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Certificate Course Compendium

on

"Recent Advancement in Reproductive and Infertility Management of Dairy Animals"

Organized by:

College of Veterinary Science & Animal Husbandry

U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura-281001 (U.P.) (INDIA)

Under

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(From 24 January to 06 February, 2023)

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BOOSTING REPRODUCTIVE PERFORMANCE OF DAIRY ANIMALS BY NUTRITIONAL INTERVENTION

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Introduction

The diet-hormonal-gonadal axis is a well-established, highly explored, but one of the least-understood links in livestock science. Feed quantity, frequency, composition, processing and quality affect the reproductive performance of the livestock directly and indirectly. These effects are mediated by metabolic by-products, feed-regulated neurotransmitters, immunity and antioxidant status of the animal. The relationship exists in both sexes and is more pronounced under critical deficiency or excess of any specific nutrient.

A high level of milk yield in cattle requires high levels of dietary protein and energy. Protein quantity, its fermentation rate in the rumen and its composition affect the circulating concentrations of progesterone. This affects the uterine environment and fertility. As dietary protein utilization is energy-dependent, the negative energy balance (NEBL) frequently encountered after parturition in dairy animals may have a damaging cascading effect on the reproductive performance of the animal.

Energy balance and reproduction

A significant portion of the nutritional requirements of a lactating animal is due to its milk and milk fat yield. There is an abrupt increase in nutrient outflow post-parturition, and as milk production rapidly increases, the feed intake fails to meet the increasing requirement. This results in NEBL. The dairy cows go under NEBL a few days before calving and usually reach their most negative level about two weeks later. The severity and duration of NEBL during the first month postpartum are highly correlated with the days to first ovulation, which is an important reproductive parameter. It is well known that cows over-conditioned at calving will exhibit decreased appetite and develop more severe NEBL than cows of moderate conditioning. Over-

conditioned cows undergo increased mobilization of body fat and accumulate more triacylglycerols in the liver. This is associated with a longer interval to first ovulation and reduced fertility. Each 2400 Kcal net energy NEB may delay the first ovulation by 1.25 days.

The first ovulation postpartum reflects the recovery from the hormonal conditions of late pregnancy. Following parturition, multiple follicular development occurs in a week as a response to an increase in FSH concentrations. The follicular wave is not affected by NEBL. However, three possible outcomes of follicular development are 1. Ovulation of the first dominant follicle in 16–20 days postpartum; 2. Non-ovulation of the first dominant follicle followed by a turnover and a new follicular wave; 3. The dominant follicle becomes cystic. The second and third scenario prolongs the interval for first ovulation to 40 or 50 days postpartum. Ovulation of a dominant follicle during early lactation depends on the re-establishment of pulsatile LH secretion. The NEBL represents a physiological state of under-nutrition which impairs LH secretion and deters ovulation. The follicles emerging after the NEBL exhibit more remarkable growth and diameter, enhanced estradiol production and are more likely to ovulate. Low energy availability during NEBL suppresses pulsatile LH secretion and reduces ovarian responsiveness to LH stimulation.

During postpartum NEB, glucose is preferentially partitioned to the mammary gland, pancreatic insulin secretion in response to glucose is suppressed, peripheral tissues exhibit insulin resistance, and cows are susceptible to metabolic disorders. Glucose is known to have a direct effect on the hypothalamus, which causes the release of GnRH, which in turn causes LH release from the pituitary. Decrease in plasma glucose and insulin levels are observed in NEBL cows, and insulin stimulates bovine follicular cells. In addition, plasma levels of IGF-I are directly related to energy status, and IGF-I is critical to ovarian follicular development. Furthermore, plasma estradiol concentrations were highly correlated with plasma IGF-I levels. During the NEBL period, the capacity of follicles to produce estradiol required for ovulation is compromised as it depends on the insulin and IGF-I levels in serum.

Since the extent of NEBL depends upon dietary energy intake relative to requirements, nutritional strategies to minimize NEBL are a priority. Increasing dietary energy intake by feeding more concentrates is not practical after a certain level due to problems with rumen fermentation, milk

composition and health. Alternatively, increasing dietary energy density by increasing the lipid content could alleviate the limitation of feed intake. Increasing concentrate supplementation during the last two weeks pre-partum and supplementation of bypass fat postpartum may help increase postpartum resilience to lactation feed and high outflow of nutrients.

Direct assessment of energy balance is not always possible under field conditions, but changes in body condition score (BCS) provide an indirect measure. Cows losing one unit or more BCS on a five-point scale during early lactation are at higher risk for low fertility. Minimizing the interval to first ovulation provides sufficient time to complete multiple ovarian cycles before insemination, which improves the conception rate.

Another important link between NEBL and fertility may be the carryover effects on blood progesterone concentrations. The rate of the increase in progesterone levels is affected by NEBL in the early postpartum period. The progesterone level from one cycle has a carryover effect over the succeeding cycle. As optimum progesterone level is vital for fertility, diminished progesterone levels during NEBL condition negatively affect the progesterone levels during follow-up cycles as well. The progesterone levels, therefore, stay sub-optimal even after the animal has recovered from NEBL.

Dietary protein and reproduction

Diets high in crude protein 17% to 19%, are typically fed to stimulate and support high milk production in dairy animals. However, high protein diets have been associated with reduced reproductive performance. Successful embryo development depends upon the nature of the uterine environment. Intake of high protein diets by lactating animals alters the pH and the concentrations of other ions in uterine secretions during the luteal phase.

High dietary protein intake elevates the serum concentrations of ammonia and urea. This depends on the degradability of protein in the rumen and the availability of fermentable carbohydrates. High concentrations of urea nitrogen in dairy cows inhibit follicular development and fertilization and decrease the binding of luteinizing hormone to its receptors on ovaries and progesterone binding capacity to ovaries receptors. Further, plasma urea is inversely related to uterine luminal pH. Increased plasma or milk urea nitrogen concentrations are highly correlated with a reduction in uterine pH. This also interferes with progesterone's inductive actions on the uterus's microenvironment. This causes suboptimal conditions for support of embryo

development. Urea also increases the secretion of $PGF_2\alpha$ which may interfere with embryo development and viability.Supplementation of protected protein and amino acids helps in keeping BUN in optimum range.

Micronutrients and reproduction

Calcium is generally adequate in forage-based diets but is often included in commercially available mineral supplements because many phosphorus sources contain calcium. Much debate and research have been conducted on the effects of phosphorus supplementation on reproductive function. Phosphorus and crude protein content generally parallel each other in pasture or rangeland. Mature forages are usually deficient in phosphorus, and impaired reproductive function has been associated with phosphorus-deficient diets. Therefore, diets should be evaluated for phosphorus content and supplemented accordingly. Magnesium, potassium, chlorine, and sulfur deficiencies and excesses can contribute to suboptimal reproductive function.

Most of the vitamins are either synthesized by rumen microorganisms, synthesized by the body or are available in common feeds and are not of concern under normal conditions. Vitamin A deficiency, however, does occur naturally in cattle grazing dry winter range or consuming low-quality crop residues and forages. The role of vitamin A in reproduction is well established, and supplementation before and after calving can increase conception rates.

Conclusion

The interactions of nutrition on reproductive performance in dairy cattle involve energy and protein quantity and proportion and their adequacy relative to requirements for high milk yield. Therefore, the observed decline in fertility may be attributed to the combined effects of a uterine environment dependent on progesterone but has been rendered suboptimal for embryo development by antecedent effects of NEBL and may be further compromised by the impact of urea resulting from intake of high dietary protein.

LUTEAL INSUFFICIENCY AND ITS REMEDIAL MEASURES FOR IMPROVING CONCEPTION IN BOVINES

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The establishment of pregnancy following insemination is the primary goal in dairy business (Lucy, 2019), at this juncture, clinical procedures to improve fertility in high-producing dairy herds leave much scope for improvement. It is one of the most important causes leading to infertility; it is defined as reduced progesterone production by the corpus luteum, either in the amount or duration, or both characterized by primary infertility and pregnancy loss during the early embryonic (day 8-18 post breeding or early fetal period (30–50 days post-AI). Low plasma P4 concentrations during the luteal phase post-artificial insemination (AI) are associated with lower conception rates. Treatments post-AI with P4, gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) improve fertility in few conditions. Low plasma P4 concentrations during the luteal phase post-AI have been extensively associated with lower conception rates (Lukaszewska and Hansel,1980; Lukaszewska and Hansel,1999). This demands treatments post-AI with P4, gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) to improve fertility. In effect, the results of recent meta-analyses, including data from a total of 59,584 cows, indicate that P4, GnRH or hCG treatment in the early luteal phase of pregnancy improves fertility, particularly in cows of lower fertility (Nascimento et al.,2016;Yan et al., 2016; Besbaci et al., 2020). Certainly, poor luteal activity following ovulation may turn a cow into a repeat breeder (Gustafsson et al., 1988 & 2002), pregnancy diagnosis is commonly performed in the late embryonic period, and up to 20% of pregnancies are lost within 30–50 days of gestation (Grimard et al., 2006; López-Gatius et al., 2012). Beyond this time interval, the risk of losses is much lower. In a similar way to the luteal period post-AI, reduced plasma P4 concentrations at pregnancy diagnosis have often been associated with pregnancy loss (Ealy, 2019;Szenci, 2021). The CL regression

causes embryonic death, or if vice versa, luteal regression is detected at least 3 days after the detection of the embryonic death.

The main bottleneck in the success of Indian dairy industry is the reduced reproductive efficiency of our animals. A calving interval of 12 or 13 months is usually recommended to be optimal for the high milk yield and reproductive efficiency of dairy animals, and is economically viable to the dairy farmers (Murugavel, 2003). Repeat breeding (RB) syndrome continues to be a major problem in cattle and Buffalo breeding, leading to substantial economic losses to the dairy farmers (Lafi et al.,1992). Being multi-factorial etiological concern, luteal deficiency is one of the predisposing factors causing low fertility due to early embryonic mortality (Diskin and Morris, 2008). Low plasma progesterone may affect reproductive processes before and after insemination. The situation has aggravated in recent years because rise in milk production is associated with a gradual decline in plasma progesterone, consistent with the negative relationship between milk production and progesterone concentrations (Lucy, 2001). High environmental temperatures (Wolfenson et al.,2000), bacterial endometritis (Bouters, 1985), nutritional problems (Stevenson, 2001), high milk production, and excessive dry matter consumption (Vasconcelos, 1998) may cause accelerated metabolism of progesterone in liver, thus leading to low plasma progesterone during luteal phase of estrous cycle triggering early embryonic losses. Corpus luteum is a transitory endocrine gland and a main source of progesterone secretion in bovines. During a normal luteal phase, the CL increases in size along with its ability to secrete progesterone. Once it has reached its mature size and attained its maximum potential for secretion of progesterone, luteal function is maintained for a few to several days depending on the species, and then if the animal does not become pregnant, luteal regression occur to initiate the next cycle (Niswender et al.,2000). The preovulatory surge of gonadotropin (LH) induces ovulation and differentiation of residual follicular cells that form the corpus luteum and begins to produce progesterone at high rates (Niswender et al.,2000).

There are two types of the cells in corpus luteum i.e. Small luteal cells (SLC), which appear to be of thecal origin, contain most of the LH receptors and are more sensitive to LH stimulation, leading to increased progesterone production. Large luteal cells (LLC) are of granulosa cell origin and have few of the LH receptor sites and most of the PGF2α receptors. LLC also produce and store oxytocin. Small and large luteal cells differ in their basal secretion rate of progesterone with LLC producing 2 to 40 fold more progesterone than

SLC. Granulosa and theca cells function co-ordinately to produce estradiol during follicular phase (Fortune and Quirk, 1991). Theca cells produce androgens from cholesterol, which is converted to estradiol by granulosa cells (Bao and Gaverick, 1998). The synthesis of progesterone is accomplished by increased expression of enzymes necessary for conversion of cholesterol to progesterone and decreased expression of enzymes that convert progesterone to estrogen. Estrogen from the developing dominant follicle activates oxytocin (OT) release from the posterior pituitary which in turn causes PGF2α release from the uterus (McCracken et al.,1996). Estrogen also lowers the action potential of uterine smooth muscle tissue, rendering it more sensitive to oxytocin. Prostaglandin $F_{2\alpha}$ synthesized by uterine endometrium reaches the ovaries through a counter-current exchange system from the uterine vein to the ovarian artery (Senger, 1999) where it acts to both initiate luteolysis and up-regulate further OT and PGF2α release from luteal and uterine sources, respectively (Niswender et al., 2000). Functional luteolysis begins primarily as a result of reduced blood flow to the CL through apoptosis of luteal tissues and corresponding reduction in capillary density. In addition to release of oxytocin from luteal cells, prostaglandin F2α also stimulates endothelin-1 production from luteal endothelial cells. Thus, at early stages PGF2α initiates a positive feedback mechanism on oxytocin and subsequently itself (Flint et al., 1990). Endothelin-1 acts to constrict ovarian capillaries and inhibit steroidogenic activity of luteal cells (Girsh et al., 1996). As blood flow is decreased and cellular machinery stopped or destroyed, there is a marked increase in ovarian populations of leucocytes, T-lymphocytes, and macrophages which facilitate apoptosis of the luteal tissues. As luteolysis progresses circulating progesterone is decreased, removing the negative feedback on the hypothalamus and anterior pituitary. Estrogen is secreted from the developing preovulatory follicle and signals an increase in the release of GnRH and LH, Thus cascade of events continue again.

In buffalo heifer, plasma progesterone was around 1 ng/ml till day 6, followed by a gradual increase to 4.89+0.40 and 5.12+0.41 ng/ml, respectively on day 15 post-estrus (Takkar et al., 1983). In another study, peak plasma progesterone was observed on day 16 of estrous cycle (3.47 ng/ml; Pahwa and Pandey, 1983). Infertile buffaloes had a combined pattern of delayed rise and low plasma progesterone indicative of insufficient luteal function (Kavani et al., 2005). Moreover, in repeat breeding buffaloes, plasma progesterone was higher on day 0 than the regular breeding buffaloes (1.04+0.57 vs 0.14+0.01 ng/ml), whereas, on day 7 plasma progesterone was lower in repeat breeder buffaloes (2.63+0.95 vs 4.05+0.01 ng/ ml (Venkatesan et al., 2005).

Major causes of luteal insufficiency in bovines

1. Genetic or congenital defects of ovaries

Hereditary defects are usually due to single gene effects, some are sex limited and others may cause adverse effect on both sexes. Severity of the abnormality decides the effect. Most important conditions are as following;

a. Ovarian hypoplasia: It is a condition where the ovary undergoes incomplete development and a part or whole of ovary lacks a normal number or compliment of primordial follicles. Normally both ovaries in cattle have 50,000 to 100,000 primordial follicles but partial hypoplastic heifers have 500 primordial follicles or no follicles. It is caused by single recessive autosomal gene with incomplete penetration. The affected ovary may be partially or totally hypoplastic. If it is unilateral then results in infertility and if bilateral, sterility may develop.

b. Agonadless condition: It is caused by inherited autosomal dominant gene and lacks one or both gonads. The heifers apparently looks normal until breeding age and no normal development of udder and exhibition of estrum occur, on examination it was found that genital tracts were juvenile and underdeveloped.

c. Segmental aplasia of Mullerian duct or paramesonephric duct and imperforate hymen (White Heifer Disease): White heifer disease is a congenital defect of the reproductive tract where there is segmental aplasia of the Mullerian or Paramesonephric ducts, especially an imperforate hymen, this condition is associated with white coat color. It has been recorded to occur in 10 % of Shorthorn breed, also reported in Holstein, Jersey, Ayrshire and Guernsey breed. It is caused by Single Recessive Sex Limited Gene with linkage to the gene for white coat color, such animals are invariably sterile.

2. Nutritional deficiency

During early lactation in cattle and buffaloes, usually it is not possible to achieve adequate energy intake to sustain production. It is maintained by using body reserves, primarily fat thus, there is considerable weight loss during early lactation. This period of weight loss precedes and sometimes overlaps the time during which the cow must be bred successfully to achieve calving intervals of 12-13 months. Energy status has frequently been thought to be related to post partum fertility. In one of the studies, it

was found that those cows gaining weight at the time of breeding resulted in 67% conception with 1.5 services per conception and those losing weight, 44% conception with 2.32 services per conception. Animal deficient in energy and protein may result in deficiencies of growth hormone or insulin with resultant low IGF-1 secretion. Low IGF-1 account for poor development of a pre-ovulatory follicle ultimately results to inadequate luteal tissue and subsequently lower progesterone production compromising optimum conception rate. Good fertility is associated with higher blood progesterone concentrations. There is evidence that progesterone concentration is related to energy balance. Cows on a high plane of nutrition required fewer inseminations and conceived earlier than cows on standard level of nutrition with earlier return to cyclic ovarian activity. There was tendency for animal gaining weight to have higher progesterone concentration preceding the first insemination as well as higher conception rates. Heifers on the lower energy diet had lower serum and corpus luteum progesterone concentration and elevated serum LH suggesting that LH release was not impaired and there may be decreased ovarian response to LH with restricted dietary intake. It is suggested to maximize dry matter intake of high energy feed early in lactation both for maximum milk production and for best reproductive performances.Under our field conditions, over conditioning of the animal is not a problem whereas most of the animals are suffering from under nutrition where dry matter intake does not meet increased energy requirements, a status of negative energy balance develops. Negative energy balance may begin pre-partum in association with declining feed intake. During the first three weeks of lactation, negative energy balance delays early ovulation and recovery of postpartum reproductive function and provides the major nutritional link to low fertility in lactating dairy cows.

3. Hormonal deficiency

Many studies concluded that average skim milk progesterone concentration at mid-luteal phase in cows was ≥2.0 ng/ml (Kawata et al., 1987); progesterone concentrations that increase a few days after estrus and remain elevated for approximately 2 weeks are the strong indicator of a normal estrous cycle (Lamming, and Darwash, 1998). Any deviation from this pattern is likely to be associated with reduced fertility (Kawata et al., 1987). Delayed CL formation was associated with a marked and progressive reduction in pregnancy rate in cattle (Lamming and Darwash, 1995). Perhaps due to asynchrony between the uterus and the embryo. In

contrast, cows with an early postovulatory increase in progesterone and high luteal phase progesterone concentrations had larger embryos 15–17 days after AI; these embryos produced larger amounts of interferon tau (INF-τ) (Walton et al., 1997), (Mann and Lamming, 2001) and therefore the pregnancy was more likely to be maintained. Mated cows with a short luteal phase failed to maintain pregnancy, although fertilization, early embryo development and transport of the embryo into the uterus appeared normal (Inskeep, 1995). A major factor associated with the short luteal phase was premature luteolysis (Hunter, 1991). This earlier development of luteolytic mechanism would not allow enough time for the embryo to develop and produce sufficient amount of INF-τ to adequately block luteolysis.

4. Environmental stress

It has been reported by several workers that heat-stressed dairy cows do not eat much dry matter which affects plasma insulin, glucose and IGF-1, and this decrease in hormone concentrations due to a prolonged negative energy balance may compromise follicular development, resulting in a reduced estrus intensity and induction of poor quality oocytes. It was clear that the conception rate of the lactating dairy cows decreased during the summer season. It is thought that an increase in the vaginal temperature along with an increase in the THI to over 80 on day 1 before AI may have caused the reduced conception rate, and the THI>80 during days 15–17 after AI may be a risk factor for early embryonic development. In addition, it was estimated that mTHI 69 was an appropriate index value for the start of heat stress management during the summer season, and this management may be needed for at least five months from June to October.

5. Functional disturbances of ovaries.

Many times it happens that apparent cause of failure of infertility is not known and difficult to diagnose possible reason as it related to functional disturbances of the either ovarian secretion of hormones in response to gonadotropic stimulation or delayed response of the ovary or it may not respond to specific releasing factors acting on the ovary and it becomes refractory to certain level and we understand that it is a case of repeat breeder. Repeat breeders cattle are typically cows that fail to become pregnant in a 60-90 day breeding season or to three or more artificial inseminations (AI). These cows can represent a substantial economic loss to the dairy industry and with increased production becoming a necessity

for current cattle production industries, new methods to improve pregnancy and calving rates to fewer breeding attempts will be required in such conditions to improve conception rates. Earlier studies have demonstrated that P4sup-plementation initiated prior to day 6 improved pregnancy rates over controls while supplementation after day 6 did not yield any significant results (Mann and Lamming, 1999). From various studies it can only be concluded that early P4is beneficial. Supplementation of P4 to repeat breeders' cows on days 3-5 following breeding resulted in increased pregnancy rates. These increased pregnancy rates are likely the result of an induced rise in P4between days 3 and 5. These results indicate that an increase in plasma P4from day 3 to day 5 of early pregnancy in the cow is beneficial to early embryo development

6. Miscellaneous causes.

i. High prolactin concentration.

ii. Injudicious use of oxytocin for milk letdown.

iii. Subclinical uterine infections.

iv. Excessive manipulation of genitalia at the time of reproductive interventions.

Remedial measures for luteal insufficiency: Luteal deficiency can be addressed either at post breeding window of day 0-12 with hormonal combinations or non-hormonal anti-luteolytic strategies.

Hormonal Approaches

1. Administration of GnRH: GnRH has primary effect at pituitary gonadotropes to stimulate pulsatile release of gonadotropins i.e. LH and FSH. LH is essential for the maintenance of progesterone production by luteal cells. Pre-ovulatory LH surges induce chain of events in the ovulatory follicle that are essential for the formation of a normal CL.

(i). GnRH administration on day of estrus: It has been demonstrated that overall advantage in pregnancy rate of 18-25%.In repeat breeding dairy cattle, after treatment with 10µg GnRH at the time of insemination, the conception rate for treated and control was 48.1 and 31.0 %, respectively (Bon Durant et al., 1991). About 54.2 % repeat breeder conceived after treatment at the time of insemination. Similarly, an improvement in conception rate (50% vs 20%) after 20µg GnRH in repeat breeders.

(ii). GnRH administration on day 5 of estrous cycle: Plasma progesterone concentrations have been positively correlated to volume of uterine secretions, conceptus development and ability of embryo to secrete interferon- tau (IFN-ι), embryo viability and ultimately conception (Campanile et al., 2008). Administration of GnRH on day 6 of estrous cycle induced accessory CL development in 75% cattle, higher luteal tissue development and increased plasma progesterone concentration.

(iii). GnRH administration on day 12 of estrous cycle: GnRH administration between days 11-13 post-insemination can boost the function of CL followed by increased progesterone. This response is due to stimulation of small luteal cells to LH that is released in response to GnRH. On an average 17% increased in conception rate was recorded. In normal cyclic animals first service conception in buffaloes was 70% when administered 10µg on day 12 from 50%.

2. Administration of hCG: It has biological activity similar to LH and hCG and can bind to LH receptors on small luteal cells to enhance progesterone production.

(i). hCG on day of estrus: Administration of hCG at specific time coincident with presence of dominant follicle stimulates CL function. First service conception was 62.5% vs 18.75% in hCG treated and non-treated repeat breeder cows (Selvaraju et al., 2004). Administration of 1500 IU hCG lead to significant improvement in conception rate.

(ii). hCG on day 5 of estrous cycle: On day 5 of estrous cycle, granulosa cells of the dominant follicle contains LH receptors, thus it may induce ovulation and formation of an accessory CL. Administration of hCG on day 5 @3000IU lead to presence of more than one CL in 86.2% cattle and led to increased progesterone in circulation inturn producing more concentration of Interferon-tau.

(iii). hCG on day 12 of estrous cycle: LH like activity of hCG may provide luteotropic stimulation to CL through conversion of small luteal cells to large luteal cells. Conversion of small luteal cells to large luteal cells and also conversion of stage-two luteal cells to stage–three luteal cells is reduced. These stage–two luteal cells secrete more progesterone compared to stage–three luteal cells. Administration @1000IU day 12-14 results in induced accessory CL, thus leading to 35% increases in conception rates in lactating cows.

3. Supplementation of progesterone between days 4 -10 of estrous cycle: Histotrophic environment for optimum nourishment and growth of free floating conceptus is created due to immediate action of exogenous progesterone. Supplementation of progesterone may also enhance pregnancy rate by blocking luteolysis mechanism due to embryotrophic effect which may be injected @750 mg weekly I/M for about three weeks.

4. Nutritional strategies: Majority of the reproductive disturbances could be alleviated by supplying the feed with balance nutrients as fat is one of the essential nutrients which have positive effect on the fertility in all the farm animals especially in dairy cattle. Dietary fats increase concentration of circulating cholesterol, progenitor of progesterone. Polyunsaturated fatty acids (PUFAs such as Omega -3 and Omega-6 are essential component of fatty acids. Omega -3 fatty acids play an important role in follicular development, oocytes maturation, MRP, embryonic survival and increasing conception rates. Omega-6 fatty acids have been associated with ovulation, parturition and post partum ovarian rebound phenomenon. Cows receiving Omega-3 fatty acids had the greatest average CL size and PUFA has ability to inhibit the action of COX-1 and COX-2 enzymes. It has been reported that cows fed with fishmeal had reduced plasma PGFM concentrations.

5. Miscellaneous remedial measures: Non-steroidal anti-inflammatory drugs like flunixin meglumine @ 1.1mg/kg body weight i/m twice on the evening of day 15 and morning of day 16 after insemination improves conception rates (Guzeloglu et al., 2007). They opined that two doses of the COX-inhibitor at critical period delayed the PGF2α synthesis and luteolysis of corpus luteum. Administration of Meloxicam @0.5 mg/kg body weight i/m once daily on days 15 and 16 of the estrous cycle increased conception rate by 20% than control i.e. 53.33 vs 33.33% (Rajkumar, 2008). The hormone levels were depressed as the temperature increased from May to September, but were always higher in the cooler months. Conception rate during this period was 31 % in the experimental group and 14% in the control group. High humidity increased the effect of high temperature (Ingraham et al., 1974) and the average temperature-humidity index of the 2^{nd} day before insemination was most important for conception.

TECHNOLOGIES FOR IMPROVING REPRODUCTIVE EFFICIENCY IN DAIRY ANIMALS

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Productivity largely depends upon reproduction, which in turn is influenced by several factors including genetic, nutritional, hormonal, physio-pathological and management practices. The reproduction efficiency is determined by the combined effect of heredity and environment. Reproductive efficiency has generally a low heritability value indicating that most of the variations in this trait are due to non-genetic factors. Reproductive disorders and associated Infertility (transient loss of fertility) among cattle and buffaloes pose serious economic loss to farmers in terms of low returns and veterinary expenses. Due to impaired reproduction ability, the calving to conception (days open) period is prolonged leading to extended calving interval, which jeopardize the aim of obtaining a calf per cow per year. To realize the dream of "one calf per cow per year" sincere and concerted efforts are required to apply "promising reproductive technologies" at field conditions in large scale. Maximizing reproductive efficiency requires the matching of genotypes to the production environment, together with appropriate husbandry practices, in order to ensure that the intervals from calving to conception are short and the rates of conception to natural or artificial breeding are high. For better economic efficiency and competitive superiority of dairy farming, a thorough knowledge on reproductive efficiency indicators, their application in the routine farm operations and overall improvement of specific reproductive parameters are at most important. The information contained in the following sections of this paper is essentially a compilation of facts published by different researchers including the author in various seminars and conferences for the purpose of teaching and training.

Factors influencing herd reproductive efficiency

1. **Puberty and sexual maturity:** Attaining puberty and sexual maturity at appropriate time is a foremost requirement for reproductive success in dairy animals. Onset of puberty and sexual maturity are due to complex

interplay between genetic and environmental factors, including nutrition, disease, temperature and season of birth. Estimates of age at puberty in dairy animals in the tropics and subtropics range between 16 and 40 months. Bos indicus cattle reach puberty later than exotic and cross breed animals. On average, the zebu reaches puberty 6 to 12 months later than *Bos taurus* cattle. Heritability of age at puberty, at first conception and at first calving are generally low indicating that these traits are highly influenced by environmental factors. Improper nutrition and exposure to stress environment during growing period affect onset of puberty. Animals with average daily weight gain (ADG) of 500 – 600 g attain puberty and sexual maturity at right time than those that gain less ADG.

2. **Estrus detection efficiency and accuracy:** Accurate and efficient detection of estrus plays important role in reproductive management of dairy animals. The duration and intensity of estrus, varies between and within breeds of cattle and as many as 1/3rd of dairy herds have significant oestrus detection accuracy problem. Selection of animals for high milk yield has declined the percentage of overt signs and duration of estrus. Over the past 30 to 50 years, the percentage of animals showing standing oestrus and duration of standing estrus has declined from 80% to 50% and 15 h to 5 hr respectively that occurs in parallel with a reduction in conception rate from 70% to 40%. Increased incidences of lameness and mastitis have negative effect on intensity of estrus expression. Heat stress associated with poor expression of estrus in cows and silent heat or summer anestrus in buffaloes. Wise and efficient use of heat detection aids along with visual observation improves the heat detection rate. Synchronization of estrus with use of reproductive hormones also increases the probability of detecting estrus at appropriate time.

3. **Body condition score:** Body condition score (BCS) provide an indication of the energy status of dairy cattle and is linked with reproductive performance in dairy animals. Negative energy balance during peripartum period and the resulting low BCS cause a delay in the time to first ovulation and thus increase the percentage of cows that are anovular and also increase the incidence of postpartum uterine diseases. BCS measured 30-60 days postpartum has the strongest relationship with reproductive performance and multiple BCS measurements can increase this relationship. Maintaining BCS between 3-3.5 in dry cow and postpartum period increases the postpartum reproductive efficiency by increasing the postpartum conception rate. For heifers less than six months old, their

body condition score should range from 2.0 to 3.0. Usually heifers should not exceed 3.5 in body condition score. A body condition score of 2.5 to 3.0 is desirable for heifers from six months old up to breeding age. At breeding, and shortly thereafter, their body condition scores may gradually increase from 3.0 to 3.5.

4. **Post-partum uterine health:** Bacteriological contaminations (both Gram-positive and Gram-negative aerobes and anaerobes) of the uterus occur 1–4 weeks after calving in most of the cows but self-cure usually occurs due to action of uterine defense mechanism within 6 weeks postpartum. Cows unable to eliminate infection lead to persistent of infection resulting metritis-endometritis- pyometra complex. If infection persist beyond 3 week postpartum, results in endometritis and it has been reported 15%–20% of cattle have clinical endometritis and about 30% have subclinical endometritis. The incidence rate of uterine infection in cattle and buffalo in India is quite high i.e. 24.7-38.54 %. Negative energy balance in early post-partum dairy cows is associated with altered metabolic environment such as reduced concentrations of glucose and IGF1 and raised concentrations of non-esterified fatty acids (NEFAs) and β-hydroxybutyrate (β-BHB) that may alter the secretion of inflammatory cytokines lead to onset of uterine diseases and also have a negative effect on ovarian activity. Delay onset of ovarian cyclicity hamper the process of microbial elimination from uterus.

