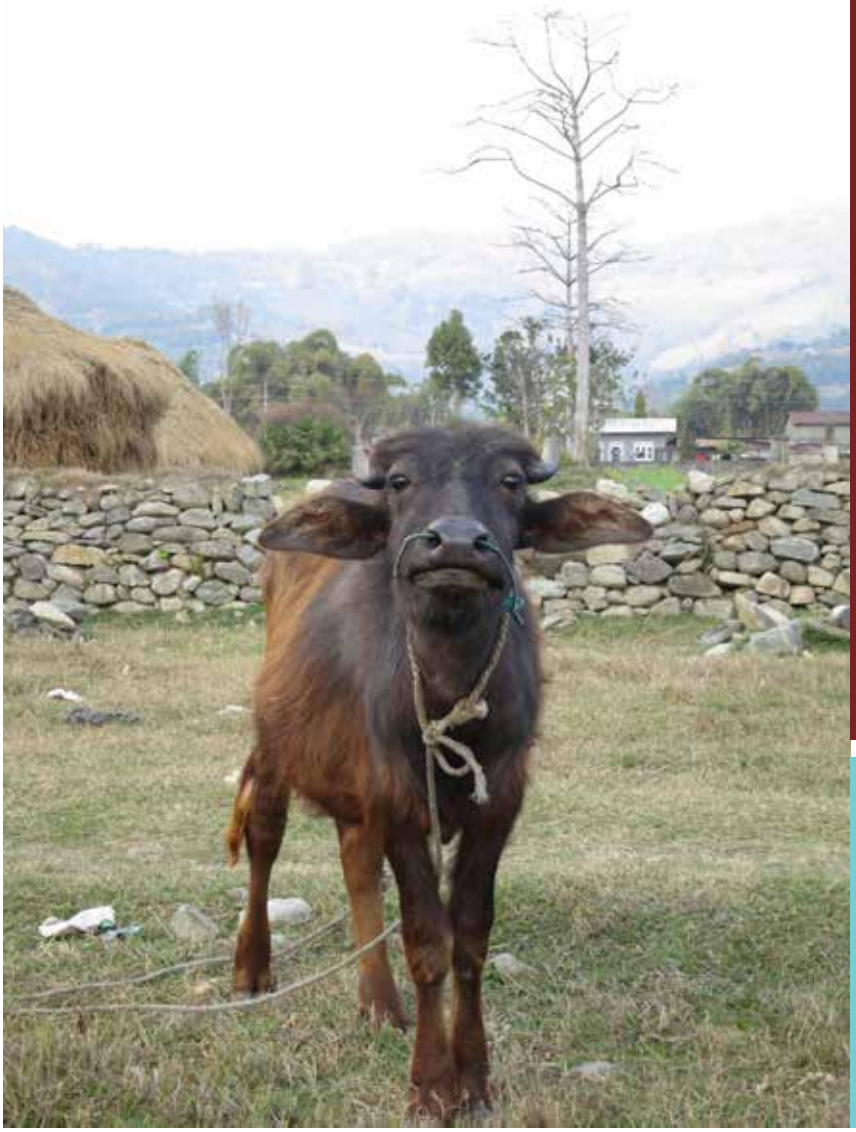

ILRI MANUAL

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INITIATIVE ON
Sustainable Animal Productivity

Manual for addressing major reproductive problems and mastitis in dairy animals in Nepal zones

Manual for addressing major reproductive problems and mastitis in dairy animals in Nepal

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Abbreviations

AI	Artificial Insemination
CCL	Cystic Corpus Luteum
CL	Corpus luteum
CMT	California Mastitis Test
COD	Cystic Ovarian Degeneration
CS	Caesarean Section
DCP	Digestible Crude Protein
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
GnRH	Gonadotrophin Releasing Hormone
HCG	Human Chorionic Gonadotrophin
LH	Lutenizing Hormone
MRT	Milk Ring Test
MoALD	Ministry of Agriculture and Livestock Development
NDDB	National Dairy Development Board
PCL	Persistent Corpus Luteum
PCR	Polymerase Chain Reaction
PG F2α	Prostaglandin F2α
RB	Repeat breeding
RBPT	Rose Bengal Plate Test
ROP	Retention of Placenta
SCC	Somatic Cell Count
SCM	Subclinical mastitis
TDN	Total Digestible Nutrient

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1. Background

A cross sectional study was conducted from May 2016 to January 2017 in Rubavu and Nyabihu districts, Western Rwanda, aiming at estimating the prevalence of subclinical mastitis (SCM) and identifying its causative bacteria. Managing practices and milking procedures were recorded through a questionnaire. One hundred twenty-three crossbreed milking cows from 13 dairy farms were randomly selected and screened for SCM using California Mastitis Test (CMT). Composite CMT positive milk samples were processed for bacterial isolation and identification.

The overall SCM prevalence at cow level was 50.4%. Sixty-eight bacterial isolates were identified by morphological and biochemical characteristics. They included, Coagulase Negative Staphylococci (51.5%), Staphylococcus aureus (20.6%), Streptococcus species (10.3%), Bacillus species (10.3%), Streptococcus agalactiae (5.8%) and Escherichia coli (1.5%). About 67.1% of the farmers checked for mastitis; of these, 58.9% relied on clinical signs and only 6.8% screened with CMT. Only 5.5% and 2.7% of the farmers tried to control mastitis using dry cow therapy and teat dips, respectively. Thus, to reduce the prevalence of SCM, farmers in the study area need to be trained on good milking practices, including regular use of teat dips, applying dry cow therapy and SCM screening. This will improve their sales and their financial status.

Dairying plays an important role in the livelihood and nutrition of Nepali people. In Nepal, dairy sector contributes about 9% to total GDP (NDDB, 2021) wherein buffalo production contributes 57.23% of the total milk and and 36.13% of the total meat (MoALD, 2020) As such, buffalo is considered as one of the most important livestock species in the country.

In Nepal, a considerable number of households are involved in dairy farming. Many of them consider buffalo farming as a main source of livelihood. In Nepal, 65% of the buffaloes are indigenous and rest 35% are cross breeds (Neopane, 2006). Based on their breed characteristics, buffaloes are broadly divided into three groups viz. Hill buffalo, Terai buffalo and Indian breeds (Rasali, 1997). Among the Hill buffaloes, Lime, Parkote and Gaddi are considered to the important breeds. Lime buffaloes are generally characterized by grey colour with chevron marks around the brisket and hugely found in northern hills whereas, Parkote buffaloes are predominantly dark in colour with no chevron marks and localized more towards the southern midhills (Rasali et al. 1998; Amatya et al. 2000). Gaddi buffaloes are found in the far Western Development Region (Pokhrel et al. 1998).

Terai buffaloes are largely considered as nondescript type. Apart from indigenous buffaloes, about 10% in the hills and little over 10% in the Terai are said to be of Indian Murrah breed or their crosses which have come to exist in the various pocket areas of the country as a result of crossbreeding program and occasional imports of buffaloes from India (Lamsal et al. 2020).

The productivity of buffaloes is lower than that of dairy cows. It is mainly due to lower reproductive efficiency. Delayed sexual maturity, postpartum anoestrus, silent oestrus and the seasonal breeding pattern are some of the reasons behind reduced reproductive efficiency of buffaloes. Among these, infertility, manifested by anoestrus, remains as a major problem in buffaloes. There is paucity of reports in Nepal pertaining to economic impact of anoestrus in buffaloes.

In India, Kumar 2013 reported an estimated loss of 372.90 Indian rupees (INR) per day from each buffalo due to anoestrus (USD 1.00 = INR 81.6694 at 16 January 2023). As the incidence of anoestrus has been reported high, the above figure shows great economic impact at the country level. Considering the total buffalo population in Nepal,

its milk production should have much higher than the existing one. This could not have achieved mainly because of higher incidences of various reproductive diseases/disorders and higher prevalence of mastitis.

Reproductive health of a dairy buffalo is a condition in which buffalo cow is physically sound enough to meet the normal parameters of reproduction such as attaining puberty in time, conceiving at the first attempt of natural service or artificial insemination (AI) (in the second heat), maintain full term of pregnancy with optimal weight gain of the calf, parturition without difficulty, shedding of placenta on time, coming into next heat within the specific time, maintaining normal inter calving period etc. Any deviation from this may be due to reproductive diseases/disorders such as anoestrus, repeat breeding (RB), abortion, retained placenta, vaginal discharge, dystocia etc. These invite serious attention of the owners as well as of the veterinarians. If not addressed properly in time, the reproductive disease/disorders will cause poor productive and reproductive performances, resulting in higher economic loss.

Mastitis is the most widespread and major disease of dairy cattle, causing huge loss to the dairy farmers. Mastitis not only reduces milk yield but also has a negative effect on milk composition. Because of mastitis, the cost of milk production and spoilage increases. Moreover, mastitis has a serious threat of zoonosis associated with shedding of bacteria and their toxins in the milk. Mastitis requires proper management at both clinical and subclinical stages for its control and prevention.

To bring these two harmful elements under control for augmenting milk production, a treatment and management protocol is the urgent need of the hour so that these two issues can be addressed uniformly across the nation.

Frequently encountered reproductive problems in dairy animals in Nepal

A fairly good number of reproductive problems/disorders of the female reproductive organs are commonly observed in dairy animals which are responsible for significant loss to the dairy farmers. These reproductive problems/disorders include:

a. **Anoestrus**

- Silent heat
- Delayed onset of puberty
- Delayed postpartum oestrus

b. **Repeat breeding (RB)**

- Delayed ovulation
- Anovulatory heat
- Endometritis

c. **Prolapse** (vaginal or uterine)

d. **Abortion**

e. **Stillbirth**

f. **Retained placenta**

g. **Vaginal discharge**

h. **Dystocia**

Some important terminologies used in the manual

In this protocol, some important terminologies have been used quite commonly which needs some clarity at this stage. The terminologies have been stated below:

- **Mastitis:** It is inflaming the mammary gland (udder and teat).
- **Uterine involution:** Restoring the uterus to its normal nonpregnant size and function after parturition is termed as uterine involution. Involution process depends on the rate of myometrial contractions, eliminating bacterial infection and the histological regenerating the endometrium.
- **Puerperium:** Puerperium is the postnatal period beginning from birth of a calf up to six weeks of age.
- **Metritis:** It is inflaming the uterus.
- **Endometritis:** It is inflaming the endometrium.
- **Cervicitis:** It is inflaming the cervix.
- **Abortion:** Expulsion or loss of a dead or live recognizable size of foetus at any stage of gestation from the age of 45 days up to parturition from the uterus before full term of gestation is called abortion.
- **Early embryonic death:** Pregnancy lost before 42 days of pregnancy is considered as early embryonic death.
- **Stillbirth:** Expulsion of dead foetus between 260 days and parturition is called stillbirth.
- **Mycotoxins:** Toxins produced by certain species of fungus.
- **Metabolic diseases:** Metabolic disease refers to a group of conditions caused by certain essential nutrients resulting in disturbance of the animals' normal metabolic processes.
- **Pyometra:** Forming and accumulating pus in the uterus. This inhibits secreting Prostaglandin F2α (PG F2α), which block regression of the corpus luteum (CL) and prevent oestrus. There are generally no accompanying clinical signs other than anoestrus.
- **Mucometra:** It is accumulating thin or viscid fluid in the uterus. It results from cystic ovaries leading to cessation of heat.
- **Corpus luteum (CL):** A corpus luteum is a mass of cells that forms in an ovary and is responsible for producing the hormone progesterone during early pregnancy.

