial insemination, pregnancy diagnosis, estrus synchronization, live ovum pick-up and embryo transfer. It will also enable producers to better understand and control production and reproduction related problems.

The female reproductive system consists of gonad (paired ovary), ducts (oviduct, uterus, cervix, vagina) and external genitalia (Vestibule, vulva and clitoris). The reproductive system is bordered by:

- Dorsally = rectum, sacrum and few coccygeal vertebrae; Laterally = Ilium bone
- Ventrally = bladder and floor of the pelvis, I .e, pubis
- Cranially = abdominal organs; Caudally = the perineum area/ Ischium

Ovary

It is paired, small oval almond shaped situated in the abdominal cavity just ventral to the kidneys (caudo-ventral from abdomen). It is a primary female reproductive organ (gonad). A portion of peritoneum fuses and form double layered connective tissue to suspend ovary, uterine tube, uterus by broad ligament (houses vascular, lymph and nerve), i.e mesovarium, mesosalpinx, mesometrim, respectively. in addition uetro-ovarian ligament connects uterus and ovary. It functions:

- Producing the female reproductive cell (the egg or ovum)
- Hormone production (estrogen, progesterone, activin and inhibin).

Oviduct/Fallopian tube/ Salpinx

It is ciliated and convoluted pathway from ovary to uterus. Ends of oviducts flare out as a fringe of finger-like projections called fimbriae. Cilia disappear almost completely after hypophysectomy and develops exogenous E2 administration. Cilia coupled with oviductal contraction keeps constant rotation of egg and bring egg and sperm to fertilize and prevent implantation in salpinx. At ovulation, contractions become most vigorous and the fringe like folds contract rhythmically and massage the ovarian surface. Secretory granules accumulated during follicular phase and released to lumen after ovulation. It has three part namely; Infundibulum; Ampulla (site of fertilization) and isthmus. It functions:

- Site for fertilization
- A way for sperm, ovum and embryo transportation
- To provide regulation check points for unfertilized oocytes.
- The mucosal glands produce oviduct secretions integral for supporting egg, sperm and embryo:

Uterus

It consist of two horns (coruna), a body (corpus) and neck (cervix). Supported by mesometrium. In cows and ewe there are about 100 caruncles important for placental formation. It functions:

- Sperm transport and sperm capacitation
- Luteolytic mechanism: regression of CL by producing prostaglandin in endometrium
- Implantation: adapted to accept the conceptus
- Nourish the fetus till term & expel during parturition
- Metabolism: CHO, lipids, proteins
- Synthesis: of hormones and enzymes

Cervix

A heavy, smooth muscle sphincter like structure, fibrous tissue characterized by a thick wall and constricted lumen. Serve as a benchmark for rectal palpation. It is tightly closed except during estrus and parturition. Secretes outward flowing mucus from goblet cells to prevent infective materials from entering the uterus from lower reproductive tract. Fornix; blind sac formed by the cervix protruding into vagina which makes it difficult for artificial insemination. The cervical canal has various prominence, forms 3-4 annular rings in cows. Functions:

- Isolates the uterus from the external environment
- Passage for sperm
- Sperm reservoir.
- During pregnancy, filled with a thick secretion that serves as a plug to protect the uterus from infective material entering through the vagina.

Vagina

It is well supplied with blood, nerves. It etends from cervix to vestibule. The immunologic response: local antibodies may produce against sperm cells; IgA and IgG are produced by mast cells to prevent bacterial infections and against spermatozoa. Functions:

- It serves as a copulatory organ

- Prevent coagulation of sperm until it is transported
- The dilated bulbous/bulging vagina provides a post coital semen pool to supply sperm for cervical reservoirs
- Excretory duct for secretions of the cervix, endometrium and oviduct
- Serves as birth canal during parturition and used for voiding of urine

Vestibule

The junction of vagina and vestibule is marked by the external urethral orifice and frequently by a ridge (the vestibular hymen). In some cattle, the hymen may be so prominent; interfere copulation. The vestibule of the cow extends inwards for approximately 10cms, where the external urethral orifice opens into the ventral surfaces

Vulva:

The external opening of the vagina is called the vulva.

Clitoris

The ventral commissure of the vestibule masks the clitoris, has the same embryonic origin as penis. It is composed of erectile tissue and well supplied with sensor nerve endings. In the cow, the greater part of the clitoris is buried in the mucosa of the vestibule.

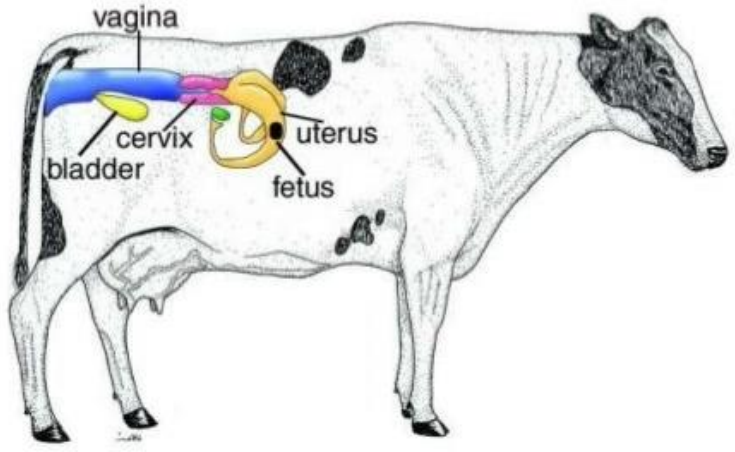


Fig. 1.3 Female reproductive system

1.3 Puberty

Puberty is the time when an animal becomes sexually capable of producing fertile gametes and exhibiting complete sexual behavior (oocytes/ spermatozoa). In female puberty is the age at first heat/ovulation. Puberty is the gradual adjustment between increasing gonadotropic activity and the ability of gonads for steroidogenesis & gametogenesis. Sexual maturity is the age at which the female become capable for insemination and supporting pregnancy.

Puberty occurs at a specific physiologic age. In preupbertal animal, the hypothalamus (HT) is sensitive to the inhibitory effect of small amount of steroid hormones (E2 in female & T2 in male) secreted by the prepubertal animals. As the animal matures/attain physiologic age, the hypothalamus becomes less sensitive to the negative feedback effect of gonadal steroids and begins to respond by secreting GnRH.

Factors affecting Age at Puberty

A. Hormonal

In prepubertal period there is minimal GnRH released, minimal FSH and LH low due to which there minimal or no folliculogenesis. However, in near to puberty age the sensitivity of the hypothalamic tonic center reduced and sensitivity of GnRH surge center in hypothalamus increased and finally, GnRH and gonadotropins secretion increased.

During puberty, there is increase in pulse frequency and amplitude of GnRH release. FSH and LH pulse will be increased and there is commencement of follicullogenesis/ Spermatogenesis.

Note: There is a difference in an endocrine secretion in male and female. In male fetus, testosterone converted to estradiol which then crosses blood brain barrier (BBB) and defeminizes the surge center of HT due to which there is no surge secretion of hormone. whereas in female, fetal ovaries produce estradiol in female offspring, it doesn't cross BBB due bindings of alpha-protein to estradiol that prevents it from crossing BBB. So there is both surge and tonic center of HT is active and there is a cyclical surge production of Luteinizing hormone (LH) in female.

B. Genetic

Breed can influence age & size at puberty. E.g. Holstein breed reached puberty in 8.5 months which is lower than other breeds.

C. Nutrition

As the animal develops energy needed for growth decreases and shifts for other processes like reproduction. Though minimal age should be reached, body weight is more important than age in determining puberty. In dairy cows, 60% of adult body weight has to be considered while breeding heifers. This precautious is recommended for artificial inseminators (AI).

D. Environmental

Birth month affects seasonal breeders in which a proper size should attain during the right season. The presence of intact adult males can decrease age at puberty. Effects of prepubertal females due to exposure to adult males are caused by pheromones (a chemical substance used to communicate between members of the same species).

Review Questions:

1. Why the testis descends and reside in scrotum?
2. Describe the importance of sertoli and Leydig cell?
3. What is BTB (Blood Testis Barrier)?
4. How could you rule out inguinal hernia during closed castration?
5. How can one differentiate CL from follicle.

2. BREEDING SOUNDNESS EVALUATION (BSE) AND BREEDING SYSTEMS

2.1 BREEDING SOUNDNESS EVALUATION (BSE)

Breeding soundness evaluation (BSE) is a method developed to certify breeding potential of sire/bulls for natural mating and artificial insemination. Bulls differ in reproductive capabilities. Various studies showed that about 20% (1 in 5 beef bulls) examined were not satisfactory potential breeders. These bulls can be identified before the breeding season with a complete BSE. There are 5 components of breeding soundness evaluation (BSE). This are:

A. History and Health Status

In multipara bull review the size, number and viability of the offspring of the sire and his daughters for any evidence of hereditary disease factors and record history of umbilical hernia, deviated penis, and other defects. In addition, herd health history/ conception rates, disease, & vaccination history is very important and has to be considered to proceed further assessment.

B. Physical Examination

This begins at a distance assessing identify the bull, size relative to age, body condition score, checking the eyes, examine the hooves (e.g. interdigital fibrosis, abscesses, Abnormal hoof growth), examine conformation and leg structure and locomotion and gait. Noting signs that might be consistent with the presence of any infectious or contagious disease. Some structural defects may have little or no influence on immediate mating ability but may predispose animals to early development of arthritis or injuries. Hoof and sole problems result from poor conformation and may require trimming and other treatments to maintain serving capacity. In addition, the bull needs to be able to eat, see and smell properly.

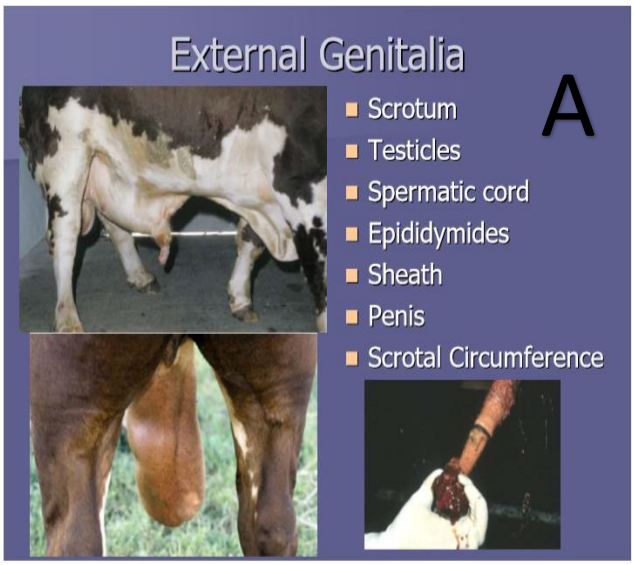
Other physical traits considered during a BSE include the degree of muscling and body size measurements such as hip height, frame score and weight. These traits usually do not result in a bull being classified as unsatisfactory but may be a factor in his selection for breeding purposes.

Fig. 2.1. Physical exam during BSE

C. Examination of the reproductive tracts

Done by visualization and palpation. The prepuce will be examined for any evidence of constriction or discharge. The penis will be palpated to ensure its freely moveable within the prepuce and that there is no evidence of any abnormal swellings/growths. Look for evidence of inflammation, tumors, adhesions, and congenital short penis, Phimosis and paraphimosis, Penile deviations. Examination of the scrotum, testes, epididymis and spermatic cord and observe the size and shape of the scrotum; size correlate with output. Looking for positions of the testes in the scrotum and palpate testes for size, shape, consistency and adhesion with scrotum. Lastly but not least, the head, body and tail of the epididymis should be carefully palpated for inflammation, enlargements, missing segments, granullomas or abscesses.

A thorough examination of the male reproductive system follows the general health examination. Developmental defects, inflammation and other deviations from normal should be observed. The vesicular glands, ampullae and prostate can be examined by rectal palpation, while the spermatic cord, scrotum, testicles and epididymides can be palpated externally. Some groups of young bulls have a high incidence of infection of the vesicular glands (these produce accessory fluid in the semen ejaculate). This is generally a temporary infection, but occasionally it will cause the discharge of pus into the semen making fertility questionable.



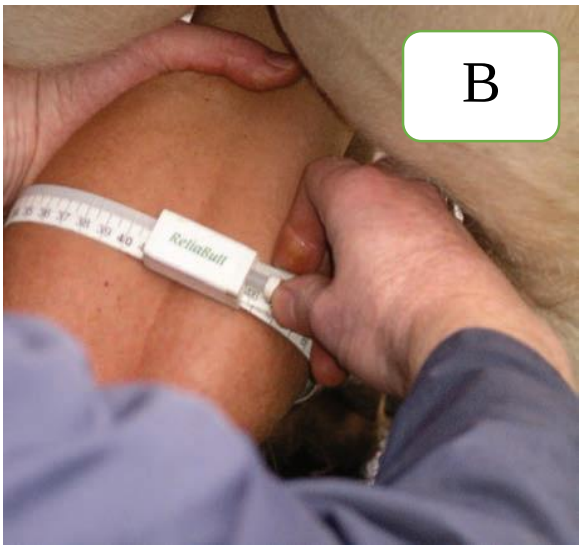


Figure 2.2. A=abnormal externa genitalia; B=testicular size measurement; C=Persistent frenulum

D. Semen Collection and Examination

The best quality samples can be obtained by teasing the bull with a cow that is in oestrus and then using an artificial vagina. The use of electro-ejaculation will also usually result in an acceptable quality sample being obtained more quickly and safely. Assessment will be made of ejaculate volume & density. Then sample is viewed using a microscope to assess sperm motility and the number of abnormal or damaged sperm. In general, semen has to be checked for volume, color, gross morphology, live-dead ratio and motility.

Appearance

- The volume should be checked by the degree of stimulation and the order of stimulation (that is 1^{st} , 2^{nd} , 3^{rd} etc).
- Repeated frequent ejaculations may lower the volume of the ejaculate.

Color

The amount of sperm in semen determine the color of semen. E.g.

- Creamy semen: $1x10^6$ -$1.2x10^6$ spermatozoa/mm3
- Milky semen: $0.5x10^6$ -$0.6x10^6$ spermatozoa/mm3
- Watery semen: $<0.3x10^6$spermatozoa/mm^3

Note: The presence of bloody segment suggests orchitis. The presence of yellow color suggests riboflavin secretion by the bull. Green color indicates Pseudomonas aeroginosa infections

Sperm concentration

It is expressed as number of sperm cell/ml. Using artificial vagina is better than electro-ejaculations in terms of collecting concentrated semen. Concentration can be determined directly in haemocytometer or indirect **(**Spectrophotometery) which uses light absorbance.

Sperm motility

Individual or mass motility could be evaluated in microscope to estimate the fertilizing ability of semen.

- Individual motility: Motility ranges from 0-80% (Multiples of ten 40, 50, 60, 70% and the so on are used in reporting normal type motility. Samples with less than 40% initial motility are not suitable
- Mass motility: examining the cloud movement of semen as a whole.

Sperm morphology

Semen may show as few as 5% abnormal sperm (100% fertility may not be affected until the level of abnormal sperm exceeds 20-25%). Abnormalities could include: head (primary abnormality), tail (tertiary abnormalities) and cytoplasmic droplets (secondary abnormalities). Heads: asymmetrical, tapering, pyriform, giant, microcephalic, double, knob etc. Tail: tightly coiled tail, Bent tail, double tail, absent tail, broken tail.

Live-Dead Ratio

This is used to determine live/dead spermatozoa. This can be done using Eosin nigrosin staining. Nigrosin provides a blue black background, while Eosin stains the sperm cells. The dead ones stain pink/red color whereas the live ones remains colorless. Implemented out of 200 spermatozoa.

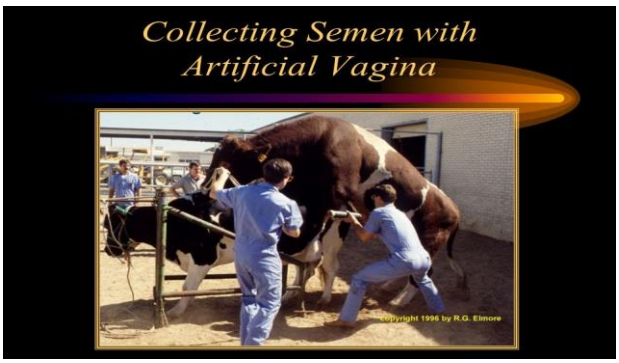


Fig. 2.3. Semen collection

Computer Assisted Semen Analysis (CASA)

In manual semen analysis intensive specialist training and quality control programs are needed and unfortunately still seminological assessment remains problematic in many laboratories due to lack of repeatability, time consuming and inaccurate as it is subjective estimation. In addition, accurate pipetting and dilution are needed. Bearing the fore mentioned drawback, currently, the traditional manual semen analysis is now replaced and assisted by an automated computerized system via CASA. CASA which saves time, accurate and can estimate the speed of semen motility, concentration, sperm density, percent motility, morphology and linear velocity precisely over manual methods.

E. Libido and Serving Assessment

Watching the bull's behavior to allow an assessment to be made of his libido (how keen he is to serve cows) and of his ability to serve:

- To appreciate the degree of sexual desire,
- Poor and exaggerated libido are undesirable
- Exaggerated results: premature ejaculation (outside female genitalia)

It is particularly important during this part of the examination to watch carefully for any penile deviations which may prevent intromission (the penis entering the vagina) and for evidence of an ejaculatory thrust. Libido is associated with testosterone production in which there is good serving habit when there is adequate testrstone production from Leydig cell.

2.2 Breeding System

Broadly breeding systems classified into inbreeding and outbreeding

A. Inbreeding

The mating of animals more closely related than the average of the breed or population. There are two types of inbreeding called intensive inbreeding and line breeding.

- Intensive inbreeding: Mating of closely related animals for several generations.
- Line breeding: A mild form of inbreeding that maintains a high genetic relationship to an outstanding ancestor.

B. Outbreeding

The mating of animals not as closely related as the average of the population. There are four types of outbreeding called species cross, crossbreeding, outcrossing, and grading up.

- Species cross: Crossing of animals of different species. (Example: Horse to donkey); Crossbreeding: Mating of animals of different established breeds; Outcrossing: Mating of unrelated animals within the same breed.
- Grading up: Mating of purebred sires to commercial grade females and their female offspring for several generations.

Review questions

1. What are the components of BSE?
2. Which type of animals certified for breeding.
3. Define inbreeding and cross breeding.
4. Why it is not recommended to mate a bull with his full sibs?

3. NEUROENDOCRINE CONTROL OF REPRODUCTION

At the end of this chapter the trainees could be able to:

- ☞ Explain the effect of nerves and endocrine on reproduction
- ☞ Describe hormones important for reproduction
- ☞ Explain the implication of each reproductive hormone

3.1 Neuroendocrine Control

Reproductive function is initiated, coordinated and regulated by both endocrine & nervous function through hypothalamo-hypophyseal portal system. The nervous system brings immediate responses through rapid electric nerve impulses. Endocrine glands uses chemical messengers, or hormones to regulate slow body processes, e.g growth & reproduction. The endocrine system regulates slower processes that takes from days to months by the action of hormones. Hormones are physiologic, organic & chemical substances secreted and released by ductless glands (i.e. endocrine) uses blood vessel with exception of uterus, placenta & Hypothalamus (neural to posterior Pitutary) that did not use blood circulation.

Hormones inhibit, stimulate and regulate the functional activity of target organ/tissue by binding to receptor proteins within the cell or on the surface of the cell. Feedback mechanisms regulate production of hormones. There are two types of feedback mechanisms:

- **Negative feedback**
  - Hormone inhibits the release of another hormone
  - E.g. Increasing inhibin slows FSH release from the anterior pituitary
- **Positive feedback**
  - One hormone stimulates the release of another hormone
  - E.g. Increasing E2 stimulates the release of GnRH, LH, FSH & prolactin

General Characteristics of Hormones

- Produced in a very small amount and most hormones are not species specific
- There is rise and fall in production, which could be related to physiological, pathological or homeostasis
- Disintegrated/degraded soon in relation to their secretion; half-life ranges from seconds to hrs

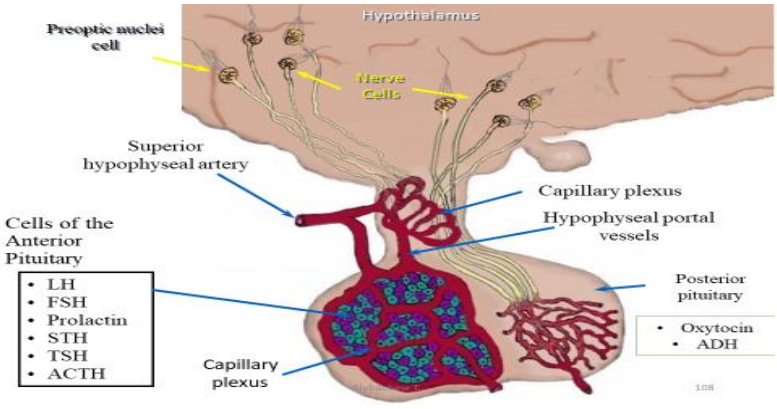


Figure 3.1. Connection of HT to Pituitary gland

3.2 Receptors for Hormones

A hormone to exert its biological effect it must interact with its own specific receptor. Steroid & Thyroid Receptors are found either in the cytosol/ nucleus in which hormones that are lipid soluble can reach these intracellular receptors by diffusing into the cell through the cell membrane. Whereas, peptide hormone receptors are found in the cell membrane. Peptides cannot freely diffuse through the lipid bilayer of the cell membrane, so their receptors must be in the outer cell membrane to be available to the hormones in the extracellular fluid.

3.3 Glands

Hypothalamus (HT) Gland

Found in 3rd ventricle extending from the optic chiasma to the mammillary body. It regulate the release of hormones from pituitary gland. There is a neural connection b/n hypothalamus & posterior pituitary (neurohypophyseal) through hypothalamic-hypophyseal tract and vascular connection b/n hypothalamus and anterior pituitary. Arterial blood enters the Pituitary by way of the superior artery & inferior hypophyseal artery.