5. **Metabolic disorders:** Metabolic diseases like milk fever and ketosis are important predisposing factors that lead to increased incidence of several other transition cow disorders like mastitis, reproductive problems (dystocia, RFM) compromising reproductive efficiency. Increased incidences of milk fever and sub clinical hypocalcemia (5-33%) increased days to first service and increased services per conception. Negative energy balance during transition period increased incidences of subclinical and clinical ketosis adversely affecting cow fertility leading to increase days to first insemination and days open and decrease conception rate.

6. **Mastitis:** Mastitis is one of the most costly diseases in dairy animals because of its direct impact on milk production. Mastitis indirectly impair reproductive performance in dairy cows due to alteration of inter-estrus intervals and shortening of the luteal phase due to premature luteolysis, increased days to first service and days open and greater risk of abortion and increases culling rate and decreases reproductive efficiency in dairy cows.

Milk production vs. reproduction

Milk production and fertility are two economically important traits affecting profitability in dairy cattle. During the recent decades, no doubt, intense genetic selection has increased milk yield. However, selection has also changed the reproductive physiology of the cow and led to a decrease in reproductive efficiency. There is a long history of associating greater milk production with reduced reproductive performance in dairy cattle. Tracing back to the history of selection of dairy animals, one can evidently note that animals were selected mainly for milk production ability and reproduction parameters have not been given due importance. Based on the analyses of large datasets, there is clearly an antagonistic relationship between milk production and reproduction in dairy cattle; however, further studies are needed to confirm this trend. Based on the available information, it is reasonable to suggest that demands of high milk production negatively impact a number of physiological pathways to reduce the likelihood of the concomitant establishment of pregnancy. Although the decline in dairy herd reproductive efficiency is chiefly related to changes in management of the female, it is logical to question the portion of this decline that can be attributed to the males. Most scientific surveys on the dairy industry, suggest that fertility of bulls has declined over a period.

What could be the approach to improve fertility in dairy animals?

- **Long-term:** Since selection for the single trait of milk production with little consideration for traits associated with reproduction in the modern dairy cow has produced an antagonistic relationship between milk yield and reproductive performance, due consideration should be given to traits associated with reproduction while selecting the animals for high milk production.
- **Short term:** Fertility in dairy animals can be improved using potential reproductive technologies like semen from high fertility sires, controlled breeding, post-insemination fertility enhancement treatments and assisted reproductive technologies. However, these practices do not address the fundamental need for correcting the underlying genetics for reproduction in high-producing dairy cows.

Potential reproductive technologies for field application

Multiple ovulation and Embryo Transfer: Embryo transfer has played an important role in genetic improvement of dairy cattle over the past several

decades. In USA 99% of currently available Holstein AI sires and 95% of currently available Jersey AI sires were produced via ET. Developments in superovulation protocols and non-surgical embryo transfer have made MOET technology viable for commercial application. NDDB established Embryo Transfer (ET) facility at SAG, Bidaj in 1987 and ET technology has been used in bull production programs. Similarly, several research and development institutes also started MOET, although not in large scale, and shown considerable progress. But in between there was a time lapse when the technology was not in use. The technology needs to be standardized to specific breeds and then implemented at field conditions. For instance, it is said that older cows (>10 years) are generally not to be used for super ovulation as the expected embryo recovery is low. However, recently we carried out MOET in Deoni breed at Southern Regional Station of ICAR-NDRI, Bengaluru and obtained 14 transferable embryos from a old Deoni cow (13.6 years age) indicating that indigenous breeds may have higher oocyte reserves and owing to their reproductive longevity old animals may also be a good donor, a finding that needs to be confirmed on large sample size.

In vitro embryo production and transfer: As an alternative to collecting embryos from donor animals, methods have been developed recently to produce embryos in vitro. In vitro embryo production (IVP) involves collection and in vitro maturation (IVM) of the oocytes, in vitro fertilization (IVF) of matured oocytes and in vitro culture (IVC) of embryos up to a stage that is compatible with its transfer to the recipient uterus. Interestingly, Ovum Pick-up (OPU) and in vitro embryo production gained momentum during recent years. Ovum pick up is a nonsurgical technique that uses ultrasound and a guided needle to aspirate immature oocytes from the ovaries. One of the recent breakthroughs in the practical world of animal reproduction is the combined application of the existing IVF technology and the state-of-the-art OPU technique in cattle. Although, a cow may ovulate only about 200 oocytes in her life time, there are lakhs of oocytes in her ovaries. While a cow normally produces one viable oocyte during each estrus cycle, up to 50 antral follicles exist on the ovary at any given time of the estrus cycle. Via OPU, potentially a valuable donor cow may yield 15-20 oocytes each week or about 700-1000 oocytes/year/cow. Assuming a 30% blastocyst rate from those oocytes, and a 40% pregnancy rate, a cow may potentially offer 200-300 blastocysts or 80-120 pregnancies each year. The birth of “Holi” a Sahiwal calf through OPU technique at NDRI, Karnal and a buffalo calf at GBPANT University, Pant Nagar is living evidence the OPU can be used as a tool for conservation of important species of animals. Besides this few private players

are also into this technology at field conditions. However, to exploit its full potential, a reliable IVF system and a dedicated OPU team are needed.

The above-said two technologies are well proven and the scope of these technologies is immense in faster multiplication of superior germ plasm. However, as mentioned earlier, the cost involved, necessity for continuous funding and skill development and import of almost every component used in MOET/IVEP from other countries are few constraints for its less frequent use. But the time has come that the application of this potential technology needs to be expanded at field conditions in mission mode so that the superior germ plasm that is in great demand but limited in availability can be multiplied at faster rate.

Use of sexed semen: Use of sexed semen for artificial insemination is recognized as more pragmatic and easy way to pre-select the sex of the offspring. Selective use of sexed semen in breeding will not only increase the genetic progress from the daughter-dam path but would also help in producing good male germplasm from elite bulls for future breeding. Combining MOET/IVEP with sexed semen is further advantageous to multiply superior germ plasm in a shorter time. The major reasons demanding use of sexed semen in our country include, but not limited to, limited number of high producing cows, limited availability of elite bulls, large number of unproductive animals and shortage of inputs. In the given situation, wherein the males (especially crossbred) are not used for draught purpose and the incidence of infertility is quite high, the farmers do not rear males and let them free on the roads causing environmental, social and ethical concerns in the country. Thus application of this technology in crossbreds is an immediate need. In our preliminary and tentative assessment, it is observed that an additional 14 million tones of milk can be obtained just using sexed semen in crossbred cattle to skew the sex ratio towards female with 90 per cent accuracy. Further, using sexed semen, the number of male calves produced can be brought down to 1.9 million from 9.7 million, thus reducing the burden on the farmers who are baffled with the crossbred male calves. Further, use of sexed semen in dairy animals will facilitate production of required number of daughters for progeny testing programme in shortest time, thus increasing the genetic gain.

Controlled breeding programs: In the recent past, several protocols have been developed and/or modified to allow timed inseminations so as to circumvent the practical difficulties associated with estrus detection. Some of the protocol has advantage of synchronizing ovulation as well and they can be

applied on large scale to improve the fertility in dairy animals. Ovsynch protocol is one such, in which GnRH is administered on day 0 of start of treatment (irrespective of the stage of estrous cycle), prostaglandin F2α is administered on day 7 and again GnRH is administered on day 9. The animal may be inseminated at 16 to 22h after the administration of second GnRH. The success rate of this protocol is quite high and being practiced regularly at several farms. A modification of Ovsynch protocol, called as Doublesynch protocol has been developed by incorporating an additional $PGF_{2\alpha}$ administration at 48 hour before the start of Ovsynch protocol and claimed to yield superior results. The CIDR - GnRH – Based protocol involves insertion of the CIDR on day1and withdrawal of the CIDR on day 8. An injection of GnRH is given on day of CIDR insertion. On the day of CIDR withdrawal, an injection of prostaglandin is given. The second GnRH injection is given after two days of prostaglandin injection. The advantage of inclusion of the CIDR in GnRH-based programs is that the animal is exposed to progesterone during the period between day 1 and day 8 which will prevent early onset of estrus and ovulation between days 1 and 9 that are inherent to the GnRH-PG systems. Although all these protocols have been shown to improve fertility, it is important that nutritional status of the animal, sanitary management and skill of the can influence significantly on the success rates.

PRODUCTION AND METABOLIC DISEASES OF DAIRY ANIMALS

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The "production diseases" are disorders related to production or management factors, but their pathogenesis is primarily related to alteration in metabolism. The term "metabolic diseases" in true sense refer to those disorders that arise due to error in metabolism; the error may be either inherited or acquired due to an increased demand for a specific nutrient that has become deficient under certain conditions. Thus in true sense they are "Production-related metabolic disorders". Then the question arises that what is the difference between production related metabolic diseases and nutritional deficiencies? Generally, nutritional deficiencies are long term, steady-state conditions that can be managed by dietary supplementation. Metabolic diseases are largely acute conditions that quickly respond to the systemic administration of the deficient nutrient or metabolite.

Though production related metabolic disorders also occur in draft and other animals (e.g. hyperlipidemia in ponies, exertional rhabdomylysis in race/ draft horses, protein-energy malnutrition of beef cattle etc.), the diseases are of more importance in dairy animals. Milk is a rich source of calcium, phosphorus and other nutrients. High producing dairy animals mobilize large quantity of body reserves of calcium, phosphorus, fat and other nutrients to sustain their milk production and to prevent a state of negative energy balance. Whenever, the dietary intake and mobilization of body reserves fail to fulfil the basic metabolic and production requirement, production diseases occurs. On the basis of nutritional and managemental factors production and metabolic diseases can be classified into different groups as follows in table 1.

Table 1. Classification of production and metabolic diseases

A. Energy metabolism associated diseases	C. Calcium/ Phosphorus metabolism abnormalities:
1. Fat cow syndrome/ Downer cow syndrome	1. Hypocalcemia (Milk fever)/ Downer cow syndrome
2. Ketosis	2. Post-parturient hemoglobinuria

3. Reproductive problems: Retained placenta, Infertility	3. Hypomagnesemic tetany
B. Diseases associated with low fibre/ acidosis 1. Bloat 2. Laminitis 3. Indigestion/ off-feed 4. Displaced abomasum 5. Low milk fat content	D. Other feeding management-related disorders: 1. Hardware disease 2. Indigestion 3. Acidosis 4. Udder oedema

Among above listed production diseases, milk fever (parturient paresis), downer cow syndrome, ketosis, postparturient haemoglobinuria and hypomagnesemia are of greater significance due to higher incidence rate and greater production/ economic losses. In this chapter, a brief description about important production and metabolic diseases will be undertaken. Readers can refer to standard medicine textbooks for their detailed description.

Parturient paresis (Milk fever, Hypocalcemia)

Parturient paresis is an acute to peracute, afebrile, flaccid paralysis of mature dairy cows caused by calcium deficiency that occurs most commonly in adult cows within 48-72 hours after parturition. It is manifested by muscular weakness, depression of consciousness, generalized paresis and circulatory collapse. The disease may be seen in cows of any age but is most common in high producing dairy cows >5 year old. Incidence is higher in the Jersey breed than Holstein Friesian or any other exotic breeds.

Onset of lactation results in the sudden loss of large quantity of calcium into milk. Bone calcium is mobilized under the influence of parathyroid hormone to fulfil this increased calcium demand. Serum calcium levels decline from a normal of 10-12 mg/dL to 2-7 mg/dL. If dietary intake plus body Ca mobilization fails to fulfil Ca requirement, the disease occurs. Commonly serum magnesium level is increased, serum phosphorus is decreased, and cows are hyperglycaemic.

Parturient paresis usually occurs within 72 hours of parturition. The disease can contribute to dystocia, uterine prolapse, retained fetal membranes, metritis, abomasal displacement and mastitis. There are 3 clinical stages of parturient paresis. In stage 1, animals are ambulatory but show signs of hypersensitivity and excitability. Cows may show ataxia, fine tremors over the

flank, ear twitching and head bobbing. Restless, shuffling of rear feet and bellowing are also observed occasionally. If calcium therapy is not instituted, cows are likely progress to the second and the more severe stage. Cows in stage 2 are unable to stand and usually remain in sternal recumbency. They show anorexia, dry muzzle, subnormal body temperature and cold extremities. Auscultation reveals tachycardia and decreased intensity of heart sounds. Pulse is weak and smooth muscle paralysis may lead to GI stasis, which is manifested as bloat, failure to defecate and loss of anal sphincter tone. Cow often tucks their head over flank, or the head is extended making S shaped curve. In stage 3, cows lose consciousness and reaches to the point of comma. They go in lateral recumbency, have complete muscle flaccidity, are unresponsive to stimuli, and can suffer from severe bloat. Heart rate can reach up to as high as 120 bpm. Cows may die in this stage within few hours, if no treatment is given.

Differential diagnosis includes peracute mastitis, metritis, TRP, traumatic injury of joints, ligaments, bones and nerve damage (damage of L6 lumbar roots of sciatic and obturator nerves).

Treatment should be started as soon as possible after the disease is diagnosed. Efforts should be made to avoid muscular and neuronal damage and to restore normal Ca levels. Recommended treatment is IV injection of a calcium gluconate salt, although SC and IP routes can also be used. A general rule for dosing is 1g Ca/45 kg BW. Most solutions are available as single dose, 500 mL bottles that contain 8-11 g calcium. In large heavily lactating cows, a second bottle given SC may be helpful since it provide sustained release of Ca. But SC route Ca therapy should not be the sole therapy. Strict asepsis should be maintained during Ca administration to lessen the chance of infection at the injection site. Many solutions (like Mifex) contain P and Mg in addition to Ca. Although administration of P and Mg is usually not necessary in uncomplicated parturient paresis, detrimental effects of their use have not been reported. Mg may protect against myocardial irritation caused by Ca administration. Ca is cardiotoxic; therefore, Ca containing solutions should be administered slowly (10-20 ml/ min) while cardiac auscultation is performed. If severe arrhythmia or bradycardia develops, Ca administration should be stopped until the heart rhythm returns to normal. Endotoxic animals are especially prone to arrhythmia caused by IV Ca therapy. Administration of oral Ca avoids risks of cardiotoxic side effects and may be useful in mild cases of parturient paresis. Ca propionate in propylene glycol gel or powdered Ca propionate (0.5kg dissolved in 8-16 L water administered as a drench) is

effective and avoids the potential for metabolic acidosis caused by Ca chloride. Hypocalcemic cows typically respond to therapy immediately. Tremors are seen as neuromuscular function returns. Improved cardiac output results in stronger heart sounds and decreased HR. Return of smooth muscle function results in eructation, defecation, and urination once the cow rises. Approximately 75% of cows stand within 2 hours of treatment. Animals not responding by 4-8 hr should be re-evaluated and retreated if necessary. Incomplete milking is advised to reduce the risk of relapse. Historically, udder inflation has been used to reduce the secretion of milk and loss of calcium. However, it is not without risk and is not used now a day.

Feeding low calcium diets during the dry period to stimulate intestinal absorption and enhance skeletal resorption after parturition reduces incidence of milk fever in dairy cows. Other methods for prevention of milk fever include delayed or incomplete milking after calving, which maintains pressure within the udder and decrease milk production. Administration of Vitamin D3 and its metabolites is also effective. Large doses of vitamin D3 (20-30 million U, SID), can be helpful if given in the feed for 5-7 days before parturition. After calving, a diet rich in calcium is required. Administering large doses of calcium in gel (PO) is commonly practiced. Doses of 150 g of calcium gel given 1 day before, on the day of parturition, and 1 day after calving is highly effective.

Downer cow syndrome

Downer cow syndrome is usually a complication of milk fever. Ischemic necrosis of the large muscles of the pelvic limbs and injuries to the tissues around the hip joint and of the obturator muscles are common in cows that remain recumbent following treatment for milk fever. The disease occurs most commonly within the first 2 or 3 days after calving. Prolonged recumbency before treatment for milk fever (> 4-6 Hours) results in ischemic necrosis due to obstruction of the blood supply. Cows with retention of placenta and dystocia are more likely to develop downer cow syndrome. A marked increase in the CPK levels in blood of cows with milk fever and failure to stand after repeated treatments are supporting evidences for ischemic necrosis associated with downer cow syndrome. Traumatic injuries to the nerves of the pelvis and hind limb are present in 25% or more of downer cows. Prolonged, low grade hypomagnesemia has been associated with the downer cow, especially when it accompanies hypocalcemia. In typical cases, the animal either makes no effort or is unable to stand following treatment for parturient paresis. About 30% of cows treated for milk fever

develop into downer cow. Downer cows are usually bright and alert and although the appetite is reduced, the cow eats and drinks moderately well. The temperature is normal and the heart rate is normal or elevated to 80-100 bpm. Tachycardia and arrhythmia occurs in some cows especially immediately after the administration of IV calcium. Respiration, defecation and urination are usually normal. The frequent attempts to stand result in crawling or creeping along the ground with both hind legs in a partially flexed position and displaced posteriorly the frog-leg attitude. About 50% of the downers stand within 4 days or less if cared properly. The prognosis is poor for those which are still recumbent after treatment. The CPK and AST levels are usually markedly elevated by 18-24 hours after the onset of recumbency and continue to elevate within next few days.

The diagnosis of downer cow syndrome is made after all other known cause of recumbency has been eliminated in a cow which had milk fever and failed to stand within 24 hours following two successive courses of treatment.

The use of parenteral solutions containing K, Ca, Mg and P has been recommended. Large quantities of fluid and multiple electrolytes can be added to the drinking water if the cow is drinking normally. Provision of comfortable bedding and to roll the cow from side to side several times daily to minimize the extent of ischemic necrosis is recommended. With good clinical care most cows may stand within 12-24 hours. Several different kinds of cow lifting devices have been used to assist downer cows to stand. Lifting cows which make no effort to stand on their own is usually unsuccessful. A water floatation tank has been designed for the management of downer cows. The early detection and treatment of milk fever will reduce the incidence and severity of downer cow syndrome. Dairy cows should be placed in a comfortable well bedded box stall prior to calving and should be left in the stall until at least 48 hours after parturition.

Ketosis (Acetonemia, Ketonemia)

Ketosis is a common disease of adult cattle that are in good health condition or are fatty. It typically occurs during early lactation and is characterized by partial anorexia and depression. Rarely, it occurs in cattle in late gestation, resembling pregnancy toxaemia of ewes. In addition to inappetance, signs of nervous dysfunction, including pica, abnormal licking, incoordination, abnormal gait, bellowing and aggression are occasionally seen. The condition is most common where the dairy cows are bred and managed for high production. All dairy cows in early lactation (first 6 week) are at risk of

ketosis. The incidence in lactation is estimated at 5-16%, but incidence in individual herds varies substantially.

Ketosis develops due to intense adipose (fat) mobilization to meet increased glucose demand and hypoglycemia. Both of these conditions are present at early lactation, at which time negative energy balance leads to adipose mobilisation. Adipose mobilization is accompanied by high blood serum concentrations of nonesterified fatty acids (NEFA) which is converted into ketone bodies in liver. High serum concentrations of NEFA and ketone bodies and low concentrations of glucose are strong indications of ketosis. The serum ketone bodies are acetone, acetoacetate and beta hydroxy butyric acid (BHB). Ketosis occurs in all parities. Cows with excessive adipose stores at calving are at increased risk of ketosis.

In cows maintained in confinement stalls, reduced feed intake is usually the first sign of ketosis. In group-fed herds, reduced milk production, lethargy, and an "empty" appearing abdomen are usually signs of ketosis noticed first. On physical examination, cows are afebrile and may be slightly dehydrated. Rumen motility is variable. CNS disturbances are noticed in minority of cases which includes abnormal licking and chewing. Incoordination and gait abnormalities occasionally are seen, as are aggression and bellowing.

The clinical diagnosis of ketosis is based on presence of risk factors, clinical signs, and presence of ketone bodies in urine or milk. Cow-side tests for the presence of ketone bodies in urine or milk are critical for diagnosis. Caution should be exercised in the use of such tests within 48 hours after calving. Due to the large surge in plasma NEFA at calving, a positive test for ketones is very common during this period. A positive milk tests for acetoacetate and /or acetone usually indicate clinical ketosis.

Treatment is aimed at re-establishing normoglycemia and reducing serum ketone concentrations. Bolus IV administration of 500 ml of 50% dextrose solution is a common therapy. Care should be taken during its administration because SC administration may cause sepsis. Administration of glucocorticoids including dexamethasone or isoflupredone acetate @ 5-20 mg/100 kg b.wt., IM, generally results in a more sustained response. Propylene glycol @ 250-400 g/dose, PO, acts as a glucose precursor and may be effective as ketosis therapy, especially in mild cases or in combination with other therapies. This dose may be given twice a day. In cases refractory to abovementioned treatment, a long acting insulin preparation may be given IM @ 150-200 IU /day. Insulin suppresses both adipose mobilisation and

ketogenesis, but should be given in combination with glucose or a glucocorticoid to prevent hypoglycaemia.

Nutritional management is very important in prevention of ketosis. Lean body condition should be maintained in late lactation and during pregnancy. But, nutritional management should also be aimed at minimizing changes of nutritional deficiencies. Cows tend to reduce feed consumption in the last 3 weeks of gestation. After calving, energy and fibre rich diets should also be added in the ration. Some feed additives containing niacin, calcium propionate, sodium propionate, propylene glycol, and rumen protected choline may be beneficial in preventing and managing ketosis.

Post parturient hemoglobinuria

Post-parturient haemoglobinuria (PPH) is a clinical disease syndrome of dairy cows and buffaloes characterized by intravascular haemolysis, haemoglobinuria and anaemia. The condition is common among animals in their third to sixth lactation during the period from calving to fifth week postpartum. The etiology of PPH is believed to involve hypophosphataemia associated to primary dietary deficiency. Hypophosphatemia inhibits erythrocyte ATP production, causes a loss of normal deformability and predispose cells to increased fragility. The disease in some countries like Canada is also known as "red water" and is supposed to predisposed by several factors like: a) recent parturition, b) heavy milk production, c) dietary phosphorus deficiency and d) consumption of turnips, rape, kale, green alfalfa and sugar beet pulp (contains haemolytic saponins). Copper deficiency is also reported as etiological factor in countries like New Zealand.

Hematologically, PPH has the features of an acute intravascular hemolytic anemia. Urinalysis can be helpful in the diagnosis of PPH. Hemoglobinuria is the most remarkable clinical sign of PPH. Microscopic examination of the urine sediment is imperative to differentiate hematuria from hemoglobinuria. Ketones, bilirubin and protein can be expected in the urine depending on the course of the disease. Another important finding is very low levels of serum phosphorus (0.4- 1.5 mg/dL) during the hemolytic crisis. In affected herds, lactating but clinically normal cows have been moderately hypophosphatemic (2-3 mg/ dL); non-lactating cows usually have normal serum phosphorus concentrations. Calcium levels usually remains within the normal range.

PPH causes a significant drop in milk production and can pose a series risk of mortality in severe cases. Hence, the condition is considered as a

disease of significant economic interest. Prevention of clinical disease requires the proper nutritional management of pregnant and puerperial buffaloes. Early intervention with phosphorus supplementation and supportive therapy can alleviate clinical syndrome. In clinical cases therapy comprising of phosphorus supplementation and non specific supportive therapy based on the clinical findings and severity of the disease should be given. Sodium salt of 4-dimethylamino-2 methylphenyl-phosphinic acid 0.2 gm (15 ml preparation), may be given by intramuscular (IM) route, twice a day for three consecutive day. Sodium acid phosphate 40.3% weight/volume (w/v) (equivalent to elemental phosphorus 8 % w/v), 50 ml suspension can be given in 1 litter Normal Saline as intravenous drip, once a day for 5 to 7 days. A combination of livotropic drugs and antioxidants should also be given to support liver and promote normal body metabolism. Some clinicians also suggest use of steroids like Dexamethasone in early stage or acute cases for one or two days.

IMPORTANT HELMINTHIC DISEASES OF DAIRY ANIMALS

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Livestock are vital to the lives and livelihoods of two-thirds of the world's rural poor- close to 700 million, majority of who are landless and marginal farmers of developing and underdeveloped countries. Livestock contribute to the livelihoods of five main groups: owners, hired caretakers, vendors, consumers and those who work in related industries such as tourism and crafts. The sustainable livelihoods framework places great emphasis on five capital assets as a source of livelihood, namely natural, social, human, physical and financial capital. Natural capital refers to the natural environment, social capital to access to a variety of social resources, human capital to the health and capability of human beings, physical capital to infrastructure and means of production, and financial capital to savings, income and access to credit. A reduction in access to any one of these capital assets may reduce the sustainability of a livelihood (Minjauw and McLeod, 2003). Parasitic infections negatively affect the productivity of animals either in the form of loss of weight gain, reduced milk production, hide quality, condemnation of carcass and reduced reproductive performance, which have a direct bearing on the livelihood of the five categories of people mentioned above. Most of the parasitic diseases are chronic in nature and the economic losses due to these diseases continue for a prolonged time until the animal dies or it is disposed off at a throw away price. Parasitic diseases are said to be less dramatic and more insidious, thus complicating the situation. Clinical signs are non-specific and barring few diseases, there is hardly any pathognomonic sign, which can help in instant diagnosis. Thus, therapeutic or preventive measures are delayed, aggravating the situation further.

The impact of parasitism on livestock production can best be evaluated in controlled experimental studies. Few such studies have been carried out in developed countries and the results may proved to be stunning for many of us. For example tropical fasciolosis, caused by tropical liver fluke, *Fasciola gigantica*, is one of the most important single helminthic infections in Asia and Africa. The prevalence of the infection in some countries may reach upto

80-100%. It has been conclusively proved that a moderate burden of parasite adversely affect the body weight gain, milk yield, draught performance, age at puberty and calving interval. A linear relationship between fluke numbers and their effects on weight gain has been reported. In one study conducted in Indonesia, a mean potential annual weight loss per fluke in Bali, Ongole and buffalo calves of 987gm, 234 gm and 114 gm, respectively, was noticed. In an unpublished study in Indonesia in 1991, J.A. Roberts, B. Barkkie, D.B. Copeman and E. Teleni measured the work output of five pairs of water buffaloes infected with *F. gigantica* in comparison to that of three other pairs that were not infected. It was estimated that anaemia from fasciolosis in this study reduced work output by 7-15%. A study conducted in Uttarakhand reported that treatment of *F. gigantica* infected buffaloes with albendazole increased milk yield by a mean of 5.5 litres per animal per week and also improved the quality of milk. Significantly longer intercalving intervals and packed cell volume were observed in *F. gigantica* infected cows than in those treated with triclabendazole each year for two years. Treated cows had mean intercalving intervals of 18.5 months whereas; in untreated cows the interval was 31.5 months.

A variety of helminth parasites infect dairy animals. The situation is grave in tropical and subtropical countries, India included. Environmental conditions in tropical countries are conducive for rapid development and long time survival of the free living infective stages of helminth parasites round the year and the animals are continually exposed to heavy infection. Classically, the problem is tackled by chemical intervention. However, due to tremendous biotic potential and short regeneration time coupled with anthelmintic resistance, the animals get reinfected within no time. Thus repeated medication is required to keep up the animal health. On the other hand, there is now increased demand for foods of animal origin, which are free from chemical contaminants. The situation thus becomes very much complicated. Economic animal production further gets a jolt due to absence of any effective vaccine against helminth parasites.

Most of the helminthic diseases are known to be less dramatic and more insidious. The clinical signs are non-specific and pose a threat to confirmative diagnosis. Fortunately, most of the helminthic diseases can be diagnosed by demonstration of parasite stages in excretions/ secretions of the affected animals. However, in some instances, viz. cerebrospinal nematodosis, immature amphistomosis and fasciolosis, it may not be possible to demonstrate the parasite stages in excretions/secretions, as immature stages

are responsible for the pathogenesis. In such cases, diagnosis is only possible by demonstration of circulating antigen/antibodies and necropsy findings. Barring few helminthic diseases (fasciolosis and dirofilariosis), sensitive and specific immunodiagnostic tests are not available. In such a situation, necropsy findings are of immense help for confirmative diagnosis and control of the disease. Carcass of animals died of any disease or affected moribund animals are good materials for arriving at a conclusive diagnosis. Sacrificing one moribund animal can save hundreds of affected animals. Apart from this, epidemiological information on parasitic diseases in a particular geographical region provides valuable information to a clinician to arrive at a conclusive diagnosis. Important helminthic diseases of dairy animals are tabulated below.

Table 1: Important helminthic diseases of dairy animals.

Host	Diseases	Aetiology
Cattle/Buffalo	Fasciolosis	*Fasciola gigantica* *F. hepatica*
	Immature amphistomosis	Various Amphistomes
	Schistosomosis (hepatointestinal and nasal)	*Schistosoma indicum* *S. spindale, S. nasale* (nasal granuloma)
	Toxocarosis	*Toxocara vitulorum*
	G.I. Nematodosis	*Haemonchus, Oesophagostomum, Trichostrongylus, Paracooperia etc.*
	Stephanofilariosis (hump-sore, ear-sore, dewclaw-sore)	*Stephanofilaria spp.*
	Parafilariosis (summer bleeding)	*Parafilaria bovicola*
	Tapeworm infection	*Moniezia, Stilesia, Avitellina etc.*

***Fasciolosis*:** Fasciolosis is one of the most important parasitic diseases of ruminants around the globe. In tropical countries *Fasciola gigantica* is mainly responsible for the disease, where as in temperate countries *F. hepatica* prevails. The disease is seen in two forms-acute and chronic. Acute fasciolosis is more common in sheep, though occasionally buffaloes, cattle and goats

may be affected. Chronic fasciolosis is generally encountered in cattle and buffaloes. Clinically, acute fasciolosis is characterized by severe abdominal pain, anorexia, distended abdomen, disinclination to move and sudden death. Blood may come out of natural orifices of dead animals. Nervous signs are occasionally seen in buffaloes. Chronic fasciolosis, on the other hand, is characterized by anaemia, oedema of dependent parts (bottle-jaw), digestive disturbances (constipation or diarrhoea), reduced milk yield and cachexia.