2. Reproductive problems/disorders

2.1. Anoestrus

Anoestrus may be defined as the failure of the heifer/buffalo cow to come to oestrus/heat within the normal range of time. It is of two types:

- delayed onset of puberty (in heifers) and
- delayed postpartum oestrus.

2.1.1. Delayed onset of puberty

- A cattle/buffalo heifer maintained under a proper nutritional plan, ideally attains puberty within 21–24 months of age which may go up to 36 months in case of indigenous heifers. When the period is further extended, it may be considered as delayed onset of puberty.

2.1.1.1. Possible causes of delayed onset of puberty

- Poor nutrition/undernutrition during the growth period
- Parasitic infestation
- Anatomical defects of the genital tract
- Improper ovarian function
- Hormonal imbalance
- Persistent hymen/white heifer disease
- Genetic effects

2.1.1.2. Approach to disease investigation

Step I: Taking animal history (in the record sheet given at Annex I)

- Age of the animal
- Breed of the animal
- System of rearing (intensive/semi-intensive)
- Deworming done in last one year
- Feeds and feeding regime (concentrate, green and dry fodder with quantity andfrequency)
- Supplementing nutrition (i.e. minerals, vitamins, probiotics etc.)
- Providing exercise if any

- Proximity of bull with the heifer
- Experiencing detecting heat symptom by the farmer.

Step II: Clinical observationexternal observation

- Colour of visible mucous membranes—pale mucous membranes indicate anaemia.
- Body coat (rough or smooth)—rough body coat indicates malnutrition, parasitic infestation etc.
- Growth in accordance with age (Figure 1).

Figure 1. Good growth of calves in accordance with age.

Figure 2. A well nourished heifer with proper growth.

Figure 3 Handling the reproductive organs of a large animal

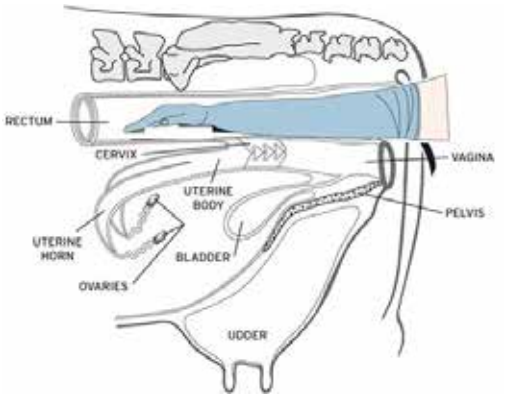


Photo courtesy: Uterine infection guide with ultrasound by John Dawson, BVMA, MRCVS, Cert CHP.

Figure 4. Various stages of follicular development during oestrus.

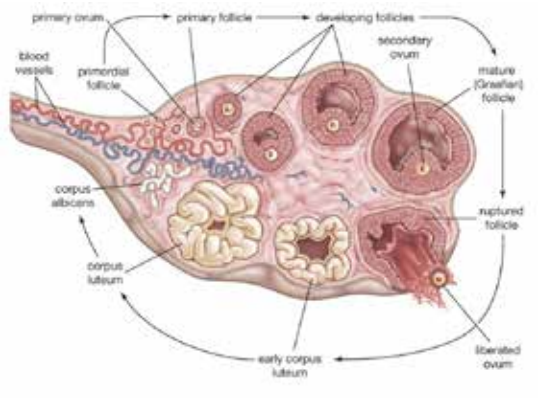


Photo courtesy: Ovary of animal and human by Robert D. Utiger, Clinical professor of Medicine, Harvard Medical School.

Per rectal examination

As showed in Figures 3 and 4, observing reproductive organs like ovaries, development of follicles, uterine horns etc. (Table 1).

Table 1. Observing various indicators of reproductive performance

Organs to be observed	Observation	Interpretation
Colour of visible mucous membranes	Pale	Anaemia
Body coat	Rough	Malnutrition, parasitic infestation etc.
	Shiny, smooth	Well nourished
Ovaries	Smaller in size and with a smooth surface	Non-functional ovaries or absence of ovary
	Uneven surface	Indicative of follicular development or CL
	Palpable growth over the ovarian surface	-do-
Uterus	Tonic uterus	Physiologically active uterus
Vulvar lips	Well developed	Nearing oestrus

2.1.1.3. Possible line of treatment

Step I: If nutritional deficiency is observed

- Should give dewormer at periodic interval.
- May provide minerals, vitamins like A, D_3, E preparations (both oral and parenteral) as per the need.
- Should provide balanced concentrate feed.

Step II: If poor follicular growth is observed

- May give utero-ovarian massage for seven alternative days followed by applying Lugol's paint into the os cervix.

Step III: If hormonal cause is anticipated

- May give hormonal treatment, if necessary.
- Review after 30 days, if any development of reproductive organs is observed, a single dose of PG $F2\alpha$ can be administered.

2.1.1.4. Advice to the farmers

- Maintain proper plan of nutrition. Concentrate feed should be given to the heifers along with addition of 50 gm of mineral mixture daily.
- May increase concentrate ration from the 18 months of age in case of heifer.
- Give deworming drugs periodically.
- Allow for regular exercise.
- Keep the heifer close to bull, it may hasten puberty.
- May cull the animal if it does not respond to treatment.

2.1.2. Delayed postpartum oestrus

Normally a buffalo cow comes to heat within 28–71 days after parturition, which may be extended up to 90 days. If it takes even more longer time, it is considered as delayed postpartum oestrus. It is of two types—suboestrus (unobserved or silent heat) and true anoestrus

Suboestrus: It is also known as silent heat. In this case, buffalo cows do not exhibit external behavioural symptoms of oestrus or heat, although the physiological symptoms of oestrus are present internally. Because of this, farmers fail to go for AI or natural service. This could be detected only if a bull is present in the vicinity.

True anoestrus: In this case, buffalo cow fails to come to heat because of physiological, hormonal, or organic changes that are required to bring the animals into heat. Internally, ovary is found subfunctional and corpus luteum and follicle are not palpable.

2.1.2.1. Possible causes of delayed postpartum oestrus

Improper involution of the uterus: Involution of the uterus is necessary for the buffalo cow to conceive again at postpartum. Time required for complete uterine involution in a normal cow generally ranges from 26 to 52 days which may be extended up to 90 days. Sometimes, because of some factors, it goes far beyond. These factors include:

- Weak myometrial contraction
- Incomplete removal of the placental tissues/membrane
- Slower regeneration of the epithelium of the endometrium
- Seasonal influence (involution is rapid in spring and summer)
- Nutritional status
- Uterine infection
- Metabolic diseases (milk fever increases the uterine involution period).

Cystic ovarian degeneration (COD): On clinical examining a buffalo cow showing cyclic irregularity characterized by anoestrus or nymphomania may have one or more fluid-filled ovarian structures (cysts). These cysts may be of two types:

Follicular cyst—causes nymphomania and repeat breeding (Figure 5).

Figure 5. Follicular cyst.

Luteal cyst—It causes anoestrus. Luteal cysts may disappear spontaneously because of the release of endogenous PG (Figure 6).

Figure 6. Luteal cyst.

Cystic corpus luteum (CCL)—A CL (corpus luteum) with a cavity or lacunae having fluid arises after normal ovulation (Figure 7). It makes actual size of CL will be small that's why progesterone hormone will be less which causes embryonic death and anoestrus or repeat breeding.

Figure 7. A cystic corpus luteum (CCL).

Pregnancy diagnosis

Pregnancy diagnosis is a difficult task at the initial stage of pregnancy. It becomes even more difficult, when buffalo cow gets pregnant by bull service without the notice of the owner. Under such circumstances, farmers may wrongly consider the case as a delay of postpartum oestrus which may invite unnecessary concern/care/treatment. Therefore, proper pregnancy diagnosis is essential at the initial stage. Following pregnancy diagnostic methods could be employed for this purpose.

Rectogenital palpation—It is the oldest method of pregnancy diagnosis and is the easiest, cheapest and fastest method of pregnancy diagnosis with little or no harm to the dairy animal and its foetus when performed carefully.

Ubio Quickvet bovine pregnancy spot assay—It is a qualitative enzyme immunoassay for detecting progesterone in milk in 15–20 minutes within 21 days of pregnancy. It measures progesterone levels in milk or serum (for heifers) using a rapid Enzyme-linked immunosorbent assay (ELISA) technique.

Limitation: The test should be conducted between 19th and 23rd days after insemination.

Doppler ultrasonography machine—It is a portable ultrasonography machine. Using it, pregnancy can be detected in a cow/buffalo cow starting from the 28th day with 100% accuracy (Figure 8).

Figure 8. A Doppler ultrasonography machine.

Limitation: The cost is high.

Persistent Corpus luteum (PCL): When Corpus luteum fails to regress spontaneously within the specified time, there is continuous secretion of progesterone which inhibits postpartum oestrus (Figure 9).

Figure 9. Persistent corpus luteum.

Buffalo cow–calf association: Late weaning causes delay in postpartum oestrus and vice versa. If suckling is continuously practiced during lactation, it inhibits ovulation, elongates period of postpartum anoestrus and contributes to delayed postpartum oestrus.

Subnutrition/undernutrition—A poor plan of nutrition with mineral deficiency delays postpartum oestrus.

Parasitic infestation—Parasitic infestation in buffalo cause anaemia and weight loss and ultimately results into anoestrus.

Pyometra—Inhibits secretion of PG F2α which in turn blocks regression of the CL to cause anoestrus. There are generally no accompanying clinical signs other than anoestrus.

