

LABORATORY MANUAL ON ANIMAL GYNAECOLOGY

S.K. Agarwal

Senior Scientist

U. Shanker

Principal Scientist

M.R. Ansari

Principal Scientist

Division of Animal Reproduction



Indian Veterinary Research Institute

Izamnagar - 243 122 (U.P.) India

Cover Photo :

Ovarian follicles and CL in gonadotrophin treated goat

Back Cover Photo :

Non-surgical embryo transfer in a buffalo cow

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भारतीय पशु-चिकित्सा अनुसंधान संस्थान
इज्जतनगर - 243 122 (उत्तर प्रदेश) भारत
INDIAN VETERINARY RESEARCH INSTITUTE
IZATNAGAR - 243 122 (U.P.) INDIA



डा. एम.पी. यादव
Dr. M.P. Yadav, M.Sc., Ph.D.
निदेशक
DIRECTOR

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FOREWORD

Productivity is the key to growth and reproduction is the back bone of animal production. Livestock production is an important source of income for the rural poor in developing countries. India has a very large livestock population and majority (70%) of them are in the hands of small and marginal farmers. Most of our indigenous stock of productive animals are low to medium producers. Reproductive problems marginalize their potential. Of the many technological advances around which explosion of knowledge has occurred, the recent advances in the diagnosis and management of reproductive technologies undoubtedly are central to sustainable and rapid growth of livestock sector. There is scope for further improvement in the reproductive status of our farm animals. The advent of Biotechnology has created a new impetus for exploring new reproductive technologies. The recent scientific developments in this area have made it possible to manipulate the reproductive processes in many ways to revolutionize world animal agriculture. To harvest the benefit of these contemporary developments, the technical know-how needs to be passed on to the practitioners of animal reproduction and other entrepreneurs.

I am pleased to learn that a Laboratory Manual on Animal Gynaecology is ready for release. I congratulate the authors for their valuable contributions in bringing out this manual on Animal Gynaecology which provides an in depth practical know-how on different animal gynaecological techniques, diagnostics and therapeutics for infertility control and augmentation of fertility in livestock. I hope, the manual will be of immense use to the students, teachers, scientists, practising veterinarians and all other personnel concerned with animal reproduction.

(M.P. YADAV)

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Chapter-1

GYNAECOLOGICAL EXAMINATION OF FEMALE MORBID GENITAL ORGANS

OBJECTIVES

- * To study gross features and location of different parts of genital organs
- * To study functions of different parts of genital organs
- * To learn method of examination of genital organs

MATERIALS REQUIRED

Morbid genital organs, SS tray for keeping genital organs, Scissors, Forcep, Gloves, BP handle and blade, Scale, Thread and Balance.

DESCRIPTION OF SUBJECT

The female reproductive system is consist of ovaries, fallopian tubes, uterus, cervix, vagina and vulva.

Ovaries : Primary organ of reproduction, two in numbers.

Functions :

- * Production of gamete i.e. ovum.
- * Production of hormones
 - Estrogen
 - Progesterone
 - Inhibin
 - Relaxin

Location :

- * At the upper portion of the abdominal cavity.
- * Near the ventral floor of the pelvic inlet.
- * Supported by uteroovarian and mesoovarian ligaments

Gross Features

Cattle and buffalo

Shape : Oval or almond shape
Size : 3.5 x 2.5 x 1.5 cm - cattle
 2.9 x 1.3 x 1.1 cm - buffalo

Weight of each ovary

Cattle	: 5 - 10 g
Buffalo	: 2.5 - 4.5 g

Remarks :

- * Size of buffalo ovaries are generally smaller than cattle
- * Right ovary of both the species is slightly bigger than left.

Sheep and goat

Shape	: Almond shape
Size	: 1.6 x 1.1 x 0.77 cm
Weight	: 1 - 2 g / ovary

Remarks :

Sizes of sheep and goat ovary is approximately half of the cattle ovary

Mare

Shape	: More or less kidney shape
Size	: 8 x 3 x 2 cm
Weight	: 70 - 100 g per ovary

Remarks :

- * Ovaries of mare is 2-3 time larger than cattle ovaries.
- * Ovaries in mare has a notch (a concave portion) called ovulation fossa - ovulation occurs here.

Sow

Shape	: Oval
Size	: 2.5 x 1.4 x 1.5 cm
Weight	: 3 - 5 g per ovary

Remarks :

Ovary appears like bunch of grapes due to development of more number of follicles and corpus luteum.

Bitch

Shape	: Oval - small
Size	: 2 x 1 x 0.5 cm
Weight	: 2-3 gm

Remarks :

Resemblance of ovary is similar to sow due to multiple follicular development and formation of CL.

Graafian follicle

Soft fluid filled raised structure on the surface of ovary.

Diameter :

Cattle	: 12 - 20 mm
Buffalo	: 10 - 12 mm
Sheep and goat	: 5 - 8 mm
Sow	: 8 - 12 mm

Corpus luteum (CL)

Transitory endocrine gland formed following ovulation of graafian follicle.

TERMINOLOGY USED

Corpus luteum haemorrhagicum : Freshly formed CL

- * Brick red coloured body

- * Soft on palpation

- * Starts projecting out of ovarian surface

Corpus luteum spurium - Cyclic CL

- * Well projected out of surface of ovary
- * In buffaloes it is invariably embedded in nature and creates problem during per rectal palpation.

Corpus luteum verum - Pregnancy CL

- * Occupies maximum (75%) area of the ovary.
- * Gets embedded in the ovarian cortex due to its increased weight.

Corpus luteum albicans - Regressing CL

- * Hard scar like structure
- * Whitish coloured body

Cattle and Buffalo

- * Usually one CL is formed at each cycle.
- * The corpus luteum after its formation following ovulation increases in size and reaches to its maximum by day 11-12 and remains upto day 14-16 of the cycle.
- * The average diameter and weight is 15-20 mm, 4-7 gms in cattle and 14-15 mm, 2-3g gm in buffaloes, respectively.

- * It starts regressing by day 16/17 losing its yellowish colour leaving a small white scar on ovarian surface known as corpus albicans. Animal, if gets pregnant corpus luteum persists.

Mare

- Corpus luteum is cauliflower shaped.
- Diameter is 3/4 to 1"
- Difficult to palpate
- Remain mostly within the surface of ovary in the region to ovulation fossa.
- * Accessory CL are formed during day 40-140 of gestation.
- * Acc. CL are followed due to secretion of PMSG (eCG) from endometrial cups of pregnant uterus.
- * Acc. CL regresses by day 140-150 of gestation.

Sheep and goat

- * 1-3 CL are formed following ovulation depending upon breed and plane of nutrition.

Sow and bitch

- * Multiple CL are formed.
- * Ovary appear like bunch of grapes.

Fallopian tubes (oviducts)

Gross features

- * A pair of convoluted tubes extending from near to the ovaries (for receiving ovum) to the tip of uterine horns.
- * Oviducts are embedded in the bursa (derived from lateral layer of broad ligament mesosalpinx).
- * The diameter of oviduct is 1-3 mm and length is 20-30 cm in most of the farm animals. In dog and cat it is of 4-8 cm long.
- * It has three major segments i.e. infundibulum, ampulla and isthmus. The funnel shaped opening of fallopian tube of ovarian end is known as infundibulum which has fimbria for capturing the ova following ovulation.
- * Isthmus of oviduct joins the tip of uterine horn (uterotubal junction).

Functions

- * Picking up ova following ovulation.
- * Provide the site of fertilization (Ampullary - isthmic junction)
- * Gamet (egg and sperm) transport to the site of fertilization.
- * Transport of zygote/embryo from the site of fertilization to uterus.
- * To provide micro environment (nutritive and protective) to oocyte, sperms (capacitation of sperm) zygote and to early cleaved fertilized eggs.

Uterus

It consists:

- * Two uterine horns (cornua)
- * A uterine body

Location

- * Pelvic cavity except during advanced stages of gestation when it descends into the abdominal cavity.

Size:

Cattle, mare, sow

Length - 35-60 cm

Ewe and goat - Size is half of the cow.

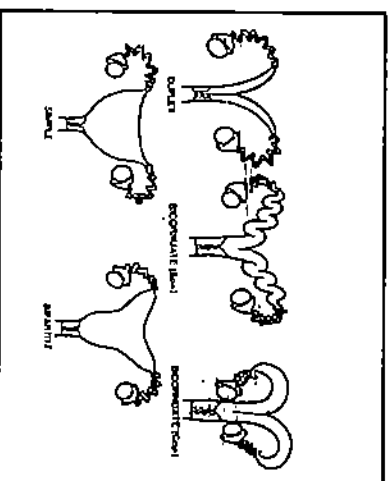
Dog - uterus has a short body (2.5 cm) and long horns (15-20 cm and its dia. is 0.5 to 1 cm.)

- Uterus lies entirely within the abdominal cavity.

Types of uterus

Bicornuate

- * Found in sow, cow, buffalo, goat and sheep.
- * Characterized by a small uterine body and two long uterine horns.
- * Sow has longer and convoluted uterine horns than cattle, buffalo, ewe and doe.
- * Uterine horns fuses near to uterine body and gives impression of larger uterine body than actually exists that why some people classify it as bipartite.



Types of uterus

Remarks:

Body of uterus in buffalo (1 - 1.5 cm) is much smaller than cattle (2.5 to 4 cm).

Bipartite

- * Found in mare
- * Prominent uterine body anterior to cervix and two horns not as long and distinct as in bicornuate type.

Duplex

- * Found in rat, rabbit, guinea pig.
- * Consists of two uterine horns each with a separate cervical canal opening into vagina.

Simplex

- * Found in primates and human being.
- * Consists of a pear shaped body with no uterine horns.

Cotyledons

- * Button like structure in the uterus.
- * Presence of cotyledons in the uterus is characteristics to cattle, buffalo, sheep and goat. The average number of cotyledons are 60-120. They help in placentaion during pregnancy :

Cattle	-	90-120
Buffalo	-	60-90
Sheep & goat	-	88-96

Remarks :

- * No. of cotyledons in buffalo are less than the cattle.
- * Size of cotyledons are smaller in sheep and goat.

Functions of uterus

- * Transport of spermatozoa to the site of fertilization.
- * Capacitation of spermatozoa.
- * Nourishment and development of early embryo.
- * To provide site for implantation of embryo.
- * Protection and development of foetus.
- * Control the life span of corpus luteum by the secretion of prostaglandin F₂ alpha.
- * Play a major role in expulsion of foetus and involution of uterus thereafter.

Cervix

- * A tubular structure with a constricted lumen.
- * Door way to the uterus.
- * Located caudal to uterus either in pelvic cavity on pelvic brim or in the abdominal cavity.
- * Length 5-10 cm, diameter 2-6 cm in most of farm animals.
- * In cattle, buffalo, sheep and goat cervix has transverse interlocking ridges known as annular rings, to prevent entry of contaminants to the uterus.
- * No. of annular rings are 3-5 in cattle and 1-3 in buffaloes (mostly only 2).
- * In sow cervical canal is cork screw type in configuration to adjust the cork screw type penis of boar.

- * In mare cervical canal is more open and mucosal folds project outside into vagina for preventing contamination.
- * In dog cervix is poorly defined and is short (0.5 to 1 cm in length).

Functions

- * Facilitates sperm transport.
- * Act as sperm reservoir.
- * Prevent entry of contaminants by getting tightly closed during pregnancy.

Vagina

- * Organ of copulation.
- * Thin walled, elastic tubular structure.
- * Located in pelvic cavity, dorsal to urinary bladder.
- * Length is 20-30 cm in cattle, buffalo, mare, sow and 8-15 cm in sow, doe and ewe.
- * In bitch vagina is long (10-15 cm) and narrow. The muscular coat is thick.

Functions

- * Site of copulation.
- * Facilitates process of parturition.

Vulva

- * External genitalia.
- * Provide protection to internal genitalia.

Clitoris

- * Erectile structure, located 1 cm inside the vulva.
- * Well developed in mare.

Procedure of examination

- * Procurement of genital tract from local slaughter house.
- * Washing and cleaning of genitalia in running water.
- * Placement of genitalia in the enamel/stainless steel tray.
- * Hold cervix (the tubular structure) first between index and middle finger of left hand.
- * Make free the thumb and palpate the external OS of cervix for any enlargement or deformity. Try to insert the thumb into OS cervix to have an idea whether OS is open or closed.
- * With the help of index, middle finger and thumb palpate whole surface of cervix to know whether it is hard/firm or flacid/relaxed. Palpate for any normal growth or enlargement of cervix, if any.
- * Proceed further by palpating body of uterus and fix index finger between the intercomal junction.

- * Palpate whole surface of left horn with the help of three fingers. Now catch ovary of the left side and fix in between index and middle finger and palpate its whole surface for the follicles (elevated soft fluid filled structure) and corpus luteum (projected hard structure).
- * Stretch the bursa using index and middle finger to palpate its freeness and adhesion, if any.
- * Fallopian tubes are embedded in the bursa but difficult to palpate. This is palpable while palpating bursa. It is more prominent, palpable, enlarged if there is hydrosalpinx and pyosalpinx.
- * The same way right side of uterine horn, ovary, bursa and fallopian tube are palpated.
- * Dissect the cervix with the help of BP blade, BP handle and scissor longitudinally to note number of annular rings and arrangement of muscle fibre.
- * Dissect the body of uterus and horns to note the number and arrangement of cotyledons in cattle, buffalo, sheep and goat.
- * Measure the length and diameter of all parts of genitalia with the help of scale and thread.

RECORDING OF OBSERVATIONS

OVARY

Right ovary Left ovary

Shape
Size
Follicle

CL

Fallopian tube and bursa have abnormal structure.

Uterus :

- * Length and diameter
- * No. and arrangement of cotyledons
- * Tone of uterus
- * Any gross abnormalities

Cervix

- * Shape
- * Size
- * No. of Annular rings

Vagina and vulva

- * Appearance

- * Abnormalities, if any

REMARKS

Write down status of genitalia.

Chapter - 2

DIAGNOSTICS FOR CONFIRMATION OF DIFFERENT PHASES OF OESTROUS CYCLE

OBJECTIVES

- * To study changes in the genital organs during different phases of the oestrous cycle.
- * Confirmatory diagnosis of different phases of oestrous cycle

MATERIALS REQUIRED

Animal, animal crate, cover all apron, gum boots, arm sleeves, liquid paraffin, soap, water and towel.

DESCRIPTION OF SUBJECT

In female animals, with the onset of puberty a distinct behaviour of sexual receptivity develops which is called oestrus (heat). There is development of definite physiological functional rhythm of the reproductive system called oestrous cycle. The duration and intensity of oestrus vary according to the species, breed, nutrition, environment and managerial practices.

Interval between first and last acceptance of male or teaser is known as duration of estrus. The period between two oestrous is known as oestrous cycle. The oestrous cycle is comprised of four phases :

- * Proestrous
- * Oestrous
- * Metoestrous
- * Dioestrous

Duration of different phases of oestrous cycle

Animal species	Proestrus	Estrus	Metestrus	Dioestrus	Length of oestrus cycle
Cattle and buffalo	3 days	12-24 hr	3-5 days	12-13 days	20-22 days
Mare	3 days	4-7 days	3-5 days	6-10 days	20-23 days
Sheep	2 days	24-36 hr	3-5 days	7-10 days	16-19 days
Goat	2 days	24-48 hr	3-5 days	7-10 days	17-20 days
Sow	3 days	48-72 hr	3-4 days	9-13 days	19-20 days
Bitch	9 days	9 days	Pregnancy	Pseudo pregnancy/anoestrus	

Time of ovulation and AI/service

	Time of ovulation	Time of AI/Service
Cattle and buffalo	10-12 hr after end of estrus	10-12 hr after onset of estrus (mid to end of estrus)
Mare	1-2 days before end of estrus	2-4 days after onset of estrus
Sheep	24-30 hr after onset of estrus	12-24 hr after onset of estrus (Towards the end of estrus)
Goat	24-40 hrs (Towards end of estrus)	24-36 hr after onset of estrus
Some	30-40 hr after onset of estrus (Towards the end of estrus)	24-36 hr after onset of estrus

Changes in the genitalia during different phases of the cycle**Proestrus**

- * Increased vascularity of vaginal mucosa. It appears reddish in colour.
- * There is increased thickness of vaginal epithelium.
- * Relaxation of anular rings, cervical musculature and opening of OS of cervix.
- * Increased secretion of mucus from goblet cells of uterus, cervix and vagina.
- * Increased vascularity of uterine mucosa and development of uterine tone.
- * Proestrus bleeding in dogs due to increased endometrial vascularity.
- * Development of palpable follicle on any one of the ovary.
- * Regression of previous cycle corpus luteum. It undergoes degeneration and decreases in size. It is palpable as scar like structure (corpus albicans).

Estrus

- * It is fairly well defined period characterized by sexual desire and acceptance of male (note the changes in the behaviour of estrus females).
- * Well developed graafian follicle is palpable on the surface of ovary.
- * Animal remains under the influence of estradiol 17-beta produced by the maturing follicle.
- * The uterus is erect, tonic and turgid.
- * Increased secretion of mucus from uterus, cervix and vagina. The clear stringy mucus hangs from the vulva.
- * Cervix is relaxed, os of cervix open.
- * The mucosa of vagina is thickened and many cornified epithelial cells being desquamated.

- * In dog and cat thickening of vaginal epithelium is more pronounced and the time of mating/ovulation can be predicted by studying vaginal exfoliative cytology.
- * Increased vascularity of vaginal mucus membrane.
- * Vulva become edematous.
- * In most of the farm animals, ovulation occurs towards the end of estrus except cattle and buffalo where ovulation takes place 10-12 hrs after the end of estrus.
- * Cat is induced ovulator. Coitus is prerequisite for ovulation. In absence of male estrus may be prolonged to 7-10 days.

Metestrus

- * Poorly defined period following estrus.
- * Ovulation takes place early during this period in cattle and buffalo.
- * Formation and growth of corpus luteum.
- * Metestrus breeding occurs in some cows due to hemorrhage from hyperemic blood capillaries of uterine endometrium.
- * Mucus secretion from uterus, cervix and vagina decreases.
- * OS cervix gets closed.
- * Dog and cat if not conceived, pseudopregnancy develops which lasts for 50-60 days.

Diestrus

- * Longest phase of oestrus cycle.
- * Well developed corpus luteum may be palpable on the surface of ovary.
- * Animal remains under the influence of progesterone.
- * Uterus becomes flacid.
- * Cervix firm and OS and cervix closed.
- * Mucus membrane of vagina is pale.
- * End of this period if animal does not conceive is beginning of proestrus in polyestrus animals otherwise anestrus may develop.

Procedure of perrectal gynaecological examination

- * Secure the animal in cattle crate.
- * Put full arm sleeve, wash with warm water and soap and get it lubricated using liquid paraffin.
- * Insert the index finger first, make cone like appearance of fingers and thumb and insert the arm into rectum.
- * Remove the dung from the rectum to make palpation of genital organs easy. Don't remove the hand from the rectum till the palpation is completed.

- * Hold the cervix between index, middle finger and thumb. Palpate the surface of cervix and its straightness or kinkiness, if any. Note firmness and relaxation of cervix. Palpate the external OS of cervix to get an idea of its openness. Try to insert the thumb into external OS of the cervix. If, the OS is closed it will resist the thumb to be pushed into it.
- * Proceed forward and fix the index finger between the intercornual junction. Palpate the uterus for its tone, size and for any enlargement or outgrowth.
- * Proceed further and palpate whole of the uterine horn with the help of index, middle finger and thumb.
- * Now catch the ovary of one side of the genitalia, fix between index and middle finger and palpate whole ovarian surface for any adhesion of ovary (ovary will not be free, palpated as adhered with surrounding bursa). Palpate the soft elevated, fluctuating area i.e. the follicle on the surface of ovary (follicular phase, estrus or approaching to estrus).
- * Palpate for projecting, hard structure over the surface of ovary it is the corpus luteum. In buffalo corpus luteum is always not well projected as in cattle. This is invariably imbedded in the ovarian cortex. It is felt like flattened hard structure. During the pregnancy, also the CL gets imbedded in the ovary. The mare's ovary is pear shaped. The follicle and CL are palpated within the area of ovulation fosa. The corpus luteum is cauliflower in appearance and difficult to palpate due to its imbedded nature.
- * Using index, middle finger and thumb try to palpate bursa and the oviduct in the surroundings the ovary. It is difficult to palpate normal oviduct but in case of inflammation, infection, the oviduct enlarges and it may be palpated. Bursa is normally free and oviduct is imbedded into it. In case of bursal adhesions area surrounding to ovary is palpated as adhered, encapsulating the ovary.

RECORDING OF OBSERVATIONS

Vulva and vagina

- * Nature of mucus membrane
- * Edematous or dry.

Cervix

- * External surface
- * Firm/relax.
- * OS cervix open/closed
- * Any enlargement or kinkiness

Uterus

- * Size and tone
- * Symmetry of horns
- * Any enlargement

Ovary

- * Size and shape
- * Follicle/CL
- * Any abnormalities (Adhesion or outgrowth)

Bursa and Fallopian tube

Nature of vulvar discharge, if present

REMARKS

Write down gynecological status of animal.

Chapter - 3**DETECTION OF ESTRUS IN CATTLE, BUFFALO, MARE, SHEEP, GOAT, PIG AND DOG****OBJECTIVES**

- * To study different methods used for detection of estrus.
- * Confirmation of estrus for timely AI/Service.

MATERIALS REQUIRED

Animals, teaser, glass slide, scissor, microtube, arm sleeves, gum boots, soap, towels etc.

DESCRIPTION OF SUBJECT

Estrus is derived from a Greek word 'Oistros' meaning mad desire. This is the period of mating. Increased spontaneous activity, changes in the behaviour, male like mounting activity and copulatory responses are developed during the estrus. The duration of estrus is 16-24 hr in cattle and buffalo, 24-36 hr in ewe, 24-48 hr in goat, 40-72 hr in sow and 4-8 days in mare and bitches.

IMPORTANCE

- * Estrus detection is one of the most important managerial tool for the success of AI programme.
- * This helps in timely breeding of females
- * Accurate estrus (heat) detection is a problem in buffaloes where estrus symptoms are weak.

Endocrine basis of oestrus

Action of estrogen produced by the ovarian follicles on the receptor cells in the hypothalamus is responsible for the phenomenon of oestrus. Synthesis of estradiol (estrogen) requires the coordinated activities of two ovarian cell i.e. granulosa and theca cells and two gonadotrophins i.e., FSH and LH. Theca cells produces androgen under the influence of LH which gets aromatized to estradiol in the granulosa cells under the influence of FSH.

METHODS OF DETECTION OF ESTRUS**Cattle and Buffalo****A. Behavioural signs**

- * Restlessness, nervousness and excitement.

- * Increased movement and female remains away in an attempt to search a male partner.
- * Jump on her fellow animals. Allows other animals to mount. This is clear cut symptom of estrus and called as standing estrus. This homosexual behaviour is prominent in cattle but not in buffaloes.
- * Hanging of clear transparent mucus discharge from the vulva. The discharge is scanty in buffaloes.
- * Sudden reduction in milk yield.
- * Belowing but not in buffaloes.
- * Frequent urination - more common in buffaloes.

Remarks

External behavioural signs of estrus as a whole are less intense and more or less weak in buffaloes. The help of teaser is needed for the detection of estrus in buffalo.

B. Use of a teaser**Preparation of teaser**

- * Vasectomy of male.
- * Lateral deviation of penis.
- * Penile block.
- * Penilectomy.
- * Use of androgenized female.

Remarks

- * Vasectomized male and lateral deviation of penis are most common method for preparation of teaser for heat detection.
- * Use of teaser is most reliable and successful method for estrus detection.
- * Parading of teaser in the herd (females) twice daily i.e. early in the morning and late in evening hours is a method for effective detection of estrus in cattle and buffaloes.

C. Vaginal electrical resistance (VER)

- * Conductivity of vaginal mucus changes with the stage of oestrous cycle. This change in electrical resistance provide the basis for detection of estrus by monitoring electrical conductivity of vaginal mucus using vaginal probe and resistance conductometer.
- * Electrical conductivity of vaginal mucus at estrus is low.

D. Chin ball marker

- * The chin ball marker device is fitted at the brisket region of the teaser.

- * The lever gets pressed during mounting of teaser to female and releases dye. The dye gets spread over the rump of the female. Such marked animals are estrus females and can be sorted or drafted out for A.I. or mating.
- * This method is more applicable for estrus detection in sheep and goat. In large animals there are more chances of lost of such devices.

E. Fern pattern of cervical mucus

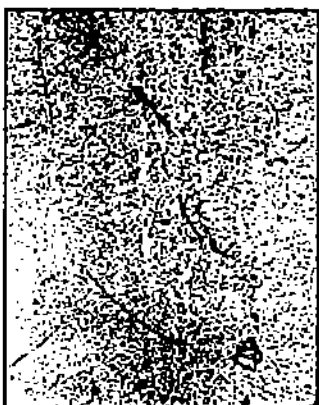
- * Cervical mucus at estrus during drying gives fern like appearance.
- * There are three types i.e. typical (clear fern leaf like appearance), atypical (mixed type appearance), nil type (no fern like appearance) fern pattern appears on drying of cervical mucus. Typical and atypical types are indicative of estrus.
- * This property of cervical mucus may be used for detection of estrus.



Typical fern pattern



Atypical fern pattern



Nil type

F. Progesterone assay

- * Progesterone concentration may be measured in blood plasma, serum or milk using radio-immunoassay (RIA) or enzyme linked immunosorbent assay (ELISA).
- * The progesterone concentration at estrus fluctuate below 1 ng/ml (at basal level).

G. Per-rectal palpation of genital organs

- * Presence of mature follicle on the surface of either right or left ovary.
- * Erectness or turgidity of uterine horns.
- * Relaxation of cervix and opening of external OS of cervix.
- * Hypemic and congestion of vaginal mucus membrane.
- * Presence of clear stringy cervical mucus discharge hanging from vulva and vagina following massage of uterus and cervix during per rectal palpation.

Sheep and goat

The symptoms of estrus in goat are comparatively conspicuous than ewe. In both the species a teaser is needed for detection of estrus.

Behavioural signs

- * Estrus ewe and goat are restless.
- * Homo-sexual behaviour is exhibited occasionally in goat but not in sheep.
- * A estrus ewe and doe stands upon mounting to a ram or buck or a teaser.
- * Estrus goat bleats and wags her tail constantly.
- * Vulva will be edematous moist with presence of mucus discharge.
- * The most practical method for detection of estrus in sheep and goat is parading of teaser twice a day (morning and evening), if flock size is small.
- * In bigger flock teaser fitted with coloured earryons on the brisket region are run @ 2% along with females.
- * Estrus females are drafted (sorted out) every day morning and evening by seeing colour marking on the rump of estrus female.

Progesterone assay

Progesterone level remains below 1 ng/ml.

Confirmation of estrus

- * On the basis of teaser mounting.
- * Progesterone concentration below 1 ng/ml.

Sow

Behavioural signs

- * Swelling and redening of vulva.

- * Restlessness.
- * Peculiar receptive grunt.
- * Remain still upon mounting/coitus by a boar.
- * The estrus sow stand motionless while pressing the loin region. She assumes mating posture.

Confirmation

By observing the behavioural signs as stated above.

Mare

Behavioural signs

- * Irritable and restlessness during estrus.
- * Exposure of clitoris and frequently adopts micriturition posture.
- * Vulva is slightly edematous with mucoid discharge.
- * Estrus mare shall allow approach of a teaser or stallion.

Per-rectal examination of genitalia

- * Presence of 25-35 mm sized mature follicle in the region of ovulation fosa.
- * Turgidity of uterus.
- * Opening of OS cervix.

Ultrasonographic imaging of ovary

- * Visualization of mature tense follicle of 25-35 mm in dia. on any one of the ovary.

Confirmation of estrus

- * By the behavioural signs.
- * Presence of follicle on the surface of ovary on rectal examination or by ultrasonography.
- * Turgidity of uterus.

Dog

A. Behavioural signs

- * The bitch accepts the male and adopts the breeding stance. She accepts coitus until the end of estrus.
- * Swelling of vulval lips. Vulval swelling is progressive during the proestrus period.
- * Continue of proestrus bleeding during early part of the true heat phase.
- * Restlessness and irritable behaviour.

B. Vaginal exfoliative cytology

- * Vaginal smears are prepared and stained with leishman's stain.
- * At proestrus large number of 'RBC' and intermediate cells are present.

- * At estrus, the number of RBC is reduced and in place of intermediate cells, cornified cells and superficial cells from the stratified squamous epithelium such as anuclear cells and cells with pyknotic nuclei are present.
- * Towards the end of estrus number of polymorphonuclear neutrophil leucocytes appear in the smear. These cells along with parabasal cells become the dominant cell.
- * Presence of abnormally large number of polymorphonuclear cells (PMN) is indicative of infection.

Confirmation of estrus

- * By observing behavioural changes i.e., restlessness and acceptance of male.
- * Presence of stratified squamous epithelial cells - superficial cells in the vaginal smears.

Procedure and recording of observation

1. External signs of estrus

- * Observe the behavioural changes of the females.
- * Allow teaser parading in the herd and record the teaser behaviour.
- * Note down external signs of estrus.
- * Observations on teaser parading.
- * Fern pattern of cervical mucus ✓
- * Take cervical mucus on a clean glass slide and allow it to get dry.
- * Examine the fern pattern (typical, atypical and nil type under the microscope.