On post mortem examination, large quantity of blood-tinged exudate is observed in the peritoneal cavity of the acutely affected animal. Thoracic cavity may also contain exudates. The carcass is anaemic and the liver shows characteristic lesions of traumatic hepatitis produced by simultaneous migration of large number of immature flukes. It is enlarged in size, pale and friable and shows numerous haemorrhagic tracts on the surface and throughout the liver parenchyma. Blood accumulate under the liver capsule, at times rupture of liver capsule may occur with haemorrhage into the peritoneal cavity. At the proximal part of each tract, an immature fluke can be demonstrated. Immature flukes can be squeezed from the cut surface or may be obtained from the peritoneal fluid. After the initial penetration of the liver parenchyma, hepatic cells are destroyed and the immature flukes lie in a pool of blood, fibrin and cellular debris. Neutrophils, eosinophils and lymphocytes infiltrate around the flukes. Macrophages and epitheloid cells become increasingly numerous in older lesions.

Chronic fasciolosis is characterized by presence of adult flukes in the bile duct and resulting hyperplastic cholangitis and hepatic fibrosis. The presence of the flukes in the bile ducts excites considerable tissue reaction. The biliary epithelium is stimulated to papillary glandular hyperplasia in some places and is eroded in others. The walls of the ducts eventually become greatly thickened from fibrous proliferation and calcification. The scarring around bile ducts often extends deep into the hepatic lobules, producing severe fibrosis in the perilobular connective tissue. In cattle and buffaloes, calcification of the fibrotic lesions may gradually develop and encrustation of calcium is frequently seen. Complete blockage of the bile ducts is quite common. The calcified bile ducts become distended and protrude markedly from the surface of the liver. It is difficult to cut this type of liver with a knife. Excessively fibrosed and calcified bile ducts resemble the stem of a clay pipe commonly referred to as “pipe-stem liver”

***Immature Amphistomosis*:** Adult amphistomes in the rumen are considered to be almost non-pathogenic. On the other hand, young flukes coming out of the

metacercariae just after excystation are highly pathogenic. The immature flukes penetrate the mucosa of duodenum and upper part of the jejunum and also draw a plug of mucosa with the help of the oral sucker and digest it. A mucosal plug is also drawn by the ventral sucker resulting into strangulation, necrosis and sloughing off of the mucosa. The parasites gradually migrate towards the stomach and ultimately reach adulthood in the fore-stomach. Migration is delayed if large number of flukes is present. Tissue invasive immature flukes cause severe enteritis clinically characterized by anorexia, profuse watery foetid diarrhoea, dehydration, abdominal pain and death.

The pathognomonic gross lesions of acute immature amphistomosis are located in the pyloric end of the abomasum and first 60-120 cm of the small intestine where large numbers of immature amphistomes are found attached to and embedded in the mucosa. Often the flukes penetrate deeper layers of the intestine touching *muscularis mucosae* layer. Immature flukes can occasionally be found in the mesenteric lymph nodes also. The affected part of the abomasum and small intestine show patchy congestion, petechial haemorrhages, erosion, oedema and hypertrophy and covered with mucoid exudates. Highly oedematous small intestine leads to partial occlusion of the bile ducts resulting into partial occlusion of the bile duct which ultimately results in the necrosis of the epithelium of the gall bladder Superficial intestinal scraping may be examined under low magnification for identification of the fluke.

Gastrointestinal nematodosis: A number of round worms parasitise ruminants and cause considerable economic losses. The worst affected groups of animals are sheep and goat. In tropical countries the important nematodes are *Haemonchus contortus* (sheep and goat), *H. placei* (cattle and buffalo); *Trichostrongylus axei* (abomasum of sheep, goat, cattle and buffalo and stomach of equines), and other species of Trichostrongylus, *Oesophagostomum spp., Paracooperia spp.* (nodular worm of buffalo and rarely cattle). Clinically, the condition is characterized by anaemia, weight loss, diarrhoea, and emaciation.

The post mortem lesions of haemonchosis depend upon the severity of infection. In general the mucous membranes are pale as are the internal organs. Hydrothorax and fluid in pericardium and ascites are common. In extreme cases cachexia is present, the fat being replaced by gelatinous tissue. The abomasal mucosa is pale, swollen and carries many bite-marks and reddish worms, which can be easily recognized with naked eye.

Histotrophic migration of the larvae causes hyperaemia of the mucosa which progresses to a catarrhal inflammation with necrosis and erosion or ulceration of the epithelium. Numerous parasites may be found associated with, or partially embedded in the mucosa in raised plaque lesions comprising grayish flat areas with sharply demarcated borders, commonly known as "ring-worm lesions". Mucosal scraping should be examined for the presence of adult worms. Raised plaque lesions similar to those seen in ostertagiosis, may also be encountered in some cases. *Oesophagostomum* spp. cause nodulation (pimply-gut) in the intestine. Migrating larval stages and cell-mediated immune response are responsible for the formation of these nodules. Though most numerous in the large intestine, nodules may be found in any part of the intestine. Teasing of these nodules in luke warm normal saline results in recovery of larval stages of the parasite. Similar types of lesions, caused by *Paracooperia nodulosa*, are also seen in the intestine of cattle and buffalo calves. In this case adult worms are found in the nodules.

Toxocara vitulorum is the most dangerous roundworm of large ruminants. It is exclusively found in the intestine of cattle and buffalo calves aged upto 6 months (1-3 month old calves are the worst sufferers). Stephanofilariosis and parafilariosis are not wide spread in India, they are restricted to certain parts of the country (Assam, West Bengal, Bihar, Jharkhand, Chhatisgarh, Orissa, Eastern parts of U.P., Parts of M.P and Maharashtra.

Chemotherapy and Control: Fasciolosis is the most important helminthic disease of large ruminants followed by immature amphistomosis. Triclabendazole (12 mg/kg, po.), Rafoxanide (7.5mg/kg, po.), Oxyclozanide (10mg/kg, po.) are suitable for the treatment of chronic fasciolosis. Out of these, triclabendazole is highly effective against all stages of flukes, particularly very early stages present in the liver parenchyma. It is worth mentioning here that this drug is not very effective against bubaline fasciolosis and the dose rate in buffaloes is 24 mg/kg.

Immature amphistomosis in large ruminants is generally encountered in young stock and is characterised by foul smelling watery diarrhoea, severe dehydration, and oedema in dependent parts, anorexia and colic. Diagnosis is based on demonstration of tiny reddish/pinkish immature flukes (pinhead sized) in diarrhoeic faeces. The disease may be successfully treated using Niclosamide (90 mg/kg po.), Closantel (10 mg/kg po.), and Triclabendazole (12 mg/kg, po.). Oxyclozanide (10 mg/kg, po.) is effective only against adult flukes.

Both hepato-intestinal and nasal schistosomosis are seen in cattle and buffaloes. However, buffaloes generally do not show overt clinical signs of nasal granuloma caused by *Schistosoma nasale*. Praziquantel is highly effective against hepato-intestinal schistosomosis, but the drug is too costly to be used in large ruminants. There is no effective treatment of nasal granuloma excepting the old timer, Anthiomaline.

Toxocara vitulorum is the most dangerous roundworm of large ruminants. It is exclusively found in the intestine of cattle and buffalo calves aged upto 6 months (1-3 month old calves are the worst sufferers). Prophylactic medication (with any of the benzimidazole derivatives or piperazine) of calves starting at 15 days of age and repeated twice at 3 weeks interval is quite effective in controlling the infection. Pregnant dams may treated during the last trimester of pregnancy by some larvicidal anthelmintic (Ivermectin- 0.2 mg/kg, sc., Levamisole- 7.5 mg/kg, po., Pyrantel- 10-20 mg/kg, po.) to kill the migratory larvae. This will greatly reduce transcolostral transmission of the parasite to the newborn calves.

Mature dairy cattle and buffaloes develop acquired immunity to nearly all common protozoal and helminthic infections of the gastrointestinal tract. However, the protection afforded by this immunity is not absolute, and many animals harbour small populations of trichostrongylid nematodes. Some animal owners and veterinarians presume that these modest trichostrongylid burdens cause disease or production losses. In recent past there is a sea change in the concept of deworming dairy cattle/buffaloes. In fact, the modern concept is that deworming of dairy animals is not required at all, until there are exceptional situations, where the animals have high eggs per gram of faeces. Deworming the entire herd is not recommended. Young stock put to pasture for the first season, however need continuous monitoring and treatment.

Stephanofilariosis and parafilariosis are not wide spread in India. They are restricted to certain parts of the country (Assam, West Bengal, Bihar, Jharkhand, Chhatisgarh, Orissa, Eastern parts of U.P., Parts of M.P and Maharashtra. Both these two diseases are economically important, as they are responsible for reduced milk yield and daft power. Levamisole hydrochloride (7.5 mg/kg, sc.) and ivermectin (0.2 mg/kg, sc.) are highly effective in treating these two affections.

HAEMOPROTOZOAL INFECTION, THEIR TRANSMISSION, DIAGNOSIS AND MANAGEMENT IN BOVINES

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Introduction

India is predominantly an agricultural country where livestock and agriculture are closely associated with each other. In India, 70% of the rural households own livestock for generating additional employment through milk, meat, tools, leather, subsisting oncrop residues, draft power and farmyard manure. The total livestock population is 535.78 million in country with increase of 4.6 percent over Livestock census 2012. In India total bovine population is 302.79 million. In the world, India is the highest milk producer with an annual production of 162.7 million tones (GOI, 2019). The livestock sector especially the dairy sector comprising of approximately 199 million cattle in India is an important part of the rural agribusiness in Indian economy (Ghosh et al., 2014). Cattle have been the source of income of small, marginal and landless farmers, a majority of whom live below the poverty line (Gandhi et al.,2015). Even though the livestock sub sector contributes much to the national economy, its development is hampered by different constraints. The most important constraints to bovine productions are widespread endemic diseases including parasitic infestation, poor veterinary services etc. The parasite affecting production performance of milch animal is protozoa, helminths and arthropods. Amphistomes (stomach fluke), *Fasciola* spp. (liver fluke), blood parasite, coccidian and tick infestation are the important parasitic infections causing enormous economic losses. The livestock are susceptible to number of haemoprotozoan and vectors borne diseases such as Theileriosis, Trypanosomiasis and Babesiosis (Singh et al., 2012). As per Annual Report of National Institute of Veterinary Epidemiology and Disease Informatics, five parasitic disease, viz., Trypanosomiasis, Theileriosis, Babesiosis, Fasciolosis and Coccidiosis were listed among the top ten diseases of livestock reported from all states of India responsible for decrease in the production traits of the animal (Anonymous, 2015). The infection is mainly transmitted by arthropod

vectors and through blood transfusion. The impact of diseases caused by these organisms on health and productivity of farm animals and human beings is huge, though a fair economic assessment on the quantum of incidental economic loss is yet to be worked out from India. The clinical manifestation of the disease varies from fever, anorexia, anemia, threatened abortion and death in the acute form of infections. The diagnosis of haemo-protozoan infections largely depends on various laboratory-based diagnostic methods as the clinical manifestations are often inconspicuous and non-specific. Traditional diagnostic methods rely on microscopically demonstration of infective stages in blood or tissue fluids. However, it is laborious, lesser sensitive and cannot differentiate between morphologically similar organisms. Recent development in the technologies has opened new avenues for improvement in the accurate diagnosis of parasitic infections. Serological tests are simple, fast but lack specificity. With advent of molecular techniques, as DNA hybridization assays, polymerase chain reaction and its modifications ensure the detection of infection in the latent phase of the disease. Nucleic acid-based assays are highly sensitive, free from immune-competence and can differentiate between morphologically similar parasites. Other approaches include- chemotherapy, vector control with chemicals and vaccines (Maiti, 2021). Also, epidemiological surveillance is an important aspect to control haemoprotozoan infections in the area. This part highlights various methods that can be adopted to manage important haemoprotozoan infections.

Important haemo-protozoan disease and their transmission

Theileriosis is a common protozoan parasitic disease of dairy cattle and buffaloes in India and the World. *Theileria* showed great pleomorphism and appeared in annular, oval, comma, dot and cornet shaped forms in stained blood smears and earlier identification of species was purely based on the proportion of different morphological forms (Krishnamoorthy et al., 2021). As a whole, various Theileria species affect cattle and buffaloes the most pathogenic and economically important species are *T. annulata* (Bovine tropical theileriosis), *T. parva* (East Coast fever), *T. orientalis* (Oriental theileriosis) and are transmitted by Ixodid ticks of the genera *Hyalomma* and *Rhipicephalus* species. The bovines in East India are reported to suffer from theileriosis by two species i.e. *T. annulata* and *T. orientalis*. The transmission of *T. annulata* is by infective sporozoites in the saliva of ticks of the genus *Hyalomma*. These are mostly two host ticks, preferring hot and humid climate for completion of their life cycle. *T. orientalis* causing 'oriental theileriosisis' reported to be transmitted by ticks of the genus's *Rhipicephalus microplus*

and *Haemaphysalis* spp. (Kumariet al., 2019) and it has also been detected in other arthropods such as mosquitoes and lice, though evidence of transmission by their bite is lacking. Clinical manifestations in animals suffering from tropical theileriosis may vary (polymorphic) depending on the severity of disease as acute, subacute and chronic. It also depends on the degree of parasitised cells of the host, virulence of the *T. annulata* and risk factors like previous exposure to the disease, physiological state and, species of the host affected and concurrent infection of other pathogens as well. The organisms cause marked pathogenicity which may even lead to mortality. Acute status of the disease is commonly occurred. The phase of the disease may be as prolonged as up to 4 weeks or more. During this period several characteristic clinical sign having significant diagnostic importance is enlargement of prescapular lymphnode, swelling of the eyelids and ears and high rise of temperature (40-41. 5°C) is another associated sign (Bhatia et al.,2010). Necropsy findings depict petecheal haemorrhage in the G. I. tract which comprises ulceration in the abomasal wall and intestinal wall. This ulcer is called as punched-out ulcer in calf.

Babesiosis

Babesiosis is caused by an infection of erythrocytes by tick-borne protozoan members of the phylum Apicomplexa, order Piroplasmida and genus *Babesia*. Several species of the genus *Babesia* are involved, with the two most important species in cattle; are *B. bigemina* and *B. bovis* that are mainly transmitted by ticks through the transovarial route. The vector role of ticks for these parasites was discovered by Smith and Kilbourne in 1893, who were the first to demonstrate arthropod transmission of a disease agent. *Babesia* species is transmitted biologically by the tick vector via transovarian transmission which transmits from egg of mother tick (first generation) to the next stage. Bovine babesiosis is transmitted by one host tick vector (*Boophilus* spp.). *B. bigemina* and *B. bovis* are transmitted biologically by *Boophilus* ticks in which nymphs and adults transmit *B. bigemina* but only tick larvae transmit *B. bovis* (Esmaeil et al., 2015). It is also transmitted mechanically by infected needles and syringes, blood transfusion and surgical instruments. The economic impact of babesiosis is considerable, particularly in bovines (including buffalo), small ruminants and companion animals are also affected. Furthermore, there is growing interest in *Babesia* spp. as zoonotic agents. Disease occurs when the rate of erythrocyte infection and loss exceeds the rate of their replacement, giving rise to anemia with its attendant health issues. Additionally, debris and toxins released as a result of erythrocyte destruction

may adversely affect organ systems. Erythrocytes infected by some species *B. bovis* can be sequestered in brain capillaries, causing cerebral babesiosis. The host immune response plays an important part in the pathogenesis of babesiosis through immune-mediated erythrocyte lysis and overproduction of pharmacologically active agents, especially cytokines. These can cause many circulatory effects, including vasodilatation, vascular stasis, lowered blood pressure, edema and intravascular coagulation.

Trypanosomosis

Trypanosomosis is a disease complex caused by several species of protozoan parasites of the genus *Trypanosoma. Trypanosoma evansi* is the most widely distributed pathogenic and mechanically transmitted vector borne haemoprotozoan disease of domestic livestock and wild animals in India. In tropical countries like India the disease is also called as Surra.It is described as monomorphic, but certain strains are pleomorphic, with a length of 15 to 34 µm and a width of 1.5 to 3 µm. Trypanosomes are elongated leaf-like parasites with a single nucleus in the centre of the body. The blepharoplast towards the back of the body gave rise to a single flagellum. Kinetoplast is a DNA-containing elongated organelle found just posterior to the blepharoplast. *T. evansi* does not require a biological vector. This organism, which can be found in blood and tissues are transmitted mechanically by biting insects. Members of the deer fly and horsefly family, Tabanidae (e.g., the genera *Tabanus, Chrysops, Lyperosia*, *Hippobosca* and *Haematopota*) and flies in the genus *Stomoxys* are thought to be the most important vectors (Juyal, 2011). Additional mode of transmission includes iatrogenic spread on contaminated needles or surgical instruments. Vampire bats can both maintain *T. evansi* and act as mechanical vectors in South and Central America. Transplacental transmission has been demonstrated in ruminants and transmission in milk and colostrum was reported in experimentally infected sheep. Trypanosomes cannot survive for long periods outside the host, and disappear relatively quickly from the carcass after death. In most domestic and many wild animals *T. evansi* is highly pathogenic with clinical signs depending on strain pathogenicity, host species and general stresses on the host and local epidemiological conditions. *T. evansi* infected animals manifest signs and symptoms of progressive anaemia, rapid weight loss among animals, rapid decrease in milk production, persistent fever up to 105°F, circling, in-coordinated gait and in terminal stage lateral recumbency with kink neck and finally death. The disease is underestimated in cattle and buffaloes due to cryptic nature.

Clinical symptoms

Common clinical symptoms as described the tick infestation and high fever can be suggestive of theileriosis and babesiosis. Enlargement of superficial lymph nodes usually the parotid and Turning sickness which is characterized by circling movements due to occurrence of necrosis of brain is a typical sign found in *T. parva* and *T. mutans* infection. *B. bigemina* and *B. bovis* are the main causative agents of the Protozoa disease in cattle. Anaemia and haemoglobinuria (coffee coloured urine) are the common clinic-pathological features of the disease. Intermittent fever, anaemia, oedema, neurological sign and corneal opacity in a buffalo tested positive for *Trypanosoma evansi*. The clinical signs of diseases are indicative but are not sufficiently pathognomonic, thus, diagnosis must be confirmed by laboratory methods.

Diagnosis

The diagnosis of diseases is basically divided into clinical, microscopically, serological as well as molecular techniques. Diagnosis of bovine haemo-protozoan by direct microscopic examination of blood or lymph node material, are not highly sensitive, but a number of techniques, including enrichment of the sample, mice inoculation, serological and DNA methods may increase the sensitivity and molecular tools are then very useful for species specific diagnosis.

Microscopy-based method

From many years, microscopy has been the only tool available for the detection of parasites through inspection of blood smears, tissue specimens, feces, lymph node aspirates, bone marrow and even cerebrospinal fluid. However, sample preparation for direct observation is time-consuming, labor intensive, and proper diagnosis depends on qualified laboratory technicians. Microscopy-based detection methods are economically cheaper and considered the gold standard for diagnosis of parasitic infections. However, due to limitations such as technical expertise, occult/ acute infection status of animal etc. may reduce the sensitivity of this test.

Chemical methods

Biochemical test, including the stindime bromoid test, thymus turbidity test and formal gel test depend on increase in serum immuno globulins following infection. These tests used were used in all diagnosis of *T. evansi* in bovines but are not specific for trypanosomes.

Serological tests

Serological tests that prove the immune contact between the host and the parasite are quite useful. Serological tests can be applied for prevalence or incidence studies, seasonal or inter-annual variations and for vector control. Serological tests are used to detect specific humoral antibodies and circulating antigen.

Latex agglutination test

Latex agglutination is observed when a sample containing the specific antigen is mixed with an antibody which is coated on the surface of latex particles. This test has been used for diagnosis of *Babesia bigemina* (Deepak and Singla, 2016). Card agglutination for trypanosomosis tests (CATT) was originally developed for the diagnosis of Trypanosoma later on for *T. evansi* (Surratex based on trypanosome- antigen detection in blood or serum) infection in livestock using latex beads coated with native RoTat 1.2. Recently, the N-terminal fragment of VSG RoTat 1.2 has been expressed as a recombinant protein in the yeast *Pichia pastoris* and incorporated in a latex agglutination test, the rLATEX/ T. evansi (Verma et al., 2018).

Immuno-chromatographic assays

Immuno-chromatography is a combination of chromatography (separation of components of a sample based on differences in their movement through a sorbent) and immunochemical reactions. This test detects the presence (or absence) of a target analyze (antigen or antibody) in the sample (matrix) without the need for specialized and costly equipment. In the last decade, many immune-chromatography tests have been developed using recombinant antigens such as merozoite surface antigen-2 (rMSA-2), spherical body protein-4 (SBP-4), rhoptry-associated protein 1 (RAP-1) and *Theileria annulata* (TaSP-1) antigen *for Babesia bovis*, *Babesia bigemina* and *T. annulata* infections, respectively (Guswanto et al., 2017). Surra Sero K-SeT test developed for detection *of Trypanosoma evansi i*nfection in domestic animals and the test is based on recombinant variant surface glycoprotein (rVSG) RoTat 1.2.

Enzyme linked immuno sorbent assay (ELISA)

Enzyme-linked immunosorbent assays (ELISA) are being used increasingly for the detection of parasite-specific antibodies. The principle of this technique is that specific antibodies to trypanosomes can be detected by enzyme-linked anti-immunoglobulin‘s using solid-phase polystyrene plates

coated with soluble antigen. The enzyme may be peroxidase, alkaline phosphatase or any other suitable enzyme. The enzyme conjugate binds to the antigen/antibody complex and then reacts with a suitable substrate to yield a characteristic color change either of the substrate itself or of an added indicator (chromogen). It has been successfully adapted for the detection of antibodies to *T. annulata* and has been shown to work without cross reacting between *T. annulata* and *T. parva.* Tests used for *T. parva* and *T. mutans* are indirect ELISAs based on parasite-specific antigens, recombinant polymorphic immunodominant molecule (PIM) and p32 antigens, respectively (Kiara et al., 2018). Both schizont antigen and piroplsmic antigen can be used in ELISA. These ELISAs provide higher (over 95%) sensitivity and specificity than the IFA tests and are soon expected to be available commercially. ELISA using variable surface glycoproteins from a *T. evansi* RoTat 1.2 has been expressed in *Spodoptera fugiperda* insect cells and clone successfully differentiated *T. evansi* from *T. brucei.* Protocols are available water buffaloes (Lejon et al.,2005). In addition, the ISG-75 gene of *T. evansi* has been cloned, sequenced and expressed in *E. coli* and *Pichia pastoris* and used in the development of antibody detecting indirect ELISA as well as competitive ELISA with diagnostic sensitivity of about 98% and specificity of about 99%.

Loop-mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a unique amplification method with extremely high specificity and sensitivity able to discriminate between a single nucleotide differences. Amplification and detection of gene can be completed in a single step using LAMP that take just 15-60 minutes, by incubating the mixture of samples, primers, Bst DNA polymerase with strand displacement activity and substrates at a constant temperature (about 60-65°C). LAMP is an isothermal nucleic acid amplification technique so it does not require expensive thermal cyclers. In-tube detection of DNA amplification is possible hence there is no need to run gel electrophoresis after amplification. Recently, parasitologists have adapted the LAMP technique to detect several parasitic diseases, viz., *Trypanosoma* spp, *Theileria* spp. and *Babesia* spp. (Verma et al., 2018).

Molecular analysis

It is an amplification technique to make numerous copies of the specific or targeted parts of a DNA sequence. The most commonly employed genes are the small ribosomal subunit RNA (ITS-1, ITS-2, 18s rRNA) and

mitochondrial DNA (Cox-1). The method is used for accurate diagnosis, give species confirmation, allow mass production of recombinant DNA and allow mass production of antigens. *T. annulata* and *T. parva* can be detected using a variety of PCR methods (targeting sequences TqR, P^{67}, P^{104}, PIM, rTaSP/TaSP, rTams- 1/ Tams-2 and Spag-1). Molecular detection of babesiosis by using of RAP-1 (rhoptry associated protein-1) and AMA-1 have been widely used for detection of *B. bovis* and *B. bigemina*, respectively (Bastos et al., 2021). It is an amplification technique to make numerous copies of the specific or targeted parts of a DNA sequence. PCR assays targeting the TBR1/2, ITS-1, 18s rRNA and RoTat1.2 VSG genes (Pruvot et al., 2010). TBR gens showed higher sensitivity and specificity than other gene sets for the detection of *T. evansi.*

Management of haemo-protozoan disease

The control of a vector borne disease is classically divided into two sections: pathogen control and vector control. There are also various alternative means of controlling transmission, which can be combined as "means to prevent the infection.

Pathogen control

Control of haemo-protozoan can be either by immunization, anti-parasitic drugs or by a combination of these approaches. Chemotherapy of haemo-protoza is important for controling the disease either to treat field cases or to control the diseases. In endemic areas, sick animals should be treated as soon as possible with an anti-parasitic drug. The success of the treatment depends on early diagnosis and the prompt administration of effective drugs. A large number of chemical compounds have been reported to be effective against bovine haemo-protozoan parasites. Some of them were very specific and effective, but many have been withdrawn for several reasons. In addition, supportive therapy such as blood transfusions, anti-inflammatory drugs, tick removal, iron preparations, dextrose, vitamins (B complex), purgatives and fluid replacements, may be necessary in severe cases of haemoprotozoan. The first specific drug used against bovine babesiosis and trypanosomosis was trypan blue, which is a very effective compound against *B. bigemina* and *T. evansi* infections, however, it did not have any effect on *B. bovis* and it had the disadvantage of producing discoloration of animal's flesh, so it is rarely used. For many years, the babesiacides: quinuronium sulfate, amicarbalide, diminazene aceturate and imidocarb diproprionate were used against bovine babesiosis in most of Europe; however, quinuronium sulfate and amicarbilide

were withdrawn because of manufacturing safety issues, and diminazene, which is widely used in the tropics as both a babesiacide and a trypanocide, was withdrawn from Europe for marketing reasons. The indiscriminate use of anti-Babesia prophylactic agents, including the administration of the drug at sub lethal blood levels to animals, can produce the development of drug resistant parasites, a problem that will require the development of new drugs. New drugs with a chemotherapeutic effect against haemoprotozoan with high specificity to the parasites and low toxicity to the hosts are desired to control the disease. Identification of novel drug targets is usually based upon metabolic pathways and cell structure. *Babesia* spp. and *Theileria* spp. are apicomplexan parasites that invade erythrocytes and multiply asexually with a reproductive phase, which differ from other apicomplexan that are able to invade and replicate within nucleated cells. In addition, most members of the phylum Apicomplexa harbor a semi-autonomous plastid like organelle called apicoplast, which was derived via secondary endosymbiotic events from eukaryotic alga. The apicoplast is essential for long term parasite viability and has been an attractive target for development of parasiticidal drug therapies. Buparvaquone (a quinine derivative) is a drug of choice in bovine theileriosis. It is effective against both schizontal and piroplasmic stages. This drug can be used @ 2.5mg/Kg b.wt. i/m. Besides, some antibiotics e.g. oxy- tetracycline and chlor- tetracycline are effective agains the schizontal stage as they cause the arrest of growth of schizonts. So they can be used only in early stages of the disease. For treatment of trypanosomiasis Diminazene aceturate is the most widely used drug in ruminants followed by isometamedium chloride and quinapyramine in India. It's use in horses and dogs is limited due to poor efficacy and tolerance. Antioxidant supplementation following diminazene injection showed superiority over diminazene alone in reversing pathological condition caused by trypanosome infection (Eghianruwa and Olayinka, 2018).

Breeding by the tolerant animals

Certain indigenous taurine breeds of cattle, namely N'Dama are significantly more resistant to trypanosomiasis than *Bos indicus* breeds. This trait, termed trypanotolerance is an innate characteristic. Despite their small size, trypanotolerant breeds are at least as productive as other indigenous breeds. Genetic differences in resistance to trypanosomiasis have also been found in some *B. indicus* types of cattle although to date the level demonstrated is much less than that of the trypanotolerant breeds (Murray and Trail, 1984). Inverse age resistance is found in babesiosis. It means that the older animals are more susceptible in comparison to young animals. The passive

transfer of maternal antibodies via the colostrum is probably responsible in part for this resistance. The natural resistance of the young calf to infection usually disappears at 9-12 month of age.

Immuno-prophylaxis

An in-vitro attenuated schizontal cell culture vaccine is available with trade name of " Rakshavac T" manufactured by Indian immunologicals limited, Hyderabad (Singh et al., 2014) to prevent Bovine tropical theileriosis. So, it is further recommended that the high valued animals should be vaccinated with this vaccine to prevent them from bovine tropical theileriosis. Administration of vaccine cattle and calves (>2 months age), 3.0 ml S/C at mid neck region (10^5-10^6 infected macroschizonts). Immunity generally develops at 6 weeks after vaccination and provides immunity up to 3 year against *Theileria* infection.

Tick control method

Use of chemical acaricides act as the mainstay for tick control programme in India. Most commonly used acaricidal drug includes organophosphates, pyrethroids, formamidines (amitraz) and macrocyclic lactones (ivermectin) falling into 4 main groups. The potential problem associated with acaricides use is the environmental, milk and meat products contamination with harmful chemical residues. There have been frequent reports of acaricide resistance in ticks globally. Cases of *R. (B.) microplus* developing resistance to organophosphates and synthetic pyrethroids are well-documented. The development of resistance against OP and SP acaricides has resulted use of formamidines (amitraz) and macrocyclic lactones (ivermectin) by the farmers (Maiti, 2021)

Vaccines for ticks

As vector control by using chemicals has several drawbacks thus it is an important option to control protozoan infection. This method is environment friendly and cost-effective that allows control of several VBDs by targeting their common vectors. Examples include- Two recombinant vaccines (GavacTM and TickGARDPLUS) against *R. (B.) microplus* commercially available (Willadsen, 2008).

Phytoacaricides

In the present era focus is on developing herbal acaricides that are safe to use and has less chances of resistance development known as phytoacaricides.