Mucometra—It results from cystic ovaries leading to cessation of heat.

2.1.2.2. Approach to disease investigation

Step I: Taking animal history

- Date of last calving
- Whether primipara or pluripara
- History of retained placenta if any

- Nutritional plan offered
- History of metabolic diseases if any
- Date of weaning
- Date of deworming

Step II: Observing clinical findings

External observation:

- Vaginal/uterine discharge

Per rectal examination

- For presence of ovarian cyst or PCL
- For presence of pus/mucus in the uterus
- Pregnancy diagnosis

Step III: Laboratory investigation

Possible tests to differentiate among follicular cyst, luteal cyst and cystic corpus luteum.

- ELISA test is performed for estimating progesterone concentrations by an enzyme immunoassay before and at 10 days after cow/buffalo cow is treated with Gonadotrophin releasing hormone (GnRH).

Sample required: Blood serum sample.

Differential diagnosis

To make differential diagnosis between pregnancy and pyometra, the following comparisons could be made (Table 2).

Table 2. Differential diagnosis from pregnancy

Criteria	Pregnancy	Pyometra
Uterine horns	Horns remain asymmetrical	Both the uterine horns remain equally distended
Fremitus	Fremitus is present	Fremitus is absent because there is no need to supply extra blood
Slippage of foetal membrane	Slippage of foetal membrane is present	No slippage of foetal membrane
Consistency of uterine wall	Thin and tonic uterine wall	Thick uterine wall and lack of tone

Differential diagnosis of follicular cyst, luteal cyst and cystic corpus luteum

To make differential diagnosis among follicular cyst, luteal cyst and cystic corpus luteum, milk progesterone concentrations should be estimated by an enzyme immunoassay before and at 10 days after buffalo cows are treated with gonadotropin-releasing hormone (Table 3).

Table 3. Differential diagnosis between follicular cyst, luteal cyst and cystic corpus luteum

Criteria	Follicular cyst		Luteal cyst		Cystic corpus luteum	
	Before 10 days	After 10 days	Before 10 days	After 10 days	Before 10 days	After days 10
External symptoms	Nymphomania		Anoestrus		Anoestrus	
Progesterone in concentration skim milk	Less than 1.0 ng/ml		1.0 ng/ml or higher	Increased value	1.0 ng/ml or higher	Decreased value
Consistency on rectal palpation Farin et al. (1992)	Soft, thin walled structure		Firm, thick walled structure		Very soft, thin walled in the top of CL	

2.1.2.3. Possible line of treatment

Step I:

In case of PCL

- May perform manual enucleation of CL
- May inject PG F2α intramuscularly (i/m)
- May give single i/m injection of GnRH analogue (10 to 20 µg).

In case of pyometra/mucometra

- Conservative treatment such as uterine irrigation could be done.
- Oestrogen/PG F2α could be injected followed by uterine irrigation.
- Intrauterine antibiotic and antiprotozoal drug combination like Ciprofloxacin andTinidazole, Ofloxacin and Ornidazole, Povidone iodine (50:50) etc. could be used.
- Parenteral antibiotics could be used if any infection is suspected.
- Some buffalo cows may fail to evacuate pus completely after the first treatment. In such case, 2nd injection of PG F2α may be repeated 8 to 12 days after the first dose.

In case of COD

- Manual enucleation: Manual enucleation of cystic structures could be done by palpation perrectum. However, it may cause ovarian haemorrhages and adhesions may follow manual rupture, so it should be avoided.
- GnRH analogue: Receptal (Buserelin) could be injected and cow/buffalo cow may show sign of oestrus in between 18 to 23 day of post treatment.
- Mineral supplementation could be given.

2.1.2.4. Advice to the farmers

- Go for early weaning.
- Call a veterinarian/paraveterinarian at time of/after calving so that proper postpartum care can be taken.
- Complete the course of treatment of metabolic disease, if any, as advised bythe attending veterinarian.
- Make deworming plan with appropriate medicine and proper dose.
- Perform faecal test before and after deworming to find out the efficacy of medicine.

- Maintain a proper plan of nutrition along with mineral supplementation.
- Call a veterinarian for palpation to find out the condition before culling.
- Cull the animals if it does not respond to treatment within six months.

2.2. Repeat breeding (RB)

RB may be defined as a buffalo cow's failure to conceive from three or more regularly spaced natural services/AI without exhibiting any detectable abnormalities.

2.2.1. Possible causes of RB

Subnutrition/undernutrition during the growth period

- Undernourished cows/buffalo cows show poor rates of conception. Buffalo cows not receiving balanced ration may become repeat breeder.
- Trace minerals particularly copper, cobalt, iron etc. are essential for steroidogenesis, i.e. formation of sex hormones, deficiency of which may lead to RB.

Delayed ovulation

- In majority of buffalo cows, ovulation normally takes place approximately 24–48 hours after the onset of oestrus or preovulatory Lutenizing Hormone (LH) surge, i.e. 15–18 hours after external signs of oestrus have ceased.
- In some buffalo cows, ovulation normally takes place beyond 6–21 hours (average 18 hours) after external signs of oestrus have ceased. In such case, the spermatozoa fail to fertilize the ova as it loses its viability beyond the specific time.

Anovulatory heat

- The ovary fails to release ovum at time of heat.

Early embryonic death within 16 days after service

- Stress such as pain, long transportation etc.
- Malnutrition
- Seasonal stress such as summer stress
- Progesterone deficiency
- Uterine infection.

Age

- The older the buffalo cow, the higher is the chances of RB. Fertility in dairy buffalo cows get better after the 1st or 2nd parturition and then declines from the 6th and 7th parturition.

Uterine infection

- There should be a favourable uterine environment for normal embryonic development. Any adverse condition such as cervicitis, endometritis, pyometra, metritis, trichomoniasis, vibriosis (now, campylobacterosis) etc. adversely affects the normal uterine condition causing embryonic death which is one of the major causes of RB.
- Uterine infections negatively influence the postpartum uterine involution and follicular development.

Anatomical defects of the genital tract

- Blocked fallopian tubes due to infection or physiological defect.

Improper ovarian function

- COD is a cause of RB in dairy animals.
- Delayed ovulation.
- Luteal inadequacy resulting into progesterone deficiency.

AI

- Poor quality of semen may reduce the conceiving rate.
- Thawing in appropriate temperature 35°C for 35 seconds.
- Depositing semen in body of cervix or last ring of cervix for higher conception.
- After completing AI, watch inside of the sheath, so that all semen is deposited or not.
- If animals are not inseminated at accurate time (12–18 hours from onset of heat), it may cause conception failure.
- Poor management and handling of frozen semen.
- Storing frozen semen in insufficient level of liquid nitrogen.
- Breaking cold chain.
- Contaminated AI instruments.

Prolonged oestrus period

- In some dairy animals, the oestrus period continues for more than the normal 24 hours, even up to 72 hours. In such case, AI timing should be scheduled as required and number of AI shall be more than once or twice. If buffalo is in heat after 6 hours of service, repeat the service after 10 to 12 hours from the 1st service.

Service by an infected breeding bull

- A bull infected with sexually transmissible disease like—Trichomoniasis, Brucellosis, Vibriosis etc. will transmit the disease to the cow/buffalo cow during service and results in RB of the cow/buffalo cow.

2.2.2. Approach to disease investigation

Step I: Taking animal history

- Age of the cow/buffalo cow.
- General health condition of the cow/buffalo cow (poor/good/very good).

- Any chronic disease being suffered by the animal (e.g. diarrhoea, cough, fever etc.).
- Normal appetite of the animal.
- Nutritional plan (concentrate, green and dry fodder, minerals and vitamins supplementetc.) offered.
- Number of times the animal has repeated.
- Sign of heat observed.
- Date and time of each services given to the animal (to see whetherrepeating in regular or irregular oestrus).
- Difference between the time of sign of heat observed and insemination/servicegiven.

Step II: Clinical observation of the animal

External:

- Examining vulvo vaginal area for presence of any abnormal discharge.
- Examining vulvular mucosa by opening the vulva after three days of breeding to find out 1st degree of metritis (pus flakes is observed).
- Per rectal examination for presence of abnormal discharge from vagina, cervix anduterus (cloudy, bloody, watery, mixed with pus etc.).

Per rectal examination:

- Per rectal examination for presence of abnormal discharge (in minute quantity) in the cervix/vaginal canal/uterus using AI gun and AI sheath.

Procedure of examination:

- Get the uterine catheter properly sterilized.
- Open the vulvar lips and insert the uterine catheter into the cervix of the cow/buffalo cow and push gently up to the 1st one-third.
- Holding in the grasp of the uterus with the left hand, gently make short to and frommovement of the catheter with the right hand.
- Gently withdraw the uterine catheter and observe for presence of any discharge—cloudy, bloody, watery, mixed with pus etc.

Or, in case a uterine catheter is not readily available

- Insert an AI gun into the cervix of the cow/buffalo cow and push gently up to the 1st one-third.
- Withdraw the AI gun carefully leaving the sheath inside the cervix.
- Grasp the uterus with the left hand and gently move the sheath with the right hand.
- Gently withdraw the sheath and observe the presence of any discharge— cloudy,bloody, watery, mixed with pus etc.
- If it is not transparent, it may be assumed to have some kind infection in thereproductive tract.

Or observe the discharge by back racking

- Per rectal examination of reproductive organs like— ovaries for presence of cyst etc.

- Per rectal examination of ovaries on 9th or 10th day of oestrus for presence of corpus luteum. If corpus luteum is not present on the ovary, it is a case of anovulatory heat.

Step III: Laboratory investigation

Possible tests

- Culture and sensitivity test
- ELISA
- Polymerase Chain Reaction (PCR)

Sample required

- Culture and sensitivity test— Aseptically collected vaginal/uterine discharge.
- ELISA— Blood serum sample.
- PCR— Blood samples collected using tubes with Ethylenediaminetetraacetic acid (EDTA).
- Transporting to the hospital in cool temperature using cool box/thermosflask.
- Packaging and dispatching to laboratory following standard protocol as early aspossible in thermocol box adding ice pack/gel pack.