3. Per rectal gynaecological examination

Palpate the genitalia by per rectum and record the observation on :

Ovary
Uterus
Cervix
Vagina and vulva

4. Presence of cell type and their number in the vaginal smear (vaginal exfoliative cytology)

- Prepare vaginal smear, fix with either : alcohol mixture (1:1) and stain it with leishman stain.
- Examine the slide under microscope for the cell type and their number i.e., superficial, cornified, intermediate, parabasal and basal cells.

5. Progesterone concentration in the blood plasma, serum or milk

P₄ - ng/ml

REMARKS

Write down nature of estrus and reproductive status of animal.

Chapter - 4**PATTERN OF OESTROUS CYCLE IN
CATTLE AND BUFFALO****OBJECTIVES**

- * To study the occurrence of normal estrus cycle.
- * To study prevalence of different aberrations of estrous cycle.

MATERIALS REQUIRED

- * Breeding dates and records.
- * Data sheet, calculator.

DESCRIPTION OF SUBJECT**Pattern of estrous cycle**

The length of normal estrous cycle in cattle and buffalo has been considered to be 21 ± 4 days (17-25 days). On analysis of data majority of normal cycles fall between 20-24 days. The oestrous cycle below 17 days has been categorized as short cycles, which may be due to either ovarian cysts, luteal insufficiency and aberration in the endocrinological constitution and secretion of hormones. The oestrous cycle length of more than the length of the normal cycles are termed as long cycles. There are two types of long cycle. One is multiple of normal cycles (double, triple of normal cycles) and other is irregular long cycles. These are those cycle lengths not following under the category of multiple of normal cycle. Multiple of normal cycles are due to missed heat either due to weak or silent estruses or shorter duration of estruses. This may also be due to poor heat detection programme. The irregular long cycles are mainly due to early embryonic mortality. The estrous cycle length prolongs, if the death of embryo takes place after the mid of the normal cycle (after day 9-10). Embryonic death if occurs before middle of the normal cycle (before day 9-10) the estrous cycle length usually doesn't prolong.

- * Normal cycle (21 ± 4 days).
- * Short cycles (below 17 days).
- * Multiples of normal cycle (34, 36, 38, 40, 42, 44, 46, 48 days).
- * Irregular long cycle (26, 33, 35, 37 etc.)

PROCEDURE AND RECORDING OF PATTERN OF ESTROUS CYCLE

- * Note down date of estrus of individual animal.
- * Calculate the length of estrus cycle.

- * Categorized the length of cycle and compile them under normal, short, multiple long and irregular long cycles group
- * Calculate mean and mode of such cycles.
- * Calculate percentages of such cycles and find out incidence of normal and different aberrations of estrous cycles.

OBSERVATIONS AND ANALYSIS OF DATA

Type of cycle	No. of cycle studied (days)	Mean length	%
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Normal cycle

Short cycle

Multiple long cycle

Irregular long cycles

REMARKS

Write down incidences of normal and aberrations of oestrus cycle.

Chapter - 5**ARTIFICIAL INSEMINATION IN
CATTLE AND BUFFALO****OBJECTIVES**

- * To study different methods of A.I. in cattle and buffalo.
- * To perform AI by rectovaginal method in cattle and buffalo.

APPLICATIONS

1. To obtain maximum number of offsprings of desired trait in least possible time.
2. To obtain offsprings from the outstanding male.
3. Unequal or intraspecies mating.
4. To prevent transmission of venereal diseases.

MATERIALS REQUIRED

Animals, apron, full arm obstetrical gloves, gum boot, semen (liquid/frozen), microscope with thermostal, glass slide, cover slip, pasture pipette, A.I. gun, Insemination catheter with rubber connector, AI sheath (medium/mini straw), tissue paper, water bath, thermometer, stop watch.

DESCRIPTION OF SUBJECT

Artificial insemination is a process by which semen is deposited in the genital tract of an estrus female with the help of an instrument. This is one of the simplest technique for raising healthy and economical livestock in least possible time. So far cattle and buffalo breeding programmes are concerned, the technique of artificial insemination has contributed much, judicious implementation of this technology is likely to contribute more. Genetic improvement of animals depends upon the good sire, who can transmit their inherited characters of dairy, draught and meat to their progeny. As such in a herd a sire is always valued more than the cow, as the cow can hardly produce a dozen of progeny in her entire span of reproductive life, while a sire has the potentiality of producing several thousand of progeny. There are two methods of insemination.

- (a) Vaginal speculum method.
- (b) Rectovaginal method

a) Vaginal speculum method

The speculum which is made up of hard glass or metal is first lubricated aspectically and carefully inserted into the vagina. The cervix is located either with

the aid of flashlight or sunlight. The inseminating catheter is inserted into the OS uteri or posterior part of the cervical canal on an average 1 to 3 cm deep. In speculum technique the semen is deposited up to the mid cervix only that is why low conception rate is recorded. As such recto-vaginal technique of A.I. has been preferred and universally accepted.

b) Recto-vaginal technique

In recto-vaginal technique the genital tract is caught in one hand by introducing the hand within the rectum. A.I. catheter/gun is inserted through vagina and directed towards OS uteri. With this technique semen can be deposited on desired site e.g. mid of the cervix/body of the uterus or uterine horn. The proper assessment of reproductive tract can be done before insemination.

PROCEDURE

- * Animal in heat is placed in the service crate and secured tightly.
- * Wear the gum boot and apron.
- * Wear the rubber/plastic full arm obstetrical gloves in one hand depending upon the convenient of the operator, if possible left hand should be used and keep the right hand free.
- * Lubricate the gloves with any lubricants or soap and water.
- * Carefully insert the hand into the rectum and remove the feces.
- * Confirm the estrus and suitability of the animal for A.I.
- * Withdraw one inseminating dose of chilled/liquid semen in catheter connected with rubber connector and 2 ml syringe.
- * Clean the exterior of the vulva with cotton or paper towel and the inside edges of the lip of the vulva with clean cotton.
- * With one hand (preferably right) insert the catheter into the vulva as far as possible without touching the side wall.
- * With left hand in rectum, press the rectal wall and grasp the cervix and with the help of finger and thumb around its posterior end, guide the catheter point into the OS-cervix.
- * With manipulation of the cervix, the desired penetration is obtained (site of deposition).
- * Deposite the semen in the mid cervix or in the body of uterus.
- * With frozen semen after conforming the oestrus the mini/medium straws are taken out from goblet of the canister. After thawing it is loaded on A.I. gun. Sheath is placed and locked.
- * The method of A.I. using frozen semen is same as discussed above.

RECORDING OF OBSERVATIONS

To maintain the proper record of A.I. the following few parameters are to be recorded.

- * Daily number of the case.
- * Monthly number of the case.
- * Yearly number of the case.
- * Name and address of the owner.
- * Species/age
- * Number of calving and date of last calving.
- * Breed/bull number
- * Collection number and date.
- * Quality of semen.
- * Neat/diluted/frozen.
- * Diluent used.
- * Proportion of dilution
- * Age of semen
- * Date and time of onset of estrus
- * Last date of insemination if any
- * Number of sperms per insemination
- * Result of insemination.

REMARKS

A.I. is to be done in strict aseptic and hygienic condition with a quality semen.

Chapter - 6

ARTIFICIAL INSEMINATION IN SHEEP, GOAT AND PIGS

OBJECTIVES

- * To study different methods of A.I.
- * To perform AI in sheep, goat and pigs.

MATERIALS REQUIRED

Animal, apron, gum boot, semen (liquid or frozen), microscope with thermostat, glass slide, cover slip, AI catheter/catheter with attached syringe, tissue paper, water bath, thermometer, stop watch, speculum (metallic or hard glass), flash light or speculum light or head lamp.

AI IN SHEEP AND GOAT**DESCRIPTION OF SUBJECT**

Artificial insemination in sheep and goat could not get much impetus in America and western countries, but, AI has been used very extensively in Russia and other Asian countries. The biggest advantage of AI in sheep and goat is that it permits intense selection of sires of exceptional merit and thus provides the opportunity to exploit the superior sire to its maximum. The process of A.I. in sheep and goat is very simple.

APPLICATIONS

- * It is valuable because of the wide spread use of outstanding individuals where the require trades are to be obtained in least possible time.
- * The progeny testing is possible only with AI techniques.
- * The labour cost and cost of management can be minimized in a sheep breeding farm by this technique.
- * A minimum generation interval could be achieved and minimum selection pressure be exerted on the ram.
- * The transmission of venereal disease can be prevented.
- * The genetically superior sterile ram or crippled male can be used.

PROCEDURE

A special crate or breeding stall for larger flock may be constructed to hold the ewe for insemination. A simple crate for a small number of ewes can be used to hold the ewe firmly and permit the rear quarters to be elevated.

- * To inseminate the ewe the pipette is filled with one inseminating dose of semen.
- * The speculum is lubricated with liquid paraffin, vaseline or oil.
- * Flash light or speculum light or head light is on.
- * Speculum is carefully inserted into the vagina and manipulated to locate the cervix.
- * The pipette is then introduced through the speculum and inserted into the cervix.
- * It is usually not possible for pipette to enter more than 1/8 to 1/4th inch deep into the cervix.
- * Semen in the pipette is slowly and gently expelled into the cervix.
- * Inseminating catheter and speculum is withdrawn after depositing semen.

RECORDING OF OBSERVATION

To maintain the proper record of A.I. the same parameter are to be followed which has been indicated in the previous chapter.

ARTIFICIAL INSEMINATION IN PIGS

Apart from the genetic aspects of the use of AI in pig the main factor which attracted the breeder is mostly removal of the risk of viral pneumonia and other infectious diseases which are known to be carried by the travelling boar. The other important factor is the cost of labour and transport which may be more than the cost of insemination even if a male is located few miles away from the flocks.

MATERIALS REQUIRED

Animals, protecting cloths, semen, spiral rubber catheter with narrow tip, a collapsable polyethylene bottle connected to the catheter, diluted semen.

SUBJECT DESCRIPTION

In the modern method of pig production and maintaining the large herd with an objective of crossbreeding programme it is essential to have optimum health control and tested boar for breeding purpose. The advantage and disadvantage of AI in pig is similar to those of cattle. Although, the A.I. services in pig are more costly in comparison to large animals. In spite of that many of the herd managers preferred A.I. programme to maintain different blood line in their herd without the risk of introducing diseases from other herds. The greatest limitations in the use of AI techniques are (a) difficulty of detecting the proper time of insemination in the sow and short life span of diluted semen (boar), (b) the poor concentration of spermatozoa in a ejaculate and larger volume of semen required.

Mostly, use of chilled semen are in practice upto 72 hours of storage. The use of boar frozen semen is not commercially accepted as yet, although, it is possible to obtain successful farrowing rates from the frozen semen.

PROCEDURE

- * Estrus sows should be inseminated 50 to 60 hours after the onset of estrus.
- * A positive reactions to "back pressure test" is an indication of correct time of A.I."
- * The spiral rubber catheter with a narrow tip passed into the dorsal aspect of vagina.
- * The catheter should be rotated anticlockwise until it become locked in the fold of cervix.
- * A collapsable polyethylene bottle containing semen connected with a catheter is allowed to flow the diluted semen by gravitations with gentle pressure on the container, for a period of 7 to 10 minutes.

RECORDING OF OBSERVATIONS

To maintain the records of A.I. the same parameters are to be recorded which has been discussed with cattle and buffalo.

Chapter - 7

THAWING OF FROZEN SEMEN

OBJECTIVE

- * To study methods of thawing of frozen semen.
- * To study post thaw characteristics of spermatozoa.

MATERIALS REQUIRED

Straws (mini or medium), scissor, filter paper or blotting paper, long forceps, gloves, water bath with thermometer, stop watch.

DESCRIPTION OF SUBJECT

To bring the spermatozoa at body temperature from ultra low temperature without affecting its characteristics and fertilizing potentiality is called process of thawing. The effect of thawing from the vitreous state is equally crucial as that of cooling to avoid the crystallization zone, thawing should be rapid. It is unlikely that a semen sample on warming follows the same thermodynamic path that of at cooling.

Adverse effect of temperature shock on rapid warming of semen is far less (or) even negligible when compared to fast cooling. Warming should be progressive (uniform at all stage). The spermatozoa of thawed semen do not withstand refreezing (or) repeat cooling and warming. The time allowed for this operation depends on the temperature of water and the packaging system. The fertility results obtained by different workers on various thawing methods are inconsistent. The basic principle in thawing is to raise the temperature more than zero, faster, but not exceed the atmospheric temperature or body temperature of the animals which ever is less. Where the atmospheric temperature is sub zero the semen is to be thawed upto 5°C and the chamber of AI gun could be raised above zero before the semen straw is loaded.

EVALUATION OF SEMEN QUALITY AFTER THAWING

There is no single effective test by which the fertilising potential of the thawed spermatozoa can be predicted. However, the fertilizing power of spermatozoa are generally judged by its progressive motility.

Progressive motility

- * A grease free dried glass slides is taken on thermostat stage of microscope.
- * A small drop of thawed semen is put on the slide and covered with coverslip.

- * Examine under the 40X objective for the progressive motility.
- * In case the progressive motility is less than 40%, it is not recommended for AI.

Acrosomal Integrity

- * Acrosomal integrity is assessed by Giemsa staining method.
- * A drop of semen is smeared on a prewarmed glass slide and dried.
- * The smear is fixed by immersion in buffered formal saline for 15 minutes and then washed in running tap water for 15-20 minutes.
- * The smear is then dried and immersed in the buffered Giemsa solution for 90 minutes after which it is rinsed briefly in distilled water and dried.
- * Giemsa working solution is prepared by mixing 3 ml of Giemsa stock solution with 2 ml of saline buffer, pH 6.8 and 45 ml of distilled water.
- * At least 2-3 smears need to be prepared from each straw or semen sample, stained and examined under oil immersion of the microscope.
- * A total of 100 sperms are counted per smear and categorized into sperms with intact, altered and completely lost acrosome.

Live and dead spermatozoa percentage

- * Live and dead percentage of spermatozoa are calculated by using Eosin and Nigrosin staining.
- * One drop of semen and 4-5 drops of eosin-nigrosin should be placed on a clean, grease free, pre-warmed glass slide and mixed gently with a blunt fine glass rod.
- * After 30 sec. a thin smear should be made from semen-stain mixture, and air dried and examined under oil immersion.
- * A total of 200 sperms should be counted per smear and total live and dead spermatozoa are calculated.
- * The sperm showing pink colour are considered as dead and the sperm which are not taken any stain are considered as live. partly stained sperms are also considered as dead.

PROCEDURE

- * There are several methods for thawing the frozen semen packed in ampoules, straws, polythelene tube, pellets etc.
- * After confirming the proper time of insemination one straw is taken out from the goblet by lifting the canister upto the neck with the help of long forceps.
- * The frost on the straw is wiped out quickly with the help of a blotting paper.
- * The straw is plunged into the water bath having a temperature of 37°C for half a minute.

- * Take it out from water bath, dry it with filter paper.
- * Load it on insemination gun towards the cotton plug (factory end) and laboratory end is cut with the help of scissor.
- * AI sheath is placed on the AI gun and locked by the device provided in the base of the gun.

Thawing can be done even at ice water or pocket for 2 minutes or 75°C in water for 7 second 95°C to 135°C for 3 sec. However, under the Indian conditions it is dangerous to advocate very high temperature thawing technique inspite of the fact that it has got the edge over the body temperature thawing techniques.

THAWING OF FROZEN SEMEN AND A.I.



Handling of frozen straw



Thawing of frozen semen



Female reproductive tract- buffalo



Artificial Insemination

MORPHOLOGICAL CHARACTERISTICS OF SPERMATOZOA

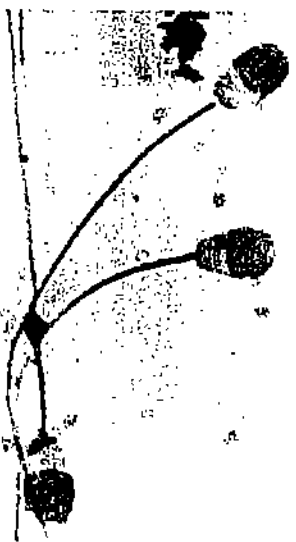


Spermatozoa

Live and Dead spermatozoa



Acrosomal Integrity



Chapter - 8

EVALUATION OF SEMEN FOR ARTIFICIAL INSEMINATION (AI)

OBJECTIVES

- * To study different methods of evaluation of semen.
- * To study norms of semen for optimum fertility.

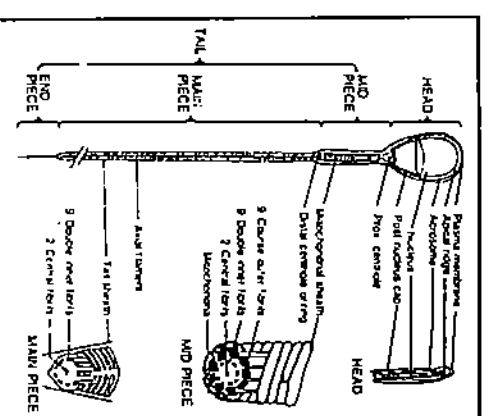
MATERIALS REQUIRED

Semen, microscope with thermostat, glass slide, cover slip, collection tube, pasture pipette, haemocytometer set, diluting fluid, different stains, etc.

SUBJECT DESCRIPTION

The development of artificial insemination was done with the major objective to use single ejaculate from one bull for covering the large number of cows. This has been done by high dilution and preservation at various temperature. This has prompted many workers to conduct the research to develop an accurate and objective method of assessing the potential fertility of a given semen sample.

Suitability of a semen samples for artificial insemination programme depends upon various characteristics. Science has made several wonders but it has not become



Structural diagram of sperm (a)

possible even today to evaluate the quality of semen on the basis of a single parameter. The important semen characteristics and the methods of their evaluation are as follows.

1. Macroscopical/physical
2. Microscopical
3. Bio-chemicals/chemical
1. Macroscopical/physical

The colour, consistency and volume of semen are the important parameters. Each mammalian species have got the fixed range of colour, consistency and volume of the semen depending upon the species and breeds. Any deviation from the normal characters give an indication of pathological condition.

Colour : The colour of semen is noted by visual appearance, soon after collection by keeping the collection tube against a white paper.

Mostly the semen samples of ruminants are milky white, or creamy white. sometimes it may be of yellow colour due to presence of riboflavin in semen. Any deviation from this is an indication of pathological condition of genital tract.

Volume : It should be noted directly from the graduated collection tube which is connected with the rubber cone and covered with insulating bag. The reading should be taken up to the tenth of millilitre. The volume of semen varies in different species and breeds. Any substantial increase or decrease in volume of the semen from the normal range may be an indication of poor quality which can be confirmed by further tests. Moreover, the volume of semen plays an important role in artificial insemination programme. With higher volume large number of population can be covered if other tests certify the quality.

Microscopical

a) **Mass activities :** Scoring of fresh semen and motility of diluted semen is done to assess its quality and detect the gross difference in the neat and stored semen. The nature of the wave depends upon the activity and concentration of spermatozoa when the spermatozoa suspended in a small area as under a coverslip, the speed of movement of these streams of spermatozoa creates local disturbance of equilibration in the medium and form the wave motion which results mostly a light and dark areas or waves may be due to the variations in the amount of scatter of lights of the different streams of spermatozoa. This is done by rating the fresh semen sample by the character of the wave observed in a fresh semen smear of uniform thickness. Spermatozoa is stored in the epididymis in a non motile stage but during the course of ejaculation when they come in contact with the secretion of accessory sex glands, they gain vigorous motility. The scoring of the initial motility is done by taking a small drop of freshly collected semen spread uniformly on a clean and dry glass slide at

37°C under the low power of microscope. This can also be done in hanging drop micro slide of 0.5 mm depth and 20 mm diameter. In both the techniques there is continuous movement of dark streaks in the field. The Swirls appear and disappear to the exterminities very rapidly. Therefore, on the basis of wave motion, swirling activities and nature of eddies, the quality of semen is graded into the five categories.

I) ++++ - **Motility :** Under this category a dense, quick wave motion is found in the field like a cloud moving in sky. There are waves after waves with a swirling movements and it is difficult to trace when the eddies are formed and when they are disintegrated.

II) +++ **Motility :** The wave motions are less, swirling and eddies are comparatively slower than ++++. The swirling can be observed to move towards the exterminities.

III) ++ **Motility :** The swirling movements are very slow and scattered in the entire field. The eddies are absent and wave of individual spermatozoa can be seen. Grossly it gives an indication of 40 to 60% motile spermatozoa in the sample.

IV) + **Motility :** No swirling movements, eddies are absent. They may have good number of spermatozoa with oscillating, backward and circular movements. Hardly 40% of motile spermatozoa are visible. This kind of semen samples are not fit for A.I. work.

V) + **Motility :** Complete absence of waves only few sporadic spermatozoa may have the motility which can be scored hardly upto 20%. This sample is not fit for A.I. work.

The initial motility of fresh semen must be examined under the low power (10X) at 38°C preferably on a thermostat. Higher temperature more than 38°C will increase the irritability of spermatozoa which leads to a false picture of semen sample. Similarly on low temperature the motility of spermatozoa goes down. Therefore, to get the real picture of semen sample it is essential that it must be examined under suitable and uniform temperature. The thermoregulatory stage for microscope is a much efficient method for this purpose, but in absence of this facility the hot water bag can be used for warming up the slide.

b) **Individual motility :** Estimation of semen quality based on mass activity may give a misleading picture because a substantial percentage of weakly motile spermatozoa may be exaggerated under the influence of actively motile spermatozoa. The quality of semen depends upon the percentage of progressively motile spermatozoa present in it. The simplest method for estimating the individual motility of spermatozoa is by dilution technique. The semen is diluted 100 - 400 times in an isotonic solution/physiological saline or in buffer solution. A drop of diluted semen is taken on a warm grease free slide, the coverslip is applied and examined under the

(40X) high power of microscope. The different fields are observed and percentage of motile spermatozoa is ascertained.

Another method of estimating the motility of individual spermatozoa is by taking a drop of 2.94% sodium citrate dihydrate solution on a clean slide maintained at body temperature (37°C). A small drop of fresh semen is mixed with a thin glass rod and cover it with coverslip. Examine under the high power (40X) objective of microscope. Count the total number of non-motile spermatozoa in 3-5 different fields. Keep this slide on a flame to give heat shock, so that all spermatozoa are killed. Count the total number of spermatozoa randomly in 3-5 different fields. Calculate the motile percentage of spermatozoa :

Percent motile spermatozoa =

$$\frac{\text{Total number of spermatozoa} - \text{Number of non motile spermatozoa}}{\text{Total number of spermatozoa}} \times 100$$

The number of non-motile spermatozoa can also be counted with the help of haemocytometer. The RBC pipette is used for dilution of semen. The dilution can be done in buffer solution of 2% sodium citrate dihydrate. The dilution rate will be 1:200. The diluted semen is charged on a clean haemocytometer covered with coverslip by a small drop of diluted semen. Total number of non-motile spermatozoa is counted in all the five big square (400 small square). Haemocytometer is then placed before a flame for few minutes or freezing chamber of refrigerator for one hour. The number of non-motile spermatozoa are counted in five big squares. The differences of non motile spermatozoa after exposure to cold shock or hot shock minus number of non-motile spermatozoa in five big squares gives the total number of motile spermatozoa in a sample. The individual motility of spermatozoa is presented in the term of percentage. There are several other methods also to find out the individual motility of the spermatozoa viz., (a) impedance change frequency (b) electric motility counter. With impedance change frequency technique a very low voltage of electric current is passed through the fresh neat semen. The impedance caused by the motile spermatozoa is recorded in the meter. This technique has got certain limitation such as it can not be used for diluted semen and also in a semen sample which has got low density.

In electrical motility counter instrument the image of sperm is projected on a screen which has an aperture, just behind the aperture a photo-multiplier is placed. When the spermatozoa cross the aperture the light falls on the multiplier, which in turn gives a voltage pulse. More motile the spermatozoa are, more sperm cells pass the opening of the diaphragm in a fixed time. The voltage fluctuations originating from the spermatozoa, while crossing the opening are counted with an electric counter. Moreover, in routine practice the conventional method of a drop of fresh semen on slide under cover slip examined under the high power is most popular which is

denoted in term of percentage. The chances of error can not be ruled out but long practice and experience of an individual is very important to give more accurate results. Temperature higher than 38°C or less than 30°C will give the wrong picture of the sample. Therefore, use of a thermostat with warm stage is essential to know the true picture of semen sample.

Percentage of live spermatozoa

Vital count is an important parameter to assess the quality of semen samples. In ideal condition a quality semen should not have more than 20% dead spermatozoa. The result of AI conducted on a large population of beef cattle indicated that semen samples containing less than 50% live spermatozoa were of doubtful fertility. A significant relationship exists between the percentage of dead spermatozoa in the ejaculate and fertility. Several workers have advocated the different staining technique to assess the live and dead spermatozoa in the semen. Eosin-opal blue staining technique where the dead sperm takes the red colour of eosin while those which are alive do not take any colour while the opal blue provides the blue background. The stain is prepared as follows :

Stain A : 2% water soluble Eosin x M/8 phosphate buffer solution

Stain B : 1 part of opal blue solution (undiluted)

and 1 part of M/8 phosphate buffer

Mix both the stains in equal parts, heat and filter.

M/8 phosphate buffer is prepared by mixing 80.4 ml of M/8 Na_2HPO_4 and 19.6 ml of M/8 KH_2PO_4 solutions.

PROCEDURE

Semen sample and stain should be maintained at same temperature preferably between 30 to 35°C.

1. Take 8-10 drops of stain in a small watch glass.
2. Add one drop of semen in the stain and mix with a fine glass rod.
3. Prepare smear from the semen stain mixture on a clean, dry and grease free glass slide. Smear should be thin, homologous and dried in the air. Putting the smear on flame or hot plate is contra-indicated.
4. Smear of the semen mixture should be prepared on two glass slide.
5. Minimum 100 spermatozoa from each slide should be counted randomly.
6. Live and dead percentage in a semen sample should be calculated as a mean of two counts made from two slides.

The live percentage of spermatozoa can also be counted by Eosin-Nigrosin compound stain. The stain is prepared as given below.

Eosin (water soluble)	-	1.0 gram
Nigrosin (water soluble)	-	5.0 gram
Sod. citrate dihydrate	-	3.0 gram
Distilled water to	-	100 ml

This solution is warm up in a water bath to 30 minutes to one hour and filtered after cooling. It should be stored in refrigerator for further use. The semen and stain should be mixed in a small watch glass. A smear is prepared on a glass slide.

Eosin-aniline blue stain can also be used to differentiate live and dead sperms. The stain is prepared by dissolving 1 gram of eosin, 4 gram of aniline blue in 100 ml of M/8 phosphate buffer and pH is maintained at 7.2. In general, the stain should be isotonic with semen because the hypertonic or hypotonic sol. will increase the morphological abnormalities of the spermatozoa. To obtain the optimum results the temperature of stain and semen should be same between 30-35°C and uniform smear should be prepared on a grease free slide which should be dried up in the air.

Percentage of abnormal spermatozoa

Morphological abnormalities of spermatozoa are evaluated with the staining techniques. The slide used for live and dead count can also be used for morphological studies. Besides Eosin-nigrosin compound stain, there are several other stains used by different workers. Some of the stains are very specific for a particular abnormalities.

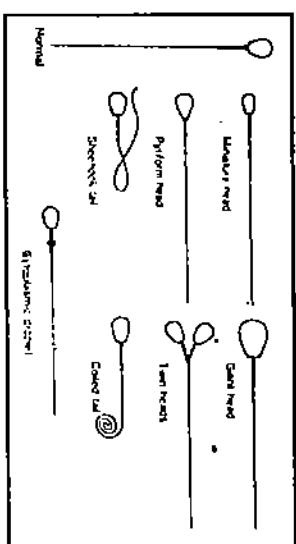
In a commercial semen bank where frozen semen is manufactured on large scale normally most common type of abnormalities are taken into the consideration. The types of abnormalities commonly found can be divided into two viz.,

- Primary* : It is due to defective spermatogenesis
- Secondary* : This is due to poor handling of semen at the time of collection, evaluation, dilution and preservation.

Primary defects are like, micro-head, mega head, detached head, double head, pyriform head, double tails etc.

Secondary defects : The detached heads, bent tail, broken tail and detachment of galea capitis. A semen sample having more than 15% primary type of defects the fertility is impaired. Although, the published reports show that morphological examination of semen is of limited value in the assessment of fertility but scientifically it is well established that the morphological abnormalities has got the co-relation with infertility. So, it must be carried out in all the breeding bulls whose semen is recommended for A.I. programme. The type of morphological abnormalities commonly detected in buffalo spermatozoa are more or less similar

to those observed in bull semen and may involve the head, mid piece or the tail. It is generally accepted that high percentage of abnormality denote poor quality of semen. Therefore, the percentage of abnormal spermatozoa should not exceed more than 5 to 15% in a good semen sample.