The superior hypophyseal artery forms capillary loops at the median eminence and pars nervosa. From these capillaries, blood flows into the hypothalamo-hypophyseal portal system, which begins and ends in capillaries without going through the heart. Part of the venous out flow from the anterior pituitary is by way of a retrograde back flow, which exposes the hypothalamus to high concentrations of anterior pituitary hormones. This blood flow provides the pituitary gland the negative feedback mechanism of regulating the functions of the hypothalamus. E.g. GnRH, prolactin releasing & inhibiting hormones, oxytocin, ACTH.

Gonadotropin releasing hormones (GnRH): structurally, it is short chain polypeptides (3 - 44 amino acids). Provides humeral link between the neural and endocrine systems. In response to neural signals, pulses of GnRH are released in to hypophyseal portal systems for the release of LH and FSH from the anterior pituitary gland.

Prolactin releasing hormones (PRH): Neurons containing dopamine in the arcurate nucleus. Stimulates prolactin release. Whereas, prolactin inhibiting factor (PIF**).** PIF is a catecholamine, dopamine that is an amine of low molecular weight synthesized from L-tyrosine. PIF is secreted from nerve terminals mostly in the arcurate nucleus to inhibits prolactin secretion from adenohypophyeal.

Anterior Pituitary Hormones

Follicle stimulating hormone (FSH): stimulates the growth and maturation of ovarian or graffian follicle, sperm development (testes). FSH itself does not cause secretion of E2, instead it needs the presence of LH to stimulate E2 production.

Luteinizing hormone (LH): Tonic & basal levels of LH act in conjunction with FSH to induce E2 secretion from the large ovarian follicle. Preovulatory surge is responsible for ovulation. Stimulates corpus luteum formation and secretion of androgens (testes)

Prolactin: It has luteotropic properties maintenance of CL in rodents, however in domestic animals LH is the main luteotropic. It initiates and maintains lactation (mamotropic).

Neurohypophyseal Hormones

There are two (Oxytocin, ADH) hormones synthesized in hypothalamus, however stored and secreted from posterior pituitary. The hormones transferred from the hypothalamus to posterior pituitary not through vascular system, but along the axons of nervous system.

Oxytocin: stimulates uterine contraction during follicular phase of estrous cycle to facilitate sperm transport and during late gestation to facilitate parturition. Stretching of the cervix at parturition caused by the passage of the fetus stimulates results a reflex release of oxytocin (Ferguson's reflex). It is also important for milk let down. Ovarian oxytocin is involved in luteal function and it acts on endometrium to induce PGF2

Gonadal and Uterine Hormones

Estrogen/Estradiol/: is a glycoprotein hormone secreted from ovary (follicle). Act on CNS to induce behavioral estrus. Act on uterus to increase both amplitude and frequency of contraction. Stimulate duct growth & the development of mammary gland. Exert both negative and positive feedback controls on LH and FSH.

Inhibin: produced by granulosa cells of growing follicles which inhibits FSH release. It is a peptide hormone.

Activins: it is a peptide hormone found in follicular fluid. It modulate the secretion of FSH.

Progestin/Progesterone: Secreted by CL, placenta and adrenal gland. Prepare the endometrium for implantation and maintenance of pregnancy by increasing activity of secretory glands. Acts synergistically with E2 to indicate behavioral estrus. Develops secretory tissue (alveoli) of mammary gland. Inhibits estrus, ovulatory surge of LH at high level and uterine motility.

Relaxin: Secreted by CL during pregnancy. It dilates cervix and vagina before parturition.

Prostaglandins F2α: secreted in endometrium. Once secreted picked up in uterine vien and directly transferred to ovarian artery by counter current exchange which bypass heart. Its action is the regression of the corpus luteum from ovary. It also promotes smooth muscle contraction in the uterus for sperm transport, ovum transport, parturition and ovulation. It promotes gonadotropin release. Moreover, it is used in clinic to treat endometritis/pyometra, induction of estrus, and induction of abortion/parturition.

3.4 Folliculogensis

Is the progression of recruited primordial follicles into large graafian follicles and followed by regression/ovulation under the influence of FSH, little LH known as maturation of the ovarian follicle. There are two major structures found in ovary (follicle &corpus luteum). A follicle is a blister like structure containing oocyte and produces hormones like estrogen in which high amount of E2 causes standing het and ovulation. Whereas, corpus luteum looks like a hard yellow structure which is responsible for progesterone production.

Follicular fluid is a liquid composed primarily of hormones, enzymes, anticoagulants, electrolytes, reactive oxygen species & antioxidants, which fills the follicular antrum & acts as an important mediator in the communication b/n cells in the antral follicle while bathing and carrying nutrients to the oocyte.

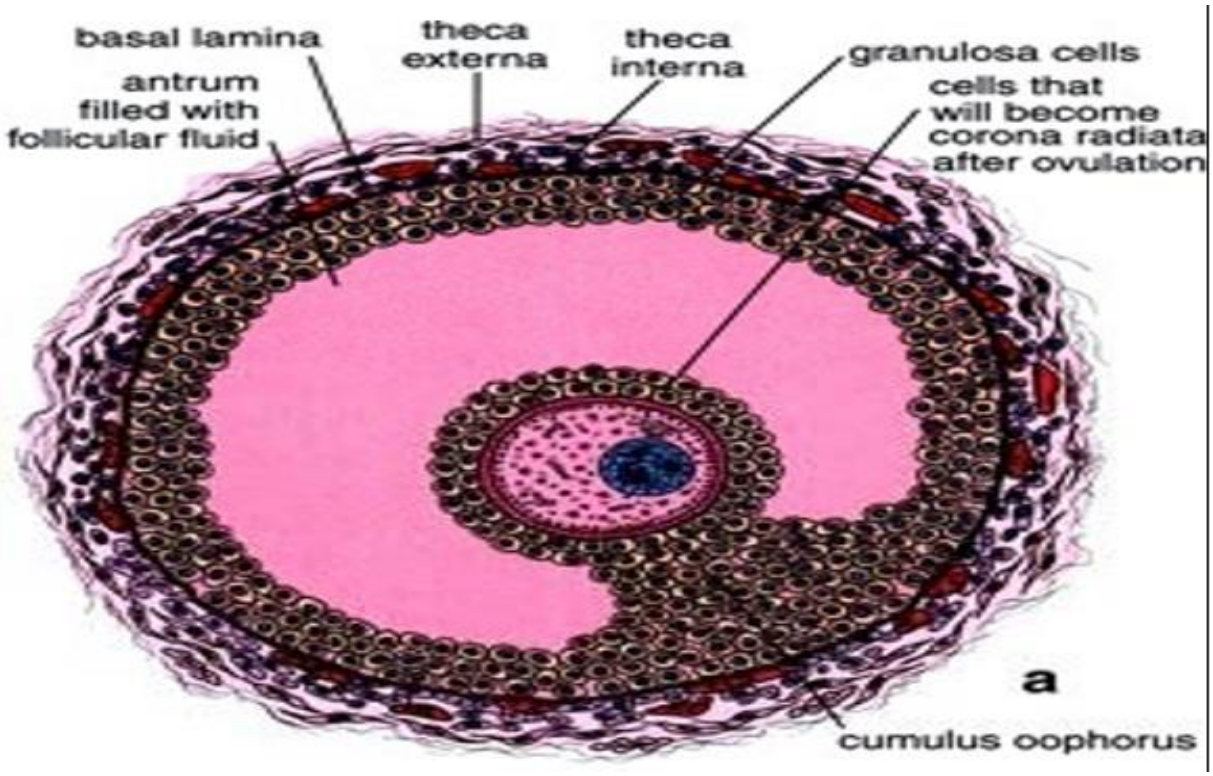


Fig. 3.2 Morphology of mature follicle

Ovarian follicular growth and development in ruminants is characterized by 2/3/4 consecutive follicular waves per oestrous cycle. Each wave involves the recruitment of a cohort of follicles and the selection of a dominant follicle. The dominant follicle continues to grow and mature to the preovulatory stage while others undergo atresia. A dominant follicle from the last wave has the chance to be ovulated due to surge production of LH. GnRH release from tonic appears to be spontaneous but is influenced by P4 Whereas, GnRH release from the surge center is controlled by high E2 accompanied by low P4. The preovulatory surge of GnRH is controlled by the combination of high E2 and low P4 in mammals, estradiol in the presence of low P4 exerts a deferential effect on GnRH. E2 in low concentrations causes a -ve feedback (suppression) on the preovulatroty center. That is, low E2 reduces the level of firing GnRH neurons in the preovulatory center. However, when

E2 level are high, as they would be during the mid-to late follicular phases, the preovulatory center responds dramatically by releasing large quantities of GnRH. This stimulation in response to rising concentrations of estradiol is called +ve feedback.

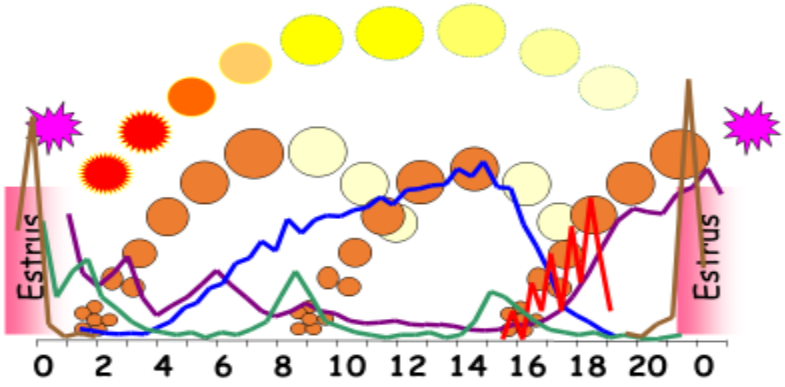
Even though the follicular phase comprises only about 20% of the estrus cycle, the process of follicular growth and degeneration called follicular dynamics which occurs continuously throughout the entire estrous cycle. Follicular dynamics is controlled by FSH & LH, involves both growth of follicles. Antral follicles of various size develop in response to tonic levels of FSH and LH. GnRH is released from the hypothalamus to promote the release of FSH and LH from the adenohypophysis. The release of GnRH can be modulated by steroid (estradiol & P4) and peptide (inhibin) hormones from the ovary, but its basal release is determined by neural inputs to the hypothalamus. The pulsatile nature is physiologically important, b/c continuous infusions of GnRH do not result in the continuous release of FSH & LH

As the follicle grow and develop there is formation of granulosa and theca interna cells which develops cellular receptors for FSH and LH, respectively. From this point, the coordinated effects of FSH & LH are both needed for normal follicular development. Under the influence of LH, thecal cells proliferate and produce androgens (androstenedione & testosterone) that diffuse into the granulosa. FSH promotes further granulosa cell proliferation, the development of cellular enzymes necessary for the conversion of androgens to estrogens (estradiol), and the secretion of several other paracrine agents necessary for follicular development. E2 produced by the granulosa cells acts as a paracrine agent on the developing follicle and also enters the systemic circulation to affect other sites throughout the body. Locally, E2 acts on granulosa cells to increase FSH & LH receptors, and, together with these gonadotrophins, it promotes further granulosa cell replication, growth, and secretion. The overall effect is that locally produced E2 promote the development of the follicle from which they are being produced. This is characterized as a local +ve feedback effect of the E2. This +ve feedback effect is one factor in the selection process that determines which of the developing follicles will ultimately produce the ovum and ovulate.

A second factor is that circulating E2 have a -ve feedback effect on FSH secretion from the adenohypophysis. Decrease in FSH during this period contributes to the atresia of more slowly developing follicles. The selection process also involves another follicular hormone, inhibin, are peptide hormones secreted by granulosa cells of developing follicles. Circulating levels of inhibin increase with follicular development, and inhibin have a -ve feedback effect on FSH release but not LH. By this means, a developing DF can suppress the development of competing follicles in non–litter-bearing animals. In litter-bearing animals, the combined -ve feedback effect of inhibin from multiple follicles can suppress other follicles to prevent litter sizes.

Estrogen from developing follicles are also necessary to prepare the follicles & the hypothalamic–adenohypophyseal axis for ovulation. Within the ovary, E2 promote an increase in LH receptors in thecal cells so that these cells increase their production of androgens and appropriately respond to LH at the time of ovulation. Circulating E2 promote an increase in LH within the adenohypophysis and condition the hypothalamic–adenohypophyseal axis so that short-term LH surge for ovulation can be delivered. Non–litter-bearing animals typically have one/two follicles per estrous cycle that develop faster and grow larger than the rest which is called dominant follicle which continue to produce increasing amounts of estradiol

as well as the hormone inhibin. Recall that inhibin is a protein hormone produced by dominant follicle that selectively inhibits the release of FSH from Anterior Pituitary. Due to this FSH does not surge to the same extent as LH. The DF exerts inhibitory effect on other antral follicles from recruited and selected cohort. Such inhibition is created in combination of inhibin and E2 by the DF and reduced blood supply to some follicles which results atresia.



NB: Red color=PGf; Green color=FSH; Blue color= P4; Purple color=E2; Grape Purple color=LH

Fig. 3.3 Hormone profile of follicular development

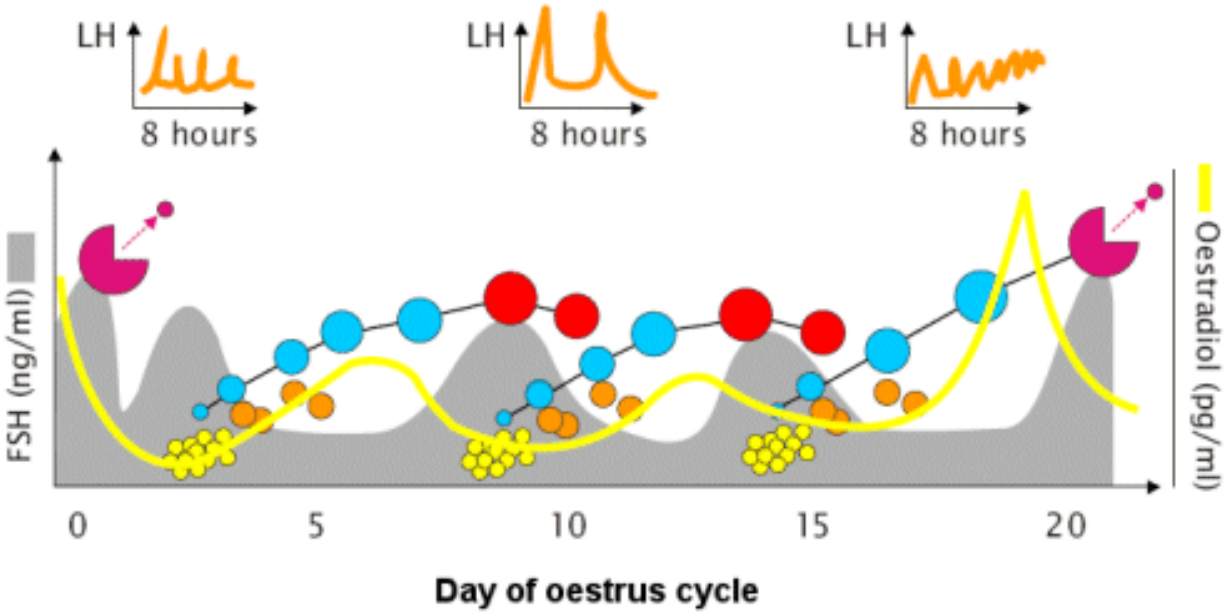


Fig. 3.4 Three wave follicular dynamics

Review questions

1. What is the endocrine difference between female and male
2. Describe hormones which have effect on folliculogenesis

4. ESTROUS CYCLE

By the end of this chapter, the learners should be able to:

- Illustrate different phases of estrous cycle
- Describe stages of estrous cycle
- Explain follicular growth, maturation and ovulation
- Describe heat detection methods and estrus synchronization in cattle

Estrous cycle (noun)/ Ovarian cycle: refers to series of changes in the ovary during which the follicle matures, the ovum is shed and the CL develops. Whereas, estrus/heat is a receptivity for mate. In dairy cattle reproduction, estrous cycle and associated hormones are the corner stones. In females, once the cow attains puberty, the reproductive cyclicity begins and it continues for lifetime. Estrous cycle is characterized by physiological events occurring internally and associated expression of sexual behavior female occurring between beginning and end of estrus (heat) (Fig. 4.1) The length of estrous cycle in cow is 21 days in average (18-24 days). The estrus (standing heat) ranges from 8 to 30 hours. Ovulation approximately 30 hours after the beginning of standing heat (or 12-18 hours after the end of standing heat).

After puberty, the females entered in a reproductive cyclicity that continuous throughout most of their life. Estrous cycle begins with estrus(heat) and ending at the subsequent estrus. Estrous cycle is characterized by repeating periods of receptivity to the male and provide females with repeated opportunities to become pregnant. Estrous cycle results from a complex hormonal interaction among hypothalamus, pituitary and ovary.

The first hormone involved in the estrous cycle is FSH**,** secreted by the anterior pituitary gland. It stimulates the follicle to develop. As the follicle matures granulosal cells begin to secrete the hormone E2, this stimulates the mammary glands to develop. It also prepares the lining of the uterus to receive a fertilized egg. Ovulation is initiated by a surge of another hormone from the anterior pituitary which is LH.

LH influences the development of the corpus luteum, which produces P4, a hormone that prepares the lining of the uterus for the fertilized ovum and readies the mammary glands for milk production. If no pregnancy takes place, the corpus luteum shrinks and the production of progesterone decreases. This causes FSH to be produced again and a new estrous cycle begins. During the estrous cycle the lining of the uterus (endometrium) thickens ready for the fertilized ovum to be implanted.

4.1 Phases of Estrous Cycle

The estrous cycle is characterized by two distinct phases; luteal and follicular phases based on the major hormones and/or ovarian structural changes in a single estrous cycle (Fig. 4.1).

A. Follicular Phase (4-6 days)

The period following luteolysis (regression of corpus luteum) to ovulation. This phase is characterized by animal expressing heat symptoms culminating into ovulation. This phase is dominated by estrogen hormone, which is secreted mainly from

developing follicles/preovulatory follicles. Relatively short in length (~ 20% of the length of the estrous cycle). Characterized by growth and maturation of ovarian follicles (follicles produces E2). Low concentration of blood progesterone as compared from luteal pahse. This phases further divided in to proestrus and estrus.

B. Luteal Phase (14-18)

This is the longest period (~ 80%) of the length of estrous cycle, which occurs between ovulation and subsequent formation and regression of corpus luteum: a temporary endocrine gland, which secretes mainly progesterone hormone. Characterized by growth and maturation of the corpora lutea. There is relatively low FSH and LH concentrations. This phase is further divided into metestrus and diestrus period.

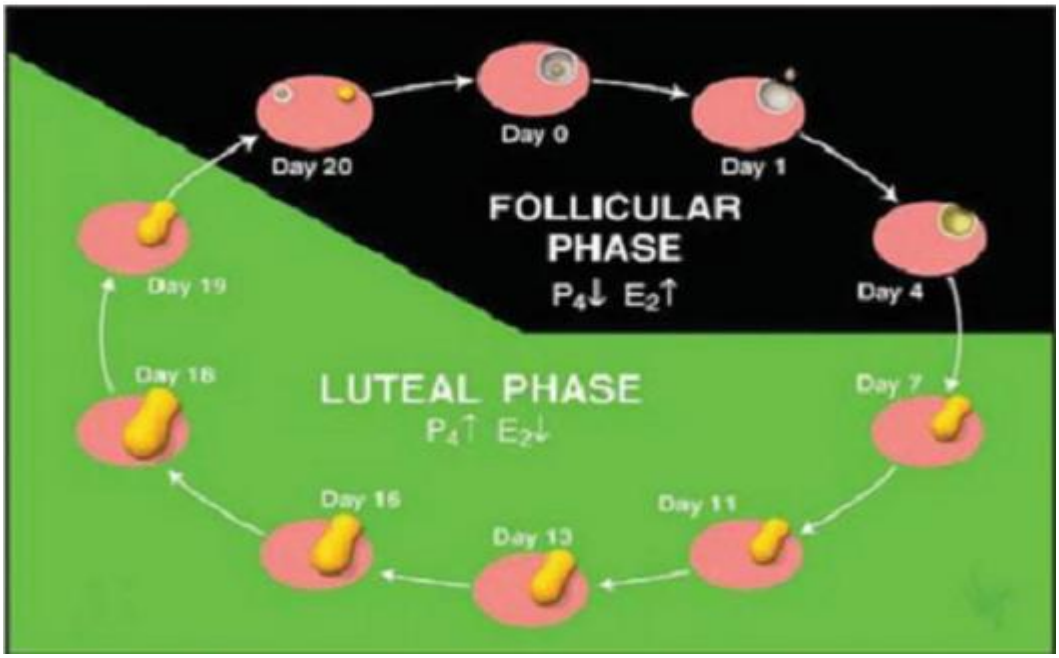


Fig. 4.1. Bovine ovarian changes in estrus cycle

4.2 Stages of Estrous Cycle

The follicular and luteal phases further subdivided in to four distinct stages (Fig. 4.2).

Proestrus (2-5 days)

Proestrus is the first and called building-up phase of estrous cycle. It begins with luteal regression and ends with onset of estrus. There is rapid follicular growth under the influence of FSH and LH secretion and characterized by increasing levels of estrogen hormone which prepares uterus and oviducts for sperm transport and fertilization. Progesterone level decreases. Late in proestrus the vaginal wall thickens, external genitalia may increase in vascularity (e.g., swelling and redness). The uterus enlarges very slightly; the endometrium becomes congested and oedematous, and its glands show evidence of increased secretory activity. The vaginal mucosa becomes hyperemic; the number of cell layers of the epithelium starts to increase, and the superficial layers become cornified.

Estrus (18–24 hr)

Estrus is the time when female is receptive for mating due to peak E2 production (dominant steroid hormone). The main reproductive hormone responsible for estrus behavior and physiological changes in the reproductive tract is estradiol. There is surge production of LH surge: causes ovulation & initiates corpus luteum formation in most species with exceptions: Cow = 25-32 hours after onset of estrus in metestrus. First day of estrus called either Day 1 or Day 0. The uterine and cervical glands secrete increased amounts of mucus. Moreover, vaginal epithelium and endometrium become hyperemic & congested; the cervix is relaxed.

