Review article

Animal Reproduction Update
Year 2025, Volume-5, Issue-2 (Jul-Dec)

Early Embryonic Mortality in Bovines: Current Insights and Interventions

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ARTICLE INFO

Keywords:
Early embryonic mortality, Early Pregnancy Factor, Endometrial receptor modulators, Luteal phase defects, Preimplantation factor, Recombinant interferon-tau.

doi:10.48165/aru.2025.5.2.1

ABSTRACT

Early embryonic mortality (EEM) is a critical reproductive challenge in bovines, significantly impacting fertility, milk production, and farm economics. Despite high fertilization rates, embryonic losses during the first 24 days of gestation remain a major bottleneck in achieving optimal conception rates. This review synthesizes current understanding of the multifactorial aetiology of EEM, encompassing genetic, endocrine, immunological, nutritional, environmental, and managerial factors. Species, breed, age, parity, milk yield, and stress are all associated with increased embryonic loss. Endocrine disruptions such as luteal phase defects and inadequate progesterone levels are frequently implicated. Immune system dysfunction, including maternal-fetal immunological incompatibility and subclinical infections, further compromises embryo viability. Diagnostic tools such as early pregnancy factor, preimplantation factor, progesterone assays, ultrasonography, and pregnancy-associated glycoproteins (PAGs) allow early detection of EEM. Therapeutic interventions include hormonal supplementation (GnRH, hCG, progesterone, kisspeptin), immunomodulators, endometrial receptor modulators, and nutritional strategies such as omega-3 fatty acid supplementation and insulin-based regimens. Assisted reproductive technologies like embryo transfer and embryo co-transfer offer promising alternatives for repeat breeder animals. Preventive strategies emphasize precise AI timing, estrus detection, heat stress mitigation, and improved nutritional management to enhance uterine receptivity and embryo survival. This review underscores the need for integrated, individualized approaches to managing EEM, combining diagnostics with targeted therapeutic and environmental interventions. Future research directions include identifying novel biomarkers, refining genomic selection for fertility traits, and leveraging AI-based tools for real-time reproductive monitoring. A comprehensive understanding of EEM pathophysiology, coupled with evidence-based interventions, is essential to improve reproductive efficiency and sustainability in dairy production systems.

Introduction

Intensive genetic selection for milk yield over the past 60 years has led to a global decline in dairy cow fertility. Reproductive efficiency is crucial for dairy productivity, national economic growth, and rural livelihoods. In India, cattle and buffaloes account for more than 95% of total milk output, yet a considerable gap persists between demand and supply. This

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Received: 28.06.2025 Accepted: 16.09.2025

gap can be reduced by minimizing infertility and enhancing fertility in productive animals. A study by Yusuf et al. (2010) estimated that each open day in a cow result in an economic loss of approximately €5 per animal. In India, milk production losses due to infertility are estimated at ₹366.0 million annually in the northeast alone (Paul et al., 2013). Early embryonic mortality (EEM) is a major cause of reproductive failure and is responsible for significant economic losses due to repeat breeding, extended calving intervals, and reduced conception rates. Fertilization failure is often not the limiting factor; most losses occur during the early stages of embryonic development. EEM has a multifactorial aetiology involving endocrine dysfunction, immune suppression, nutritional deficits, heat stress, and oxidative imbalance (Lobodin et al., 2017). Factors such as high milk yield, body condition score, and heat stress significantly influence embryo survival (Lepesheva et al., 2020). Immunological markers, including cytokines like IL-10 and progesterone-induced blocking factor (PIBF), are emerging as critical regulators of maternal tolerance and embryo viability (Mohapatra et al., 2020). This review aims to report the different therapeutic and preventive measures proposed in the literature for the treatment of early embryonic loss in bovines, based on the knowledge of possible risk factors and causes. In the first part of the review, an overview of the possible risks and aetiological agents is proposed, followed by an evaluation of the specific diagnostic approaches and therapeutic and preventive measures suggested by current research.

Fertilisation failure: Fertilisation rates in dairy cows are generally high, typically exceeding 80% (Sartori et al., 2010) and even reaching 90–100% in some studies (Sreenan & Diskin, 1986). Despite this, calving rates remain around 55%, indicating that approximately 35% of pregnancies are lost due to embryonic or fetal mortality (Diskin et al., 2006). This suggests that while fertilisation itself is not a major limiting factor in dairy cow fertility, subsequent stages of pregnancy are far more vulnerable. Subtle declines in fertilisation success can still occur, often linked to ovulatory dysfunction, impaired sperm transport, or suboptimal insemination window (Diskin & Morris, 2008). However, the primary challenge lies in early embryonic development and survival. Studies indicate that most reproductive wastage in high-producing dairy cows stems from early embryonic loss rather than fertilisation failure.