The herbal formulation of these agents leads to their rapid degradation, bioaccumulation in environment and lack of persistence and proves to be more beneficial for synthetic chemical use. *Ageratum houstonianum*, *O. minutiflorum* against *R. (B.) annulatus* essential oils from leaves and flowers has acaricidal activity as reported by Cetin et al. (2009). Recently, NBRI and IVRI have developed an herbal acaricides that are eco-friendly in nature to control ticks and mite infesting livestock and pets. (Maiti, 2021)

Conclusion

To control the haemo-protozoan diseases confirmatory diagnosis at the initial stage is the most important key factor. Immuno-diagnosis should be quick, fast and reliable with high sensitivity and specificity. Sometimes this test gives false negative results due to persistent carrier thus traditional methods needs to evolve from time to time. Modern Nucleic acid based diagnostic tests are highly sensitive and quick to perform. Various PCR types for species specific diagnosis are useful and even help in conducting study of molecular epidemiology of parasites. These diagnostic tools would help in detecting carrier animals in endemic area. So, an integrated approach for controlling the haemo-protozoan diseases should be based on early diagnosis by treatment of sick animals by efficient chemotherapeutic agent, advanced diagnostic techniques, application of chemo-prophylactics, identification of endemic area, vector control and vaccination if available. Besides, prompt diagnosis and treatment of diseased animal, for control of diseases the main emphasis should be focused towards control of tick vectors and chemo-prophylaxis wherever possible.

USE OF ULTRASONOGRAPHY IN FEMALE BOVINE REPRODUCTION

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Ultrasonography provides an excellent method for evaluating the reproductive tract of the animal to gather a significant amount of information and thus help to maximize the reproductive efficiency. For this reason, the modality has been used to its maximum extent in the study of animal reproduction. With the use of ultrasound scanner an operator can visualize organs which were previously reachable by tactile sense.Ultrasonography has several advantages over other imaging modalities. It is non-invasive, free from radiation hazards, provides instant diagnosis, and determines shape, size, location and internal consistency of a structure. Further, repetitive examinations can be done and it is well tolerated by the animals. The clinical uses of ultrasonography in female involve assessment of pubertal status, seasonal status of ovaries, stage of cycle, prediction of ovulation, pregnancy diagnosis, fetal viability, fetal age and sex, amnio-allantocentesis, ovulation failure, ovarian and uterine tumors, follicular/luteal cyst, pyometra, mucometra, hydrometra, embryonic loss, postpartum involution, ovarian response to hormonal treatment. The clinical uses in males involve evaluation of external and internal genitalia, inflammatory conditions and tumors of testes and accessary sex glands and routine method for breeding soundness evaluation.The technique has various advanced applications viz. vascular flow dynamics (colordoppler), serial examination (follicular and luteal dynamics), qualitative assessment (visual scoring), quantitative assessment (image analysis) and transvaginal ultrasound guided ovum-pickup/follicle and cyst ablation.

Echotexture of normal ovarian structures

(i) Ovaries: The ovarian stroma appears as a mixed echotexture of hypo- and hyper- echoic display. Various types of structures can be imaged within the stroma depending on the physiological status of the ovary. In a small, inactive ovary the cortex can be seen to contain small anechoic (black) follicles (2-8 mm in diameter) whereas, medulla appears free of follicular activity. In a

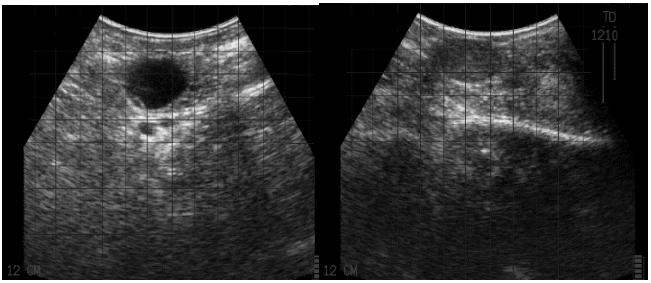
large, active ovary the differentiation in two zones is less distinct, and stroma is imaged as narrow.

(ii) Follicles: Ultrasound is a more sensitive method than palpation per rectum for detecting and measuring ovarian follicles especially, those within the ovarian stroma. Future use of computer assisted image analysis may improve the diagnostic potential of ultrasound to determine the health of a large follicle in a single examination. Ovulation is detected by ultrasonography as the acute disappearance of large follicle (9-20 mm) that was present at a previous examination.

Several studies have been conducted to test the superovulatory response of various treatments. If an embryo transfer donor has failed to respond a standard superovulation regimen, the use of ultrasonography to characterize the activity of the dominant follicle prior to beginning FSH treatment may be beneficial.

(iii) Corpus luteum: The ultrasonic detection and evaluation of corpus luteum (CL) provide valuable information to the diagnostician and biologist. The presence and stage of the luteal gland cannot be ascertained readily during the developing and regressing stages by trans-rectal palpation. Progesterone assays are not convenient for immediate consideration. Therefore, ultrasonography renders the immediate detection and evaluation of luteal gland. Ultrasonographic detection of CL may be more sensitive than detection by palpation but this is dependent on the experience of individual performing rectal palpation. The CL in buffaloes is smaller than cattle in size, deeply embedded and has less pronounced ovulation papilla. Palpation of CL by per-rectum is thus difficult and ultrasonography provides correct picture of ovarian status. The echogenicity of CL depends on the stage of CL development. A mature active CL appears as large circular structure with a relatively homogeneous echotexture. The young, newly formed CL (corpora haemorrhagicum) is difficult to distinguish in its first four days of life, being imaged as a hyperechoic folded structure with a faint dark surrounding line. By six days post-ovulation, the CL is well defined in outline and this appearance will persist until 16 days post-ovulation. If pregnancy does not occur, the CL will regress and appeared as hyperechoic, heterogeneous structure with flattening of the outline. The presence of central cavity (lacuna) within the CL is a common feature. These cavities are distinguished from follicles by non-spherical, often lobulated appearance and by surrounding borders of luteal tissue. A CL with a fluid filled cavity is a normal condition and usually replaced by a dense, solid core of luteal tissue

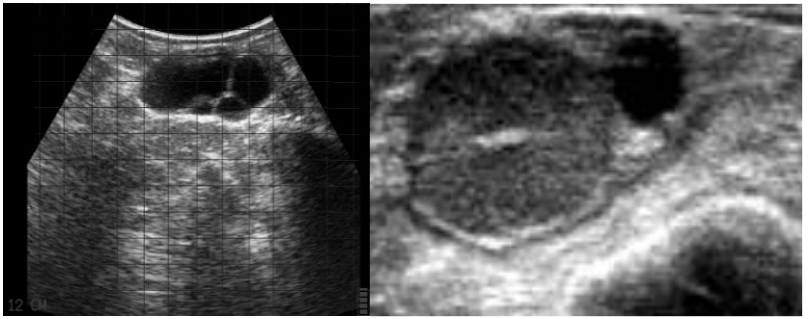
late in estrous cycle or during the first 25 days of pregnancy.Ultrasonography may provide a better method of evaluating CL in embryo transfer recipient. It is recommended that if there is a question about suitability of CL after performing rectal palpation, the ovary can be scanned with ultrasound and decision made on whether to transfer to that recipient.



Dominant follicleMature CL

Echotexture of abnormal ovarian structures

In contrast to the natural and frequent occurrence of fluid filled cavities in the corpus luteum after ovulation, pathogenic cysts also form following failure of ovulation. Cysts are common in post-partum cows and buffaloes. Since these structures are anovulatory and may be persistent (≥ 25 mm). They are considered pathologic and are a source of transient infertility. Some cysts form a distinct luteal lining and are called luteal cyst, whereas others form little or no obvious lining and are called follicular cysts. Variation in the amount of lutinization of the cyst wall is difficult to assess by rectal palpation. The treatment of cows with ovarian cyst is dependent upon an accurate diagnosis of the condition and in particular whether the cysts are follicular or luteal .The failure to detect luteinization of follicular cyst by palpation per-rectum leads to unnecessary treatment in many cows. The therapeutic success can be confirmed quickly by visualization of cyst with ultrasonography. Thus, early diagnosis of cysts by ultrasonography helps in guiding for appropriate treatment and for preventing economic loss. Ultrasonography provides a method for measuring wall thickness and is valuable for diagnostic purposes. Generally a firm thick walled structure is diagnosed as luteal cyst and a soft, thin walled structure as a follicular cyst.



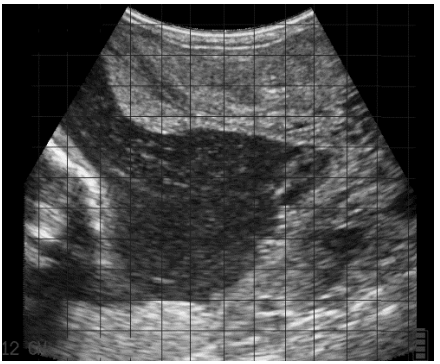
Follicular Cyst Luteal Cyst

Echotexture of female tubular genitalia

The cranial portion of the vagina is normally observed as a hyperechoic line close to the transducer face, but when it is fluid filled, it is seen to have an ovoid, anechoic lumen with enclosing hyperechoic lines. Various changes in vagina that occurs during estrous cycle can be visualized by ultrasonography. Vaginal fluid first increases on day 17 (equivalent to 4 days before ovulation) and decreases to base line (by day 6 or 7) after ovulation. The imperforate hymen (persistent hymen) can also be visualized through ultrasonography as accumulation of fluid in cranial vagina. The echoic specks float on ballotment. The annular rings of the cervix appear as hyperechoic and fluid (anechoic) between them are more distinct. The cervix is thicker during estrus than during diestrus. The zigzag course of the cervical canal can be discerned by rotating the transducer, with the identification of external os and portio-vaginalis being possible within the cranial portion of the vagina.

Ultrasonic appearance of the uterus of the cattle and buffaloes is dependent on stage of the oestrous cycle. Variation in the appearance of the uterus involves changes in endometrial thickness, vascularity and the presence of intraluminal fluid. The Ultrasonographic appearance of abnormal uterine fluid can vary from anechoic fluid with floating particles (referred to as 'snowy specks') to homogenous, purulent exudates that can appear similar to the echogenicity of the surrounding uterus. In endometritic uterus, the fluid containing echogenic particles can easily be distinguished from the clear anechoic fluid of the peri-ovulatory period or early pregnancy. The presence of a thickened uterine wall associated with endometrial infection can also be identified with ultrasound. In the animals diagnosed with pyometra the fluid contain diffuse, echogenic particles within the distended uterus and a

thickened uterine wall. The viscous fluid may resemble the uterine tissue but can be distinguished by the flowing motion of the exudates within the lumen. Mucometra and hydrometra are often associated with segmental aplasia of the uterus and thin walled uterus appears to be full of echogenic particles. Ultrasound offers an objective method to assess treatment progress and to differentiate tissue characters associated with pathology of the reproductive tract.



Pyometra in buffalo

Early pregnancy diagnosis

Under most on-farm conditions pregnancy diagnosis can be rapidly and accurately diagnosed using ultrasound as early as 26 days post AI. Pregnancy confirmation at early stage allows pregnant animals to be moved to a separate management group and managed less intensively (continued heat checking and/or a recheck 60-90 days later is highly recommended as there will be a normal 60 % pregnancy wastage or loss between 21and60 days post conception). It also allows open cows and buffaloes to be short cycled and re-inseminated or set up as recipients, decreasing the number of days open.

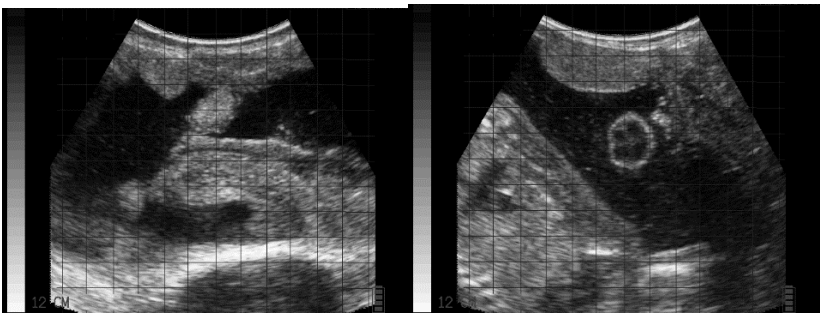
The embryo proper is defined as a distinct echogenic structure within the non-echogenic, fluid filled vesicle. Presence and vitality of the embryo initially can be confirmed by the detection of a heartbeat as early as 19 to 24 days of gestation. The embryo initially appears as a short, straight echoic line (20-22 days), later becomes C-shaped (22-30 days) and finally, by 30-32 days of gestation assumes an L shape. The potential advantages of using ultrasonography for pregnancy diagnosing are that the presence of an embryo can be detected earlier than by palpation per rectum and that direct physical manipulation of the gravid reproductive tract is not necessary with

ultrasonography. The latter fact should reduce the risk of inducing embryonic mortality. Use of ultrasonography rather than per rectal palpation may also improve consistency of early (<45 days) pregnancy diagnosis by reducing the variation in accuracy among practitioners. The efficiency of detecting early pregnancy with ultrasound is markedly increased when the embryo can be detected more easily. Although the embryo can first be detected between the days of 19 and 24 of gestation, when scanning large number of cattle, it is most practical to scan females which are expected to have embryos >24 days of age. The ability to identify open cows with ultrasonography earlier than by rectal palpation can be an economic benefit to dairy and beef producers.

Determination of fetal viability and age

The growth of embryo proper from day 20-60 can be characterized and determined through ultrasonography when the characteristics such as the heart beat (day 22), spinal cord (day 28), placentomes (day 35), split hooves (day 44) and ribs (day 52) first become detectable. Measurements of crown rump length, head diameter and trunk diameter are the easiest predictive measurements to use for estimation of gestational age. In addition, the use of these measurements in formulas to estimate age results in the least variation between the estimated and actual ages. Crown rump length is that measured from the tailhead to the greater curvature of the skull. It is most easily measured in embryos presented in the frontal or sagittal view. Head and trunk measurements are recorded at their maximal diameters. A cross sectional or frontal presentation is required to record head and trunk measurements.

Macerated fetuses may appear as distorted images surrounded by purulent fluid characterized by anechoic background fluid containing echogenic particles. Degenerating embryonic tissues within the vesicle increases the echogenicity of the amniotic fluid surrounding the embryo, which also may appear distorted. Frequently, these changes are too difficult to be detected by per rectal palpation. Sometimes, the fetus may retain its shape but a heartbeat cannot be detected and the amniotic vesicle may appear gray due to the degenerating debris from the dead fetus while the surrounding allantois maintains its non-echogenic appearance. Mummified fetuses often appear only as a poorly defined echogenic intrauterine mass without surrounding fluid. Occasionally, the bones may be identified as dense echogenic tissues shadowing the tissue below. A thickened uterine wall may also be apparent.



Abnormal pregnancy (Fetal maceration at 3 M) Fetal loss at 45 days of gestation in buffalo

Determination of fetal sex

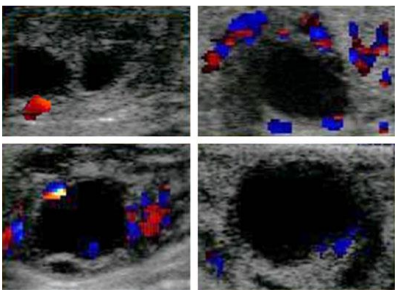
The genital tubercle is embryonic tissue that gives rise to the clitoris in the female and to the glans penis in the male. Sex of the fetus can be accurately determined via ultrasound between 60-85 days post-conception. The genital tubercle is visualized and the determination of the male or female can be made by relative location of the tubercle (caudal to umbilicus in male and ventral to the anus in female). Ultrasound imaging of fetuses on day 48 to 119 has been performed to determine fetal sex. The accuracy of fetal sexing can be optimized by proper timing. Sex determination prior to day 60 is more difficult because the relative migration of the tubercle is not complete.

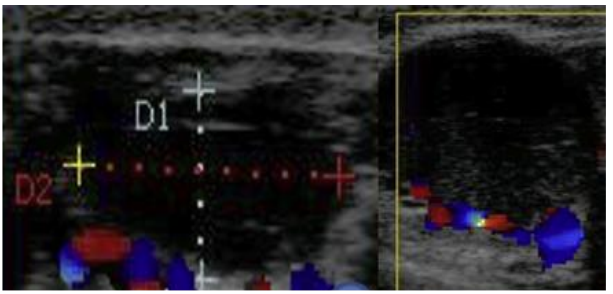
Determination of abnormalities of male reproduction

The abnormalities of external and internal male genitalia can be easily visualised by ultrasound viz. orchitis, testicular cyst, hydrocele, abscesses, hypoplais, tumors and seminal vasculitis.

Color Doppler ultrasonography: Color Doppler ultrasonography is a tool for evaluating vascularity of an organ or structure. For ovarian examinations, it allows visual observation of the blood flow in a demarcated area in the wall of preovulatory follicles, within the corpus luteum and changes in the uterine blood circulation in cows during the estrous cycle. Assessment of the vascularity through color doppler provides useful information on the status and future success of an ovarian structure. The local blood flow usingcolor doppler ultrasonography in individual ovarian follicles and the corpus luteum (CL) in the cow is closely related to follicular growth, atresia and ovulation, CL growth, maturity and its regression. Normal pregnancy is much associated with high vascularization of preovulatory follicle. The animals with deprived

vascularization of follicle remain non pregnant or have complicated pregnancy (Embryonic death and fetal growth retardation).





Color Doppler images of dominant follicle and mature CL (Varughese et al., 2014**)**

Besides detecting aforesaid ovarian and uterine structures, Colloton (2021) also demonstrated the use of ultrasonography for diagnosing granulosa cell ovarian tumors, abembryonic vesicles, fetal umbilical hernia, amorphous globosus, fetal anasarca, fetal ascites, schistosomusreflexus, vaginal urine pooling, uterine abscesses and lymphosarcoma in bovines.

AMELIORATING INFERTILITY IN DAIRY ANIMALS BY MEANS OF MICRONUTRIENTS

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Introduction

While milk productivity drives the major on-farm revenue in dairying, reproductive performance contributes to both profitability and sustainability. Hence, it is imperative to ensure optimum reproduction at farm. Some of the most common practical yardstick to measuring reproductive efficiency in dairy cows are: inter-calving period (i.e., one calf per year) and the number of services per conception (i.e., ideally less than two) etc. However, as productivity of cows is in increasing trend worldwide due to intense genetic selection for high dairy merit, reproduction is reported to be somewhat compromised as nutrients are primarily channelised for milk synthesis as against reproduction. It has been reported that the average conception rate in India is low, which is about 35%, representing a serious impediment to dairy sustainability. Unless the reproduction is optimised, it may lead to higher incidence of culling rates (up to 30%) in the farm and consequently cause a potential shrink in farm profits. It should be noted that, besides endocrine (hormonal) factors, day-to-day "management" also has a significant role in achieving reproductive success.

It is well-recognised that "balanced feeding" that is adequate in major/macronutrients (energy, protein and fats) as well as minor/micronutrients (minerals and vitamins) has been the cornerstone of productive and reproductive functions in dairy cows. Whereas forages and concentrates supply major nutrients, for the dietary adequacy of micronutrients, extraneous supplementation is necessary. This paper comprehensively discusses some aspects of the micronutrients as relevant to reproduction of dairy animals within a practical framework of Indian dairy husbandry.

What are the common reproductive abnormalities?

The following are some of the most common conditions seen as reproductive abnormalities in dairy animals:

- **Infertility:** Temporary loss of fertility.
- **Sterility:** Permanent failure of fertility.
- **Anoestrus:** Failure of normal cycling to return after calving.
- **Repeat breeding:** Failure to conceive despite at least three or more consecutive inseminations.
- **Retention of foetal membranes:** Failure to expel foetal membranes (placenta) within 24 hours after parturition.

Micronutrients

Out of several factors influencing reproductive process in dairy animals, micronutrient deficiency is one of the main causes of infertility leading to reproductive failure. Broadly, there are two nutrient categories within micronutrients, as below:

(i) Minerals

These are classified into two groups

a. Major/macrominerals: Calcium, phosphorus, magnesium, potassium, sulphur, sodium and chlorine.

b. Trace/microminerals: Zinc, copper, manganese, iron, iodine, cobalt, selenium, chromium, fluorine and molybdenum

(ii) Vitamins

These are classified into two groups

a. Fat-soluble vitamins: A, D_3, E and K.

b. Water-soluble vitamins: B-complex and vitamin C.

Although both minerals and vitamins generally constitute not more than 3% of total dietary dry matter, these do play a pivotal role in cellular metabolism as cofactors/coenzymes as well as other roles regulating reproductive functions.

Table 1. Potential roles of various mineral elements in dairy animals

Mineral	Physiological function	Concentration inthediet	Deficiencysymptoms	Commonfeedsources
Calcium(Ca)	Bone and teethformation, bloodclotting, musclecontraction,0.12%inmilkand0.23%in colostrum	0.65-0.80%	Milk fever in adultcows,ricketsincalves,slowgrowthand bone development,decreased milkproduction	Leguminous forages,limestone(38%) anddicalciumphosphate(20%)
Phosphorus(P)	Bone and teethformation, energymetabolism, component ofDNAandRNA,phospholipidsynthesis,0.09%inmilk	0.35-0.45%	Bone fragility, poorgrowth, depravedappetite(pica),reproductivefailure(anestrous)	Cerealgrains,grainby-products(bran),oilseedmeals and dicalciumphosphate(18.5%)
Magnesium(Mg)	Enzyme activator,found in skeletaltissue and bone,important formuscle relaxation,cofactorinsecondmessengersystems in cellcommunication	0.25-0.35%	Irritability,hypomagnesaemictetany (cows), milktetany (calves),increasedexcitability	Magnesiumoxide(54-60%),magnesiumsulphate (10-17%) andforages
Sodium(Na)	Acid-basebalance, musclecontraction, nervetransmission	0.28-0.45%	Craving forsalt,reduced appetite,severe cases followincoordination,weakness,shiveringanddeath	Commonsalt(40%)andsodiumbicarbonate
Chlorine(Cl)	Acid-base balance,HCl production	0.28-0.35%	Cravingforsalt,reducedap	Commonsalt(60%)

	inabomasum		petite	
Sulphur(S)	Rumenmicrobialprotein synthesis,found in cartilage,tendonsand aminoacids	0.20-0.22%	Growth retardation,decreased milkproduction,reducedfeedefficiency	Elemental sulfur,sodium sulphate (10%),potassium sulphate (28%), proteinsupplements andlegumeforages
Potassium (K)	Maintenance ofelectrolyte balance,enzyme activator,muscle and nervefunction	1.0-1.6%	Decreasedfeedintake,loss of hair glossiness,lower blood and milkpotassium	Legume forages, oathay,potassiumchloride(50%)andpotassium sulphate(41%)
Iodine (I)	Synthesis ofthyroid hormones,regulationofbasalmetabolicrate	0.45-0.60mg/kg	Goitreincalves	Iodisedsalt,potassiumiodide (69%)and potassiumiodate(58%)
Iron(Fe)	Part ofhaemoglobin andmanyenzymes	50mg/kg	Nutritionalanaemia	Foragesandgrains
Copper(Cu)	Needed for thesynthesis ofhaemoglobin,part ofmanyenzymes	12-16mg/kg	Severe diarrhoea,abnormalappetite,poorgrowth,coarsegraying hair coat,osteomalacia	Coppersulphate(25%)
Cobalt (Co)	Partof vitamin B_{12},needed for growthofrumenmicrobes	0.11mg/kg	Failure of appetite,anaemia, decreasedmilkpr	Cobalt sulphate andcobaltchloride

			oduction,roughh aircoat	
Mang anese (Mn)	Growth, boneformation, enzymeactivator	45-55mg/kg	Delayedsignsofe strus,poorconce ption	Manganousoxide (52-62%)andmangan ous sulphate(27%)
Zinc(Zn)	Enzyme activator,influences immuneresponse	45-55mg/kg	Decreasedw eightgains, lowered feedefficien cy, skinailment s	Forages,zincoxid e (46-73%),zincsulphat e (22-36%)andzincmet hionine
Selenium(Se)	Componentof glutathione peroxidase,cellularant ioxidantfunctions withvitaminE	0.3-0.5mg/kg	Whitemuscledis easein calves, retainedfoetal membranes	Oil cakes, forages,s odiumsel enite
Molybd enum(Mo)	Part of enzymexanthineo xidase	-	Loss of weight,emaciatio n,diarrhoea	Widely distributed infeeds

Adapted from Risco and Retamal (2011)

Table 2. Potential roles of various vitamins in dairy animals

Vitamin	**Function**	**Deficiency**	**Requirement***
Fat soluble A(retinol)	Vision,genetranscription,i mmune function,reproduction, bonemetabolism, epithelialintegrity, antioxidant activity	Nightblindness,ca lvesbornblind	110IU/kgBW(N RC, 2001)or100000I U/d

D_3 (1,25 dihydroxy cholecalciferol)	Calcium homeostasis,induction of calciumbinding protein forintracellular Ca transport,secretion of insulin andprolactin,musclefunctionandimmuneresponse	Rickets in young andosteomalaciainadults	20,000IU/d
E(αtocopherol)	Antioxidant,involvedin innate immunity andphagocyticcellactivity	Muscle dystrophy, uterinediseases,retainedplacenta,risk of mastitis, impairedneutrophilfunction	2000-4000IU/d in first 3-4 weekspostpartumfollowedby1000IU/d
K(quinine)	Synthesisofbloodclotting proteins	Delayedbloodclotting andinternalbleeding	Synthesised byrumen and intestinalmicrobes
Watersoluble B_1(Thiamin)	Coenzyme rolein energymetabolism, synthesis ofneurotransmitters,passivetransport of Na in nerveimpulses	Polioencephalomalacia (cerebrocorticalnecrosis)	Synthesised byrumen and intestinalmicrobes
B_2(Riboflavin)	Component of flavinadenine dinucleotide(FAD) and flavin adeninemononucleotide(FMN),transfer of H in cellularreactions	-	Synthesised byrumen and intestinalmicrobes
B_3 (Niacin)	Coenzymes of nicotinamide, NAD and NADP, role in carbohydrate, protein and lipid metabolism, causes vasodilation	Dermatitis, hepatic lipidosis	Synthesised by rumen and intestinal microbes

B_6 (Pyridoxine)	Pyridoxal phosphate participates in metabolism of carbohydrates, amino acids and lipid metabolism, incorporation of iron into haemoglobin, antibody production	Reduced growth, dermatitis, alopaecia, anaemia, neurological symptoms, immunosuppression	Synthesised by rumen and intestinal microbes
B_{12} (Cobalamin)	Cofactor in single-carbon transfer, propionate metabolism and incorporation into TCA cycle, RBC synthesis, neural integrity	Deficiency occurs if diets are deficient in Co or if rumen microflora are destroyed, loss of myelin in nerve cells, causes megaloblastic anaemia, poor appetite, weakness	Synthesised by rumen and intestinal microbes
Folic acid	Cofactor, cell division, DNA methylation	Megaloblastic anaemia, neural tube defects in new borns	Synthesised by rumen and intestinal microbes
Biotin	Cofactor for carboxylase enzymes, involved in TCA cycle, gluconeogenesis and fat synthesis, participates in the production and deposition of keratin in horn and hooves	Dermatitis, weakness, paralysis of hind legs, reduced integrity of hoof and horn tissues	Synthesised by rumen and intestinal microbes
Pantothenic acid	Component of coenzyme A, activation of fatty acids for oxidative metabolism in the mitochondria	Impaired fatty acid metabolism, increased ketogenesis and metabolic acidosis	Synthesised by rumen and intestinal microbes

C (Ascorbic acid)	Cofactor for enzyme activity, antioxidant, regenerates vitamin E, synthesis of collagen, phagocytic activity of leukocytes, synthesis of carnitine and adrenal cortical steroids	Deficiency is rare, impaired synthesis of collagen	Synthesised from glucose by the liver
Choline	Phospholipid synthesis, cell membrane integrity, absorption and transport of fatty acid and cholesterol, synthesis of acetylcholine and transmethylation reactions	Hepatic lipidosis, ketosis	Not a typical vitamin. No requirements established although beneficial effects are observed when rumen protected forms are fed at 15-50 g/d

AdaptedfromRiscoandRetamal (2011); * As specifiedbyNRC(2001)

From the above tables, some of the micronutrients are directly associated with reproduction, whilst others have indirect roles.

How to ensure optimum micronutrient adequacy in the diet?

The requirements for macrominerals like Ca, P, Mg etc. as well as vitamins like vit. A, E etc. can be considerably fulfilled through the basal diet of forage and concentrates to a varying extent. On the other hand, microminerals need to be supplied through external sources to the extent of about 75% of the requirements since their bioavailability remains questionable from feed ingredient sources.

- Let us consider an example of fulfilling zinc requirement in a cow producing 10 kg of milk and having a dry matter of 11 kg/d.

  -Requirement of Zn: 55 mg/kg diet. i.e., 55×11=605 mg/d.

-If we supplement the cow with 50 g of BIS type-II mineral mixture (Table 3) containing 0.8% Zn, then:

The amount of Zn supplied will be: 50×0.8%=400 mg/d, which is about 66% of total daily Zn requirement, and with 75 g of this mineral mixture, complete Zn requirement can be accomplished without considering Zn supplied through the basal diet.

On the other hand, if this mineral mixture is incorporated at 2% of concentrate mixture and the cow is fed 4 kg of concentrate mixture, then:

The amount of Zn supplied will be: 4×90%×2%×0.8%=576 mg/d.

Alternatively, dairy farmers may be recommended to use area-specific mineral mixture, wherever it's locally available to augment production, reproduction, immunity and overall health status of animals. By whatever means, it should be remembered that on a 365-day basis, dairy animals must receive adequate micronutrient supplementation.

Table 3. BIS specifications for complete mineral mixture (BIS: fourth rev.)

Sl. no.	Characteristic*	Type-I	Type-II
1	Moisture, % by mass (Max.)	5	5
2	Calcium, % by mass (Min.)	16	20
3	Phosphorus, % by mass (Min.)	9	12
4	Magnesium, % by mass (Min.)	4	5
5	Salt (as NaCl), % by mass (Min.)	22	0
6	Iron, % by mass (Min.)	0.3	0.4
7	Iodine, % by mass (Min.)	0.020	0.026
8	Copper, % by mass (Min.)	0.078	0.100
9	Manganese, % by mass (Min.)	0.10	0.12
10	Cobalt, % by mass (Min.)	0.009	0.012
11	Fluorine, % by mass (Max.)	0.05	0.07
12	Zinc, % by mass (Min.)	0.64	0.80
13	Sulphur, % by mass	1.4-2.3	1.8-3.0
14	Acid-insoluble ash, % by mass (Max.)	2.4	3.0
15	Lead, % by mass (Max.)	16	20
16	Arsenic, % by mass (Max.)	5	7

17	Total ash, % by mass	81-88	78-85
18	Presence of proteinous/organic impurities	Shall pass the test	

*on a dry basis

How micronutrients help mitigate stress and contribute to better reproduction?