Metestrus (3–5 days)

It is the period from ovulation and formation of CL. Ovulation occurs during this phase in cows, about 12 hours after the end of estrus. Ovulated follicle goes under structural remodeling and starts forming a corpus luteum. During ovulation small blood vessels rupture, and the cavity of the ruptured follicle fills with a blood clot, a corpus hemorrhagicum and granulosa cells also continue to undergo luteinization then developed to yellowish adult corpus luteum. The cellular transformation of the follicle to the CL is called luteinization There beginning of p4 secretion and estrogen levels decreased. The endometrial lining of the uterus thickens; uterine glands enlarge; and uterine muscles show increased development.

Diestrus (10–12 days)

Diestrus is the longest phase, which is the period when corpus luteum is fully functional and dominated by high levels of progesterone hormone secreted from corpus luteum. During this period, animals do not express heat symptoms and sexual receptivity. The uterus is prepared to receive the developing embryo. If the animal will pregnant, the animal stays in diestrus.

Note: A follicle is a sac filled with fluid that has the appearance and feel of a blister whereas, CL looks & feels solid

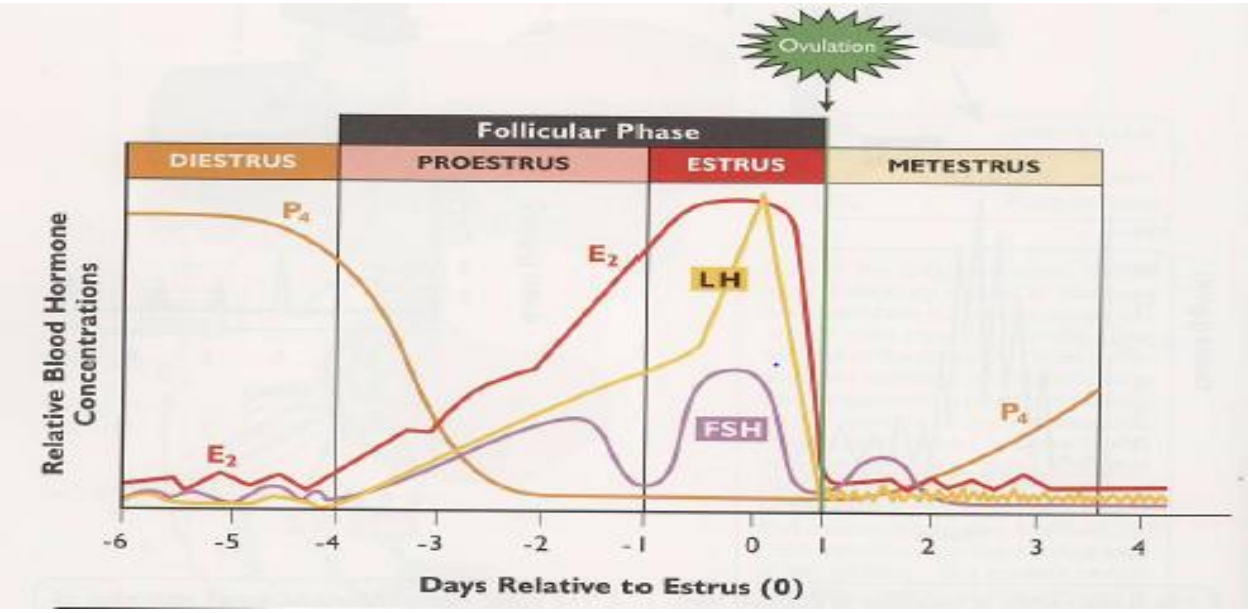


Fig. 4.2. Hormonal changes in estrous cycle

4.3 Estrus (Heat) Detection Methods

The current reproductive technologies (like Artificial insemination and embryo transfer) management programs are highly dependent upon knowing stage of estrous cycle and accurate heat detection procedures for achieving successful outcomes. Conducting two to three daily (AM-PM))visual heat detection observations of the cattle herd during the AI breeding season could lead to economic benefits to dairy producers. Efficient heat detection, is time-consuming, labor-intensive, and requires good management and recordkeeping. Undetected heats play a significant role in lowering reproductive efficiency by increasing the number of open days which in turn results in longer calving intervals and ultimately reduces the net return to the producer. In Ethiopia, identifying animals in heat and determine the time of AI are a bottlenecks for dairy producers. This CPD module has been developed to alleviate the fore mentioned bottleneck factors.

Several factors which are essential for maintain herds` reproductive health. Among different factors; maintaining accurate record, identify animal in estrus or heat using proper detection aids, and maintaining herd health program. The onset of estrus is indicated by primary and secondary signs:

- Primary signs: most reliable
- Secondary signs: less reliable because (vary in length and intensity and confused with symptoms of minor health problem

Estrus detection better when the animals:

- Relaxing during normal activities and better in the coolest times of the day
- Early in the morning and early afternoon (before feeding and milking or after) (6 PM & 6 AM)
- Avoid times of increased excitement or stressful situations, such as feeding or milking

There are two heat detection methods which visual and non-visual methods.

4.3.1 Visual methods

A. Observation of estrous behaviors (sign)

Standing to be mounted is the primary heat sign as it is the surest heat indictor. It is the best sign of a cow's fertile period.

Fig. 4.3. Primary heat sign

There are secondary heat signs considered while observing females:

- Mounting other cows
  - Cows in heat try to ride females not in heat
  - Remember, only cows standing for mounting are in heat and cows that ride may or may not be in heat
- Roughened tail head
  - After standing to be ridden hair on the tail head is rough or rubbed off
  - Easier to notice during winter when hair is longer
  - Muddy forefeet of rider animal may leave marks on lower hips, sides or shoulders
- Friendly/ soliciting
  - Cows in heat follow/ stand beside, put their head on backs or rumps and sometimes they will sniff, nuzzle and lick
- Nervous and restless, Clear mucus from vagina (stringy, clear mucus)

Figure 4.4. Secondary heat sign

Note: Bloody discharge is NOT a sign of heat rather it mean an animal was in heat several days ago. It is better to watch for next heat cycle.

B. Observation for heat mount indicators

There are several aids are available for use in detecting standing heat; nevertheless, none are substitutes for visual observation. If a producer is not able to observe the herd at least two or three times a day during the AI breeding season, the following could be helpful in detecting standing heat.

- Chin-ball markers on vasectomized bulls, Paint on the tail head of cows suspected of heat
- Video tape recorders, Pedometer (activity measurements)
- Other mount signal indicators (heat watch system), Androgenized Animals/ teaser bull

4.3.2 Non-Visual Methods

It is possible to detect animals in heat by measures p4 Level in milk in which p4 is at low level just before, during & just after heat. The plasma concentration of p4 has been reported to be less than 1.0 ng/ml at around estrus which has not been raised appreciably until day 5. Progesterone assay is simple and highly effective in determining the efficiency of herd man in detecting estrus.

4.3.3 New Heat Detection Approach

Eerdenburg et al (1996), recommended that lists of heat signs recorded in 12 hours a day (24hrs) for 30 minutes. During observation, if one observe 12 times per day for 30 minutes, a score of 100 points is reached, the animal is considered to be in heat and can be inseminated if desired. When the cows are observed two or three times per day for 30 minutes, a threshold of 50 points can be applied.

Table 4.1. Eerdenburg Scoring scale for estrous behavior.

Behavior	**points**
Mucous vaginal discharge	3
Cajoling	3
Restlessness	5
Being mounted but not standing	10
Sniffing vagina of other cow	10
Resting with chin on other cow	15
Mounting (or attempting) other cows	35
Mounting head side of other cow	45
Standing heat	100

Review questions

1. What are the phases and stage of estrous cycle
2. Describe at what stage cows ovulate
3. Which hormone is peak in diestrus stage of estrous cycle
4. Describe heat detection methods

5. PHYSIOLOGY OF FERTILIZATION AND PREGNANCY DIAGNOSIS

By the end of this chapter, the learners should be able to:

- Describe methods of pregnancy diagnosis
- Describe the physiology of fertilization
- Describe the common disorders of gestation
- Confirm pregnancy in rectal palpation and ultrasonographically

5.1 Physiology of Fertilization

Fertilization is the process of union of mature male gamete (sperm) with mature female gamete (ovum) to produce new cell which is called (zygote) through chain of events in the oviduct (fallopian tubes). There are sequence of events that occur before fertilization are:

- Egg and sperm transport, Ovum maturation, Capacitation of spermatozoa, Acrosome reaction and
- Cortical granule reaction/ Block of polyspermy

During ovulation, ovum is in metaphase II of the second meiotic division in which ovum maturation and meiosis is not completed until fertilization completed. Sperm requires maturational changes during 10-15 days transport through epididymis

The transport of ovum is enhanced by:

- The ciliated fimbrae collect the ovum during ovulation
- Fluids of abdominal cavity & fluids escaping from follicle during ovulation serve as medium for free-floating ovum
- Cilial action & muscular contraction assists downward transportation of ova to site of fertilization
- Ova transport is under control of ovarian steroid hormones since E2 increases and P4 reduces the speed of passage of ova

Sperm transportation is assisted by:

- Motility: Pass through the cervix by its own movement
- Myometrium contraction is responsible for sperm transport in the uterus
- Before spermatozoa are able to fertilize the ovum, they undergo maturational changes in the female tract
- Maturational changes of spermatozoa known as capacitation and the acrosome reaction are thought to require about 6 hours in the cow

Freshly ejaculated sperm are unable to fertilize an egg that requires series of changes known as capacitation. Capacitation is associated with the removal of adherent seminal plasma proteins, reorganization of plasma membrane lipids and proteins. One of the effects of capacitation is the removal of glycoprotein, meant for sperm protection, from the surface of the sperm cell. The site of sperm capacitation in the female is in uterus or oviduct. This content has been identified as heparin-like glycosaminoglycan. However, events of sperm transport further complicate determination of the site. Fertilization is a complex process and involves a cascade of events.

Closing up: soon after capacitation, there is a hyperactive motility of spermatozoa. The motility patterns changes from a progressive, linear motility in it swim a straight line, into frenzied, dancing motion that is not linear and is localized in a small area. Hyperactive motility occurs throughout the oviduct and is thought to be brought about by the specific molecules produced by the epithelium of oviduct. This type of motility thought to facilitate sperm-oocyte contact.

Zonal Reaction: binding of sperm to zona pellucida requires specific zona-binding proteins on the spermatozoa membrane. Spermatozoa has specific proteins on their the plasma membrane surfaces overlying the acrosome that binds specifically with zonal pellucida proteins. Theses specific proteins is exposed during capacitation process occur before binding to zonal pellucida. On the other hand, zona pellucida consists of three zonal proteins (ZP1, ZP2, and ZP3). The first two are structural protein in which they provide a structural integrity for zona pellucida. ZP3 is a receptor and serve a binding site for proteins on spermatozoa membrane. Such bindings requires 10000-50000 ZP3 molecules. The sperm plasma membrane has two zonal binding sites. The first, primary zonal binding region, is responsible for adherence of spermatozoa to zonal pellucida. The second is believed to be acrosome reaction promoting ligands, in which it initiates acrosome reaction when binding with ZP3. Acrosome reaction is an orderly fusion of the spermatozoa plasma membrane and the outer acrosomal membrane. This acrosome reaction enables the spermatozoa to penetrate the zona pellucida and modifies the equatorial segment so that it can later fuse with the plasma membrane of oocyte.

In the cow, it occurs exclusively in the ampulae of the oviduct to the side ipsilateral to ovulation only near or immediately after ovulation. The acrosome reaction begins the plasma membrane of the spermatozoon forms multiple fusion sites with the outer acrosomal membrane. During such fusion, many small vesicles are formed and this process called vesiculation. After vesiculation, the acrosomal contents dispersed and the sperm nucleus is left with the inner acrosomal membrane surrounding it. The penetration of zona pellucida by spermatozoon is believed to be a few minutes. Following attachment to zona pelucida, the acrosome reaction allows the release of enzymes (acrosin,) that hydrolyze/ penetrate cumulus oophorus, corona radiata and zona pellucida. This small regional dissolution leaves the zona pellucida predominately intact which very important to prevents blastomeres in the early embryo from separating during embryogenesis. When the Spermatozoon completely penetrates the zona and reaches the privitilline space (the space between the zona and the oocyte plasma membrane), it settles into a bed of microvilli formed from the oocyte plasma membrane. The plasma membrane of oocyte fuses with the membrane equatorial segment (by fusion protein) and fertilizing spermatozoon is engulfed. Prior to the acrosome reaction, this fusion protein is inactive. After vesiculation and release of acrosomal contents, the fusion protein is activated, enabling the sperm membrane to bind with the oocyte membrane.

After membrane fusion, the oocyte undergoes a series of changes that prepare it for early embryogenesis eg. Cortical reaction. During first and second meiotic division of oogenesis, small, dense granule called cortical granules move to the periphery of the oocyte cytoplasm. It is made up of mucopolysaccharide, proteases, plasminogen activator, acid phosphatases and peroxidase. After membrane fusion between the oocyte and spermatozoa, the cortical granules undergo exocytosis and their contents are released into the periviteline space. Exocytosis of cortical granules results in the zonal block, a process whereby the zona pellucida undergoes biochemical changes so that further sperm cannot penetrate it. In

addition to alteration of zona pellucida, the cortical reaction reduce the ability of the oocyte plasma membrane to fuse with additional spermatozoa, called vitelline block.

Pronuclei formation: in the cytoplasm of egg, the sperm nucleus undergoes a series of changes, including chromatin decondensation and formation of a new nuclear envelope, to form a male pronucleus. The male pronucleus uses microtubules to migrate to the center of the cell, where it fuses with the female pronucleus to reconstitute a diploid nucleus, called syngamy. On penetration of the vitelline membrane by the spermatozoon, the activated ovum completes meiosis and expels the first and/or second polar body into the perivitelline space. The remaining maternal haploid chromosomes are then enclosed by a pronucleus. Male and female pronuclei migrate to the ovum center, for rearrangements in the cytoskeletal framework of the ovum after activation. Other sperm organelles (e.g. mitochondria) persist during early cleavage stages of the embryo and it is conjectured that they may play a role in development.

Amphymixy: the union of pronucli of male female forms zygote (unicellular), undegoes a series of mitotic divisions which is called cleavage divisions. The first cell divisions produced two-cell embryo, individual cell called blastomere.

Following fertilization:

- The zygote become multicellular and the one-cell zygote rapidly cleaves into 2, 4, 8 and more cells
- The zygote undergo differentiation
- The embryos start to secrete hormones that ensure their survival a process called maternal recognition of pregnancy
- the embryo moves down the oviduct and into the uterus.

5.2 Implantation/Embryo Attachment

The attachment of embryo to uterus is called implantation. There are four steps achieved before embryo attach to uterus:

- Development of embryo within confinement of zona pellucida
- Hatching of the embryonic cells (blastocyst) from the ZP
- Maternal recognition of the pregnancy
- Formation of extra embryonic membranes (e.g placenta)

5.2.1 Embryo Development (Cleavage Divisions)

Mitotic divisions transform zygote into a multicellular embryo

A. Zygote to 2-cell

- Each cell of embryo is called a blastomere, its size decreases with cell divisions
- There is no cell growth, only division of the cytoplasm

B. Two-cell to 4- & 8- cell

- Each blastomere undergoes subsequent division yielding 4 and then 8 daughter cells
- No cell growth: Blastomeres are 1/4 and 1/8 original size
- Divisions continue with embryos for the number of cells present

NB: embryo from 2-8 cell, each cell can be fully developed to individual i.e called Totipotent

C. Morula (Mulberry) (16-32 cells)

- Solid ball of cells (too many blastomeres to count)
- Individual blastomeres become smaller but the size of the embryo remains the same (still no embryo growth)
- In cattle it occurs around day six after fertilization and when embryo is non-surgically recovered (flushing embryo) for embryo transfer
- During late morula stage blastomere cells begin to differentiate into two distinct populations, the inner and outer cells
  - Inner cells: develop gap junctions which is important for intercellular communication
  - Outer cells: develop tight junctions. Alter permeability of the outer cells allowing for fluid accumulation inside of the embryo
- Development from morula to blastocyst
  - Intracellular fluid creates a distinct cavity (hollow) inside of the embryo
  - Hollow center of blastocyst called the blastocoele
- Balstocyst have two cell types present
  - Trophectoderm (Outer single layer of cells). Develop into chorion and contributes to the placenta
  - Inner cell mass (ICM): Develop into fetus
- Blastocyst is the first stage where embryo grows in size

5.2.2 Maternal Recognition of pregnancy (MRP)

It is a functional relationship between the uterus, CL, and embryo itself. . During early pregnancy, the blastocyst (before attachment) must signal its presence to the maternal system to stimulate CL maintenance for establishment of pregnancy. Once MRP established luteolysis is prevented and pregnancy continued. MRP is estabilished when the length of the estrous cycle exceeds than normal cycle. The main events during MRP are prolongation of the life span CL and conceptus enforcement of maternal immune response (production of interferon tau).

The signal which originates from the pre-attached blastocyst acts either directly at the endometrial level cow to block the action of $PGF_2\alpha$. The developing embryo enters the uterus between day 2 and 5 after ovulation depending on the species. For the early embryo to become an established pregnancy, luteolysis must be prevented (CL maintained). The conceptus must provide a timely (before luteolysis) biochemical signal:

- Conceptus signals its presence to the dam
- Signals enable pregnancy to continue
- If a signal is not delivered quick enough, luteolysis will occur, progesterone will decline, and the early embryo will die

Second events of MRP is that the blastocyst begins to secrete trophoblastic protein known as bovine interferon -tau (bIFN τ) as a means of MRP in cows. The trophoblast produces bIFNτ between day 13-21 days as the conceptus elongates

(spherical to tubular to filamentous). The bIFNτ do not enhance progesterone production directly. Rather the secreted bIFNτ bind endometrium and inhibit endometrial oxytocin receptor synthesis which prohibit pulsatility PGF2α secretion therefore luteolysis does not occur (remember, oxytocin, oxytocin receptors, progesterone, estradiol, and PGF2α all play a role in luteolysis). The fore mentioned, mechanism prevents the rejection of allogenic conceptus and maintained till parturition. In addition, bIFNτ also promotes protein synthesis thought to be critical to pre-attachment embryonic survival

5.3 Pregnancy Diagnosis

Early pregnancy confirmation is very important in dairy management and minimizing economic loss. In general, pregnancy diagnosis is necessary for:

- Combating infertility by early bred non pregnant animals
- Avoiding blanket feeding i.e. animals should be fed based on their physiological status
- Reducing waste in breeding programs using expensive hormonal protocol
- Certifying animals for sale

Methods of Pregnancy Diagnosis

1. Visual Method

A. Non-return to estrus (wait till 24 days)

Once conceptus formed regression of corpus luteum is inhibited and prevent further returning to estrus. Therefore, an animal not returning to estrus after service is assumed to be pregnant. Producers, several artificial inseminators and professional used this methods as an indicator of pregnancy. However, there is inaccuracies due to:

- Cows may fail to come return to estrus other than pregnancy like at anestrus stage
- Needs efficient and accurate heat detection
- Failure in regression of prior CL due to abnormalities (endometritis, endocrine insufficiency etc)
- Silent estrus

B. Observing changes in genital system

As pregnancy advance there is growth and development of udder, enlargement of belly and external genital organs.

2. Clinical Methods

A. Rectal Palpation

This is the oldest and most widely used method. In most large domestic animal, it is the easiest, cheapest and fastest method of pregnancy diagnosis with minimal invasive to the animal and its fetus when performed cautiously. As a drawback, it is difficult to identify the sex of fetus. Structures used as indicator of pregnancy:

Cervix: Transrectal palpation begins identification of cervix, which is thick walled, firm, cartilaginous and feels like chicken neck due to 3-4 annular rings in cows. The cervix is chiefly a landmark serving as a guide for locating other structures. Its position gives an indication of the stage of pregnancy, but a diagnosis should never be based on the cervix alone. In the nongravid or early pregnant uterus, the cervix is typically found within or just at the brim of the pelvis. In advanced pregnancy, the cervix found pulled (by the weight of the uterus and contents) more cranial and ventral.

It's vital to keep in mind that the cervix is movable, so finding it can require sweeping the pelvic canal and working across the brim of the pelvis. Once located, the cervix can be used to further access and examine these structures by pulling the body, horns, and uterus caudally. The palpator can also locate the bifurcation of the horns by following the cervix cranially to the body of the uterus. To allow for comprehensive probing of each horn at this point, the ventral intercornual ligament can be utilized to retract the horns and lay them along the pelvic floor. Just beyond the tip of each horn, the ovaries can be palpated in dorsolateral in the region of ilium bone.

Uterus: Mostly diagnosis is based on the uterus and its contents. Size of the uterus (asymmetry) influences its position in relation to the pelvis. Uterine wall becomes thinner as pregnancy progresses and is very resilient to touch compared with the uterus of the open cow.

Foetal membrane slip: Gently grasping of uterine wall b/n the thumb & forefinger & lifting slightly can detect chorionic membrane as early as 30 days of pregnancy. If pregnant, a distinct popping sensation can be felt as the membrane slips from the grasp within the uterine walls. At 32 days of pregnancy it gives the impression of a thread and by day 45 a small string slipping from the fingers in the gravid horn. By day 60, it is palpable in both horns as a somewhat larger string and by day 75 is approaching the feel of a piece of yarn slipping from the fingers. It is used up to day 90 of gestation period.

Amniotic Vesicle: It is somewhat turgid, fluid-filled sac surrounding and protecting embryo/fetus peculiarly in early pregnancy. It can be palpated as a small knot/bump within the uterine horn nearly 6–7 mm in diameter by 32–35 days (5 weeks) of gestation. By 6 weeks of gestation it will be 1.5 cm in diameter and at 7 weeks it will be 3.5–5 cm in diameter. By 8 weeks, the vesicle will be 6–7 cm, will be losing its turgidity, generally allowing palpation of the fetus around day 60. Early in pregnancy, the vesicle is turgid, but as the pregnancy progresses, it becomes flaccid until days 65 to 70, when it becomes very difficult to identify.