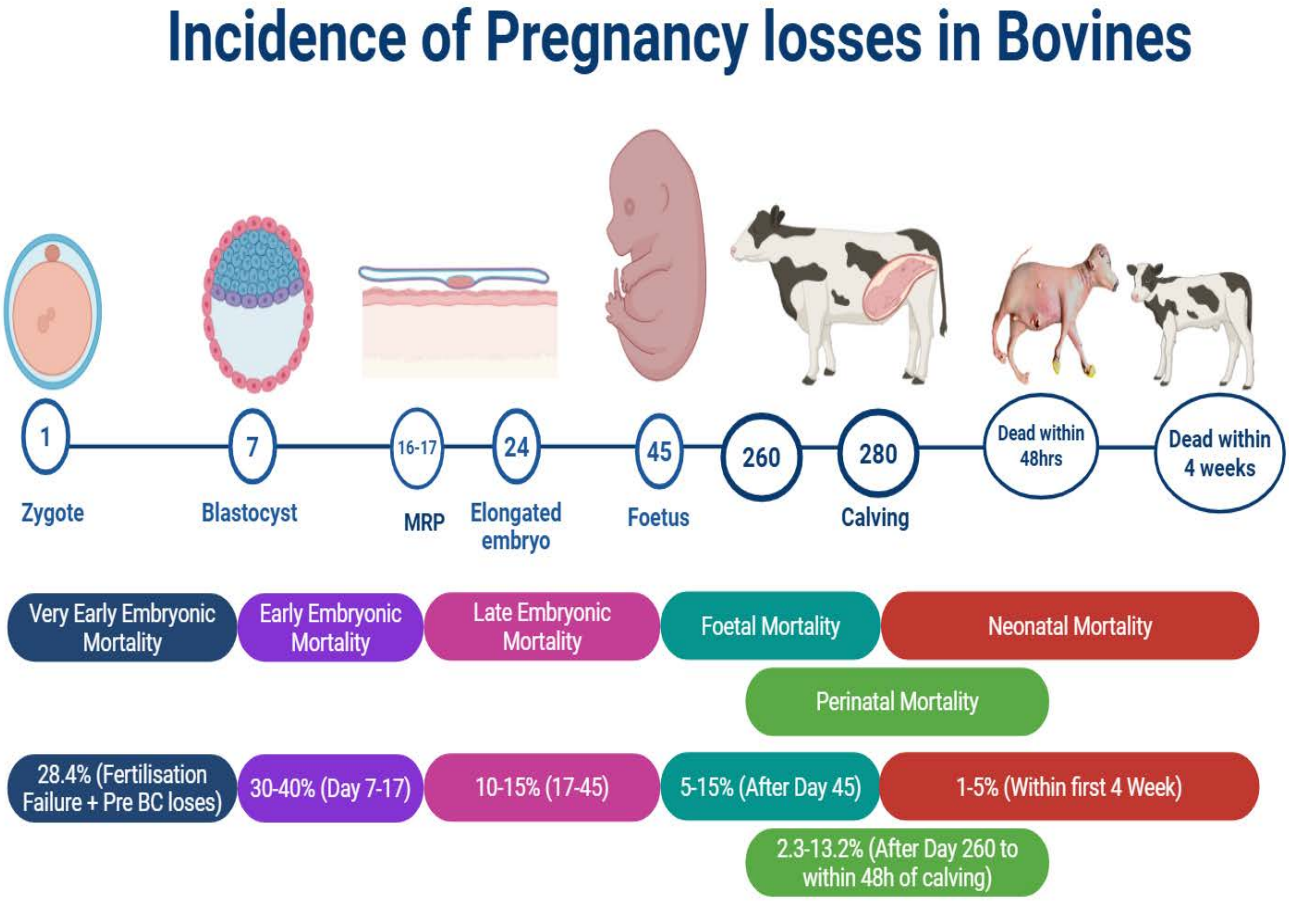

Fig. 1. Incidence of embryonic and foetal mortality in bovine

Early embryonic mortality (EEM): In bovines, the embryo is defined as the product of conception up to approximately 42–45 days, marking the period of cellular differentiation (Committee on Reproductive Nomenclature, 1972), This period is used to assess embryonic mortality (EM) (Inskeep and Dailey, 2005). EM is classified as: very early embryonic mortality (VEM, days 0–7), early embryonic mortality (EEM, days 7–24), late embryonic mortality (LEM, days 25–45), and fetal mortality (FM) from day 46 to parturition (Committee on Bovine Reproductive Nomenclature, 1972). Perinatal mortality refers to fetal death after >260 days or within 48 hrs post-parturition, while neonatal mortality is defined as death within 7 days of calving. Reported incidences of embryonic mortality are 80% (days 8–16), 10–15% (days 17–42), and 5% beyond day 42 (reviewed by Parmar et al., 2016), with 30–40% loss between days 7–17 post-fertilization in cattle (Campanile et al., 2005). In buffaloes, EEM ranges from 16–35%, LEM from 20–40%, and FM from 9–15% (Neglia et al., 2008; Campanile et al., 2007). EEM typically results in a normal return to estrus, whereas late embryonic loss is marked by irregular estrous intervals (Silke et al., 2002). Although less frequent, late losses incur higher economic costs, especially in seasonal calving herds, as rebreeding opportunities are limited, leading to increased culling. The incidence of perinatal mortality ranges from 2.3-13.2% from various findings (Meyer and Berger, 2004; Rodriguez-Campos et al., 2020). While 1% to 5% for neonatal mortality in well-managed herds, with higher rates in herds with poor colostrum management or hygiene (Mee, 2023; Fig.1).