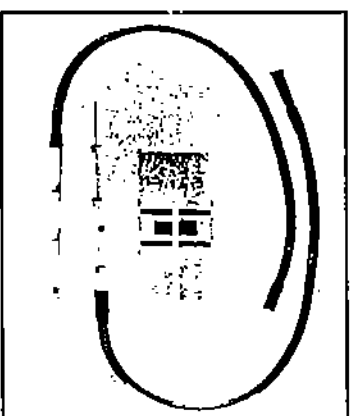


Morphological abnormalities of spermatozoa

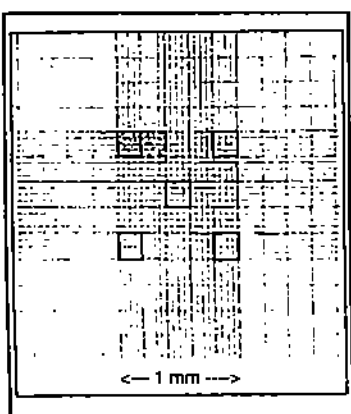
Sperm concentration

Sperm density or concentration of spermatozoa in one cubic mm of semen sample must be estimated for all the breeding males which are to be used for A.I. programme. The rate of dilution depends to a greater extent on the concentration and percentage of motile spermatozoa. There are four different methods to count the sperm concentration. Some of these methods are quick but chances of errors are more where as other method is a bit time consuming but accuracy is more.

- Haemocytometer method* : In practice two types of haemocytometer are in use.
 - Fuch's Rosenthal
 - Neubauer



Standard haemocytometer slide and dilution pipettes



Neubauer haemocytometer for sperm counting. Sperms are counted in the dark 5 chambers

i) On the Fuch's Rosenthal haemocytometer has got two counting chambers. Each counting chamber is divided into the 16 big square and each square is further sub-divided into the 16 small squares.

ii) Most of the laboratories are using Neubauer haemocytometer which has got two counting chambers. Each counting chamber is divided into 25 big square and each big square are further sub-divided into the 16 small squares. So altogether 0.1 cubic square area is having 400 small squares. When the diluted semen is charged in these square under the coverslip. The thickness of film is 0.1 mm. The dilution of the semen can be done 1:200 in red beaded RBC pipette or it can be diluted with the help of micro-pipette in test tube. But most of the AI Lab or semen bank prefer RBC (Read beaded) pipette for dilution of semen for routine work.

METHOD

The diluting fluid is prepared by dissolving 0.05 g of eosin Y (water soluble) and 1 gram of sod. chloride in 100 ml of distilled water. Formal saline (1 ml of formalin in 99 ml of normal saline) can also be used for diluting the semen. The objective of mixing the formalin in sodium chloride solution is to kill the spermatozoa before counting and eosin is added as colouring agent for more convenient and better visibility in the field. The entire assembly of haemocytometer should be nicely cleaned and washed with distilled water, xylene and alcohol. It should be dried up with ether. Semen sample should be gently shaken up and a small quantity is taken on a watch glass. The RBC pipette should be washed thoroughly and dried up. The sample should be sucked up in red headed pipette upto the 0.5 mark. The knob of the pipette is clean with the filter paper. The diluting fluid is sucked till it reaches upto 101 mark. Mix the contents (semen and diluting fluid) gently inside the pipette by rolling it between the palms of the hands. Discard the few drops of fluid from pipette since it does not contain semen. Since 0.5 cubic mm semen is diluted 100 times to make a dilution rate of 1:200. The counting chamber is focused under the microscope and cover slip is apply over it. The chamber is charged with a small drop of diluted semen by the side of cover slip. Over flowing and under charging should be avoided which will give the wrong picture. Focus the left top, right top, right bottom, left bottom and centre squares one by one under the high power objective of microscope. Number of spermatozoa counted in five big squares (80 small square). In each big square a uniform protocol is followed and those sperms whose head is within the middle bright line of the chamber should be counted.

Calculation of sperm concentration.

Number of spermatozoa counted in 5 big squares
(80 small squares) = S

So, Number of spermatozoa in 25 big squares (400 small squares)
which contain 0.1 cubic mm of diluted semen = 5S

So, Total number of spermatozoa in 1 cubic mm of diluted semen = 5S x 10 = 50 S

So, Total number of spermatozoa is 1.0 cubic mm of undiluted semen = $\frac{200 \times 50S}{10000S}$

The concentration of spermatozoa in most of the domestic animals like bull, buffalo, bull, goat and sheep are very high. The semen dilution rate should be increased upto 1:1000 to facilitate in counting of spermatozoa.

b) *Opacity tube*: Brown's opacity tube have been used satisfactorily for estimating the sperm density. It is a rapid method and can be used at a semen bank for routine field work. It is based on the principle of opacity as we know the higher is concentration of spermatozoa the more will be the opacity. But sometime it may give wrong picture due to the presence of epithelial cell, dirt or vaseline in the semen sample. The opacity tubes are standardized for varying concentration of spermatozoa with the help of haemocytometer. The evaluation of sperm density is done by matching the semen sample with standard opacity tubes. The per cubic concentration of spermatozoa are equal to opacity tube No x dilution rate x 1000 x 5.

c) *Photo-electric colourimeter*: This is also a very rapid method of estimating the concentration of spermatozoa. It is based on the principle of variation in the light absorbing power of semen which increases or decreases with the variation in the concentration of spermatozoa. Moreover, the estimation may be erroneous when semen contains epithelial cell, dirt, gel or vaseline. The photo-electric colourimeter used for sperm count is the same as one used for micro-analysis. It has been found that the estimate of sperm concentration with photo-electric colourimeter was equivalent to the mean of duplicate haemocytometer count for estimating the concentration of spermatozoa in a semen sample. It is necessary to standardize the number of spermatozoa in varying dilution with the help of haemocytometer. In general practice for estimating the concentration of spermatozoa in domestic animals a dilution rate of 1:40 to 1:400 are done in 3% sodium citrate dihydrate solution to make a volume of 10 ml. Semen sample in sodium citrate dihydrate solution should be thoroughly mixed before placing them in the mounting chamber.

METHOD

- Check the instrument for correct deflection of needle from zero to infinity.
- Red filter should be used with light wave length of 625 milli μ .
- The blank tube with 3% sodium citrate dihydrate (10 ml volume) put on the mounting chamber at 30°C.
- Current should be switched on and reading on galvanometer is adjusted to zero. Switch off the instrument.

- e) Blank tube, should be replaced with the diluted semen sample. The temperature should be maintained at 30°C and switch on the current and record the deflection of the needle.
- f) Repeat the process of all the samples whose sperm concentration are to be estimated.
- g) A graph is plotted to find a straight regression line connecting reading point. If straight line is not obtained it should be repeated again. All readings are thus standardized for the equivalent concentration of spermatozoa in a diluted semen sample.

In routine work 0.1 ml of fresh semen is diluted in 9.9 ml of 3% sodium citrate dihydrate. Thus, 10 ml of diluted semen (1:100) is matched against the blank tube. The reading recorded after the deflection is recorded and converted to per cubic mm concentration of spermatozoa in that sample. However, the chances of error due to the presence of epithelial cell and dirt is inevitable. Few workers have used 4% chlorazene suspension in place of sodium citrate dihydrate buffer to minimise this problem. At present photo-electric colourimeter is the best and most practical method of sperm counting.

Characteristics of semen

	Cattle				
	Dairy	Beef	Sheep	Swine	Horses
Volume (ml)	4	4	1	225	60
Sperm concentration (billion/ml)	1.2	1.0	2.0	0.2	0.15
Total sperm (billion)	7	4	3	45	9
Motile sperm (%)	70	65	75	60	70
Morphologically Normal sperm (%)	80	80	90	60	70
pH	6.5-7.0	6.5-7.0	5.9-7.3	6.8-7.5	6.2-7.8

3. Biochemical/chemical

Several bio-chemical tests have been advocated to assess the quality of semen. These tests consider the metabolic, glycolytic, respiratory mechanism and dehydrogenase activity of the spermatozoa. Some of these metabolic tests have shown the direct co-relation with the keeping quality and fertilizing potential of the spermatozoa. There are good number of bio-chemical/chemical tests that can be conducted to evaluate the quality of semen but few of them are complicated and time consuming. As such majority of the semen bank conducted only those bio-

chemical tests which are simple and quick.

- i) **pH (Hydrogen ion concentration)** : Evaluation of pH of a semen sample just after collection has not shown any practical value, although the pH of a semen sample varies with the concentration and metabolic activity of spermatozoa. The pH of poor quality semen is generally neutral or slightly alkaline but good quality semen is having the pH less than 7 that is slightly acidic. The average pH of good quality semen ranges between 6.6 to 6.8. There are several methods to evaluate the pH of semen. Best measured with the help of pH meter. For routine A.I. work BDH pH paper cover with range from 5.5 to 8.5 are generally used. The method is very simple quick but rough.

Method : A paper strip is torn off from the indicator paper and dip into a drop of semen. Look for the first change of colour and match this colour on the cover of the book corresponding to the pH noted against it which gives the pH of the sample. Other method for measuring the pH is with the help of BDH-capillary tube whose accuracy is more or less similar to the pH paper. The capillary tubes of equal diameter are being used. In one capillary tube semen is sucked upto a fixed mark. In another capillary tube Bromothymol blue is sucked upto the same mark, so that the volume of semen and the dye should be equal. Both semen and dye mixed together gently in a small watch glass. This mixture is sucked in another capillary tube of similar bore and match with the Bromothymol capillater ranges from 6 to 8.2. The colour which match with standard tube gives the pH of sample.

- ii) **Cold shock resistance test** : It has been reported that the semen with higher percentage of cold shock resistant spermatozoa keeps longer under preservation and also predict higher fertility rates. The different methods employed to find out the percentage of cold shock resistance are as follows :

Method

- a) This method is employed by Tomar *et al.* (1966). In this technique 0.25 ml of fresh semen is kept in a small vial. It is suddenly chilled from 37°C to 0°C and kept at 0°C for about 10 minutes. Thawing is done at 30°C, smear is prepared in eosin-nigrosin compound stain and percentage of live spermatozoa is counted as discussed earlier.
- b) The method to estimate the percentage of cold shock resistance spermatozoa was described by Hancock (1951). Ten drop of eosin-nigrosin compound stain is taken in small test tube and kept at 0°C. One drop of freshly collected semen sample is added in cooled stain and maintained at 0°C for 15 minutes. Smears on two clean, grease free slides are prepared and percentage of live spermatozoa is counted.

iii) Millovanov's resistance test ("R" test)

"R" test is measured by the ability of the spermatozoa to withstand the action of 1% sodium chloride solution. The resistance is denoted as the millilitre of 1% NaCl solution required to stop the progressive motility of all the spermatozoa in 0.02 ml of semen.

Method : In a five litre bottle 1% sodium chloride solution is prepared. A 250 ml burette is fixed on a stand and filled with 1% sodium chloride solution. A 500 ml flask containing 200 ml of 1% NaCl solution with 0.02 ml of semen is taken. From the burette containing 1% NaCl solution, 10 ml is added each time and progressive motility of spermatozoa is assessed. This should be continued till the progressive motility of spermatozoa is completely ceased. A good quality semen shows the R value not less than 5000.

iv) Methylene Blue Reduction Test (MBRT)

This test denotes the dehydrogenase activity of the spermatozoa. It is assessed by the time required by a semen sample to decolourise the solution of methylene blue under standard condition of incubation. More the hydrogen ions liberated per unit of time less is the time taken by methylene blue to change the colour. A semen sample with higher concentration of active spermatozoa will reduce methylene blue in less time. There are several methods reported by different workers to conduct this test. But the method used by Beek and Salisbury (1943) is much popular.

Method

- Methylene blue is prepared by dissolving 50 mg of methylene blue in 100 ml of 3% sodium citrate dihydrate solution.
- Freshly collected semen is diluted at the ratio of 1:4 in egg yolk sodium citrate (EYC) diluent.
- In a small test tube one ml of diluted semen is taken and added 0.1 ml of methylene blue, mix it thoroughly.
- The content of this tube is covered with 1 to 1.5 cm thick layer of paraffin liquid or mineral oil. Incubate the tube in a water bath at 46.5°C.
- Note the time when colour of the mixture is changed. Sometimes, blue colour on the top does not change, it is mostly due to failure of creating a complete anaerobic condition in the tube. In good quality semen sample the colour of methylene blue changes within 3 to 5 minutes where as an average sample takes about 9 minutes. The average methylene blue reduction time for buffalo semen is 8.75 minutes.
- The different methods adopted by different workers demonstrated that the rate of decolourisation could be influenced by several substances like glucose, citrate, lactate and bacterial load. Branton *et al.* (1951) has used a modified technique

in which semen was diluted to give a concentration of 300 million spermatozoa in each ml. Most of the worker accepted that the reduction time is directly related to motility and concentration of the spermatozoa. This is the bottleneck which undermines the importance and accuracy of this test. There is divergent opinion regarding the co-relation between MBRT and fertilizing potential of the spermatozoa.

v) Resazurin Reduction Test

This is also a test for determining metabolic activity of semen. This test was used for the first time by Erb and Ehlers (1950) to assess the quality of semen. The reduction times required for the colour to change first to pink and then, to white are significantly related to reduction time of MBRT.

Method

- Dissolve 11 mg of resazurin in 200 ml of distilled water.
- In a small test tube 0.2 ml of neat semen is taken and 0.1 ml of resazurin solution is added and mixed thoroughly.
- This mixture is covered with 1 cm thick layer of liquid paraffin to make an anaerobic condition.
- Incubate this mixture at 45°C.
- Note the time for change in colour to pink and then to colourless.

Good quality semen reduces resazurin to pink colour in about one minute and it requires about 4 minutes to make it colourless. The average reduction time of resazurin was reported about 4.39 minutes. Although, a correlation between resazurin reduction time and fertility has been demonstrated by several workers but variation in motility and concentration of spermatozoa in different samples create problem in interpretation of the result.

vi) Other test

In addition to the above several other tests have been used by different workers to evaluate the quality of semen viz.,

- Fructolytic index
- Ascorbic acid estimation
- Oxygen utilization test
- Pyruvate utilization
- Hyaluronidase content

Majority of the above tests are not only time consuming but also require a good infrastructural laboratory facilities. At a semen bank for routine evaluation, it is not necessary to conduct all these tests. Few simple tests which have been discussed in the text will meet the requirement of semen bank for routine evaluation.

Chapter - 9

PREGNANCY DIAGNOSIS IN CATTLE AND BUFFALO

OBJECTIVES

- * To study different methods of diagnosis of pregnancy in cattle and buffalo.
- * To monitor changes of genital organs for pregnancy diagnosis by per rectal palpation of genitalia.

MATERIALS REQUIRED

Animal, arm sleeves, cattle crate, soap, water braker, towel, gum boots, syringe, needle, test tube etc.

DESCRIPTION OF SUBJECT

Importance

- * Better management of pregnant animals
- * Early identification of non-pregnant animals to reduce production time losses as a result of infertility in non-pregnant animals by initiating early treatment or culling.

Principle

An essential feature of establishment of conception is the prolongation of luteal phase of the oestrous cycle. The cyclic corpus luteum doesn't regress and persists resulting elevation of luteal progesterone. This exerts a negative feed back on anterior pituitary, inhibiting secretion of FSH/LH interfering in the maturation of follicle and ovulation, thus prevention to return to oestrus. The presence of viable developing embryo interferes in release of PGF₂ alpha from the endometrium preventing regression of corpus luteum. Maternal recognition of pregnancy thus results from anti-luteolytic signalling between trophoblast of embryo/conceptus and mother uterus. These signals are certain proteins which have been identified in majority of farm animals. These signals insures continuation of corpus luteum for the production of hormone of pregnancy i.e. progesterone. The day 16/17 is the time for maternal recognition of pregnancy in cattle. Development of conceptus following fertilization and implantation produces certain changes in the uterus and other parts of genital organs which are monitored for the diagnosis of pregnancy.

METHODS OF DIAGNOSIS OF PREGNANCY

- * Non-return to estrus.
- * Per rectal palpation of genital organs.

- * Progesterone assay
- * Ultrasonography
- * Pregnancy specific protein assay

Non-return to estrus

- * It is called visual method of diagnosing pregnancy and depends upon efficiency and accuracy of estrus detection.
- * The onset of estrus 21 ± 4 days post service is fairly a good indicator to consider the animal as non-pregnant. It is generally assumed that if a cow or buffalo fails to return to estrus 17 to 25 days after the service or insemination it is probably pregnant.
- * A few animals display estrus during pregnancy i.e. gestational estrus and animal who are neither pregnant nor come into estrus (anestrus) affects reliability of this method.
- * In this method there will be some percentage of animals that are not returning to estrus and also not pregnant but incorrectly be assumed as pregnant on the basis of non return rate. Thus, it over estimate pregnancy at least 10-20% than the actual.
- * In buffaloes return to service are difficult to detect due to silent or weak external signs of estrus. The inability to detect estrus precisely limits use of this method for pregnancy diagnosis in buffaloes.

Pre-rectal palpation of genital organs

- * It is one of the most practical, accurate and quick method of diagnosis of pregnancy in cattle and buffalo.
- * In this procedure uterus is palpated through the rectal wall to detect uterine enlargement occurring during pregnancy. Fetus and foetal membranes are also palpated for the confirmation of pregnancy.
- * The earliest diagnosis of pregnancy in cattle and buffalo can be made between 35-45 days post service or insemination. Palpation at this stage, however, requires a greater expertise.

Normal gestation period in cattle and buffalo are 280 and 305 days, respectively. The gestation period for practical conveniences in cattle and buffalo is divided into three trimesters viz., first trimester (upto 3 months), second trimester (three months to 6 months) and third trimester (more than 6 month to term). Certain physiological changes in the female reproductive tract occur which are monitored by rectal palpation for the diagnosis of pregnancy.

First trimester of pregnancy

- * Unilateral enlargement of uterine horn. The enlargement of uterine horn is due to the development of conceptus (foetus, foetal fluid and foetal membranes).

The amount of foetal fluid largely determine the size of uterus. The size of foetus is small during 1st trimester of pregnancy.

In early pregnancy diagnosis i.e. 35-45 days, enlargement of uterine horn in palpated at the tip of the uterine horn. This is a pronounced dorsal bulging due to formation of amniotic vesicle.

- * Alive feeling of uterine horn can be palpated. This is due to thinning and stretching of uterine wall. The uterus is felt fluid filled like tubular structure.
- * The uterine horn develops a tone due distension and tenseness. This is known as pregnancy tone.
- * Slipping of foetal membranes upon genital pinching of uterus with thumb and fore fingers between day 60-90 of pregnancy.
- * Palpation of foetus by keeping palm and pressing against the outer curvature of enlarged uterus. The foetus is felt like a wooden piece floating inside the uterus.
- * Corpus luteum of pregnancy can be palpated on the ovary adjacent to the pregnant horn. Corpus luteum gets embedded in the ovarian stroma during pregnancy makes difficult to palpate by per rectal examination. This situation is more difficult in buffaloes due to embedded nature of corpus luteum.
- * Uterus during first trimester of pregnancy remains in the pelvic cavity. In older, pluriparous animals it start descending into abdominal cavity early by the end of this trimester. In all cases by four months of pregnancy uterus lie on the floor of the abdominal cavity.

Second trimester of pregnancy

- * Uterus is fully distended and descending into abdominal cavity.
- * The cervix is pulled as uterus sinks into abdominal cavity. The position of cervix gets verticle instead of horizontal.
- * The foetus can be easily palpated and identified. At 5 1/2 to 6 1/2 months of pregnancy foetus sinks down into the capacious abdominal cavity and makes difficult to palpate through per rectal palpations. This is the period where most of the mistakes are committed if one is not careful.
- * Foetal bumps and foetal movement can be palpated by keeping palm and pulling the uterus.
- * In the beginning, small placentomes can be identified which become larger in size measuring about 5-7 cm in diameter as pregnancy advances. The placentome resemble to mushroom like structures.
- * The freemites/pulse of pregnancy can be palpated by pressing middle uterine artery on the lateral wall of the pelvis.
- * The ovaries are difficult to reach but a CL will be present on the ovary ipsilateral to the pregnant horn.

Third trimester of pregnancy

- * The cervix will be at the top of pelvis, verticle and bent over the edge.
- * Uterus reside deep into the abdominal cavity. The dorsal wall of the uterus is tight and difficult to palpate.
- * Freemites is pronounced.
- * Foetal bumps can be easily palpated. Foetus at 7 months onwards can easily be palpated. Head and legs are usually palpated easily.
- * Movement of foetus can frequently be demonstrated at 7 month onwards by grasping and pulling foetal legs and other parts of foetus.

Points to be remembered

- * Pregnancy is the greater cause of uterine enlargement. But by no means it is the only cause.
- * At every stage, pregnancy must be differentiated from one or more of the other causes of uterine enlargement viz., pyometra, mummification and maturation of foetus.

Progesterone assay

Establishment of conception following mating or insemination prevents regression of corpus luteum in the ovary. The corpus luteum secretes progesterone which can be assayed in the blood or milk. The progesterone concentration in the peripheral circulation remain elevated, if CL persists because of pregnancy. A marked difference between blood levels of progesterone in pregnant and non-pregnant cattle and buffalo 19-21 days post insemination, hypothesized the basis for early pregnancy diagnosis by progesterone assay.

- * 21-25 days post AI/service is the more appropriate time to carry out progesterone assay for pregnancy diagnosis in cattle and buffalo. At this time progesterone will be high in pregnant animals (> 3.5 ng/ml) and low (1 ng/ml) in nonpregnant females.
 - * It detects non pregnancy with almost 95-100% accuracy and pregnancy 75-80%.
 - * Progesterone can be assayed blood plasma/serum and milk using RIA and ELISA.
- Points to be remembered*
- * Progesterone, although, is not pregnancy specific but can be used as marker of pregnancy because it is the secretion of CL which persists during pregnancy.

Ultrasonography

Ultrasound waves are inaudible to the human ear and operate at frequencies of 1-10 mega hertz. In 1970s although 'A mode' ultrasonic technique i.e., Doppler and amplitude depth analyser became popular for the diagnosis of pregnancy in pigs.

but it was only the advent of real time ultrasound (B-mode) to be used for scanning purpose commercially. Real time ultrasound scanning involves the use of a rectal probe which transmits ultrasonic waves through body tissue are reflected to the transducer and converted to produce image on display screen. These images are interpreted for the diagnostic purposes.

Salient features

- * The commonly used transducer in domestic animals are of the linear array type and have a frequency range 3.5 to 7.5 mHz.
- * In bovine pregnancy diagnosis 5 to 7.5 MHz transrectal transducer is used.
- * The image of urinary bladder, embryonic vesicle, fetal fluid etc. appear black (anechoic). The foetal skeleton appear white, foetal membranes and maternal tissues appear in various shades of grey (ecogenic).
- * Embryonic vesicle can be detected as early as day 9-10 but chances of mistake is more.
- * Embryo proper, embryonic heart beat and foetal membranes can be visualized in cattle and buffalo by day 30 - Accuracy is high during this period.
- * Genital tubercle can be detected as early as day 55-60 for the diagnosis of sex of foetus.
- * Scrotal sock and memmary teals can be diagnosed by day 90 for better diagnosis of foetal sex in bovine.

Pregnancy specific protein

Several proteins and protein like substances secreted by the conceptus have been identified during pregnancy. These substances have been used as marker of pregnancy in bovine and other farm animal species. These pregnancy specific proteins are secreted by the binucleate trophoblastic cells and can be detected in maternal circulation as early as 3-4 weeks post conception.

IMPORTANT FEATURES

- * Pregnancy specific protein can be detected in test serum either by RIA or ELISA.
- * Accuracy rate is 90% at 30 days of pregnancy.
- * Pregnancy, twinning, stage of gestation, embryonic/foetal mortality can be detected.
- * Presence of pregnancy specific protein is an indicator of viable foetus in the uterus.

PROCEDURE FOR PER RECTAL PALPATION OF GENITAL ORGANS FOR PREGNANCY DIAGNOSIS

Secure the animal in the animal crate. Put coverall and arm sleeve, gel lubricated using liquid paraffin. Insert the hand into rectum. Examine the cervix

by grasping in between index and middle finger. Examine the uterus, uterine horns for the enlargement, alive feeling, foetal membranes, foetus freematus and foetal movements etc. Note down the location and position of cervix and uterus.

RECORDING OF OBSERVATIONS

- * Animal number.
- * Species
- * Date of birth/age
- * No. of calvings
- * Date of last calving
- * Date of last AI/Service
- * Gynaecological findings
 - Vagina and vulva
 - Colour of mucus membrane
- * Cervix
 - OS of cervix
 - Tone of cervix
- * Uterus
 - Size
 - Location
 - Feeling on uterus
 - Foetal membrane
 - Foetal movement
 - Foetal bump
 - Corydons
 - Fremitus

Take 5 ml blood, separate serum and send the sample for progesterone assay.

P₄ - ng/ml

REMARKS

Comments on status of pregnancy.

Chapter - 10**PREGNANCY DIAGNOSIS IN MARE****OBJECTIVES**

- * To study different methods of diagnosis of pregnancy in mare.
- * To diagnose pregnancy in mare by per rectal palpations.

MATERIALS REQUIRED

- * Animal securing crate, sleeves, liquid paraffin, soap, water bucket, towel.

SUBJECT

The gestation period in mare is 320 days. One of the important feature of establishment of conception in mare is development of temporary hormone producing structures in the pregnant uterus, the endometrial cups. These structures are formed somewhat day 40 of gestation. It secretes equine chorionic gonadotrophin (eCG) or commonly known as pregnant mare serum gonadotrophin (PMSG). The presence of eCG can be well demonstrated at day 40 of gestation and it disappear by day 140 of gestation.

METHOD OF PREGNANCY DIAGNOSIS

- * Non return rate
 - Failure of return to estrous is good sign that a mare is pregnant.
- * False positive
 - If the mare has a silent heat.
 - If mare become anestrus
 - If mare has prolonged diestrus yet has not conceived.
 - If mare has prolonged luteal phase associated with embryonic death - pseudopregnancy.
- * False negative
 - Some mare are pregnant but show estrus during this period.

PER RECTAL PALPATION OF CHANGES IN GENITAL ORGANS**Stage of pregnancy**

1st month	Changes to be monitored
	* Contracted and firm cervix.
	* Turgid uterine horn

2nd month

- * Chorioallantoic sac bulges ventrally in lower third of uterine horn
- * Uterine horns as turgid
- * Practically pregnancy can be diagnosed with good accuracy of this stage.

3rd month

- * Chorioallantoic sac grows rapidly and descends into uterine body.
- * The bulging of chorioallantoic sac become ovoid from spherical.
- * Uterine tensus is gradually
- * Uterus descends into abdominal cavity

4th month

- * Dorsal surface of uterus is felt as distended dome
- * Foculus and foetal parts are palpable.

5th to 7th month

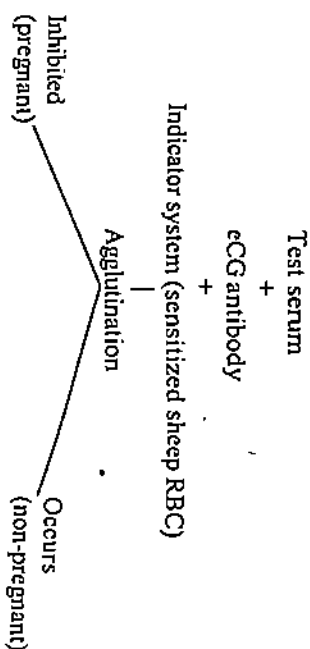
- * Uterus lies deep into abdominal cavity
- * Difficult to palpate foculus
- * Uterus ascends
- * Fetus is easy to palpate

7th month to term**Ultrasonography**

- * B mode ultrasound scanner is used for pregnancy diagnosis in mare.
- * 5 MHz transducer, rectal probes are used.
- * Earliest pregnancy diagnosis (90-100% accuracy) at 9-11 days by visualizing conceptual vesicle in the uterus as a black sphere of about 3 mm in diameter.
- * Detection of foetal pulse from day 40 to 240. Best time to detect foetal pulse is day 90 of pregnancy.

Haemagglutination test for detection of eCG**Equine chorionic gonadotrophin (eCG or PMSG)**

Equine chorionic gonadotrophin appears in the maternal circulation as early as day 40 following conception and remain in the circulation upto day 140. The highest concentration of eCG is recorded at day 90-100. Detection of eCG in the blood is suggestive of pregnancy. Immunological diagnosis of pregnancy in mare is based on the principle that eCG when present in the test blood sample, prevent agglutination of sensitized sheep RBC by anti eCG (HI test). Agglutination of red cells means negative result no pregnancy. Inhibition of agglutination is positive result means pregnancy. Accuracy is high between day 50-100 of gestation.

HI TEST**Procedure for per rectal gynaecological examination for pregnancy diagnosis**

- * Secure the animal in the crate.
- * Put on cover all, arm sleeves and wash it with soap and water.
- * Lubricate arm sleeve with liquid paraffin.
- * Insert the hand into rectum, remove the faeces.
- * Examine the cervix for its firmness and closure of OS cervix.
- * Examine uterus for ventral bulging during early pregnancy. In later parts of pregnancy palpate whole uterus for the tone, enlargement and presence of foetus.
- * Examine vagina and vulva from the out side to note its dry and paleness and wrinkling of vulva.

RECORDING OF OBSERVATIONS

- | | |
|---|---------------------|
| <ul style="list-style-type: none"> * Animal No. * Date of birth/age * Date of last foaling * Total number of foaling * Date of last service * Gynaecological findings <ul style="list-style-type: none"> - Vagina and vulva - Cervix - Uterus | Date of examination |
|---|---------------------|

REMARKS

Write down the status of pregnancy on the basis of above examination.

Chapter - 11**PREGNANCY DIAGNOSIS IN SHEEP AND GOAT****OBJECTIVES**

- * To study different methods of pregnancy diagnosis in sheep and goat.
- * To diagnose pregnancy in sheep and goat.

MATERIALS REQUIRED

Animal, biopsy catheter, glass slides, test tube, hypodermic needle, microscope.