Fetus: Around days 55 to 60, the fetus will be palpable and will be around 5 to 6 cm long. It can be felt floating in the gravid horn fluid by gently cupping the horn with the fingers and gliding them along the length of the pregnant horn. A wave of fluid might also be created by lightly tapping the uterine wall with the fingers, which would cause the fetus to bump up against the fingers (ballottement). As the pregnancy advances, fetal parts may more readily be identified such as the head, feet and legs. By 3-4 months of pregnancy, the uterus and fetus will drop over the brim of the pelvis into the abdominal cavity and by 4–5 months of pregnancy the fetus may descend into the abdomen far enough making palpation difficult, particularly in large deep-bodied cows. Usually by 7 months to term, the fetus has grown large enough that it will be readily accessible and comeback into pelvic cavity.

Placentome: Placentomes (button like formed by dam caruncle and fetal cotyledon) is another positive sign of pregnancy & detectable from about 75 days to term. It can be detected as soft, thickened lumps in uterine wall & more easily detached as pregnancy advances. Ensuring that the ovaries are not mistaken for placentomes

Ovaries: The ovaries can be palpated up to about 120 days for the presences of CL. However, one must remember that a corpus luteum is not always accompanied by pregnancy.

Pulse of pregnancy (Fremitus): This is helpful in confirming a diagnosis and the viability of calf, particularly at certain stages of pregnancy. The middle uterine artery will have expanded to a sufficient degree by 120 days of pregnancy to be used as a differential diagnostic in pregnancy determination. After 80 to 90 days of pregnancy, the uterine artery ipsilateral to the pregnant horn enlarges.

Stage of Gestation

Palpation at 35 to 40 days: Uterus on the floor of the pelvis, except in large cows with elongated reproductive tracts. There is a slim enlargement of one horn with detectable dorsal bulging. Foetal membrane slip and CL can be felt adjacent to the gravid horn.

Palpation at 45 to 50 days: Foetal membrane slip, the uterus still on the pelvic floor and CL is palpable ipsilateral to gravid horn.

Palpation at 60 days (2 months): The gravid uterine horn will be dropping slightly over the brim of the pelvis and feels like balloon filled water. Foetal membrane slip. Corpus luteum on the ovary adjacent to gravid horn.

Palpation at 90 days (3 months): The uterus will be pulled well over the pelvic brim and will be 8 to 10 cm in diameter. The foetus will be 10 to 15 cm long and easily palpated. Corpus luteum on the ovary adjacent to gravid horn.

Palpation at 120 days (4 months): The uterus will be well over the brim of the pelvis with the cervix pulled almost to the pelvic brim. The foetus can be easily palpated and will be from 25 to 30 cm long. Small palcentomes can be identified. The ovaries may be difficult to reach, but a corpus luteum will be present on the ovary adjacent to the gravid horn.

Palpation at 150 days: (5 months): The uterus will be pulled well into the abdominal cavity and the cervix will be located at the brim of the pelvis. Distinct placentomes about the size of ovaries can be identified. The foetus is well formed and will be 35 to 40cm in length but may be difficult to reach in larger cows. The pulse of pregnancy (fremitus) will be quite distinct with the artery being 6 mm to 1.25 cm in diameter

Palpation at 170 to 230 days (5.5 to 7.5 months): Cervix will be at the brim of the pelvis and may be bent over the edge. The dorsal wall of the uterus will be tight and difficult to palpate. The placentomes will vary in size and may be difficult to palpate because of the tight uterine wall.

Palpation at 170 to 230 days (5.5 to 7.5 months): The foetus will be large enough to extend back within range of the hand. The head and front feet are usually the structures palpated. Movement of the foetus can frequently be detected

https://www.youtube.com/watch?v=w-j8HL2OHEY&t=132s

https://www.youtube.com/watch?v=c7XWi91SuTE

B. Ultrasound

There are two types of ultrasound which are Doppler phenomenon and pulse-echo principle. The first is used to appreciate movement of fetal heart flow in the fetal (umbilical vessels) or maternal (uterine artery). The second one, pulses are generated by piezoelectric crystals in a transducer, on contacting tissues of varying acoustic impedance (resistance to the transmission), are reflected (echoed) to the transducer, then converted into electrical energy and displayed on a cathode ray oscilloscope in various ways. Under pulse-echo principle, A-mode (amplitude) is a one dimensional display of echo amplitude versus distance. B-mode (brightness) ultrasound produce an accurate two dimensional image of tissue cross section.

Transrectal ultrasound is most accurate and useful gadgets for pregnancy diagnosis. To generate sound of high frequency and extremely small object is made to ring or vibrate. For ultrasound, a piezoelectric quartz crystal about the size of match head is used. When a voltage is applied to this crystal it changes shape and when the voltage is switched off it resumes its original shape. Frequent voltage pulses cause this crystal to change shape rapidly and vibrate. A piezoelectric crystal in addition to giving off ultrasound it also receives returning sound waves (echoes). Thus it converts applied electrical energy into ultrasound energy (vibrations) and alternatively mechanical energy (echoes) into electrical energy.

The probe or transducer that house the crystal(s) can be used to transmit energy and detect the returned echoes. When ultrasound waves meet a boundary or interface between two substances of varying density/stiffness such as tissues, bones, fluid air, etc, some of the sound wave are echoed back to the probe. Substances with similar characteristics such as muscle and liver reflect only small proportion of sound waves. The remaining waves continue on the body to provide information about the underlying interface. This is important because any interface that reflects a large proportion of sound waves will provide information about deeper lying structures. It determines the number and sex of the fetuses. Coupling gel prevent interference of air between the organ and the probe.

In summary, the image created by ultrasound imaging

- White for bone
- Black for fluid like amniotic, follicular
- Grey for tissue

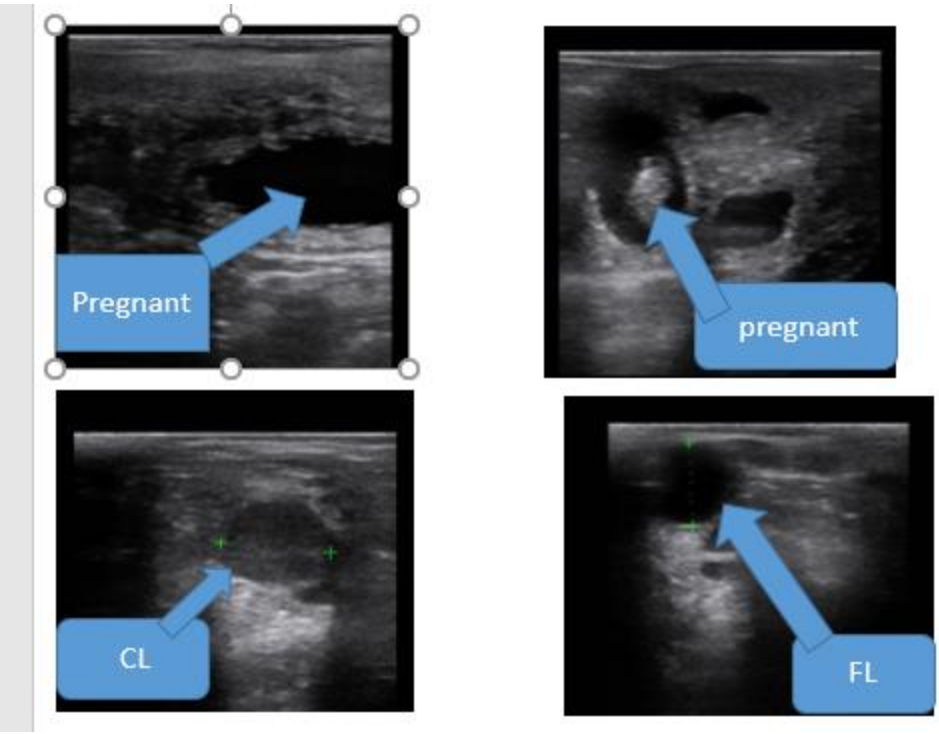


Figure 5.1. image of conception at day 32, FL=follicle; CL = Corpus luteum April 2018 @ Asella, Alebachew *et al*., 2018

https://www.youtube.com/watch?v=0MoprQ6tEhY

https://www.youtube.com/watch?v=-pWJ-qjsPpM&t=116s

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https://www.youtube.com/watch?v=-pWJ-qjsPpM

https://www.youtube.com/watch?v=QPt04WA9Ixg

3. Immunological Pregnancy Diagnosis

This techniques rely on detecting and measuring the level of pregnancy associated hormones/ pregnancy specific molecules in the conceptus, the uterus, or ovaries that enter to dam circulation. Substances found in urine, milk or blood. Unlike rectal palpation and transrectal ultrasound, this cannot be used to estimate the stage of gestation.

Progesterone (P4): Collecting milk/plasma/serum during 19-24 days post service. If the female is open, progesterone level will decrease to baseline as CL is regressed and new follicular growth triggered. We can seldom find when the animal is not pregnant and its accuracy is 86%. The rest 14% might be due to failure of CL regression in cases like uterine inflammation. The level of P4 is determined in radioimmunoassay (RIA), ELISA and electro immunoassay (EIA). Though the level of P4 varies in estrous cycle lengths between individual animals, less than 1 ng/ml indicated the animal is open.

Pregnancy Specific Substances: Pregnancy specific protein B (PSPB) or bovine pregnancy associated glycoprotein 1 ($bPAG_1$): Produced by fetal trophoblast binucleate cell (BNC) in bovine placenta. PSPB and $bPAG_1$ detected by radioimmunoassay (RIA) or EIA in pregnant cow serum from day 24 of pregnancy until parturition, peak at parturition. They are inactive member of aspartic acid proteinase family. Both have identical gene nucleotide sequences but differ in

carbohydrate and sialic acid content. However, there is a limitation to use these as a confirmatory of pregnancy due to its long half-life (slow decline in concentrations in maternal serum) i.e mislead during:

- After embryonic/fetal death
- During early postpartum

Estrone sulphate: In all domestic animals esterone sulfate is produced by placenta during pregnancy. It is detected after 72 days of pregnancy in cows. Its secretion progressively increases until parturition. This progressive increase in concentration enables monitoring of feto-placental development.

5.4 Accident of Gestation

The conceptus may be exposed to harmful agents during the pre-attachment, embryonic or fetal stages of development, and vulnerability to these agents varies with these different stages. During the pre-attachment stage the embryo is very resistant to teratogens and the zona pellucida is an efficient barrier to many viruses. In contrast, the embryonic stage, with rapid cell growth and differentiation, is most susceptible to teratogens. Furthermore, each organ has a critical period of development. For example, the palate, cerebellum and urogenital systems develop relatively late in the fetal period. It should also be remembered that the membranes are part of the conceptus and so any impairment to their development will affect the fetus.

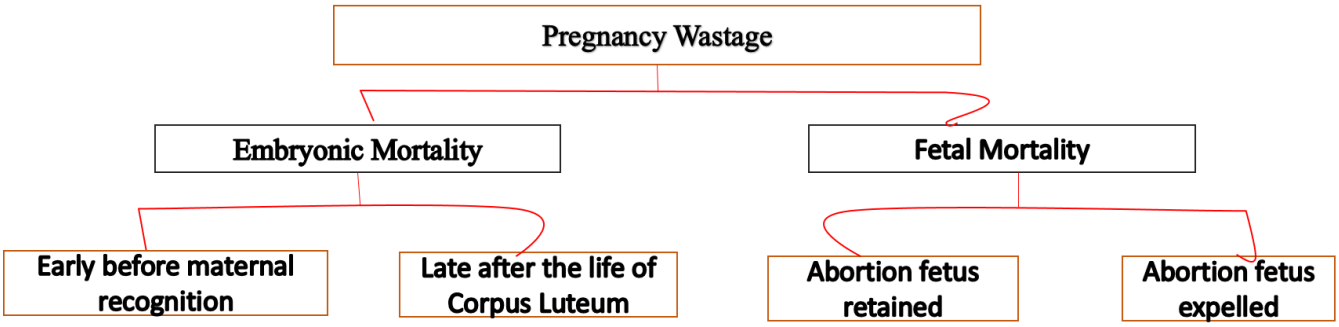


Fig. 5.2 Pregnancy wastage

Embryonic Mortality: embryonic loss before fetal maternal recognition, and did not involve the elongation of the life of the corpus luteum called early embryonic death (EED). Embryonic loss after the life of the corpus luteum has been extended is referred as late embryonic death (LED). In beef cattle most loss are occurred before day 15 post-service, and in dairy heifers losses plateau after about day 19 post-service. Generally speaking, embryonic loss may be due to either genetic or environmental factors or a combination of the two. The exact effect of each factor depends upon when, during gestation, it is encountered and how it exerts its influence.

Environmental factors causing embryonic/fetal loss: These include climate, nutrition, stress, ovulation rate, failure of the normal fetomaternal recognition factors, uterine conditions, hormones, infectious agents, and teratogens. Some infectious agents causing embryonic or fetal loss in cow in many ways:

- Impaired sperm survival or transport in the female tract, leading to reduced fertilization rate
- Direct effects upon the embryo. Leads to embryonic death, and those that infect the more advanced fetus or its placenta, resulting in abortion, stillbirths or the birth of weak calves.
- Indirect effects upon embryo survival. Exert adverse effects on uterine function and those that infect the maternal component of the placenta and results in embryonic death, fetal death with abortion, mummification or stillbirth.
- Systemic illness causing fetal losses (e.g. pyrexia-induced abortion) or a direct impairment of reproductive cyclicity

Embryonic mortality could be caused by a number of viruses, bacteria and protozoa specific pathogens. These pathogens enter the uterus by the haematogenous route (primary infection of the female with T. gondii) or via the vagina at natural service (C. fetus, vibriosis, T. foetus) or at insemination like in Bovine viral diarrhea virus (BVDV). Whereas, non-specific pathogens mainly bacteria that enter the uterus by ascending infection or at the time of insemination leads endometritis could leads to embryonic loss. Subclinical endometritis is a silent cause of embryonic mortality and development of uterine infections have been reported to be associated with an increased incidence of COD (cystic ovarian disease).

Regarding nutritional; following parturition, the animals are in negative energy balance which affects the subsequent reproduction. Some of plant toxins that leads to reproductive problems include mycotoxins, endophyte infected fescue, nitrates, locoweed, and ponderosa pine. Mycotoxins developed in moldy feed could cause abortions in cattle by decreasing progesterone concentrations. Crude protein in the total diet greater than 17 -20% has been implicated in lowering conception rates with increases seen in the number of services per conception and days open. Some studies have indicated that blood urea nitrogen above 20 mg/100 ml may decrease the chances of pregnancy.

Endocrinological causes like low concentrations of progesterone in the cycle preceding oestrus on subsequent embryo survival is premature oocyte maturation, which compromises the ability of the embryo to continue normal development after fertilization.

The main natural physical environmental factors affecting livestock system includes air temperature, relative humidity, solar radiation, atmospheric pressure and wind speed. The environmental factor like heat stress seems to have the greatest impact on embryo survival. Heat stress decreases blood progesterone concentration, which is a major cause for abnormal oocyte maturation, implantation failure and finally early embryonic death in dairy cattle

Teratogenic agents in cows are: Virus; Akabane virus, Bluetongue virus, Bovine viral diarrhoea virus, Rift valley fever virus, Wesselbron virus; Plants: Lupins and others (Hyperthermia, Iodine deficiency).

Genetic causes of embryonic/fetal loss: due to chromosomal defects, individual genes and genetic interactions. Genetic causes of embryonic/fetal loss include single-gene defects, polygenic abnormalities and chromosomal anomalies. A few single-gene mutations are lethal and result in the death of the conceptus. If the gene is dominant, a single copy may be sufficient to cause death, whilst in other instances it is only the homozygous state that is lethal (e.g. the dominant Manx gene (M) in the cat). Recessive genes only exert their effect in the homozygous state. Not all genetic defects are lethal. Some abnormal fetuses survive to term that are biologically and economically wasteful. E.g. Achondroplasia, Hairless condition, Amputates, Syndactyly, Arachnomelia, polyspermia etc.

Detection of embryonic/fetal loss: a cow might be suspected when there is an irregular extension of the interoestrous period. However, this will be an underestimate of total loss because it will not detect that which is occurring early on, before the maternal recognition of pregnancy and the resultant extension of the life of the corpus luteum. Some scholars concluded that increase in the interval between service and return to oestrus beyond the usual range of 17-25 days reflects embryonic mortality. Determining p4 in blood/milk is also important to estimate embryo mortality.

Examining embryos collected by in vivo flushing of the reproductive tract at different days after breeding. 4. Determining P4 in blood or milk

More accurate estimations of embryonic loss can be made by slaughtering at different times during gestation and correlating the number of embryos with the number of corpora lutea. However, this method requires the sacrifice of the animal and hence the loss of the pregnancy. A non-invasive method is preferable, but one such as the per rectum examination of the fetus has the disadvantage that it can be carried out only in the larger domestic animals. Furthermore, since the pregnancy can be palpated only relatively late, early embryonic loss goes undetected. More recently, ultrasonic scanning, such as Doppler, A-mode and real time B-mode techniques, has allowed the very early detection of pregnancy and embryonic loss in a non-invasive manner.

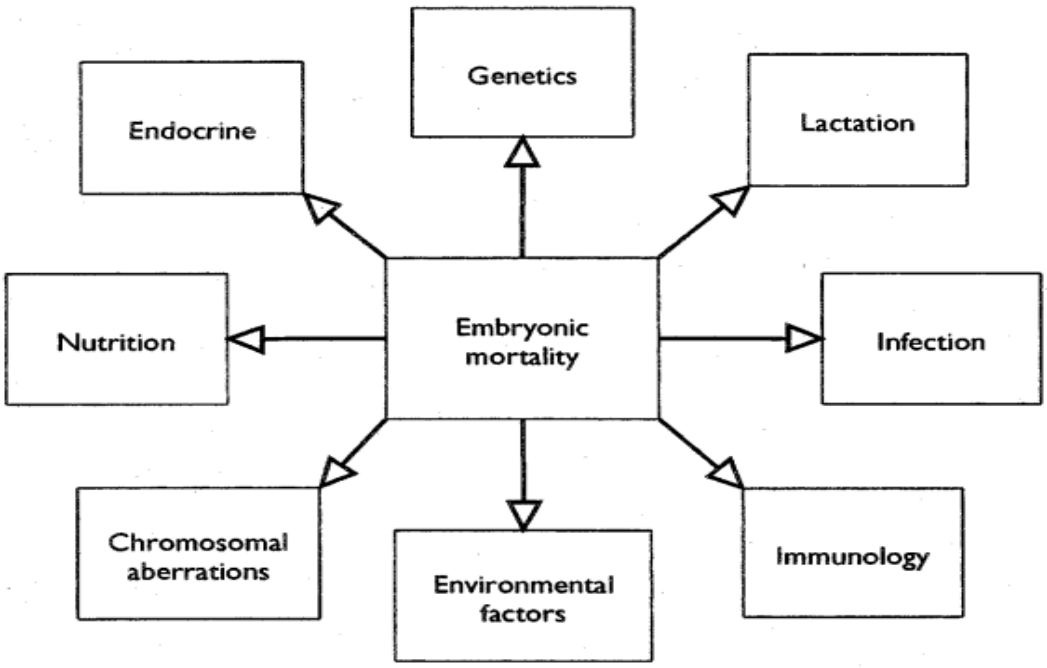


Fig. 5.3 Causes of embryo mortality

Abortion: Termination of pregnancy with the expulsion of the fetus before it is viable**.** Abortion after five months of gestation in cows is usually followed by retention**.** In most abortions, the fetus is expelled within 24-72 hours**.** During this time postmortem decomposition or autolysis occurred. Agents/factors that affect the fetus**, f**etal membranes**, m**aternal placenta or endometrium or combinations of these causes abortion. Broadly, abortion can be caused by infectious or noninfectious.

Table 5.1 Infectious causes of abortions

Cows	Transmission	Clinical finding
Trichomonas	Venereal	Abortion in 1st trimester, pyometra and repeat breeding
Brucellosis	Ingestion/venereal	Abortion in last trimester (90% rate in susceptible herd
Vibriosis	Venereal	Abortion at 3-4 months, 5-10% infertility
Leptospirosis	Cutaneous/mucous Membrane abrations	Abortion in late trimester, 25-30% fetal death is common
Listeriosis	Contaminated feed Low	Abortion 4-last trimester, associated with septicemia, placentitis
IBR	Aerosol	Abortion in 2nd half of gestation (with a rate of 25-50%)
Mycosis	Inhalation	Abortion at 3-4 months (the rate is less than 10%)

Non-infectious causes of abortion

- Heat stress: cause fetal hypotension, hypoxia, acidosis
- High maternal temperature in case of pyrexia
- Trauma
- Toxins: plant (Astragalus spp contain alkaloids that could affects:
  - Corpus luteum, Chorioallantois
  - Fetal neurons; all these lead to abortion or abnormalities
  - Grasses or moldy sweet clover
  - Mycotoxicosis: particularly those having estrogen

Investigation of abortion

Investigating every abortion may be considered uneconomical. scholars Caldow and Gray (2004) suggest an interference level of 3 % for abortions that occur after pregnancy is confirmed at around six weeks, based on reports that quote unavoidable fetal losses at around 1.7 to 2%. Each abortion should be considered in the light of other clinical findings, such as whether there has been a cluster of abortions or if the aborting cow(s) is/are ill. Rectal palpation and ultrasonographic images of embryo/fetus can confirm fetal loss. It must be remembered that many abortifacient agents are potential zoonoses and, a farmer has a legal responsibility to protect staff from avoidable health hazards.A detailed examination to be undertaken, the entire fetus and placenta should be submitted to the laboratory for an abortion investigation. Appropriate tests will be authorised at the discretion of the veterinary investigation carrying out the investigation. Placenta (or placentome) is often not submitted, but is frequently very useful in establishing a diagnosis, particularly for fungal, B licheniformis and Chlamydophila infections. The following information is also helpful in assessing the extent of the problem and providing clues about possible pathogens:

- Age of the dam (heifer/cow), Approximate stage of gestation, Approximate date of first abortion;

- Number of abortions occurred so far, Number of normal calvings, Any illness in dams or other disease problems, Whether animals are housed or at grass, Diet; Recent purchases, stock movements or feed changes, Herd serological status (from bulk milk or individual cow tests), Vaccination status
- History of previous disease/infections in the herd.
- Serological tests necessary for confirmation; in BVD; Rising titers to BVD virus are rarely demonstrated in cases of abortion because of the delay b/n infection and abortion. High titres can be maintained for many years after infection and, therefore, are not necessarily an indication of recent infection. Serology could be considered to rule out BVD virus infection, if the aborted cow is sero negative (and antigen negative), it is unlikely BVD was involved.
- Serology for *Leptospira* species is usually unhelpful in individual aborting animals, as antibody titers are falling or absent at the time of abortion. Further investigation is suggested for unvaccinated herds.