Embryo development and maternal recognition of pregnancy

In bovines, the first cleavage occurs ~30 h post-insemination, followed by the second at ~48 h. Over the next 4–5 days, successive blastomere divisions yield 4-, 8-, 16-, and 32-cell stages, progressing to tight morula and blastocyst within the oviduct (Senger, 2004). The morula enters the uterus around days 4–6 post-mating and forms a blastocyst comprising an inner cell mass and a fluid-filled blastocoel surrounded by trophectoderm. After hatching from the zona pellucida (days 9–10), the blastocyst transitions into an ovoid conceptus and begins elongation on day 12 in sheep or day 15 in cattle. In bovines, the conceptus is ~2 mm on day 13, ~6 mm by day 14, ~60 mm by day 16, and exceeds 20 cm by day 19. This exponential growth between days 9–16 is marked by ~10-fold increases, from days 12–15 (Berg et al., 2010). Elongation is accompanied by secretion of interferon tau (IFNT) from mononuclear trophectoderm cells between days 10–25, peaking at day 14 in sheep and day 20 in cattle. IFNT prevents luteolysis by suppressing endometrial ESR1 and OXTR expression through IRF2-mediated pathways, establishing MRP (Hansen et al., 1988; Stewart et al., 1989; Peterson et al., 2025). Fibroblast growth factors (FGF1, FGF2, FGF10) also regulate IFNT production and conceptus development during this phase (Cooke et al., 2009). Early attachment, initiated by apposition and interdigitation of trophectoderm and uterine microvilli, begins around day 19, with firm adhesion by days 21–22.

Risk factors associated with early embryonic mortality

Species: EM is a significant issue in both cattle and buffaloes, with early losses (up to day 16) being more frequent in buffaloes due to higher heat sensitivity and hormonal imbalances (Kumar et al., 2021). Differences in species-related physiology (e.g., INFT secretion and luteal function) impact embryo survival, making cattle more responsive to hormonal treatments for reducing mortality (Huynh et al., 1997).

Breed: Studies indicate that Holstein cows experience greater EM than breeds such as Simmental and Brown Swiss, likely because of their elevated metabolic requirements for high milk yield (Voljč et al., 2017). Additionally, crossbred cows (17.57%) show a higher incidence of RB compared to indigenous breeds (8.64%) (Verma et al., 2018).

Age: Younger cows (2–2.4 years) experience the highest EM (45.5%) likely due to reproductive immaturity, while optimal rates are seen in cows aged 3.5–4 years (Puklová et al., 2011). Furthermore, the likelihood of EM increases with age, older cows show higher EM due to diminished ovarian reserve, mitochondrial function, and increased genetic mutations (Kordowitzki et al., 2024).

Parity and senility: EM is significantly higher in primiparous cows than multiparous, likely due to reproductive immaturity and metabolic adaptation issues (Chetoui and Slimane, 2012). Fertility tends to decline with increasing parity beyond the third calving, which may be linked to cumulative reproductive complications such as dystocia, caesarean section, retained fetal membranes, postpartum metritis, pyometra, and delayed uterine involution and stress etc. (Eshete et al., 2023; Kaneda et al., 2024).

Fetal plurality: Twin pregnancies significantly increase the risk of early and late embryonic/fetal mortality. Loss rates are higher in twin pregnancies (33.6%) compared to singlet ones (20.7%) (Mellado et al., 2016). EM is more pronounced in unilateral twin pregnancies than bilateral ones (López-Gatius and Hunter, 2005; Silva-Del-Río et al., 2009).

Milk yield: High-producing dairy cows and buffaloes are more susceptible to EM due to prolonged energy demand, increased metabolic demands, which accelerate hepatic metabolism of steroid hormones and negatively impact reproductive function (Kaneda et al., 2024).

Breeding method: Artificial insemination (AI) following hormonal synchronization is associated with higher rates of EM compared to estrus-based AI (Kim et al., 2017). Contributing factors include poor heat detection, mistimed AI, substandard semen quality, improper handling (e.g., thawing, insemination site and timing), and operator errors (Eshete et al., 2023).

Seasonality: EM is significantly higher during hot seasons due to heat stress impairing embryo development and uterine environment (Ryan et al., 1993). Heat stress affects ovarian follicle dynamics, reduces dominant follicle viability, shortens the luteal phase, and impairs oocyte competence (Manoj et al., 2015). Fertility and embryo quality improve in cows inseminated during autumn and winter, indicating seasonal climate plays a strong role in reproductive outcomes.

Body condition score (BCS) and energy balance: Animals with medium (BCS 2.75–3.5) or poor condition (<2.5) experience higher LEM than those with optimal scores (>3.75) (Tameoka et al., 2012). Negative energy balance from deficiencies in energy, protein, vitamins, minerals, and antioxidants is directly associated with reproductive failures (Eshete et al., 2023).

10.Pre- and postpartum complications: Conditions such as prepartum cervico-vaginal prolapse, and postpartum issues like RFM, dystocia, caesarean section, uterine prolapse, metritis, endometritis, and laminitis strongly linked to poor BCS changes and increase the risk of infertility and embryo loss (Hadef et al., 2021, Sahu et al., 2024).

Causes of early embryonic mortality

Chromosomal, cytological and genomics alteration:

Genetic causes of EM include chromosomal and cytological defects, individual genes and genetic interactions (VanRaden and Miller 2006). In cattle, genetic defects may account for up to 20% of EM and FM (Vanroose et al., 2000; Diskin and Morris, 2008). Gametes quality is one of the main factors involved in the phenomenon of EM in domestic animals. Approximately 5% of embryos die because of gross chromosomal abnormalities preventing development (Peters, 1996). Robertsonian translocation (fusion of chromosomes 1/29) in RB reduces chromosome number to 59 instead of 60, leading to fertility issues (Gustavsson, 1979). Additionally, polyploidy particularly triploidy (3n) from polyspermy, aged ova, or meiotic errors causes EM, due to disrupted normal fertilization and embryo development (Hafez & Hafez, 2013). The deficiency of uridine monophosphate synthase (DUMPS), a monogenic autosomal recessive disorder, causes EM at 40-50 days gestation (Robinson et al., 1984). Testing of AI sires has effectively eliminated this infertility cause. Similarly, complex vertebral malformation (CVM), another recessive condition, leads to late fetal death (VanRaden and Miller, 2006). Furthermore, A genomic analysis on RB cows revealed altered expression of nine genes in cumulus-oocyte complexes, affecting oocyte maturation, fertilization, and embryo quality. Upregulated genes (e.g., phospholipase A2, glutathione peroxidase) and downregulated genes (e.g., Annexin A1, lactoferrin) were linked to oxidative stress, meiotic defects, and impaired metabolism. These changes contribute to ovulation defects and EM (Puglisi et al., 2013). Further, Sahu, (2025) reported transcriptomic dysregulation with higher apoptotic index and lower proliferation in cattle oocyte affected with SCE, indicating detrimental effect of oocyte on the fertility outcome.

Endocrinedysfunctions: Endocrine imbalances are a leading cause of early embryonic mortality (EEM) in cattle, disrupting hormonal regulation essential for pregnancy establishment and maintenance. Key dysfunctions include:

Luteinizing hormone: A subdued secretory pattern of LH, insufficient LH release, or incorrect timing of the LH surge results in either anovulation or delayed ovulation, leading to failure of fertilization (anovulation) or delayed ovulation resulting poor embryo quality due to aging of gametes, subsequently early embryo death (Kimura et al., 1987).

Estrogen: High E2 levels, which can increase due to delayed or overmature follicles due to delay in the secretion of LH, lack of LH receptor expression or LH refractoriness etc. have been found to cause asynchrony between the embryo and uterine environment, leading to pregnancy failure (Sood et al., 2015).

Progesterone: Suprabasal P4 level >1 ng/ml (basal P4 level <1 ng/ml or 0.1–0.3 mg/ml) before ovulation causes delayed, irregular ovulation patterns or anovulation due to a decrease in the frequency LH. Basal P4 post-estrus due to anovulation has been reported in 2-16% of RB cows.

Luteal phase dysfunction (LPD): This term was first used for human corpus luteum (CL), defined as a reduction in P4 steroidogenesis by CL in terms of amount and duration, or both. It also occurs in domestic animals (Kimura et al., 1987; Jisna, 2024; Nivetha, 2024). Causes include inadequate luteal development, premature regression, or functional defects (Campanile et al., 2007), such as disrupted P4 synthesis, PR downregulation, or shortened luteal phases. Further, LPD in 67% of cases of buffaloes (Jisna, 2024) and 65% cases of Crossbred cattle (Nivetha, 2024) with conception failure was characterized by Suprabasal, delayed rise, or early fall of P4 and lowered CL area, volume, and vascularity on day 5

suggested the early sign of LPD. Furthermore, upregulation of ISGs (37-68 folds on day 18) indicated pregnancy, while moderate ISG15 (2.7-3.5-fold on day 18 to 27) and OAS1 (9.41-fold on day 27) were associated with EM. Furthermore, LPD affected buffaloes had a 10x lower conception odds than normal (Jisna, 2024). Suboptimal levels of P4 in the first week after ovulation make it difficult for the development and implantation of embryos, causing EEM. In addition, certain pregnancy-related factors or proteins may decrease, such as INFT, involved in early MRP are related to P4 levels. Low levels of P4 fail to down-regulate large MUC1 glycoprotein molecules on the epithelial surface of the uterus at day 15 post-fertilization because the loss of MUC1 from the epithelial surface allows the conceptus to contact its integrin to bind its integrin receptors to initiate implantation (Hafez and Hafez, 2013; Moqbel and Al-Ramadan, 2024). Reduced peripheral P4 in high yielder dairy cows has been also reported due to high feed intake, which elevates hepatic steroid metabolism, increasing EM (Sangsritavong et al., 2002).

AMH: Low AMH are associated with a higher incidence of RB in cattle. Since AMH reflects antral follicle count and ovarian reserve, diminished fertility in low-AMH cows may stem from fewer available oocytes, reduced theca, granulosa, and luteal cell sensitivity to FSH and LH, impaired embryonic developmental competence, and low P4 during the estrous cycle (Akbarinejad et al., 2020).

Endogenous opioids and neuropeptides: Stress-induced overproduction of β-endorphins and free radicals impairs CL function. Additionally, neuropeptide Y inhibits the GnRH pulse generator, reduces FSH and LH release, and elevates PRL secretion, collectively contributing to EM (Rizzo et al., 2007; Nestor et al., 2018).

4.Subclinical genital infections:

Genital infections contribute to 2–8% of total EM caused by various pathogens, which may enter via hematogenous or ascending infection through the vaginal tract (Table 1). A wide range of subclinical infectious agents can cause pregnancy loss in cattle, often without obvious clinical signs. Bacterial pathogens such as Campylobacter fetus, Brucella suis, and Brucella canis; viral agents including BVDV, BHV-1, Cache Valley virus, border disease virus, swine fever virus, pseudorabies, and porcine parvovirus; protozoa like Tritrichomonas fetus and Toxoplasma gondii; and mycotic organisms such as Aspergillus spp., Mucor spp., and Mycoplasma spp. are all implicated in EEM (within 21 days), late embryonic death (21–45 days after MRP), and fetal Additionally, increased infiltration of macrophages (CD14+) in the stratum compactum and spongiosum suggests an