Transition management

Transition period (3 weeks before and after calving) represents a stage of enormous stress to dairy animals, and the aim of transition management is to augment peak production, boost immunity, achieve early resumption of ovarian cyclicity as well as minimise calving-associated complications. Specific micronutrients such as rumen-protected choline, biotin and niacin are considered as critical transition nutrients along with vitamin D_3 and vitamin E. Organic (chelated) trace minerals like glycinates, proteinates, propionates, amino acid chelates etc. are useful to increase mineral bioavailability in animals, which in turn may help in improved reproductive performance post-calving. Additionally, during close-up transition (-15 days of calving), anionic mineral salts should be supplemented to achieve dietary cation-anion difference of -50 to -100 mEq/kg of diet to stimulate calcium homeostasis aiming at minimising the incidences of milk fever. Pre-partum transition feed should have a low Ca content (not exceeding 30 g/d), just to support maintenance function.

Summer (heat) stress

As the dry matter intake will be relatively low in summer stress, the corresponding intakes of micronutrients as well as mineral retention will also be low. NRC (2001) suggests higher dietary levels for some of the specific mineral elements for feeding during summer, as below:

Table 4. Select mineral recommendations (% dry matter) during summer stress

Mineral	NRC (2001)	Summer stress
Sodium	0.18	0.4-0.6
Magnesium	0.20	0.3-0.35
Potassium	0.90	1.2-1.5

Cost-benefit ratio of micronutrient supplementation

One calf per year is a practically ideal proposition for optimum reproductive performance in cows. To achieve this, a cow must successfully conceive within 90 days post-calving (i.e., 305 days of lactation + 60 days of dry period=365 days). If there is a miss of one heat or post-partum heat is delayed by one month or if cows fail to conceive after insemination, there will be additional cost of rearing, which is approximately INR 4,500 per cow and loss will be even more on using sexed semen. Therefore, when micronutrients are supplemented regularly, it acts as "investment" for better farm returns against treatment for infertility at a later days that may represent "expense".

Conclusions

Micronutrients play an important role in ameliorating infertility, and thereby improving reproductive performance of dairy animals. With optimum production and reproduction, dairy operations become more efficient, financially lucrative and sustainable.

MANAGEMENT OF ANOESTRUS IN BUFFALOES

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Introduction

Buffalo is the mainstay in the dairy industry in India. Despite its high productive potential the species has been neglected in past because of our limited knowledge about the basic process of buffalo reproduction. Anoestrus is the most common infertility problem in dairy animals in our country. It causes significant losses to the dairy farmers in general and buffalo farmers in particular. Theoretically, absence of periodic manifestation of behavioural signs of estrus is termed as anoestrus. It is characterized by the absence of palpable follicular or luteal structure (true anestrus), or with the presence of a corpus luteum indicating ovarian cyclicity (silent oestrus or subestrus) based on trans-rectal examination. Anoestrus may also be diagnosed with a persistent CL associated with uterine pathology like pyometra, mummification etc. Inherent weakness in the exhibition of behavioural signs of oestrus complicates to detect buffaloes in oestrus. For the said reason, many buffaloes are failed to detect in oestrus because of the absence of males and by the ignorance of the farmers about the proper oestrus signs, individual variation and variation with parity, breeds and geographical locations.Inadequate nutrition, high ambient temperature, high parasite burdens, and diseases exacerbate the condition. In India, it causes huge economic loss to the farmers and the dairy industry. Losses are great because it affects female buffaloes at all breed able age groups and reproductive phases starting from pubertal age (delayed puberty or pubertal anoestrus) to post partum period (post-partum anoestrus) including the period following service (post service anoestrus).All the three types of anoestrus are found commonly under field condition of which delayed puberty and post partum anoestrus is most common but post service anoestrus cannot be ignored. The underlying etiology of anoestrus is multi-factorial in nature hence often difficult to manage the condition. Although the gross economic impact of the condition has been acknowledged worldwide, the exact economics of the associated losses has not been estimated to the best of our knowledge.

Incidence

The incidence of anoestrus varies with the different managemental system and also with different agroclimatic regions. It increases with the age and parities in buffalo. The incidence of anoestrus in buffaloes is reported to vary between 9.1 and 69.4% and can be as high as 82.5%. A classified study on the incidence of anoestrus in buffaloes reported that around 50 to 60% cases were sub-estrus, 30.5% to 40.0% were post partum anoestrus and a high proportion of anoestrus (26.6%) animals had genital infections. In another study based on the clinical survey among the cases of reported anoestrus , 58.4% were true anoestrus , 33.3 % silent estrus and 8.3% of buffaloes had infantile genitalia.

Characteristics of buffalo ovaries

The number of primordial follicles in buffalo ovaries is ranged between 12,000 and 19,000. In non-cycling buffaloes, the number is reported to be 10,000 while in cycling buffaloes it is 12,000. The average number of pre-antral follicles in adult buffalo ovaries is 19,819. The rate of follicular atresia is very high in buffalo. Studies have shown that about 92-95% buffalo ovarian follicles are oestrogen inactive. The rate of atresia is very high in small (92%) and medium (97%) follicles than large follicles (74%).

Follicular Wave Dynamics

In normal course of development, ovarian follicle progresses through growing, static and regression phases. The process of continual growth and regression of antral follicles leading to pre-ovulatory size and subsequesnt ovulation is termed as follicular dynamics (FD). The FD of antral follicles is characterized by the recruitment of a cohort of small antral follicles at the emergence in a wave, subjected to grow for some time (2-3 days) with equal opportunity (growth phase) leading to selection of a future dominant follicle (selection), deviating other sub-ordinate follicles for atresia, finally reaches in the process of dominance (only structural in anovulatory wave and both structural and functional in ovulatory wave) and at last regress (anovulatory wave) or ovulates (ovulatory wave). Each wave emergence is preceded by a transient rise of circulatory FSH.

Follicular Wave Dynamics in Buffaloes

Like other species of animals, follicular growth in buffaloes occurs in a wave like fashion during each oestrous cycle. In each wave, antral follicles undergo a dynamic process of development through growth and regression phase. In buffaloes, the number of follicular waves may vary between 1 and 3 in each

estrous cycle. In general, the 2-wave follicular dynamics is the most common pattern in buffalo which constitutes about 63 – 83 % cases followed by 25-33 % cases of 3-waves and 3.3% cases of 1-wave pattern. There is normally one (in 2-wave pattern) or two (in 3-wave pattern) non-ovulatory follicular waves followed by an ovulatory wave in each follicular wave during oestrous cycle. Buffalo heifers show a tendency of 2-wave cycles, whereas buffalo cows tend to have 2 or 3-follicular wave cycles**.**

Follicular Dynamics in pre-pubertal buffaloes

Recent literature suggests that pre-pubertal buffalo heifers show variable degree of ovarian activity and the follicle turnover follows a typical wave-like pattern characterized by continuous growth and regression with follicles reaching >9 mm diameter and present either of the ovaries on 85% of days of scanning. The largest dominant follicle (DF) of each wave attains a mean diameter of 9.55±0.24 mm (range 7-11 mm) and also may attain the pre-ovulatory size (10-16 mm) but fails to ovulate and finally undergoes atresia. This indicates a possible absence of follicular dominance (functional dominance) in those buffaloes which is yet to be established. The regression of DF is followed by emergence of a new follicular wave in which a variable number (8.48±0.28) of small follicles emerged in a cohort and one of these subsequently developed to be the largest DF. The remaining follicles in a wave ceased to grow after a certain stage and started regressing without ovulation.

Follicular Wave Dynamics in Anoestrus Buffaloes

Some early and recent reports based on trans-rectal examination it was opined that most of the buffalo ovaries remain smooth and inactive without any maturing Graafian follicle during summer. Further, studies on slaughter house ovaries revealed that the number of visible surface follicles present in buffalo ovaries was lower (6.1) during summer compared to that of winter (12.6). This indicates that perhaps the follicular growth continues during summer. Ultrasonographic studies on follicular dynamics confirm that follicular growth and atresia are continuing in summer anoestrus buffaloes. Two types of ovarian follicular dynamics have been shown in postpartum anoestrus buffaloes during summer. One with the presence of CL indicating silent or unobserved oestrus and the second type was true anoestrus characterized by a dynamic follicular activity with a smaller population of follicles, failure of the largest follicle to reach the pre-ovulatory size without ovulation in the majority of the animals. Another study had shown that

dominant follicle attained the preovulatory size (as in cyclic buffaloes) during summer but failed to ovulate and remain as anovulatory follicle and subsequently undergoes atresia.

A major problem in buffaloes appears to be the poor expression of overt oestrus (silent oestrus) which often complicates estrus detection in proportion to the number of buffaloes. The follicular dynamics of silent oestrus buffaloeshas been appeared in current literature. The size of the ovulatory follicle was smaller on Day 0 in buffaloes showing silent oestrus than the buffaloes showed overt oestrus. Further, the growth rate of the dominant follicle was slower in silent oestrus buffaloes than it's normal cyclic counterpart.

In early post-partum period, greater follicular activity occurred in ovary contra-lateral to previously gravid uterine horn in buffalo**.** Largest diameter attained by first postpartum ovulatory follicle ranged from 13-14 mm with an average growth rate of approximately 1mm /day. Postpartum anoestus may be due to a lower growth rate of dominant follicle thereby not able to reach the requisite preovulatory size and to induce behaviour estrus in such buffalo**.**

Etiology of anoestrus in buffaloes

Causes or etiology of anoetrus in buffalo are multi-factorial in nature. Delayed puberty is caused by the factors like breeds, poor nutrition (deficiency of energy, protein, vit-A, vit-C, major minerals, altered Ca: P and trace elements), environmental stress, management etc. Similarly, postpartum anoestrus is also triggered due to many causative factors like nutrition (especially NEB), environmental stress, suckling, infection, management etc.

Management / Treatment of Anoestrus in buffaloes

Multi-factorial etiology often makes difficulties in treating anoestrus in buffaloes. Many a times it is realized that majority of the field infertility problems are nutritional in origin. Anoestrus in rural buffaloes perhaps is one of the best examples for the above views.

Management practices

Protection from thermal stress by providing loose housing system, proper ventilation in intensive system, sprinkling water over body of animals, provision of wallowing has shown to increase the conception rate in buffalo cows.

Nutrition

Among the causative factors, nutritional factors are most important for causing anoestrus condition both in buffalo heifers as well as buffalo cows. Adequate amount of green fodder and concentrate with hay should be provided. Along with this, chelated mineral mixture and trace mineral supplementation has shown to increase reproductive efficiency of the animals. Also, feeding of dietary fatty acids during the dry period can also reduce the interval from calving to the first post-partum ovulation.

Under field condition, in many cases the delayed pubertal heifers was observed to be apparently healthy. Trans-rectal examination revealed either pea shaped ovaries along with normal sized genitalia, or apparently normal size ovaries with infantile genitalia and the third extreme cases pea shaped ovaries accompanied with infantile genitalia. Therefore, apparently healthy buffaloes with infantile genital organs indicate specific deficiencies of micro nutrients. In such cases oral feeding of chelated mineral mixture @30-40 g daily for 21 to 30 days along with trace elements (Cu, Co, Mn, Zn, I, Fe and Se) feeding for 20 days and antioxidant vitamins like vit-A, C and E would be worth to bring the animals in to estrus except extreme summer months where response may not be optimum. In a report it is shown that one Kg concentrate and 100 g sprouted grains daily for 7 days followed by chelated mineral mixtures @50 g/day for 20 days resulted in 90% (9/10) estrus induction and 70% conception rate in 4-5 years aged buffalo heifers.

During early post-partum period, buffalo cows suffers from negative energy balance (NEB). Nutritional management can help in such situation but not the sole for recovering from the condition. Some recent studies indicated a reduction in the length of dry period or its elimination can attenuate the detrimental effects of NEB. Feeding certain dietary fatty acids during the dry period (without altering its length) can also reduce the interval from calving to the first postpartum ovulation. Suckling is one of the major causes for post-partum anoestrus in buffalo cows. Weaning has also been tried to initiate ovarian activity during postpartum but with a limited success.

Plant based heat inducers

Many plants such as *Murraya koenigii* (curry leaves), *Aegle marmelos* (Bel leaves), *Nigella sativa* (kalonji), *Trigonella foenum–graecum* (Methi), *Bambusaaruninacea, Carica papaya, Asparagus recemosus, Leptadeniareticulate, Courupita guianesis, Pergulacia daemia, Semecarpusanacardium cucumber, and jute plants* are reported to be used for induction of oestrus. The shade dried leaf powder of single plant as well as in

combination reported to good estrus response. The above plants contain certain phyto-chemicals and the neutraceuticals present in the different components of those plants perhaps responsible for such response. A number of herbal heat inducers like prajana, janova, fertina, bucofert etc. available in the market which are used commonly for induction oestrus in buffaloes.

Genital massage

Utero–ovarian massage (genital massage) is the oldest, cheapest and effective method for induction of oestrus in anoestrus buffaloes. Genital massage daily / on alternate day / weekly for 3–4 weeks About 40- 80% estrous induction has been reported in buffalo following. The exact mechanism for induction of cyclicity through genital massaging is unknown, however, probable mechanisms include enhancement of blood circulation into the ovaries and uterus post massage that increases the availability of hormones and growth factors; stimulation of local oxytocin production by the ovaries which consequently influence local blood circulation and luteolysis, if CL is present.

Biostimulation

Biostimulation is another method to treat anoestrus bugffaloes. Female buffaloes primed with pheromones from intact or vasectomized male influence the induction of puberty, termination of seasonal anestrous and shortening of postpartum anestrous in buffaloes. It is reported that exposing the buffaloes continuously to vasectomized bull from day 3^{rd} to 60^{th} post-partum reduced the incidence of short cycle (33.3 vs. 46.15 %), silent estrus (57.14 vs. 85.71 %) and a significantly greater conception rate in bull exposed than non-exposed (81.18 vs. 40.0 %) buffalo cows.

Hormonal treatment

Hormonal treatments are based mostly on estradiol, progesterone, melatonin, eCG/PMSG, GnRH (10-20 µg), and PG. The GnRH @20 µg i/m alone or in combination with long acting insulin @0.25 IU/ Kg b. wt. s/c for 5 days has been used successfully for reasonable oestrus induction (60-64%) and fertility (60-69%) in anoestrus buffaloes. Various combinations of GnRH analogues and $PGF_2\alpha$ have been used to initiate ovarian cyclicity. Subestrus should be treated with $PGF_2\alpha$ (natural-25 mg, i/m or synthetic-250-500 µg i/m) followed by AI at the observed estrus when estrus detection is good, or with GnRH + $PGF_2\alpha$ + GnRH (Ovsynch) when estrus detection is poor. Besides, intra-uterine infusion of $PGF_2\alpha$-1 mg for 2-3 days and 5 mg of natural PGF2α through IVSM routes also tried in some studies for oestrus induction in

anoestrus buffaloes. In a study, intra-uterine infusion of 20 ml sterile solution has been shown to release the uterine PGF2α.

A number of progesterone preparations have been tried to induce oestrus in anoestrus buffaloes. Melengesterol acetate (MGA), Crestar, Progesterone releasing intra-vaginal device (PRID), Controlled Internal Drug Release (CIDR) and Syncromate-B (3 mg norgestomet) etc. and also in combination with eCG/PMSG have been used in the treatment of postpartum anoestrus buffaloes with variable success rate for oestrus induction and to achieve subsequent conception.

Conclusion

Buffalo is an important dairy animal in Indian sub-continent. The species have hidden productive potential but farmers are often failed to exploit the species because of her inherent weakness in reproductive behavior and physiological processes. The species suffers from many reproductive disorders, of which anoestrus is the most important one. The production loss as a result anoestrus is unlimited and we do not have a precise estimate for the loss across the country or zone wise. Multi-factorial etiology of anoestrus in the said species often throws challenge to the dairy farmers, veterinarians, researchers/academicians, planners and scientists. A number of managemental strategies are employed to counter buffalo anoestrus both at farm as well as field condition. Most of those show variable response. The condition is still lying as challenge in coming time. Better management avoiding breeding during hot summer months along with antioxidants minerals and vitamins may be good choice to overcome such condition. Anti-stress preparations with good management may be helpful to overcome such condition during summer months.

COMMON INFECTIOUS DISEASES THEIR DIAGNOSIS, PREVENTION AND CONTROL STRATEGIES IN RELATION TO REPRODUCTIVE DISORDERS IN DAIRY ANIMALS

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Campylobacteriosis

It is a widespread bacterial venereal disease in the world that leads to infertility, early embryonic death and abortion in females. Vibriosis is caused by *Campylobacter fetus*, formerly called *Vibro fetus venerealis*. The disease causes high economic losses to the bovine industry. There are two subspecies of Campylobacter fetus: *C. fetus subsp. fetus* and *C. fetus subsp. venerealis*. *C. fetus subsp. venerealis* is an obligate parasite of the bovine genitalia while *C. fetus subsp. fetus* is found in the intestine and causes abortion in sheep and cattle.

Transmission: *Campylobacter fetus subsp. fetus* is transmitted by ingestion in cattle, sheep and goats. Animals can become infected after contact with feces, vaginal discharges, aborted fetuses and fetal membranes. This organism and *C. fetus subsp. venerealis* are also transmitted venereally in cattle. Genital *C. fetus* infections can be spread on foamites including contaminated semen, contaminated instruments and bedding. Bulls may transmit *C. fetus* for several hours after being bred to an infected cow; some bulls can become permanent carriers. Cows can also become carriers for years.

Clinical Signs: In cattle, *C. fetus subsp. venerealis* and *C. fetus subsp. fetus* can cause bovine genital campylobacteriosis which is characterized by infertility, early embryonic death and a prolonged calving season. Abortions are also occasionally seen. Infected cows may develop a mucopurulent endometritis but do not usually have other systemic signs. Bulls are asymptomatic.

Diagnosis: Bovine genital campylobacteriosis can be diagnosed by detecting specific IgA in the cervical mucus. These antibodies are present for several months in half of all infected cows. Most commonly used tests include a vaginal mucus agglutination test (VMAT) and enzyme-linked immunosorbent assays (ELISAs). Sheath washings taken twice from bulls, approximately one week apart, can be submitted for culture or immunofluorescent testing. Vaginal cultures can also be collected immediately after abortion or infection, but this method may be unreliable. *Campylobacter fetus* is fragile and usually present in low numbers. A realtime PCR assay has been developed to detect the causative organisms of bovine genital campylobacteriosis, including one that can differentiate C. fetus subsp. fetus and C. fetus subsp. venerealis.

Treatment: Many cases of campylobacteriosis are self-limiting and require only supportive therapy. Antibiotics may be useful for some cases of enteritis, especially those that are severe. Macrolides and fluoroquinolones are commonly prescribed for campylobacteriosis.

Brucellosis

Brucellosis is a zoonotic bacterial disease caused by several species in the genus *Brucella*. Reproductive losses are the most common syndrome in animals, while humans may suffer from a debilitating nonspecific illness or localized involvement of various organs. Brucellosis in bovine is caused by the organism *Brucella abortus*, However *Brucella melitensis* and *Brucella suis* can also cause infection in cattle.The disease in cattle, water buffalo is caused almost exclusively by *Brucella abortus*; however, *B suis* occasionally is isolated from seropositive cows but does not appear to cause clinical signs and is not contagious from cow to cow.

Transmission: The infection of Brucella abortus inCattle is often acquired by contact with organisms in vaginal discharges and birth products (e.g., placenta, fetus, fetal fluids) from infected animals. Ingestion and transmission through mucous membranes are thought to be the major routes, but organisms can also enter the body via broken skin. In many cases, cattle remain infected for years or indefinitely. They can shed *B. abortus* whether they abort or carry the pregnancy to term, and reinvasion of the uterus can occur during subsequent pregnancies. *B. abortus* is also shed in milk, urine and semen. Shedding in milk may be intermittent. Some calves acquire *B. abortus* when they nurse, and a small percentage may be born infected. Persistently infected young animals can remain undetectable by diagnostic tests, including serology, until they give birth or abort. Natural mating does not seem to be a

major route of transmission in cattle, but venereal transmission appears to be more efficient when *B. abortus*is deposited in the uterus, and contaminated semen could introduce this organism during artificial insemination.

Clinical Signs: Abortion is the most obvious manifestation. Infections may also cause stillborn or weak calves, retained placentas, and reduced milk yield. Seminal vesicles, ampullae, testicles, and epididymides may be infected in bulls; therefore, organisms are present in the semen. Agglutinins may be demonstrated in seminal plasma from infected bulls. Testicular abscesses may occur. Longstanding infections may result in arthritic joints in some cattle. Deaths are rare except in the fetus or newborn. Infections in nonpregnant cows are usually asymptomatic

Diagnosis: *B. abortus* may be detected by microscopic examination of stained smears from tissues, secretions and exudates (e.g., placenta, vaginal discharges or the contents of the fetal stomach), using modified Ziehl-Neelsen (Stamp) staining. This can provide a presumptive diagnosis of brucellosis, especially if supported by serology. Definitive diagnosis requires culture and/or the detection of nucleic acids by PCR. *B. abortus* may be isolated from aborted fetuses (stomach contents, spleen and lung), the placenta, vaginal swabs, milk, colostrum, and the secretions of nonlactating udders, semen, the testis or epididymis, and sites of clinical localization such as infected joints or hygroma fluids. Serology can help diagnose clinical cases or screen herds. Serological tests can determine that an animal has antibodies to a Brucella species with "smooth" LPS in the cell wall, such as *B. abortus*, *B. melitensis* or *B. suis*; however, they cannot distinguish reactivity to different organisms within this group. Commonly used tests in cattle include the buffered Brucella antigen tests (Rose bengal plate test), Milk ring test (MRT), Serum tube agglutination test (SAT), ELISA, FPA & CFT. Moelcular tests like PCR and Real time PCR has also been used for detection of the organism in clinical sample.

Prevention: Vaccines has been used to control and eradicate the disease caused by *B.abortus*. Strain 19 and RB51 vaccines are generally used, The *Brucella abortus* strain 19 vaccine is usually given to 3-5 month-old female calves but this vaccine interfers with commonly used serological tests. The RB51 vaccine, which is based on a rough *B. abortus* strain, does not interfere with the commonly used serological tests, and it can be used in older calves. In some situations, adult cattle have also been vaccinated or given boosters. One issue with adult vaccination is that all of the currently available vaccines contain live attenuated organisms, which can cause abortions in pregnant

animals. The disease is controlled by vaccination and entire herd test and slaughter of reactors.

Leptospirosis

Leptospirosis is the most widespread zoonotic disease in the world, and an economically important bacterial infection of livestock that causes not only reproductive losses due to abortions, stillbirths, and infertility but also non-reproductive losses due to septicemia and nephritis. It is caused by infection with a spirochete *Leptospira* sp. Approximately 7 species of *Leptospira* are known to be pathogenic to cattle and approximately 200 different serovars of pathogenic *Leptospira* sp.have been identified throughout the world with the predominant serovar varying from one geographic region to another in cattle. The most common cause of leptospirosis among cattle is infection with *Leptospira* that belong to the serovar hardjo group. Cattle appear to be the primary maintenance hosts of this serovar. Other common causes of leptospirosis include the serovars pomona and grippotyphosa in cattle.

Transmission: Leptospirosis can be transmitted either directly between hosts or indirectly through the environment. *Leptospira* spp. can be ingested in contaminated food or water, spread in aerosolized urine or water, or transmitted by direct contact with the skin. The organisms usually enter the body through mucous membranes or abraded skin. They might also be able to penetrate intact skin that has been immersed for a long time in water, although this is controversial.

Clinical signs: With serovar hardjo infection, clinical signs include abortions, stillbirths, or birth of weak calves may occur, but the symptoms are generally seen only when a female is infected during the first pregnancy. The first signs of illness, in many cases, are abortions, stillbirths, and occasionally, weak calves with increased neonatal mortality. Because only cows infected for the first time during pregnancy are usually affected, reproductive losses may occur sporadically or as an outbreak. Some aborting cows may retain the placenta, and infertility can be a sequela. The infection is associated with an abortion that may occur several weeks without obvious illness in the females, and infertility.

Diagnosis: Diagnostic tests of *Leptospira* sp. infection include fluorescent antibody testing (FAT), culture of bacteria, PCR and silver staining and immune-histochemistry with tissue samples. Serological tests include ELISA, Microscopic Agglutination Test (MAT) are established for the diagnosis of leptospiral infection.

Prevention: Vaccination – The use of inactivated vaccine is the most reliable method of controlling leptospirosis.

Listeriosis

Listeriosis is caused by several species of Listeria, bacterial organisms that live as saprophytes in the environment but occasionally cause disease in a wide range of vertebrates including mammals, marsupials, birds and reptiles. These organisms are most often ingested in food, where they can proliferate even at refrigeration temperatures. Most illnesses are caused by Listeria monocytogenes, but L. ivanovii is found occasionally, and there are rare reports of clinical cases caused by other species of Listeria. Listeriosis is caused by members of the genus Listeria, a Gram positive bacterial rod in the family Listeriaceae. *L. monocytogenes* is the primary pathogen in humans and animals, but L. ivanovii is found occasionally

Transmission: Listeria spp. are mainly acquired by ingestion, but they can also enter the body by other routes including inhalation or inoculation into broken skin or the eye. Their primary reservoirs are soil and decaying vegetation, where they grow as saprophytes, but they can also be found in many other environmental sources, such as plants and water. In ruminants, listeriosis is most often linked to eating silage with pH > 5-5.5. This can occur in poorly fermented silage or, more often, in areas of aerobic deterioration in otherwise good silage. Clinical cases are described occasionally in animals at pasture or eating other feed, such as moist brewers' grains or hay bales that spoiled after becoming wet.

Clinical Signs: Reproductive losses are one of the most common syndromes in cattle and small ruminants. Animals may abort, typically late in gestation, or give birth to stillborn offspring, and some live neonates may develop septicemia. Abortions in cattle usually occur in the second half of their pregnancy.

Diagnosis: *L. monocytogenes*, its nucleic acids and antigens may be detected in the placenta, fetus (e.g., fetal stomach contents) or uterine discharges after an abortion; in the blood of septicemic animals; in samples from sites of localization, such as cerebrospinal fluid (CSF) or ocular swabs; and in postmortem tissue samples such as the liver, kidneys, spleen and brain. Feed or other environmental samples may be tested in some investigations. Organisms are often undetectable in the CSF of animals with rhombencephalitis, and, in this syndrome, a definitive diagnosis may only be available at necropsy. Serology is not used routinely for diagnosis. Many

healthy animals have high *Listeria* titers, and cross reactions can occur with enterococci, Staphylococcus aureus and other organisms in some tests. An antilisteriolysin ELISAs for IgG is more specific than other assays, but rising titers would need to be seen, and this test is often negative or inconsistent in cases of encephalitis.

Prevention & Control: Feeding good quality silage is important in preventing listeriosis in herbivores. Although silage with Listeria growth can appear normal, the superficial few inches exposed to air and any spoiled or moldy silage should not be fed, as they indicate areas where conditions may have allowed Listeria to grow. Any leftover silage should be removed after feeding and silage handling tools cleaned to prevent cross-contamination. Animals should also be kept away from rotting vegetation, which may contain high levels of organisms, and opportunities for fecal contamination of food should minimized. Live attenuated vaccines have been used in ruminants in a few European countries, but no vaccine is available in North America.

Infectious Bovine Rhinotrachietis, infectious pustular vulvovaginitis (IBR-IPV)

Infectious Bovine Rhinotrachietis, infectious pustular vulvovaginitis is caused by Bovine herpes virus 1. The disease is characterized by rhinotracheitis, pustular vaginitis, balanoposthitis, conjunctivitis, abortion, , enteritis. Infectious bovine rhinotracheitis (IBR) is a disease characterized by acute inflammation of the upper respiratory tract. BoHV-1 infection can also sporadically cause abortion in cattle. BoHV-1 infection affects animal health and productivity causing significant economic losses to cattle producers.. IBR is caused by bovine herpesvirus 1 (BoHV-1). There are two subtypes of Bovine Herpesvirus 1:BoHV-1.1,BoHV-1.2.

Clinical Signs: BoHV-1 infection in cattle is manifested as upper respiratory tract disease and disease of the reproductive tract. Clinical signs are influenced by the age of the animal, the dose of virus, route of infection and whether other agents are also present. The reproductive tract disease is mainly characterized by abortion. In Infectious pustular vulvovaginitis (IPV). Initially oedema of the lining of the vulva and vagina is seen. Pustules then form which often coalesce, giving rise to a yellowish-white membrane. Lesions usually heal within 10-14 days, in some animals a purulent discharge may persist. In Infectious pustular balanoposthitis (IPB) the prepuce may be swollen and a mucopurulent discharge may be seen. Often lesions are only

obvious on extrusion of the penis. Some bulls lose their libido and find erection and ejaculation painful.

Transmission: The cows generally get infected from semen of infected bulls either from natural service or through artificial insemination from infected bulls.

Diagnosis: Laboratory diagnosis is done by rapid methods such as nucleic acid detection by polymerase chain reaction, electron microscopy of vestibular fluids and immunofluorescence staining of the smears or tissue sections. Typical cytopathic effect i.e syncytia formation and characteristic eosinophilic intra cytoplasmic inclusion bodies are diagnostic features of the disease

Prevention: Both inactivated and live attenuated vaccines are available for IBR. The use of live vaccines is preferred above the inactivated ones because of the superior efficacy in clinical protection and more importantly in reduction of the virus circulation in newly infected animals. Proper hygiene and management in farms can reduce the incidence of the disease.

Bovine Viral Diarrhoea (BVD)

Bovine viral diarrhoea and mucosal disease is a sub-acute, acute, in apparent contagious disease of bovines characterized by high fever, diarrhoea and erosive lesions on mouth, oesophagus, rumen, abomasum and intestine. It is caused by Bovine Viral Diarrhoea Virus. Itis one of themost significant infectious diseases in livestock industry with high prevalence, persistence and clinical consequences. It was first recognized in USA. BVDV-1 strain is the most predominant strain in the world. BVDV-2 was first isolated in UK in 2000. BVDV-3 has also been reported recently. Principal host of BVD is cattle of age group 6-24 months. BVD occurs in all seasons but most common in rainy and winter season.