Actions During Occurs of Abortion Case

If abortion occurred in a farm/herd; the producers should do the following:

- Call veterinarian advisors, careful in handling the cow as well as the aborted materials
- Dispose of all the aborted materials away from the farm/ homestead by deep burial
- Keep the affected animals separated from the healthy animals
- Cull the cow if found infected with brucellosis (should be confirmed by serological test)

Prevention of Abortion

Preventing abortion is suggested for herd management than control and treating abortion. The following techniques are important to prevent abortion:

- Biosecurity practices could minimize the incidence of introducing diseases onto the farm, and the spread of disease within the herd. This may include quarantining purchased animals for a period of time, or maintaining a completely 'closed' herd. In addition, visitors to the farm could also be required to wear clean clothing and to disinfect footwear and any equipment that may have been in contact with other cattle.
- Maintaining the general health & immune function of the cattle is also important in minimizing the risk of abortion problems. Providing an adequate amount of a properly formulated and delivered ration, and providing a clean, comfortable and minimal-stress environment are essential to accomplishing this task. Special attention to possible contamination of the ration with molds and toxins is likely warranted.
- Although vaccination is not a remedy for poor management, it is an integral component of a complete herd health program. Safe and efficacious vaccines are available for many of the infectious diseases that can cause abortions in cattle
- Health status of bulls in herds that utilize bulls for natural mating or AI should be evaluated to prevent venereal disease transmission.
- Keep the floor, manger & watering trough, farm utensils, drainage system and all the surroundings clean and disinfected
- Do not expose the pregnant heifer/cow to extreme environ mental temperature. Make provision for proper air circulation in the shed during summer so that heat stress could be minimized;

- Do not transport the pregnant cow/heifer for a long distance causing exhaustion
- Do not administer any medicine without consultation of a qualified veterinarian

Mummification: It is a retention of dead fetus in uterus in which fetal fluid resorption with formation of dry mass of fetus**.** Dehydration of the fetus and fetal membranes. Fetal membranes wrapped around the fetus in the uterus. It is more common in cattle and swine

Fetal Macerations

It is a sequella to mummification & fetal emphysema. It occurs in all species, most frequently in cattle. Occurs as a consequence to the failure of aborted fetus to be expelled, may be due to uterine inertia and bacteria enters through the dilated cervix and causes putrefaction. The soft tissue are digested by autolysis and mass of bones remains unaffected and embedded in the uterine wall, difficult to remove and could perforate the uterine wall. If the case is chronic hysterectomy is a best intervention.

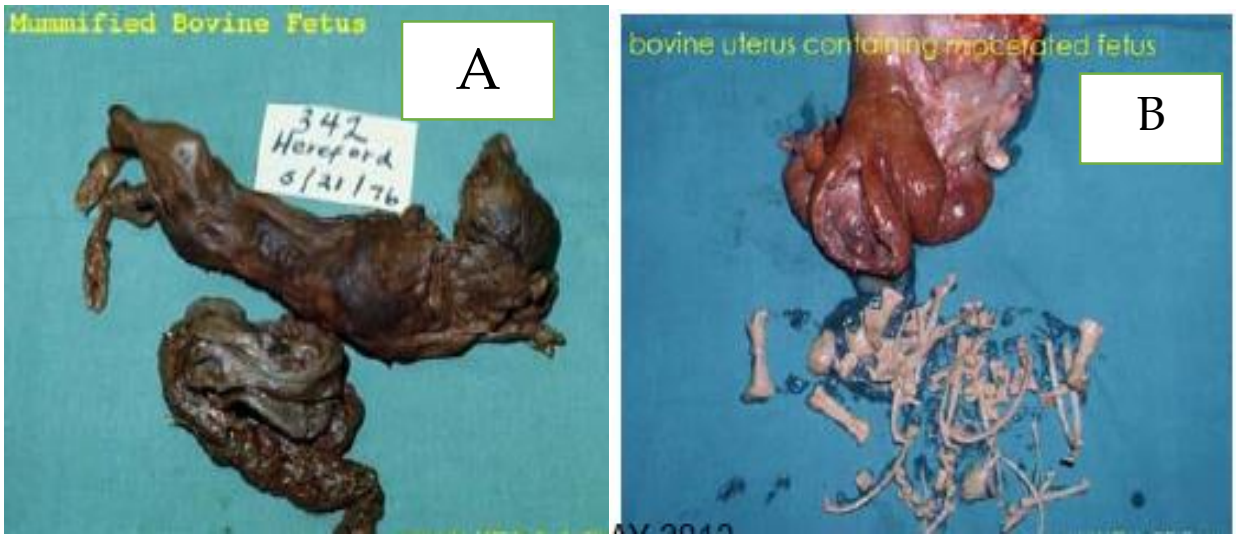


Figure 5.4. Mummified fetus (A); Macerated fetus (B)

Cervico-Vaginal Prolapse

It is a disorder of ruminants normally in late gestation. Occasionally it is seen after parturition and rarely it occurs unconnected with pregnancy or parturition. It can be recognized by the protrusion of varying parts of the vaginal wall and the cervix through the vulva so that the vaginal mucosa is exposed. The exact cause of the disorder has not been ascertained but several factors are generally believed to play a part; An excessive deposition of fat in the perivaginal connective tissue and ligamentous relaxation may increase the mobility of the vagina. These effects might be due to a state of endocrine imbalance, in which oestrogenic hormones predominate; the administration of stilboestrol has been shown to soften the suspensory ligaments of the genital tract. Where oestrogenic substances are present in inordinate amounts in the diet, as in subterranean clover pastures or in mouldy maize and barley which are considered to have a high oestrogen content, this can

result in a high incidence of prolapse. When heifers are fed these in their diet they may show vulvovaginitis with oedema of the vulva, relaxation of the pelvic ligaments, tenesmus and vaginal prolapse.

Postparturient prolapse of the vagina of cattle is usually due to severe straining in response to vaginal trauma, infection, following a serious dystocia. Vaginal contusion at parturition, followed by *Fusobacterium necrophorum* infection, exerts a high degree of irritation with frequent exhausting expulsive efforts.

REVIEW QUESTIONS

1. Describe different methods for pregnancy diagnosis
2. List down the differential diagnosis for pregnancy
3. What are the common disorders of gestation?
4. How could it possible to estimate gestation stage.

6. PARTURITION AND POSTPARTUM COMPLICATIONS

By the end of this chapter, the learners should be able to:

☞Explain physiology of parturition
☞Identify different postpartum complications
☞Describe different causes and methods of correction of dystocia

Parturition is an act that involves various physiological processes which result in the birth of offspring's & placenta. It begins with softening and initial dilation of the cervix along with the start of uterine contraction & ends when the fetus and associated membranes are expelled. Parturition is accompanied by lactogenesis that help to adequately supply the neonate with nutrients immediately after birth

6.1 Initiation of parturition

Parturition is triggered by fetus and maternal mechanisms completed by a complex interaction of endocrine, neural and mechanical factors and it is obligatory for initiation. The primary precursor for initiation of parturition appears to be interplay of prostaglandins and fetal cortisol in the cow. Fetus controls day of parturition based on the stress response for lack of space, gas exchange and nutrients.

Fetal changes

Increasing stress (requirement of space and nutrition) on placenta during rapid growth phase (last trimester) stimulate production of cortisol which initiates delivery through increased secretion of PGE_2 and, as a result, induce PGF2α. The increasing output of fetal corticoids stimulates the maternal conversion of P4 to E2 by activating the enzyme 17α-hydroxy1ase. Sequentially, the increased maternal E2 has a direct effect on the cotyledon &caruncle complex to stimulate the production & release of PGF2α. Slow decline in P4 and rise in E2 is due to activation of the enzyme phospholipase A2 that stimulates the release of arachidonic acid from phospholipids. Then, the arachidonic acid converts to PGF2α via cyclo-oxygenase (COX) path-way.PGF2α induces myometrial contraction, which increases intrauterine pressure and moves the fetus towards the cervix, causing further cervical dilation.

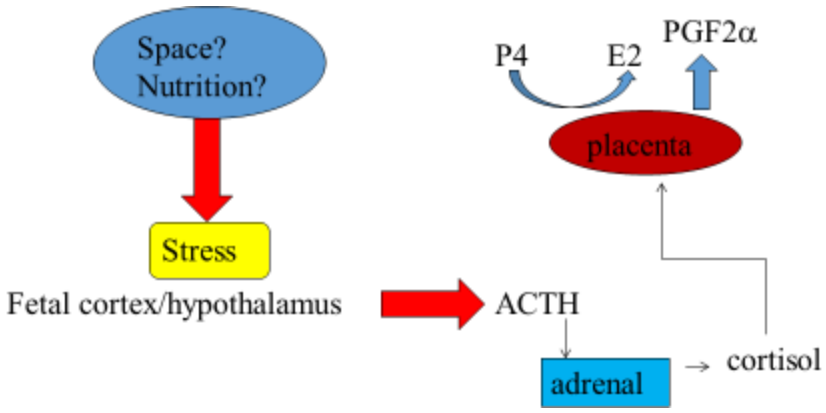


Fig. 6.1 Fetal changes during parturition

Maternal mechanisms for parturition

Maternal presence in parturition is evident. Anxiety, fear and stress prolong the act of parturition through a decrease in myometrial contraction due to release of epinephrine. The essential prepartum events in the mother are those which ensure myometrial contractility, cervical dilation, lactogenesis and specific behavioral changes including nest making.

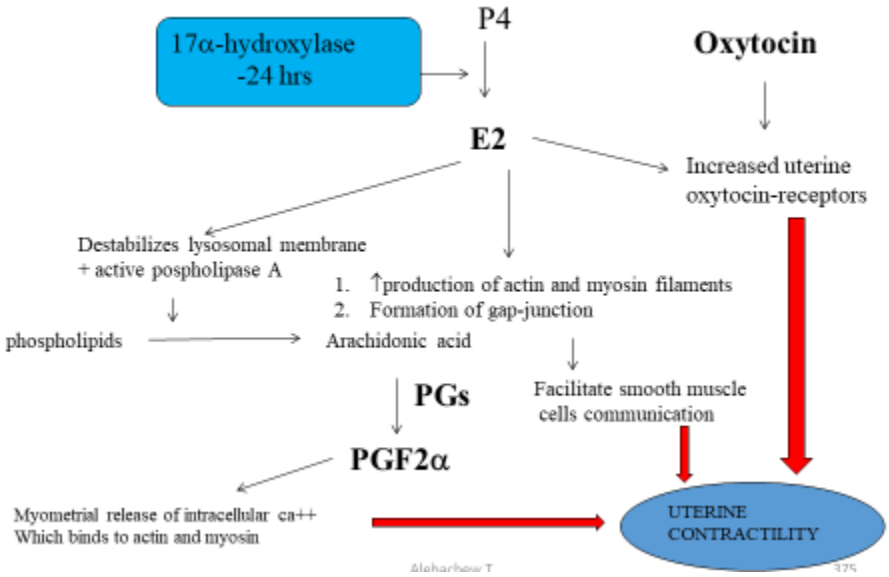


Fig. 6.2 Uterine change during parturition

The Fergusson reflex (fetal ejection reflex)

It is the neuroendocrine reflex comprising the self-sustaining cycle of uterine contractions initiated by pressure at the cervix or vaginal walls i.e +ve feedback. Pressure on the cervix brought about by increased myomerial contractions activates pressure sensitive neurons located in the cervix that synapse in the spinal cord & eventually synapse with oxytocin producing neurons in the hypothalamus. Oxytocin, released into the systemic circulation, acts to facilitate the myometrial contractility initiated by estradiol and by PGf2a. As the pressure against the cervix continues to increase, so does the oxytocin secretion, and thus the force of contraction of the myomerial smooth muscle begins to peak. When this occurs, the fetus enters the cervical canal & the first stage of parturition is complete.

Changes in the cervix (cervical ripening)

Smooth muscle component of the cervix reacts to changes in steroids similar to myometrium. Dilation is induced by maternal steroids and prostaglandins released locally. Cervical softening (ripening) involves edema & softening because of enzymes. Increased hydration & release of enzymes at parturition that loosens the tightly packed collagen fibers during gestation. PGF2α induces myometrial contraction, which increases intrauterine pressure and moves the fetus towards the cervix, causing further cervical dilation. Oxytocin is released by the maternal posterior pituitary gland as the cervix is dilated by the fetus (Ferguson's reflex). Oxytocin induces further myometrial contractions.

6.2 Stages of Parturition

6.2.1 Preparatory stage (Stage 1)

This stage is initiated by fetus which consists of preparation of the dam and fetus for the actual birth process. Normally, it lasts a few hours to 24 hours. It is characterized by active contraction of both longitudinal & circular muscle fibers of myometrium. Placental attachments begin to 'loosen' and cervix shortens & fully dilated. As the pressure inside the uterus continues, change in fetal position and posture (front feet and head positioned to posterior of the dam). Restlessness anorexia, pains, sweating (flank/stomach), lying down and getting up, elevated pulse and respiratory rate, back is often arched, tending to blow and kick abdomen. Finally, in the end rupture of the "water bag"(allantois) may occur.

6.2.2 Expulsion of the fetus (Stage 2)

It a period from complete cervical dilation to end of delivery of fetus. It lasts from ½-4 hrs in primipara and ½-1 hr in pluripara. There rupture of umbilical cord, amniotic sac and allantochorion. The placenta, along with head & forelegs, are forced into the vagina that stimulates the stretch receptors that activate neural reflex to release oxytocin from posterior pituitary gland (Ferguson's reflex)

6.2.3 Expulsion of fetal membrane (Stage 3)

Fetal membrane normally expelled within 12 hours. It is associated with uterine contractions but the contraction decreased with amplitude. Maternal straining ceases, inversion of chorioallantois and loosening of chorionic villi from maternal cryps. After the fetal membranes have been expelled, myometrial contractions continue as well as the release of oxytocin and PGF2a

6.3 Fetal Orientation

There are 3 fetal orientations: presentation, Position and Posture

Presentation: Is the relationship b/n long (spinal) axis of fetus with long axis of dam

- Longitudinal presentations
  - Anterior/ or posterior (breech); depending on which fetal extremities entering the pelvis
- Transverse presentations (the long axis of fetus makes 90^{o} angle with long axis of mother with transverse present.
  - either dorsal/ventral, depending upon which portion of the fetus is towards the birth canal.

Position: The relationship of the dorsum of fetus with case of longitudinal presentation with the quadrants of the maternal pelvis.

- The quadrants are the sacrum, the right ilium, the left ilium, and the pubis.
- Thus positions can be dorso sacral, right or left dorso ilial, dorso pubic and right or left cephalo ilial..

Posture: Is the relationship of b/n the moveable part of fetus (head, neck and limbs) with its own body.

- The extremities or head may be flexed or extended or retained on the left or right side, or above the fetus.

6.4 Dystocia and Postpartum Complications

6.4.1 Dystocia and its management

Dystocia is a difficult/ prolonged parturition with assistant frequently required. The term dystocia comes from Greek and means ‘’difficult birth’’ which is the opposite condition of eutocia referring to safe, easy, natural or physiological parturition. It occurred in 1st, and more commonly the 2nd stage of parturition. Prolonged straining becomes difficult for the dam without artificial aid i.e assistant. Dystocia is categorized into basic/general and immediate/specific causes.

A. Basic/General Causes of Dystocia

Basic causes are those factors contributing for higher incidence of dystocia and can be anticipated before the time of parturition.

1. **Hereditary causes** (e.g. Defects like double cervix, uterus or hypoplasia of the reproductive tract, inguinal hernia)

2. **Nutritional and management factors**

Debility(undernourished), stunted growth low energy reserve needed for labour. Overfeeding (high level of feed intake) in heifers due to excessive fat deposition in pelvic region resulting in narrowing of the birth canal

3. **Level of exercise** (e.g. Short term confinement due to lack of exercise, weakness of smooth muscles then uterine torsion and uterine inertia)

4. Diseases

5. Traumatic causes

B. Immediate/ Specific Causes

These are causes which appear during time approaching parturition. Needs veterinarian correction activities. It may arise from defects in one of the three components of the birth process. It could be maternal cause (expulsive force & birth canal) and fetal cause (fetal conformation).

Maternal Cause: Failure of expulsive forces

A. Uterine

Primary uterine inertia

- Myometrial defects: overstretching, degeneration, uterine infection, systemic illness, small litter size, oversized litter
- Biochemical deficiencies; estrogen/progesterone ratio, oxytocin, prostaglandin F2a, relaxin, calcium, glucose level
- Oligoamnion (deficiency of amniotic fluid)

Secondary uterine inertia

- The consequence of another causes of dystocia e.g. uterine rupture, uterine torsion

B. Abdominal

- Inability to strain (because of age, pain, diaphragmatic rupture, tracheal/laryngeal damage)

C. Obstruction of the birth canal

- Bony pelvis: fracture, breed, diet, immaturity, neoplasia, disease
- Soft tissue:
  – Vulva congenital defect, fibrosis, immaturity
  – Uterus torsion, deviation, herniation, adhesion, stenosis

Fetal Cause

- Hormone deficiency ACTH/cortisol: initiation of birth
- Fetopelvic disproportion due absolute fetal oversize (due to high energy feed intake during **last trimester**), fetal hydrocephaly

Fetal Maldisposition

- Malpresentation (transverse, lateral, vertical)
- Malposition (ventral, lateral, oblique)
- Malposture (deviation of head and limbs) (abnormal extension or flexion of limbs and head)

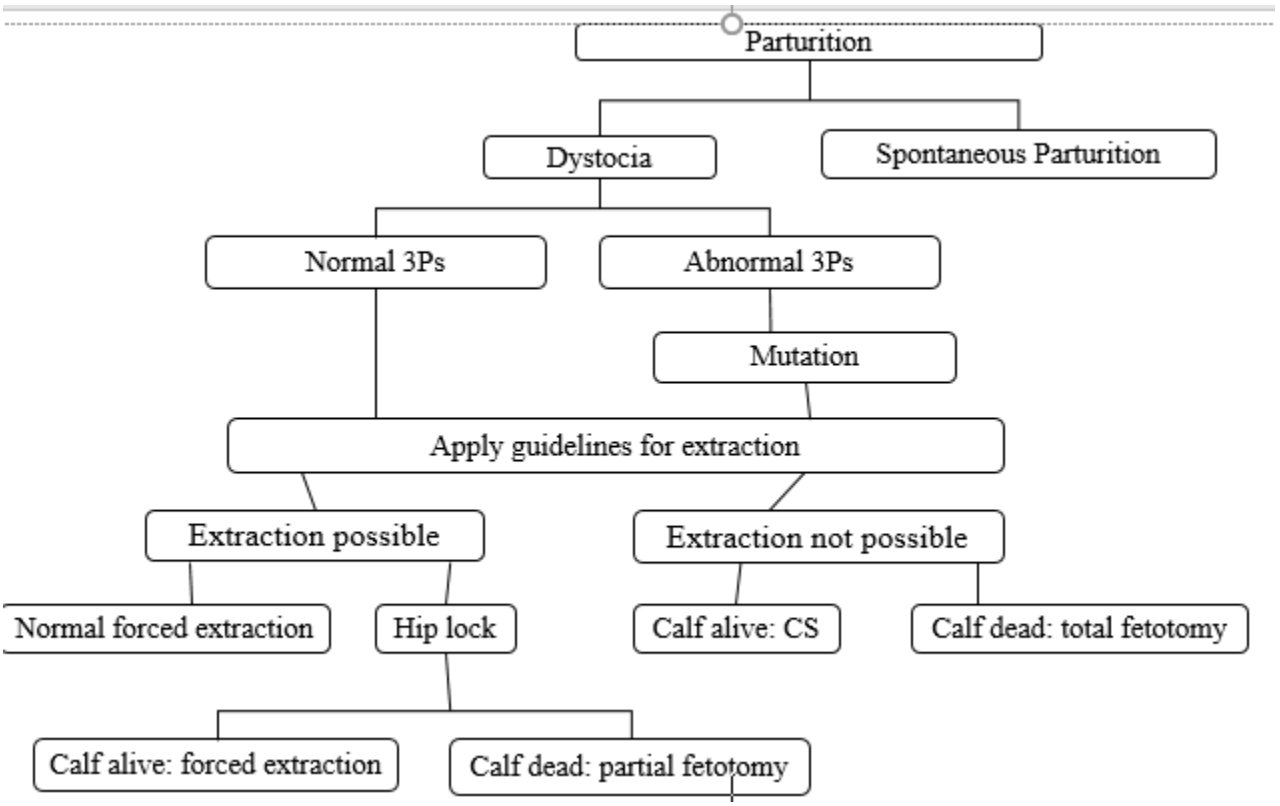
Management of Dystocia

To consider intervention, when the 1st stage of labor is longer than 6 hours and there is no abdominal press, 2nd stage of labor for 2/3 hours and progress is very slow or absent, hanging anmiotic sac is observed from the vulva or separating its lips and delivery is not completed in 2 hours, the cow should be examined. To proceed further correction survival of the fetus has to be checked by eyes reflex, the mouth and tongue palpation to elicit suckling/ tongue withdrawal reflexes. The anal sphincter may be felt to contract if a finger is placed in the rectum. Finally, in the absence the fore mentioned reflexes, one should palpate the thorax for a heartbeat/ the umbilicus for a pulse before making a final decision on the vital status of the fetus. For a live fetus, mutation and forced extraction or Cesarean section. For the dead fetus, mutation and forced extraction or fetotomy is recommended.

Lubricant: It is better to apply a lubricant in managing dystocia, Expanding polymers derived from cellulose, e.g. carboxymethylcellulose is the best ideal lubricant than other. Mineral oil is a short- lived, requiring frequent reapplication, and they tend to remove the natural lubricants from the birth canal. Mineral oil has good lubricating qualities but has caused oil granulomas in the inner part of uterus. Carboxymethylcellulose can overcome the side effect the fore mentioned lubricants.