[3.] **Immunological causes:** Infertility may stem from autoimmune conditions such as autoimmune oophoritis, anti-sperm antibodies (IgG and IgA), or allergic reactions to semen extenders containing egg yolk, enzymes, or antibiotics. The presence of anti-zona pellucida antibodies in the blood of mares and cows further demonstrates how immune dysregulation can contribute to EM. A properly regulated immune environment at the placental–uterine interface is essential for pregnancy maintenance. During early gestation, INFT and local P4 collaborate to induce tolerogenic immune responses (Chełmońska-Soyta, 2002; Oliveira et al., 2012), supported by the selective expression of non-classical BoLA class I antigens and absence of BoLA class II in cotyledonary tissues (Davies et al., 2004). A shift from Th1 to Th2-dominant maternal tolerance arises for survival of foetus as an allograft (Oliveira et al., 2013). However, intrauterine infections can shift this balance toward Th1 responses, increasing abortion risk. EEM may result from maternal-fetal immunological incompatibility, inadequate INFT production by underdeveloped embryos, low P4 levels, or defective maternal T cell apoptosis, leading to embryo rejection. Studies reinforce these mechanisms, highlighting that the shift toward Th2 immunity is associated with higher pregnancy success (Zhang et al., 2015; Corpron et al., 2021). Elevated interferon-stimulated genes (ISGs) and IL-10 expression in the uterus and peripheral blood during early pregnancy indicate critical immunomodulation (Mohapatra et al., 2022; da Silva et al., 2024). Moreover, the early maternal immune response is fine-tuned even before embryo implantation, with implications for fertility prediction and embryo survival (Ott, 2019).

fetal death (45–90 days) in cattle. For instance, BVDV can induce EEM in the first trimester, cerebellar hypoplasia and fetal inflammatory response syndrome with multi-organ failure in the second trimester, and abortion in the third trimester (Givens and Marley, 2008; Jawor et al., 2021). These pathogens compromise embryonic viability by direct cytotoxic effects on the embryo or MUC1, IGF1, and IGFBP2 upregulation in endometrium, which plays a protective role by inhibiting bacterial adhesion results hindered embryo implantation due to its anti-adhesive properties (Kasimanickam et al., 2014; Wagener et al., 2017). Additionally, increased infiltration of macrophages (CD14+) in the stratum compactum and spongiosum suggests an inflammatory contribution to EM (Janowski et al., 2013). Uterine dysfunction in bovines has also been associated death (45–90 days) in cattle. For instance, BVDV can induce EEM in the first trimester, cerebellar hypoplasia and fetal inflammatory response syndrome with multi-organ failure in the second trimester, and abortion in the third trimester (Givens and Marley, 2008; Jawor et al., 2021). These pathogens compromise embryonic viability by direct cytotoxic effects

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inflammatory contribution to EM (Janowski et al., 2013). Uterine dysfunction in bovines has also been associated with altered endometrial production of heparin-binding epidermal growth factor (HB-EGF), which is critical for conceptus glucose metabolism, endometrial proliferation, and early embryonic development (Katagiri et al., 2004; Hai Fan Yu et al., 2019). The subclinical nature makes diagnosis and control particularly challenging.

5. Nutritional causes and negative energy balance:

EEM is significantly influenced by nutritional status, which affects reproductive physiology via metabolic, endocrine, and oxidative pathways. Both overnutrition and undernutrition disrupt the HPG axis, impairing ovarian function, oocyte competence, and uterine receptivity (Guzel et al., 2014). In polytocous species like swine, excessive energy intake may cause superovulation leading to uterine crowding and EM (Hafez & Hafez, 2013). Elevated rumen degradable protein increases BUN, negatively affecting uterine conditions for embryonic development (Boakari et al., 2020). NEB elevates non-esterified fatty acids, reducing INFT secretion and compromising maternal immune tolerance (Leroy et al., 2020). Recent evidence shows that periconceptual undernutrition in beef cattle impacts placental development and long-term offspring performance (Serrano-Pérez et al., 2019; Caton et al., 2020). Additionally, maternal nutrient restriction increases risk of LEM without affecting peri-implantation ISGs (Serrano-Pérez et al., 2019). Endocrine dysfunction, such as leptin deficiency in metabolically stressed animals, further disrupts signaling to the reproductive axis (Guzel et al., 2014). Oxidative stress due to trace mineral deficiencies (Cu, Zn, Se, Fe) diminishes antioxidant enzyme activity (e.g., SOD, GPX), impairing embryo viability (Mikulková et al., 2020). Some studies emphasize that paternal nutrition also influences early embryo development, where high-energy diets in bulls reduce blastocyst quality despite normal

Class	Species	Disease	Infection Route	Persistence/Transmission	Embryonic mortality
Viral	BVDV	BVD (Bovine Viral Diarrhoea)	Hematogenous	Persistent infection	++
	BoHV-1	IBR (Infectious Bovine Rhinotracheitis)	Hematogenous	Viral latency	+++
Bacteria	M. bovigenitalium	Mycoplasmosis	Genital	Asymptomatic carrier cow	+
	Campylobacter sp.	Campylobacteriosis	Genital	Asymptomatic carrier bull	+
	U. diversum	Ureaplasmosis	Genital	Asymptomatic carrier cow	+
	Leptospira spp.	Leptospirosis	Hematogenous	Renal carrier	+++
Protozoal	Neospora caninum	Neosporosis	Vertical	Oocyst shedding	+
	Histophilus somni	Histophilosis	Hematogenous		+
	Tritrichomonas foetus	Trichomoniasis	Genital	Asymptomatic carrier bull	+

Table 1. Embryonic mortality associated with different infectious agents in beef and dairy cattle herds (Givens and Marley, 2008; Kumar et al., 2011; Gates et al., Sanhueza et al., 2013)

fertilization rates (Fontes, 2024). These findings underscore the need for precise energy, protein, and micronutrient regulation to support endocrine balance, optimal uterine environment, and embryo survival.