Differential diagnosis (Table 4)

Table 4. Differential diagnosis from repeat breeding

Conditions	Observations	Interpretation
Interval between two consecutive heat	Normal cycle (18–22 days)	Anovulatory heat, delayed ovulation, faulty AI, progesterone deficiency
	Beyond 22 days	Uterine infection such as cervicitis, endometritis, pyometra, metritis etc.
	35–55 days	Trichomoniasis
	Less than 18 days	Follicular cyst
Type of vaginal discharge	Transparent and watery	Early heat
	Transparent and viscous, cuts off in the midway	Late heat
	Profuse watery	Mucometra
	Cloudy and with variable degree of consistency	Infection in the vagina, cervix or uterus
	White or whitish-yellow, mucopurulent	Severe endometritis
	Frequent and copious	Follicular cyst
	Bloody	Acute and severe endometritis
External symptoms of heat	No bellowing, not restless	Silent heat
	Tries to mount on other cow/buffalo cow, profound signs like bellowing,running, restlessness, vaginal discharge	Normal heat
	At frequent intervals, very restless	Follicular cyst, buller cow
Appearance of the ovary on palpation	Smooth, small	Anovulatory heat
	Presence of soft, thin walled cyst	Follicular cyst
Duration of oestrus	More than 24 hours	Prolonged oestrus period

2.2.3. Possible line of treatment

Step I: Curative treatment

In case of undernutrition

- Prescribe a proper plan of nutrition.

In case of delayed ovulation

- Inseminating the cow/buffalo cow two or three times at 12 hours interval.
- Injecting Human Chorionic Gonadotrophin (HCG) 4 hours before AI.
- Providing vitamins A, D3, E and minerals like phosphorus, selenium, iron, copper, cobalt etc. parenterally and orally one week prior to breeding.

In case of anovulatory heat

- GnRH analogues or HCG (2000 LU i/m or 1000 LU i/v) at the time of insemination to promote ovulation.
- Provide vitamins A, D3, E and minerals like— phosphorus, selenium, iron, copper, cobalt etc. parenterally and orally one week prior to breeding.

In case of early embryonic death due to external or environmental factors

- Proper management may help to overcome this defect.

In case of early embryonic death due to heat stress

- Inseminated cow/buffalo cow should be kept in a cool place or in a shed for 15 days after insemination.
- Administering GnRH analogue (2.5 ml) on day 11 after insemination.
- Providing adequatedrinking water and may cool the shed with water.

In case of progesterone deficiency

- Administer progesterone injection 3 to 5 days after insemination and continued for avariable period of 2 to 3 weeks.

In case of mild/subclinical uterine infection (oestrus cycle is generally regular)

- Intrauterine deposition of Levofloxacin, Ornidazole and Alpha Tocopherolcombination, Ciprofloxacin and Tinidazole combination, 5–6 hours prior to AI.
- Parenteral use of Enrofloxacin, Ciprofloxacin, Ceftriaxone etc. as per body weight forthree days.
- Inject 500 mg progesterone i/m after 5 days of insemination, repeat the same doseon day 10.

In case of severe uterine infection (oestrus cycle is irregular/prolonged)

- May irrigate the uterus with potassium permanganate solution or isotonic saline solution; if required, clean the uterus of any pus, debris, caseated/necrotic materials etc.
- May inject Oxytocin i/v to induce uterine contractions for complete evacuation of uterus.
- May deposit intrauterine—Levofloxacin, Ornidazole and Alpha Tocopherol combination, Ciprofloxacin and Tinidazole combination.

- May irrigate uterus with povidone iodine 1–2% solution in 1st degree, 2–3% solution. In 2nd degree 3–4% solution for 3rd degree of metritis @ 50–100 ml daily for 3 days. Povidone iodine is highly effective in the presence of pus, debris and blood in the uterus while other antimicrobials lose their effectiveness. Povidone iodine always uses in luteal phase for better response.
- May administer Enrofloxacin, Ciprofloxacin, Ceftriaxone etc. as per body weight for three days.
- May give a course of anti-inflammatory drug in case of severe inflammation and pain.
- May inject vitamins A, D3 and E weekly for three weeks or alternate days.
- Should allow sexual rest in the subsequent heat.
- Should allow service on the 3rd heat, followed by injecting 1500 IU HCG after AI.
- May inject 500 mg progesterone i/m after 5 days of insemination, may repeat the same cow/buffalo cow dose on day 10.

In case of severe/acute uterine infection (oestrus cycle is generally irregular and they may have fever)

- May inject Enrofloxacin, Ciprofloxacin, Ceftriaxone etc. as per body weight.
- May use anti-inflammatory drug in case of severe inflammation and pain.

Note: Never use intrauterine infusion in acute metritis or when genital discharge is mixed with fresh blood or when inflammation is so severe that the cow/buffalo cow shows an arched back posture.

In case of COD—(follicular cyst in this case)

- Manual enucleation: Manual enucleation of cystic structures by palpation per rectum may be done. Complications like ovarian haemorrhage and adhesions may follow manual enucleation, so it should be discouraged.
- May inject GnRH hormone. Generally, the cow/buffalo cow will show oestrus 18 to 23 days post treatment.
- May administer HCG: Inject Chorulon: 3000–5000 IU i/v. Cow/buffalo cow generally develops oestrous within 20 to 30 days post treatment. A second or third dose may be required in few cases.
- May administer progesterone 50 to 100 mg i/m for 14 days or a single dose of 750 to 1000 mg repository progesterone.

If due to faulty AI practices

- Animal should not be excited 15 minutes prior to, during or 15 minutes after AI. Excitation causes the release of adrenaline thereby lowering the action of Oxytocin which is required for sperm transport.
- Proper thawing of frozen semen and AI should be done within few minutes after thawing.
- Proper insemination technique should be followed to deposit the semen just after the anterior end of cervix or in the body of uterus otherwise it will cause traumato the genital tract.

2.2.4. Advice to the farmers

- Should maintain proper plan of nutrition. Should provide good quality concentrate feed along with addition of 50 gm of mineral mixture daily. In addition, should provide sufficient good quality green and dry fodder and ad lib clean drinking water.
- Should go for AI and in case AI facility is not available, go for natural service with a good quality bull with a satisfactory breeding record.
- Should not use a breeding bull for natural service to a cow/buffalo cow that is already infected with genital disease and having a history of RB. It will make the bull infected with sexually transmissible disease.
- Should serve the cow/heifer properly during heat. A prolonged heat may create confusion to the attending veterinarian and would lead to RB. Should narrate the history properly to the attending veterinarian.
- Should do AI at proper timing. It is the farmer who can give the knowledge on timing ofoestrus.
- Never hide any information on previous history of RB, conception failure, service by infected bull etc. from the attending veterinarian. Many farmers think that on giving the true information, the cow/heifer would not be inseminated by him/her, rather a course of treatment would be prescribed which would make the conception further delayed and costlier.

2.3. Abortion

The expulsion of a dead or live recognizable size of foetus at any stage of gestation (45–60 days onwards to parturition) from the uterus is called abortion (Figure 10).

Figure 10. An aborted foetus.

Source :https://agritech.tnau.ac.in/expert_system/cattlebuffalo/Abortion.html

2.3.1. Possible causes of abortion

May be of noninfectious or infectious type

Noninfectious (Ahmed 2015; Hovingh 2009):

- Heat stress causes foetal hypotension, hypoxia and acidosis that may lead to abortion.
- High maternal temperature due to pyrexia is more lethal than environmental heat stress.
- Mycotoxins, especially those having estrogenic activity leads to abortion.
- Use of steroids or other drugs contraindicated in pregnancy. Never use steroids during pregnancy. If situation calls for use of such drug, use only the pregnancy safe steroids.
- Severe trauma, kicking or hitting with horns by adjacent cows/buffalo cows, falling during transit etc.
- Over exhaustion because of driving pregnant cow/buffalo cow for a long distance on foot, running out of fear or being chased by dogs etc.
- Threatened/habitual abortion—abortion may take place when there is insufficient release of maternal progesterone due to a weak CL.

Infectious:

- **Brucellosis or Bang's disease**
    - Abortion mainly takes place in between 6–9 months of gestation.
    - May be accompanied with retention of placenta or RB.
- **Campylobacter foetus (previously Vibrio)**
    - Abortion may take place in between 5–8 months of gestation.
    - Rate of abortion may be less than 10%.
- **Corynebacterium pyogenes**
    - Abortion may take place in last trimester of gestation.
    - Rate of abortion may go up to 64%.
- **Listeria species**
    - Abortion takes place in 7th month of gestation.
    - Rate of abortion may go up to 50%.
- **Trichomonas foetus**
- It is one of the most common protozoa causing many early embryonic deaths and foetal deaths leading to abortion.
    - Abortion may take place in 1st half of gestation.
- **Leptospirosis**
    - Abortion may take place in the last trimester of gestation.
    - Rate of abortion may be 5–40%.
- **Mycotic abortion** caused by various species of fungus like—Aspergillus spp etc.
    - Abortion may take place from 4 month to full term of pregnancy.
    - Most common in winter.

2.3.2. Approach to disease investigation

Step I: Taking animal history

- Age of animal
- Number of lactations
- Breed of animal
- System of farming (intensive/semi-intensive)
- Month of abortion during the time of gestation
- Appearance of foetus (lathery/dry/fresh)
- Any chronic debilitating diseases suffered by the animal
- Any abnormal symptom shown by animals
- History of any injury/trauma
- History of any stress
- History of abortion in the herd

Step II: Clinical observation

- General health of the animal

Step III: Laboratory investigation

Possible tests

- Milk ring test (MRT)
- Rose Bengal Plate Test (RBPT)
- ELISA/PCR
- Culture and sensitivity test

Sample required:

- MRT—aseptically collected milk sample
- RBPT —blood serum sample
- ELISA/PCR—blood serum sample
- Culture and sensitivity test—vaginal/uterine discharge

Differential diagnosis (Table 5)

Table 5. Differential diagnosis of causes of abortion

Conditions	Observations	Probability of cause
Stage of gestation	Any time	Over exhaustion, severe trauma, mycotoxins
	First trimester	Trichomonas foetus
	First and second trimester	Mycotic abortion habitual abortion, *Listeria* spp
	Second or third trimester	*Leptospirosis*, *Listeria* spp, *Brucella* spp
	Third trimester	*Corynebacterium pyogenes*
Season	Summer, with higher temperature and humidity	Heat stress
Proximity to horned cow/buffalo cow		Severe trauma
Association of retained placenta	Yes	Brucellosis
Pyrexia	Yes	High maternal temperature
Transportation	Yes	Stress due to transportation
History of medication	Given	Use of steroids or other drugs

2.3.3. Possible line of treatment

Step I: Curative treatment: If placenta is not shredded, may remove the placenta as per procedure described in section 2.5 Retained placenta of this protocol.