There are 5 obstetrical delivery methods that involve the fetus/ dam. These are Cesarean section, pelvic symphysiotomy, Fetotomy, forced extraction, and correction or mutation (repulsion, rotation, version, and reposition of extremities. It

involve change of presentation, position or posture so as to bring fetus in normal presentation, position or posture or expulsion. *Repulsion* and *reposition of extremities* are self-explanatory. *Version* is accomplished by turning the fetus end-for-end, i.e. on a transverse axis. *Rotation* is sometimes required when the fetus is in the dorso-pubic position, version when it is presented transversely.



Note: 3Ps=Presentation, Position and Posture; CS= Cesarean Section

Fig. 6.2. Dystocia Decision Tree

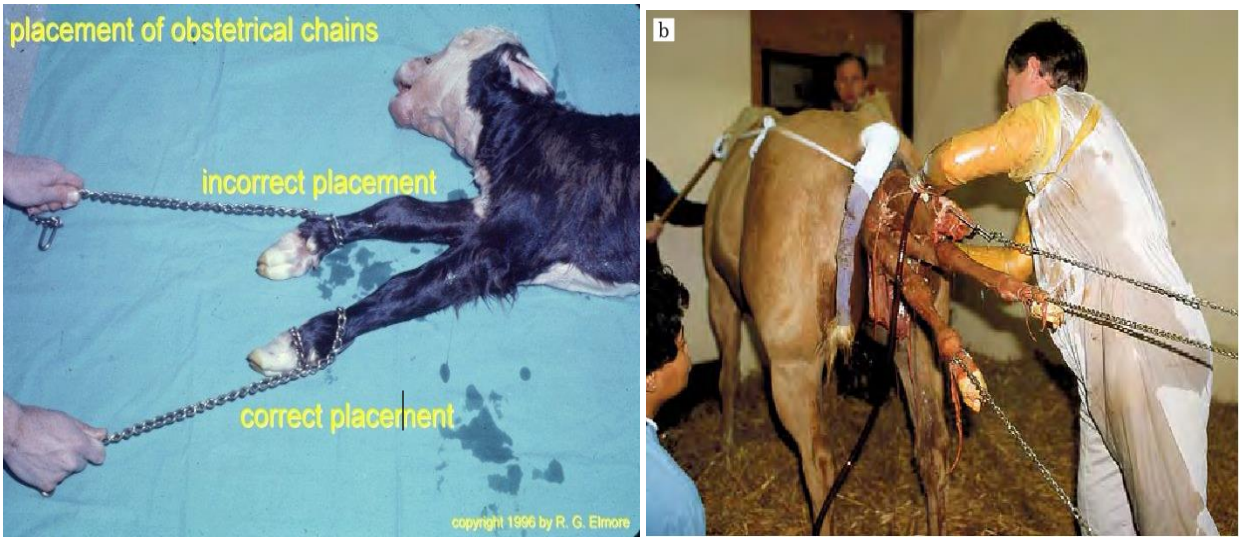


Fig. 6.3. Assisted delivery

1. Fetotomy /Embryotomy

Signifies any obstetrical operation which has the object of reduction in the volume of the fetus either by mutilation or by division to be extracted in parts. It is rapid reduction in size of fetus facilitating safe delivery per vaginum and exposure of dam to major surgery is avoided. There might be partial or complete fetotomy depending on the case.

2. **Caesarean section**

Caesarean section: is the surgical delivery of a calf through a cut (incision) made in the mother's abdomen and uterus. Procedures of C-section:

A. Preparation: Pre-medication Pre-operative medications

- Once a decision to perform a cesarean section has been made NSAIDs should be administered.
- An epidural, if not already performed, should be performed at this stage.

B. Dietary preparation

- If an elective cesarean is performed, feed restriction for 12 hours may help to prevent an enlarged rumen.

C. Site preparation flank approach:

- Left flank preferable due to presence of rumen reducing risk of abdominal contents being expelled.
- Large surgical clip; caudal to greater trochanter, cranial to last rib, dorsal to vertebral processes, ventral to milk vein
- Aseptic preparation of surgical area with suitable product (ie chlorhexidine or povidone iodine) followed by surgical spirit.
- Local anesthetic blocks administered at this point. Paravertebral, line or inverted L blocks are all suitable. If Line block or inverted L block Line block are used then the surgical field should be prepared aseptically again
- Sterile drapes can be placed around surgical site or disposable drapes placed and a window cut to fit the surgical site
- Other preparation; aseptic preparation of surgeon, sterile gowning, gloving and preparation of surgical kit

Restraint and incision procedure

If flank approach then cow should be haltered to a solid structure and the non-surgical flank should be against a solid wall or gate. Surgical procedure:

Step 1: Skin incision; a vertical skin incision is selected, starting approximately 10cm ventrally to the transverse processes and 10 cm caudal to the last rib. This is typically extended to a length of 30-40 cm ventrally. Incisions might extend up to 70cm downward for large calves.

Step 2: Incision through muscle layers; under here cutaneous, external abdominal oblique, internal abdominal oblique and transverse abdominal muscles will be incised. Incision can be by scalpel or once an initial entry point has been made the surgeon may switch to the use of surgical scissors. This reduces the likelihood of cow movements leading to trauma of internal tissues/ organs but may cause slower tissue healing. Hemorrhage has to been minimized by avoiding cutting larger

vessels and if bleeding encountered ligation is recommended. As a complications in entry into the peritoneum there is a sound of air entering the abdominal space, peritoneal fluid which is blood-tinged may apparent.

Step 3: Exploration of the abdomen; Identify uterus and disposition of distal extremity of the calf with gentle traction of this limb the uterus can be exteriorized. To aid exteriorization of a hindlimb the tarsus and foot can be held and used to lever the uterus into the incision. The tarsus and foot then can effectively lock the uterus at the dorsal and ventral aspects of the incision.

Step 3. Uterine incision; incision should done over the calf's leg from toe to carpus/tarsus. It is advised to follow the greater curvature of the uterus and will ideally run parallel to the longitudinal muscles of the myometrium.

Note: The incision should be far from cervix and avoid incising through any cotyledons as this can lead to profuse hemorrhaging. In addition, it is vital that the incision is long enough, as if it is too short there may be uncontrolled tearing of the uterus during extraction of the calf.

Step 4: Extraction of the calf; The allantochorion & amnion can be ruptured manually and the calf's legs grasped by the surgeon. It is advisable to direct the head of the calf out of the incision before too much traction is placed on the forelimbs to prevent situations similar to a head back presentation which may lead to damage of the uterus. If any, check for the presence of a second/ third calf.

Step 5: Care of the calf; the live calf should immediately be attended by an assistant and care should be given.

Step 6: Closure; The uterus should be held by a sterile assistant or sterile uterine forceps should be applied. Uterine closure should start at the cervical end of the incision with absorbable suture material (eg. Catgut or Polyglactin). Better to apply Lembert or Cushing pattern. Especially if Cushing, care should be taken not to include fetal membranes within the closure. Excess free abdominal fluid can be removed and in the case of gross contamination can be diluted with saline and procaine power. Absorbable suture material on a round bodied needle should be used in a continuous pattern for peritoneal and muscle closure. It is important to include a good bite of peritoneum in each throw of suture for the first layer. To reduce dead space between muscle layers occasional deeper bites into the underlying muscle layer can be made. The skin incision is closed using non-absorbable suture material on a large cutting needle and follow interrupted suturing pattern.

As a post-operative care, antibiotic spray or wound healing sprays can be applied to the incision line. Blood on the skin should be washed off to reduce the urge of the cow to rub the area as it dries and minimize the attraction of flies. After 10 days the non-absorbable suture materials from the skin should be removed. If involution of the uterus was not occuring at point of closure then oxytocin can be administered. Calcium borogluconate can be administered intravenously or subcutaneously if there are concerns about Milk fever milk fever. If evidence of shock detected then intravenous fluid therapy can be administered. A broad spectrum antibiotic is indicated for parenteral route.

Advantages of performing the C-section procedure

- Increased chance of live calves if intervention performed early.
- Reduced chances of secondary complications to cow, eg peripheral neuropathies.

Disadvantages of C- section

- Cost, Risk of surgical and post-surgical complications in dam, including death
- May reduce subsequent fertility of dam, Anesthetic depression of calf (if sedative used).

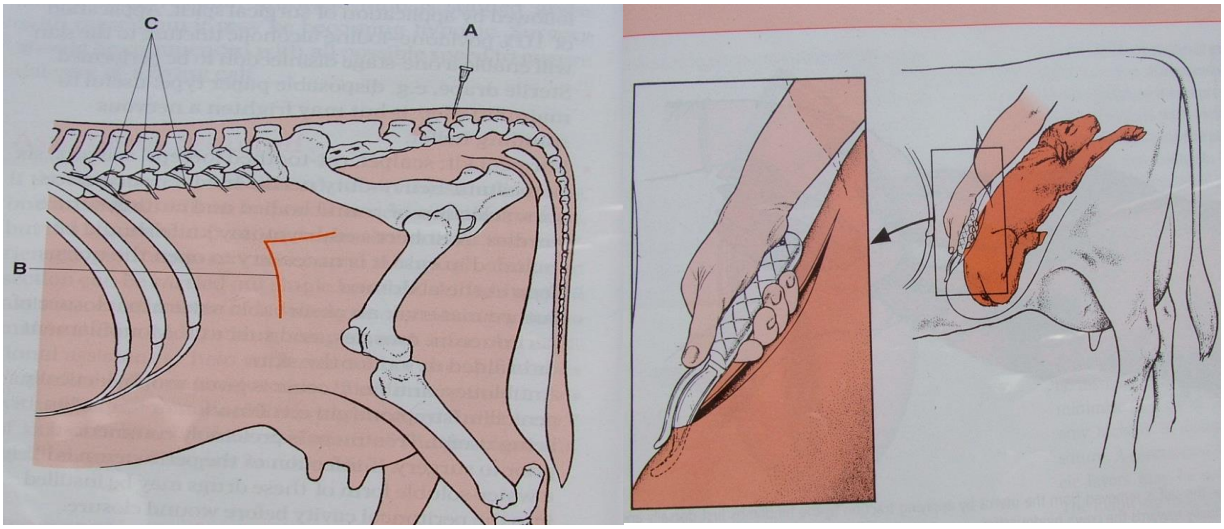


Fig. 6.1. Site of Cesarean section

Ways to reduce the occurrence of Dystocia

It is possible to reduce the incidence of dystocia in a given farm or herds if the following crieterias are implemented by producers:

- Selection of heifers with optimal pelvic width and culling heifers with the narrowest pelvic width i.e reproductive tract measure for replacement heifers
- Selecting bulls on the basis of estimated breeding values (EBVs) or expected progeny differences (EPDs) to ensure acceptable birth weights
- Appropriate nutrition management of cows during pregnancy to prevent feto-maternal disproportion i.e avoid underfeeding of pregnant animals
- Avoid breeding cows having hip fracture
- Feed heifers well enough to weigh at least 85% of their expected mature weight at first calving.

6.4.2 Hemorrhage

- Hemorrhage from severed umbilical cord → fairly transient
- Persistent or copious hemorrhage is indicator of trauma to birth canal (severe with arterial rapture)
- Mostly branches of posterior uterine artery
  - Explore and ligate
- General lacerations in non-arterial bleeding
  - Use pressure packs

6.4.3 Prolapse of the uterus, cervix, vagina

- An eversion or slight prolapse or protrusion of uterus, cervix and vagina
- This is caused by an excessive relaxation of the pelvic ligaments brought about by relaxin
- Vaginal prolapse could be accompanied by cervical prolapse, called cervico-vaginal prolapse.
- Causes:
    - Increased intraabdominal pressure, Increased size of the uterus, Intraabdominal fat
    - Rumen distensions, mucosal irritation, infection and Swelling

Treatment procedures:

- Place the cow in sternal recumbency
- Extend both hind-legs (frog position)
- Remove placenta if it separates, if not cut dependent part
- Caudal epidural anesthesia
- Suture any obvious wound
- Clean with plain warm water
- Replace by applying pressure to the part of everted uterus nearest to the cervix until horn tip
- Administer oxytocin, Calcium borogluconate and systemic antibiotic
- If not replaceable, amputation or slaughter

https://www.youtube.com/watch?v=9EqtSP7meKk

https://www.youtube.com/watch?v=qFKtCUanebM

6.4.4 Endometritis

It is inflammation of endometrium. The effects of endometritis:

- Reduced milk yield, delayed return to oestrus
- Intentional delay of first service-discharge
- Reduced pregnancy rate, increased culling rate
- Cause
    - Infections*; C. fetus* and *T. fetus*
    - Nutrition; Fatty Liver Disease → reduced resistance
    - Route; at coitus, insemination, parturition/postpartum

NB; A. *pyogens (*produce substances that interfere with the phagocytosis and killing of Bacteria) and *F. necrophorum* (has leucocidal endotoxin which interferes with the host's ability to eliminate) have a synergistic effect.

Diagnosis of endometritis

- Manual examination of the vagina like discharge or inflammation (Metricheck)
- Vaginoscopy, ultrasound and uterine cytology

Signs; a white or whitish-yellow mucopurulent vaginal discharge, reduced appetite, slightly depressed, doughy in rectal palpation

Treatment of endometritis;

- Delayed until at least 4 weeks after calving
  - Prostaglandins
  - Intra-uterine antibiotics
- Putting antiseptics, such as Dettol however, will cause infertility

6.4.5 Retained Fetal Membrane (RFM)

It is a failure of the fetal membranes to be expelled during the third stage of labor. RFM beyond 12 hours in cattle is considered pathologic and is primarily due to either uterine inertia or inflammation of the placenta, which in turn results in a failure of the fetal villi to detach themselves from the maternal crypts. RFM invariably accompanies abortions in late gestation due to:

- Infections such as brucellosis, leptospirosis, and IBR
- Premature births associated with twinning, and
- Induced parturition with corticosteroids, obstructive dystocia, cesarean operation and due to subsequent endometritis

Reason for retention of fetal extramembrane:

- Failure of placental maturation (reduced collagen in placetome)
- Presence of placentitis (during infected with *B.abortus, C.fetus, Aspergillus*)
- Inadequate uterine contraction
- Odema of fetal villi and maternal crypts (site of attachment)
- Abortion (Faye et al., 1986)
- Multiple births (Muller and Owens, 1974)

Treatment of RFM

- Manual removal (gently to avoid excessive traction damaging endometerium)
- Administration of ecbolic agents like PGf2α, E2 and oxytocin have all proven ineffective
- No treatment if there is no infection
- So better to identify sick cows with signs of metritis (high temperature, reduced appetite and poor milk yield) and inject parenteral antibiotics and anti-inflammatories

6.4.6 Pyometra

Accumulation of large amounts of pus in the uterus**.** It might be opened/closed**.** The pus prevents the growth of the embryo.

Causes of pyometra

1. A sequel to chronic endometritis and persistent CL

2. Death of fetus and invasion by A. pyrogens, CL exists

3. Infections with *T. fetus* (causes embryonic death)

4. Insemination after pregnancy or luteal phase

Diagnosis of Pyometra:

- Absence of cyclical activity, Might be intermittent vaginal discharge
- uterine horns are enlarged and distended, uterine wall is thicker than at pregnancy
- The uterus has a more 'doughy' and less vibrant feel
- Impossible to 'slip' the allantochorion, No uterine caruncles can be palpated
- Transrectal ultrasonography will demonstrate the absence of a fetus
- Rx; PGF-2α (regression of the CL, dilatation of the cervix and expulsion of the purulent flui**d**

REVIEW QUESTIONS

1. Explain the physiology of parturition?

2. What is uterine involution?

3. Describe postpartum complications?

4. What is dystocia and how could treat it?

7. REPRODUCTIVE TECHNOLOGY

At the end of this chapter, the trainee will be able to:

- Describe hormones used for multiple ovulation
- Inseminate cows/ heifers properly
- Explain why it is recommended to synchronize cows/heifers
- Adopt sexed semen for heifer replacement

There are different reproductive technology have been innovated and exercise.

Table 7.1 Generation of Reproductive Technology

S/N	Generation	Technologies
1	First generation	AI, Cryopreservation of gamete, estrus synchronization
2	Second generation	Multiple Ovulation and Embryo Transfer (MOET)
3	Third generation	Invitro Embryo Production (IVEP)(OPU, IVM, IVF and IVC) embryo cloning, sex selection
4	Fourth generation	Nuclear transfer, Cloning and transgensis, Stem cell biology

NB: AI=Artificla insemination; OPU=Ovum Pick-Up; IVM=Invitro Maturation; IVF=invite fertilization and IVC= inviro culture

7.1 Artificial Insemination

Artificial insemination (AI): is manual placement of semen collected from a male in the female reproductive tract using equipment designed for the purpose other than natural mating. In Ethiopia, AI was introduced in 1938 in Asmara then part of Ethiopia, which was interrupted due to the Second World War and restarted again from 1952.

Advantage (Pros) of AI

- Maximum use is made of outstanding sires. Semen can be used after the sire has died or can be shipped anywhere in the world.
- Uniformity of offspring is increased.
- Sire cost can be reduced and danger and cost of keeping a sire is eliminated.
- Cost and delays involved in using infertile sire are reduced.
- It prevents the spread of certain diseases and sterility due to genital diseases
- Pride of ownership is increased
- Less wastage of semen
- The progeny testing can be done at an early age

Disadvantage (Cons) of AI

- Skilled technicians are required.
- Physiological principles must be followed.
- Sire market may be limited
- Need to know Estrous Cycle of animal

- If the bull is not properly tested, the spreading of genital diseases will be increased.
- May accentuate the damage of a poor sire.
- Requires large investment, As it requires N2 gas, Sire, tank, cost for some for AI and other equipment

Artificial Insemination Techniques

The technique of inseminating a cow is a skill requiring adequate knowledge, experience and patience. Semen must be deposited within the tract of the cow at the best location and at the best time to obtain acceptable conception rates. In the recto-vaginal technique a sterile, disposable catheter containing the thawed semen is inserted into the vagina and then guided into the cervix by means of a gloved hand in the rectum. The inseminating catheter is passed through the spiral folds of the cow's cervix into the uterus. Part of the semen is deposited just inside the uterus and the remainder in the cervix as the catheter is withdrawn. Expulsion of the semen should be accomplished slowly and deliberately to avoid excessive sperm losses in the catheter. The body of the uterus is short; therefore, care should be taken not to penetrate too deeply which might cause physical injury.

Proper Timing of Insemination for Maximum Conception

The conception rate is lower when the animal is bred prior to mid-estrus and later. Maximal conception is obtained when cows are inseminated between mid-estrus and the end of standing estrus. This time period is when the female ovulates, sending an ovum out to the fallopian tube to await fertilization from sperm from a bull. In cows estrus may last from 10 to 24 hours. Success in insemination timing depends upon good heat detection. A successful heat detection and subsequent proper timing of insemination will increase reproductive efficiency. inseminated 6 to 24 h after onset of estrus as fertility was lower with earlier or later insemination Practical recommendation for timing of insemination are:

In Morning Next day Morning; In Evening Next day afternoon

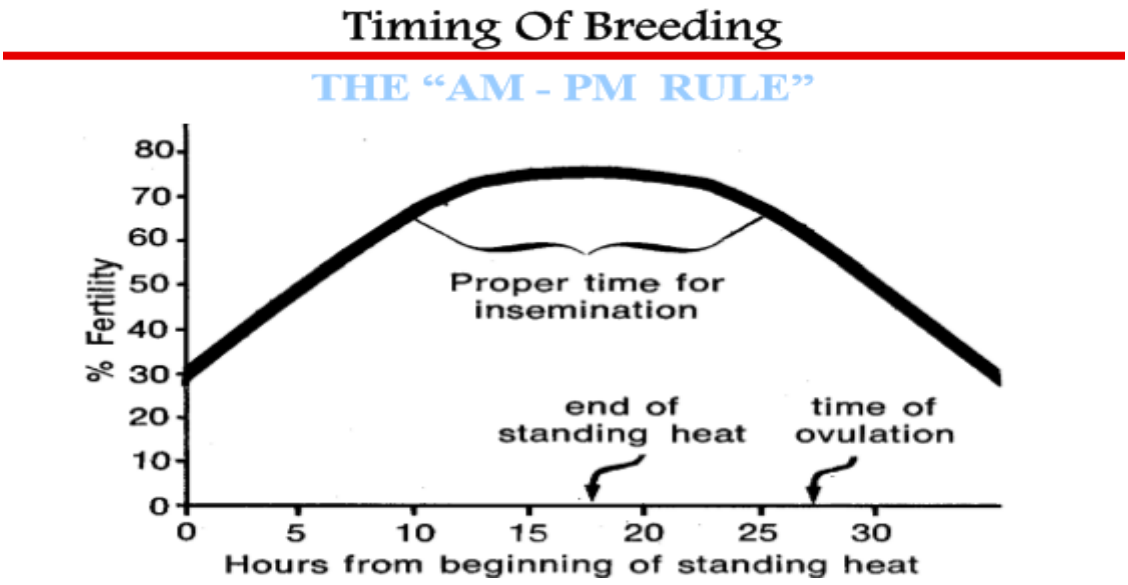


Fig 7.1 Time of breeding /AI

Proper AI requires the following procedures:

A. Positioning the candidate

Place the candidate in a relaxed, stand on a level surface with plenty of grips and restrict the movement in crush/chute. In addition, use familiarize area and facility should be provided with provision made for food and water.

B. Thawing the straw

Thawing: Once the candidate in proper time to be inseminated, wipe and disinfect external genitalia before inserting AI Catheter. Thawing has been conducted at 35^{O}c warm water for 20-30 seconds for 0.25ml straw and 30-40 sec for 0.5ml or following instructions from the semen company. If sexed semen is used, require a slightly longer and warmer thawing temperature.

https://www.youtube.com/watch?v=WtL_hRxFrdk

Thawing steps

- Better to use tweezers and quickly remove from N2 tank. Don't use your finger as it potentially leads to thawing the semen too quickly.
- Shake the straw to remove excess liquid nitrogen (nitrogen quickly goes into a gaseous state when exposed to air and warmer temperatures) otherwise it can burst the semen straw when put in water bath
- Inserting in warmer water having 35^{O}c
- Remove the straw from the thermos and wipe it dry with a paper towel and avoid hand contact.
- Warm the AI gun by stroking vigorously 5-6 times.
- After withdrawal, wipe it dry and place it in the gun, which should have been pre-warmed by rubbing b/n the hands. Cotton-plug end should be inserted in AI gun
- Cut one-fourth inch from the end straw end at a 90^{O} angle, then slide on to the plastic sheath and secure with the collar. Hold the gun vertically and gently press the plunger upwards, until the semen rises to the top.
- Place the sterile sheath over the gun and push on until it seals
- Place the gun between your body and shirt to maintain optimal temperature until you are ready to breed the cow.

