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Embryonic Mortality in Cattle- A Review

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A B S T R A C T

Cattle is one of the major livestock species for milk production, contributing significantly to the economy of our country and continuous milk production needs regular conception and successful calf crop production. World-over, for dairy economy, there is emphasis on one calf per year per cattle. Successful calving follows survival of the conceptus through embryonic and fetal development. Embryonic mortality is a major source of economic loss with mortality rate upto 40% in animal production through repeat breeding and increased cost of artificial insemination, cost of treatment, extended calving intervals and prolonged dry period resulting in reduced milk production. Factors involved in embryonic mortality are multifactorial and can be summarized into three intrinsic, extrinsic and embryonic categories. The present review on embryonic mortality in cattle deals with majority of the etiological factors.

Introduction

The cattle and buffaloes are known for their milk production and they contribute approximately 96% to total milk production in India. Though milk production in India has been reached to 132.4 million tonnes in 2012-13 with a growth rate of 3.5%, but there is high demand of milk (BAHS, 2014) and it is projected that by 2030 India will be able to produce 200 million tonnes of milk (NDRI Vision, 2030). This target will be achieved if there is the optimum balance between conception, embryonic nourishment and successful calving. Embryo mortality is a major cause of economic loss in dairy production systems. An incidence of 20-50% embryonic and fetal death has been noticed in apparently normal healthy animals of all domestic species including bovines (Arthur *et al.*,1989) whereas 15% early embryonic mortality between day 23 and 29 in repeat breeding *Holstein Friesian* cows using ultrasonography and progesterone profile has been recorded (Patel *et al.*, 2005).According to a study early and late embryonic death in Holstein cows in 44 herds in France after first insemination were 31.6 and 14.7%, respectively (Humblot, 2001). Late embryonic deaths after day 27 of gestation ranged from

3.2% in dairy cows producing 6000-8000 kg of milk per year upto 42.7% in high producing cows under heat stress (Silke *et al.*, 2002). In dairy cows, rate of pregnancy loss between day30 and day45 of gestation is 0.85% per day or approximately 12.8% which is higher than that observed for beef cows (Beal *et al.*, 1992). Some studies had reported that highest incidences of early pregnancy loss occur during first 56days post-insemination (Fricke *et al.*, 1998).

Fertilization rate in cattle

Fertilization rate in cattle, in heifers and in moderate yielding dairy cows, are in the order of 90-100% following the use of high-quality semen. In contrast, for higher producing dairy cows there is less quantitative information on fertilization rate (Sreenan and Diskin, 1986). It is observed that embryo resulting from fertilization of compromised oocyte have a low probability of successful development (Hansen, 2002). In a study on the effects of ambient temperature on fertilization rate it was reported that fertilization rates are 82.4 and 79.5% for high and low temperatures, respectively whereas fertilization rate of 55.6% in lactating dairy cows compared with 100% for heifers under high ambient temperature (Ryan *et al.*, 1993). In a subsequent study during the winter season, fertilization rates were 87.8 and 89.5% for lactating and non-lactating dairy cows, respectively (Sartori *et al.*, 2002). Based on milk production, some studies concluded that fertilization rates of 83 and 88% were recorded in high-producing dairy cows and it appears that fertilization rate may be a little lower in high than in moderate producing dairy cows, at least during the hot season (Cerri *et al.*, 2009). Embryonic and fetal mortality rate in cows with fertilization rate of 90% had an estimated loss of 70-80% sustained between days 8 and 16 after AI (Sreenan and Diskin, 1986). The overall loss rates and the pattern of loss between days 28 and 84 of gestation were similar for cows producing on average 7247 kg of milk (7.2%) and heifers (6.1%) and almost half (47.5%) of the total recorded loss occur between Days 28 and 42 of gestation (Silke *et al.*,2001). Another studies also showed similar overall late embryo or fetal loss rate of 7.5% between days 30 and 67 of gestation in dairy cows that were managed under pasture based systems of production (Horan *et al.*, 2004). The extent of late embryonic loss is less than early embryonic loss and causes serious economic losses, particularly in seasonal calving herds because it is often too late to rebreed cows, which results in increased culling rates and replacement cost (Grimard *et al.*, 2006).