DESCRIPTION OF SUBJECT

Methods of pregnancy diagnosis

- * *Non return rate*: Animal following mating if does not come to estrus is considered as pregnant.

- * **Palpation of foetus through abdominal wall**

- Ballote the foetus by lighting the abdomen in front of the udder. Foetus can be felt at 100 days of pregnancy.

- * **Development of udder**

- More pronounced in primipara.
- Gives better judgement during late pregnancy.

- * **Ultrasonography**

- Use of B-mode ultrasonographic scanner using 5 MHz abdominal or transrectal transducer.
- Foetus and placenta can be visualized at day 45-50 with 100% accuracy using transrectal probe. Accuracy rate is low at this stage of pregnancy using external probes.

- * **Plasma progesterone assay**

- Diagnosis of pregnancy is more accurate using progesterone assay.
- Best time to take blood sample for progesterone estimation is 20-22 days after mating.
- Progesterone concentration in pregnant sheep and goat will be in between 3-4 ng/ml where as in non-pregnant animal it will be around 1 ng/ml.

- * **Radiography**

- Dorsoventral and lateral radiograph of lower abdomen is taken.
- Foetal skeleton can be seen on radiograph.

- Detects pregnancy from 70 days onwards.
- Accuracy is better at 100 days of gestation.

* Vaginal biopsy

- Secure the animal in standing position.
- Sterile biopsy instrument is introduced into the vagina.
- A piece of tissue is removed by rotating the inner rod of the biopsy instrument.
- Tissue is fixed, sections are cut and stained with haematoxylin and eosin stain.
- Observe under microscope.
- In pregnant animal vaginal epithelium is 3-4 layer thick.
- In non-pregnant animals and during estrus vaginal epithelium is composed of 10-12 layers of stratified squamous cells.

PROCEDURE

- * Examine the animal for udder development.
- * Examine the focus by lifting the abdomen in front of udder and palpate the presence of foetus.
- * Take 2 ml blood, separate the serum and go for the progesterone assay.
- * Take vaginal biopsy by biopsy instrument. Stain it and record the vaginal cell layer thickness and types of the cells.

RECORDING OF OBSERVATIONS

- * Animal No.
- * Age/Date of birth
- * Date of last heat/service
- * Observation on abdominal ballotment
- * Observation on udder development
- * Vaginal smear examination
 - Cell type
 - Height of vaginal epithelial cell

REMARKS

Write down status of pregnancy in the above animals.

Chapter - 12

PREGNANCY DIAGNOSIS IN SOW

OBJECTIVES

- * To study different methods of pregnancy diagnosis in sow
- * To diagnose pregnancy in sow.

MATERIALS REQUIRED

Animal, biopsy instrument, glass slide, haematoxylin-eosin stain, microscope, soap, towel.

DESCRIPTION OF SUBJECT

Methods of diagnosis of pregnancy

Diagnostic technique

Earliest day of diagnosis of pregnancy

Failure to return to estrous	18-25
Vaginal biopsy	18-25
Plasma/serum progesterone	21
Ultrasonography	25
Estrone sulphate	26-30
Frenitus of middle uterine artery	30-60

Vaginal biopsy

The method is based on histological assessment of number of cell layers type of squamous epithelium of vaginal mucosa. The cells of vaginal mucosa become multilayered under the influence of estrogen during proestrus and estrus. The cells are only 2-3 layer thick during pregnancy.

PROCEDURE

- * Restrain the sow in service crate
- * Sterilize biopsy instrument.
- * Clean the vulva with 70% alcohol.
- * Insert the biopsy instrument into the vagina.
- * Press the tip against the vaginal wall.
- * Rotate the inner rod for a piece of vaginal mucosa.
- * Withdraw the instrument and vaginal pieces are transferred to 10-20 ml to 10% buffered formal saline.

- * Section it and stain with hematoxylin-eosine stain.
- * Examine under microscope for the vaginal squamous epithelial cell type and number of cell layer thickness.
- * In pregnant animal vaginal epithelial cell are 3-4 layer thick with condensed dark staining nuclei.

OBSERVATIONS

- * Animal number
- * Date of service
- * Vaginal epithelial cell layer height
- * Type of cells

REMARKS

Write down status of pregnancy.

Chapter - 13

PREGNANCY DIAGNOSIS IN BITCH

OBJECTIVE

To diagnose pregnancy in the bitch.

MATERIALS REQUIRED

Animal, x-ray and ultrasonography unit, soap, towel etc.

DESCRIPTION OF SUBJECT

Bitch is not a polyestrous animal, therefore, failure to return to estrus is not considered a method for diagnosing pregnancy in bitch. Development of pseudopregnancy which is very common in dog also creates problem in diagnosis of pregnancy. Changes in body weight, distention of abdomen and characteristic changes of mammary glands occurs during pregnancy but the similar changes do occur at pseudopregnancy.

Methods of pregnancy diagnosis

- * Abdominal palpation
- * Radiography
- * Ultrasonography

Abdominal palpation

- * Optimum period for pregnancy diagnosis is day 40-45.
- * Spherical and elongated distension of uterine horns can be palpated on tilting of bitch by putting both the paws at abdomen.
- * Fetus can be readily palpated at day 45 onwards.

Radiography

- * Help to differentiate pregnancy from pseudopregnancy.
- * Gives information about total number of foetuses in the uterus.
- * Dorsoventral view of radiography is more appropriate to identify number of foetus and their presentation.
- * Identification of pregnancy is based upon presence of foetal skeleton.
- * Foetal sac can be identified at 25 days, skull 25 day and foetal skeleton at 40-45 days of gestation.

Ultrasonography

- * B- mode ultrasonography using 2.4 MHz linear array transducer probe is used for diagnosis of pregnancy in bitch.
- * Transducer is placed on abdominal wall.
- * Earlier diagnosis of pregnancy is at 28 days with a high-accuracy rate, conceptus are readily identifiable.

OBSERVATIONS

- | | |
|---|---|
| <ul style="list-style-type: none"> * Animal Number * Breed/species * Age * Date of mating * Findings of abdominal palpation * X-ray findings * Ultrasonographic findings | <ul style="list-style-type: none"> * Date of pregnancy diagnosis |
|---|---|

REMARKS

Write down status of pregnancy.

Chapter - 14

SIGNS APPROACHING TO PARTURITION AND STAGES OF PARTURITION

OBJECTIVES

- * To provide gynaecological aid to the parturient dam/animal by understanding the signs approaching to parturition.
- * To record major events taking place during different stages of parturition.

REQUIREMENT

- * Breeding date/breeding records.
- * Animal
- * Beddings etc.

PRECAUTIONS

- * Just prior to parturition, animal should be separated from the other animals.
- * The sow, bitch and cat should be provided bedding.
- * Animal should be properly fed prior to parturition.

DESCRIPTION OF SUBJECT

Signs approaching to parturition

Cattle and Buffalo

1. Changes in the pelvis, genital organs and mammary glands along with changes in the behaviour of animal occurs.
2. The pelvic ligaments, specially sacrosacral become progressively more relaxed, causing a sinking of croup ligaments and muscles.
3. The caudal border of sacro sciatine ligaments between the coccygeal vertebrae and ischial tuberosity becomes more relaxed and flaccid.
4. Relaxation of the pelvic ligaments, cervix and possibly the structures around the perineum is due to edema and changes in the collagen fibres in connective tissue.
5. The relaxation of the ligaments is also noted by the elevation of tail head.
6. The vulva becomes progressively edematous and more flaccid. The udder becomes enlarged and edematous but in heifers the udder enlargement begins about 5th month of gestation. Sometime edema may extend forward on the abdominal floor to the xiphoid region.

7. Just prior to parturition the udder secretion changes from a honey like dry secretion to a yellow, turbid yellow, opaque cellular secretion called "COLOSTRUM".
8. The animal usually exhibit a tenacious, whitish, stringy type of mucus coming out from the cranial part of the vagina, starting at about 7-8 months of pregnancy.
9. During the last few hours before calving the animal may exhibit restlessness.
10. Heifer may show sign of abdominal pain as kicking at the abdomen, treading, switching their tails and lying down and rising.

Mare

1. The sinking of the sacrospinic ligaments is not so pronounced, due to heavy croup muscles.
2. The vulva does not become as edematous as in cow but edema and flaccidity are noted in the last few weeks of gestation.
3. No discharge of vaginal mucus prior to foaling is noticed in mare.
4. The udder starts to develop noticeably about 3 to 6 weeks before foaling.
5. The oozing of colostrum from the teats called "waxing".
6. Within 4 hours of parturition slight sweating in the flank region and behind the elbows is frequently observed.
7. Mare becomes restless, anorexia, shows slight colic symptoms, switches her tail and lies down and gets up frequently.
8. Mare greatly prefers solitude and quiet at parturition and seems more able than other animals to control or suppress parturition until the night hours, when stable activities are at a minimum.

Ewe and goat

1. Condition are similar to the cow with the exception that udder development is not so great.

Sow, bitch and cat

1. Parturient animals become quieter and as parturition approaches, the vulva becomes flaccid, enlarged and edematous.
2. The mammary glands becomes enlarged and edematous and milk may be present in the udder several days before parturition.
3. Animal becomes restless and shows anorexia for a few days before delivery.
4. Environmental disturbances, especially those caused by other animals, will delay or interrupt the birth process.

OBSERVATIONS TO BE RECORDED

1. Prediction of the exact time of parturition : Calculated from the gestation period.

	Bovine	Equine	Ovine	Canine
1.				
2.				
3.				
4.				
5.				
2. Symptoms				
1. Croup ligaments				
2. Vulva				
3. Udder/teats				
4. Abdominal floor				
5. Secretion from teats				
6. Mucus				
7. Anorexia				
8. Abdominal pain				
9. Switching of tail				
10. Environmental disturbances during process of parturition				

B. MAJOR EVENTS DURING DIFFERENT STAGES OF PARTURITION

First Stage

- ◆ First stage is apparent for longer period in primiparous than pluriparous.
 - ◆ Active contractions of both longitudinal and circular muscles fibres of uterine wall, and the dilation of cervix.
 - ◆ Contractions are directly proportional to fetal resistance.
 - ◆ Maximum contraction 1 to 2 hours before birth.
 - ◆ Cervix was not dilated by the allantois chorion but was pulled open by the contraction of longitudinal uterine muscles.
- Uterine contractions occurs about every 10 to 15 minutes and last 15 to 30 seconds in cattle. In mare and bitch the fetus is rotating from its dorso pubic or dorso-lateral position into the dorso sacral position.
- During the first stage of parturition which lasts about 1 to 4 hours, straining or labour is not exhibited in the mare but later part mare may roll back.
- In the cow and ewe the symptoms of abdominal pain or discomfort may not be evident especially in pluriparous.
- In the bitch, sow and cat nervousness, anorexia and an increase in pulse and respiratory rates usually occur and this may result in panting. This stage usually lasts for about 2 to 12 hours.

Second Stage

- * This stage is characterized by the entrance of the fetus or fetuses into the dilated birth canal; rupture of the allantoic sac; abdominal contractions or labour; and the expulsion of the fetus through the vulva.
- * In cow abdominal contractions occurred only after the feet of the foetus were in the cervix and vagina. Following the rupture of the allantoic sac, the amnion is pushed through the cervix and may appear at vulva as a translucent distended membrane.
- * Uterine contractions occurred in cow about 4 to 8 times every 10 minutes and lasted 80 to 100 seconds.
- * Almost all animals, as soon as straining commences, lie down. Occasionally the foal or calf may be born with the dam standing.
- * The mare and sow usually lie out flat with the legs extended whereas the cow, bitch and ewe are more likely to lie on their sternum.
- * In the mare straining is usually characterized by several 2 to 5, strong expulsive efforts followed by 2 to 3 minutes of rest and this process is repeated at fairly regular intervals.
- * In the cow the straining is not as forceful or as frequent as in the mare.
- * In the ewe the second stage of birth is similar to that in the cow.
- * In the bitch straining begins after 0.5 to 1 hour or more and another pup is expelled. Although it may take about 1 hour of labor for the first pup to be born, the period of straining for the second and third pups and usually progressive shorter. The rate of expulsion of the fetuses is very irregular.
- * During this stage the contracting and shortening uterine walls force and direct the fetus into the birth canal and pelvis and abdominal contractions or labor, drives the fetus through birth canal.
- * The time for the second stage of birth in the cow is from 0.5 to 3 to 4 hours depending upon breed of the animal in pluripara. This second stage usually require 0.5 to 1 hour while primipara may take longer upto 3 hours or more. In ewes and goats the second stage is completed in 1 hour (range 0.5 - 2 hours or slightly longer if twins or more kids/lambs are present). In mares the second stage is normally completed in 5 to 40 minutes with an average of 20 minutes. The average total time for the second stage of parturition in bitch and cat is 3 to 6 hours. Twelve hours would be a maximum. In the sow the second stage is usually completed within 1 to 5 hours but occasionally may last upto 8 hours normally.
- * When the umbilical cord ruptures, the two umbilical arteries together with the urachus retract into the abdominal cavity of the fetus, and by the contractions of arteries into the body tissues, provision is made for the prevention of the bleeding from the maternal. The umbilical vein collapses, the blood drains from it and the

fluids in the umbilical cord drain out, often aided by the licking of the blood by the mother and becomes necrotic, dries up and drop away in 7 to 21 days.

Third stage

- * The third and last stage of parturition is the expulsion of the fetal membranes and the involution of the uterus.

A. Expulsion of foetal membranes

- * The expulsion of foetal membranes is normally completed within few hours.

- * After the expulsion of foetus the uterus contracts strongly for 48 hours and less vigorously, but more frequently help shrinking of the villi and the dilation of the maternal caruncular clefts probably play a major role in the preparation of the foetal trophoblast and cryptal epithelium of the maternal placenta. The middle uterine artery immediately contracts following parturition. The weight of the amnion and the portion of the allantois in the birth canal tends to help remove the after birth from the uterus. Normal expulsion of the foetal membranes can be said to be a complex process involving both mechanical and hormonal factors.

- * In dogs and cats, the foetal membranes are usually expelled irregularly between the fetuses; or one foetus may be expelled with its own placenta and that of a foetus expelled earlier.

- * In the sow, since a number of the allantois chorions may be fused, the foetal membranes may be expelled at only 2 to 3 intervals during parturition.

- * In the cow and buffalo, the length of time required for the expulsion of the foetal membranes is normally 1/2 to 8 hours. The ewe is similar to cow and buffalo but the mare normally expels its foetal membranes within 0.5 to 3 hours after the birth of the foal.

- * Domestic animals, with exception of the mare, usually eat the expelled foetal membranes which causes indigestion.

- * After the expulsion of the foetal membranes in a normal birth, the cervix secretes a rather thick tenacious mucus that tends to seal the cervix and thus prevents infection gaining entrance to the uterus.

B. Involution or regression of the uterus

- * In cow, buffalo time for involution varies from 26 to 56 days.

- * In mare, regression of the uterus proceeds at a rapid rate. The endometrium largely restored by the third to fifth day, the foal heat occur 9 to 12 days post partum and conception often occurs from service at the estrum. Exercise appears to hasten involution.

- * In multiparous animals and in the ewe, estrus usually does not occur until after the young are weaned, but involution of the uterus rapidly take place.

- * In the bitch the uterine horns are back to nearly normal size in 4 to 5 weeks.

OBSERVATIONS TO BE RECORDED

(i) Study the different stages of parturition in different species.

	(A) Time	(B) Symptoms
First stage	Second stage	Third stage
Cow		
Mare		
Sow		
Ewe/goat		
Bitch/cat		

(ii) To study the involution of uterus

	Cattle	Buffalo
At 10 days		
At 20 days		
At 40 days		
At 60 days		

(iii) To study the uterine discharge

	Colour	pH
Cow		
Mare		
Sow		
Goat		
Bitch		

Chapter - 15**CARE AND MANAGEMENT OF POST-PARTUM ANIMALS****OBJECTIVES**

1. To identify the post partum complications.
2. To provide the therapeutic management.

REQUIREMENTS

1. Recently calved animals
2. Protective clothing and lubricant
3. Cattle chute
4. Register for recording.

DESCRIPTION OF SUBJECT

- * The animal following parturition should be allowed to lick and nurse her young.
- * Undue excitement, noise or unusual happenings should be eliminated or prevented.
- * Rest and quiet following parturition is imperative.
- * Most of the animals the amount of grain should be increased gradually during the first three weeks after parturition to prevent acetonemia or ketosis.
- * Attention should be paid for diarrhea in pig, constipation in the mare following foaling.
- * Cow should be watched carefully for several days for symptoms of milk fever.
- * Excess edema of the udder should be controlled by massage, frequent milking or preferably diuretics such as carbonic anhydrase inhibitors with or without the glucocorticoids.
- * Moderate and light daily exercise is advisable in animals after parturition.
- * Mares should be turned out for about one hour a day for the first week and then for longer periods thereafter.
- * In domestic animals, retention of the fetal membranes occasionally occurs. The membranes are considered as being retained if they are not expelled within 8 to 12 hours in cow, 3 to 6 hours in the mare and 8 to 12 hours in the ewe.
- * In multipara, membranes usually cannot be observed hanging from the vulva and they frequently go unnoticed until they are expelled in 1 to 2 days or decompose and a mucopurulent discharge is noted in 4 to 10 days.

- * Mares should be treated earlier, within 12 to 24 hours, to avoid septicemia, toxemia and parturient laminitis.
- * If genital discharge persists beyond 14 to 20 days post partum or if they are abnormal in amount, purulent in nature, the genital tract of the animal need treatment under uterine infections. Early treatment is necessary, if the animal is to conceive promptly.
- * Agalactia or lack of milk after parturition, usually due to the failure of milk let down due to adrenalin secretion specially in heifers.
- * Some time animals lost their newborns which hasten the mammary involution can be treated mild application of liniments such as camphor in oil to the udder.

OBSERVATIONS TO BE RECORDED

- * Behaviour of post parturient animals with their new born.
- * Type of feed and green provided to the post parturient animals first three weeks.
- * General health and uterine lochia for providing sexual health control measures.

Chapter - 16

UTERINE INVOLUTION IN CATTLE AND BUFFALO

OBJECTIVES

1. To study uterine changes following parturition.
2. To record ovarian changes and cyclicity during post-partum period.

PRINCIPAL

The reduction in the size and shape of the genital tract following parturition is called uterine involution. It occurs in a decreasing logarithmic scale, the greatest change occurring during the first few days after calving.

MATERIAL REQUIRED

Advanced pregnant/recently calved animals, protective clothing/chute and lubricants.

DESCRIPTION OF SUBJECT

- A. Involution of uterus
- B. Restoration of endometrium
- C. Return of cyclic activity (ovarian rebound)

A. Involution of uterus

- * Stimulus for changes that occurring during the puerperium is primarily due to the removal of the fetus, oxytocin and prostaglandin $F_{2\alpha}$.
- * The hormones increases after the end of parturition to reach peak values three days post-partum and does not return to basal levels until 15 days.
- * The diameter of the previously gravid horn was halved by five days and its length halved by 15 days.
- * After the initial rapid phase of involution the subsequent changes proceed more slowly.
- * A reduction in the rate of involution between four and nine days post-partum, with a period of accelerated change from day 10 to 14 and a gradual decrease thereafter.
- * The whole of the uterus is usually palpable per rectum by eight and ten days post-partum in primipara and pluripara.

- * The speed of involution of the non-gravid horn is more variable than that of the previously gravid horn, which depends upon the degree of involuement in placentation.
- * In dairy cattle the time taken for complete involution ranged from 26 to 52 days but the changes after 20-25 days are imperceptible.
- * The cervix constricts rapidly post partum within 10-12 hours of a normal calving it becomes almost impossible to insert a hand through it into the uterus and by 96 hours it will admit just two fingers.
- * The mean external diameter range 15 cm at 2 days post partum, and by 9-11 cm at 10 days, 7-8 cm at 30 days and 5-6 cm at 60 days.
- B. Restoration of the endometrium
 - * There is usually a noticeable loss of fluid and tissue debris.
 - * Pluripara usually void upto a total 2000 ml but in primipara it is rarely more than 500 ml and some animal even it is less.
 - * The greatest flow of lochia occurs during the first two to three days; by eight days it is reduced and by 14-18 days post-partum it has virtually disappeared.
 - * Regeneration of the epithelium of endometrium occurs immediately after parturition.
- C. Return of the cyclic activity (ovarian rebound)
 - * Because of the prolonged period of inhibition during pregnancy, due to the continuous negative feedback effect of progesterone secreted by the corpus luteum and placenta, the pituitary is refractory post partum, as demonstrated by a lack of response to the administration of GnRH.
 - * The mean interval from calving to first oestrus ranged from 33 to 85 days.

RECORDING OF OBSERVATIONS

- A. Changes that occur in the caruncles of the cow during the puerperium
1. 48 hours post partum
 2. 5 days post-partum
 3. 10-15 days post partum
 4. 20 days post-partum
 5. 25+ days post partum

Chapter - 17

CARE AND MANAGEMENT OF NEW BORNs

OBJECTIVES

1. To identify the problems of the newborns.
2. To provide the therapeutic management to the newborns.

MATERIALS REQUIRED

Calving pen and new born, oxygen tank and short rubber tubes.

DESCRIPTION OF SUBJECT

- * At the time of birth of the young, an attendant should be present to remove the amnion around the muzzle and nose to check asphyxiation.
- * If respiration in the newborn is delayed various procedures should be used to stimulate this activity like use of oxygen in the pharynx/nostriil after removal of mucus and mouth are closed tightly.
- * Other practices such as artificial respiration, injection of adrenaline or cardiac stimulant should be provided.
- * Holding the young up by their hind legs to aid cleaning mucus from the large bronchi, throat and nose.
- * Umbilical disinfection is not necessary if births occur on clean pasture.
- * Clean sanitary, well bedded stalls for the young and parturient cow help greatly in preventing naval infection.
- * The new born should nurse and thus get the colostrum within 1 or 2 hours after birth.
- * The use of the antibiotics help in bringing these animals lacking immune bodies through the first critical week.
- * The problem of handling and feeding of orphan animals are substituted/adopted by another post-parturient female of the same species at the same time.
- * Retained meconium is seen more commonly in colts. All new born foals may be given an enema soon after birth.
- * Rupture of the bladder may occur in new born foal laparotomy on the mid-line and suturing of the ruptured bladder is desirable.
- * Many congenital or hereditary conditions such as atresia ani cleft palate, contracted tendons, blindness, corneal hy poplasia and other may affect new born animals.

- * Inbreeding and recessive factors causing the condition. If the defects may recognize early situation can be controlled.
- * Parasites transmitted in utero from the dam to fetus can be controlled by systematic deworming.
- * Nutritional deficiency causing diseases in the newborn can be controlled with replacement of minerals.
- * Management and environmental conditions may cause or predispose to the death of new born animals. Pups, pigs and lambs are poikilothermic nature and are often unable to respond to cold by increasing their metabolic rate until 3 to 7 days of age.
- * Injury or death of the newborn caused by the dam can be controlled by management specially in sow, dogs.

RECORDING OF OBSERVATIONS

	Calf	Foal	Kid/lamb	Pup
* Study the expulsion process.				
* Behaviour of the new born.				
* Emergency help for survival.				
* Control of feeding and management.				
* Therapeutic management.				

Chapter - 18

RETENTION OF FOETAL MEMBRANE AND ITS THERAPEUTIC MANAGEMENT

OBJECTIVES

1. To study therapeutic approaches for the removal of retained foetal membrane.
2. To study the effect of retention of placenta on future fertility.

PRINCIPAL

The after birth (foetal membranes) in cows and buffalo dropped away within 3-8 hours following calving. If the placenta is retained longer than 8 to 12 hours the condition is considered pathological.

REQUIREMENT

Animal suffering with retained placenta, protective clothing and gynaecology kit, antibiotic, syringe, needle etc.

DESCRIPTION OF SUBJECT

Retention of the foetal membranes or placenta (ROP) is basically because of failure of the villi of the foetal cotyledon to detach themselves from the maternal caruncles of the caruncles. After the fetus is expelled and the umbilical cord ruptures no blood is pumped into the foetal villi and they shrink in size. In the dam uterine contractions continue and the large amount of blood formerly going to the uterus is markedly reduced. The maternal caruncles become smaller in size, due to a reduced blood supply and the maternal crypts dilate. There is no muscle in the caruncle, during contractions of the uterine wall following parturition the shape of the caruncles may change from oval to round. Detachment of the foetal cotyledons from the maternal caruncles will be much easier during these contraction waves than between them. Incidence of retained placenta is high in births occurring several days before the expected date, because the placenta has not undergone the degeneration necessary for normal detachment. There are evidence to show that infections of the uterus during gestation may be a cause for retained placenta. Infectious agents such as *Brucella abortus*, tuberculosis, *Vibrio fetus* and various molds infections cause placentitis and cotyledonitis, resulting in abortion or pathological parturition with retained foetal membranes.

ETIOLOGICAL FACTOR

Infection, progesterone deficiency, nutritional deficiency.

Symptoms

- * A portion of the fetal membranes hang from the vulva.
- * Occasionally the fetal membranes do not hang from the vulva but are entirely within the vagina or uterus.
- * About 75 to 80% of cattle with retention of the fetal membrane show no marked illness.

Prognosis

- * Good. Mortality rate in retention of fetal membranes is very low 1 to 2 percent.
- * The effect of retained placenta on subsequent fertility is important to consider, as delayed involution and chronic endometritis, following retention of membranes is common.
- * After 2 to 3 months and a number of oestrus periods most cattle having a retained placenta eliminate the infection and the uterus becomes normal.
- * In most of the animals affected with ROP, the major loss is loss of milk/ subsequent conception.

Treatment

- * Variety of techniques employed the drug and hormones are to be recommended and in the time lapse between parturition and the attempt to remove the placenta.
 - * Manual attempt to detach the fetal placenta from the maternal caruncles should not be made upto 24 hours.
 - * Clean manual examination of the uterus can be tried with disposable gloves.
 - * The perineal region of the cow should be carefully cleaned and washed with soap and mild antiseptic.
 - * If the fetal membranes fall away readily from the caruncles they should be removed.
 - * Douching of the uterus is contraindicated.
 - * In some cases enough autolysis occurred, the villi will be separated readily from the crypts and placenta can be removed easily after 24-48 hours of calving.
 - * Placenta should be removed after 48 hours.
 - * The use of epidural anesthesia controls straining and defecation and makes possible a cleaner, more hygienic and gentler operation.
 - * Put antibiotics in the uterus and ecoblle should be recommended.
- The use of Pitocin (Pitocin or oxytocin (50 IU) has the value in preventing the ROP when given shortly after the expulsion of foetus or after its retention.

- * Oestrogen has been widely used for increasing the tone and muscular activity but reduces the milk production, should be avoided.
- * Antiseptic can be used locally to control the bacterial flora.
- * Broad range of antibiotics have been used intravenously and parentally.
- * If symptoms of septic metritis develops, local and parental antibiotic should be given.
- * All cows affected with ROP should be examined 20 to 30 days post calving to determine the involution of the uterus and for sexual health control measures.
- * ROP is less in mare, sow and bitch but in ewe and doe it occurs and may be handled similar to that of cow except that removal from the uterus is seldom possible.

RECORDING OF OBSERVATIONS

Handle the cases of retained placenta and observe the following.

- * Time of dropping of placenta from calving.
- * Involution of uterus in days.
- * First heat after R.O.P.
- * First insemination after ROP (in days).
- * Conception results

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Discussion

COLLECTION OF BIOLOGICAL MATERIAL

- * Abomasal contents
- * Focal membranes
- * Material from liver, spleen, kidney and heart
- * Brain tissue in case of small and decomposed fetus
- * Blood serum

COLLECTION OF MATERIAL FROM ABORTED FOETUS

Procedure

★ The focus is opened ventrally.

- * Stomach (abomasum) is scared with a hot spatula

COLLECTION OF MATERIAL FROM FEMALE ANIMALS

1. Vaginal washing

2. Cervical mucus

- Prepuceial washing
- Semen
- Blood

Prejudicial washing

- ## Semen

• Blood

- I. Foetal age in days

$$2.5 \times (\text{CRL cm} + 21)$$
 If CRL is 10 cm

$$\text{Foetal age in days} = 2.5 \times (10 + 21)$$

$$= 2.5 \times 31$$

$$= 77.5 \text{ days}$$
- II. Foetal age in months = $\sqrt{2} \times \text{CRL inches}$
 If CRL = 4.5 inches

$$\text{Foetal age} = \sqrt{2} \times 4.5$$

$$= \sqrt{9}$$

$$= 3 \text{ months}$$

Therapeutic Management

Animals with abnormal discharge from vulva should be treated parenterally and intra uterine with the antibiotics. In cattle and buffaloes abortion occurring after fourth month of gestation frequently follows puerperal metritis and retention of foetal membranes. In such case placenta need to be taken out. Intra uterine antibiotic needs to be given. Antihistamine and antibiotic should also be given parenterally to overcome infections.

Procedure

- * Blood, foetal membrane, uterine discharge and foetus, liver, kidney, tissues and abomasal contents of foetus should be collected for despatch to the laboratory for the diagnosis of causative agents of abortion.
- * Examine the affected animal for the presence of any abnormal vulval discharge.
- * Animal should be given antibiotic intrauterine.
- * Parenterally antibiotic and antihistamine should be given to the affected females.

Recording of Observations

Date of abortion
 Species of animal
 Age/date of birth
 Parity
 Date of last calving
 Date of last service/AI
 Appearance foetus, foetal membranes and cotyledons
 C-R length
 Age of foetus
 Appearance of vulval discharge, if any

Remarks

Write down status of aborted foetus and dam.