6. Management errors and AI technique:

Timely AI ideally 12–18 hrs after the onset of standing heat with efficient detection of oestrus is essential maximize conception rates. However, this has become increasingly difficult. Over the past 60 years, the proportion of cows showing standing heat has dropped from 80% to 50%, and the duration of oestrus has shortened from 15 to just 5 hrs (Dobson et al., 2008) resulting 30–40% of oestrus to go unnoticed. Estrus detection becomes even more unreliable in larger or semi-confined systems, where cattle may have

limited pasture access (Noakes et al., 2019; Eshete et al., 2023). Increasing herd size has mixed effects: while it raises the number of cows in the sexually active group enhancing behavioural cues it can also reduce overall detection accuracy if staffing levels don't keep pace (Noakes et al., 2019). Unlike natural mating, which ensures ideal timing, sperm delivery, and transport, AI demands precise coordination. AI, though widely used, presents several technical hurdles such as improper semen thawing, prolonged exposure to ambient temperatures, inaccurate deposition, and poor hygiene may all impair sperm function and the uterine environment leading to higher incidence of EM (Noakes et al., 2019; Chandrika et al., 2024). Inseminating too early can cause sperm to age before ovulation; too late increases the risk of polyspermy (15–20%) and poor embryo quality (Ouedraogo et al., 2016). However, EM rates of 44% have been reported with some bulls, despite normal fertilization rates (Bulman, 1979). The use of sexed semen in cattle breeding is associated with a reduced conception rate (30–40%) compared to conventional semen (50–60%), primarily due to lower sperm viability and altered uterine compatibility (Sahu et al., 2025). This decline increases the risk of early embryonic mortality (EEM), as fewer viable embryos reach critical developmental stages.

7. Environmental causes:

The thermoneutral zone for dairy cattle is 5–25°C (McDowell et al., 1976). Heat stress occurs above 25°C and 70% humidity, severely reducing fertility. Buffaloes are more susceptible due to darker skin and fewer sweat glands. Summer heat increases prolactin, suppressing LH and correlating negatively with P4 (Roy and Prakash, 2007). Each hour with THI >85 in early gestation raises pregnancy loss risk 1.57-fold (Santolaria et al., 2010). Grazing cattle EEM can exceed 50% when average THI >70 (Souto et al., 2010). Heat stress increases ROS, causes cellular damage, suppresses INFT, and disrupts uterine signaling (Kumar et al., 2021; Parra-Bracamonte et al., 2024). Global warming is expanding its impact. Cold stress also impairs reproduction, reducing nighttime estrus behavior and shortening its duration (Noakes et al., 2019).

Diagnostic measures for EEM:

Identifying reproductive failure in cows without obvious signs is vital to reduce economic losses. A cost-effective, systematic approach includes history, clinical exams, hormonal tests, and breeding evaluation.

1.Planned slaughter inspection post-AI:

Planned slaughter studies post-AI revealed that over 50% of embryonic mortality occurs between Days 16–34 (Hawk et al., 1955), with major losses before Day 18 due to failed MRP or poor uterine environment (McGeady et al., 1981). PSPB testing has since proven more sensitive for early loss detection (AUC = 0.92) (Abubakar et al., 2019).

2.Embryo removal on defined dates post-AI:

Uterine flushing post-slaughter (Days 14–30) permits recovery of embryos to assess morphology, viability, and development (Leeuw, 1992). It also reveals embryo–maternal signaling, as shown by altered uterine blood flow and gene expression on Day 7 (Scarlet et al., 2024), and helps detect uterine infections linked to repeat breeding and embryonic mortality.

3.Early pregnancy factor (EPF):

One of the earliest and most specific signs of fertilization is early pregnancy factor (EPF), first discovered in mice (Morton et al., 1974) and later in women (Morton et al., 1977), sheep (Morton et al., 1979), cattle (Nancarrow et al., 1981), and pigs (Paisley et al., 1982). EPF appears in serum within hours of fertilization and disappears if the embryo dies (Yamazaki et al., 1995), making it a potential marker for embryo survival (Koch, 1986). The rosette inhibition test was proposed to detect EPF within the first week (Laleh et al., 2008), but its reliance on bioassays limits practicality. Polyclonal antibodies against the immunosuppressive form of EPF (~200 kDa) enabled development of the Early Conceptus Factor (ECF) test (Threlfall, 1994), though it cannot confirm pregnancy before Day 21 (Gandy et al., 2001; Whisnant et al., 2001). A related protein, chaperonin 10 (10.84 kDa), with immunosuppressive and growth-promoting properties, has also been identified (Cavanagh, 1996; Morton, 1998). Despite these advances, a rapid, accurate, and farm-level test for early pregnancy is still unavailable.