Factors related to embryonic mortality

The main factors implicated in embryonic or fetal loss are normally categorized as those of genetic, physiological, endocrine or environmental origin.

Genetic causes

Genetic causes of embryonic death include chromosomal defects, individual genes and genetic interactions (VanRaden and Miller, 2006).Chromosomal aberrations are major cause of early pregnancy loss in animals (King, 1990).A range of misalliances can occur during the pairing of the haploid parental chromosomal sets at the time of fertilization, which are subsequently lethal to the embryo. Chromosomal abnormalities may also originate by penetration of ovum by more than one sperm cell (polyspermia). Mixoploidy, polyploidy and haploidy are all aberrations that are encountered frequently *in-vitro* produced embryos (Viuff *et al.*, 1999) but it has not been investigated yet whether this could be a cause for the higher embryonic mortality rates, which are observed after the transfer of *in-vitro* produced bovine embryos

(Van Soom *et al.,* 1994). Chromosomal abnormalities may account for approximately 20% of the total embryonic and fetal losses (King, 1990). In the Holstein breed, two major recessive defects are responsible for embryo or fetal deaths (Robinson *et al.,* 1984). Also the DUMPS (Deficiency of Uridine Mono-Phosphate Synthase) a homozygous recessive condition, causes fetal death at days 40 to 50 of gestation (Shanks and Robinson, 1989). Some studies have also reported that, testing of AI sires for DUMPS reduce the frequency of heterozygous sires and of homozygous recessive embryos and has now almost eliminated this as a cause of infertility (VanRaden and Miller, 2006). Apart from these genetic causes, several reproductive traits are adversely affected by inbreeding. Maternal inbreeding has been reported to decrease the 56 to 70day non-return rates by 1 to 2% per 10% inbreeding of the dam (Wall *et al.,* 2003). Inbreeding of the embryo has been reported to reduce the 70 days non-return rate by 1% for each 10% increase in the level of inbreeding of the embryo. Genetic variation in embryo survival may be attributable to the genetic constitution of the embryo itself or the genetic differences among dams with respect to their ability to provide an appropriate intra-ovarian and intra-uterine environment (Cassell *et al.,* 2003; VanRaden and Miller, 2006). Another factor is the age of the animal which is responsible for fluctuations in conception and embryo survival rate. In heifers, conception rate is maximum at 15 to 16 months of age and breeding heifers at 26 months of age or older result in a 13% reduction in conception rate, presumably due to a lower embryo survival rate (Kuhn *et al.*, 2006).