Transmission: It can be spread by direct and indirect contact.Incidence of BVD is more in crowded herd. Virus is normally present in nasal secretionsoral dischargeurine. Other sources like transport vehicles, farm appliances, contaminated feed and water etc can also serve as a potential means to spread the virus.Ingestion of contaminated materials of diseased animals can also spread the virus**.** Sheep and swine no role in disease transmissionCalves harboring cytopathic strain act as principal source of infection.

Clinical signs: BVDV infection can result in a wide spectrum of clinical disease varying from sub-clinical infection to fatal disease. Different clinical signs can be seen simultaneously in a herd. The most common clinical signs are: Infertility or abortion – foetal abnormalities can occur with infections later in pregnancy resulting in brain abnormalities. Mucosal disease – a condition that can vary in severity from mild to severe. Signs include ill-thrift, diarrhoea, ulceration in mouth and gastro-intestinal tract and lameness (from ulceration of feet). Infection from approximately nine days prior to service until 120 days of gestation may result in: Failure to conceive, • Early embryonic death / abortion / congenital deformity, Foetal loss & Persistently infected calves

Diagnosis: Diagnosis is made by observing the typical signs and syndromes, gross and microscopic lesions, leukopenia etc. Confirmatory diagnosis can be made by isolating and identifying the virus from suspected materials like blood, spleen and lymph node. Other tests like Viral Neutralization Test, CFT, and ELISA can be used.Prevention and control

Prevention and Control: The elimination of persistently infected animals is of utmost importance to keep out infection and prevent it from continuing to spread. An additional control tool is to ensure animals are immune prior to becoming pregnant to prevent the formation of new PI's.This is best achieved by the appropriate use of a BVD vaccine to prevent foetal infection during the danger period when PI's are formed. Modified live viral vaccine/Killed vaccine used to prevent the disease (first vaccination **~ 6** months**, booster** required for proper immune response).

USE OF ASSISTED REPRODUCTIVE TECHNOLOGIES IN REPRODUCTIVE MANAGEMENT OF BOVINE

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The dairy industry plays a very crucial role in Indian agriculture by offering sustainable livelihood and nutritional security to more than 60% of the rural masses. In India ART is mainly being used for breed improvement and conservation of Indigenous cattle under the RashtriyaGokul Mission (RGM) and also for the production of superior bull for semen production at various semen stations in the country. Further in a country like India, where in some parts, cattle slaughter is prohibited and now agriculture is predominated by mechanization and almost no use of male animals and becoming a menace. Therefore the government is supporting insemination with sex semen.

The primary aim of ART to maximize the number of offspring from genetically superior animals and their wide spread dissemination(Hansen, 2006; Berglund, 2008) in addition to conservation and propagation of the germplasm from threatened species and native breeds. Primarily used for augmentation of fertility and production in farm animal and to address infertility in human being

What is ART?

Assisted reproductive technique is manipulation of sperm and eggs or embryos in a laboratory for producing pregnancy.

Some important assisted reproductive techniques are mentioned below

- **Artificial insemination:** Artificial insemination (AI) is the process of collecting sperm cells from a male animal and manually depositing them into the reproductive tract of a female.
- **Multiple ovulation & embryo transfer (MOET):** ET involves the removal of an *in-vivo* derived embryo/ embryos from a female of superior

genetics and the placement of the embryo into the reproductive tract of a female of average genetics.

- **Ovum-pickup-In vitro fertilization and embryo transfer**(OPU-IVF-ET): It involves collection of oocytes from female of superior genetics and finally develop *in-vitro* embryo/ embryos and the placement of the embryo into the reproductive tract of a female of average genetics.
- **Cloning:**Somatic cell nuclear transfer called cloning; it is a type of biological copying. Cloning prize cow/bull, breeding through clones and keeping their offspring, the farmer can introduce the natural positive characteristics into the herd quickly. It would take several more years to achieve these same improvements by conventional breeding.
- **Sexed semen AI**: Deposition of sexed spermatozoa's into the reproductive tract of a female.
- **Intra-cytoplasmic sperm injection**: Intra-cytoplasmic sperm injection is an in vitro fertilization procedure in which a single sperm cell is injected directly into the cytoplasm of an egg. This technique is used in order to prepare the gametes for the obtention of embryos that may be transferred to a maternal uterus.
- **Transgenic:** AnOrganism or cell whose genome has been altered by the introduction of one or more foreign DNA sequences from another species by artificial means.
- **Frozen embryo transfer (FET):** Embryo are frozen/ vitrified embryo were transferred into suitable recipients
- **Gamete intra-fallopian tube transfer (GIFT):**GIFT is a tool of assisted reproductive technology against infertility. Eggs are removed from ovaries, and placed in one of the fallopian tubes, along man sperms.
- **Zygote intra-fallopian transfer (ZIFT):** A technique in which a woman's egg is fertilized outside the body, then implanted in one of her fallopian tubes. This technique is one of the methods used to overcome infertility, the inability of couples to produce offspring on their own.
- **Pronuclear stage tubal transfer (PROST):** is a technique that involves in vitro fertilization (IVF) of oocytes, followed by the transfer of pronuclear oocytes into the fallopian tubes.

- **Tubal embryo transfer (TET):** Tubal embryo transfer (TET) is a part of the process of the assisted reproductive process (ART), which helps to implantation of the created embryo in laboratory set-up through the IVF process is transferred into the fallopian tube of the mother/gestational carrier for further growth of the embryo.

1. Multiple Ovulation and Embryo Transfer

1.0 Multiple Ovulation and Embryo Transfer (MOET) Technology is used to increase the reproduction rate of superior female dairy animals. Normally, one can get one calf from a superior female dairy animal in a year. But by using MOET technology, one can get 10-20 calves in a year from a cow/buffalo.During the last 40 years hormonal regimens forsuperovulation in cattle have been refined and combined withsubsequent transfer of fresh or cryopreserved embryos, a combination of techniques is called multiple ovulation and embryo transfer(MOET). Today, this technique has become a routine procedurein many countries, and in 2015 more than 520,000 *in vivo* derivedbovine embryos were transferred to recipients worldwide, mainlyin North America and Europe (Perry 2016).ET can increase the genetic potential of a herd in a relatively short period of time. ET can increase milk production in dairy herds.ET can increase weaning weights in beef and dairy herds. ET allows other producers to take advantage of superior genetics because frozen embryos can be shipped almost anywhere.ET preserves superior genetics for future generations due to embryo freezing. Procedure involves in multiple ovulation and embryo transfer are mentioned below

- Selection of donor
- Selection of recipients
- Superovulation of donor
- Synchronization of recipients
- Flushing of the donor
- Searching & grading of embryo
- Donor & Recipient management
- Transfer of embryo to recipients
- Freezing/storage of surplus embryo
- Pregnancy diagnosis recipients

1.1 Selection of Donor

Few salient points to be taken while selecting the donor

- Donors merits based on her outstanding qualities such as production performance, market values of her progeny and her genetic background
- Donor should be healthy and having body conditions score > 3.0 and also avoid obese animal
- At least 60 days postpartum, regular breeder, devoid of any conformational or detectable genetic defects and should have sound reproductive organs
- It is preferred the donor must have calved at least once to prove its potential as a good breeder and producer.
- Donor selection can be made in advance before calving and should be monitored closely for post partum care and can be programmed 60 days post calving.
- It is also appropriate that donor should be programmed during the favorable breeding season
- Higher producer but a repeat breeder should also not be included as donor. In most of instances such repeat breeder donor are poor responder in super ovulation.
- There should be a minimum of 60 days interval between two super ovulations.
- Based on genomic selection the heifer can be inducted under MOET programme.
- A.I gun shall be used to ensure the patency of cervix before the FSH injection.
- The donor selected should be young (<8 years for HF and JERSY <12 years).
- Donor cow must be II to IV lactation however older animal can be consider based on production and reproduction performances.
- Remarks: Elite animals that are poor responder to SOV on two or more occasions should not be used for further superovulation (SOV)
- Infertile anestrous obstetrical or gynecological problems must be avoided.
- Ovaries with cyst, very long pendulous uterus must be avoided.

- The ultrasonography can be used on 7th day of the estrus of donor to assess the pool of antral follicle that would be available for recruitment the following the FSH injection if dominant follicle is available on 7th day than that follicle must be ablated.
- The super ovulatory protocol can be started in donors with the minimum time laps of 20 days post vaccination programme.
- The donors in the field may be given additional concentrate of 2 kgs per day and mineral mixture for at least 1 month prior to super ovulation.

1.2. Selection of recipients

Few mentioned points should be taken in consideration while selecting the recipient

- Repeat breeder, cystic animals, irregular cyclic, anestrus or animals having other reproductive abnormalities are not suitable recipients as ET is not practiced to treat the infertility conditions in the farm animals.
- Animals to be selected as recipient may be genetically inferior to their contemporaries to whichever trait they are been selected for.
- Heifers as well as regular breeder cow results in highest conception rate following transfer.
- Mature heifers can be used as recipients.
- The recipient cow should have completed minimum 60days post partum, so as to allow the complete involution of uterus and attain normal reproductive hormonal profiles.
- AI gun shall be used to ensure the patency of the cervix on the heat date.
- Recipient and donor having synchronized estrus on same day result in highest pregnancy rate.
- Recipient and donor having estrus synchrony variation of ± 1day produces average result

1.3. Synchronization of recipient

- Single dose PG
- Double dose P.G

(Animal on standing estrus is preferred as recipient)

1.4. Super ovulation of Donor

Type of Animals Total FSH Dose (Folltropin –V/ Stimufol)

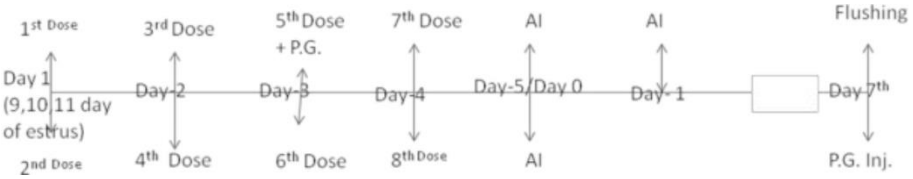
Species	Stimufol	Folltropin –V
Bosindicus	150 - 250mcg	150 - 250mg
Bosindicus X Bostaurus	250 - 350mcg	250 - 350mg
Bostaurus	300 - 400mcg	300 - 400mg
Bubalusbubalis	400- 600 mcg	400 - 600 mg

1.5. Dose regimeFSH is injected intramuscular for four days morning and evening with total of eight doses. The FSH doses can be given in as below mentioned

- Constant dose – Eight equal doses of FSH
- Tapering dose- Uniformly tapering dosage

There is linear correlation between the FSH dose and super ovulatory response, however the FSH dose versus recovery is not a linear co-relation, and the recovery may decrease drastically. Therefore the dose standardization is must to conduct the MOET work.

1.6. Dose schedule skeleton for FSH



1.7. Recovery of embryo (Non surgicalflushing)

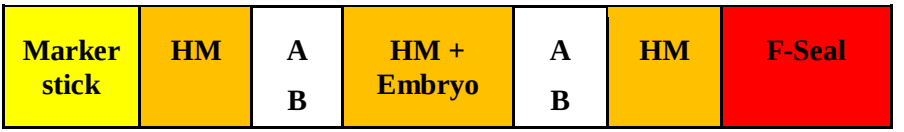
Non surgical flushing should be done under caudal epidural anesthesia

1.8. Searching of embryo

Searching should be done under steriozoom microscope (10 -20 X magnification) by adjusting the light and proper focusing and ZonaPellucida is a good land mark for embryo identification and minimum three searching should be done per petridish and at last content of embryo filter should also be scanned the microscope for embryo.

1.9. Loading of straw for fresh transfer

Take sterile 0.25 cc French straw, and rinse it twice with holding medium to remove any dust or toxic contaminant, taking care not to wet the cotton plug.



1.10. Transfer of embryo

Transfer should be done under epidural anesthesia ipsilateral to the corpus luteum and surplus embryo must be frozen. Sometimes, sufficient numbers of viable embryos are not available as the recipients. In such recipients, frozen embryo can be transferred after thawing and evaluation.

1.11. Donor aftercare

Immediate after flushing of donor, one dose of sterpto penicillin should be infused intrauterine to reduce the possibilities of uterine infection. Simultaneously, one dose of $PGF_2\alpha$ should also be administered immediately after flushing to cause lysis of the CLs.

2. In vitro embryo production

The collected oocytes (Either through USG-OPU or abattoir bases ovaries) are filtered through emcon filter with PBS and five percent fetal calf serum. The cumulus oocyte complex is then observed and classified into

Grade 1) > three compact morula layers

Grade 2) One layer of cumulus cell

Grade 3) Denuded oocyte

Grade 4) COC with degenerated cytoplasm.

Prior to maturation, washing of (COCs) should be done with TCM 199 and 10 % fetal calf serum and gentamycin. Different COCs should be cultured for 24 hrs in maturation media at 39°C with 5% CO_2. The frozen semen is usedfor invitro fertilization is thawed and centrifuged by means of Percoll gradient, density gradient method, swim up technique, using BoviPure and BoviDilute or BO- Semen washmedia. The heparin is used (30ug/ml) for capacitation of spermatozoa. After maturation COCs washing is done 2-3 times with TCM-199 containing HEPES and BSA. Fertilization is done using fertilization media (BO-IVF-Fertilization medium) and 2×10^6 sperm per mL in a 100 µL droplet covered with mineral oil in petri plate for 18 hours. Following fertilization, the COCs is shifted to culture medium of size (100 µl drop) a synthetic oviductal fluidwith BSA under mineral oil in petri plate is used for culture, the embryo is cultured for a week (seven days) at 39°C with 5% CO_2. On 7^{th} day after IVF the blastocyst rate can be determined.

2.1. Brief about factors affecting oocyte quality and recovery rate

Nutritional health, parityand adequate estrous cycle regulation are factors that influence a donor's ability to produce embryos. These characteristics when considered collectively play a critical role in the development of a successful embryo transfer programme. The success rate of IVF majorly depends on quality and quantity of oocytes retrieved at the time of OPU. The quantity and quality of collected oocytes depends on frequency of treatment, use of superstimulation, developmental stage of aspirated oocyte.If collected oocyte may not completed maturation at the time of collection it may decrease blastocyst yield. The embryo production rate was largely reduced when collected from superstimulated donor and matured in in-vitro medium than in-vivo. The quality of oocyte referred as the ability of the oocyte to form blastocyst, this not only depends on oocyte but also on method of collection, source of oocyte, media used,

2.2. Session timings- at the time of OPU different follicles of size (> two-three mm) are aspirated which resulted in growth of other follicles in later days. For ex- If ovum pick up (OPU) is performed in 7 days interval it will result in yield of more COCs when compared with day 3 collection, but the quality of oocyte is goodif aspirated at 3-day interval than day 7. May be the dominant follicle affects the developmental competence of other follicles. Also, if Coasting (FSH stimulation) is done before aspiration of oocytes, this results in development ofmore follicles, more COCs recovery and more embryo production.

2.3. Different OPU procedures –The collection of COCs is more in slaughter house ovaries as compared to aspiration from live animal may be due to better assessment of all follicle through naked eyes. The machine used in OPU should be of good quality for better visualization of follicles this also helps in good recovery of embryo.

2.4. Oocyte maturation duration -The maturation time may affect the embryo yield some reports suggest more is the maturation time less is the embryo recovery this may be due to oocyte ageing. Average maturation time is 16-24 hrs.

2.5. Personals involve in OPU- The collection of COCs may vary when done by different personnel's

2.6. Season- For seasonal breeding area the embryo production through IVF may be helpful to make embryos and store for use in favourable season. The oocyte itself can be stored which later thawed and fertilized invitro with frozen thawed semen to make embryo in favourable season. The freeze- thawed oocyte yield less embryos of low quality as compared to fresh embryos.

3.0. Animal cloning

Somatic cell nuclear transfer called cloning; it is a type of biological copying. Cloning prize cow/bull, breeding through clones and keeping their offspring, the farmer can introduce the natural positive characteristics into the herd quickly. It would take several more years to achieve these same improvements by conventional breeding.

3.1. Steps involved in somatic cell cloning

- ❖ Isolation of oocytes
- ❖ Maturation of oocytes
- ❖ Enucleation of oocytes
- ❖ Transfer of somatic cell
- ❖ Fusion and Activation
- ❖ In Vitro culture
- ❖ Transfer into foster mother

3.2. Problems in cloned animals

- Lower pregnancy rates
- Higher abortion rates
- Fetal & placental abnormalities
- Large offspring syndrome
- Changes in gene expression pattern

4.0. Transvaginal aspiration of cyst as a therapeutic measure for COD in Bovines

Prior to the advent of hormonal preparations, manual rupture of the cyst during rectal palpation was practiced to treat the condition at postpartum routine reproductive examination. This method of treatment cannot be recommended because it can cause trauma or haemorrhage, which might result in ovario-bursal adhesions. Hormones that induce the release of LH from the anterior pituitary (e.g. GnRH), or have LH-like action (e.g. hCG), or LH itself can be used to treat follicular cysts and also now people are combining CIDR/PRID along with GnRH and prostaglandin treatments.

Alternatively, emptying of the cystic fluid may be advantageous in such cases (Cairoli et al., 2002). In this regard, Ultrasound guided trans-vaginal follicular aspiration (TVFA) in cattle has been used for oocyte collection in vitro embryo production (ovum pick-up, OPU), dominant follicle ablation (Bergfelt et al., 1994). After removal of the cystic content the cows are deprived from the main source of oestrogens along with other possible locally acting factors that leads to a new follicle development and finally ovulation (Amiridis, 2009) and also space occupation. It has suggested that removal of steroids or metabolites produced by cystic ovaries could be involved in the success of these mechanical treatments. So, transvaginal ablation can be effectively used as an alternative to hormones at field level for prolonged duration cystic ovarian follicles. Treatment with buserelin after needle aspiration of the cyst, produced an overall recovery rate of 75.6% within 30 days of treatment and a conception rate of 64.7%.

IMPACT OF HEAT STRESS ON REPRODUCTION IN DOMESTIC ANIMALS

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Thermal stress challenges an animal's homeostasis or equilibrium, resulting in a stress response. The impact of thermal stress on the physiological, biochemical, endocrine, immune and nervous systems compromises animal performance and prevents the realization of optimum reproduction. High ambient temperature, relative humidity, and radiant energy compromise the ability of lactating animals to dissipate heat, and coupled with metabolic heat, it makes it challenging to maintain thermal balance. Environmental factors and animal factors, including thermoregulatory mechanisms, significantly affect the energy exchange between the animal and the environment (Nienaber et al., 1999). Animals reared in subtropical and tropical regions are subject to high ambient temperature and/ or high relative humidity for extended periods, reflecting their low productivity (Abi Saab and Saleiman, 1995). In these agroecological zones, Temperature-Humidity-Index (THI) reflecting extreme stress persists for 5-6 months of the year. This severely hampers the animal's productive and reproductive capability.

The THI (combined effects of temperature and humidity) is used as a guide to measure heat stress. There are three stress categories: alert, THI 75-78; danger, THI 79-83 degrees; and emergency, THI 84 and above.

In mammals, the high environmental temperature may cause delayed puberty, delayed onset of the sexual season, irregular cycle length, duration of estrus, ovulation rate, frequency of anovulatory estrus, morphological abnormalities in ova, embryonic mortality, fetal abnormalities, abortion and abnormal semen characteristics. Thermal stress challenges livestock's reproductive performance through various altered physiologic means, including altered follicular development, lowered oestrus activity and impaired embryonic development. The effect of a hot environment on reproduction may occur through a direct action of hyperthermia upon the reproductive tissues or indirectly due to lower nutrient intake and impairment of hypothalamic, pituitary, gonadal and endometrial secretions. Thermal stress

and nutritional stress negatively affect most factors related to reproductive performance in sheep (Hansen and Aréchiga, 1999; Maurya et al., 2004). Further, undernutrition of livestock results in lower body weight and BCS, which has a negative effect on oocyte quality, which results in lower rates of cleavage, and numerous reproductive functions, including hormone production, fertilization and early embryonic development (Sejian et al., 2010b).

Growth is characterized by increased live body mass or cell multiplication and is controlled genetically and environmentally. The available nutrients, hormones and enzymes, and elevated ambient temperatures are considered some of the factors that can influence average daily gain (ADG) (Habeeb et al., 1992). In addition, thermal stress drastically affects the overall growth performance of animals, i.e. growth rate, daily weight gain and live body weight. The calculated loss in the body solid due to heat stress conditions was found to be 14-29% in cattle (Kamal and Johnson, 1971). The effects of elevated temperature on growth performance are the products of decreased anabolic activity and increased tissue catabolism. The decrease in anabolism is essentially caused by the decrease in voluntary feed intake of essential nutrients, particularly metabolizable energy, for both maintenance and weight gain. Exposure of the pregnant animals during mid and late gestation to warm ambient temperatures significantly decreases the embryo cell number and placentome size. In addition, calf birth weight, live body weight gain or growth rate, as well as total body solids and daily solids gain, are impaired by exposure to elevated temperatures, which may lead to severe economic loss.

Proper assessment of sexual behaviour and receptivity before breeding of animals may increase the possibility of fertile mating. High environmental temperature or the rapid and sudden fluctuations of temperature that often occur in arid and semi-arid regions have a detrimental effect on the sexual behaviour of animals (Maurya et al., 2005). The implication of thermal stress is such that even if an animal has a normal ovarian development leading to ovulation, there is a reduction in the full expression of estrus behaviour that might lead to the failure of the animal to mate and conceive. A higher incidence of silent heat and anoestrus is one of the main problems in cows and buffaloes during summer or on exposure to high ambient temperatures. Heat stress compromises oocyte growth in cows by altering hormonal secretions during the oestrus cycle (Ronchi et al., 2001) as well as impairing embryo development and increased embryo mortality (Wolfenson et al., 2000). Heat

stress affects the development of follicles (Badinga et al., 1993; Wilson et al., 1998). The pre-implantation embryos are susceptible to maternal heat stress, but the susceptibility declines as development proceeds. In cattle, exposure of lactating cows to heat stress at day 1 after oestrus (2-cell embryo stage) reduced the proportion of embryos that developed to the blastocyst stage at day 8 after oestrus (Ealy et al., 1993). However, heat stress at day 3 (8–16 cells), day 5 (morula) and day 7 (blastocysts) was observed to have no effect on the proportion of embryos that were blastocysts on day 8.

It has been reported that heat stress suppresses thefunctions of the largest follicle, i.e. the dominant follicle. Under heat stress, the size of the dominantfollicle is reduced during the first and second follicular waves,and the numberof follicles of the next largest size is increased.Thermal stress during folliculogenesis could lead to the ovulation of low-quality oocytes with lowered developmental competence. The circulating concentrations of gonadotrophin, which plays anessential role in regulating follicular dynamics, are also alteredby heat stress. In addition, heat stress induces alterations in the follicular LH receptors. Alfujairi et al. (1996) reported the adverse effect of hot summer on the ovulation rate in cows.

Most cells in the body produce Heat Shock Proteins (HSPs) in response to heat stress that limits the damaging effects of elevated temperature on cell function. Unfortunately, in cattle, around the time of ovulation, the oocyte and /or the resulting early embryo are unable to produce such proteins. Consequently, embryo viability is compromised, resulting in lower conception rates. Embryonic loss can occur when there is a disruption in the physiological regulation of oviductal and uterine function. In cattle, the exposure of pregnant females to heat stress during the embryonic period leads to embryonic loss (Ealy et al., 1993). Fetal growth within the uterus is a complex biological event influenced by genetic, epigenetic, and environmental factors and maternal maturity. These factors impact the placenta's size and functional capacity, uteroplacental blood flow, transfer of nutrients and oxygen from mother to fetus, conceptus nutrient availability, the endocrine milieu, and metabolic pathways (Wu et al., 2006).

Heat stress drastically reduces animal pregnancy rates (Hahn et al., 2003). The reproductive efficiency of the animals in the environment they are kept should be fine-tuned to produce the maximum number of offspring most efficiently (Lindsay, 1996). During heat stress, redistribution of blood flow from the viscera to the periphery increases for dissipation of heat, which leads to reduce blood supply to the placentas and retards fetal growth (Collier et al.,

1982). The fertility of animals is markedly affected by exposure to high ambient temperature immediately before estrus or after mating. The calf born from heat-stressed cattle has a lower birth weight than those born from control cows maintained under comfortable environmental conditions. Exposure to thermal stress reduces the body condition score of animals, affecting the birth weight of newborns (Sejian et al. 2010a).

The male animals are the most important factor for the overall reproductive efficiency and productivity of herd/flocks, which is sometimes overlooked. A highly fertile and adapted bull will settle a greater number of cows early in the breeding season and will be more likely to fertilize a higher proportion of eggs than a less adapted bull with poor fertility. Sephadex filtration of semen may increase the seminal quality of buffalo bulls (Maurya et al. 2003). Bulls with relatively rapid ejaculation rates can inseminate a greater number of cows per unit of time than males with poorer libido. Tests for ranking bulls on sexual performance are repeatable and reliable predictors of sexual performance under field conditions, potentially allowing breeders to evaluate the mating competence of individual males before they are employed in a breeding programme. The stage of spermatogenesis is most susceptible to elevated temperature is the primary spermatocytes, although damage to B spermatogonia can occur in bulls and prolonged exposure to heat can damage dividing spermatocytes and spermatids (Thatcher and Collier, 1982). Heat stress occurs when the scrotum is not able to reduce the temperature of the testicles below normal body temperature and results in loss of motility, an increased proportion of abnormal sperm, decreased concentration of sperm and ultimately, cessation of spermatogenesis. In addition, there was a higher percentage of abnormal spermatozoa and acrosomal damage. Blanc et al. (1981) reported that heat stress on the scrotum drastically affects the function of Sertoli cells and the release of androgen. The body condition score drastically affects the seminal attributes of animals (Maurya et al. 2010).

Ruminants primarily adjust evaporative heat loss to maintain homeothermy during brief exposures to adverse weather but will reduce feed intake to lower heat production during prolonged hot weather. In a hot environment, energy exchanges by radiation are dominant. To alter the microclimate of an animal effectively through housing or environmental modification, we must consider altering one or more of the following factors: temperature of the surroundings; air temperature; air velocity; air vapour pressure; radiation or shade factors; and conductivity of surfaces that animals might contact. Grazing animals or animals giving birth will seek shelter from

strong winds. Structures or trees can markedly reduce wind speed and benefit exposed animals' survival (especially newborns). However, windbreaks are important beyond these benefits, especially in tropical and subtropical regions. A windbreak acts as a barrier lowering the wind speed near the ground surface, deviating and splitting the air stream. Using leguminous trees or shrubs can be a practical means to counteract the effects of wind and heat stress.

Shades and other minimal measures should be considered a form of insurance for protecting farm animals in hot climates. As a matter of fact, when dairy cows are given access to adequate shade, milk production is increased (Davison et al., 1988). The radiant environment in the shade has three constituent parts: the cold ground in the shade, the hot ground outside the shade, and the roof's lower (inner) surface. As for the materials used, hay or straw shades are the most effective and low-cost artificial materials. The ground cover around a shade is a factor of importance. The level of thermal radiation above the grass field is less than above dirt ground (Bond et al., 1969). Thus, shades are essential for animals in stallfed animals in a hot, sunny environment. The most effective shades are trees, as they protect from sunlight.

Shades, sprinklers and fans are very effective methods of improving the thermal environment for animals in hot, humid climates. Genetic variation in cattle for cooling capability suggests that more heat tolerant cattle can be selected genetically, and cross-breeding may also offer opportunities. Developing suitable strategies is required to reduce the adverse effect of thermal stress and economic losses to the farmers for large and small ruminants reared in tropical environments.

INFRARED THERMOGRAPHY: A MODERN APPROACH FOR MANAGEMENT OF ANIMAL HEALTH AND REPRODUCTION

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Introduction

The evolution of infrared thermography (IRT) as a precise, efficient, non-invasive, and simple non-contact tool to monitor the body surface temperature at the farm level more precisely at the animal level brings new dimensions to dairy animal management. Any object releases infrared energy; the infrared camera captures such energy and produces a thermogram depending on the article's emissivity, conductivity, and temperature. Different colour palates depict the thermogram; one denotes the coolest area with a blue or black region and the warmest area with a white or red region, and it can detect various physiological and pathological events through skin surface temperature change. The body temperature change is associated with the blood flow change in animals, depending on the physiological, climatic, and biochemical changes during metabolism. The skin's temperature is one of the critical indicators of health status, and animal dissipates their excess heat by the skin from the core body, and peripheral blood circulation helps in heat dissipation (Collier, 2006). The temperature change can be precisely measured by IRT (Mcmanus et al., 2016). The thermographic cameras can measure body surface temperature as heat exchanges with the surroundings, whereas the rectal thermometer measures the conductive heat transfer through the sensor. Researchers are exploring such opportunities in animal science, where temperature change can be an essential phenomenon in the case of feed efficiency, disease diagnosis, and climatic and physiological stress in animals. It can also be used to monitor inflammation, reproductive efficiency (Talukder et al., 2014; Talukder et al., 2015), hoof (Alsaaod et al., 2015), and udder (Polat et al., 2010), health and ectoparasite infestation in livestock animals. IRT can monitor thermal profile and animal welfare in various

climatic conditions more precisely (Stewart et al., 2005; Paim et al., 2013). Animals maintain homeostasis through conduction, convection, radiation, and evaporation, but the thermoregulatory mechanism gets affected due to elevated environmental temperature, and IRT can detect body surface temperature change, which primarily occurs due to blood flow change (Knizkova et al., 2007).

Unlike pros use of IRT has certain limitations under field conditions regarding the capture of the image in a controlled environment under shaded conditions; otherwise, sunlight, moisture, dirt and weather conditions, etc., can affect the accuracy of the IRT. Besides that, the distance of the handler from the object while capturing the image and stability of both and trained handler are the pre-requisite requirements. Extensive study under controlled and real-time farm situations is needed regarding the efficient use of IRT for various conditions across different species to open up new avenues toward precision livestock farming.