C. Preparing the cow

- Clean the cow's vulva with a paper towel and put on a full-arm glove and lubricant.
- Insert your arm into the cow, by forming a cone with your fingers while keeping place the tail on the back side of your left arm.
- Gently work out any excess dung and if the rectum becomes distended with gas or the cow strains excessively, withdraw the arm and consider reserving a few hours later.
- The cow must be relaxed during the procedure to avoid injury, as the rectum wall is a delicate structure.

- If the bladder is full, wait and try again once the cow has urinated.
- With your left hand make a fist and press down directly on top of the vulva. This will spread the vulva lips allowing easy access to insert the gun.
- The insemination gun should be inserted into the vulva upwards at a 30°-40˚angle with the left hand inserted in rectum, to prevent accident insertion of the gun into bladder

D. Finding the Cervix

- The initial landmark is the cervix and this should be located before inserting the gun.
- When the gun reaches end of vagina, raise the rear of the gun to level position and slide forward until it contacts the beginning of the cervix. If the gun is getting caught in the folds of the cervix, try stretching the cervix away from you with your left hand to free the gun and allow easier passage to the cervix
- The cervix is normally found on the pelvic rim, but in older cows, it may have moved slightly to one side.
- The anterior portion of the vagina, termed the fornix vagina, tends to stretch rather easily when the insemination rod is pushed forward and beyond the cervix. This may give the false impression that the rod is advancing through the cervix, when indeed it is above, below, or to either side of the cervix. The inseminator should be able to feel the rod within the vaginal fold, but unable to feel the rod tip within the cervix.
- After locating the cervix, use the elbow to exert downward pressure on the vagina. However, never force the gun through a tough part of the cervix
- Once the gun is just through the cervix, you should feel a release in resistance to the gun

E. Depositing Semen

- Ideally, semen should be deposited just beyond the cervix into the uterine body.
- Depositing the semen in the uterine body allows the semen to evenly distribute between both horns.
- Slowly deposit semen, it might take 5 seconds. Confirm that the finger did not block the flow of semen to uterine horns.
- Currently ipsilateral insemination is effective, depositing in horns on the side of active ovary (presence of Graffan follicle)
- Gently remove the gun and check for any abnormal discharge.

Last, but not least, clean your equipment. Before taking off your breeding glove, remove the used sheath. Then dispose glove and clean equipment thoroughly.

https://www.youtube.com/watch?v=AC4HRcUDWsI

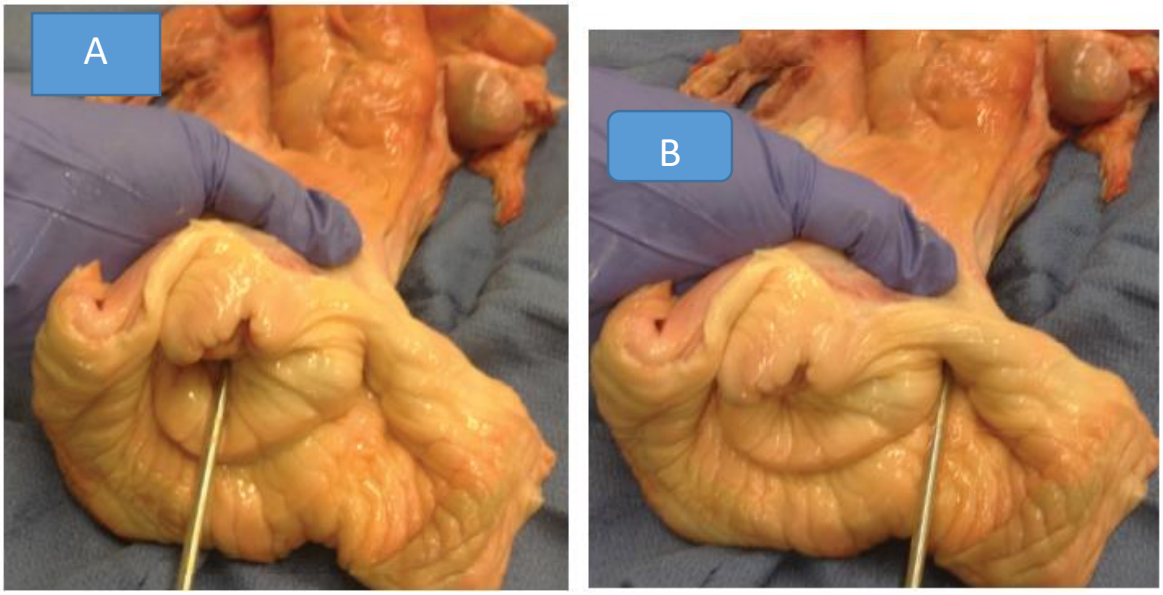


Fig. 7.1 A=correct insertion; B=incorrect insertion

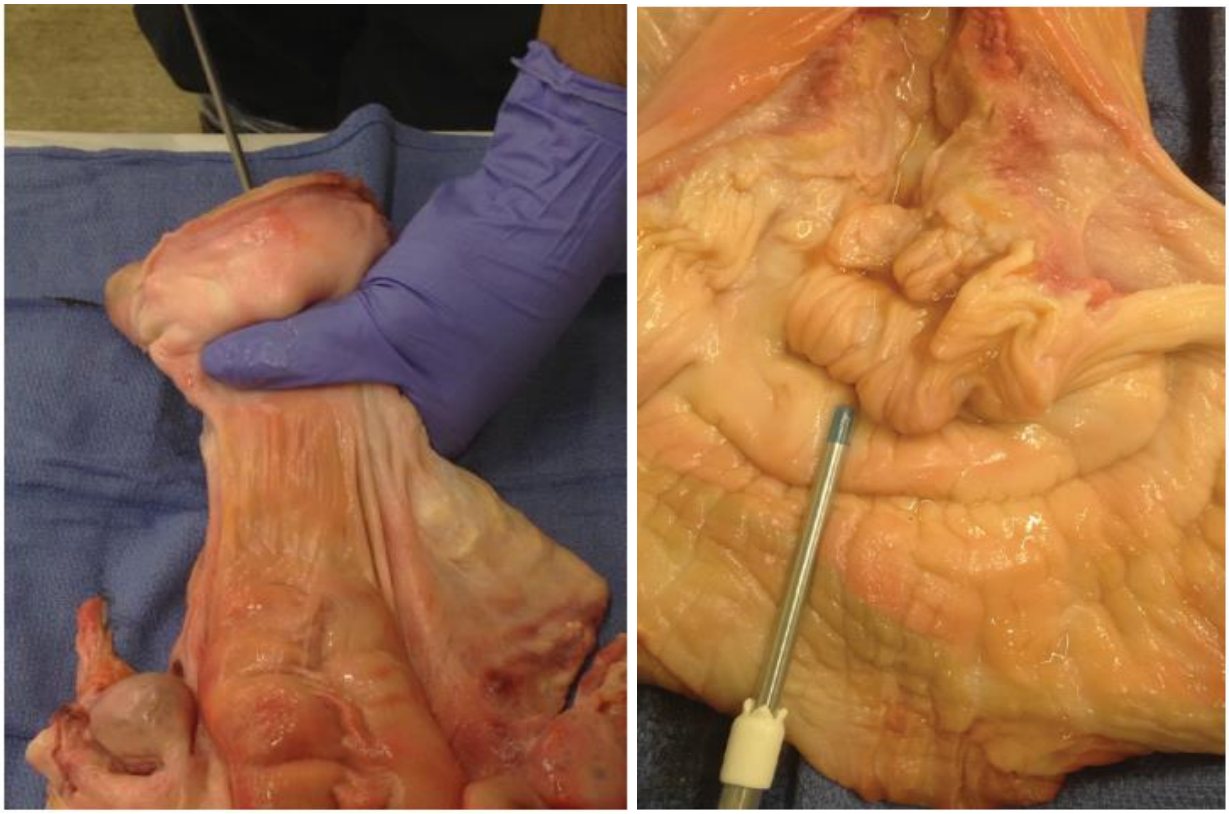
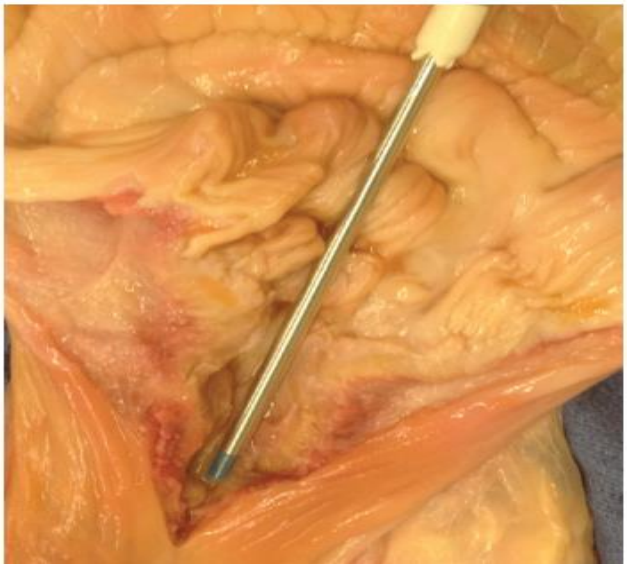


Figure 16. Insertion of AI catheter



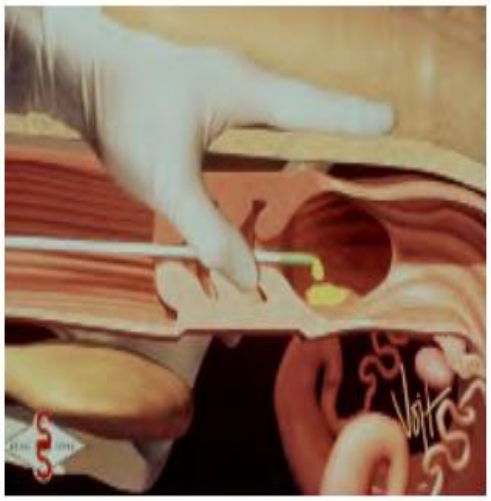


Fig.7.2 Site of Semen deposition

https://www.youtube.com/watch?v=vDIghmEiVW4

https://www.youtube.com/watch?v=H_SvuiT3GTk

Fixed Time Artificial Insemination (FTAI): In cattle, estrus synchronization and artificial insemination (AI) availed to exploit the reproductive potential of cows by incorporating superior genetics. Artificial insemination (AI) promotes genetic and economic gains through the use of 24 superior genetic bulls. Despite the technological advances of AI programs, the application of AI programs based on estrus detection is hampered by postpartum anestrous and estrus detection (ED) failure. Fixed timed artificial insemination is currently applied in GnRH and PG based synchronization (Ovsynch and Co-Synch). This reproductive biotechnology alleviate heat detection issue by inseminate the animal at a specific hours after the protocol. No need to detect heat in FTAI.

7.2 Semen Preservation

The primary goal is to maintain the fertilizing potential of spermatozoa over a long period of time in a pathogen-free environment. Secondly, efficiency of male gamete use should be increased by dilution with semen extenders & producing multiple insemination doses. The principle features of semen preservation are:

- Nutrition and protection of spermatozoa: energy substrates, buffers, membrane protectants (e.g., antioxidants,cryoprotectants)
- Concentration of spermatozoa and removal of seminal plasma (freezing only).
- Microbial control: antibiotics
- Portioning spermatozoa into AI doses and mechanical protection: filling in plastic semen tubes or bags (5–100 ml) for liquid storage or in plastic straws (0.25 or 0.5 ml) for frozen storage.
- Prevention of cold shock: controlled cooling regime to storage temperature.

- Immobilization and reduction of sperm metabolism at low storage temperatures, i.e., liquid semen at 16 °C (boar) or 5°C (most other species), frozen semen at −196 °C in liquid nitrogen

Semen Cryopreservation: Cryopreservation is the preferred preservation method allowing indefinite storage of male gametes, international trade of superior genetics, and screening for pathogens prior to use. Biobanking, storage in the frozen state is indispensable. However, there are limitations:

- The distinct composition & thermotropic phase behavior of membrane lipids render spermatozoa susceptible to cold shock, the extent of which varies b/n species.
- The vast majority of commercially marketed bull semen is cryopreserved

Damage to cells during freezing and thawing strongly depends on the cooling rate: slow cooling (about 5 ^{O}C/min) results in dehydration due to hypertonic conditions induced by extracellular ice formation, whereas rapid cooling (>100 °C/min) leads to the formation of damaging intracellular ice crystals (Mazur 1963). Cryoprotectant **s**elected based on minimal cell toxicity, high efficiency, and low risk of introducing contaminants. It increases the biological complexity of the cellular response to cooling and freezing. E.g glycerol and dimethyl sulfoxide (DMS).

Glycerol and DMS: are membrane-permeable (MP) agents exert their effect mainly by inhibition of intracellular ice formation. Whereas, non-membrane-permeable (NMP) cryoprotectants include osmotically active molecules such as disaccharides, e.g., sucrose & trehalose, and osmotically inactive macromolecules, e.g., polyvinylpyrrolidone (PVP), hydroxyethyl starch, and dextran. The NMP promote cell dehydration, lower the freezing point, & increase the viscosity of media, thus together inhibiting ice crystal formation.

Semen extenders is a diluent that provides membrane stabilization in cool temperatures, ions for membrane and cell balance, and antibiotics to prevent growth of microbes that can cause disease and compete for nutrients. The primary purpose of extending semen is to maximize the number of females that can be inseminated with a single collection as it increases the volume of semen. Components of extenders:

- Nutrients **(**Provide energy for sperm); Glucose, fructose
- Lipids **(**Cold shock prevention); Provides protection of sperm membranes from temperature changes e.g. Milk, skim-milk, & egg yolk
- Buffer **(**control pH 6.7 to 7.0, keeping osmotic pressure of semen**)**; e.g. Sodium citrate, Tris, egg yolk
- Antibiotics (Inhibit bacterial growth)
- Cryoprotectant
    - It protects against the lethal effects of freezing to prevent crystallization of water within the sperm cells, which eventually allows sperm cells to be frozen rapidly.
    - Formation of ice crystals results in puncture of cell membranes resulting in decrease in membrane integrity; e.g**.** glycerol

Liquid Semen Preservation: It is preservation of semen in the liquid state at temperatures above 0 °C. Preferred in species with cold-shock-sensitive sperm (e.g., porcine), in males with poor semen quality but high genetic value, or in sires with the best genetics to extend the number of insemination doses. Might be beneficial for sperm stressed by sex-sorting. Due to bacterial risk; a single inseminations doses bearing 60–100 ml semen for sow. Its low cost, avoidance of chilling injury thus preserving higher sperm quality, and low carbon footprint. Preservation are for limited life span in vitro (typically a few days) & the higher risk of bacterial growth.

7.3 Estrus Synchronization

Synchronization is inducing a number cows to come into heat in a group of females at a confined period of time. Optionally, it is a method to condense heat detection and breeding efforts into a tighter time frame. The goal is making all the cows to come to heat in a confine time

There are two principle of synchronization

A. **Regress active Corpus Luteum at day 5-17 of estrous cycle**

Cows will be in estrus 2-5 days after injection. About 60-65% of the herd responds to injection. To get the whole herd synchronized give 2 injections 11-14 days apart.

Prostaglandin based injection synchronization protocol

Prostaglandin (PGF) is a naturally occurring hormone. During the normal estrous cycle of a non-pregnant animal, PGF is released from endometrium of uterus 16 to 18 days after the animal was in heat. This release of PGF from the uterus is the triggering mechanism that results in the animal returning to estrus every 21 days. The major limitation of PGF is that it is not effective on animals that do not possess a CL. This includes animals within 6 to 7 days of a previous heat, prepubertal heifers and postpartum anestrous cows. E.g. Commercial available PGF (Lutalyse, Estrumate, Prostamate).

One time PG injection protocol: This system is used where heat detection is practiced, and drug costs are a concern. Sixty to seventy percent of cows will be bred AI using this system.

Two PG Injection protocol: This system is used to bring more cows in heat during the AI period (90%). Heat detection must be practiced. If extended heat detection is not a concern but drug costs are a problem, then animals can be heat checked and bred after the first injection of PG. Animals not showing heat following the first injection would then be administered a second PG injection 11-14 days later and then bred.

B. **Maintenance of CL Regresses**

This principle prolong the lifespan of CL. Done by exogenous insertion of progesterone source for a certain days and then remove to decrease progesterone level. Animals will respond for low level of P4 and show estrus 2-5 days later. Administration of progestogens source: Injection, feed, implant and or Control Internal Drug Release (CIDR).

Estrous Synchronization Using MGA & PG: The synthetic progesterone, Melengesterol acetate (MGA), has been used with prostaglandins for synchronization. This protocol takes advanced planning and requires bunk feeding. This program works best with larger groups of heifers that are already fed grain and where heat detection is practiced. For orally active MGA feed animals for several day blocks estrus and ovulation then sharp deprive leads to come to heat. It acts similar to progesterone from CL. MGA can induce estrous cyclicity in peripubertal (within 30 days to attain puberty) heifers and postpartum anestrous cows. Currently, MGA and CIDR are the only progestins commercially available.

Fig. 7.3 MGA in feed additive

Estrous Synchronization Using A CIDR (control internal drug release): A progesterone-containing device which is inserted into the cows vagina has become commercially available. They are T shaped devices with silicone coated nylon core that impregnated with P4. This CIDR appears to offer some advantages due to ease of insertion and removal. CIDR is inserted intra-vaginally using a specialized applicator and removed after 7 days. CIDR provided slow release administration of P4 which artificially extends the luteal phase. Following removal P4 level decreases rapidly. This causes them to cycle and can help fertilization to occur during a time of the year when they are not normally cycling.

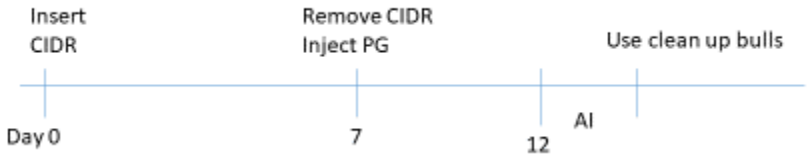


Fig. 7.4 CIDR plus PG system

C. Combination of GnRH and PG based synchronization

Combination of GnRH and PG could synchronize follicular and luteual phases of the estrous cycle. A naturally occurring hormone, GnRH is more popularly known by the commercial brand names of Cystorelin, Factrel, and Fertagyl. Each GnRH-based protocol uses the same basic framework, which involves an injection of GnRH followed 7 days later with an injection of PGF. The way animals are subsequently handled for heat detection and breeding is where the protocols begin to vary. To understand the benefits of GnRH-based synchronization protocols and how they work, first understand the concept of follicular waves and dynamics in cattle.

Ovsynch: The primary synchronization of ovulation protocol, which consists of first injection of GnRH followed 7 days later with an injection of PGF, followed in 48 hrs by a second injection of GnRH; timed artificial insemination (TAI) could be performed 0 to 24 h (optimally 16 to 18 h) later. Although Ovsynch allows for satisfactory pregnancy rates without heat

detection, it does not necessarily eliminate the need for heat check. Ovsynch-treated animals should be observed closely for returns to estrus 18 to 24 days later. Additionally, up to 20 % of treated cows will display standing estrus between days six and nine of the Ovsynch protocol.

Fig. 7.5 Ov-synch protocol

Co-Synch: It eliminates one animal handling by breeding cows coinciding with the second GnRH injection. Most field trials indicated only a small reduction in conception rates in co-synch than Ovsynch. As with Ovsynch, pregnancy rates are maximized if early heats (± 24 hours of PGF) are visually detected and bred using the A.M.-P.M. rule.

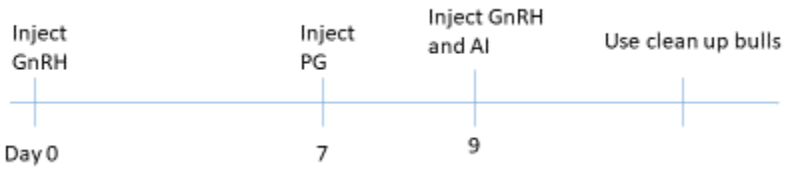


Fig. 7.6 Co-synch protocol

Select Synch: Select Synch is a breeding option for those herds with good heat detection programs and that prefer to breed cows based to standing estrus. Cows are either bred to detected estrus for three to five days after PGF. This approach allows most cows (50 to 70 %) to be bred at standing estrus and gives all cows an opportunity to conceive with the clean-up AI at 72 hours. The Select Synch approach saves additional hormone costs because only those cows that fail to show estrus receive the second GnRH injection. It facilitates more efficient use of expensive or genetically valuable semen by targeting its use in cows at estrus, whereas less expensive semen can be reserved for the timed AI services.

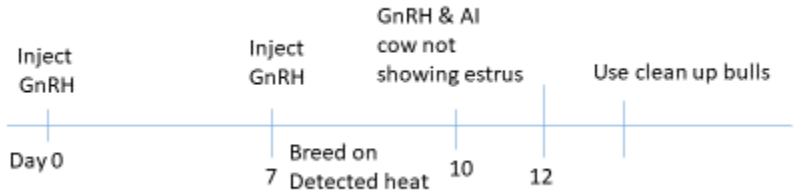


Fig. 7.7 Select synch program

7.4 Multiple Ovulation & Embryo Transfer (MOET)

Embryo transfer is a technique by which embryos are collected from a donor female and are transferred to recipient females, which serve as surrogate mother for the remainder of pregnancy. It is a second-generation innovated reproductive

technology in which done by induction of multiple ovulations by ovarian super stimulation using fertility drugs. It increases supply of embryos from animals of superior genetic merit. The most favorable and optimal time for superovulation treatments is between the 8th and 14th day of the cycle. The advantage is a female (donor) can increase the number of offspring produced in her life time (ova, embryo).Superovulation can be done in conjunction with estrus synchronization of both donor and receipts. Superovulation is induced using FSH, eCG or GnRH. In using eCG, a single injection is enough, whereas, FSH has a shorter half-life. Thus, the total dose should be divided and injected at 12 hrs interval for 3-4 days.