Chapter - 20

Mummification of Foetus - Diagnosis and Therapeutic Management

Objectives

1. To study the gynaecological changes of genital tract for the diagnosis of mummification of foetus.
2. To provide therapeutic management in cases of mummification.

Materials Required

Prolonged gestation animal, cattle crates, protective clothing, PGF₂ alpha and Dexamethasone.

Description of Subject

- * Fetal mummification occurs occasionally in animals following fetal death. The corpus luteum persists and there is no outward sign of trouble.
- * The fetal membranes becomes shrivelled and dried until they resemble parchmen and the fluid of the allantois, amnion and fetus are reabsorbed.
- * The uterus contracts on the fetus and moulds it. It is of two kinds :
 - # Papyraceous : Both fetus and membranes undergo disiccation and the hard and surrounded by fetal envelopes which resembles parchmen, e.g., sow, bitch and cat.
 - # Haemate : Similar process takes place but between the uterus and chorion there is present a peculiar adhesive substance, apparently derived from, blood which resembles melted chocolate.
- * In cattle mummification occurs following fetal death when the parturition-abortion mechanism fails to occur. The corpus luteum of pregnancy remains although the fetal fluids are resorbed and the uterus, which is always in a state of tone, contracts and causes severe distortion of fetus.
- * The cervical mucus seal remains intact and gestation apparently continues until it is recognized to be abnormal at a pregnancy diagnosis check or until it becomes obvious at the end of the gestation period that cow shows no sign of approaching parturition.
- * Occasionally there is spontaneous expulsion but some time it happened in the grazing field; the episode may be unobserved. Such a termination only occurs following regression of the corpus luteum and is usually occurred by oestrus.

DIAGNOSIS

- * Per rectal gynaecological examination of genital organs.
- * Foetus small, lack of fetal fluid and OS is closed. Focal membrane are wrapped over inner fetal mass.
- * No cotyledons and uterine artery small and without fremitus.
- * No signs of approaching parturition are seen. CL not palpable.

TREATMENT

- More rational, is to initiate the parturition mechanism.
 - To perform hysterotomy.
- Use of PGF₂ alpha and sometime corticosteroids drugs. It causes cervical relaxation and foetus is expelled out.
in horse, dog and sheep, mummification is occasionally observed, but in sow it is an important cause of prenatal losses. Foetuses dying in utero from 40 to 90 days of gestation usually mummified and are expelled at parturition.

Prognosis

The treatment of bovine foetal mummification by hysterotomy presents some technical difficulty as compared with caesarian section at term and it also carries a risk of subsequent adhesions of the uterus, leading to sterility.

RECORDING OF OBSERVATIONS

To handle a cases of mummification to observe.

- * Presence of fetus in uterus.
- * Type of coylet zone.
- * Middle uterine artery fremitus.
- * Fluid in uterus.
- * Type of foetus mass.
- * Recovery after hysterotomy.

Chapter - 21

MACERATION OF FOETUS - DIAGNOSIS AND THERAPEUTIC MANAGEMENT

OBJECTIVES

- To study gynaecological changes for the diagnosis of maceration of foetus.
- To provide therapeutic management in cases of maceration.

MATERIALS REQUIRED

Animal, cattle crate, apron, arm sleeves, soap, liquid paraffin, water, bucket, antibiotics, syringe, needle.

DESCRIPTION OF SUBJECT

- * Following fetal death and regression of the Corpus luteum - parturient phenomena - abortion fails to occur. The fetus then undergoes a gradual bacterial digestion in the uterine fluid by a process known as maceration. The cervix remains patent and there is a chronic mucopurulent vaginal discharge, but the animal is not ill.

- * Maceration may follow mummification in cases where natural or induced 'parturition' has failed to expel the fetus but the CL has regressed.
- * Maceration may occur at any stage of gestation and has been observed in all species.
- * In multipara, maceration of early embryo and foetuses usually ends. The other foetuses develop normally.
- * In the cow fetal maceration occurs after the third month of gestation by which time fetal bones are fairly well developed.
- * This condition may be more serious and fatal due to the presence of decomposing fetus(es), failure of cervix and genital canal to dilate normally.
- * Two factors of an open cervix and a dead fetus at body temperature cause a rapid bacterial invasion, the foetus develops emphysema.
- * Cases have a history of intermittent straining for several days, anorexia, temperature and pulse are often elevated.

PROGNOSIS

Fair to poor
There is no satisfactory treatment.

TREATMENT

1. Cervix may be carefully dilated manually prior to removal of the decomposing fetus.
2. After removal of the fetus, uterus should be re-examined to make certain another fetus is not present and to remove the placenta.
3. Large doses of antibiotics should be given parentally as well as intrauterine.
4. Oestrogen preparation 5 to 10 mg daily for 5 days or until the cervix is relaxed.
5. PGF₂ alpha and dexamethasone injection may be given for dilation of cervix.
6. Caesarian section should be considered as a last resort in the cow.
7. Diagnosis in the dog is aided by abdominal palpation and x-ray/ultrasonography.
8. On rectal palpation in cattle/mare, fetal bones may be palpated. There will be crepating sound on per rectal palpation.
9. The uterine wall is thick and heavy the prognosis is, poor.

RECORDING OF OBSERVATIONS

To handle the case of maceration of fetus.

- * Presence of C.L.
- * Uterine discharge/cervix dilatation.
- * Uterine wall.
- * Condition of foetus.
- * Fluid in uterus.
- * Thickening of the uterine wall.
- * Crepating sound of uterus on rectal palpation.

Chapter - 22

DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF ANESTRUS

OBJECTIVES

- * To study different methods for the diagnosis of anestrus
- * To study use of different hormonal and non-hormonal preparations for induction of estrus in anestrus animal.

MATERIALS REQUIRED

Animal, test tube, syringe, hypodermic needle, arm sleeves, gum boot, hormonal preparations, (GnRH, eCG, progesterone, insulin) non-hormonal preparations (Projana, Alves compound, Fertivet tab, Sajni cap, Janova, CoCu Tabs etc.).

DESCRIPTION OF SUBJECT

* Absence of periodic manifestation of estrus is termed as anestrus. On gynaecological examination, ovaries of such animals are smooth, quiescent and do not reveal presence of palpable follicle or corpus luteum. It is the most common single cause of infertility in cattle and buffaloes. There are several causes of anestrus. Existence of pregnancy is one of the cause of anestrus. No females should be treated for anestrus until she is checked for pregnancy. Anestrus may be post partum or post service. Most cows and buffaloes exhibit a period (35-45 days) of anestrus following parturition. Sheep and goat remain anestrus following kidding till the onset of breeding season. The sow usually remain anestrus till piglets are weaned. The sows exhibit estrus within a week of removal of piglets. Mares show heat 9-12 days following foaling and cycle again. Mares are also seasonal breeders and are stimulated to sexual activity by increasing day length in spring. Low energy intake and lower plane of nutrition affect adversely the cyclicity in livestock following birth of offsprings. Occurrence of anestrus is higher in buffaloes. This may be due to the fact buffalo has

- * Lesser number of primordial follicle
- * Higher rate of follicular atresia
- * Lower level of circulatory gonadotrophins.

CAUSES OF ANESTRUS

A. Ovary containing corpus luteum

- * Anestrus due to pregnancy
- * Anestrus due to retained or persistence of corpus luteum.
 - Due to early embryonic mortality on foetal death.
- Gross uterine pathology
 - # Post service pyometra.
 - # Foetal maceration
 - # Foetal mummification
- * Anestrus due to subestrus, weak or silent estrus
 - Frequently occur during 1st 60-90 days following calving in cattle and buffalo.
 - Ovary contains a cyclic CL
 - There is no overt estrus.
 - This may be due to weak estrus or shorter duration of estrus
- * Anestrus due to unobserved estrus
 - Weak or shorter duration of estrus.
 - Poor sexual health control measures, including heat detection programme.

DIAGNOSIS

- * Presence of corpus luteum during per-rectal palpation in cattle, buffalo and ewe and under ultrasonograph in sheep and goat.
- * Higher (more than 1 ng/ml) plasma/serum progesterone concentration.

THERAPEUTIC APPROACHES

Anestrus animals having CL and not pregnant can be treated using normal luteolytic dose of PGF₂ alpha.

- * The uterine pathology (pyometra, mucometra, maceration and mummification of foetus) may be treated using estradiol, parenteral and intrauterine injections of antibiotics.
- * Better application of sexual health control measures, specifically heat detection programme.
- * Improving the managemental conditions.

Prognosis : is usually good in this category of anestrus.

B. Ovaries without a corpus luteum or inactive ovaries - True anestrus

- * Weakness, debility or loss in weight
 - Lower plane of nutrition

- # Carbohydrate
- # Protein
- # Mineral
- # Vitamins
- Chronic or debilitating diseases
 - # External or internal parasitism
 - # Arthritis
- # Chronic diseases like TB, JD and metabolic disorders
 - Senility or old age
- Lactational stress and suckling.
- * Seasonal influences
 - Summer anestrus in buffaloes.
 - Anestrus in sheep, goat and mares.
- * Cystic ovaries - luteal cyst
- * Miscellaneous conditions
 - Non functional ovaries due to parturition stress and uterine diseases.
 - Prematurisms.
 - # History of colwin birth with a male calf
 - # Per rectal examination reveal thred like cervix, uterus, ovaries are flat, small and hard without any palpable follicle.
 - Hypoplasia of ovary and infertile genitalia.
 - Ovaries may be very small and poorly developed
 - Tumor of ovary - very rare

DIAGNOSIS

- * History of no symptoms of estrus.
- * Per-rectal and sonographic examination reveal flat, smooth ovaries without any palpable structure ie. follicle or CL at 2-3 palpations at 6-7 days interval.
- * Plasma/serum progesterone at the basal level in cases of smooth ovaries. In luteal cyst the progesterone level would be high.

TREATMENT

- * Anestrus condition is if due to cystic ovary-luteal cyst, administration of PGF₂ alpha will be effective for restoration of cyclicity.
- * In free matingism or hypoplasia of ovary, no treatment is suggested looking the hereditary nature of the anestrus condition.
- * In case of ovarian tumor - affected ovary may be removed surgically.
- * Treatment must be given for restoration of general health of the animals.
- * Adequate nutrition in respect to CHO, protein and minerals should be provided.

There is no value of use of hormones in cases of deep or true anestrus with non functional small, smooth ovaries due to poor physical conditions.

Massage of ovaries, uterus and cervix through per rectal palpation in cattle and buffalo has been found very effective.

Painting of OS cervix using Iugol's iodine through a tamponing forcep. These practices should be combined by additional supplementation of feeding of mineral mixture daily for 15-20 days.

The best hormonal treatment is the use of progesterone through either injection, oral, implant alone or in combination with oestradiol benzoate or smaller dose of eCG.

GnRH and eCG either alone or in combination.

Insulin injection for 3-5 days @ 0.25 IU/kg b.wt.

Certain non normal preparations viz., Prajama, Aloes compound, Janova, Sajini may also be used as they are cheap and easily available.

Commercial preparation of progesterone

- * Duraprogen Unichem
- * Proluon Depot German Remedies
- * Lutogen Depot Servier Pharma
- * Synchromate-B ear implant Animal Health Inc., USA
- * CRESTAR implant Intervet, Holland
- * MGA oral Upiolm

Commercial preparation of estrogen

- * Oestradiol Benzoate Intervet
- * Progynon German Remedies

PROCEDURE

- * Take the history of animal about age, total calvings, date of last calving and any complication occurred during last calving.
- * Examine the general health of the animal to rule out suffering from any chronic disease and internal or external parasitism.
- * In case of cattle, buffalo and mare examine on per rectal palpation at least twice at 6-7 days interval. The anestrus animal would have smooth ovaries without any palpable structure of follicle and CL. Uterus will be flaccid and OS of cervix closed.
- * Take 5 ml blood, separate plasma/serum. Progesterone concentration is determined using RIA/ELISA Kit. The progesterone concentration in anestrus animals will be at the basal level (around 1 ng/ml).

RECORDING OF OBSERVATIONS

Animal No.

Date of examination

Species of animal

Age

Total calving

Date of last calving

Date of last estrus/AI and total number of estrus

Gynaecological findings

Ovary - Follicle and CL

Uterus - Tone and Enlargement

Cervix - Tone and opening of OS cervix

Vaginal and Vulvar mucus membrane

Progesterone concentration mg/ml

TREATMENT GIVEN

- * Deworming along with supplementation of mineral mixture.
- * Utero ovarian massage
- * Lugol's iodine tamponing.
- Progesterone injection - 50 mg/ml for 5 days I/M
- Oral progesterone - 1mg OD
- Crestar implant - 9 days
- Insulin - 0.25 IU/kg B.Wt. 5 days I/M
- eCG - 500 IU I/M.
- GnRH - 50-100 µg i/m
- PGF₂ alpha - 25 mg or 500 mg I/M

REMARKS

Write down gynaecological status of animal.

Chapter - 23

DIAGNOSIS AND TREATMENT OF ANOVULATION

OBJECTIVES

- * To diagnose a case of anovulation
- * To provide suitable treatment in cases of anovulation.

MATERIALS REQUIRED

Animals, arm sleeves, animal crate, gun boot, hCG, GnRH, syringe, needle, test tube.

DESCRIPTION OF SUBJECT

Anovulation is a condition where animal comes into estrus but ovulation does not occur. Animal shows estrus but this not followed by release of ovum. The consequence is failure of conception. Such animal if not being treated become infertile. The condition occur due to inadequate release of luteinizing hormone (LH) from the anterior pituitary. This may be due to lower levels of oestradiol 17-beta secretion from the maturing follicle or higher threshold of anterior pituitary to oestradiol 17-beta for the adequate and timely release of LH.

DIAGNOSIS

The diagnosis may be made either by per rectal palpation of ovaries or by ultrasonographic scanning of ovaries 10-12 days following estrus/service/Al.

Treatment

The condition may be treated using hCG or GnRH.

PROCEDURE

- * Take the breeding history of animal including total number of Al and last day of estrus and A.I.
- * Secure the animal in the crate.
- * Examine the animal (cattle, buffalo and mare) by per rectal method or ultrasonography (sheep and goat). The examination is made 10-12 days following estrus/service.

- * The ovaries will be smooth without any palpable corpus luteum.
- * Take the blood sample on day 10-12 and go for progesterone estimation either by RIA or ELISA.
- * The progesterone concentration shall be fluctuating around 1 mg/ml, indicating non formation of corpus luteum following estrus/Al.
- * Animals should be treated at next estrus by injecting 1500-3000 IU hCG or 100-200 µg GnRH intramuscular at the time of service/Al.

RECORDING OF OBSERVATIONS

Animal No.	Date of examination
Species	
Age	
Total calving	
Date of last calving	
Total Al and Date of Al or estrus	
Cycle length (days)	
* Date of last heat/Al * Date of present heat * Gynaecological findings	
Ovary	
CL - Present/Not present	
Ultrasonographic findings	
Ovary	
CL - Present/Not present	
Treatment	
Given at estrus	

REMARKS

Write down the gynaecological findings and response to the treatment.

Chapter - 24**DIAGNOSIS AND THERAPEUTIC MANAGEMENT
OF OVARIO-BURSAL ADHESION****OBJECTIVES**

- Diagnosis of a case of ovario-bursal adhesion
- Therapeutic management of such cases.

MATERIALS REQUIRED

Animal, cattle crate, arm sleeves, coverall, soap, bucket, water and gum boots.

DESCRIPTION OF SUBJECT

Ovario-bursal adhesion involves ovary and surrounding bursa. Ovary becomes encapsulated within the surrounding bursa. Fallopian tubes may also get involved. The condition may occur due to rough handling of ovary, bursa, rupture of cyst and removal of corpus luteum manually. Infection either from uterus or peritoneum may lead to ovario-bursal adhesions. Peritoneal tuberculosis may involve ovaries and bursa and lead to their adhesions.

Diagnosis is made by per rectal palpation of ovary. The ovaries are not free on palpation and get encapsulated with bursa. The animal shows cyclically but fail to conceive. The treatment in such cases are not successful. The animal should be given breeding rest. Prognosis is poor.

PROCEDURE

- Take the history of animal
- Examine the animal gynaecologically by per rectum or on ultrasonography.
- Ovaries are not free and encapsulated with bursa. The condition can be demonstrated under ultrasonography.
- Animal shows normal cyclicality but fails to conceive as ovary fails to release ova into the fallopian tube.
- It is difficult to palpate follicle or CL on the surface of ovary due to encapsulation of ovary with the bursa.

RECORDING OF OBSERVATIONS

Animal No.
Species of animal

Date of examination

Age

Total no. of calving

Date of last calving

No. of heat/AI

Date of last AI

Gynaecological findings

Ovary

Uterus

Cervix

REMARKS

Write down the gynaecological status of animal, prognosis and treatment response.

Chapter - 25**DIAGNOSIS AND THERAPEUTIC
MANAGEMENT OF OVARIAN CYST****OBJECTIVES**

- * To study diagnostic approaches for ovarian cyst.
- * To study therapeutic management of ovarian cyst.

MATERIALS REQUIRED

Animal, Animal crate, Coverall, gum boots, syringe, needles, GnRH, hCG, PGF₂ alpha

DESCRIPTION OF SUBJECT

Cystic ovarian degeneration is one of the common affection of ovary in dairy cattle and buffalo but its incidence has been found to be low in buffaloes.

Types of cyst

- Follicular cyst
- Luteal cyst
- Cystic corpora lutea
- * Follicular cyst is the persistence of single or multiple fluid filled structure on the ovary. The follicle does not ovulate and persists for longer period. The cyst is soft on palpation and usually bigger than the graafian follicle. The animal usually show intense estrus of persistent in nature of shorter interval (5 to 7 days interval). Such condition is said nymphomania. Animal may show normal cyclicity or may be anestrus. The cyst is formed usually on the surface of ovary but some time it may be central or embedded also.
- * The luteal cyst is also anovulatory. The follicle does not ovulate and gets partially or totally retained and persists for a longer period. The animal remain anestrus.
- * Cystic corpora lutea is another type of cyst its occurrence is very low. It is not considered pathogenic and usually follows normal cycle.

PRINCIPLE

- * The follicular and luteal cyst arises due to inadequate secretion of luteinizing hormone from the anterior pituitary. The cystic corpora lutea is due to inadequate secretion of PGF₂ alpha from the endometrium. The conditions, therefore, may successfully be treated using hCG, GnRH and PGF₂ alpha, respectively. Spontaneous recovery can also occur within 45-50 days post calving in cows.

DIAGNOSIS

The diagnosis is based on :

- * Frequent onset of estrus with the history of failure of conception and infertility.
- * Appearance of animal - Buller type in follicular cyst.
- * Gynaecological examination on 10-12 days post estrus. The affected animals would not have cyclic corpus luteum development on any one of the ovary.
 - Fluid filled structure i.e. persistence of bigger follicle will be palpated in follicular cyst.
 - Luteinized hard follicle may be palpated in luteal cyst.
- In cystic corpora lutea the well developed CL will present on day 10-12 post estrus.

PROCEDURE

- * Take the history of estrus and cyclicity.
- * Secure the animal.
- * Examine the animal by per rectal palpation method. The ovaries should be examined for the presence of persistent soft fluid filled structure i.e. follicular cyst on the surface of ovary. In case of luteal cyst the hard luteinized follicle can be palpated.
- * Animals ovary may be examined under ultrasound and laparoscopy, if facilities are available for visualizing the follicular cyst and luteal cyst and cystic corpora lutea.
- * Examine vagina and vulva. It will be moist, hyperemic and edematous.
- * Treatment using hCG 3000 IU or GnRH 400 µg may be given to follicular cyst condition in cattle and buffalo. In luteal and cystic corpora lutea normal luteolytic dose of PGF₂ alpha may be given intramuscularly.
- * Follicular cyst may ruptured through manually by applying digital pressure through per rectal examination in cattle and buffalo. It, however, should be avoided as it frequently lead to haemorrhage and adhesion of ovary.

Some cysts are so hard that it is difficult to rupture by digital pressure. Follicular cyst may also be ruptured using ovarian puncturing needle through the vaginal wall. These methods are now not in practice and are not safe. The use of hCG and GnRH are much better approach for the treatment of follicular ovarian cyst.

Commercial preparations of LH

- * Chorulon. Interval 1500 IU per vial
- * Chorionic gonadotrophin. Univet. 1000 IU/vial.

Commercial preparations of GnRH

- * Receptal Hoechst, India
- * Fertagyl Intervet, Holland
- * Ovalyse Upjohn, USA
- * Cystorelin CEVA Laboratories Inc.

RECORDING OF OBSERVATIONS

Animal No. _____ Date of examination _____

Animal species _____

Age _____

Total calving _____

Date of last calving _____

Total no. of estrus since calving _____

Date of last heat/AI _____

Gynaecological findings _____

Ovary _____

Uterus _____

Cervix _____

Vagina and vulva _____

Treatment given _____

hCG _____

GnRH _____

PGF₂ alpha _____

Treatment schedule in cattle and buffalo using GnRH & PGF₂ alpha

Follicular cyst

Day 0 = day of treatment GnRH 200-400 µg IM

Day 12 = PGF₂ alpha 25 mg on 500 µg IM

Day 15 = Onset of estrus, do AI inject GnRH 100 µg IM at AI.

Luteal cyst or cystic corpora lutea

Day 0 = PGF₂ alpha 25 mg or 500 µg IM

Day 3-4 = Onset of estrus, do AI, inject GnRH 100 µg IM at AI.

REMARKS

Write down status of animal based on gynaecological findings.

Chapter - 26

DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF OVARIAN HYPOPLASIA AND INFERTILE GENITALIA

OBJECTIVE

- * To study diagnostic procedure for the ovarian hypoplasia, infantilism and under development of genital tract.
- * Therapeutic management of such cases.

MATERIALS REQUIRED

Animal, gun boot, soap, towel, syringe, needle, test tube.

SUBJECT DESCRIPTION

Ovarian hypoplasia and infantilism of genital tract are congenital abnormalities. This may be unilateral or bilateral, partial or total. The affected ovaries are very small, hard with reduced number of primordial cells. The genital tract is also very small and thin. In partial cases of hypoplasia/infantilism, the tract is in the mid way and remain under developed.

In some animals, the whole reproductive tract remain underdeveloped. This may be due to poor feeding and managerial condition. The chronic diseases and parasitism may also lead to under developed genitalia.

Such animals usually remain anestrus and don't show cyclicity since birth. The gynaecological examination reveal very small flattened ovary and thin atonic small genital tract. Progesterone assay twice or thrice at 7 days interval will not show any increase or decrease pattern. Conformational diagnosis is made by the histopathology of ovary and genital tract by observing reduced number of cells and their cell types. Ultrasonographic studies would reveal very small ovaries and genital tract. Treatment in such cases is not successful and as such animals are required culling from the herd. Supplementation of protein, energy and mineral mixture may be helpful in cases of underdeveloped genital tract.

PROCEDURE

Animal number: _____

Species _____

Date of examination _____

* History of animal _____

* Date of birth _____

- * Calving if any
- * No. of heat
- * Date of last heat
- * Gynaecological findings
- Ovary
- Uterus
- Cervix
- * Ultrasonographic findings
- Ovary
- Uterus
- Cervix
- * Progesterone profile
- * Treatment if any

REMARKS

Write down gynaecological status of animal.

Chapter - 27

DIAGNOSIS AND TREATMENT OF ENDOMETRITIS AND METRITIS

OBJECTIVE

- * To study different approaches for the diagnosis of endometritis and metritis.
- * Therapeutic management of endometritis and metritis.

MATERIALS REQUIRED

Animal, glass slides, test tube, syringe, needle, A.I. gun, catheter, antibiotics, PGF, alpha, arm sleeves, gun boots, coverall, bucket, towels, soap and water.

SUBJECT DESCRIPTION

Endometritis, commonly known as chronic uterine catarrh is the inflammation of the endometrium. Metritis is an advanced stage of endometritis involving the inflammation of muscular layers, the entire thickness of uterus. Peri or para metritis, involves inflammation of the serosa and broad ligaments and other pelvic and abdominal organs. The condition occurs due to septic metritis, douching and perforation of uterus, careless rectal palpations at frequent interval, haemorrhage during calving, rough handling of uterus during removal of retained placenta and excessive massage or manipulation of infected uterus. Tuberculous infection also leads to peri or para metritis.

CAUSES OF ENDOMETRITIS

- * The causative organism reaches to uterus at Alveolus or during and following parturition.
- * Microorganisms involved are, *Trichomonas foetus*, *Vibrio foetus*, *Brucella abortus*, *C. pyogenes*, *Ps. aeruginosa*, *E. coli*, *Streptococcus* and *Staphylococcus*.
- * The condition usually follows abortions, retained placenta, dystocia, traumatic lesions of uterus, cervix and vagina.
- * Use of infected semen or service by the infected male may lead to endometritis.
- * The condition affects fertility by:
 - Affecting normal development of embryo i.e. embryonic cleavage.
 - Affecting attachment of embryo to the endometrium i.e. implantation of embryo.
 - Affecting microenvironment of uterus and nutrition to the embryo.
 - Involution of uterus in post partum animals.

- Post partum onset of estrus.
- Prolongation of service period and calving interval.

DIAGNOSIS

Endometritis is of two types

- Subclinical
- Clinical

The subclinical endometritis may not be diagnosed by rectal or vaginal examination. The animals shows history of normal oestrous cycle with failure of conception. The oestrous mucus discharge usually appears normal. The infection in the uterus is mild in nature and condition usually goes undetected may be diagnosed by white side test and by isolation and culture of uterine contents.

- White side test.

METHODOLOGY

- * Take 2.3 ml cervical mucus and mix it with 2-3 ml 5% NaOH in a test tube, mix it and boil.
- * Colour of mucus if turns yellow is indicative of uterine infection. The intensity of yellow colour indicates severity of endometritis.
- * Degree of yellow colour indicates the concentration of neutrophils indicative of infection.

CLINICAL SIGNS

- * Repeated service.
- * Failure of conception
- * Turbidity in oestrus mucus discharge.
- * Presence of mucopurulent vaginal discharge in the post partum animals.
- * On per rectal palpation the uterus is usually normal. In severe case it is felt thick walled, slightly large and heavy.
- * Oestrous cycle and period is usually normal. Occasionally the oestrous cycle length may be shortened (8-10 days) due to inflammation of endometrium, preventing development of corpus luteum. The fertilized egg dies because of infected endometrium and animal repeats to estrus.
- * Sometime longer cycles occurs due to embryonic mortality and persistence of corpus luteum.

PROGNOSIS

- * In most cases prognosis is fair to good.

TREATMENT

The principle of treatment is based upon :

- * Stimulating uterine muscle tone.
- * Stimulating uterine defence.

- * To overcome intrauterine infection.
- * The treatment used to stimulate the uterine muscle tone, blood supply and flow of mucus involves uterine flushing/washing with mild warm chlorine solution, physiological saline, dilute iodine solution, acriflavin solution and povidone iodine solution. Intramuscular injection of estradiol 3 to 5 mg for increasing uterine tone and evacuation of uterine contents. Use of PGF₂ alpha for luteolysis also help for increasing uterine tone by bringing animal into estrus. Antibiotic both intrauterine and parenteral are helpful to overcome uterine infection. Lower doses of flavamisole and hipopolysaccharide have also been used as immunomodulator for increasing uterine defence.

PROCEDURE

- * Take the history, age, date of last calving, date of last heat/AI and about the nature of oestrus mucus discharge.
- * Examine the animal on pre rectal palpation. Examine the ovary for the CL or follicle, uterus for tone or texture. Examine vagina and vulva for the mucus discharge.
- * Give the treatment to the animal using intra uterine antibiotic infusion and parenteral antibiotic.
- * Repeat intrauterine antibiotic treatment at alternate day till the recovery (3-5 infusions are usually sufficient).

RECORDING OF OBSERVATIONS

Animal No.	Date of examination
Animal species	
Age	
Total calvings	
Date of last calving	
Date of last heat	
Nature of vaginal discharge	
Gynaecological examination findings	
Ovary	
Uterus	
Cervix	
Treatment given	
Parenteral and intra uterine	
Antibiotic treatment	
Any other treatment.	

REMARKS

Write down status of animal and response to treatment.

Chapter - 28**DIAGNOSIS AND TREATMENT OF PYOMETRA****OBJECTIVES**

- * To diagnose a case of pyometra.
- * To provide treatment to such type of case.

MATERIALS REQUIRED

Animal, AI Gun, AI catheter, syringe, needles, antibiotics, PGF₂ alpha, gum boot, arm sleeves, coverall etc.

DESCRIPTION OF SUBJECT

Progressive accumulation of pus in the uterus is termed as pyometra. There is persistence of corpus luteum.

- * The condition arises as a
- * Sequel to endometritis
- * Embryonic or foetal death

Inflamed uterus ceases to produce or release of endogenous luteolysin (PGF₂ alpha). The corpus luteum thus persists. The genital tract remains under influence of progesterone and infection progress. The cervix remain fairly closed. Occasionally there is dribbling of purulent discharge from vagina and vulva. Pyometra may follow septic metritis during post partum period. There is large amount of accumulation of pus in the uterus. The pus come out during per rectal massage of uterus and cervix. Post service pyometra is characteric of trichomonas infection. The pus during such infection is more copious and potato soap type.