Nutritional causes

Following parturition, the nutrient demands of the dairy cow increase dramatically as peak lactation yield is approached and typically exceed dietary intake, resulting in a state of negative energy balance (NEB). During this period, body reserves are mobilized to meet the combined demands of maintenance and lactation. Reproductive performance decrease in high producing dairy cows especially when animals are under severe NE (Nebel and McGilliard, 1993).Gene expression in uterine tissue and spleen of cows with severe post-partum NEB (SNEB) indicate that these cows have increased expression of many key genes known to be involved in inflammatory responses, which is consistent with a re-modelling of the post-partum uterus and the clearance of microbial infections. Cows in SNEB has a delayed immune response and the pattern of gene expression in the spleen indicate that this is because immune cells are exposed to an environment of increased oxidative stress, causing a reduction in genes encoding cytokines, which are essential for a normal immune response cascade (Morris *et al.,* 2009; Wathes *et al.,* 2009). So, the importance of maximizing feed intake and minimizing NEB in the immediate post-calving period in order to sustain high embryo survival rates was emphasized (Patton *et al.*, 2007). Follicles exposed to adverse conditions such as NEB during their initial stages of growth seems to impair the development resulting in the production of inferior quality oocytes and dysfunctional corpora lutea, though the hypothesis has not been adequately tested (Britt, 1994).There is a great relationship between EB (energy balance), DMI (dry matter intake) and peripheral concentrations of insulin-like growth factor-1(IGF-1) measured during the first 28 days of lactation and subsequent conception. First service conception rate is associated positively with all the three variables because there may be long term carry-over effects of nutrition and EB on conception rate, which is an observation, but effects on fertilization and types of embryo mortality remain to be documented (Patton *et al.,* 2007). High circulating concentrations of insulin have negative effects on oocyte quality (Garnsworthy *et al.*, 2008). In some studies, it has been examined that feeding strategies with glucogenic-lipogenic substances to dairy cows have some beneficial effects on reproductive functions (Garnsworthy *et al.*, 2009; Friggens *et al.*, 2010). Lipogenic diets increase the oestradiol secreting capacity of the pre-ovulatory follicle, provided enhanced substrate for progesterone production and improved blastocyst development rates which reduces embryonic mortality rates (Leroy *et al.*, 2008). Also the increased milk production resulting from concentrate supplementation may be associated with increased hepatic blood flow and increased metabolism of progesterone with the predisposition to greater risk of embryonic death (Sangsritavong *et al.*, 2002).

Some of the more common plant toxins that can cause reproductive problems include mycotoxins, endophyte infected fescue (Porter and Thompson, 1992), nitrates (Brownson and Zollinger, 2003), locoweed, and ponderosa pine (Ford *et al.*, 1992). Mycotoxins can occur in moldy feed and mycotoxin 'zearalenone' is also suspected to cause abortions in cattle by decreasing progesterone concentrations (Parmar *et al.*, 2017). Crude protein in the total diet greater than 17 to 20% has been implicated in lowering conception rates with increases seen in the number of services per conception and days open. Some studies have indicated that blood urea nitrogen (BUN) above 20 mg/100 ml may decrease the chances of pregnancy (Blanchard *et al.*, 1990; Elrod and Butler, 1993; Elrod *et al.*, 1993).

Infectious causes

Infection of the uterine and oviductal environment can be caused by specific and non-specific uterine pathogens. Specific uterine infections are caused by a number of viruses, bacteria and protozoa. These pathogens enter the uterus by the haematogenous route (primary infection of the female with *Toxoplasma gondii*) or via the vagina at natural service (*Campylobacter fetus)* or at insemination like in *Bovine viral diarrhea virus* (BVDV). Non-specific pathogens are mainly bacteria that enter the uterus by ascending infection or at the time of insemination. Sometimes, may cause endometritis. The infection and the resulting inflammatory products must be eliminated before the embryo descends into the uterus.

Specific infectious causes

Among specific infectious causes numerous bacterial, viral, protozoan and fungal pathogens have been associated with embryonic mortalities, abortion and infertility in cattle. *Corynaebacterium pyogenes* is responsible for abortion, retention of placenta and vaginal discharge (Griffin *et al.*, 1974). *Campylobacter fetus* (commonly known as “vibrio”) is easily transmitted from cow to bull or vice-versa and cows can remain infected for up to six months. *Vibriosis* is responsible for infertility and causes early embryonic mortality in cows (Adler, 1959). Some protozoa like *Tritrichomonas foetus* (flagellated protozoa) causes venereal disease in cattle. Infected cows can experience early embryonic death, infertility and abortion in the first trimester of gestation. Some cows develop post-coital pyometra (Onyango, 2014) and the infection is generally, the infection is cleared within 90 days (Peter, 1997). After fertilization, the zona pellucida can be considered as an effective barrier for virus penetration (Vanroose *et al.,* 1999). Passive migration of virus through the meshes in the zona pellucida is highly unlike to occur, since particles with a diameter of 40 and 200 mm comparable size as BVDV and BHV-1 remain stuck in the peripheral part of the zona pellucida (Vanroose, 1999). Among viral causes, *Bovine herpesvirus-1* (BHV-1) is a group of viruses that includes IBRV (Infectious bovine rhinotracheitis virus) and IPV (Infectious pustular vulvovaginitis). This group is responsible for more abortions than any other infectious agent. Also, *Bovine viral diarrhea* (BVD) has been shown to cause early embryonic loss, but is not a major cause of embryonic mortality in cattle (Whitmore *et al.,* 1981).