- May irrigate the uterus with potassium permanganate solution or other antiseptic solution of normal saline or 1–2% of potassium iodine solution or normal saline + antibiotic solution.
- If suspected abortion due to Brucellosis, should not touch the aborted materials and placenta with bare hands. Placenta should be removed taking all possible aseptic precautions and it should be disposed off by deep burial and blood sample should be collected and sent to veterinary laboratory for confirmatory diagnosis.

In case of bacterial infection

- Antibiotic injection like—Ceftriaxone and Sulbactum, Amoxycillin and Cloxacillin, Enrofloxacin etc. may be given for 3–5 days. Duration of antibiotic therapy depends upon whether there is preexisting infection or it is to prevent further infection.

In case of Trichomonadal abortion

- May inject Oxytocin i/v to induce uterine contractions for complete evacuation of uterus.

May perform intrauterine deposition of Levofloxacin, Ornidazole and Alpha Tocopherol combination. Ciprofloxacin and Tinidazole combination, Povidone iodine (50:50 in water) etc. may be given daily for 3 days. Povidone iodine is highly effective in presence of pus, debris and blood in the uterus while other antimicrobials lose their effectiveness:

- May use Enrofloxacin, Ciprofloxacin, Ceftriaxone etc. parenterally as per body weight.
- May inject vitamins A, D3 and E weekly for three weeks.
- Should allow sexual rest in the subsequent heat.
- May go for AI/natural service in the 3rd heat, followed by injection of 1500 IU HCG immediately after AI/natural service.
- May go for oral ecbolic preparations—200 ml on the first day followed by 100 ml for 3 consecutive days. Should double the dose for animals weighing above 400 kg. It induces uterine contraction and hastens involution of the uterus.

If there is history of threatened/habitual abortion

- May inject progesterone 500–750 mg i/m. First injection on 45th day, followed by 4–5 injections on every 10 days interval for animals aborting in early pregnancy.
- Or, 2 ml i/m for 3 days in probable calving month, followed by 3 injections at weeklyintervals for abortion in mid/ late pregnancy.

Step II: Preventive measures

- Should vaccinate against brucellosis
- Offer good quality feed to pregnant cow/buffalo cow
- Feed contaminated with moulds should not be offered.

2.3.4. Advice to the farmers

- Maintain good hygiene and cleanliness in the farm and surroundings.
- Keep the pregnant cows/buffalo cows a bit away from the other cows/buffalo cows so that there is least possibility of injury/trauma from other cows/buffalo cows.
- Avoid slippery floor.
- Arrange for proper air circulation during summer so that heat stress could beminimized.
- Not to drive the cow/buffalo cow for a long way.
- Not to transport pregnant cows/buffalo cows.
- Keep close contact with the local veterinarian if there is history of threatened/habitualabortion.
- Bury at 6 feet deep all the aborted materials away from the farm/homestead.
- Segregate the affected animals from the others.
- Cull the cow/buffalo cow if infected with brucellosis.
- Be very careful in handling the cow/buffalo cow as well as the aborted materials because brucellosis is one of the major causes of retained placenta and brucellosis is a very easily communicable zoonotic disease that can be transmitted to the farmer while handling the cow/buffalo cow as well as the aborted materials. It causes undulant fever and sterility in humans.

2.4. Stillbirth

Expulsion of dead foetus at the time of parturition is called stillbirth.

2.4.1. Possible causes of stillbirth

- Difficult calving: Trauma caused by technical assistance at delivery such as subcutaneous, subdural or internal bleedings and external lesions. Abundant amounts of mucus in the respiratory tract which may be a sign of ceased placenta function before the birth process was completed, resulting in suffocation of the calf.
- Intrauterine death: Subcutaneous oedema and hydropsies, dehydration/mummification. The state of decomposition as an indicator of how long the calf has been dead in uterus.
- Foetal malformations: Calves with visible malformations.
- Unspecified infections: Calves with unspecified infections (born after normal gestation length).
- Infectious causes: It's important to rule out infectious causes of stillbirths, such as *Neospora caninum, Salmonella spp, Listeria monocytogenes, Q fever, Campylobacter foetus venerealis, Chlamydia and Brucella*. Mycotoxins have also been incriminated.
- Genetic defect.
- Clinically normal at fullterm, unknown cause of death: Clinically normal (wellformed) calves. Lack of external signs of difficult calving. No malformations.

2.4.2. Approach to curative and remedial measures: Same as in case of abortion.

2.4.3. Advice to the farmers

- Maintain good hygiene and cleanliness in the farm and surroundings.
- Keep the pregnant cows/buffalo cows a bit away from the other cows/buffalo cows so that there is least possibility of injury/trauma from other cows/buffalo cows.
- Avoid slippery floor.
- Arrange for proper air circulation during summer so that heat stress could beminimized.
- Not to drive the cow/buffalo cow for a long way.
- Not to transport pregnant cows/buffalo cows.
- Keep in touch with the local veterinarian if there is history of threatened/habitualabortion.
- Bury the aborted materials away from the farm/homestead.
- Segregate the affected animals from the others.

2.5. Retained placenta

As showed in Figure 11, retained placenta is defined as nonseparation of the placenta/foetal membranes by 24 hours after calving. It results from nondetachment of cotyledons from the maternal caruncle.

Figure 11. A case of retained placenta.

Source: Journal of Entomology and Zoology Studies 2018; 6(6): 603-605

2.5.1. Possible causes of retained placenta

- Uterine inertia: Uterine muscles become exhausted after prolonged contraction against an obstructing or oversized foetus. There is usually subclinical hypocalcaemia and/or hypoglycaemia.
- Abortion/stillbirth: Abortion caused by brucellosis is almost always followed by retained placenta.
- Advanced age of cow/buffalo cow: Advanced age causes weaker myometrial contractions.
- Dystocia: Dystocia causes exhaustion of uterine muscles as a result of prolonged uterine contraction.
- Vitamins A and E and selenium deficiency: Lack of vitamins A and E along with selenium may lead to reduced myometrial tonacity.
- Placentitis: When there is inflammation of the placenta, may be inflicted by injury or infection, the inflamed placenta causes retardation/inhibition of detachment of uterine caruncles and placental cotyledons.

2.5.2. Approach to disease investigation

Step I: Taking animal history

- Age of the cow/buffalo cow
- Breed of the cow/buffalo cow
- Number of lactations
- How long was the labour?
- How long it took to expel the placenta after normal delivery/abortion/stillbirth
- History of abortion/stillbirth if any in the animal and herd
- If aborted, at which stage of gestation

- History of previous treatment if any
- Appetite is normal or off fed
- Milk yield normal or reduced

Step II: Clinical observation

- Colour and smell of the placenta
- Is the placenta moist or dry?
- Body temperature and pulse
- Is there any straining?
- How much portion is hanging out?

2.5.3. Possible line of treatment

Step I: Medicine

- May inject Oxytocin, 75–100 IU i/m immediately after calving. This may loosen the attachmentbetween the cotyledons and caruncles.
- PG F2 α @ 5 ml i/m.

Step II: Manual removal

Procedure of manual removal:

- Following aseptic procedure, grasp the hanging placenta in the right hand (with gloves) and twist it like a rope so that the placenta can be more easily managed.
- Introduce the lubricated left hand (with gloves) into the uterus keeping the hand in between the uterus and placenta.
- Grasp the individual cotyledon and its caruncle between the thumb and fingers and the two structures (cotyledon and caruncle) should be gently separated by rolling, pushing and squeezing motion. This may be aided by traction with the right hand.
- Detach all the cotyledons from the caruncles trying first from the cervical area, then from nongravid horn and lastly from the gravid horn. Maintain tension on the hanging placenta during the entire procedure and try to twist the outer portion.
- Sometimes, especially in exotic and large breeds of cow/buffalo cow, the cranial end of the horn may not be accessible to hand, in such case, traction brings the apex of the horn to reach of the hand and makes it possible to separate the cotyledons and caruncles.
- Apply some traction to the placenta in a to and from motion until the entireplacenta comes out.
- Be careful not to break any uterine caruncle which may lead to severehaemorrhage.
- Irrigate the uterus with potassium permanganate solution or other antiseptic solution.
- Insert four numbers of intrauterine preparation containing Nitrofurazone and urea. It may be continued for 3–5 days in suspension form with the help of a catheter.

- Removal of all the foetal membranes is highly desirable because if any portion is left inside, it will be a potential focus of infection.
- If suspected to be due to brucellosis on clinical symptoms and history, utmost aseptic measures must be taken to avoid zoonotic transmission and all the biological waste should be properly treated with strong disinfectant and then deeply buried.

Step III: Post manual expulsion of placenta

- May give antibiotic injection such as—Ceftriaxone and Sulbactum, Amoxycillin and Cloxacillin, Enrofloxacin etc. for 3–5 days. Duration of antibiotic therapy depends on whether there is preexisting infection or it is to prevent further infection.
- May inject Siquil @ 5 ml i/m in case of severe straining.
- May give oral ecbolic preparations—200 ml on the first day followed by 100 ml for 3 consecutive days. May double the dose for animals weighing above 400 kg. It induces uterine contraction and hastens involution of the uterus.

2.5.3. Advice to the farmers

- Be very careful in handling the cow/buffalo cow as well as the aborted materials.
- Never try to pull the hanging portion of placenta which will cause removal of only the visible external portion and the remaining portion may remain inside and may cause severeinfection and even septicaemia. Pulling with force may cause uterine prolapse.
- May use oral ecbolic preparations right after calving on advice of the local veterinarian.
- Should not wait for too long, may call the nearby veterinarian/paraveterinarian for removal of placenta.
- Maintain a proper plan of nutrition.
- Bury the placenta away from the farm/homestead.
- Cull the cow/buffalo cow if infected with brucellosis.

2.6. Purulent vaginal discharge

Occasionally, there may be discharges coming out through vagina of a cow/buffalo cow at any point of time. Though it reflects disease inside the vagina, cervix or the uterus, it is not always pathological. Vaginal discharge may be of different types which are indicative of different condition of the female genital tract.

2.6.1. Vaginal discharges and possible causes

- Transparent and moderately viscous extending to ground without breach—- may indicate that the cow/buffalo cow is in heat. It should be correlated with other symptoms.
- Transparent and watery—may indicate that the cow/buffalo cow is in early heat. Should be correlated with othersymptoms.
- Transparent and viscous, cuts off in the midway—may indicate that the cow/buffalo cow is in late heat. Should be correlated with other symptoms.