Infrared thermography and its working principal

Infrared thermography is a non-contact sensor method for determining the temperature of objects' surfaces without any radiation effect. The advantages of infrared thermography sensors are less maintenance required, durable, longer service life, suitable for in-line monitoring due to small size and fast response time, ability to identify immediate and dynamic motions, and able to generate a digital image or high-speed video. The infrared radiation of mid-to long-wave emitted by any object can be captured using Infrared thermography in a non-invasive way. William Herschel made the first breakthrough in 1800 for the discovery of Infrared radiation (IR), then his son John Herschel created the first heat image in 1840, which was visible via differential evaporation of a thin oil film subjected to heat. The industrial use of Infrared thermography (IRT) as a tool took 140 years. It was made available to research and industry in the 1950s. In the 1960s United States developed IRT for military use to carry out nocturnal surveillance and heat signature identification (Rogalski, 2012).

Japan, Europe, and America are the pioneer in developing new generation thermal imaging equipment in the 1960s and 1970s. The 1970s saw the introduction of battery-operated portable IR scanning devices with television sets. The use of liquid nitrogen as a cooling agent in these devices limited some of their applications; thus, in the 1980s, the first thermo-electrically cooled systems with greater portability were developed. In the

nineteenth-century bolometer was discovered by Samuel Langley and brought improvements in IR sensing, and it identified the heat signature of the cow from 400 meters distance. By the 1990s, a handheld non-cooled camcorder had been developed. After 2000, thermal imaging technology advanced quickly, and low-cost, compact devices and automated systems are now available on the market. IRT captures the thermal image either by scanning the surface of the object point by point or by focusing on the thermal radiation of the surface using cooled or uncooled sensor arrays, and it depends on the based on temperature, emissivity, the conductivity of the articles (Knizkova et al., 2007). The IRT camera is available in the market with various levels of infrared resolution (pixel), thermal accuracy, and sensitivity. The application of thermal imaging is increasing in human health, diagnostic, agriculture, and livestock, although industrial use was prevalent. The IRT's evaluation of structures, building materials, locating the source of distress, moisture ingress and flow through pipes, etc., is typical in Industries. In agricultural operations, to evaluate seed viability, soil quality, crop water stress, water status, diseased plants, fruit yield, maturity of fruits and vegetables, etc., can be monitored by IRT. Disease diagnosis in humans using IRT is more common, and it can be helpful in the assessment of arthritis, urology problems, reflex sympathetic dystrophy syndrome, neurological disorders, vascular diseases, and cancer. IRT use has become more witnessed for quickly thermal screening of larger populations under the COVID19 pandemic. The application of IRT in animal science is more recent, and researchers used IRT for the identification of temperature change in lameness, mastitis, inflammation, fever, calf diarrhea, calf pneumonia, bovine viral diarrhoea (BVD), BRD, respiratory disorders, injuries, oedema or vascular thrombosis, foot and mouth disease, estrus and ovulation time. IRT has also been used in counting livestock, specially wildlife. Besides, IRT has been used to understand thermal comfort, scrotal thermoregulation, animal welfare, meat quality, feed utilization efficiency, heat and methane production in livestock science.

The working principle of infrared thermography is based on the physical phenomena of electromagnetic radiation being emitted by any object with a surface temperature above absolute zero (-273.15 ˚C) can be captured by Infrared sensors. The radiation of the objects is proportionate to their internal temperature. The thermographer's optics focuses the beams on a detecting element, which generates an electrical signal proportional to the amount of radiation. The signals are then processed and amplified to provide temperature-related output signals. This information can be seen on a computer or transmitted to a control system through a connecting link.

Planck's radiation law is used to calculate the infrared temperature. It describes the relationship between the temperature and wavelength of a black body's spectral radiation into space. The infrared energy of objects is detected and measured by an infrared camera (also known as a thermal imager). The camera turns the infrared data into an electronic representation of the measured object. An optical system in an infrared camera concentrates infrared energy onto a particular detector chip (sensor array) with thousands of detection pixels arranged in a grid. Each pixel in the sensor array produces an electrical signal in response to the infrared energy concentrated on it. The camera processor analyses each pixel's signal and uses a mathematical formula to build a colour map of the object's perceived temperature. A different hue is allocated to each temperature value. The generated colour matrix is saved in memory and displayed as the object's temperature picture (thermal image) on the camera.

Applications of Infrared Thermography in the Veterinary field:

Delahanty and Georgi employed infrared thermography (IRT) in veterinary medicine for the first time in 1965, and correct thermograms in specific sections of horses' bodies were identified. Changes in vascular circulation cause a rise or reduction in tissue temperature in live creatures, which is then utilized to assess the status in the given area. IRT was tremendously helpful in diagnosing various disorders before they could be seen clinically. The use of infrared thermography in multiple species by different authors are mentioned below:

Species	Applications	Reference
Cattle	Assessing ruminant methane output	Montanholi et al., 2008
	Detection of systemic infection in calves	Schaefer et al., 2004
	Assessing transportation stress	Schaefer et al., 1988
	Early Mastitis Detection	Hovinen et al., 2009; Polat et al., 2010
	Detection of estrus and prediction of ovulation	Talukder et al., 2014
	Predicting carcass quality	Schaefer et al., 2000
	Lameness Diagnosis	Nikkah et al., 2005
	Estrus detection and andrological	Turner et al., 1991

Horses	evaluation	Stelletta et al., 2012
	Detection of back injuries:	Pavelski et al., 2015
	Measuring stress levels	Moura et al., 2011
Swine	Detection of Febrile Diseases	Cook et al., 2015
	Estimation of stress level and welfare	Mitchell, 2013
	Detection of estrus	Scolari, 2010
Other mammals and birds	Assessment of feather cover in Brown Leghorn hens	Cook et al., 2006
	Assessing the birth coat's thermal insulation in 3-day-old lambs	Mala et al., 2004 and Knizkova et al., 2005
	Tool for investigating the temperature variations in rabbits	Stewart et al., 2005

Besides that, various diseases of livestock were diagnosed using IRT. The increase in temperature of the nose (3.5°C), ear (3.9°C), and eye (6.1°C), respectively, were recorded in the case of Bovine viral diarrhoea (BVD)caused by Pesti virus in dairy cows (Schaefer et al., 2004). Literature depicting that IRT can detect one of the highly contagious viral diseases, i.e., Foot and mouth disease (FMD), based on surface temperature change in the mouth, coronary band, and mammary gland area as well as fever and viremia, although the clinical manifestation of vesicular lesions is evident (Gloster et al., 2011). The increase of 4.8°C, 7.2°C, and 8.9°C of foot temperature at pre-clinical, clinical, and post-clinical stages of FMD infection was recorded (Rainwater-Lovett et al., 2009), whereas in mule deer, Dunbar et al. (2009) reported an increase of 5.7°C in case of the first appearance of FMD foot lesion.

Assessment of heat stress by IRT

IRT is a safe and non-invasive method to evaluate the skin surface temperature of animals to understand the thermal profile of the animal body. IRT can help in welfare assessment, heat production, and heat loss under various physiological processes associated with body temperature change. The body temperature of animals changes during improper handling and restraining and during adverse environmental conditions due to blood flow change (Mazieiro et al., 2012). Physiological parameters, i.e., body temperature, pulse rate, and respiratory rate, are good indicators of heat

tolerance and better adaptability (Costa et al., 2015). IRT can also identify the heat stress-affected animals by monitoring specific body regions (Costa et al., 2015) as IRT can understand the temperature change more efficiently. It is well documented that IRT temperature of muzzle, cheek, and face is the better predictor of physiological stress (Montanholi et al., 2008; Weschenfelder et al., 2014), and IRT muzzle, neck, and rump region of lambs indicate thermal stress (Paim et al., 2012). IRT eye temperature and salivary cortisol levels were reported to be a better indicator of stress due to increased heat load condition catecholamines and cortisol level increased because of activation of the Hypothalamic-pituitary-adrenocortical axis (Schaefer et al., 2002). The seasonal effect on body surface temperature is pronounced as significantly higher body surface, and scrotal temperatures were observed during the summer season in comparison to winter seasons in Sahiwal and Crossbred bull, but scrotal surface temperature gradient was higher during winter and lower during summer may be associated with poor quality semen production during summer months.

Similarly, a higher temperature gradient during winter (4.0 °C) and lower during summer (0.9 °C) was also reported by Menegassi et al. (2015). The relationship between scrotal surface temperature and temperature gradient with sperm was also understood (Menegassi et al., 2015). The adverse effect of thermal stress on semen quality was more evident in crossbred bulls.

Udder health monitoring using IRT

Udder health monitoring is generally ignored in dairy animals at the farmer's level. Therefore, the incidence of sub-clinical and clinical cases is increasing, and the country is losing approximately INR 7165.51 crores due to mastitis, and major share of INR 4151.16 crores (57.93% of total losses) is due to sub-clinical mastitis in India (NAAS, 2013). Early identification of mastitis and sub-clinical mastitis is essential for identifying causative organisms and implementing efficient management techniques. Identifying SCM is difficult as no visible sign is observed in udder and milk. The diagnosis of mastitis is laboratory-based primarily, but recently IRT as a non-invasive technique to monitor the change of udder surface temperature due to inflammation got the attention of researchers and documented udder and teat temperature change due to localized inflammation using IRT (Berry et al., 2003; Schaefer et al., 2012). In Holstein and Brown Swiss cows, 1ºC higher temperature was recorded in mastitis-affected quarters compared to healthy quarters and found that SCC is highly correlated with udder skin surface temperature (Colak et al., 2008). The literature depicts the various degree of change in udder surface

temperature in SCM and CM using IRT, and most of the studies reflected IRT can detect CM more efficiently (Scott et al., 2000; Metzner et al., 2014) as compared to SCM, because some contradictory results are available for detection of SCM using IRT (Barth, 2000; Polat et al., 2010). Therefore, IRT can be used as a supportive tool for early diagnosis and assessment of udder health in routine dairy animal management.

In our laboratory during the autumn season, Sahiwal, Tharparkar, Gir, HF crossbred, and Murrah buffaloes were screened for healthy, subclinical, and clinical quarter based on California mastitis test (CMT) and somatic cell count (SCC), and thermal images were captured using a handheld digital infrared thermal camera (DarviDTL007). An increase in udder and teat surface temperature was observed in the case of subclinical (SCM) and clinical mastitis (CM) quarters as compared to healthy quarters of Sahiwal, Tharparkar, Gir, Murrah, and HF crossbred cows. CMT and SCC values showed a significant increase ($p<0.05$) in SCM and CM and a positive correlation between the mean values of udder and teat surface temperatures. In another study increase in udder surface temperature was also recorded in case of sub-clinical and clinical mastitis affected quarter as compared to healthy in case of crossbred goats. In the laboratory a total of 2721, 3287, and 2719 thermograms of clinical, sub-clinical mastitis affected quarters and healthy quarters, respectively, of Sahiwal cows and 2493, 2749, and 2373 thermograms of clinical, sub-clinical mastitis affected quarters and healthy quarters, respectively, of Murrah buffaloes belonging different parity and stage of lactation were collected and improved deep learning Convolutional Neural Networks (CNN) was trained using the data set. The training and validation accuracy of Sequential model was 0.74 to 0.99 in Indigenous cattle and buffalo.

Lameness evaluation using IRT

Lameness is one of the costliest diseases in dairy animals, leading to production and reproduction loss and associated with various health issues. In the case of lameness, sometimes inflammation or swelling is not pronounced, but the change in surface temperature is distinct due to vasodilatation and increased blood flow (Hovinen and Pyorala, 2011). It is well documented in the literature that a change in temperature in the coronary band and surrounding area is suitable to monitor the lameness problem as the coronary band has less hair, highly vascularized, and reflects the blood circulation of the claws. The lameness can be visually assessed by scoring methods and is well accepted worldwide, but the change in posture does not occur even in the

presence of a foot lesion, or the animal does not show signs of lameness till the lesion becomes severe (Laven and Proven, 2000; Stokes et al., 2012). Therefore, IRT has added advantage in early assessment of temperature change in case of foot lesion and lameness (Alsaaod et al., 2014) even the appearance of actual clinical signs of lameness, so better management strategy can be formulated to prevent or reduce the lameness cases. Once an animal got affected with lameness, it is complicated to manage or treat the lameness cases as it may take a few days to a few months, depending on the severity of the cases. In case of lameness, 1-3 ^{O}C increase in the coronary band's surface temperature was reported.

Tick identification using IRT

Tick infestation is a prevalent problem in dairy animals, and due to which, worldwide economic loss is huge (Jongejan and Uilenberg, 1994), but the awareness among the farmers regarding the loss is less. In a year world is losing 20 to 30 billion US$ (Lew-Tabor and Valle, 2016), and India about 498.7 million US$ (Minjauw and McLeod, 2003; Chhillar et al., 2014; Geevarghese et al., 1997) due to tick infestation. Production loss, a decrease in hide quality, and disease spread among the animals are the major factors associated with the economic loss (Gracia, 2003). Manual tick counting is time-consuming, strenuous, slow, skill and labour-dependent. IRT can identify the tick's efficiently based on the principle that tick temperature is lower than the animal's body, and it can further be automatically analyzed by ImageJ software (Cortivo et al., 2016) and algorithm (Barbedo et al., 2017). Environmental temperature, long hairs, and the size of the ticks can influence the tick count. A study in the laboratory observed that the suitable time to capture the IRT image for identification and quantification of tick was from 6:00 to 10.00 AM. Identification and quantification of ticks were better in the inner thigh region, and the temperature (°C) difference was significantly higher in this region, followed by the body's perineum, dewlap, and lateral side. ImageJ analysis for auto-counting ticks was more efficient for the inner thigh region than perineum, dewlap, and lateral aspect.

Calf health monitoring using IRT

Calf mortality under field and farm conditions in India is one of the major concerns as a calf is the backbone of the dairy herd for future replacement. Not only that, if the calf gets affected by the various diseases in the initial stage of life, its future production performance is affected. In India, under field and farm conditions, 12.5 to 81 % of calf mortality was recorded (Tiwari

et al., 2007; Shakya et al., 2017; Patbandha et al., 2017; Panmei et al., 2016; Sreedhar and Sreenivas, 2015) and one of the major contributing factors is calf diarrhoea (Tiwari et al., 2007; Shakya et al., 2017; Sreedhar and Sreenivas, 2015). Few pieces of literature have reported as high as 80% calf mortality due to calf diarrhoea (Sreedhar and Sreenivas, 2015; Tiwari et al., 2007). It has been calculated that farm profitability can reduce up to 38 to 40% in case of 20% calf mortality (Singh et al., 2009). Therefore, early detection of calfhood diseases can help formulate efficient management strategies to reduce calf mortality and attain proper growth to achieve early sexual maturity (Shakya et al., 2017). Calf diarrhoea occurs due to bacteria or viruses or managemental negligence. It leads to septicemia and viremia, which is associated with increased body temperature. The emission of infrared rays, which can be captured by an IRT and eyeball region temperature, can act as a good indicator. Besides, IRT was proved to be helpful in identifying bovine viral diarrhoea (BVD) (Schaefer et al., 2004), bovine respiratory disease complex (BRD) (Schaefer et al., 2007, Schaefer et al., 2012), and Neonatal calf diarrhoea (Lowe et al., 2019). A study was designed in the laboratory for the early identification of calf diarrhoea using IRT during the natural course of infection in buffaloes. A significant increase in Orbital and rectal temperature was recorded on the day of diarrhoea as compared to two days before diarrhoea and the previous seven days' average temperature before diarrhoea in affected calves. IRT can be a supportive tool for the early identification of calf diarrhoea even before the actual clinical signs appear.

Estrous detection using IRT

The success of artificial insemination depends on accurate heat detection, which is very challenging in dairy farm management and associated with production, reproduction, and economic loss. The phenomena of temperature change in the vulval and vaginal region during estrus can be monitored by IRT (Rezac et al., 2002). The increase in vulvar temperature during the follicular phase and decrease during corpus luteum phage was recorded by Stelletta et al. (2012) may be that pre-ovulatory follicles released higher levels of estradiol. In pig peak estradiol level was observed 24 to 48 hours before the estrus when higher vulvar skin and body surface temperature was also recorded (Schmidt et al., 2013). During estrus in pigs increase of 1.58 °C (Sykes et al., 2010) and 5.3±2.4°C (Simoes et al., 2014) vulvar temperature was recorded 48 hours before estrus, whereas the increase of 1.0 to 1.4°C and 0.3 to 1.0 °C vulvar and vaginal temperature was recorded in Holstein Friesian cow (Talukder et al., 2014).

In a study in the laboratory, 14 cyclic multiparous animals and 8 cyclic heifers were synchronized by PGF2α protocol, and IRT monitored the temperature of the muzzle, eye, ear, and vulva from the day of synchronization to seven days. Estrus was confirmed by ultrasonography, progesterone hormone concentration, cervical mucus fern pattern, and spinbarkeit value. Maximum muzzle, eye, ear, and vulva temperature was recorded on the day of estrus. Eye and vulva temperature showed an increasing trend two days before estrus, then reached a peak on the day of estrus, followed by a decrease to regular after two days of estrus in the estrus synchronized Sahiwal cows. Significant ($p<0.05$) increase in the vulva, ear, eye, and muzzle temperature was recorded on the day of estrus from two days before estrus in Sahiwal cows. IRT can be a valuable tool to monitor the increase in vulvar temperature in estrus synchronized Sahiwal cows.

Application of IRT in male reproduction

Artificial insemination using frozen semen plays a vital role in the production improvement of dairy animals and countries' growth in milk production. Further bull is contributing as they are considered as more than half of the herd and their fertility is equally important to produce a good, viable and genetically potential offspring using good quality frozen semen, which is further dependent on efficient thermoregulation mechanism of the testis as it is related with spermatogenesis. IRT can be an efficient tool for understanding the testicular thermoregulation, which may get affected by environmental and vaccination stress. In breeding bulls, 2 to 6 °C lower testicular temperature compared to core body temperature is desirable for quality sperm production (Coulter and Kastelic, 1994). The elevated testicular temperature adversely affects semen quality and reduces fertility in livestock (Brito et al., 2004). Local mechanisms, such as counter-current heat exchange, blood flow regulation, the position of the testes, and sweating, play avital role in the maintenance of testicular temperature. The change or increase in testicular temperature resulting from heat stress changes the seminal and biochemical parameters leading to infertility problems in bulls. The season has an adverse effect on sexual behaviour, semen quality, testicular volume, and hormonal level, specially the effect was more pronounced on male reproductive performance during summer months (Bhakat et al., 2014; Cardozo et al., 2006). Studies in our laboratory depict the adverse effect of the summer season on semen quality parameters in dairy bulls (Bhakat et al., 2014; Bhakat et al., 2015). Coulter et al. (1988) demonstrated that IRT was equally efficient in monitoring testicular

thermoregulation compared to invasive sensors directly inserted in gonads, but the risk was associated with it. Various literature recorded an accuracy of 0.10°C in understanding the testicular thermoregulation using IRT (Purohit et al., 1985; Coulter, 1988). It was also documented that scrotal surface temperature is highly associated with inner testicular temperature (Coulter et al., 1988).

Relationship of a testicular temperature gradient with semen quality

Breeding soundness evaluation (BSE) is a mandatory requirement for the evaluation of bulls for selection for frozen semen production. But, BSE has been developed long back, and with time newer aspects have been explored extensively with better positive results and can be incorporated. During BSE, scrotal circumference has given prime importance to bull selection as it is associated with quality semen production. Many studies depict the importance of testicular thermoregulation, specially the testicular temperature gradient, which is highly correlated with quality semen production. IRT can be used asa supplementary examination tool for the reproductive evaluation of bulls (Ruediger et al., 2016). Our laboratory has conducted various studies to understand the relationship between scrotal surface temperature measured by the DarviDTL007 infrared camera and semen quality in indigenous (zebu), Crossbred and Murrah bulls. In all the three breeds, mass activity and non-eosinophilic sperm count were significantly ($p<0.01$) improved, and head, midpiece, tail, and total abnormality significantly ($p<0.01$) reduced with an increase in scrotal surface temperature gradient (SSTG) (group-I $\leq$ 4ºC, group-II- 4.1 to 6.8 ºC and group-III- $\geq$6.9 ºC) (Kushwaha, 2017). Another study on 130 Murrah buffalo breeding bulls revealed that semen quality was better in breeding bulls with a higher scrotal surface temperature gradient (SSTG) ($\leq$ 4ºC, 4.1 to 6.4 ºC, and $\geq$ 6.5 ºC) (Yadav, 2016). In another study, on 20 Sahiwal bulls, it was observed that the scrotal temperature gradient of good semen quality bulls (2.95±0.30°C) was more than poor semen quality bulls (1.7-5.7°C). The scrotal temperature gradient was negatively correlated (r -0.77) with the scrotal skin thickness (Abdullah, 2016). Usually, the testicular temperature is higher at the dorsal pole and lower at the ventral pole, and the gradient varies with different breeds and seasons. The higher temperature at the dorsal pole is due to the arrangement of the vasculature and the presence of pampiniform plexus (Brito et al., 2004);

the testicular artery ramifies from bottom to top (Kastelic et al., 1995), so the effective temperature gradient is maintained for the regulation of better thermoregulation, which is positively associated with quality semen production (Silva et al., 2017). The thermal comfort of breeding bulls gets compromised with a higher Temperature Humidity Index and adversely affects semen quality (Berry et al., 2011; Kastelic, 2014) may be due to higher scrotal surface temperature and inefficient thermoregulation (Kastelic and Brito, 2012; Santos et al., 2014). In breeding bulls, higher the testicular temperature gradient showed better quality and fertility (Lunstra and Coulter, 1997; Kastelic and Brito, 2012; Menegassi et al., 2015) might be associated with a decrease in sperm abnormality. It was demonstrated that scrotal insulation leads to a moderate increase in testicular temperature but significantly increases abnormal sperm percentage and decreases sperm production and motility (Kastelic et al., 2001; Fernandes et al., 2008).

Conclusion

Infrared Thermography with analysis software is the new generation non-invasive, noncontact, easy handling tool and can be effectively used in dairy farm management to improve dairy animals' production, reproduction, and health. IRT can be used to monitor the change of temperature in case of various physio-pathological conditions more precisely without any physical contact with the animal, but the specific limitation is associated with it while capturing the valuable information of the thermographic image due to dirt, debris, exposure of sunlight and humidity. Therefore, scientific validation of data using IRT under various climatic conditions across different parts of the country is required to formulate better management strategies to overcome the problem. The main focus of precision dairy farming is detecting various physio-pathological conditions early with higher accuracy. Therefore, in the future, IRT with the mobile-based artificial intelligent system can play an essential role in efficient dairy farm management.

HANDLING OF FROZEN SEMEN, ITS EVALUATION AND ARTIFICIAL INSEMINATION

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Introduction

Artificial insemination (AI) with frozen semen has been proved to be the best tool worldwide for genetic improvement through the dissemination of superior germplasm. This objective can be achieved only if the frozen semen used in the AI program confirms to the quality standards (Minimum standard for production (MSP) of bovine semen, 2019, Govt. of India). Maintaining the quality of frozen semen from semen stations to end-user requires an uninterrupted supply of liquid nitrogen (LN_2) to maintain cold chain. The "Cold Chain" is the system of transporting and storing frozen semen in LN_2 (-196^{0}C). The cold chain begins when frozen semen is manufactured, moves through to the state, district, and block distribution centres to AI centres, and ends with AI to the animals at farmers' door. This long-travelled frozen semen may get destroyed due to breakage in the cold chain. In this way, the frozen semen may lose its potency. The distribution of frozen semen in India is descendent from- Frozen semen station- large stakeholders- small stakeholders - AI centres-farmers' doorstep, whereas, in the government sector, semen station-main animal husbandry department- district animal husbandry department-veterinary hospital- different AI centres- farmers' doorstep. The entire distribution systems of frozen semen/ LN_2 are covered by a van carrying frozen semen and/or LN_2 through the road. Once by any means, the cold chain is broken, the frozen semen will lose its viability and cannot be regained or restored. In order to maintain viability throughout the cold chain, need to check time to time during storage, transportation, and before end user. Artificial inseminators are entrepreneurs offering 'door-to-door' insemination in cattle and buffaloes of a country like India. Factually most of the AI technicians in developing countries like India are the persons those learned the technique of AI from other AI technician and start to do AI in the field. They are not getting proper training for AI technique, storage of LN_2, storage of frozen semen, and proper handling of frozen semen resulting in thermal injury to sperm. When frozen semen straws are exposed to a

temperature of 2 inches from the top of the cryocan, within 10-12 seconds, the temperature of frozen straws reaches -100°C to -80°C, which is the beginning of ice-crystallization. Hence, unknowingly, AI technicians, expose frozen semen straws of a canister every time while doing AI. Each exposure to frozen semen straws damages the spermatozoa to some extent. The extent of damage depends upon how long and how many times the frozen straws are exposed to elevated temperatures. The thermal injury to sperm is permanent and cannot be corrected by returning frozen semen to the LN_2. For optimal maintenance of sperm viability, canisters and canes containing frozen semen should be raised into the neck of the cryo-can only for the time required to retrieve a single straw. This time should not exceed 5 to 8 seconds. Therefore, in the chapter, we will discuss the handling of LN2 container, frozen semen, semen evaluation in field condition and AI.

Handling of semen in LN_2 container

In the upper half of the neck tube of LN_2 container, high temperatures exist. Time spent in removing semen from the liquid nitrogen (LN_2) tank must be kept to a minimum to reduce semen damage. The typical temperature range in the neck of the LN_2 container is -191°C to +2.2°C (Fig. 1) Temperatures can reach +54°F (12.2°C) in the neck of the container (1 inch from the top). If the entire canister of semen (10 straws) is withdrawn above the frost line (3 to 4 inches from the top of the tank) (Fig. 1&2), all straws of semen will be damaged. Thermal injury to sperm is permanent and cannot be corrected by returning semen to liquid nitrogen.

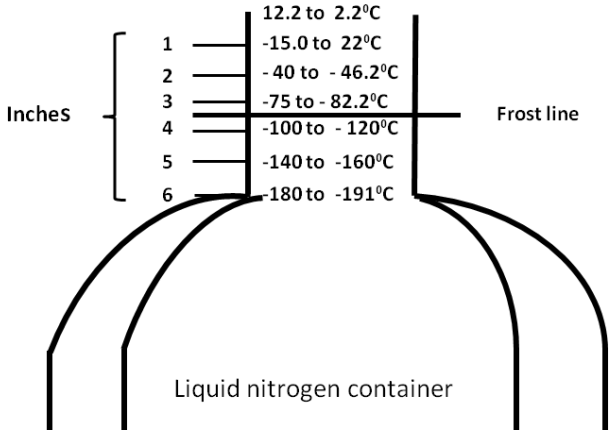


Fig. 1: Diagram showing temperature in the region of neck of the container

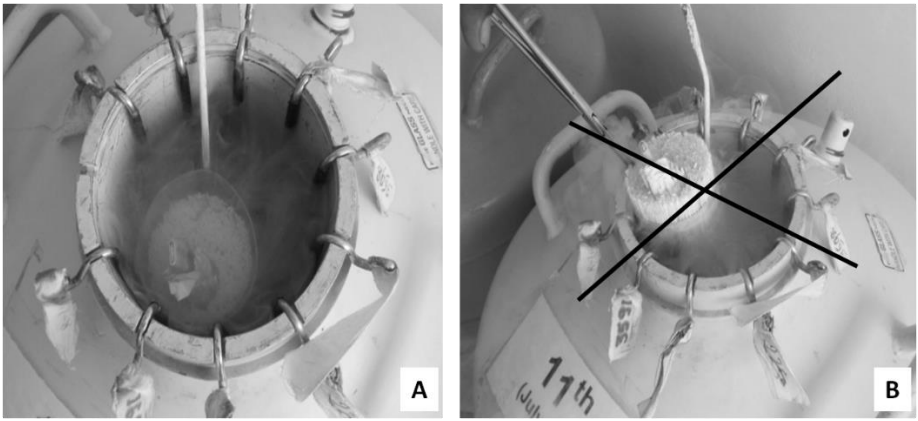


Fig.2: A. Right method to raise the canister **B.** Wrong method to raise the canister

Therefore, following steps should be remembered during the handling of semen.

- Identify which canister contains the desired semen with the help of attached tags to the canisters (Fig. 3A).
- Remove the canister from its storage position to the middle of the tank (Fig. 3B).
- Raise the canister just high enough in the neck region to grasp the desired straw (Fig. 3C).
- Grasp the straw and immediately lower the canister to the tank floor (Fig. 3D).
- Use tweezers or long pre cooled forceps to remove the straw (Fig. **4**).
- The straw should be removed within 10 seconds from the time the canister is raised into position. Then immerse the straw in 37˚C water.
- Immediately, after the straw is immersed in water, the canister should be returned to its storage position.
- Any time if it takes more than 15 seconds to take out the desired number of straws, the canister should be lowered back into the tank to cool completely and again after sometime take out the canister.

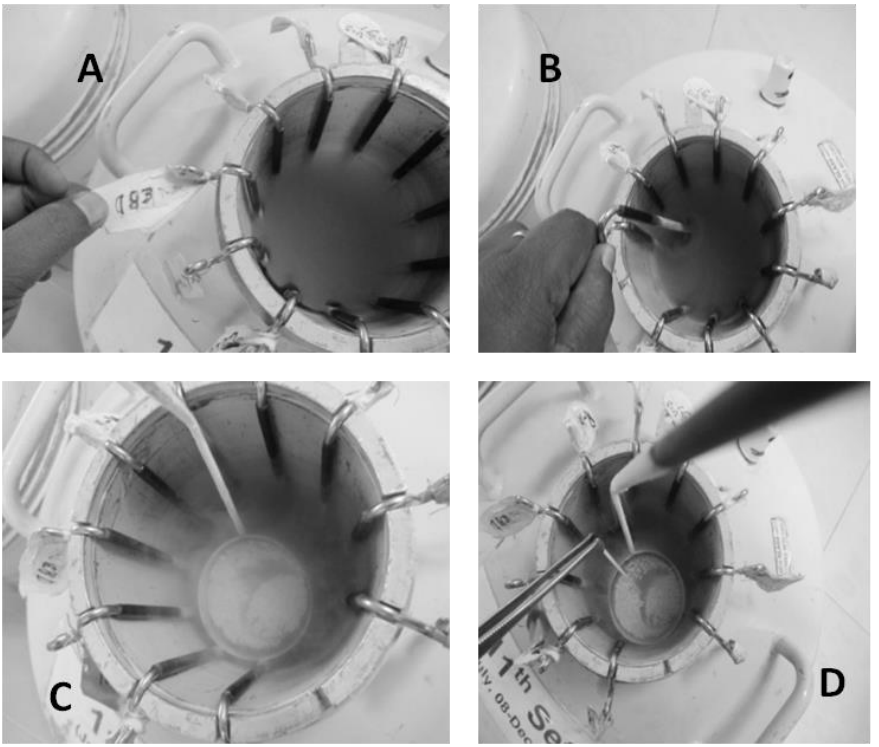


Fig. 3: Handling of semen in LN2 container A. Identify canister that contains the desired semen. **B**. Remove the canister from its storage position to the middle of the tank. **C** Do not lift the canister above the frost line to grasp the desired straw. **D.** Grasp the straw with pre-cool tweezers or haemostats and immediately lower the canister to the tank floor.