Non-specific infectious causes

Uterine infection can be caused at the time of A.I by ascending infections with facultative pathogenic bacteria present in the vagina or in the semen also impair with normal conception. Such infections do not impair fertilization but disturb the embryo-maternal interactions or disrupt the process of implantation of the embryos. This results in vaginal discharge 14-25 days after insemination (De Winter *et al.,* 1995). Whenever, the uterus is already infected before service fertilization will not take place or early embryonic development is disturbed resulting in embryonic mortality before day 11 (De Winter, 1995). The innate immune system is alerted due to the presence of pathogens by endometrial cell toll-like receptors (TLRs) detecting pathogen associated molecules such as lipopolysaccharide (LPS), DNA and bacterial lipids. The innate immune system, including toll-like receptors (TLRs), antimicrobial peptides (AMPs) and acute phase proteins (APPs) constitutes an initial defence of the mammalian endometrium against microbes. The endometrial cells secrete cytokines and chemokines to direct the immune response and increase the expression of AMPs. Chemokines attract PMNs and macrophages to eliminate the bacteria, although neutrophil function often gets disturbed in postpartum animal. Persistence of PMNs in the endometrium in the absence of bacteria is thought to be the primary characteristic of sub-clinical endometritis (Zerbe *et al.,* 2003). Subclinical endometritis is a silent cause of conception failure and development of uterine infections have been reported to be associated with an increased incidence of COD (cystic ovarian disease) (Andrew *et al.,* 2006). Post-partum endometritis in cattle is a multifactorial disease with high economic impact. Inflammation of the bovine uterus has been demonstrated to decrease fertility. Both clinical and subclinical endometritis were associated with increased days to first service as well as decreased conception and pregnancy rates resulting in an increased risk of culling (Perea *et al.,* 2005).

Endocrinological causes

Progesterone secretion by the CL is essential for arranging the histotrophic environment for nourishment of the conceptus. Indeed progesterone and estradiol act as systemic regulators leading to local oviductal and endometrial timed events and they program the uterus to regress the CL if there is sub-optimal communication between conceptus and uterus via secretion of $PGF_{2\alpha}$ (Robinson *et al.,* 2001). There is a positive linear association between the concentrations of progesterone on the day of prostaglandin induced luteolysis and subsequent embryo survival rate (Brooks *et al.,* 2014). Potential mechanisms by which low concentrations of progesterone during the preceding oestrous cycle might reduce fertilization or embryo survival rates include the production of oocytes that are at a more advanced stage of maturation at time of ovulation, and increased frequency of luteinizing hormone pulses which in turn induces increased secretion of oestradiol-17β or an alteration in endometrial morphology. The more probable effect of low concentrations of progesterone in the cycle preceding oestrus on subsequent embryo survival is premature oocyte maturation, which compromises the ability of the embryo to continue normal development after fertilization (Diskin *et al.,* 2006). Relationship between early and mid-luteal phase concentrations of progesterone and subsequent embryo survival per conception have used logistic regression techniques to model the relationship between the binomially distributed dependent variable (conception/embryo survival rate) and the continuously distributed independent variable i.e. progesterone (Diskin *et al.,* 2006). There is also a positive linear and quadratic relationship between concentrations of progesterone in milk on days 5, 6 and 7 and the rate of change in concentrations of progesterone between days 4 and 7 after insemination and embryo survival rate. Further analysis of these data reveal that 75, 72 and 56% of dairy cows have concentrations of progesterone that is optimal for conception on days 5, 6 and 7 after insemination, respectively (Stronge *et al.,* 2005). In beef heifers, a similar linear and quadratic association between peripheral concentrations of progesterone and embryo survival was also noticed (Diskin *et al.,* 2006). Some studies suggested importance of progesterone supplementation to dairy animals at risk of low embryo survival rate as a result of progesterone insufficiency to improve embryo survival rates. So for this purpose reliable, easy to use and cheap methods of identifying animals at risk of embryo death as a consequence of low circulating concentrations of progesterone are required, but supplementation of animals already adequate concentration of progesterone may cause embryo death and should be avoided (Starbuck *et al.,* 2001). Also administration of an anti-prostaglandin agent at the time of embryo transfer increased pregnancy rates (82% versus 56%). These data indicate that suppressing $PGF_{2\alpha}$ secretion favours establishment and maintenance of pregnancy in cattle by reducing embryonic mortality (Elli *et al.,* 2001). Peripheral concentrations of progesterone and oestradiol are lowered by increased plane of feed intake due to increased metabolic clearance rate of the steroids, which is related to liver blood flow. It appears that liver blood flow remains high in high-producing, lactating dairy cows, which in turn results in a lowering of peripheral concentrations of progesterone thus increasing the risk of embryo death (Sangsritavong *et al.,* 2002). Uterine expression of mRNA for progesterone and oestradiol receptors and retinol-binding protein mRNA was sensitive to changes in peripheral concentrations of progesterone during the first week after A.I (McNeill *et al.,* 2006). The transcriptome of the endometrium of cyclic heifers is sensitive to circulating progesterone concentrations in the first few days after oestrus. Under low-progesterone conditions, a sub-optimal uterine environment with reduced ability to support conceptus elongation was observed (Forde *et al.,* 2011).