DIAGNOSIS

The diagnosis is made by

- * The presence of purulent vaginal discharge.
- * Perrectal gynaecological examination.
 - Uterus will be thickened
 - Uterus will be doughy on palpation.
 - Enlargement of uterus

The condition may be differentiated from pregnancy as there will be no alive feeling of uterine horn, no slipiness of foetal membrane, no uterine tone and no palpation of foetus will be observed/palpated during pyometra.

Ultrasonography

Uterus appears as black colour fluid filled structure indicating presence of pus and no foetus.

TREATMENT SCHEDULE

- * Injection of PGF₂ alpha : It would lead regression of CL which will follow dilatation of cervix. Opening of OS cervix, increases myometrial tone and evacuation of uterine contents.
- * Evaluation of uterine contents
 - By per rectal massage
 - Using uterine catheter.
- * Inj. of estradiol benzoate 3-5 mg IM. causes proluse vaginal discharge and evaluation of uterine contents. This may be followed by 30-50 IU oxytocin to hasten evacuation of uterus.
- * Antibiotic intra uterine and parenteral.

PROCEDURE

Take the history of animal

- * For general health and infection
- * For breeding details and calving etc.
- * Secure the animal and examine gynaecologically either by per rectal examination or by ultra sonography. The uterus should be examined. Affected uterus will be thick walled and doughy. On massage there will be dribbling of pus.
- * The treatment schedule as described above may be followed.

RECORDING OF OBSERVATIONS

Animal No.	Date of examination
Animal species	
Age	
Total calving	
Date of last calving	
No. of heats after calving	
Date of last heat/AI	
Gynaecological findings	
Ovary	
Uterus	
Cervix	
Vagina	
Nature of vaginal discharge	
Treatment	

REMARKS

Write down gynaecological status of animal and progress of treatment.

Chapter - 29**DIAGNOSIS AND TREATMENT OF PYOSALPINX AND HYDROSALPINX****OBJECTIVES**

- * To diagnose a case of hydro and pyo salpinx.
- * To provide therapeutic management in such cases.

MATERIALS REQUIRED

Animal, animal crate, cover all, gum boots, soap, bucket, water, antibiotic, syringe, needle.

DESCRIPTION OF SUBJECT

Affection/diseases of oviduct occur more commonly in cattle and buffalo than other animal species. Accumulation of watery mucoid fluid in the oviduct is termed as hydrosalpinx, however, accumulation of pus in oviduct is known as pyosalpinx. The earlier diagnosis of such cases are difficult.

Infection to the fallopian tube is secondary to ascending infection from the uterus following:

- * Abortion
- * Detained placenta
- * Septic metritis
- * Pyometra

* Genital tuberculosis may also affect the fallopian tubes. Other organisms involved are:

- *Streptococci*
- *Staphylococci*
- *E. coli*
- *C. pyogenes*
- *Mycoplasmas*

- * Obstruction of fallopian tube and its adhesion usually occur following rupture of cyst, enticulation of CL and rough handling of ovary and bursa.
- * Hydrosalpinx is observed secondary to congenital anomalies i.e. segmental aplasia of paramesonephric ducts.
- * Pyosalpinx develop due to infection and usually led to bursal adhesion.

DIAGNOSIS

- * Difficult
- * Only in severe cases per rectal palpation of fallopian tube can be made.
- * By the presence of adhesion of bursa and fallopian tube.
- * In hydro salpinx-thin walled, coiled, enlarged fluctuating oviduct can be palpated.
- * In pyo salpinx - thick walled, enlarged oviduct is palpated.
- * Occlusion of oviduct may be diagnosed by the tubal patency test.

TUBAL PATENCY TEST**PSP Test (Phenolsulphathaline test)**

- * 20-30 ml 0.1% sterilized PSP solution is infused into the uterine horn to that side of fallopian tube to be tested for patency.
- * Urinary bladder is catheterized with two way Foley's catheter to take out the urine samples.
- * Epidural anaesthesia may be given before the start of the test.
- * Urine sample is collected after 30-60 minutes. Take 10 ml urine mix it to 0.2 ml 10% NaOH. The colour of solution is turn pink or red is indicative of presence of PSP. It would be indicative of opening of fallopian tube. If urine colour does not turn red or pink it means PSP has not come out through fallopian tube and will be indicative of occlusion of fallopian tube.

TREATMENTS

- * Treatment is not very satisfactory.
- * Mild cases may get spontaneous recovery.
- * Use of parenteral antibiotic may cure the condition.
- * Breeding rest is advocated.

PROGNOSIS

Poor and guarded.

REMARKS

Write down the diagnosis based on per rectal palpation and PSP test.

Chapter - 30**DIAGNOSIS AND THERAPEUTIC
MANAGEMENT OF REPEAT BREEDING****OBJECTIVES**

- * To study different approaches for the diagnosis of repeat breeding.
- * To study therapeutic management of repeat breeding.

MATERIALS REQUIRED

Animal, cattle crate, gum boots, cover all, soap, water, antibiotics, GnRH, PGF, alpha, syringe, needle, AI gun, sheath

DESCRIPTION OF SUBJECT

The repeat breeding animal has normal or nearly normal estrous cycle, period and has been bred with 2 or more times to a fertile bull yet failed to conceive. The incidence of such problem has been reported to be 10-20 per cent. The incidence varies with breed, farm, agroclimatic and managerial conditions. Incidence of repeat breeding has been found higher in older and higher milk producing dairy animals. Stress of milk production interfere in the synthesis and release of gonadotrophins leading to repeat breeding.

Causes of repeat breeding

- * Failure of fertilization
- * Early embryonic mortality

Failure of fertilization and early embryonic mortality are the major causes of repeat breeding. The condition may arise either due to pathological reasons or improper managerial conditions including nutritional deficiencies. Problems in heat detection, timing of AI, handling and quality of semen, sanitary conditions in gynaecological operations, environmental temperature and skill of inseminator largely influence the incidence of repeat breeding in cattle and buffaloes. The estrous cycle length is not affected in majority of cases where repeat breeding is due to failure of fertilization and early death of embryo (day 8 to 16). However, in cases of embryonic mortality occurring at 16 to 25 days, the estrous cycle length is prolonged. The condition may be monitored using progesterone assay.

PROCEDURE

- * Examine the animal by per rectal palpation of genital organs 10 days post estrus to confirm ovulation by palpation of CL. If corpus luteum is not palpable, the animal at next estrus should be treated with GnRH followed by AI. The treatment of GnRH would also take care of delayed ovulation and anovulation.

- * Examine the cervical mucus for the presence of turbidity or small pus flakes. While side test may also be conducted to rule out sub clinical endometritis. The uterine swab can be taken for culture of microbes and antibiotic sensitivity test. The animal should be treated with antibiotic intrauterine and parental to take care of uterine infection. Animal should be bred at next estrus.
- * Animal should be detected in heat by observing the animal closely by day morning and evening using a teaser and visual signs of estrus. The animals drafted in heat in morning should be inseminated by same day evening and animals drafted in heat in the evening should be inseminated at next morning.
- * AI should be performed under hygienic conditions by the skilled persons. Semen should be free from micro organisms and must have optimum number of viable normal spermatozoa.
- * Thawing of semen should be done by dipping straw in lukewarm water at 37°C for 30 seconds.
- * Animal should also be examined by per rectal palpation of genital tract for ovario bursal adhesion, fallopian tube, uterine and cervical abnormalities.
- * Injection of insulin during mid cycle and at estrus may improve quality of CL, and thus promotes conception rate in repeat breeding animals.

OBSERVATIONS

Animal No.	Date of examination
Animal species	
Age	
Total No. of calving	
Date of last calving	
Total AI services done	
Date of estrus since last calving	
Gynaecological findings	
* Ovary	
* Uterus	
* Vagina and vulva	
* Nature of estrus discharge	
Findings of isolation and culture of uterine swab	
and antibiotic sensitivity test	
Treatment given	

REMARKS

Write down the reproductive status of animal.

Chapter - 31**SYNCHRONIZATION OF ESTRUS****OBJECTIVE**

- * To study different methods of synchronization of estrus.
- * To synchronize estrus in cattle and buffalo.

MATERIALS REQUIRED

Animals, PGF₂ alpha, PMSG, progesterone injection, progesterone implant.

SUBJECT DESCRIPTION

Synchronization of estrus is a technique by which most of the females in a population or herd can be brought into estrus at a predetermined time.

Advantages

- * Reduces the time required for detection of estrus.
- * Allows planned breeding of more number of females at a time.
- * To breed animals at a fixed time even without detection of estrus.
- * Animals can be grouped into desired parturition pattern.

Approaches or methods of synchronization of estrus

- Prologation of luteal phase of the cycle by the administration of progesterogen.
- Shortening of luteal phase of the cycle by exogenous administration of prostaglandin F₂ alpha.

Use of progesterogen

- * Progesterone injection (17 hydroxy progesterone) or oral feeding of progesterone (MGA, MAP)
 - Degree of synchronization of estrus is low
 - Fertility is low.
- * Progesterone injection followed by single injection of oestradiol benzoate.
 - Precision of synchronization increases
 - Fertility is low.
- * Progesterone implant (morgestome)
 - Synchronization of estrus is good.
 - Fertility is better.
- * Intravaginal progesterone
 - Used in small animal
 - Chance of its loss is more.

- * Progesterone releasing intra vaginal devices (PRID)
 - Used in small animal (sheep and goat)
 - Synchronization is good.

PRACTICAL APPROACHES FOR SYNCHRONIZATION OF OESTRUS

Species	Hormones/drugs	Treatment schedule	Expected onset of estrus
Cattle & Buffalo	PGF ₂ alpha	* Give PGF₂ alpha 1/m between day 6-15 of cycle (ovary must has cystic CL) * Give two injection of PGF₂ alpha 7/m 11-12 days apart (for range cattle unknown history of previous estrus)	Majority of animals comes into estrus within 3-4 days. Animals will be in heat within 3-4 day
	Progesterogen implant	* 3 mg oestradiol valerate + 3 mg norgestome 1/m - day 1 * Next day put norgestomet implant for 9 days * Single injection between day - 7-14 days	Majority comes into estrus 2-5 days
Sheep	PGF ₂ alpha	* Give 2 injection 1/m and 9-10 days apart	Animals are in heat with 2-3 days
	Progesterogen plus PMSG	* Progesterogen implant for 12 days and one dose of PMSG at withdrawal of implant	Animals are in heat with 2-3 days
Goat	PGF ₂ alpha	* Single injection 1/m between day 6-15	Majority are in estrus with 3 days
	Progesterogen plus PMSG	* Two injections 1/m 11 days apart * Progesterogen implant for 12 days * One dose of PMSG at withdrawal of implant	Animals are in estrus within 2-3 days
Swine	Progesterogen	* Progesterogen for 14 days	Animals are in estrus within 4-7 days
	PGF ₂ alpha	* PGF₂ alpha during day 11-18	Majority of animals are in heat within 3-4 days
Mare	Progesterogen	Progesterogen for 15 days	Majority in heat with 4-7 days

Use prostoglandin F₂ alpha

- * Prostaglandin F₂ alpha has been identified as a potent luteolytic agent and has successfully been used for luteolysis.
- * Endocrine changes following luteolysis has been found similar to those occurring at natural estrus.
- * Degree of synchronization of estrus is very good most of the animals do come into estrus within 72-84 hours.
- * Fertility at synchronized estrus is better.

Remarks

PGF₂ alpha works in presence of a responsive corpus luteum (between day 6-15 of the cycle with presence of a cyclic corpus luteum).

PROCEDURE AND RECORDING OF OBSERVATIONS

- * Examine the animal either per rectum or through ultrasonography for the presence of cyclic CL.
- * Inject normal luteolytic dose of PGF₂ alpha intramuscularly.
- * Subject the animals for detection of estrus.
- * Note down date and time of onset of estrus (most of animals comes into estrus with 3-4 days).
- * If facilities for ultrasonography and per rectal examination is not available and history of previous estrus is not known two injection schedule of PGF₂ alpha is followed. Give 2 injections of PGF₂ alpha at 11 days apart. Majority of animals will be in heat within 3-4 days of 2nd injection.
- * Apply norgestomet ear implant for 9 days and give small dose of PMSG at withdrawal of implant.
- * Observe the animals for onset of estrus (majority of animal show estrus with 2-3 days).

Use of PGF₂ alpha I. Single injection regime

Animal Nos.

Total animal treated

Date of last estrus = day 0

Date of examination (between day 6-15) for presence of CL

Date of injection of PGF₂ alpha

Date of onset of heat

Total animals responded.

Onset of estrus interval

TWO INJECTION SCHEDULE OF PGF₂ ALPHA

Animal No.

Total no. of animal treated

Date of 1st injection of PGF₂ alpha (day 1)

Date of 2nd injection of PGF₂ alpha (day 11)

Date of onset of estrus

Onset of estrus interval

Total animals responded

USE OF PROGESTOGEN**I. Progestogen and OB/PMSG**

Animals treated

Dates of progesterone injection for 5-7 days.

Date of onset of estrus

Onset of estrus interval

Total animals responded

II. Progesterone implant

Animal treated

Date of application of implant (for 9 days)

Date of withdrawal of implant and injection of PMSG

Date of onset of estrus

Onset of estrus interval

Total animals responded.

REMARKS

Write down results of all the experiments on onset of estrus and degree of synchronization of estrus following use of above approaches.

Chapter - 32**SELECTION OF DONOR FOR SUPEROVULATION
AND EMBRYO COLLECTION****OBJECTIVE**

- * To study criteria for selection of donor.
- * To study reproductive soundness for suitability to a donor.

MATERIALS REQUIRED

Animals, arm sleeves, gun boots, towel, soap etc.

SUBJECT DESCRIPTION

The main purpose of selection of donor is to ensure genetic superiority and success of superovulation and embryo collection for embryo transfer programme.

Criteria for selection of donor

- * Superior individual performance.
- * Good productive performance
- * Regular cyclicity.
- * Ovaries must be free and without any adhesions.
- * Cervix must be easy and allows free passage to the catheter without any kink or abnormalities.
- * Fallopian tubes and uterus also must not have any abnormalities and infections.
- * No detectable genetic defects in the donor.
- * Comparatively younger donors are preferred.
- * Donors must have calved at least 60 days back.
- * Best time to initiate superovulatory treatment is 90-100 days post calving.
- * For repeated superovulation donors should be treated atleast 60 days after 1st superovulatory treatment.

PROCEDURE AND RECORDING OF OBSERVATIONS

Animals should be examined for general health conditions. She should not be suffering from any major disease and abnormalities of limbs, joints and muscles. She should be examined gynaecologically for the normal estrus and estrus cycle length. Ovaries should be examined on per rectum examination. It should be normal in size and shape and free from any adhesions. The uterus must not have any adhesion or endometritis/metritis etc. The cervix must be normal, without any kinkness otherwise it will be difficult to introduce catheter for flushing to this donor.

RECORDING OF OBSERVATIONS

- | | Donor No. | Date of examination |
|---|-----------|---------------------|
| * Age of donor | | |
| * Body weight | | |
| * Total number of calvings | | |
| * Date of last calvings | | |
| * In milk/dry | | |
| * Milk production | | |
| * Reproductive health of donor | | |
| * Nature of estrus | | |
| * Nature of cervical mucus at estrus | | |
| * Length of the oestrous cycle (days) | | |
| * Whether oestrus cycle length is normal or any aberrations | | |
| * Gynaecological status of | | |
| i) Ovary | | |
| ii) Uterus | | |
| iii) Cervix | | |

REMARKS

Comments on suitability of female as donor.

Chapter - 33**SUPEROVULATION PROTOCOL IN CATTLE,
BUFFALO, SHEEP AND GOAT****OBJECTIVES**

- * To study different methods of superovulation in farm animals.
- * To prepare a protocol for superovulation of cattle, buffalo, sheep and goat

MATERIALS REQUIRED

Donor animals, PMSG/FSH, PGF₂ alpha, syringe, needle, gum boots, arm sleeves etc.

SUBJECT DESCRIPTION

Superovulation is one of the critical step in embryo transfer programme. Third or more than 3 ovulations (≥ 3 CL) are considered as superovulatory response.

It ensures

- Supply of embryos from superior animals.
- Exploits use of female germ plasma of ovary.

Superovulation, biologically is divided into two phases.

- 1st phase - Development of follicles due to administration of FSH/eCG
- 2nd phase - Rupture of mature follicles under the influence of LH and release of ova.

Hormones used

- * Pregnant mare serum gonadotrophin or equine chorionic gonadotrophin (PMSG/eCG).
- * Follicle stimulating hormone (FSH).
- * Human menopausal gonadotrophin (hMG).

Pregnant mare serum gonadotrophin (PMSG or eCG)

- * A potent placental gonadotrophin.
- * Secreted by endometrial cups of pregnant mare uterus between day 40-140 of gestation.

- * It is a glycoprotein with alpha and beta subunits similar to FSH/LH but possessing higher contents of carbohydrates specially sialic acid.
- * Has longer biological half life 50-120 hrs.
- * Possesses both FSH and LH biological activities but FSH like action is being dominant.
- * Greater variation in ratio of FSH:LH from batch to batch. This forms one of the basis for variation in ovarian response following use of PMSG.
- * PMSG has been used as a single IM injection during mid luteal phase of the cycle followed by IM administration of normal luteolytic dose of PGF₂ alpha 48-72 hr after the injection of PMSG.
- * Ovulation, embryo recovery rate and number of transferable embryo per donor using PMSG has been found low.

Commercial preparations

Folligon (Intervet, Holland)
Trophover (Indian Immunological, India)

Follicle stimulating hormone (FSH)

- * An important gonadotrophin, secreted by delta basophilic cells of the anti-pituitary gland.
- * A glycoprotein having alpha and beta subunits, has lesser sialic acid contents than PMSG, this accounts for its shorter biological half life (2-4 hrs).
- * FSH stimulates growth and maturation of follicles by the initiation of granulosa cell mitosis, formation of antrum, follicular fluid and inducing granulosa cell sensitivity to LH by increasing LH receptors.
- * FSH rescues follicles to become atretic.
- * Follicle stimulating hormone is used in multiple doses, twice daily morning and evening for 3 or 4 days in constant or tapering dose schedule. Treatment is initiated during mid luteal phase of the cycle. Normal luteolytic dose of PGF₂ alpha is injected 48-72 hours following start of 1st injection of FSH.
- * Superovulation response and number transferable embryo recovery have been found good. In general superovulation response and number of transferable embryo recovery has been lower in buffalo than cattle.

Commercial preparations of follicle stimulating hormone

- * FSH-P : Schering Corporation, USA.
Burns Biotech, USA
- * Follitropin : Vetrepharm Inc., Canada
- * Superov : AUSA International Inc, USA
- * Ovagen : Immunochemical Products Ltd., New Zealand

Human mesopausal gonadotrophin (hMG)

- * Biological activity is similar to FSH.
- * Results revealed no substantial advantage of hMG over FSH-P.
- * hMG is costlier than FSH-P which limits its use.

Commercial preparations

- * Pergonal (hMG, Menotropins, Serone Laboratories Inc., Braintrres, MA 02184)

Commercial preparations of PGF₂ alpha

	Dose
Dinofertin (Dinoprost)	25 mg IM
Lutalyse (Tromethamine)	25 mg IM
Illicen (Tiaprost)	25 mg IM
Presolvin (Luprostiol)	15 mg IM
Estrumate (Clopriostanol)	500 µg IM
Carboprost	500 µg IM
	UpJohn Ltd. Sussex, England

SUPEROVULATION PROTOCOL**CATTLE****I. Superovulation treatment using follicle stimulating hormone (FSH-P)**

- * Selection of donor
 - Normal cyclic without any gross abnormality of genital tract and ovary.
 - Cervix should be free and without any kinkiness.
- * Date of estrus is considered as day 0.

Day 9

- Rectal palpation of donor to confirm presence of corpus luteum
- Donor must have a good corpus luteum prior to start of superovulation treatment.
- Prepare doses of follicle stimulating hormone (FSH-P, total dose 36 mg make 8 divided doses for 4 days treatment (4.5, 4.5, 4.5 & 4.5) each in separate syringe. Store it in deep freeze.
- Start the treatment (IM) from day 10 of the cycle.

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Superovulation protocol

	Morning	Evening	
Day 10	4.5 mg	4.5 mg	Thaw the respective dose
Day 11	4.5 mg	4.5 mg	
Day 12	4.5 mg	4.5 mg	
Day 13	4.5 mg	4.5 mg	Administer single luteolytic

± 25 mg PGF₂alpha
(luteal) IM dose of PGF₂alpha in the morning just after FSH-P injection.

Day 14

Subject the donors for detection of estrus

Day 15

Majority of donors will be in estrus today morning. This day = day 0 (superovulatory estrus) A.I., Use double dose of semen

A.I., use double dose of semen

semen

Day 1

A.I., use double dose of semen

semen

- * Day of estrus is considered as day 0.
- * Day 9 Palpation of donor for presence of good CL on any one of the ovary.
- * Day 10 Inject 2500 IU eCG I/M in the morning.
- * Day 13 Inject 25 mg lutealysate (PGF₂ alpha) I/M in the morning.
- * Day 14 Observe for the estrus symptoms
- * Day 15 Donor will be in estrus in the morning.
- = day 0 This is day = 0 (superovulatory estrus) Do A.I. in the afternoon use double dose of semen
- * Day 1 A.I. in the morning
- Day 2
- Day 6 Rectal palpation of donor's ovary for assessing superovulation response
Examine ovaries very carefully and handle the donor with utmost care.
- Day 7 Prepare flushing medium (D-PBS)
Flush the donors for collection of embryos.

Remarks

Embryo recovery and their quality are usually poor than with the use of FSH-P.

BUFFALO**I. Superovulation treatment using follicle stimulating hormone (FSH-P)**

- * Selection of donor
 - Normal cyclic
 - Without any gross abnormalities of genital tract and ovaries.
- * Date of estrus is considered as day 0.
- * Day 9.
 - Rectal palpation of donor to confirm presence of a corpus luteum on the surface of any one of the ovary (presence of a good CL is mandatory for initiation of superovulation treatment).
 - Preparation of doses of follicle stimulating hormone (FSH-P, total dose 40 mg, make 8 injections of either of 5 mg as a constant dose 5/5, 5/5, 5/5, 5/5 for four days or 7/7, 6/6, 4/4, 3/3 mg as tapering dose for 4 days.
 - Each dose is kept in separate syringe and store in deep freeze.
 - Start the treatment (I/V) from day 10 of the cycle.

- | | Morning | Evening | |
|----------|----------------------------------|--------------------------|--|
| * Day 10 | 5 mg | 5 mg | Thaw the respective dose kept in the separate syringe 5 mins. before giving the injection. |
| * Day 11 | 5 mg | 5 mg | |
| * Day 12 | 5 mg
+ PGF ₂ alpha | 5 mg
5 mg | Inject normal luteolytic dose (25 mg or 500 µg) PGF ₂ alpha I/M in the morning after FSH-P injection |
| * Day 13 | 5 mg | 5 mg | |
| * Day 14 | Donors in estrus | A.I. | |
| = day 0 | confirm on per rectal palpation | Use double dose of semen | |
| Day 1 | A.I. | - | |
| Day 2 | | | |
| Day 6 | | | Palpation of ovaries for assessing the superovulation response
Non surgical flushing of donor using D-PBS for collection of embryo
Expected stage of embryo is morula. |

II. Superovulation treatment using eCG or PMSG

- * Selection of Donor
 - Normal cyclic
 - Without any gross abnormalities of genital tract, and ovary.
- * Day 9 Rectal palpation of donor to confirm presence of CL (good CL is mandatory for initiation of superovulation treatment).
- * Day 10 Inject 2500 IU PMSG/eCG I/M in the morning.
- * Day 11
- * Day 12 Inject 25 mg lutealysate (PGF₂ alpha) or 500 µg carboprost I/M.
- * Day 13 Observe the donor for onset of estrus.
- * Day 14 Donor will be in the estrus in the morning
 - * Do AI in the afternoon confirm by rectal palpation
 - * Use double dose of semen
- This is day 0
- * Day 1 A.I. in the morning
- * Day 2
- Day 6 Palpation of ovaries for assessing the superovulation response
Nonsurgical flushing of donor using D-PBS as flushing media.

Remarks

- * Ovulation rate, superovulation response, embryo recovery and their quality have been found low using PMSG (eCG) than FSH-P.
- * Overall response of superovulation and recovery of transferable embryo per donor have been low in buffalo than cattle.

SUPEROVULATION PROTOCOL FOR SHEEP AND GOAT**1. Superovulation using Follicle stimulating hormone**

- * Selection of donor
- * Day of estrus is considered day '0'.
- * Day 9 Prepare doses of follicles stimulating hormone (super OV, total dose 20 IU). Prepare six injections of 4/4, 3/3 and 3/3 I.U. in separate syringes and keep it in the deep freeze. Start treatment on day 10.

	Morning	Evening	
* Day 10	4 IU	4 IU	Thaw the respective dose 5 mins before the injection.
* Day 11	3 IU	3 IU	
* Day 12	3 IU	3 IU	Inject 7.5 mg Luprasitol (PGF ₂ alpha) IM after giving 3 IU super OV in the morning (prolvin)
* Day 13			Observe for symptoms of estrus
* Day 14 = day 0	Donor will be in Mating	Inject 500 IU hCG (chorulon) in standing heat	hCG 500 IU IM at mating.
	This is day '0'	500 IU or GnRH 50 µg	
* Day 1	Mating		
* Day 2			
* Day 3			Surgical collection of embryo from fallopian tube.
			Expected stages of embryo 4, 8, 16 cell.
* Day 5			Surgical collection of embryo from uterus.
			Expected stages of embryo morula and compact morula.

Procedure

Select the donor and observe her cyclicity at least for two cycles. She should be a normal cyclic animal. In case of cattle and buffalo examine the ovaries, uterus and cervix on per rectal examination. There should not be any gross clinical

abnormalities of genital tract and ovary. Keep all the donor on regular heat check up by putting a teaser and visual observations of signs of estrus. Day of estrus is day 0 and observe for normal appearance of cervical mucus discharge. Examine by rectal palpation on day 9 for presence of a good cyclic CL as it is mandatory for initiation of the superovulation treatment. Prepare doses of FSH and keep them in deep freeze. Start treatment on day 10 as per the chosen protocol for superovulation. Thaw the respective dose 5 minutes earlier for injection to the respective animals. Give PGF₂ alpha as per the protocol. Following the treatment with PGF₂ alpha observe the animals for onset of estrus. Confirm the estrus and breed the donor as per the protocol. Arrange for collection of embryo on day 7 in cattle, day 6 in buffalo, day 3 in sheep and goat for collection of embryo for fallopian tube and day 5 from the uterus.

RECORDING OF OBSERVATIONS

1. Donor No.
2. Date of birth
3. Total calving
4. Date of last calving
5. Date of estrus
6. Date of CL examination
7. Hormone used and dose for superovulation.
8. Date of FSH/PMSG injection(s)
9. Date of PG injection
10. Date and time of onset of superovulatory estrus
11. Interval between PGF₂ alpha injection and onset of heat
12. Assessing ovarian response
13. Ovarian response
14. Date of flushing
15. Donors behaviour

REMARKS

Write down ovarian response following superovulation treatment

Chapter - 34**BREEDING OF DONOR****OBJECTIVES**

- * To study nature and onset interval of superovulatory estrus.
- * To breed the donors at appropriate time using suitable dose of semen.

MATERIALS REQUIRED

Animals, AI gun, frozen semen, straw, thermometer, microscope, slide, gum boot, apron arm sleeves.

DESCRIPTION OF SUBJECT

Breeding of donor forms an important aspect of embryo transfer programme. Donors need to be detected in estrus following superovulatory treatment so that timely breeding using AI or natural service may be made for fertilization of oocytes released by the multiple follicles. Ovaries should not be touched during per rectal palpation either for confirmation of estrus or at AI. Endometrium of uterus is highly vascular during estrus. It should be handled with utmost care during AI. Otherwise uterine bleeding may occur and uterus become prone to infection. This would ultimately affect recovery and quality of embryo. External mucus discharge from vagina and vulva is indicative of uterine health. The discharge should be clear and stringy. In case of uterine infections if donors are bred then it would lead recovery of poor quality and degenerated embryos. Therefore, care should be taken for the selection of donor, to maintain aseptic conditions during AI and to use quality semen free from contamination for obtaining better results.

SUPEROVULATORY ESTRUS

- * Following administration of PGF₂ alpha, donors are subjected to detection of estrus twice during morning and evening using a teaser and visual observation of signs of estrus.
- * Majority of donor (80-90%) exhibit estrus 36-48 hours following administration of PGF₂ alpha.
- * Intensity of estrus and estrus response have been found greater than non superovulated control animals.
- * Mean interval from PGF₂-alpha injection to onset of estrus is shorter in superovulated donors than the normal recipient females.

Remarks

Recipients are given PGF₂ alpha 12-24 hrs earlier than donor for getting efficient synchronization of estrus between donor and recipients for embryo transfer work.

BREEDING OF DONOR

- * Confirm estrus on per rectal examination by observing relaxation of cervix, opening of external OS of cervix, rigidity and oestrus tone of uterus.
- * Donor are inseminated twice at 10-12 hrs interval.
- * High quality semen with double dose should be used for AI (twice dose of semen than normal for fertilizing more number of oocytes released by the rupture of multiple follicles induced due to gonadotrophin treatment).
- * Semen should be free from microbial contamination.
- * To perform AI / natural service under aseptic conditions.
- * Semen should be deposited in both the horns. Therefore, only skilled and trained personnel should be allowed to breed the valuable donor.