- Profuse watery—may indicate mucometra.
- Cloudy and with variable degree of consistency—may indicate infection in the vagina, cervix, or uterus.
- White or whitish-yellow mucopurulent vaginal discharge comes out when a diseased cow/buffalo cow sits down—may indicate severe endometritis.
- Transparent and viscous in late pregnancy—may indicate calving is nearing. Should be correlated with other symptoms.
- Light yellowish green vaginal discharge—may indicate foetal anoxia with associated expulsion of meconium. Should be correlated with other symptoms.
- Frequent and copious vaginal discharge than normal, opaque or whitish grey in colour- may indicate follicular cyst.
- Bloody—may indicate acute and severe endometritis.

Diagnosis cannot be done solely on the indications given for vaginal discharge. Correlating with other clinical findings are essential. For confirmation, vaginal discharge should be collected for testing in the Disease Diagnostic Laboratories.

2.6.2. Possible line of treatment

Step I: Curative treatment

- Treatment should be given as per the primary condition/disease which is to beconfirmed in accordance with other symptoms and history which are given above.
- Give further treatment if the laboratory report is different from what was diagnosedtentatively.

Step II: Advice to the farmer

- Not to touch the discharge with bare hand.
- Isolate the cow/buffalo cow, if possible. If there is space for isolation, keep the cow/buffalo cow a bit away from the others.
- Disinfect the floor with a good disinfectant like phenyl, potassium permanganate solution etc.
- Consult with the nearby veterinarian/paraveterinarian.
- Never consider the case as the cow/buffalo cow to be in heat and go for natural service or AI.

2.7. Dystocia

When the stage I or especially, the stage I labour is markedly prolonged, i.e. more than an hour and becomes difficult or impossible for the dam to expel out the foetus without artificial aid, the condition is called dystocia.

2.7.1. Possible causes of dystocia

Foetal factors: As showed in Figures 12 to 16, developmental defect like conjoined twins, hydrocephalus, foetal monsters, foetal death, abnormal foetal position, abnormal foetal posture, abnormal foetal presentation, foetal oversize, foetal emphysema etc.

Figure 12. Developmental defect: Hydrocephalic foetus removed by foetotomy

Figure 13. Developmental defect: Conjoined twin.

Source: Buffalo Bulletin, 2007, 26(123)

Figure 14. Dicephalic monster.

Source: Indian Journal of Field Veterinarians (The), 2011. 7(1), pp.72-74.

Figure 15. Abnormal foetal presentation: Breech presentation

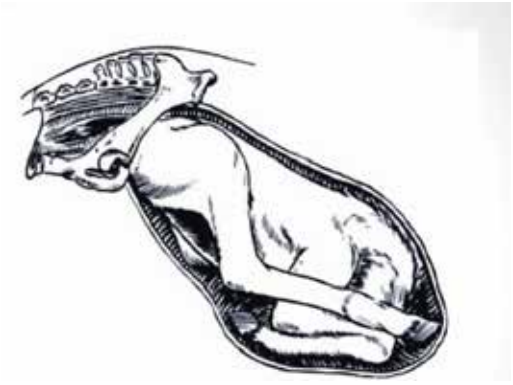
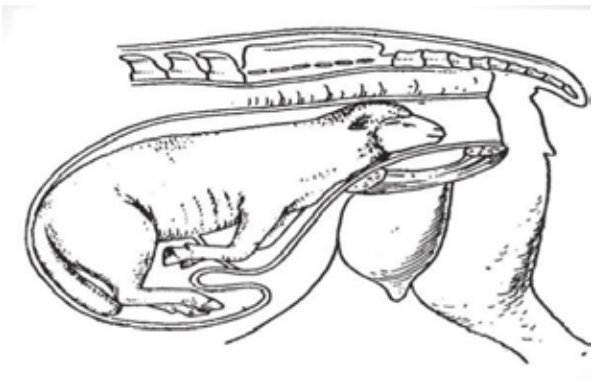


Figure 16. Abnormal foetal posture: Bilateral shoulder flexion



Source: https://www.slideshare.net/MohamedWahab2/5th-year-practical-revision-fetal-presentations

Maternal factors:

- Incomplete cervical dilation—sometimes the cervix fails to dilate properly during calvingcausing dystocia.
- Uterine torsion—it is one of the most common cause of dystocia in buffalo than cow/buffalo cow in Nepal.
- A disproportionate size of the foetus in relation to the size of the pelvis.
- Metabolic deficiency—hypocalcaemia, hypoglycaemia, hypomagnesaemia, obesity, oxytocin deficiency.
- Secondary uterine inertia—uterine muscles become exhausted after prolonged contraction against an obstructing or oversized foetus. There is usually subclinical hypocalcaemia and/or hypoglycaemia.
- Ectopic pregnancy—when the fertilized ovum is implanted in any tissue other than the uterine wall.
- Inadequate pelvis size—sometimes the pelvic girdle fails to expand during calving, or the pelvic girdle is too narrow to allow the foetus to be expelled under the influence of uterine contractions.
- Hip joint fracture/hip dysplacia—it causes obstruction to the birth canal and surgical intervention is required.
- Birth passage is blocked with neoplastic growth.

2.7.2. Approach to disease investigation

Step I: Taking animal history

Cases of dystocia should be attended without any delay.

- A thorough history regarding length of gestation, parity and problems seen during pregnancy should be investigated. Should find out when the animal started straining and if any water or fluid bags have appeared and/or ruptured. Also, should take history of previous hip dislocation/fracture.
- It is important to understand what has been done so far by the farmer including any drugs administered.

Step II: Physical examination

Physically examine of the dam who might have systemic illness, such as milk fever. Proper restraint and assistance should be sought and initial treatment may include fluid therapy, pain relief and the provision of calcium.

Step III: Vaginal examination

The vulva, vagina and cervix should be assessed for dilation, torsion or injury. Copious **lubrication** should be used at all stages of the examination.

Step IV: Foetal assessment should establish its presentation, posture and position and identify any palpable structures. Foetal viability can be assessed by feeling pulses on the limbs or tongue.

2.7.3. Possible line of treatment/correction

Anaesthesia is not essential, but an epidural can be administered to help reduce straining.

Manual correction of dystocia: It relies on traction to facilitate expulsion, repulsion of the foetus back into the uterine lumen to enable further manipulation.

- Use of lubricants is essential. Should apply lubricant liberally, especially in case of prolonged operations, two or more times or frequently so that the foetus or birth-canal does not become dry.
- Ropes can be applied to both legs and the head and traction applied while manually dilating the vagina and vulva.
- If the foetus is too large to be delivered vaginally, a caesarean operation or a foetotomy should be performed.

Possible treatments for the dam include:

- Analgesics and anti-inflammatory drugs—it can be given at the start. Short acting steroids can help reduce swelling and oedema. If cervix is not dilated properly use PGF2alpha along with valethamate bromide injection I/V or IM rout.
- Antibiotics like Ceftriaxone, Amoxycillin and Cloxacillin, Ceftiofur sodium, Enrofloxacin etc. should be given for 5–7 days after calving due to the increased risk of complications such as retained placenta and metritis.

Step II: Prevention

Foetomaternal disproportion is one of the major contributors to dystocia and this can be prevented with proper reproductive management.

Postpartum complications

Dystocia leads to an increased risk of postpartum complications in cattle, such as:

- Retained placenta: usually treated by manual removal
- Metritis: which can lead to toxaemia and laminitis and should be treated aggressively
- Uterine prolapse
- Cervical tears
- Uterine rupture
- Uterine haemorrhage
- Perineal lacerations.

2.8. Mastitis

Mastitis is inflaming the udder of a cow/buffalo cow mostly caused by bacterial infection and sometimes by other pathogens. These micro-organisms produce toxins which directly damage milk producing tissue of the mammary gland. This damage initiates inflammation process within the mammary tissue to eliminate the invading micro-organisms. The inflammation contributes to decreased milk production and is primarily responsible for the compositional changes observed in milk from infected quarters and cows/buffalo cows (Figure 17).

Figure 17. Mastitis.

Source: Chanduri Nagendra. https://curofy.com/discussion/mastitis-heifer-27cd97e6710e6a3d73f3277cadcb33ff.

There are mainly two types of mastitis—(i) clinical mastitis in which the affected cow/buffalo cow exhibits the clinical signs and (ii) subclinical mastitis in which the cow/buffalo cow does not exhibit any visible/clinical signs and the disease goes unnoticed.

Clinical mastitis: is characterized by sudden onset, alterations of milk composition and appearance, decreased milk production and the presence of the cardinal signs of inflammation in infected mammary quarters of the cow/buffalo cow and the disease becomes noticeable.

Subclinical mastitis: no visible signs are seen either on the udder or in the milk, but the milk production decreases and the somatic cell count (SCC) increases. It is more common and has serious impact in older lactating animals than in first lactation heifers. Because of absence of external manifestation, the diagnosis of subclinical mastitis is difficult. It can be diagnosed by milk test either with Mastrip paper (available in the pocket of Mastilep ointment) or by using CMT. This is a very simple test that the veterinarian/farmer can perform in the field provided the CMT solution is available.

2.8.1. Possible causes of mastitis

The main causes for developing mastitis are:

- Bacterial infection
    - Species of bacteria such as—***Staphylococcus aureus, E. coli***, ***Klebsiella*** spp, ***Streptococcus*** spp, ***Corynebacterium*** spp, coliforms, Arcanobacterium pyogenes etc. may cause mastitis in cows/buffalo cows (Jost and Billington 2005).
    - About 90% of pathogens responsible for mastitis are found in the cow–barnenvironment.
    - Except Arcanobacterium pyogenes, the etiological agent of clinical mastitis, all other micro-organisms cause both clinical and subclinical form of mastitis.
    - ***S. aureus, E. coli*** and ***Klebsiella*** spp cause the greatest losses of milk production in primiparous cows/ buffalo cows with mastitis.
    - ***Streptococcus*** spp, ***S. aureus,*** Arcanobacterium pyogenes, ***E. coli*** and ***Klebsiella*** spp cause most significant losses in older cows/buffalo cows.
    - Apart from causing mastitis, ***E. coli*** spp can lead to severe systemic disorder.
- Fungal (mycotic) infection
    - Increased incidence of udder mycosis results from mineral–vitamins deficiencies, anti-oxidant deficiencies, imbalanced diet, poor environmental conditions and even weather changes.

- In general, fungi are rare cause of mastitis but sometimes can occur in epizootic proportions.
- Usually in farms with poor environmental and hygienic conditions, poor quality of materials used as bedding (e.g. straw) with high humidity can be source of fungi causing mastitis in cattle.
- Fungi are also reason of udder inflammation when udder is washed with water but is not dried.
- Several species of yeast may cause bovine mastitis (Candida, Trichosporon, Saccharomyces and Rhodotorula).