Environmental causes

The fertility traits in dairy animals show a very low heritability value and this indicates that most of the variations in the fertility are determined by non-genetic factors or environmental effects (Thiruvenkadan *et al.,* 2010). The main natural physical environmental factors affecting livestock system includes air temperature, relative humidity, solar radiation, atmospheric pressure and wind speed (Hahn *et al.,* 2003). The environmental factor like heat stress seems to have the greatest impact on embryo survival (Sartori *et al.,* 2002). Heat stress has an adverse effect on reproduction traits of dairy cattle (Garcia-Ispierto *et al.,* 2007) and buffaloes (Dash *et al.,* 2015). There are several possible mechanisms by which heat stress can prevent the growth of oocytes. The foremost is the reduction on the synthesis of pre-ovulatory surge in luteinizing hormone and estradiol. Hence, there is poor follicle maturation and this leads to ovarian inactivity in cattle (Hansen, 2007). Heat stress also delays follicle selection and reduces the degree of dominance of the dominant follicle. Heat stress decreases blood progesterone concentration, which is a major cause for abnormal oocyte maturation, implantation failure and finally early embryonic death in dairy cattle (Khodaei-Motlagh *et al.,* 2011). The lactating cows experiencing clinical mastitis in the first 45 days after A.I were reported to be 2.8 times more likely to experience late embryonic death between 31 and 45 days of gestation (Chebel *et al.,* 2004).

Diagnostic approaches to embryonic mortality

A novel way to reduce calving to conception interval in dairy animals is the early pregnancy diagnosis and early detection of those animals that fails to conceive after service (Pieterse *et al.,* 1990).