PROCEDURE

- * Observe the donors daily morning and evening for behavioural symptoms of estrus visually and on teaser parading.
- * Draft the donors in estrus on the basis of external symptoms.
- * Examine the donors on per rectum for confirmation of estrus by palpation of uterine tone, relaxation of cervix, opening of external OS cervix, nature of cervical mucus hanging out of vulva during massage of cervix.
- * Inseminate the donors as follows :
 - Donors observed in estrus in morning - inseminate in afternoon.
 - Donors observed in estrus in the afternoon - inseminate next day early morning.
- * If frozen semen is used, thaw at 37°C lukewarm water for 30°C whip it with tissue paper. Cut its one end and load it into AI gun.
- * Use two doses of semen at each time for AI of donors.
- * Prefer to deposit semen into each horn.
- * Use protective sheath over AI gun to avoid contamination to the uterus.

RECORDING OF OBSERVATIONS

Donor No.

Date and time of onset of estrus

Time taken for onset of estrus

Interval between PGF₂ alpha injection

to onset of heat)	
Nature of estrus	Excellent/good/poor
Per rectal finding	
- Uterus	
- Cervix	
- Vagina and vulva	
Date and time of AI	
Type of semen used	Fresh/frozen
No. of doses used	
and bull No.	
REMARKS	

Write down your remarks on nature of estrus and AI of the donor.

Chapter - 35

NON SURGICAL COLLECTION OF EMBRYO IN CATTLE AND BUFFALO

OBJECTIVES

- * To perform uterine flushing and collection of the embryo by non surgical method.
- * Post flush management of donor.

MATERIALS REQUIRED

- * Flushing catheter - Foley's or Rush catheter (18 or 22").
- * Cervical dilator, stylet, Y connector, Inflow and outflow tubes, embryo concentrator (0.45 μ), measuring cylinder, catheter tip syringe, local anaesthetics (xylocain), antibiotic for post flushing intrauterine administration, Air syringe (30 cc), flushing media, holding media, syringe filter (0.22 μ), integral petri dish, tissue culture dish, sterilized gauze, alcohol (70%), hemostates, hypodermic needle (18 G), donor, cattle crate, gum boot, coverall, soap, bucket, water, towel.

DESCRIPTION OF SUBJECT

Embryo in cattle and buffalo can be collected by surgical and non surgical methods. Surgical method is more complex, full of post operation complications and invariably lead to ovariohysterectomy and uterine adhesions. For these reasons, it is not practised for routine collection of embryo in cattle and buffalo. The non surgical method of embryo recovery is preferred in cattle and buffaloes.

SALIENT FEATURES OF NON SURGICAL EMBRYO COLLECTION METHOD

- * The method is comparatively easy.
- * Allows repeated collection of embryo without any damage to the reproductive tract.
- * Avoids surgery and post surgical care and complications.

PROCEDURE

- * Secure the donor in specially designed cattle chute to avoid undue movement during flushing of uterus.
- * Administer epidural anaesthesia (2% xylocaine or procaine hydrochloride: 5-7 ml). Hold the tail and by upward and downward movement of tail locate the junction of last sacro and first coccygeal vertebrae. Insert the 18 G needle inbetween sacro coccygeal junction and inject 5-7 ml anaesthetic solution to desired levels of anaesthesia.

- * Wash the perineal region and vulva with the water and mop it to make it to dry using cotton cloth.
- * Check the balloon of Foley's catheter by inflating it.
- * Rinse the Foley's catheter, syringes, flushing tubes, "Y" connector and embryo concentrator using flushing media.
- * Stiffen the Foley's catheter with a metallic stylet.
- * Introduce the stiffened Foley's catheter into the one side of uterine horn through vagina and cervix.
- * Fix the Foley's catheter 1-2" above inter cornual junction by inflating the balloon using air with the help of 15 cc air syringe.
- * Take out the stylet and introduce 30 ml flushing media into the uterine horn through the Foley's catheter using a catheter tip syringe.
- * Connect the flushing tubes to the Foley's catheter with the help of "Y" connector.
- * The flushing medium (D-PBS) is kept in 500 ml bottle hanging on IV drip stand just above the height of the animal. The inflow tube is connected to the flushing media container bottle. The outflow tube is connected to embryo concentrator.
- * The flushing of uterine horn is done by the gravitation method. The uterine horn is allowed to be filled with flushing medium of its own capacity, thereafter, fluid is drained out and collected through out let into the embryo concentrator (0.45 μ pore size). Care should be taken to recover approximately all the fluid before another filling.
- * The uterine horn is filled up and drained out 8-10 times using 500 ml flushing medium. After completion of the flushing of one horn, the catheter is deflated and removed.
- * Similar procedure is followed for the flushing of other horn.
- * Total 1.0-1.5 ltrs of medium is required for flushing of one donor.
- * After flushing of both the horns. The embryo concentrator is taken to the laboratory and contents are poured into the embryo searching dish.
- * The embryo concentrator is cleaned gently with 30-50 ml flushing medium using 20 cc syringe with 22 G needle.
- * Donor No., dish No. and date of flushing are marked on the top of the searching dish.

Post Flushing management of donor

- * Intra uterine antibiotic infusion.
 - To overcome uterine infection.
- * PGF₂ alpha injection
 - For early return to estrus.
 - Ensure repeated use of donor.

- To destroy left over embryos in order to avoid complication of multiple pregnancy.

RECORDING OF OBSERVATIONS

- Donor No.
- Date of flushing
- Ovarian response
- Epidural anaesthesia used
- Dose
 - Type of preparation
- Problem in fixing of catheter and in flushing, if any
- Quantity of flushing media required for each horn
- Post flushing managerial donor
 - Intra uterine administration of antibiotic
 - I/M PGF₂ alpha injection.

REMARKS

Write down your comments on donors behaviour during flushing, ovarian response and total media required for collection of embryo.

Chapter - 36**SURGICAL COLLECTION OF EMBRYO IN SHEEP AND GOAT****OBJECTIVES**

- * To collect embryo by surgical method.
- * To provide post flush management of donors.

MATERIALS REQUIRED

Donor, surgical operation room with the facility of operation table, overhead lamps etc. surgical cloth, clamps and other routine surgical equipments including suturing needle and thread, antiseptic, sequil, xylocain, needle, syringe, embryo collection disc and catheter, dettol, soap, surgical gloves etc.

DESCRIPTION OF SUBJECT

Embryos can be collected by surgical method from the oviducts or uterus. The recovery rate of embryos are better using surgical method of collection of embryo. This approach is the method of choice in sheep and goat, however, in larger animals, the non surgical method of collection of embryo from the uterus is preferred. Embryo/ova remain in the oviduct. 3-4 days after estrus and ovulation and then they migrate to the uterus. Therefore, if collection is aimed from oviduct operation is performed 72 hours following mating/AI. Uterine embryos can be collected by flushing of uterus on day 5 after the mating.

SALIENT FEATURE

- * Recovery rate of ova/embryo from the oviduct is higher than the uterus.
- * Embryos collected from uterus results higher pregnancy rate.
- * Embryos collected from uterus can be frozen more successfully.

APPROACHES

- * Oviductal embryo/ova can be flushed from the fimbriated end of oviduct by putting plastic catheter from the fimbriated end of oviduct and flushing fluid is introduced using a syringe and needle from the uterotubal junction. This approach is usually preferred.
- * Embryo from oviduct can also be collected in reverse way by introducing fluid from the fimbriated end of oviduct.
- * Uterine horns are flushed by introducing flushing medium from the uterotubal junction and collection of fluid from the base of uterine horn.

PROCEDURE**Preoperative preparations**

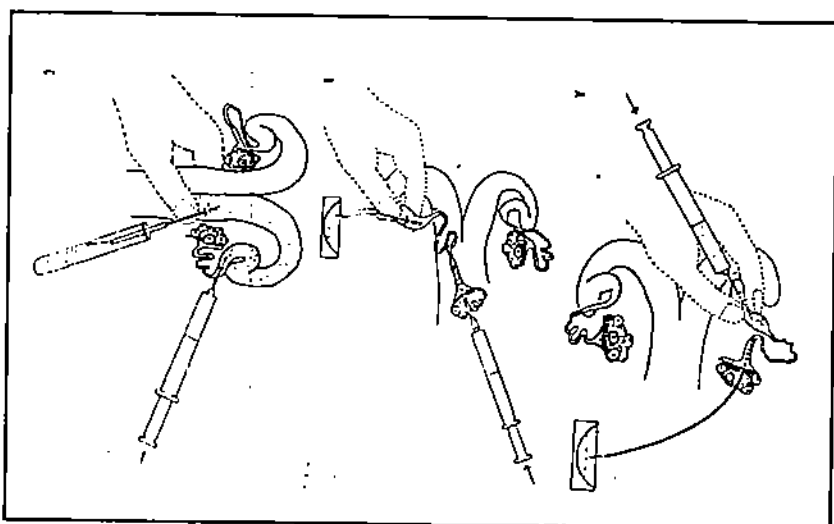
- * Withhold the feed for 24 hours before operation.
- * Shave the skin area cranial to the udder.
- * Clean it aseptically with savalene and 5% povidone iodine solution.
- * Administer triflupromazine hydrochloride (Siquil, Sarabhai, India) 40-50 mg intramuscularly (application of tranquilizer).
- * Keep the animal on the surgical table in a ventrodorsal position and restrain properly.
- * Clean the para-median laparotomy site using povidone iodine.
- * Inject local anaesthetic lignocaine HCl 2% 10-15 ml as a local infiltration at the site of incision i.e. between linea alba and mammary vein.
- * Apply sterile draps over the site of operation.

Operation procedure

- * Make a longitudinal incision of about 8-12 cm between udder and umbilicus.
- * Incise abdominal muscles and peritoneum and enter into peritoneal cavity.
- * Retract the internal genital organs by inserting the gloved hand to catch hold the uterus.

Flushing of fallopian tubes

- * Prior to flushing of fallopian tubes estimate the ovarian response by counting functional and non functional (avascular/white) corpora lutea and unovulated large follicles (≥ 5 mm) on the ovaries.



Surgical collection of embryo: (A) Flushing oviduct toward fimbria; (B) Flushing oviduct toward uterotubal junction; (C) Flushing uterus toward base of the uterine horn

- * Fimbria of left fallopian tube is spread to locate osium tubae abdominal. Insert a 10-15 cm long polythene catheter into the oviduct from fimbriated end to a distance of about 2-2.5 cm.
- * The catheter is kept in position by pressing with the help of finger and thumb. This would avoid also the leakage of flushing media and oviductal contents.
- * The other end of catheter is placed with embryo collection bowl or a small 50 cc beaker.
- * Take flushing medium (8-10 ml) in 20 ml glass syringe fitted with 20 G blunt needle.
- * Puncture the uterine horn of 3-4 cm away uterotubal junction. Confirm the position of needle in the uterine lumen. Press the caudal segment of uterine horn with the finger and thumb to prevent back flow of flushing fluid.
- * Push the flushing medium with moderate force towards the fallopian tube.
- * Collect the tubal flushing is a sterile beaker.
- * After the flushing is over, take out the catheter and flush the other side (right side) of the fallopian tube in the similar manner.

Post Flushing procedure

- * Reposition the genital organs after the flushing is over.
- * Irrigate 10-15 ml gentamycin over the reproductive organs to avoid infections.
- * Suture the peritoneum and abdominal muscles with absorbable cat gut (size No. 1) by interrupted horizontal mattress suture and superimpose with simple continuous suture. The skin layer is sutured with cotton thread by interrupted horizontal mattress suture.

Post Flush management of donor

- * Administer broad spectrum antibiotics, analgesics and anti-inflammatory drug daily for 7 days.
- * Suture wound is dressed using 5% Povidone iodine and gamma BHC ointment (Loraxane, ICI, Chennai, India).
- * Remove suture 7 days after surgical operation and observe for complete healing.
- * Inject normal luteolytic dose of PGF₂ alpha on day 7 post operation or on the day of suture removal.

SUPER OVULATION AND EMBRYO COLLECTION IN GOAT



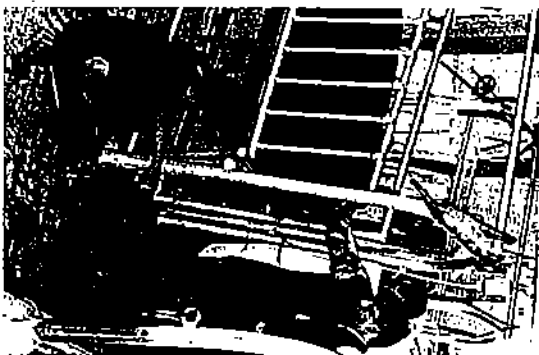
Unovulated large follicles with multiple CL

Ovary with multiple CL



Surgical collection of embryo

NON-SURGICAL COLLECTION OF EMBRYO



Cattle



Buffalo



Non-surgical embryo transfer in buffalo

Chapter - 37**PREPARATION OF FLUSHING AND
HOLDING MEDIA****OBJECTIVES**

- * To prepare flushing media Dulbeccos phosphate buffer saline for the flushing of donor.
- * To prepare holding media for short term storage of embryo after collection.

MATERIALS REQUIRED

Sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, calcium chloride, magnesium chloride, glucose, sodium pyruvate, foetal calf serum (FCS), bovine serum albumin (BSA), penicillin G sodium, pH meter, chemical balance, measuring cylinder, beaker, storage bottle, syringe, needle.

DESCRIPTION OF SUBJECT

Dulbecco's phosphate buffer saline has uninvested been used as flushing media for collection of embryo and short term storage till transfer, culture and freezing of embryo. The ingredients of DPBS is as follows :

Ingredients	Quantity (g/lit.)
Part A	
NaCl	8.0 g
KCL	0.2 g
Na ₂ HPO ₄	1.115 g
KH ₂ PO ₄	0.2 g
Part B	
CaCl ₂ .2H ₂ O	0.132 g
MgCL ₂ .6H ₂ O	0.100 g
Glucose	1.00 g
Na pyruvate	0.036 g
Streptomycin sulphate	0.05 g
Penicillin G sodium	1 lakh IU

PROCEDURE

- * Part A is dissolved in 600 ml triple glass distilled water.
- * Part B is dissolved in 200 ml triple distilled water separately.
- * Both the solutions are mixed together and final volume is made 1 litre (1000 ml)
- * Media is filtered through Seitz filter. (C₅₀₀ - 700)
- * BSA @ 0.1 - 0.2% or FCS 5% is added to the medium
- * pH should be 7.2 - 7.4 and osmolality 270-310 mOS

Holding media

Flushing media containing 4% BSA or 10-20% FCS is used as holding medium for short term storage of embryo.

Chapter - 38**SCREENING AND EVALUATION OF EMBRYO****OBJECTIVES**

- * To identify different stages of embryo.
- * To identify quality of embryo.

MATERIALS REQUIRED

Stereozoom microscope, embryo lifter, embryo searching disc, embryo culture disc, 0.22 μ syringe filter, beaker, tissue paper, absolute alcohol.

DESCRIPTION OF SUBJECT

Screening and evaluation of embryo is done under the stereozoom microscope. The embryo is located in the searching disc by examining the disc from one to another corner. Upon location of an embryo, it is lifted with the help of an embryo lifter and transferred into tissue culture disc containing 2 ml holding medium. The different stages and quality of embryo is determined morphologically.

STAGES OF EMBRYO

- | | |
|--|---|
| * Preclavage stage | * Presence of two polar bodies into the perivitelline space. |
| * 2 cell, 4 cell, 8 cell, 16 cells stage | * No indication of presence of blastomere cells. |
| * Early morula | * Embryo containing 2 to 16 blastomere cells with the zona pellucida. |
| * Compact morula | * Contains 16-32 blastomere cells. |
| * Early blastocyst | * Contains 32-64 blastomere cells. |
| * Blastocyst | * Compact arrangement of blastomere cells with undulating contours. |
| * Expanded blastocyst | * Embryo containing a small blastocoeal cavity. |
| | * Embryo with expanded blastocoeal cavity. |
| | * Expanded blastocoeal cavity and tightly stuck to zona pellucida. |
| | * The perivitelline space is very small. |
| | * Zona pellucida thin |
| | * The diameter of embryo increases 1/3 of its preliminary size. |

51-60 um - better pore size

Hatched blastocyst

- * Embryo is hatched out of zona pellucida.
- * It is spherical following hatching but afterwards it becomes elongated.
- * It is difficult to detect hatched blastocyst in the flushing fluid because most of the debris look like it. This creates problem to distinguish them.

Quality of embryo

The quality of embryo determines its fate of development either under *in vitro* culture or *in vivo* transfer to the recipients.

Parameters ascertaining the quality and stage of embryo

- * State of zona pellucida.
- * No. of visible blastomere cells.
- * Compactness of blastomere cells.
- * State of perivitelline space.
- * Size of blastocoele cavity.
- * Colour and appearance of cytoplasm.
- * Stage of development of embryo.

Morphological description of embryo

Excellent

- * Embryo perfectly symmetrical.
- * Even granulation and well defined distinct outline.
- * No blastomere extrusion.

Good

- * Embryo showed even granulation with well defined distinct outline.
- * Some blastomere extrusion.
- * Occasionally slight degeneration and asymmetric.

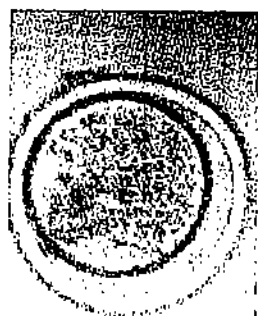
Fair

- * Embryo integral but with a hazy outline in parts.
- * Some blastomere extrusion and degeneration.
- * Asymmetric in shape and attachment of cell debris to the zona pellucida.

Poor

- * Embryo showing uneven granulation with a hazy outline.
- * Much blastomere extrusion and degeneration.
- * Sometime abnormally shaped.

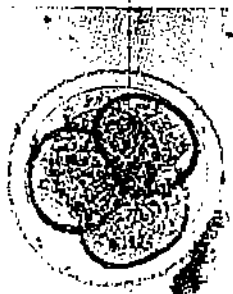
STAGES OF EMBRYOS AND THEIR QUALITY



Unfertilized egg



2-cell embryo- transferable



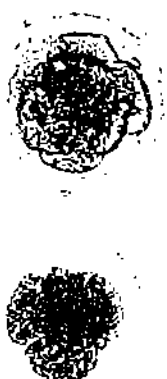
4-cell embryo-transferable



4-cell embryo with cumulus cells

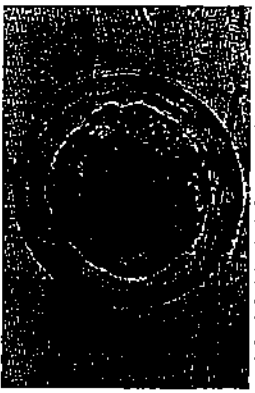


8-cell embryo-transferable



Degenerating embryos

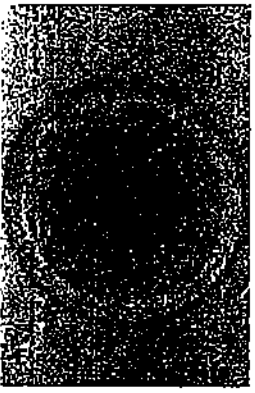
STAGES OF EMBRYOS AND THEIR QUALITY



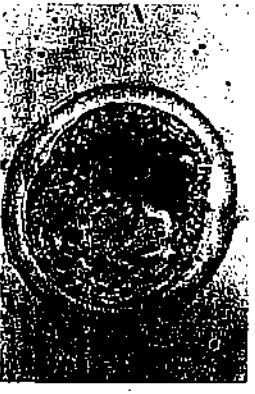
Morula-excellent



Morula-fair to good



Morula-good



Blastocyst-good



Morula-Degenerating



Degenerating embryos

Unfertilized

- * Granular appearance of cytoplasm
- * No indication of cleavage and formation of blastomere cells.

PROCEDURE

- * Keep the searching disk containing flushed fluid and examine under the stereozoom microscope for the presence of embryo.
- * Lift the embryo and transfer it to the holding media in the tissue culture disk.
- * Evaluate it for its quality and stage of development.

RECORDING OF OBSERVATIONS

- * Donor No.
- * Date of flushing
- * Date and time of screening and evaluation of embryo
- * No. of embryos recorded
- * Stage and quality of embryo
- * Draw their diagrams.

Chapter - 39**SELECTION OF RECIPIENTS AND NON-SURGICAL TRANSFER OF EMBRYO****OBJECTIVES**

- * To study criteria for selection of recipients for embryo transfer.
- * To perform non surgical transfer of embryo in cattle and buffalo.

MATERIALS REQUIRED

Embryo transfer gun, protecting sheath, stereo zoom microscope, minisraw (0.25 cc), holding medium, embryo litter, xylocain, syringe, needle, cotton, gauze, povidone, iodine, gum boots, cover all etc.

DESCRIPTION OF SUBJECT**Selection of recipients**

Following points should be kept in mind while selecting recipients for ET work.

- * Recipients should be healthy and free from infections and diseases.
- * She must be regular cyclic.
- * She must be free from any reproductive abnormalities of genital tract.
- * It is necessary to evaluate patency of cervix to allow free passage of ET gun. There should not be any kinkness or stenosis of cervix.
- * Recipients must have a good cyclic CL of desired stage at the time of embryo transfer.
- * Recipient can be selected from the natural cyclic animals in the herd. Select the recipients who are in estrus along with the donor or inject PGF₂ alpha 12-24 hours before giving PGF₂ alpha to the donors. Try to keep close synchrony of estrus between donor and recipient.

Transfer of embryo

Embryo in cattle and buffaloes can be transferred by non-surgical and surgical methods. However, in sheep and goat, the embryos are transferred by surgical method. The non-surgical method is easy and avoids surgical interventions but the results of conception is lower than surgical method.

- * Embryo is loaded in ET gun and gun is passed through cervix to deposit embryo in the uterine horn.

- * Embryo should be transferred to uterine horn ipsilateral to the ovary bearing corpus luteum.

PROCEDURE

- * Examine the recipient for the presence of corpus luteum and note down which side of ovary having CL.
- * Load the ET gun with straw containing embryo, cover it with sheath and then with the protecting sheath.
- * Pass the ET gun through cervix.
- * Recipient if strains epidural anaesthesia may be given using 2% procain hydrochloride.
- * The protecting or sanitary sheath is tearsd off while ET gun enter through cervix.
- * Embryo by pushing the plunger, is deposited deep into the uterine horn ipsilateral to the ovary bearing CL.
- * Embryo should be deposited quickly with out putting much strain on the animal and any trauma to endometrium.
- * Embryo transfer must be performed by the skilled and experienced person for getting optimum results.

RECORDING OF OBSERVATIONS

- | | |
|---|---------------------------|
| * Recipient No. | * Date of embryo transfer |
| * Date and time of last estrus | |
| * Presence of CL on right/left ovary | |
| * Quality of CL | |
| * Stage and quality of embryo transferred | |
| * Any other observation like | |
| - Site | |
| - Easiness of cervix etc. | |

Chapter - 40**SURGICAL TRANSFER OF EMBRYO****OBJECTIVE**

Transfer of embryo by surgical method in cattle, buffalo, sheep and goat.

MATERIALS REQUIRED

Embryo, stereozoom microscope, straw, langlevy pipette, embryo lifer, surgical operation table, room, surgical apparatus, suture, local anaesthetic, antibiotic, sequil, microscope, syringe, needle, blunt needle.

DESCRIPTION OF SUBJECT

Embryo can be transferred by surgical method in cattle, buffalo, sheep and goat. The conception results are always better with surgical transfer of embryo. The quality of CL and site of deposition of embryo can be judged by visual observation, facilitating better conception. In cattle and buffalo genital organs are exposed by flank surgery, however, in sheep and goat incision is made on the median to lines alba, ventral side of abdomen just before udder.

PROCEDURE

- * Withhold the feed for 24 hours before operation.
- * Shave the skin area cranial to udder in sheep and goat and on the left or right flank in cattle and buffalo.
- * Clean it aseptically with savalene and 5% povidone iodine.
- * Administer 1-2 ml sequil (Tri lypromazine HCl) to sheep and goat and 3-4 ml to cattle and buffalo as a tranquilizer.
- * In case of sheep and goat keep the animal in a ventro-dorsal position and restrain properly. In cattle and buffalo operation is performed in standing position by securing in cattle chute.
- * Inject local anesthetic 2% procain hydrochloride 10-15 as local infiltration in sheep and goat and 20-30 ml in cattle and buffalo.
- * Apply sterile draps over the site of operation.
- * Make a longitudinal incision of 8 cm between udder and umbilicus (paramedian laparotomy) in case of sheep and goat. In case of cattle and buffalo make a vertical incision on lower flank of that side, the ovary of which side contains corpus luteum.
- * Incise abdominal muscles and peritoneum and enter into peritoneal cavity.

- * Retract the genital organs, see quality of corpus luteum. Note the side of ovary containing CL. Embryo is deposited to horn ipsilateral to the ovary bearing CL.
- * Load the embryo in langlevy pipette under the stereozoom microscope.
- * Make a puncture of uterine horn at greater curvature to insert the tip of langlevy pipette filled with a syringe. By pushing the piston of syringe, embryo is deposited into the lumen of uterine horn. The langlevy pipette is then withdrawn.
- * Reposition the genital tract after transfer of embryo.
- * Suture the peritoneum and abdominal muscles with cat gut suture. Then suture skin with silk thread.
- * Remove sutures on day 7 post transfer.

RECORDING OF OBSERVATIONS

Recipient No.	Date of embryo transfer
Date of last heat	
Side of ovary bearing CL and its quality	
Quality and stage of embryo transferred	
No. of embryos transferred	

Chapter - 41**CRYOPRESERVATION OF EMBRYO****OBJECTIVE**

To freeze the embryo for long term storage.

MATERIALS REQUIRED

Embryo, glycerol, PBS, stereozoom microscope, petridishes, embryo lifter, freezing machine, LN_2 and its storage tank.

DESCRIPTION OF SUBJECT

The utilization of technique of embryo transfer on large scale depends upon development of methods for long term storage of embryos. This would help in

- * Preservation of valuable germ plasma for future use.
- * Conservation of endangered species.
- * Transportation of genetic material across the country.
- * Allows disease testing while embryos are held in quarantine.

PRINCIPLE

For successful long term preservation it is important to maintain the embryos in a metabolically inhibited state where not only cleavage is stopped, but other cellular functions also must be greatly reduced or completely inhibited. Embryo may be damaged during freezing either by formation of large intracellular ice crystals or by increased intracellular concentration of solutes resulting from dehydration of cells (solution effect).

- * Fast freezing minimizes damage from the solution effect but it leads formation of large intracellular ice crystals that causes severe mechanical damage to the embryo.
- * Slow freezing prevents large ice crystal formation but it leads damage from solution effect.
- * The optimum cooling rate would be that which is slow enough to prevent formation of intracellular ice crystals and is rapid enough to minimize exposure of cells to the higher concentration of solutes.
 - Cryoprotectant is added to the freezing medium before freezing of embryos and removed following thawing.
 - Glycerol has most commonly been used as cryoprotectant. It reduces ice crystal formation.

PROCEDURE**Protocol of freezing embryos**

- * Selection of excellent and good quality embryos (Morula or early blastocyst).
- * Three step washing in PBS and 20% FCS.
- * Equilibration in 1.0 and 1.36 M glycerol for 10 mins at room temperature.
- * Loading of embryo into 0.25 cc casson straw and sealing the end with PVC.
- * Transfer the loaded straw into the freezing machine.
- * Adjust the cooling rate from room temperature to $-6.0^{\circ}C$ (seeding temperatures) @ $4^{\circ}C/min$.
- * Perform seeding either manually or automatic at $-6.0^{\circ}C$.
- * Hold the embryo at $-6.0^{\circ}C$ for 5 mins.
- * Adjust cooling rate from $-6.0^{\circ}C$ to $-33^{\circ}C$ @ $0.3^{\circ}C/min$.
- * Plunge the straws in LN_2 ($-196^{\circ}C$).
- * Store into the LN_2 till use.

RECORDING OF OBSERVATIONS

- | | |
|---|------------------|
| * Donor No. its breed and species | Date of freezing |
| * Date of flushing | |
| * Cryoprotectant used and its concentration | |
| * No. of embryo frozen | |
| * Quality and stage of embryos | |
| * Straws identification. | |

Chapter – 42**THAWING OF FROZEN EMBRYO AND
REMOVAL OF CRYOPROTECTANT****OBJECTIVES**

- * To thaw the embryo.
- * Removal of cryoprotectant.

MATERIALS REQUIRED

Stereozoom microscope, micro pipette, tips, petri dishes, bucket, embryo lifter, sucrose, glycerol, DPBS.

DESCRIPTION OF SUBJECT

Embryos are thawed after taking out from liquid nitrogen container by direct dipping of frozen straws into water bath at 37°C for 30 seconds. The cryoprotectant is removed following thawing.

PROCEDURE OF REMOVAL OF CRYOPROTECTANT**I. Serial dilution of glycerol - five step method**

- * Take out embryo in the culture disc by cutting sealed end of straw.
- * Transfer embryo in 8% glycerol in holding media for 10 minutes.
- * Transfer EMB in 6% glycerol in holding media for 10 minutes.
- * Transfer EMB in 4% glycerol in holding media for 10 minutes.
- * Transfer EMB in 2% glycerol in holding media for 10 minutes.
- * Transfer EMB in holding media for 10 minutes.
- * Final evaluation of embryo (EMB).

II Three step method

- * Take out embryo in culture disc by cutting sealed end of straw.
- * Transfer EMB in 0.95 M glycerol + 0.33 M sucrose in holding medium for 5 minutes.
- * Transfer EMB in 0.47 M glycerol + 0.33 M sucrose in holding media for 5 minutes.