Fig.4: Use tweezers or long pre cooled forceps to remove the straw **A**. Tweezers **B**. Forceps

Shipment of semen

Shipment of frozen semen

Although some distributors transport frozen semen in liquid storage containers but shipping of frozen semen is best accomplished using containers

specifically designed for transport. The dry- shipper is specially designed for shipment of frozen semen. The various sizes of the dry- shipper are commercially available (Fig. 5). The shipper absorbs the liquid nitrogen into a porous material in its walls. These will not spill and therefore need not to be shipped as dangerous goods, which is more expensive. They should, however, always be sent as fragile goods, because they are easily broken by rough handling. The tank is usually shipped in a plastic box for protection.

Fig.5: Different sizes of cryoshipper (Courtesy of MVE Company)

Care should be taken while transferring frozen semen from storage to shipping containers. Exposure of frozen semen to room temperature should be not more than a few seconds; therefore, this should be performed with shipping and storage containers placed side by side. Always work within the neck of the storage container, below the frost line, while locating, identifying, and grasping straws.

Liquid shipper vs. vapour shipper

If liquid shippers' i.e. liquid nitrogen containers are tilted during transport, it will lose all liquid nitrogen and warm rapidly, destroying valuable semen. Also, liquid nitrogen container is a safety hazard and could cause serious injury to handlers. Because of this, liquid shippers must be classified as containing hazardous materials, which requires special paperwork and increases shipping costs. Vapour shippers, on the other hand, contain no "liquid" nitrogen per se. The liquid nitrogen vapour absorbed into the containers. Hence, vapour shippers are often referred to as "dry shippers." No

liquid nitrogen means no hazardous materials classification and fewer shipping restrictions. Vapour shippers are generally lighter than liquid storage containers and are therefore also less expensive to ship. MVE manufactures a "mushroom" shaped protective shipping carton for all sizes of shipping tanks (Fig. 8). The carton has wide base with a rounded top that reduces the likelihood of the tank tipping over or being loaded upside down or on its side. The hard moulded plastic carton also helps prevent tank damage due to normal shipping use.

Fig.6: Diagram showing cryoshipper and its plastic box for protection (Courtesy of MVE Company).

Thawing of Frozen Semen

The literal meaning of thawing is to melt or become liquid. The thawing of frozen semen is a process in which frozen semen is brought from frozen to liquid stage by keeping in warm water at particular temperature for certain time with the purpose to prevent recrystalization of the water into bigger crystals which cause cryoinjury to sperm. The procedure for thawing semen is equally important as the freezing process. As a thumb rule, rapid freezing rates require rapid thawing. Normally, straws of bull semen are thawed rapidly in a warm water bath. Temperature at which the semen should be thawed depends on the type of straw in which the semen is packaged. Straws of 0.25 or 0.5ml are generally thawed at 37^0C for 30 seconds. Thus, during thawing, temperature of semen rise from -196^0C to 37^0 C within 30 second at the rate more than 200^0C/ min. Always thaw only one straw because more

than one straw may adhere to each other resulting an uneven thawing. Semen should be deposited into the uterus immediately after thawing**. Under no circumstances thawed semen should be used beyond 15 minutes.** The thawed semen cannot be refreezed again. The following steps should follow while thawing of frozen semen.

- Pour hot water in the thawing tray and adjust temperature of water in the tray to 37^0C by adding cold or hot water or use automatic thawing unit which is commercially available (Fig.7).
- Identify the canister tag for particular bull, and straw is removed very quickly with the help of pre cooled long forceps or tweezers.
- The straw should be shaken in air one or two times to remove the excess liquid nitrogen from cotton plugs.
- Immediately the straw should be immersed in warm water at 37^0 C for 30 seconds.
- Take out the straw from the tray and wipe with tissue paper for one or two times to allow it to dry. Rubbing the straw should be avoided because heating is harmful. The exposure of the semen to as little as one drop of water results in irreversible cell injury.
- Before loading the straw in the gun, ascertain that air space in the straw is at the laboratory seal end.
- Load the straw in the AI gun and cut the laboratory end at right angle.

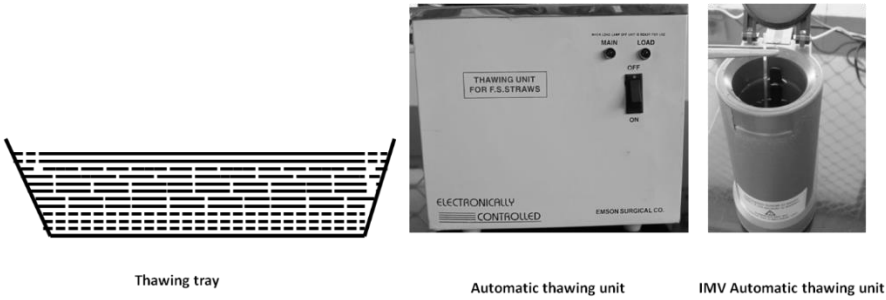


Fig. 7: Different types of thawing units.

Therefore, from time to time, one or two straws from a canister should be checked to confirm semen quality. But to check the sperm motility of frozen sperm, the microscope must be equipped with a warm stage fixed at 37°C

otherwise we cannot assess actual sperm motility. If the temperatures of the microscope' stage, glass slide and coverslip used for semen evaluation are less than 37°C, for example in winter, we will not get actual sperm motility of frozen spermatozoa. In this condition, we will not be able to distinguish or identify good or damaged lots of semen straws stored in cryo-can. If the temperature of the microscope' stage, glass slide, and coverslip used for semen evaluation are greater than 37°C, for example in hot summer temperatures reached about 45°C, all spermatozoa are killed immediately because of a very thin layer of frozen semen between the glass slide and coverslip. In the condition also, we will not be able to distinguish or identify a good or damaged lot of semen straws stored in cryo-can. For this, it requires a microscope fitted with a warm stage to maintain the temperature at 37°C, further uninterrupted power supply is required to operate and maintain the temperature of the instrument. But in rural areas uninterrupted supply of electricity is a major problem in most states of India and other developing countries. Therefore keeping these difficulties in mind we, for the first time, designed a handy microscope (Spermoscope) to evaluate the sperm motility in field condition.

Spermoscope for semen evaluation in field condition

Prior to this product, there was no such equipment that can monitor semen motility at any remote and distant location at any time. In all laboratories, sperm motility has been assessed with the use of a phase-contrast microscope fitted with a warm stage. It cannot be carried from one place to another place. Therefore, designing a device that should be simple, cheap, and have no electricity input for operating, was needed for the researchers, stakeholders, inseminators working in the field of frozen semen. The handy microscope is specially designed to test the semen quality in field condition and hence its name is given "spermoscope." It is very useful for AI workers, farmers, stakeholders, small laboratories working in rural areas. As a result of the configuration, it is possible to perform the test at any desired place and time. On account of its compact design and construction, the device can be carrying in a handbag and is essentially ready for use at any time. The spermoscope is closed from the outside and for slide insertion; one slit is given which prevents from atmospheric temperature either in very cold and very hot weather. The bull frozen-thawed spermatozoa cease moving on a glass slide at around 5°C more or less of the recommended temperature. The present design meets the required temperature for assessing semen motility optimally. The closed system of the device also protects from the direct rays of the sun,

ultraviolet, and radial rays which are detrimental to sperm. Due to the fixed single eyepiece and objective lens in the design, there is no need to focus on the object, again and again, only fine adjustment is sufficient to evaluate the sperm motility. The design having a knob which help in fine adjustment of the object for image evaluation. The design provides a total magnification of 200X sufficient to observe the sperm (Fig. 8). Through the microscope, AI technicians can evaluate the post-thaw sperm motility of their stock from time to time by evaluating one or two straws as representative samples of a lot. In addition to it, the sperm motility of the semen straw which is to use for AI to the cows/buffaloes can be assessed to show quality semen to the stockman. For this, when the semen straw is cut from one end before loading into the AI gun, the wasted cut part may be squeezed for semen on the glass slide. This will enhance the trust of the stockman that the semen dose used for AI of his buffalo is unblemished and if his cow/buffalo repeats are due to cow side accountability.

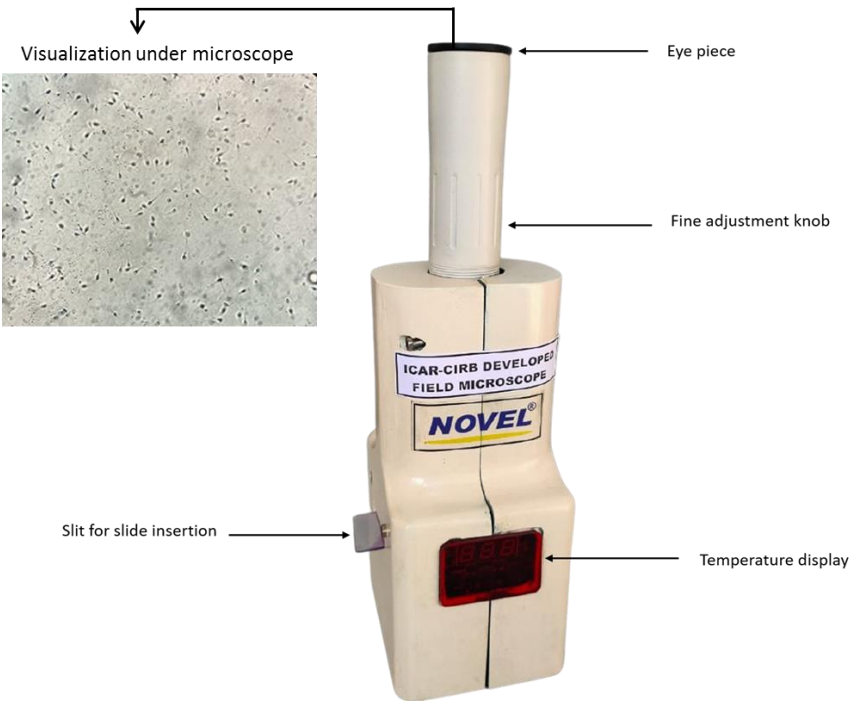


Fig 8: Spermoscope: the portable microscope for semen evaluation in the field condition.

Technique of A.I. in Bovines

Several techniques have been used for inseminating cow/buffalo artificially. These are vaginal insemination, cervical insemination and recto-vaginal technique. Vaginal insemination requires fairly large quantities of semen and has low conception rate. Therefore, this technique has now been replaced by other methods. In the cervical insemination technique, semen is deposited in the cervix by using speculum. This method has also been replaced by recto-vaginal method.

Recto-vaginal method of AI

The recto- vaginal technique is the most efficient method for bovine artificial insemination and is in use worldwide. The AI is performed in relatively unhygienic surrounding; therefore, the responsibility of the inseminator is to clean in every possible aspect. Always use a glove while inseminating. Plastic disposable gloves are preferred but reusable rubber gloves may be used.

Artificial insemination guns

The AI gun is hollow stainless steel with a plunger to expel the semen. There are commonly three types of AI gun: **O-ring, spiral and universal guns** used in India. AI guns are available to accommodate specific straw sizes: French medium and mini straw; but universal guns accommodate both the straws (Fig. 9).

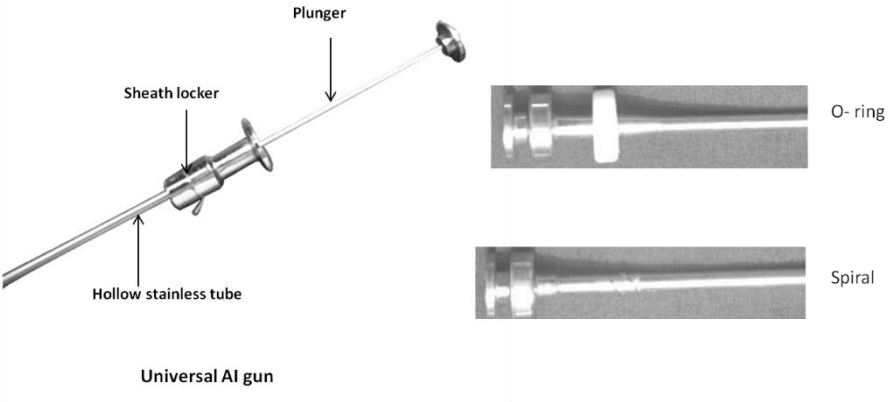


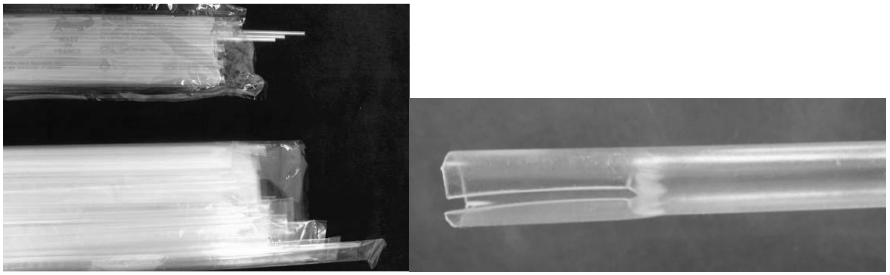
Fig. 9: Different types of AI guns.

Breeding Sheaths

It is disposal plastic tube specially designed to fit over the barrel of AI gun to secure semen straw and prevent transfer contaminants in the uterus during insemination (Fig. 10). During breeding, various contaminants can also be picked up by the open end of a sheath on the way to the uterus and released into the uterus. Therefore, the sealed sheath (i.e. sheath is individually covered with thin plastic) should be used. It keeps the inside of the breeding sheath clean until ruptured by depressing the plunger during breeding. The various types of sheaths are available for example:

• **Split sheaths** are designed for use with O-ring gun.

• **Non-split sheaths** are designed for use with spiral gun.



A **B**

Fig.10: A. Breeding sheaths in packet **B.** Split end of a sheath

Loading of A.I. gun

- Tear off approximately 20 cm of tissue paper.
- Remove the straw from the thawing tray using fingers and dry it with tissue paper.
- Drying is done by following steps:
  - ✓ Hold the straw by the laboratory seal end
  - ✓ Draw it through the paper once
  - ✓ Now, grasp the straw by the manufacture's end
  - ✓ Again draw it through the paper
  - ✓ Hold the straw by the manufacturer's end after drying is completed.

Don't dry the straw excessively as heat produced due to friction may raise the semen temperature.

- Check the name of the bull printed on the straw. If the wrong straw has been selected it must be discarded. **Never replace a thawed straw in liquid nitrogen**.
- Pull back the plunger of the AI gun.
- Holding the straw by the end, to avoid damage to the semen through temperature shocks, insert the manufacturer's end into the gun as far as it will go. There is an in-built stop preventing it going too far (Fig.11A).
- Prepare to cut off the laboratory end of the straw using thoroughly cleaned and dried the scissors.
- Hold the loaded gun vertically at eye level and using clean sharp scissors make a horizontal cut just below the laboratory seal end (Fig. 11B). The cut must be at right angle to the straw so that a perfect seal occurs between the straw and the sheath (Fig. 12).

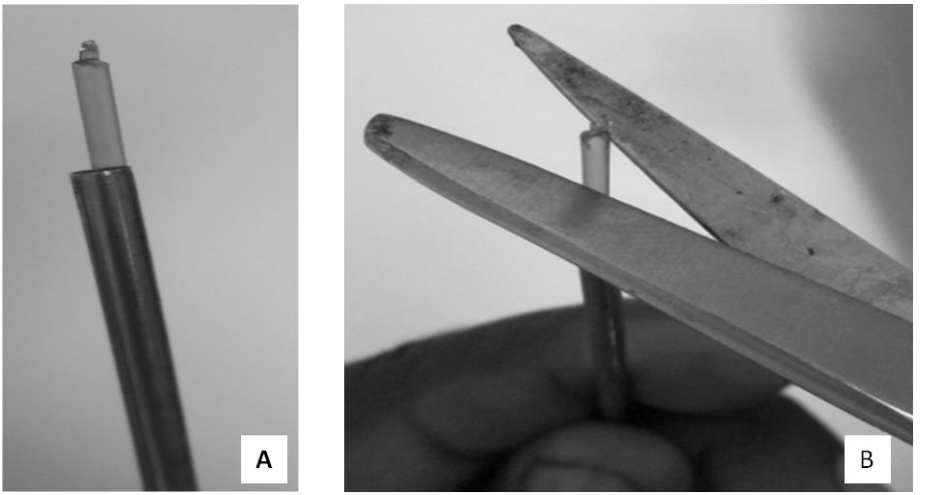


Fig. 11: A. Laboratory end outside the gun and inbuilt stopper prevent it going too inside. B. Cutting of laboratory end of straw with scissor.

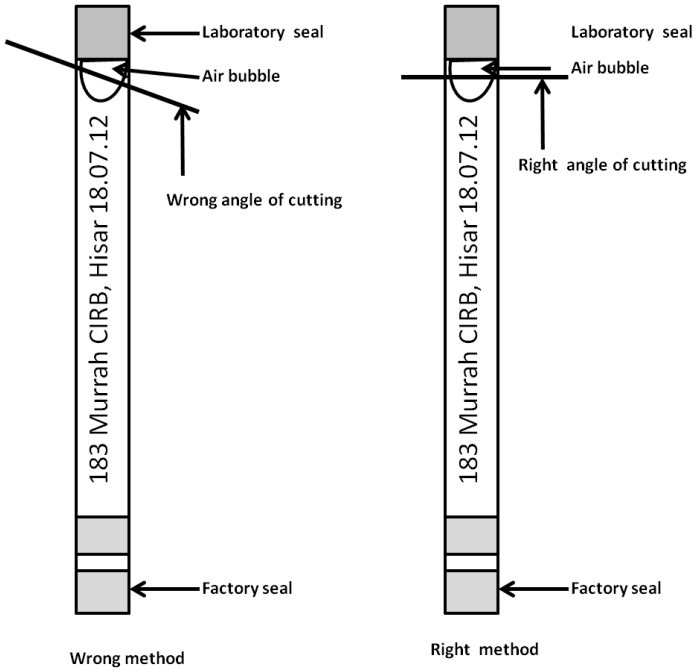


Fig. 12: Right and wrong methods of cutting straw.

- Clean and dry the scissors after cutting the straw and keep them to their correct location in the kit box.
- Place a sheath over the barrel of the gun. Handle and touch only the split end of sheath to keep the other end clean.
- Push the sheath through the central hole of the locking ring, twist it down on the conical seal of the gun and place O-ring over the sheath (Fig. 13A).

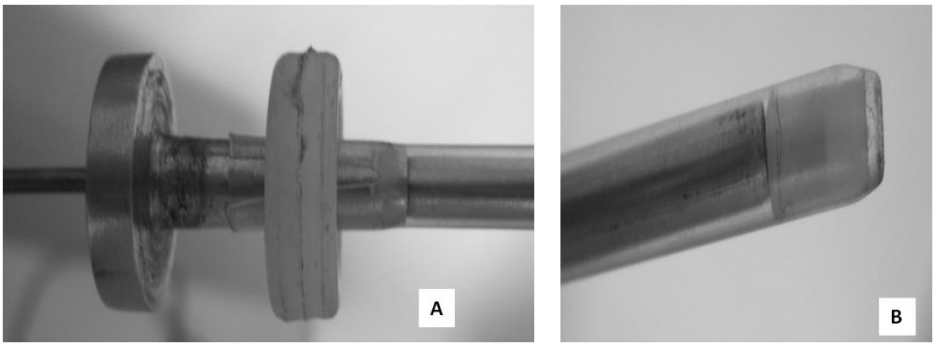


Fig. 13: A. Sheath passed through the O-ring and twisted on conical seal of the gun. B**.** Proper seal between sheath and laboratory end of straw.

- Seal between the sheath and the gun is essential otherwise semen will leak into the gun (Fig. 13B).
- Press the plunger of the gun until the semen is just visible at the end of the gun. This reduces the stretching of fingers needed during handling of the loaded gun.
- Now, loaded AI gun is ready to inseminate the animals.

Steps of artificial insemination

1. Properly secure the animal in the crate.
2. Wear the glove and wet it by dipping the gloved hand into a bucket of clean water, scooping water up in your hand and letting it run back down your arm. Apply a small quantity of lubricant to the back of your hand.
3. Make the cow/buffalo aware of your presence. A distressed animal may kick.
4. Ask to attendant to grasp the animal's tail and lift it aside.
5. Smear lubricant across the cow's anus, using the lubricated back of the gloved hand.
6. Form a cone of your gloved fingers and insert hand into the rectum. Pause at this stage and encourage the anus to relax by gently revolving your fingers. The wide part of the hand passes easily without rubbing the surrounding of anus and remove the faecal matter. This is best

accomplished by first inserting two or three fingers into the opening of the anus and allowing air to enter the rectum, which usually allows the cow defecate. If the rectum doesn't empty, the dung should be removed manually. However, complete removal of dung is not possible.

Avoid rough sudden entry which can abrade the anus and cause the release of adrenalin which reduces the conception rates.

7. Once most of the faeces are removed, relax constriction rings by placing two fingers through the rings and gently massaging back and forth (Fig. 14).

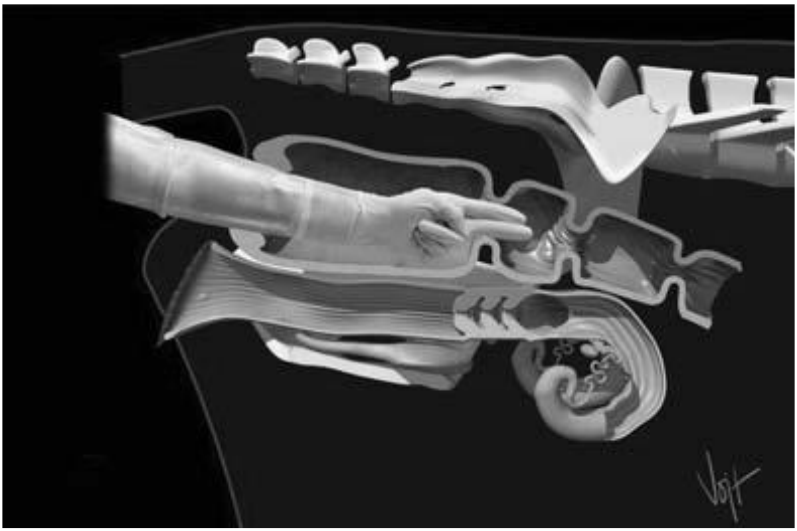


Fig. 14: To relax rectal constriction rings, insert two fingers through the centre of the ring and massage back and forth (courtesy: www.selectsires.com).

8. Thoroughly clean the vulva with water and wipe with tissue paper. Use a fresh piece if the paper is too soiled.

9. Give some pressure downwards with the wrist of the hand in the rectum which helps to apart the vulval lips presenting a clean area for inserting the gun or the vulval lips are pulled apart with the help of assistant.

10. Insert the gun clearly between the lips of the vulva into the vagina. Ensure the gun passes along the roof of the vagina thus avoiding the bladder. AI gun is passed at an angle of 45^0 through vagina.
11. Gently push the gun through the vagina up to external os of the cervix.
12. Now, hold the cervix between fingers and keep the thumb over the external os. Maintain a light forward pressure on the gun and manipulate the cervix so that the gun passes through the cervix canal.
13. Manipulate the AI gun so as to strike thumb placed over the external os and then pass the gun through the cervix.
14. While passing the gun through the cervix, feel the tip of the gun at internal os to ensure that the gun is in correct place.

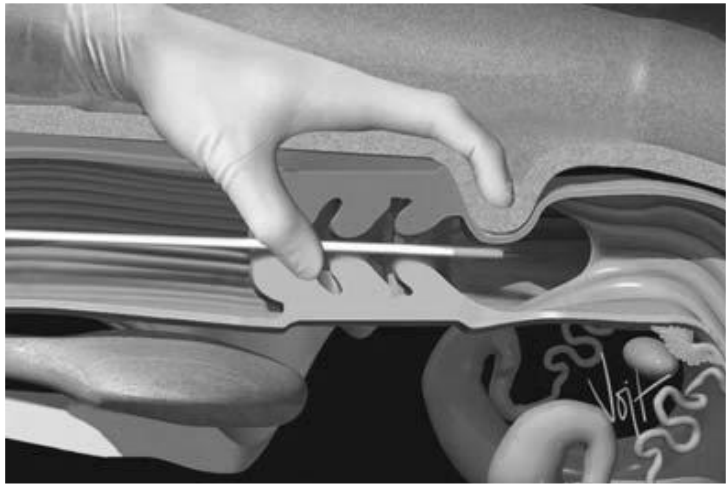


Fig. 15: Use index finger to assure the correct position of gun before depositing semen (courtesy: www.selectsires.com).

15. Gently deposit the semen in the body of the uterus being careful not to 'spit' it out (Fig. 16).

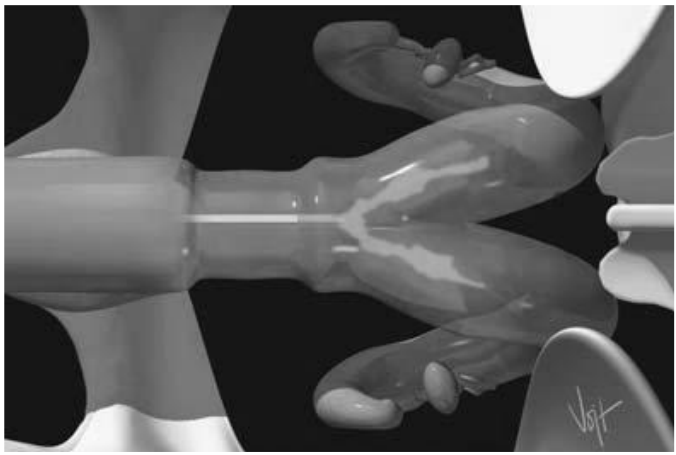


Fig. 16: Deposit semen in the uterine body and contractions will transport spermatozoa forward to both horns and oviducts (courtesy: www.selectsires.com).

16. Don't passAI gun beyond the uterine body otherwise all semen will be deposited into only one horn. (Fig. 17).

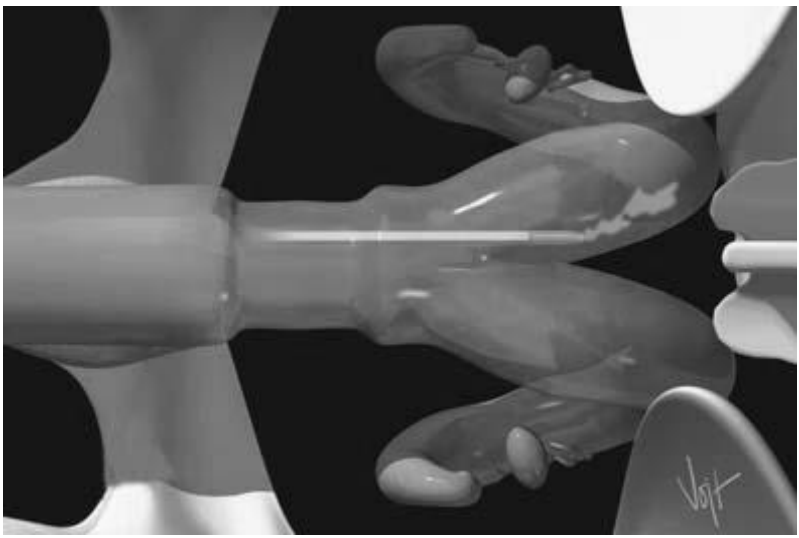


Fig. 17: Don't passAI gun beyond the uterine body (courtesy: www.selectsires.com).

17. Gently take out the gun and check for abnormal discharge and complete semen deposition.
18. Slowly withdraw the arm from the rectum of the cow.
19. Loosen the locking ring on the gun and remove the soiled sheath.
20. Dispose the straw, sheath and dirty glove properly.

Post Artificial Insemination

After AI, there are several clean - up steps. These are:

- Inspect the gun tip for sign of infection.
- Remove sheath and straw and bend at a 90^0 angle and dispose properly.
- Remove the glove and the used straw, sheath and manure put inside the glove. Tie a knot in the open end and dispose the glove.
- Clean the gun with 70% alcohol and dry it.
- Tighten the O-ring on the gun so it will not get lost.
- Clean and disinfect yours boots before leaving the farm.
- Wash your hands before leaving.
- After the last call of the day, wash outside of your AI kit.
- It is good practice to remove all equipment from AI kit and clean them thoroughly once a week.

CLINICAL POINTER

- Insemination is accomplished by pushing the plunger to deposit the semen slowly. This is accomplished by slowly counting up to 10 during expulsion.
- Clitoral massage immediately after insemination has been advocated to improve conception. In one study, a 10 second massage increases 6 percent conception rate over no massage (58 vs52 percent) in cows but there is no effect in heifers.
- Insertion of AI gun is most easily accomplished by passing the tip along the roof of the vagina for about 15 cm to bypass the **sub-urethral diverticulum** and **urethral orifice** on the floor. During the

insertion into the vagina, the hand in the rectum should grasp the cervix and stretch the vagina forward for easy passage of the AI gun.

- Once the tip of the inseminating gun passes into the uterus it is easily detected through the relatively thin wall of the uterus.
- Avoid deep penetration of the uterus as the gun may cause damage and possibly infection thus reducing the chance of the conception or in the case of pregnant cow, an abortion (up to 5 percent of pregnant cows show some signs of heat) may occur.
- Occasionally the gun cannot be passed to the proper position. In this condition, avoid bruising and other injury to the cow by depositing the semen at the position reached after reasonable effort. Prolonged and forceful struggling will have a worse effect on conception rates than incorrect semen placement.
- Rapid withdrawal of the gun can suck semen back through the cervix into the vagina.
- Sometimes the cervical opening is not in the center of the cervix but is located to either side or on the upper or lower part of the cervix. Use the forefinger of the palpating hand to feel the tip of the AI gun and to determine when it has penetrated the cervical opening.

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