Immunological methods

Serum concentrations of bPAG-1 (Bovine pregnancy associated glycoproteins) for detection of fetal survival were estimated by using a double-antibody radioimmunoassay (RIA-706) and the minimum detection limit of the RIA-706 was 0.2ng/ml (Perenyi *et al.,* 2002). Bovine pregnancy associated glycoprotein-1 concentration of 0.8 ng/ml in the plasma is taken as the cut-off point for diagnosing pregnancy and below this concentration embryo is not considered healthy (Kaufmann *et al.,* 2009). For pregnancy diagnosis, the plasma progesterone concentration ranged from 3.93 to7.68 ng/ml with an average of 6.48±0.38 ng/ml in pregnant animals on day 21(Hadiya *et al.,* 2015), which approximated well with earlier reports (Patel *et al.,* 2005; Bhoraniya *et al.,* 2011). The positive predictive value and negative predictive value of early pregnancy diagnosis on day 21 with plasma progesterone was recorded as 79.55 and 100%, respectively (Hadiya *et al.,* 2015). Contrarily it was observed that accuracy of pregnancy diagnosis based on progesterone profile on day 24 or 25 was 91% for positive and 88% for negative results (Chung and Kim, 1980). There might be gradual decline in plasma progesterone concentration resulting in loss of embryo between days 35 and 60 prior to rectal palpation (Chaffaux *et al.,* 1986).There is also a significant relationship between P_4 level during week 5 and the occurrence of pregnancy losses up to week 7 of gestation (Starbuck *et al.,* 2004).Cows producing more milk may be more likely to have sub-optimal concentrations of P_4 (Bech-sabat *et al.,* 2008). In contrast to this, there were no associations between milk P_4 concentrations at day 28 or day 42 and the occurrence of pregnancy losses between days 28 and 56 of gestation (Karen *et al.,* 2014). Similar results were reported in other studies that did not find a significant relationship between P_4 level at day 30 (Humblot *et al.,* 1988) day 35 (Bech-sabat *et al.,* 2008) or day 42 (Lopez-Gatius *et al.,* 2007) and the occurrence of pregnancy losses in dairy cows.

Cytology, uterine lavage, biopsy and cytobrush methods

Sometime the uterine environment is not conducive for embryonic development due to some infectious causes like in subclinical and clinical endometritis cases. No gold standard exists for the diagnosis of SE (Subclinical endometritis), which turns the task into a challenging one. Nevertheless, uterine cytological evaluation is the most used tool for SE diagnosis (Kasimanickam *et al.,* 2005). Endometrial and inflammatory cells may be collected by a guarded cotton swab (Studer and Morrow, 1978), uterine biopsy (Bourke *et al.,* 1997), uterine lavage (Hammon *et al.,* 2001), or cytobrush (Glenthoj *et al.,* 1986) techniques to evaluate endometrial cytology, especially as an aid in the diagnosis of subclinical endometritis. In the lavage technique, the procedure from sample collection to the preparation of the slide for cytological examination, took a maximum of 2hrs. It is possible that any delay in the cytological evaluation will affect the total nucleated cell count and may alter the cytological evaluation. Cyto-brushing is considered the best technique for obtaining endometrial cytological samples because it is easy and quick to perform (Barlund *et al.*, 2008), safe and effective (Oral *et al.*, 2009). Biopsy provides detailed information about uterine health status, and a 4-point scale has been developed for use in cows (Chapwanya *et al.*, 2009) but, studies are still lacking relating biopsy scores with future fertility of the cow. In addition, only few reports exist evaluating its use as a diagnostic tool for SE (Meira *et al.*, 2012). Cytological examination of the reproductive tract is often used to evaluate possible reproductive lesions in domestic animals. Endometrial cytological examination in cows is an accepted diagnostic technique (Gilbert *et al.*, 1998; Hammon *et al.*, 2001). Still, a technique that yields well-preserved cells representative of a large uterine surface area without causing harm to the reproductive tract is required for consistent and reliable cytological results. The uterine lavage technique harvests cells from a larger uterine surface area and provides a more representative sample of luminal contents than does either a swab or a uterine biopsy (Bonnett *et al.*,1991; Bourke *et al.*, 1997), but it may cause irritation to the endometrium (Brook, 1993). Cytologic examination by uterine lavage with low volumes of saline to recover neutrophils has recently been studied in dairy cattle as a method to define subclinical endometritis (Gilbert *et al.*, 1998) and endometritis (Hammon *et al.*, 2001). Whereas, the cytobrush technique resulted in less distortion of cells compared with the lavage technique. Even though the cytobrush technique requires specialized equipment but sample collection by this method is easier, more consistent and produce rapid results (Kasimanickam *et al.*, 2005). Rani *et al.*, (2018) reported that < 3% polymorphonuclear cell count in uterine cyto-brushing as normal on the basis of microbial culture in cows on day 45 that suffered from late embryonic mortality and were previously confirmed pregnant on day 28.