- * Transfer EMB in 0.33 M sucrose in holding media for 5 minutes.
- * Transfer EMB in holding media for washing for 10 minutes.

III. Single step method

- * Take out embryo in culture disc by cutting sealed end of straw.

- * Place EMB in 0.5 M sucrose in holding medium for 5-10 minutes.
- * Wash in holding medium.
- * Final evaluation of embryo.

In routine practice, five step method i.e. serial dilution method is preferred.

Preparation of cryoprotectant solutions for freezing and thawing of embryo

Three stock solutions are prepared :

Solution A :

1. Measure 54 ml PBS and add 6 ml of heat treated serum = 60 ml of solution of 10% serum in PBS (Solution A).

Solution B :

2. Measure 27 ml of PBS + 10% serum (solution A) and add 3 ml glycerol using an 18 gauge needle and 30 ml syringe = 30 ml of a 10% cryoprotectant solution (Solution B).

Solution C :

3. To prepare 1.0 M sucrose :
– Weigh out 34.2 g of sucrose and add PBS plus 10% serum until total volume = 100 ml. This is 34.2% (wt./vol.) solution (1.0 M). The solution requires at least 30 minutes to mix on stirring plate.
4. Pass solutions through a millipore filter to sterilize.

Volumes required for various cryoprotectant solutions (ml)

Solution desired	Solution A PBS + 10% serum	Solution B 10% glycerol	Solution C 1.0 M sucrose
8% glycerol	1.0	4.0	-
6% glycerol	2.0	3.0	-
5% glycerol	2.5	2.5	-
4% glycerol	3.0	2.0	-
2% glycerol	4.0	1.0	-
6% glycerol + 0.3 M sucrose	0.4	2.4	1.2
3% glycerol + 0.3 M sucrose	1.6	1.2	1.2
0.3 M sucrose	2.1	-	0.9

RECORDING OF OBSERVATIONS

Straw identification

Quality of stage of embryo at freezing

Date of thawing

Temperature

Time

Method of removal of cryoprotectant

Quality and stage of embryo after thawing

Chapter - 43

IN VITRO MATURATION AND FERTILIZATION OF OOCYTES AND CULTURE OF EMBRYO

OBJECTIVES

- * To study methods of collection of oocytes.
- * To study *in vitro* maturation of oocytes, *in vitro* fertilization of oocytes and *in vitro* culture of fertilized egg/embryo.

MATERIALS REQUIRED

Ovaries, stereozoom microscope, CO₂ incubator, laminar flow, micropipettes, embryo lifter, petri dish, scissor, forcep, liquid paraffin, DPBS, maturation, fertilization and culture media.

DESCRIPTION OF SUBJECT

In vitro maturation (IVM) and fertilization (IVF) are such biotechnological tools by which a substantial number of embryos can be produced in the laboratory. There exist greater demand for such embryos in the modern research. The technique is helpful for :

- * Conservation of germ plasma.
- * Sexing and cloning of embryo.
- * Production of transgenic animals.
- * Pharmaceutical farming.
- * Testing fertility of semen and screening of bulls.

Major steps involved

- * Oocyte collection.
- * *In vitro* maturation of oocyte.
- * *In vitro* fertilization.
- * *In vitro* embryo development.

Oocyte collection

Success of IVF in any species depends mainly on harvesting of good quality oocytes.

Approaches

- * By slaughter of donor.
- * By laparotomy.



Oocytes

- * Laparoscopy.
- * Intravaginal aspiration of oocytes using ultrasonography.
- * Morbid ovary.

Collection of oocytes from morbid ovary is an easy and cheaper method.

Methods of collection of oocyte from morbid ovary

- * Ovaries are obtained from local slaughter house.
- * Aspiration of oocytes from the surface follicles.
- * Slicing of ovary.
- * Isolation of individual follicles.

Aspiration and slicing of ovary are easy and quick methods and routinely used for oocyte recovery.

In vitro maturation of oocytes

- * Oocytes in the ovary are maintained as a reserve pool and from this pool a small number of follicles are recruited into the growing population every day.
- * Primary oocytes during prenatal life enter into meiosis and progress to the diplotene stage of prophase I (Germinal vesicle stage, GV) and remain arrested shortly before ovulation.
- * The arrested oocytes at ovulation under the influence of gonadotrophin hormones resume meiosis.
- * The oocyte undergoes germinal vesicle breakdown (GV-BD), extrude the 1st polar body and progress meiotically to the metaphase - II.
- * Such development from prophase I to metaphase II is referred as period of oocyte maturation.
- * The oocyte now get matured and become capable of fertilization.
- * The oocyte at metaphase II further gets arrested. This arrest is maintained until egg is fertilized by spermatozoa.
- * At this stage meiosis is completed by extrusion of second polar body.

In vitro capacitation of spermatozoa

Spermatozoa undergoes certain morphological and biochemical changes before taking part in the process of fertilization. These changes provide capacity to spermatozoa to fertilize the ovum. The process by which spermatozoa gets ability to fertilize the ovum is known as capacitation.

Capacitation occurs as a result of

- * Decreased negative surface charge.
- * Loss of membrane cholesterol.
- * An influx of calcium between the plasma and outer acrosome membrane.

Capacitation culminates in

- * Acrosome reaction leading to fusion of out acrosomal membrane with spermatozoa plasma membrane.
- * Release of acrosomal enzymes.

Capacitation normally occurs in female reproductive tract.

***In vitro* fertilization of matured oocytes**

In vitro matured oocytes are co-incubated with *in vitro* capacitated spermatozoa which results in fertilization and formation of zygote.

Success depends upon

- * Time of sperm - egg incubation.
- * Medium used.
- * Temperature.
- * No. of sperm co-incubation.

***In vitro* culture of fertilized egg/embryo**

In vitro fertilized eggs need to be cultured in a suitable medium for its further development to the desired stage of development (morula or blastocyst) so that they can be transferred into recipients or cryopreserved for further use.

PROCEDURE

1. Collection of ovary from slaughter house and transportation to the laboratory in normal saline (30-35°C) within 1-2 hours of slaughter.
2. Ovaries are washed thoroughly for 3-5 times in lukewarm saline.
3. Aspiration of oocytes from visible surface follicles (2-6 mm in dia.) using 180 needle and 10 ml syringe.
4. Aspirated fluid is centrifuged for 3 minutes at 500 rpm and pellet is transferred in a tube containing 10-20 ml Hepes-buffered tyrodes medium (TL-HEPES) and then poured into petridish for recovery of cumulus oocyte complexes (COCs) using stereozoom microscope.
5. Evaluation and selection of excellent to good quality oocytes for *in vitro* maturation.
6. COCs are washed twice in the oocyte maturation medium (Ham's F-10 supplemented with 10% fetal calf serum (FCS), 5 µg of H/ml, 0.5 µg FSH/ml and 1 µg/ml estradiol).
7. The COCs (10-12 oocytes) are placed in 50-100 µl droplets of the above maturation medium in 35 mm petridish and is covered with paraffin oil.
8. The COCs are cultured in CO₂ incubator (5% CO₂ in air) at 38-39°C for 24 hours and 95% relative humidity.
9. Assessment of oocyte maturation.

- A) Cultured COCs are denuded by repeated pipetting or vortexing for 2 minute in TL-HEPES in 1 ml microcentrifuge tube.
- B) The denuded oocyte is placed on a glass slide and fixed with methanol-acetic acid (3:1) stained with 2% giemsa or 1% acetocoin solution.
- C) Examination of oocyte under phase contrast microscope for evaluation of maturation (GVBD, MI to MII stages and presence of polar body).

***In vitro* sperm capacitation and fertilization**

1. Thawing of frozen semen.
2. Thawed semen is layered on top of percoll gradient and tubes are centrifuged at 800 g for 30 minutes.
3. Removal of top layer immediately after centrifugation with pasteur pipette.
4. Spermatozoa are washed with 10 ml sperm. TL supplemented with BSA 6 mg/ml. Tube is centrifuged at 400 g for 10 minutes. Supernatant is removed until 100 µl is left in the tube.
5. Matured oocyte are washed with TL-HEPES then final washing with tyrodes fertilization medium containing 6 mg/ml BSA (essential fatty acid free) and 0.25 mM pyruvate.
6. Matured oocytes (10-12 oocytes per drop) are then put in the 50 µl fertilization medium drop containing 10 µg/ml heparin in a 60 mm petridish. The oocytes are covered with mineral oil.
7. Infusion of 2 µl aliquot of sperm suspension @ 2 million spermatozoa/ml.
8. After insemination, oocyte and spermatozoa are co-incubated at 38-39°C (under 5% CO₂ in the air) for 20-24 hours.
9. At the end of oocyte spermatozoa co-incubation, oocytes are separated and washed in fertilized media.
10. Assessment of fertilization (The presence of two polar bodies within the perivitelline space, swollen sperm head in the ooplasm and presence of two pronuclei, etc.).

***In vitro* embryo development**

1. Probable zygotes are taken out from the fertilization drop.
2. Wash the zygote with fertilization medium followed by embryo development medium (TCM-199 + serum + epidermal growth factor).
3. Culture these zygotes alongwith oviducal epithelial cells in a suitable embryo development medium at 38-39°C under 5% CO₂ in air for 7-9 days.
4. Evaluation of embryos at 24 hour intervals for the embryonic development.

Chapter - 44

AQUINTANCE WITH LAPAROSCOPE AND ITS APPLICATION IN ANIMAL GYNAECOLOGY

OBJECTIVES

- * To acquaint with the different parts of laparoscope and its use.
- * Application of laparoscope as diagnostics and therapeutics in animal reproduction.

MATERIALS AND EQUIPMENTS REQUIRED

Laparoscope and its accessories, animal, animal crate, scissor, povidone iodine, antibiotic, procain hydrochloride, syringe, needle, gum boot, soap, towel.

SUBJECT DESCRIPTION

Laparoscope is an optical instrument developed on the principle of "seeing in believing". It is one of the endoscopic equipments to visualize the pelvic and abdominal organs. The endoscopic surgical technique performed in the abdominal cavity is termed as laparoscopy. Application of laparoscope in animal reproduction is a recent innovation.

APPLICATION IN ANIMAL REPRODUCTION

- * Inspection of genital structure.
- * Diagnosis of various abnormalities of genital organs such as cystic ovaries, ovarian tumor, endometritis, pyometra, uterine tumor, adhesions of fallopian tube and bursa.
- * Diagnosis of ectopic pregnancy.
- * Used for surgical interventions like ovariectomy, sterilization (laparoscopic assisted vaginal hysterectomy), cryptorchidectomy etc.
- * Used to take biopsy material from uterus and cervix.
- * To study ovarian follicular dynamics, ovulation and corpus luteum development.
- * Laparoscopic artificial insemination in sheep and goat.
- * Retrieval of oocytes for IVF experiments.
- * Retrieval and transfer of embryo.
- * Intra fallopian transfer of gametes (GIFT).

EQUIPMENT AND ACCESSORIES

- * **Telescope**
 - It is used to deliver light into the abdominal cavity. The telescope used for large animal is 10 mm in diameter and 60-90 cm long and for small animals 5 mm diameter and 29-30 cm long. The telescope/laparoscope provides a wide field of vision in the abdominal cavity.
- * **Fiber optic cable**
 - It is used to transmit high intensity light from a source of atleast 300 watts. It has two advantages.
 - # To get high intensity light for better illumination.
 - # Elimination of thermal injury.
- * **Trochar and Canula**
 - To make hole
 - Dimension is 10.0 mm in diameter and 23.5 cm in length.
- * **Video camera**
 - For projecting surgical field onto a video monitor.
- * **Electronic CO₂ gas insufflator.**
- * **Sterilization of equipment**
 - * Only wet sterilization is performed.
 - * Instruments are soaked in chlorhexidine for 15-20 minutes and rinsed with distilled water. The instruments are wiped and dried with sterile gauze immediately after washing.
- * **PROCEDURE**
 - Preparation of animal**
 - * Withholding of food for 24 hours and water for 12 hours.
 - * Restrain the animal in animal crush.
 - * Xylocaine may used as a chemical restrain, if required.
 - APPROACHES**
 - * There are mainly three approaches for laparoscopic examination. They are through :
 - Left paralumber fossa
 - Right paralumber fossa
 - Mid ventral approach
 - * Right paralumber fossa in large animals and mid ventral lower abdomen in small

animals are the preferred site for general diagnostic observations of genital organs.

- * The above site is prepared by clipping and shaving of hairs. Make the site aseptic using Povidone iodine and savalon.
- * Infiltration of local anaesthetic at the site of operation.
- * Make a small incision and trochar cannula assembly is introduced at a slight caudal angle into the abdominal cavity. The trochar is then withdrawn.
- * The telescope is inserted and pointed towards different directions to visualize the visceral organs.
- * Accessory probe is inserted through second incision to help in manipulating different structures while examination.
- * Gas insufflation is practiced for improving visualization. For insufflation CO₂ gas is passed in abdomen through a cannula until slight abdominal distension is evident.
- * The findings of laparoscopy can be recorded on video cassette using TV camera or still photographs using a special camera.
- * The telescope and probes are removed when laparoscopy is completed. Each incision is closed with a single interrupted suture in the skin.
- * Animal may be treated with antibiotics to overcome infections.

ADDITIONAL EQUIPMENTS

Depending upon the necessity the following instruments are used.

Palpation probe : Used to assess the consistency of the internal organs e.g. palpation of uterus for diagnosis of fibrosis, neoplasm etc. It is inserted through a separate cannula.

Biopsy punch forceps : Biopsy forceps are handled from outside for body cavity through a cannula to take biopsy as in case of uterine tumor or ovarian biopsy for hypoplasia etc.

Rotation type scissors : Scissors with the facility of rotating the operating edges from outside are used in surgical laparoscopy to cut a organ/tissue.

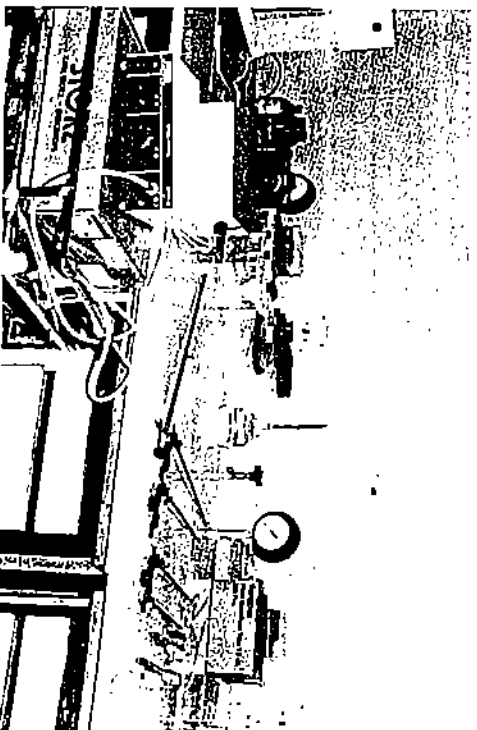
Clip applicator : Used to occlude the lumen of a small hollow organ or tube e.g. oviduct, specialized clips are applied through this applicator.

Irrigator pump : It is used to clean the surgical area after surgical intervention or to suck the excess fluid in the surgical area.

Unipolar/bipolar coagulator : To arrest the bleeding of surgical site, this is used. It can be attached to the operating instrument itself.

Endovision with colour monitor : This is attached to the telescope at one end and to monitor at other end to visualize the internal organs better.

LAPAROSCOPY EQUIPMENTS

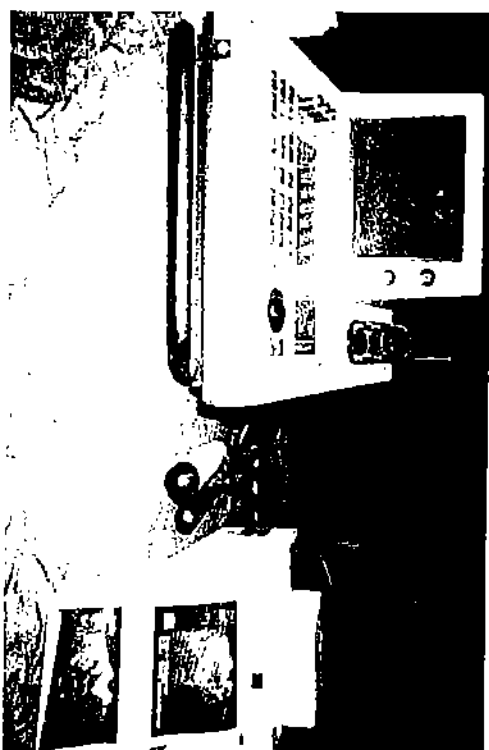


Laparoscope, fiberoptic cable, light projector, forceps, trochar and cannula



Laparoscope

ULTRASOUND MACHINE



Monitor, transducer & thermoprinter

INDICATION FOR LAPROSCOPY**1. Observation of physiological reproductive events**

- a) Ovulation.
- b) Number of corpus luteum.
- c) Follicular dynamics.
- d) Pregnancy diagnosis.

In sheep and goat, the mid-ventral site is preferred over other sites for the incision to introduce laproscope.

2. Diagnosis of reproductive abnormalities.

- a) Abscess, adhesion and tumors
- b) Cystic ovary
- c) Hydrosalpinx
- d) Inflamed uterus.

3. Surgical laprotomy

- a) Oocyte, follicular fluid aspiration
- b) Biopsy of reproductive organs to confirm disease condition
- c) Cryptorchidectomy
- d) Ovariectomy
- e) Sterilization

4. Laparoscopy in artificial insemination and embryo transfer technology**CONTRAINDICATIONS**

1. Diaphragmatic rupture.
2. Peritonitis.
3. Intrabdominal adhesions.
4. Advanced cardio-pulmonary decompensation.
5. Coagulopathies.
6. Obstructive bowel disease.

COMPLICATIONS

1. Puncture of hollow organs.
2. Subcutaneous emphysema and pneumothorax.
3. CO₂ may cause peritonitis/initiation and post operative pain.
4. Surgical field is two dimensional.
5. Thermal injury from laproscope light source.
6. GAS embolism and hemorrhage.

Chapter - 45

ACQUAINTANCE WITH ULTRASONOGRAPHY AND ITS APPLICATION IN ANIMAL REPRODUCTION

OBJECTIVE

To study use of ultrasonics as a diagnostic aid in various gynecological problems.

MATERIALS REQUIRED

Ultrasonic machine, jelly, sedative and animal/cattle crate.

PRINCIPLE

Ultrasonic waves are inaudible to the human ear and operate at frequencies of 1 to 10 mega hertz (MHz). Two types of ultrasound are employed in veterinary science.

1. Doppler phenomenon
2. Pulse echo principle

DESCRIPTION OF SUBJECT

Doppler phenomenon

- * Sound waves striking a moving object are reflected to the transmitting source at a slightly altered frequency.
- * The ultrasonic fetal pulse detector that is based on the Doppler phenomenon consist of a transducer and amplifier.
- * The transducer (probe) when applied to the animal's abdominal wall or inserted into the rectum, emits a narrow beam of high-frequency waves (ultrasonics). Movements of the fetal heart blood flow in fetal (umbilical vessels) or maternal (uterine artery) circulation alter the frequency of these waves, which are reflected back to the probe, where they are converted into audible sound and amplified (earphones or speaker) or illuminated (oscilloscope).

Pulse-echo ultrasound

- * Pulses of ultrasound, generated by piezo electric crystals in a transducer, on contacting tissues of varying acoustic impedance (resistance to the transmission, are reflected to the transducer, then converted into electrical energy and displayed on a cathod-ray oscilloscope in various ways.

- * A mode and B-mode are the basic forms currently in use.
- * A mode (amplitude) is a one dimensional display of echo-amplitude verses distance.
- * B mode (brightness) produces an accurate two dimensional image of soft tissue cross section. The brightness of the dots on the oscilloscope is projected in various shades of grey. Comparable to a black and white succession, they will reveal any motion in the tissue being imaged.

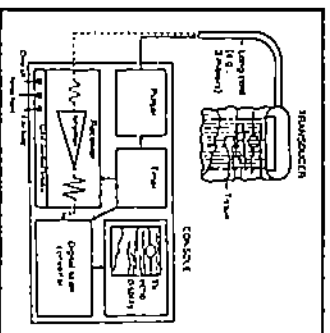
This is the basis of B-made "real-time" scanning.

B-MODE REAL TIME ULTRASOUND

The major components of an ultrasound machine are :

1. An electrical pulse generator.
2. A transducer.
3. A scan converter and
4. A video display

- * A high voltage electrical pulse of a few microseconds causes the piezoelectric transducer to vibrate and convert electrical to mechanical energy (ultrasound). The reflection of these waves (echos) from tissue surfaces on reaching the transducer, produces an electrical signal that is processed by a scan converter and displayed on a video monitor.



Basic components of ultrasonic machine: a pulse generator, transducer, a scan converter and a video display.

- * Depending on the display patterns they produce, the transducers are of two types.

A linear transducer produces a rectangular image whereas the sector

transducer produce a sector image. Similar to that of a "Slice of a pie". The linear array type transducers are commonly used and have a frequency range of 3.5 to 7.5 MHz. Transducers with low frequencies (3 to 3.5 MHz) penetrate deeper and image deeper tissues than higher frequencies (5.0 to 7.5 MHz) that image closer to the

surface. The image produced by low frequency and high frequency transducers are comparable to the view of a tissue as seen under the microscope at low and high magnifications.

- * For scanning the uterus, the transducer is placed on the ventrolateral abdominal wall (transabdominal) or in the rectum (transrectal) of the experimental animal.
- * Contact between transducer and the skin or the rectal wall is made with coupling gel or vegetable oil.
- * The ultrasound techniques, placement of transducer and the earliest day after mating, the diagnostic criteria and accuracy may be seen in the table.
- * The 3.5 MHz transducer is used for trans-abdominal approach (goat, sheep and bitch) and the 5.0 to 7.5 MHz transducers for the transrectal route (horse, cattle and pig).
- * Images of tissues seen on the screen are either black (non-echogenic) or various shades of gray (echogenic).
- * The urinary bladder, embryonic vesicle and foetal fluids appear black.
- * The fetal skeleton appears white.
- * The fetal membranes and maternal tissues appears various shades of gray.

Ultrasound techniques of pregnancy diagnosis.

Species	Technique	Placement of transducer	Days after mating	Diagnosis/criteria
Horse	B-Mode RT	Transrectal	9-12	Embryonic vesicle
Cattle	B-Mode RT	Transrectal	40-60	Foetus
Buffalo	B-Mode RT	Transrectal	22-30	Embryonic vesicle, fetus and heart beat
Sheep & goat	Doppler A-mode RT	Transabdominal	22-30	Embryonic vesicle, fetus and heart beat
	Doppler A-mode RT	Transabdominal	60	Foetal heart sound
	Doppler A-mode RT	Transabdominal	45-50	Foetal fluid
	Doppler A-mode RT	Transabdominal	45-50	Foetal placental
	Doppler A-mode RT	Transabdominal	10-40	Foetal fluid
Pig	Doppler A-mode RT	Transabdominal	60	Foetal heart
	Doppler A-mode RT	Transabdominal	60	Foetal fluid
	Doppler A-mode RT	Transabdominal	25	Allantoic fluid
Bitch	B-Mode RT	Transabdominal	30	Foetal fluid

RT = Real time

Transducer

3.5 MHz (Transabdominal)

5.0 - 7.5 MHz (Transrectal)

OBSERVATIONS TO BE RECORDED

1. Exposure to the

- A) Doppler
- B) A-mode
- C) B-mode

2. Placement of transducer

- A) Trans abdominal
- B) Transrectal

3. Type of transducer

- A) 3.5 MHz (Trans abdominal)
- B) 5.0 - 7.5 MHz (Trans rectal)

4. Pregnancy diagnosis (Earliest days after mating)

- A) Mare
- B) Cattle
- C) Buffalo

D) Sheep and goat

E) Pig

F) Bitch

5. Diagnostic criteria in different species

- A) Mare
- B) Cattle
- C) Buffalo
- D) Sheep and goat
- E) Pig
- F) Bitch

6. Exposure of transrectal transducer for

1. Follicular dynamics
2. Corpus luteum shape and size
3. Reproductive abnormalities.

7. Interpretation of findings:

Interpret the sonographs and write down the comments.



Ultrasound image of ovary - medium and large follicles



Ultrasound image of ovary - follicle and corpus luteum (CL)

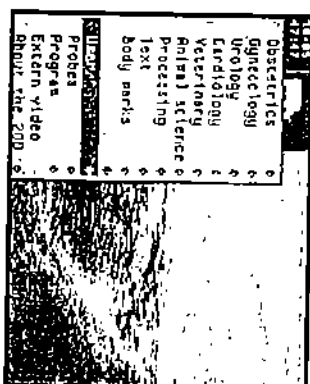
ULTRASONOGRAPH OF BOVINE UTRUS



Conceptus Heart- 35 days
pregnancy



Hydrometra/Mucometra



Fetus



Pyometra

APPENDIX

Reproductive organs and their functions

Organs	Function(s)
Ovary	1. Production of ova 2. Production of hormones i) Estrogen, (ii) Progesterone, (iii) Inhibin, (iv) Activin Relaxin
Oviduct	1. Picking up of ova following ovulation 2. Gametes and embryo transport 3. Provides optimal environment for union of gametes i.e. to facilitate fertilization. 4. Oviductal fluid provides suitable environment for early cleavage of fertilized eggs
Uterus	1. Spermatozoa transport to the site of fertilization 2. Nourishment of embryos 3. Implantation and retention of the fetus 4. Controls the life span of CL through PGF₂ alpha secretion 5. Helps in parturition 6. Post-partum uterine involution
Cervix	1. Acts as a barrier for the entry of foreign agents 2. Facilitates spermatozoa transport through cervical mucus 3. Acts as sperm reservoir 4. Selection of viable spermatozoa
Vagina	1. Organ of copulation 2. Site of semen deposition during natural service
Vulva	Provides protection to the vagina

Reproductive hormones

Source	Name	Chemical nature	Function(s)
Hypothalamus	1. Gonadotropin Releasing Hormone (GnRH)	Peptide	Stimulates FSH & LH release
	2. Prolactin Inhibitory Factor (PIF)	Peptide	Inhibits release of prolactin
	3. Corticotropin Releasing Hormone (CRH)	Peptide	Stimulates release of ACTH
	4. Growth Hormone Releasing Hormone (GH-RH)	Peptide	Stimulates release of growth hormone
	5. Growth Hormone-Inhibitory Hormone (Somatostatin)(GH-IH)	Peptide	Inhibits release of growth hormone
	6. Thyrotropin Releasing Hormone (TRH)	Peptide	Stimulates release of TSH and prolactin
Anterior pituitary	1. Follicle Stimulating Hormone (FSH)	Glycoprotein	- Stimulates follicular growth - Spermatogenesis - Secretion of estrogen
	2. Luteinizing Hormone (LH)	Glycoprotein	- Causes ovulation - Corpus luteum formation and function - Stimulates secretion of progesterone, estrogen and androgen - Promotes lactation - CL function - Release of glucocorticoids
Posterior pituitary	3. Prolactin (PRL)	Peptide	Promotes lactation
	4. Adrenocortico-trophin	Peptide	Release of glucocorticoids
	Oxytocin	Peptide	- Stimulates uterine contractions - Helps in sperm and egg transport - Helps in parturition
	Estrogen	Steroid	- Promotes sexual behaviour - Stimulates secondary sexual characteristics - Growth and maintenance of female reproductive tract - Stimulates uterine contractions - Promotes mammary duct growth - Initiates ovulatory surge of LH

	2. Progesterone	Steroid	- Maintenance of pregnancy - Feed back control of gonadotrophin release - Mammary gland growth - Controls the release of FSH - Dilatation of cervix - Relaxation of pelvis
	3. Inhibin	Glycoprotein	
	4. Relaxin	Peptide	
Testes	1. Androgen (testosterone)	Steroid	- Stimulates secondary sexual characteristics and behaviour - Development and maintenance of male duct system and accessory sex glands - Stimulates spermatogenesis - See ovary
Placenta	2. Inhibin	Glycoprotein	See ovary
	1. Estrogen	Steroid	See ovary
	2. Progesterone	Steroid	See ovary
	3. Relaxin	peptide	See ovary
Uterus	1. Prostaglandin F ₂ alpha (PGF ₂ alpha)	Lipid	- Regression of corpus luteum - Helps in parturition
	2. Relaxin	Peptide	See ovary
Adrenal cortex	Glucocorticoids(Cortisol)	Steroid	- Helps in parturition - Milk synthesis

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Symedix

Appendix

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Chandul Bhaur

Measures of reproductive efficiency

1. Age at first calving	Age in months
2. Days open (service period)	Calving to conception interval
3. First service conception rate (%)	$\frac{\text{Animals pregnant at 1st service}}{\text{Total animals bred at 1st service}} \times 100$
4. Overall conception rate (%)	$\frac{\text{Total animals pregnant}}{\text{Total animals bred}} \times 100$
5. Services per conception	$\frac{\text{No. of services in all animals}}{\text{Total conceptions}}$
6. Pregnancy rate (%)	$\frac{\text{No. of animals pregnant}}{\text{Total breedable animal}} \times 100$
7. Calving interval (days)	$\frac{\text{Days between successive calvings}}{\text{Total animal calved}}$
8. Calving percentage	$\frac{\text{No. of calves born}}{\text{Total breedable animals}} \times 100$

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