- **Algae** (Protothecal)—Mastitis can also be caused by algae (organisms from Prototheca genus) in a lesser proportion. Poor environmental conditions, inappropriate milking hygiene and prolonged antibiotic therapy can be the reason of increased protothecal mastitis occurrence.
- **Trauma**—Inflammation of the udder may be caused by various forms of trauma such as—friction with a very rough floor, threshing by hoof of other cow/buffalo cow, injury while trying to jump over a fence or concrete wall (as in a manger), fisting type of milking practice or even a polyp developed inside the teat canal.
- **Injudicious use of antibiotics**—It is a general practice of the farmers to discontinue thecourse of antibiotics against mastitis prescribed by the veterinarian. They just stop it on seeingthe disappearance of the visible symptoms. This is one of the causes of relapse of the disease and subclinical form of the disease. They also use antibiotics at their own choice without having a prescription from a registered veterinarian. This kind of practice has influence on increased incidence of mycotic bovine mastitis. Eliminating bacterial flora antagonist to fungal growth during antibiotic therapy stimulates fungal multiplication. It also develops bacterial resistance to the antibiotic given.

Sequels of mastitis:

- One of the most detrimental sequels of mastitis is fibrotic changes that take place in the mammary gland. In such case, the functional milk alveoli are replaced with fibrous tissue as a result of severe inflammation of the gland and despite the cow/buffalo cow being totally free from the infective cause, the cow/buffalo cow will be no more in a position to givea good milk yield.
- Another one is damage done to the mammary gland and, even to the body system, by the toxins produced by the pathogens which are not destroyed by antimicrobial drugs.

2.8.2. Approach to disease investigation

Step I: Taking animal history

- Duration of suffering/showing symptoms
- History of mastitis in previous lactation
- Has any medication given already and if so, the details of the same?
- History of prepartum udder oedema
- History of practicing insertion of grass stem into teat canal prior to calving (a malpractice adopted in many places of Assam) to get the cow/buffalo cow acquainted tomilking
- Use of Oxytocin in previous lactation
- Blocked teat canal of any quarter.

Step II: Clinical observation of the animals Observing for clinical findings:

In clinical mastitis:

- All the cardinal signs of inflammation—swelling, pain, redness and hotness (rubor, dolour, calor, tumour) of the udder and also, hardness; rise in body temperature
- Oedema of the udder extending to the naval
- Drop in milk yield
- Pus/blood/flakes in milk
- Offensive odour of milk
- Curdled milk
- Watery milk
- Hard milking causing reduced flow of milk
- Change in pH of milk
- A reduction in mobility, due to the pain of a swollen udder or simply due to feeling unwell and lack of appetite
- In severe cases of acute, clinical mastitis, the cow/buffalo cow may appear very ill.

In subclinical mastitis:

A cow/buffalo with subclinical mastitis may appear otherwise normal with the following signs—

- A reduction in yield potential
- A high SCC
- Milk shows flakes like or powdery residues on straining
- A hard, fibrotic appearance of the udder (any of the quarters)
- Positive result of CMT
- Change in pH of milk on pH strip test
- Milk gets sour earlier than normal, i.e. before 4 hours of milking.

Step III: Laboratory Investigating possible tests

- CMT (rapid test at farm level) (Figures 18 and 19).

Step III: Laboratory Investigating possible tests

- CMT (rapid test at farm level) (Figures 18 and 19).

Figure 18. A CMT kit.

Figure 19. Interpreting results from CMT.

- Isolating and identifying causative agent (bacteria)
- SCC

Sample required:

- CMT—Aseptically collected milk sample
- Isolating and identifying causative agent—Aseptically collected milk sample
- SCC—Aseptically collected milk sample

2.8.3. Possible line of treatment

Step I: Curative treatment

- May give conservative treatment with a course of suitable antibiotic.
- May give an anti-inflammatory drug.
- May use corticosteroid drugs in case of sever degree of inflammation.
- May use proteolytic enzymes like—serratiopeptidase, chymotrypsin etc. to heal the inflammatory tissues.
- May infuse intramammary preparations as per schedule.
- May give hot fomentation with saturated magnesium sulphate solution.
- May use externally applicable gels etc.
- In case of systemic involvement, may use DNS infusion.
- May surgically remove obstructing teat polyp, if any.
- May drain out the infected quarter with antiseptic solution by using milk siphon.

2.8.4. Advice to the farmers

- Strictly maintain hygiene in the farm.
- Strictly maintain personal hygiene.
- Handle and milk the affected cow/buffalo cow only after completing milking the other cows/buffalo cows in the farm.
- Never practice fisting method of milking.
- Never use oil etc. to make the teats slippery during milking.
- Separate the affected cow/buffalo cow from the herd.
- Complete the course of treatment, especially that of antibiotics, as prescribed by the attending veterinarian.
- Never use the milk for sale or consumption until the withdrawal period is over.
- Never drip the milk in the floor.
- Dip teats in the antiseptic solution (Potassium iodine 90 ml + glycerine 10 ml) after completing the milking.
- Provide green fodder just after milking so that cow/buffalo cow stand until the teat canal closed.
- To check the pH of milk periodically using pH paper strip for detecting subclinical mastitis.

3. Process to be followed for per rectal examination

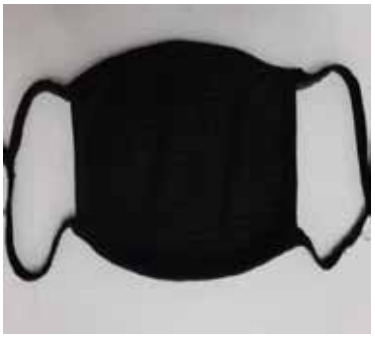
3.1. Materials required for rectal examining a cow/buffalo cow

- Full sleeve hand gloves
- Face mask
- Apron
- Shoe cover
- Soap
- Hand sanitizer
- Lubricant e.g. coconut oil, petroleum jelly etc. (Figures 20 and 21).

Figure 20. Per rectal exploring a cow/buffalo cow.

Figure 21. Materials required for rectal examining a cow/buffalo cow.

Full sleeve hand glove



Face mask

Apron

3.2. Personal precaution during rectal examination

- Wash the hands with soap and water
- Use disposable gloves, apron, mask and shoe cover
- After examining, dispose the personal protective clothing, especially gloveshygienically (burn/deep burial)
- Wash your hands with soap water
- Wash your cloth after returning to residence.

3.3. Procedure for rectal examination

- After wearing gloves, lubricate the left hand (in case of right handed person) and insert into the rectum very gently;
- The animal may put some opposing force which is due to peristaltic movement of the bowel and wait until it subsides;
- Push the hand forward and feel the uterine fundus;
- Go further and feel the horns and then the ovaries;
- Explore all the organs one by one, i.e. ovaries, horns, fundus of the uterus, os cervix and vaginal canal;
- Gently withdraw the hand;
- Extreme care should be taken so that the rectal mucosa is not torn or damaged.

4. Sample collecting, labelling, storing and dispatching

Different types of samples are to be collected for different types of tests following specific procedures and then it should be packed, preserved if required and finally, to be dispatched to the laboratory or used on the spot for the rapid tests.

4.1. Steps in collecting samples to dispatching to laboratory:

- Collecting biological sample (discharges, milk, faeces etc.) aseptically followingstandard protocol;
- Transporting to the hospital in cool temperature using cool box/thermosflask;
- Packaging and dispatching to laboratory following standard protocol as early aspossible in thermocol box adding ice pack/gel pack.

4.2. Procedure for collecting vaginal/uterine discharge:

4.2.1. Materials required:

- Uterine catheter,
- Adaptor,
- AI gun,
- AI sheath,
- Pipette,
- Cotton gauze,
- Syringe,
- Sterilized container/vial,
- Isotonic saline solution.

4.2.2. Methods

4.2.2.1. By back racking

- Put on a glove on the left hand and properly lubricate it;
- Insert the left hand into rectum, lift the uterus and cervix upward and massage ina backward direction;

- Continue to massage backwardly to the vagina through the rectal wall which willallow cervical mucus to flow down through the vulvar lips;
- Collect the discharge in a wide mouthed sterilized container/vial;
- Cut the discharge with the help of scissors if it remains hanging.

4.2.2.2. By using uterine catheter

- Put on a glove on the left hand and properly lubricate it;
- Insert the left hand into rectum, hold the cervix and uterus in the grasp of thehand;
- Open the vulvar lips and introduce the uterine catheter with the right hand into vagina (anterior part) or cervix or from where genital discharge must be collected;
- Fit a syringe to the outer end of the uterine catheter by the right hand and aspirate the mucus;
- Collect the discharge in a wide mouthed sterilized container/vial.

4.2.2.3. By using AI gun and AI sheath: (It is done in case the uterine catheter is not readily available)

- Put on a glove on the left hand and properly lubricate it;
- Insert the left hand into rectum, hold the cervix and uterus in the grasp of the hand cervix;
- Open the vulvar lips and introduce the AI gun with the right hand, with the AI sheath fitted, into vagina (anterior part) or cervix or from where genital discharge must be collected;
- Withdraw the AI gun leaving the AI sheath in place within the grasp of the left hand;
- Trim the slit outer end of the sheath with scissors to facilitate fitting of the syringe;
- Fit a syringe to the outer end of the AI sheath by the right hand and aspirate the mucus;
- Collect the discharge in a wide mouthed sterilized container/vial.

4.2.2.4. ampon method: (not a common practice)

- Take a sterile cotton gauze tampon of about 1 g and attach a string to it;
- Put on a glove on the left hand and properly lubricate it;
- Insert the sterilized gauze tampon into the vagina using the left hand;
- Leave the tampon in the vagina for 20 minutes;
- Remove the tampon from the vagina by pulling the string;
- Place the tampon in a sterilized bottle containing isotonic saline solution.

4.3. Procedure for collecting milk sample:

The veterinarian should collect milk samples adopting the following procedure for on the spot testing such as CMT kit, pH metre strip, SCC etc. or for onward transmission to diagnostic laboratories for identifying pathogen, culture and sensitivity test, antibiogram etc.