Ultrasonography

Diagnosis of viability of fetuses at early stages of gestation and detection of early embryonic death is difficult by rectal palpation. Ultrasonography provides a good tool for early pregnancy diagnosis by the study of ultrasonographic appearance of conceptus (Kastelic *et al.*, 1988). Ultrasound is a minimally invasive, accurate and efficient technique for early pregnancy diagnosis (Vaillancourt *et al.*, 1979) and may minimize the rare incidence of palpation induced abortions. Direct observation of a fetus with ultrasonography is found to be more accurate than assays for the presence of pregnancy-specific proteins in plasma but resulted in more false negative diagnoses (Szenci *et al.*,1998). Ultrasonography has been successfully used for early pregnancy diagnosis as well as for detection of early embryonic mortality in cattle (Patel *et al.*, 2005). Ultrasonographic diagnosis of pregnancy can be made as early as day 25 after insemination in cattle (Fricke, 2002). The advent of ultrasonography and other methods for early pregnancy diagnosis has allowed researchers to characterize the timing and extent of late embryonic losses in cattle (Santos *et al.*, 2004). In cattle, trans-rectal ultrasonography for pregnancy diagnosis between days 21 and 25 after breeding has sensitivity and specificity of 44.8% and 82.3% respectively, which further increase to 97.7% and 87.7% respectively, when conducted between 26 and 33 days after A.I (Pieterse *et al.*, 1990).

Linear-array, real-time, B-mode ultrasound scanners are best suited for veterinary applications involving cattle reproduction and most ultrasound machines consist of a console unit that contains the electronics, controls and a screen upon which the ultrasound image is visualized by the operator and a transducer, which emits and receives high frequency ultrasound waves. Linear-array transducers consist of a series of piezo-electric crystals arranged in a row. These crystals emit high frequency sound waves upon being energized. Linear-array transducers of 5.0 and 7.5 MHz frequency ranges are most commonly used in cattle to perform reproductive ultrasound examinations and most veterinary ultrasound scanners are compatible with probes of different frequencies. The configuration of a linear-array transducer results in a rectangular image on the field of scan (Frick and Lamb, 2002). Experienced veterinarians could easily pay back their investment in an ultrasound machine within 3 years when charging half of the breakeven cost of ultrasound while servicing 15 well-managed 100 cow dairies. Furthermore, as the proportion of pregnant cows at pregnancy evaluation decreases below 70%, the economic impact of ultrasound increased. Bovine reproductive organs are most commonly scanned per-rectum using a linear-array transducer specifically manufactured for trans-rectal use (DesCoˆteaux and Fetrow, 1998). Some studies showed that trans-rectal ultrasound scanning of cows on days 23, 28, 35 post-service was less accurate than on day 42 (Hadiya *et al.,* 2015). Whereas, in one study on repeat breeding *Holstein Fries* cows the pregnancy was detected as early as on day 23 in 12 out of 24 animals (Patel *et al.*,2005). The absence of embryonic vesicle and its fluid are reliable signs of non-pregnancy (Pieterse *et al.,* 1990).Some studies reported 16.66% cases of false negative diagnosis in cows (5/30) under field conditions when ultrasound scanning was performed between days 27 and 31 post-service (Szenci *et al.,* 1998). The inability to detect pregnancy was attributed to the location of uterus being far cranial to pelvic inlet and with transducer of 7.5 MHz frequencies most part of the uterus probably could not be visualized as transducer of higher frequency has limited penetration ability of few centimeters only. The specificity, positive predictive value and diagnostic accuracy were comparatively higher on day 35 and 42post-service, indicating that day 35-42 is the earliest possible time when pregnancy diagnosis should be attempted using ultrasound for maximum accuracy and specificity in zebu cattle (Patel *et al.*,2005; Awasthi *et al.,* 2011; Bhoraniya *et al.,* 2011). The first visible change appearing by day 21 after breeding, when fetal heartbeat can be visualized, also help to confirm a viable pregnancy though it is not a routinely assessed parameter for pregnancy diagnosis (Curran *et al.,* 1986). The fetal heart develops early in embryo genesis and displays regular beating by day 30 in cattle. The depolarization of cardiac muscle tissue results in the dissemination of an electrical signal from the foetus through the maternal tissues. The activity of the heart and movement of fluid within blood vessels generates pressure and sound wave signals, which also disseminate from the foetus through the maternal tissues. Positive diagnoses of pregnancy by trans-rectal ultrasonography depend on the detection of anechoic allantoic fluid and the embryo proper. LEM and EFM were diagnosed when the embryo was detected without a heartbeat or when a previously observed embryo with a heart beat was no longer visible during subsequent ultrasonographic examinations (Szenci *et al.,* 1998; Rani *et al.,* 2018)