Basic Steps for Embryo Transfer

- Synchronization of donor and recipients
- Superovulation (FSH, luteolysis with PGF)
- Insemination at estrus (Day 0)
- Collection of embryos using flushing media (phosphate-buffered saline) (PBS)(Day 5)
- Surgical (laparotomy) or nonsurgical (trans cervical)
- Grading of embryos-Cryopreserved
- Transfer (Fresh or Frozen) in recipients (Day 5)
  - Surgical or nonsurgical
- Pregnancy test.

Super ovulation of Donor

Induction of multiple ovulations by ovarian super stimulation using fertility drugs. It increases supply of embryos from animals of superior genetic merit. Superovulation is induced using FSH, eCG or GnRH. The most favorable and optimal time for superovulation treatments is between the 8th and 14th day of the cycle. Superovulation treatment will result in release of multiple ova at a single estrus. Superovulation can be done in conjunction with estrus synchronization. Individual response to superovulation is variable (0 - 10). Superovulation is induced with exogenous FSH or eCG injected IM or SC. FSH has a shorter half-life. Thus, the total dose should be divided and injected at 12 hrs interval for 3-4 days. While eCG, has long half-life, used in single injection. Cows or heifers that are properly treated can release 10 or more viable eggs. Nearly 85 % of the donors respond to superovulation treatment protocols.

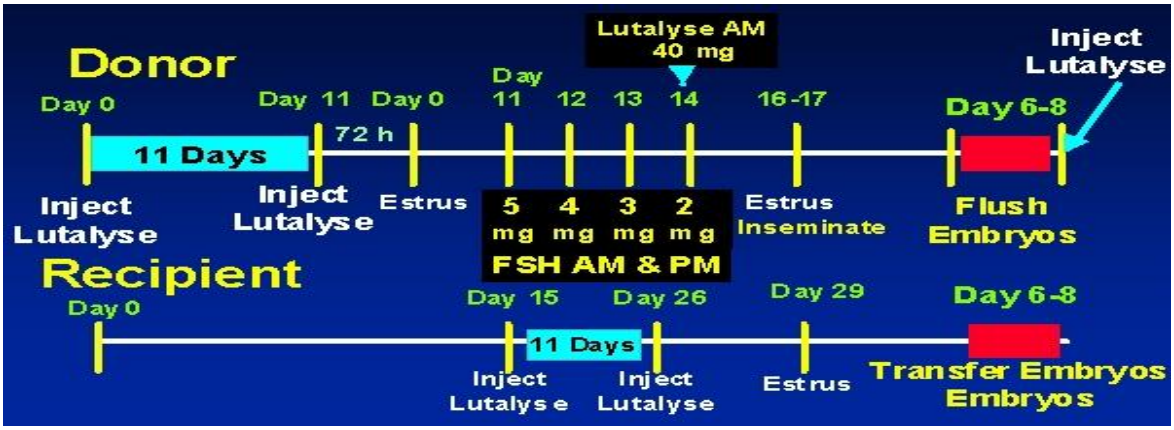


Fig. 7.8 Superovulation for embryo transfer

Insemination of Superovulated donors

- Most of the superovulated females are inseminated multiple times.
- Inseminate the superovulated cow at 12, 24, and 36 hours after onset of standing heat.
- Site for semen deposition: body of uterus or into the entrance of each uterine horn.

Method of Embryo Collection

Transcervical: To collect the embryos nonsurgically, a small synthetic rubber catheter is inserted through the cervix of the donor cow, and a special medium is flushed into and out of the uterus to harvest the embryos 7 to 8 days after estrus. A pre-steriled stylet is placed in the lumen of the catheter to offer rigidity for passage through the cervix into the body of the uterus. When the tip of the catheter is in the body of the uterus, the air cup is blown with 14 ml syringe. The fluid is a sodium chloride based phosphate that has a small amount of serum which sequentially added and removed by gravity. The fluid in the uterus is agitated rectally, especially in the upper one-third of the uterine horn. The uterus is finally filled with medium to about the size of a 40 day. Each uterine horn is filled and emptied five to 10 times with 30-200 ml of fluid each time, according to the size of the uterus. The embryos are flushed out with this fluid into a large graduated cylinder. After 30 minutes, embryos settle and can be located under a microscope by searching through an aliquot from the bottom of the cylinder. Most commonly, two-way and three-way Foley (soft and short) catheters are used in embryo transfer. Sometimes it is possible to use Modell Neustadt (stiff and long) two-way catheter.

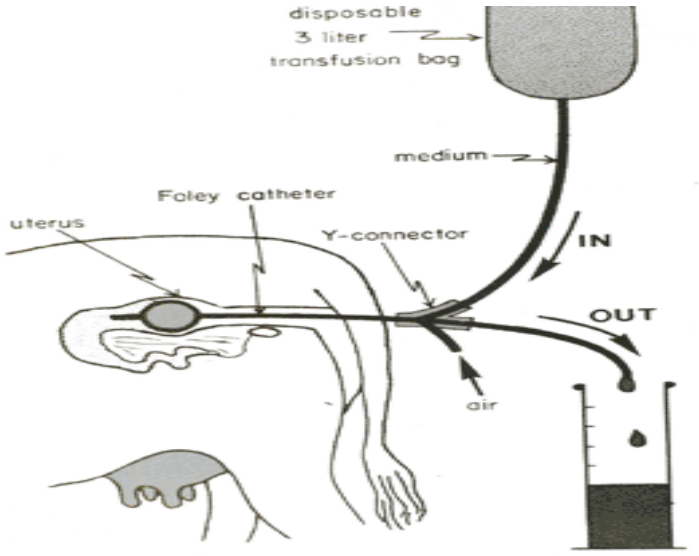


Fig. 7.9 Embryo collection with Folly catheter

Laparoscopy: The basic difference between surgical technique and laparoscopy is that instruments for flushing are inserted through stab wounds rather than by mid-ventral incision. This is inserted through one stab wound while visualizing the uterus a 2 way-Foly catheter is inserted through another stab wound and guided into one horn and ballon is inflated. Next an IV catheter is inserted into the uterine lumen close to uteri-tubal junction. About 30-40ml PBS injected through IV catheter and flushing collected through folly catheter. Repeat the procedure till 150-200 ml PBS is finished

Surgical method: Consists of exposing the reproductive tract by mid-ventral incisions under general anesthesia. Embryo collected from uterine horns 5 days after estrus. Fluid introduced into the base of uterine horn and flushed towards utero-tubal junction.

Searching of Embryo

Media after flushing is allowed to settle down for 35 min in a straight-sided cylinder. A siphon of sterile silastic tubing may then be set up to remove all but bottom 50 ml of medium. Remaining media is agitated, swirled, and aspirated into a syringe with a uterine infusion pipette. Place the media in a sterile, disposable petri dish. Observe the aspirated media under a stereo microscope.

Evaluation of Embryos

Embryos are evaluated for quality and potential likelihood of success if transferred to a recipient female. Criteria considered are:

- Regularity of shape of the embryo
- Compactness of the blastomeres (the dividing cells within the boundaries of the embryo)
- Variation in cell size
- Color and texture of the cytoplasm (the fluid within the cell wall)
- Overall diameter of the embryo
- Presence of extruded cells
- Regularity of the zona pellucida (the protective layer of protein and polysaccharides around the single celled embryo)
- Presence of vesicles (small bubble like structures in the cytoplasm)

Table 7.2 Embryo Grading

Grade	Quality	Typical characteristics of embryo
1	Excellent	Embryo perfectly symmetrical, showing even granulation, and with a well-defined, distinct outline; no blastomere extrusion. The embryo should be at the expected stage of development for its age
2	Good	Embryo showing even granulation with a well-defined distinct outline; some blastomere extrusion and some minor blastomere degeneration; occasionally somewhat asymmetric in shape
3	Fair	Embryo intact but with a hazy outline in parts; obvious defects apparent such as extruded cells, vesiculation, and some degenerate blastomeres
4	Poor	Embryo showing uneven granulation with a hazy outline; much blastomere extrusion and degeneration evident; sometimes shaped abnormally
5	Degenerate	Degeneration so pronounced that it may not be possible to determine the exact developmental stage; sometimes shaped abnormally

Selection and Preparation of Recipient

It is a critical step for embryo transfer success. Recipient must have the following characteristics:Must have proper nutrition (BCS 6 out of 9 scale); Should have gone through herd health program; Synchronization of estrus (within 1 day) between the donor and recipient cow; Must not have any problem in reproductive tract; Recipient should not have weak CL. The placement of embryo is better in ipsilateral to ovary having CL.

7.5 Ovum Pick Up

Ovum can be collected from slaughter or live animals. Ovum collected from slaughterhouse by aspiration, and ovum pick-up from live animals. Trans-cervical method is a non-invasive procedure for recovering oocytes from antral follicles in live animals. In live animals, the procedure is guided by ultrasonography for aspiration of follicular oocytes. Together with *in vitro* fertilization of oocytes, OPU has been taken as a most flexible and repeatable technique to produce embryos from any given live donor. Unlike MOET, OPU does not interfere with the normal reproduction and production cycles of the donor. From abattoir, ovaries are transported to lab in a medium containing saline, antibiotics, and kept in 32-37^{O}C. Aspirated oocytes are kept in (TCM-199+ 10%FBS).

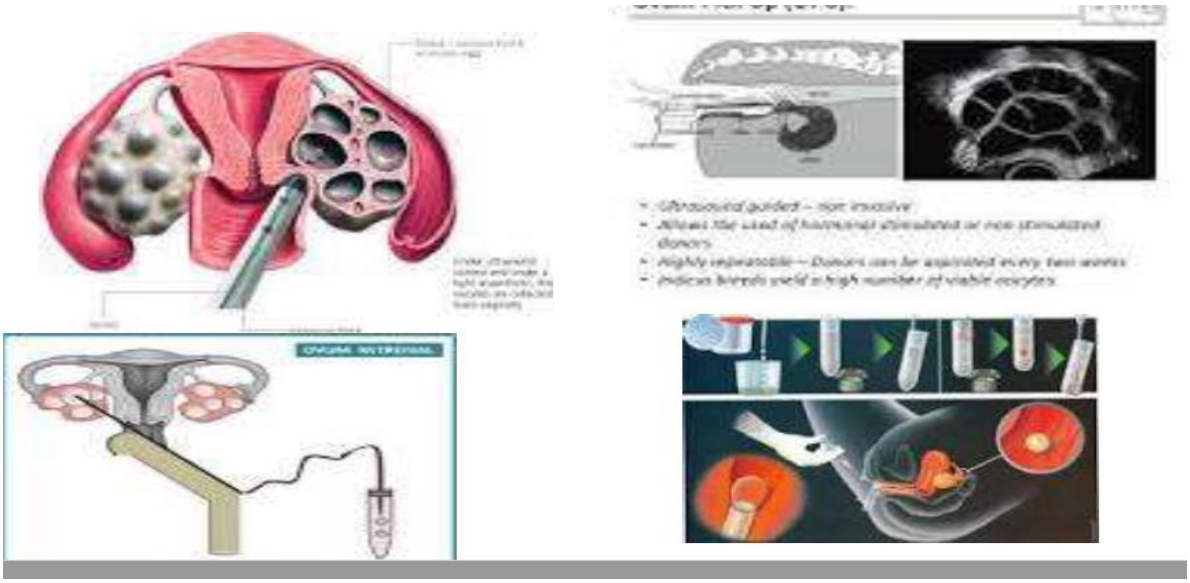


Fig. 7.10 Oocyte collection from live animal

Table 7.3 Grading of oocytes

Grade	criteria
A	Compact cumulus-oocyte-complexes (COCs) with an unexpanded cumulus mass having ≥4 layers of cumulus cells, and with homogenous evenly granular ooplasm, intact zona pelucida
B	COCs with 2-3 layers of cumulus cells and a homogenous evenly granular ooplasm
C	Oocytes partially or wholly denuded or with expanded or scattered cumulus cells or with an irregular and dark ooplasm
D	Poor quality

7.6 In Vitro-Fertilization (IVF)

The collected oocytes from a donor female that are matured in the lab and fertilization of matured oocyte in a laboratory dish. The first cattle calf was born by using IVF in 1982 by Brackett *et al*. The embryo resulting from IVF is cultured in a specific media for a few days and ultimately transferred into a female recipient. The eggs after collection are placed in 5% CO2 incubators in the IVF laboratory. Most viable spermatozoa are recovered after processing for inseminating the eggs. Because of the thick layer of zona pellucida and thousands of follicular cells around the ova, embryologists usually add approximately 100,000 spermatozoa for an egg. The addition of large number of viable spermatozoa to each ova will disperse the follicular cells and also ensure fertilization of egg by one spermatozoa. IVF has been used to treat many infertility issues, i.e., when both fallopian tubes are blocked, fertilization of the egg cell has to take place outside the body. It is effective for animals suffering from certain issues of infertility such as tubal obstruction, endometritis.

7.7 Semen Sexing

Sexing: semen and of embryos provides in species and production circumstances where X- or Y-chromosome-bearing sperm is preferred, a way to produce the wanted sex type of animal. Manipulating the sex of the animals has become of great

interest in dairy producers, due to several sex-related traits, like milking, herd replacement and growth rates. Semen having sperms of a desired sex (with 80-90% accuracy) is known as sexed semen which predetermines the sex of the offspring prior to conception. The principle of sexing is based on difference in X and Y chromosomes. The chromosomal DNA contents (higher in X than Y spermatozoa), X sperm has a negative charge and Y sperm has a positive charge and and the X sperm swim slower than Y the sperm . among different sexing methods, Flow cytometric Sorting is better different criteria.

Importance **of sexed semen**

- 90 % heifer calves
  - Greater numbers of heifer calves, Replacements born earlier
- Increase rate of herd expansion, select best cows to breed replacements from
- Reduce production of unwanted dairy cattle males

Drawback of sexed semen use

- High wastage during sorting and expensive as compared to conventional semen
- The use of an UV excitable DNA specific stain brings the risk of cytotoxicity and mutagenic effects.
- Causes different types of damage on the viability of sperm

Review Questions

1. Differentiate between one shot and two shot PG injections in bovine.
2. What you understand from superovulation and embryo transfer
3. What do you still need to know about artificial inseminations, and how could to achieve effective conception?

8. INFERTILITY

At the end of this chapter the trainees be able to:

- Define infertility
- Explain the way to combat infertility in dairy industry
- Describe key indicators of fertility

Infertility is one of the bottleneck in dairy producers that leads to reduction in calf production below the expect level.

8.1 Infertility in Male

Infertility in male is due to abnormal spermiogenesis. A high incidence of abnormal spermatozoa observed are found to be linked with testicular hypoplasia in mammals. Interval occurrence of testicular hypoplasia is a normal phenomenon in seasonal animals. However, an increased frequency of a specific type of abnormal spermatozoa is encountered in non-seasonal species in association with normal testicles, and in these cases the condition is believed to be inherited. An example associated with this type is the "knobbed acrosome". The acrosome of the defective spermatozoon exhibits acentric thickening due to differentiation failure of the proacrosome, and subsequently is unable to adorn over the nucleic surface. Generally, the affected bull is sterile. This condition is caused by a single autosomal recessive gene. It is thought that these defective spermatozoa are unable to undergo capacitation and acrosome reaction.

Table 8.1 Sperm defects affecting fertility

Defect	**Description of Defect**	**Fertility**
Knobbed	Sperm Acentric thickening of acrosome	Sterility
Decapitated	Head and tail separated at the neck region	Sterility
Dag defect	Folding of tail over the midpiece, giving an impression of a swollen midpiece	Infertility
Pseudodroplet	Rounded or elongated thickening of the midpiece	Sterility
Corkscrew	Tail defect	Infertility
Diadem effect	Nuclear pouch formation	Infertility
Sterilizing	Tail stump Tail defect	Sterility

Mesonephric/Wolffian/ duct aplasia: During ontogenic development mesonephric duct, segments of the epididymis, ductus deferens, and the vesicular gland, which are not fully developed are occasionally observed in some males. This malformation may be either unilateral/bilateral despite the gonads being well-developed.

Epididymal stenosis: resulting in spermatocoele. Stenosis of the epididymis generally has a higher incidence in bulls. In this defect, the spermatozoa which accumulate in the ductus efferent lead to the formation of a spermatocoele in the epididymal head of sexually mature males.

Testicular hypoplasia: Testes of subnormal size which exhibit inadequate growth and development are considered as hypoplastic. Although cryptorchid testes are hypoplastic, testicular hypoplasia also occurs with normal descended testes in some animals. It is commonly found in cattle, is inherited as a sex-limited recessive trait. Removing affected bulls from the herd is, however, ineffective in eliminating testicular defects. Nevertheless, to reduce the incidence, bulls with gross testicular defects are not used in natural services, and also breeding parents who had produced offspring with testicular defect are also avoided.

Cryptorchidism: is the failure of the testes to descend into the scrotum near/soon after parturition. The position of the undescended testis may be in any part of the descent path, including the inguinal region or diverted to an ectopic position after traversing the inguinal canal. The affected testicles remain smaller than normal since the germ cells fail to undergo normal prepubertal development. Orchidectomy of the maldescended testis is advised since tumors are common among cryptorchid animals.

8.2 Infertility in Cows

Fertility in cows refers as producing a calf per year (1 calf/cow/year). Whereas, infertility is animals having reduced in fertility. Sterility refers to absolute inability to reproduce offspring. The causes of infertility divided into:

- Structural/ anatomical abnormalities.
- Functional aberrations →Physiological problems
- Management factors
- Nonspecific Infectious agents

8.2.1 Structural/ anatomical causes of infertility

Grossly, divided in two:

A. Congenital abnormalities

- Ovarian agenesis →ovarian developmental problem
- Ovarian hypoplasia → incomplete growth, autosomal recessive gene
- uterine tube abnormalities →ex double cervical os
- Intersexuality → (Hermafrodism, affects genes)
- freemartins →Hormonal cause, females co-twin with male and has shared placenta, the exchange of fluid and blood between the two calves mixes the antigens responsible for carrying the unique sex. Twins develop with some sex x/ces of both the male and female
- Segmental aplasia of the tubular genital tracts → recessive genes

B. Acquired lesions

- Uterine tubes adhesions & occlusions, usually due to inflammation, some iatrogenic (surgery)
- Hydrosalpinx → cranial occlusion of the oviduct
- Pyosalpinx → inflammation mostly due to infection

- Cervical fibrosis → 'ring womb' due to trauma from assisted delivery
- Tumors → interfere with all stages of fertility ex. fertilization, conception, pregnancy, birth etc

8.2.2 Functional Aberrations

Can affect individual but may a herd problem. These are:

A. No observed estrus due to:-

- True anestrus/acyclicity →postpartum anestrus interval
- Silent estrus →ovulation without estrus, E_2 deficiency
- Non-detected estrus → missed oestrus due to mgt deficiency
- Persistent CL → chronic infections/pyometra because this damages endometrial cells which produce PGF2α, Ovarian cysts

B. Ovulatory Defects:-

- Delayed ovulation ⇒Fertility failure due to aging of the sperm cells
- Anovulation ⇒Follicular cysts, luteinized cyst, luteinized follicle
- Luteal deficiency → short-lived CL with either normal or sub-optimal P_4 level and CL of normal duration with sub-optimal P_4level
- Hormonal imbalance/asyncrony → ex, delayed LH surge → delayed ovulation
- Fatty Liver disease → affects hormone metabolism including reproduction

C. Management factors

- Poor detection of estrus
- Incorrect timing if artificial insemination
- Nutritional deficiencies and excesses

D. **Non-Specific Uterine Infectious Agent**

- Opportunist pathogens gain entry at or after parturition
- If not eliminated during puerparium ⇒hostile uterine environment leading to endometritis (purulent or mucopurulent discharge from vulva)

8.3 Fertility Parameters/ Key Performance Indicators

The following parameters are important for assessing fertility in a herd/individual animals:

- Calving interval (Interval in days from one calving to the next
- Age at first calving (Interval from birth to first calving)
- Calving rate (Fertility rate) (No. cows calving/No. cows served x 100)
- Calving to conception interval (from calving to the subsequent effective service)
- Days open (days since calving the cow to not pregnant)

- Non-return rate to first insemination
- Pregnancy rate to first service (No. diagnosed pregnant/No. of first services x 100)
- Services per conception (No. of services which resulted in a diagnosed pregnancy/Total no. of services), 1 ideal, 1.5-2 good, >2 poor

Overall Fertility Targets in Developed countries

- Mean Calving to first service interval (days) =65
- Mean calving to conception interval (days)/Days open =85 for developed country and 115 in Ethiopia
- Mean interval from first service to conception (days) = 20
- Overall pregnancy rate (%) = 58
- First service pregnancy rate (%) = 60

8.4 Repeat Breeding

A cow that has normal reproductive tract with normal estrus cycle but which does not hold during repeated services by fertile bull or quality semen. Therefore, a repeat breeder is a cow

- That has had 3 or more unsuccessful services
- Has normal oestrus cycle with approx 21 days interval between services
- Free from palpable abnormalities
- Has no abnormal vaginal discharge
- Has calved at least once before
- < 10 years old

Causes of repeat breeding;

A. Fertilization failure

- Ovulation failure → ex, occlusion of oviduct
- Defective ova → ex, due to aging of sperm and/or egg
- Endocrine dysfunction → ex, failure of LH surge
- Management → nutritional deficiency, obesity, incorrect timing of AI
- Nutritional deficiency → Energy, protein and micronutrient

B. Embryonic death

This might be due to infections which causes luteolysis of CL/or blastolysis leads to embryonic mortality. In addition:

- Endocrine dysfunction → ex, decline in progesterone, deficiency of maternal recognition of pregnancy (INFτ)
- Nutritional deficiency → Energy, protein and micronutrient deficiency

As a closing note, persistence ovarian cyst due to endocrine insuffiency can be treated using GnRH hormone and luteinized CL can be regressed by exogenous administration of PGF and it's analogs.

REVIEW QUESTIONS

1. Define infertility in cows
2. How could you treat repeat breeding animals
3. What are the key indicators of fertility

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