4.3.1. Materials required:

- For collecting sample directly from the cow/buffalo cow, take Latex or nitrile gloves (one pair per cow/buffalo cow), predip, paper or cloth towels (one per cow/buffalo cow), cotton balls soaked in 70% rubbing alcohol, 60 ml or larger sterile screw on vials (one for each quarter).
- A cool box/thermosflask to carry the sample;
- Required ice/gel packs in cool box to keep the sample cool during transportation;
- Personal protective clothing like apron, gloves, mask etc.;
- Sticker tags, marker, notepad, mask, sanitizers and biohazard bag;
- Disposal bag for carrying disposable materials like leftover milk, gloves, mask etc.;
- Spirit lamp/alcohol for sterilizing the instruments on the spot.

4.3.2. Procedure for collecting milk sample directly from the udder of cow/buffalo cow

- Ask the owner of the cow/buffalo cow to clean the udder and teats of the cow/buffalo cow thoroughly with water (Figure 22).
- Put on the clean gloves, apply predip to teats that have already been washed of manure, dirt, or sand.
- Strip two to three streams of milk from each teat to flush the teat canal and thereby to reduce contamination risk.
- Dry teats thoroughly with an individual cloth towel, paying close attention particularly to the teat end.

Figure 22. Clean udder.

- While holding the top of the teat steady, wipe the end of the teat well with an alcohol soaked cotton ball (Figure 23). Use as many cotton balls as necessary until the cotton ball still looks clean after using.

Figure 23. Cleaning of the teat.

- Open the milk vial and immediately take the sample, making sure not to touch the inside of the tube or bottom part of the lid. Hold the milk vial about 3 inches from the teat end and fill the tube half to three-quarters full of milk. Hold the vial at a 45 degree angle to prevent dirt from falling into the vial as presented in Figure 24.

Figure 24. Collecting milk sample in a sterile milk vial.

- Close the lid immediately and label the top with the date, cow/buffalo cow number and quarter sampled (Figure 25).

Figure 25. Milk sample.

- Cool samples immediately. Milk samples can be refrigerated for up to 2 days and can be frozen for up to 6 months. If mailing samples into a laboratory, mail them overnight on ice packs.
- If samples are intended for SCC evaluation, never freeze the samples because the cells will burst and you will get a false reading.

Note: Do not place the teat inside the vial while collecting samples.

4.4. Procedure for collecting sample for PCR:

- Tissues/organs: these samples, e.g. liver, lung etc. should be sent whole in individual tubes.
- Faeces: two faecal samples per animal are required. Faecal samples may be pooled, however only up to five different samples should be pooled to make up one sample for testing.
- Blood: Aseptically draw 10 ml of blood from the jugular vein and put in a sterile tube with EDTA (purple top tubes), other coagulants such as heparin or sodium citrate should not be used.
- Blood samples collected for PCR should be frozen, preferably at –8°C, immediately after collection and transported in ice (local transport) or in dry ice (interstate transport).

4.4.1. General requirement for collecting sample:

Make arrangement of all necessary materials before going to sample collection.

- Take a cool box/thermosflask to carry the sample;
- Take required ice/gel packs in cool box to keep the sample cool duringtransporting;
- Take personal protective clothing like apron, gloves, mask etc.;
- Take sticker tags, marker, notepad, mask, sanitizers and biohazard bag;
- Take a disposal bag for carrying disposable materials like leftover milk, gloves,mask etc.;
- Take a spirit lamp/alcohol for sterilizing the instruments on the spot;
- For collecting sample directly from the cow/buffalo cow: Take Latex or nitrile gloves (one pair per cow/buffalo cow), predip, paper or cloth towels (one per cow/buffalo cow), cotton balls soaked in 70% rubbing alcohol, permanent marker, 60 ml or larger sterile screw on vials (one for each quarter).

4.4.2. Information to be submitted along with the sample:

- Type of the sample (e.g. milk/whole blood/blood serum/faeces etc.);
- Species: Cow/buffalo;
- Type of animal: Exotic/crossbreed/nondescript;
- Quarter of udder: L (left) / R (right) / F (fore) / R (rear);
- Sample number;
- Weight/volume of the sample;
- Place of collection;
- Date and time of collection;
- Name of the collector;
- Purpose of collecting the samples (e.g. culture and sensitivity test, RBPT, milkprogesterone etc.);
- Name, address and signature or thumb impression of the person from whom thesample has been taken.

4.5. General considerations in sample collecting, handling and storage:

- The samples should never be touched with bare hands. Gloves and mask shouldalways be used in the process of collection.
- Outer and inner surface of the container should be cleaned thoroughly.
- Sample should not be allowed to expose to dirty materials/environment aftercollection and should not be mixed with other biological samples.
- Temperature and pH shall be recorded at the collection stage and aftertransporting to the laboratory.
- Gloves, mask and other materials in contact with the sample must be disposedproperly.
- The stopper/cover of the container shall be securely fastened to prevent leakageof the contents in transit.
- The container shall be completely wrapped in strong thick paper.
- The ends of the paper shall be neatly folded in and affixed by means of gum oranother adhesive.
- Always collect the samples as fast as possible.

4.6.Sample labelling and storing:

- Separate sterile container should be used for each sample.
- Collected sample should be distinguished from other samples by writing the details of the sample on the body of the container by a good quality marker and on a sticker tag and pasting the same on the container immediately after collection.
- The detail information of the sample should be written in a piece of paper and should put the same in a polythene zip bag and stick it at the body of the container by a transparent adhesive tape.
- The labelled container should immediately be transferred to the cool box/thermosflask filled with ice packs.
- The collected container shall be properly secured and sealed so that no tempering is possible after collection. To ensure this, signature of the milk producer/trader/sweet maker and a witness should be taken on the sealed pack.
- All samples should be transported to the laboratory by maintaining the cold chain in a cool box/thermosflask with gel/ice packs.
- No personal details of the owner of the sample should be supplied to the laboratory technicians who conduct the tests to avoid any potential prejudgement by the laboratory technicians.
- The sample should be stored at 4°C and processing should be made immediately.

In case of delay, samples should be refrigerated at 4°C and processing should be done within 96 hours of sampling. If sample needs to be preserved for more than 96 hours, it should be stored at –20°C.

4.7. Packaging and dispatching samples to other laboratories:

- For dispatching the samples to other laboratories, sample with their details shouldbe put in a thermocol box. Adequate quantity of cool ice pack/gel pack should be put in the box to keep the sample cool during the time of transportation.
- The outside of the thermocol box should be wrapped up with white paper and address of 'From' and 'To' should be clearly written on it preferably in all capital letters.
- A certificate should also be enclosed with the box stating the nature of the materials and purpose of sending.
- Packages should be marked clearly to provide information about the contents of package and nature of the hazard, if any.
- Sample should be sent by the mode of transportation that can deliver the sample at the quickest possible time in the destination. If the transportation time takes more, the ice/gel pack may come to normal temperature and the sample may get spoiled.
- The thermocol box should also be marked with 'Handle with care' an 'arrow mark' showing upside of the box, to guide the handlers during transportation.

Annex I. Animal observation check list

Name of the owner/s

address:

Contact Number

A	History	Particulars	Remarks
	Identification number/name of the cow/heifer		
	Breed		
	Age		
	Type	Cow/heifer	
	Number of lactations		
	Age at first heat		
	Type of mating	Natural/AI	
	Number of AI required per conception		
	Date of AI/natural mating		
	Months of gestation, if conceived		
	Interval between two to three successive heats, if not conceived		
	Age at first calving		
	Time interval between last calving to next heat		
	Time interval between last calving to next conception		
	History of previous diseases/ conditions	Delayed postpartum heat: How long it took?	
		RB:	
		How many times repeated?	
		Dystocia: Foetus dead or alive	
		Retained placenta: How many times?	
		Abortion: In which month of gestation?	
		How many times?	
		Stillbirth: How many times?	
		Mastitis: In how many previous lactations? How many quarters of teat affected?	
		Is there any blockage of teat canal?	
		Is there any fibrotic change in the udder?	
	History of hip dislocation/fracture		

	Duration of the existing disease/ condition		
	Previous treatment if any		
	Vaccination	Disease	
		Date	
	Deworming	Date	
	Nutritional plan	Concentrate feed/conventional feed	
	Mineral and vitamins supplement	Given/not given	
B	External appearance		
	General appearance	Dull/alert	
	Body coat	Rough/shiny	
	General health condition	Normal/emaciated	
	Body coat	Rough/shiny	
	General health condition	Normal/emaciated	
	Appetite	Normal/off fed	
	Eyes	Dull/bright	
C	Physical examination		
	Body temperature	°C	
	Presence of injury in the abdomen	Yes/no	
	Presence of external parasite	Yes/no	
	Presence of worms in manure	Yes/no	
	Appearance of udder	Normal/swollen/oedematous/hard/soft	
	Presence of injury on udder	Yes/no	
	Hanging out of placenta	Partial/not visible	
	Position of foetus	Half delivered/not visible	
	Water bag	Not visible/coming out, intact/ruptured	
	Genital discharge	Clear/cloudy/watery/with pus/bloody	
	Colour of visible mucous membrane of eye	Normal/pale/reddish	
D	Predisposing factors		
	Occurrence of disease in neighbourhood/in the same herd	Yes/no If yes, what kind of disease?	
	Number of cows/heifers affected		
	Number of households affected		
	Scarcity of food	Yes/no	
	Flood	Yes/no	
	Type of floor	Rough/damp/waterlogged/earthen	
	Unhygienic farmstead	Yes/no	
E	Rapid tests done		
	CMT	Results:	
	MRT	Results:	
	RBPT	Results:	
	pH paper strip test	Results:	
	Ubio Quickvet bovine pregnancy spot assay	Results:	
	Faecal sample for presence of worms	Results:	

	Pregnancy diagnosis by Rectogenital palpation	Results:	
	Pregnancy diagnosis by Doppler ultrasonography machine	Results:	
F	Laboratory test required if any		
	Vaginal/uterine discharge: culture and sensitivity test	Results:	
	Milk: culture and sensitivity test	Results:	
	ELISA/PCR for brucellosis	Results:	
	ELISA test for estimating milk progesterone concentrations	Results:	
	SCC	Results:	

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CGIAR's Sustainable Animal Productivity for Livelihoods, Nutrition and Gender inclusion (SAPLING) is working in seven countries focusing on livestock value chains to package and scale out tried-and-tested, as well as new, innovations in livestock health, genetics, feed and market systems. SAPLING aims to demonstrate that improvements in livestock productivity can offer a triple win: generating improved livelihoods and nutritional outcomes; contributing to women's empowerment; and, reducing impacts on climate and the environment. Its seven focus countries are Ethiopia, Kenya, Mali, Nepal, Tanzania, Uganda and Vietnam.