Managemental strategies

A significant increase in conception rate among cattle was recorded when Crestar ear implants (Norgestomet) were given on day 7 of estrous cycle (Broadbent *et al.,* 1992). Studies have supported that conception rate is better in cows with three follicular waves after insemination as compared to cows with two follicular waves and hCG induction of three-wave cycles may also contribute to higher pregnancy rates. It was demonstrated that injecting 3300 IU of hCG in lactating cows 5 days after AI resulted in increased number of CL and higher plasma progesterone concentrations and conception rates on days 28, 42 and 90 were improved. The findings of Santos *et al.,* (2001) were supported by findings of Nishigai *et al.,* (2002) as hCG administered on day 6 increased the pregnancy rates (67.50 %) with formation of accessory corpora lutea as compared to control cows (45.0 %) or cows receiving hCG on day 1 (42.50 %). Luteotropic effect of PMSG in cattle was studied and it resulted significant increase in progesterone concentration on administration of 500 IU of PMSG on day 7 after estrus (Hirako *et al.,* 1995). Administration of GnRH (250 µg) at the time of insemination increases pregnancy rates by 12.5% and effect was more pronounced (Morgan and Lean, 1993) in repeat breeder cows.

Also by supplementing nutritious diet, the conception rate can be improved by reducing embryonic losses. Protein supplementation have been reported for cattle feed as rumen by-pass protein during the breeding season (Wamsley *et al.,* 2005) had better results. The exact mechanism by which increased undegradable intake protein improves fertility is unknown, but is likely related to decreased embryonic mortality. Feeding fishmeal has also been demonstrated to suppress oxytocin induced prostaglandin secretion in heifers with low progesterone concentrations suggesting it may improve an embryo's ability to signal maternal recognition of pregnancy (Wamsley *et al.,* 2005). Evidence exists that beta-carotene, vitamin A precursor, manganese and zinc are involved in steroidogenesis (Hurley and Doane, 1989; Corah and Ives, 1991). Their deficiencies may therefore directly impair ovarian activities or indirectly through a breakdown of the hypothalamo-pituitary feedback mechanism. Both selenium and vitamin E functions as intra-cellular antioxidant scavenging for free reactive oxygen and lipid hydroperoxides and converting them to non-reactive forms, thus maintaining the integrity of membrane phospholipids against oxidative damage and peroxidation (Surai, 1999). So, diet enriched with all above mentioned nutrients can also help to improve conception rate and reduce embryonic mortality in cattle.

In conclusion, there is no single approach to target embryonic mortality. Hopefully, a better understanding of some of the factors involved and the likely causes of embryonic mortality will enable us to limit its effect in the herds. Efforts should be made to provide appropriate protection against adverse climate, ensuring effective vaccination and to provide clean environment. Sero-monitoring of diseases, isolation of ailing animals and effective treatment should also be carried out for better farm economics.

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