4.Preimplantation factor (PIF):

It is a peptide (MVRIKPGSANKPSDD) secreted by trophoblast cells and placental tissue in humans, cattle, and mice (Barnea, 2007; Stamatkin et al., 2011). In bovine and murine models, viable embryos secrete PIF, while non-viable ones do not (Stamatkin et al., 2011). Functionally, PIF supports early pregnancy by enhancing endometrial receptivity, promoting trophoblast invasion, and modulating maternal immunity (Duzyj et al., 2010; Paidas et al., 2010; Barnea et al., 2012). It also mitigates recurrent pregnancy loss by counteracting embryo-toxic serum and reducing NK cell cytotoxicity (Stamatkin et al., 2011; Roussev et al., 2013). In cattle, PIF was detectable in serum as early as Day 10 post-AI with 91.3% sensitivity, reaching 100% by Day 20, and its presence correlated with live calf birth, while non-pregnant animals tested negative (Ramu et al., 2013). These findings highlight the potential of PIF-ELISA as a practical tool for early diagnosis of embryonic viability and mortality.

5.Pregnancy associated glycoproteins (PAGs):

Compound also known as pregnancy specific protein B (PSPB) or pregnancy serum protein 60 (PSP-60), initially described as placental antigens are present in maternal circulation after implantation (Sousa et al., 2006). The PAGs are proteins synthesized in the mono-and bi-nucleate cells of the ruminant trophectoderm (Haugejorden et al., 2006; Sousa et al., 2006), from where these proteins are released into circulation and their presence used in monitoring pregnancy status. PAGs are the key biomarkers for early pregnancy detection and EM assessment in cattle. PAGs can be detected via ELISA or RIA in maternal blood from Day 24 post-insemination and are closely associated with the presence and health of the conceptus. Recent study by Pohler et al. (2022) revealed that cows with lower PAG concentrations on Day 29 were significantly more likely to experience LEM between Days 29 and 100, with a predictive sensitivity of 83% and specificity of 77% (Pohler et al., 2022). Similarly, Akköse (2023) reported that both commercial PAG tests: On Farm Pregnancy Test and Rapid Visual Pregnancy Test accurately diagnosed pregnancy but were affected by undetected EM events around testing time. Vasconcelos et al. (2018) demonstrated that PAGs levels at Day 24 post-AI differ significantly between cows that remain pregnant and those that lose pregnancies by Day 60.

6.Daily P4 assay and/or return to estrus:

Daily P4 estimation may serve as diagnostic aids for reflecting estrous cycle status, delayed ovulation, persistent luteal activity, Suprabasal P4, and LPD (Vrisman, et al., 2018). Monitoring EM in bovine can be also done by daily P4 assays, and/or observation of return to estrus. Daily P4 assays help identify pregnancies at risk. Low or declining P4 levels between days 10 and 20 post-insemination are associated with higher embryonic loss, especially in buffaloes and cattle (Prandi et al., 2005). Early detection of non-pregnancy is possible by checking P4 on day 21 post-estrus values below 2 ng/mL reliably indicate failure of implantation (Tamasaukas et al., 1998). Recent study where Jisna, (2024) found the cut-

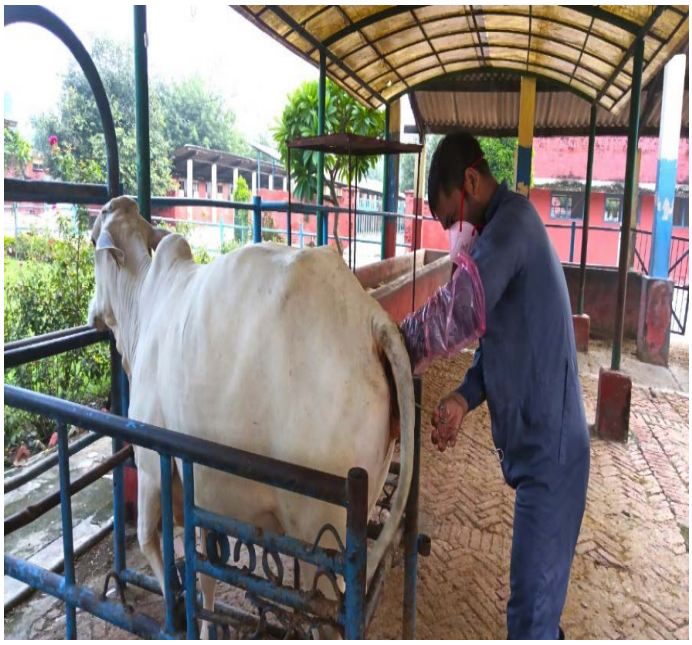

Fig. 2. Endometrial cytology sampling techniques for SCE screening by cytobrush method (Sahu, 2025)

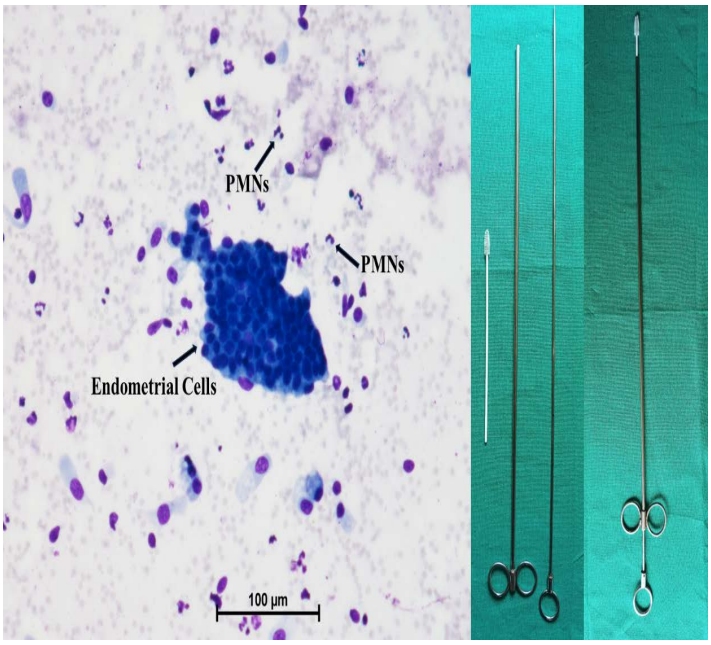

Fig. 3. Endometrial cytology stained with Modified Wright Giemsa Stain (40X), Cytobrush, Barrel, Stylet and Assembly (Sahu, 2025)

off serum P4 concentration >0.95 ng/mL on day 0, <1.86 ng/mL on day 5 and <1.98 ng/mL on day 18 of estrous cycle for the prediction of LPD in buffaloes due to supra-basal, delayed rise and early fall of P4, respectively.

7.In-vivo imaging techniques:

The clinician's role in pregnancy management ultrasound plays an essential role in managing a pregnancy. The only definitive markers of impending pregnancy failure are the absence of an embryo heartbeat, membrane detachment, and echoic floating structures including embryo remnants. Conversely, the presence of a heartbeat and amnion with a clear hyperechoic outline are signs of embryo viability (López-Gatius and Ispierto, 2010). Although pregnancy diagnosis may be accurate as early as 20 days into gestation (Kastelic, et al., 1991), the presence of both an amnion and heartbeat can be only clearly detected at around Day 28 of gestation (Curran et al., 1986). USG based evaluation of ovarian structures in relation to uterine contents: it is plausible that a contralateral gestation arises from a bilateral double ovulation and subsequent regression of the ipsilateral CL, the gestation being thereafter maintained by the contralateral CL. If so, this makes the embryo particularly vulnerable, as intra-cornual P4 levels will be reduced compared to the contralateral horn (Takahashi et al., 2016).

8.Tests to evaluate subclinical infections:

Numerous pathogens in the female reproductive tract can impair embryonic development, causing vulvitis, vaginitis, cervicitis, or endometritis. Diagnostic methods include the white-side test for screening, endometrial cytology (Fig. 2 and 3) and biopsy for histopathological evaluation of inflammation and disease. Cervical and vaginal mucus inspection helps detect genital infections in cows. In buffaloes, it is less effective due to low mucus and nocturnal estrus. Mucus can instead be collected by squeezing genital organs or using sterile pipettes.

9.*Levels* of antioxidants and markers:

Elevated MDA and NO, and reduced CAT, SOD, ASCA, GPX, and TAC in cattle and buffalo indicate oxidative stress linked to reproductive issues (Nenova et al., 2023).

10.Immunoassays:

Srivastava and Pande (2017) outlined tests for antisperm antibodies including immune-peroxidase (IPT), sperm immobilization (SIT), and sperm agglutination (SAT).

Preventive and therapeutic strategies for early embryonic mortality

Hormonal strategies

hCG:

The main luteotropic hormone in the cow is LH. So, if LH activity increases (e.g., with hCG or GnRH injection) post-ovulation, it stimulates CL development and function. In cattle, hCG treatment (Day 5–7 post-AI) elevates P4 by enhancing CL function and inducing accessory CL formation (Abdelli et al., 2020). In buffaloes, hCG (1500 IU) increases P4 by Day 10, likely via accessory CL formation rather than CL stimulation. hCG at Day 25 post-AI induces ovulation in ~57% of buffaloes (Campanile et al., 2007), comparable to GnRH agonists (62–68.6% ovulation rates; Campanile et al., 2007).

GnRH: The results of using GnRH during insemination are usually positive, thus, the use of GnRH is quite common in this way (Malmo and Beggs 2000). Lean et al. (2003) reported that the administration of GnRH during insemination, results total pregnancy risk increased by 12.5% in all cows but increased by 22.5% when used with repeat breeders. An alternative approach is to administer hCG or GnRH 11–13 days after breeding. The rationale behind this approach is to induce accessory CL or luteinized follicles, or to augment the P4 secretion from existing structures.

P4: The importance of P4 during the first weeks of pregnancy for reducing EM has been demonstrated in cattle (Maillo et al., 2013). According to some reports the presence of an early P4 peak (within 5 days after mating or AI) facilitates the elongation of the conceptus and, consequently, the secretion of adequate INFT (Lonergan, 2023). In cattle, INFT extends the lifespan of the CL (Plante et al., 1991) by suppressing ER and OXTR genes and by attenuating the endometrial secretion of PGF2α (Basavaraja et al., 2017; Hansen et al., 2017). Exogenous P4 (PRID®, Vetem) on day 5 after AI gave the lowest pregnancy rate and highest incidence of EM, suggesting that exogenous P4 can have had a detrimental effect on conception. After removal of the exogenous source of P4 the CL may not be able to secrete P4 in the amount required to maintain pregnancy (Villarroel et al., 2004).

Kisspeptin: The administration of Kisspeptin (Kp10) at the dose rate of 10 µg/kg IV at estrus and day 5 promoted accessory CL formation or had a direct effect on augmenting P4 production and improved the conception rate by 50 (4 out of 8 cows became pregnant) percent in luteal insufficient cows. Further, Kisspeptin induced increased P_4 production in in-vitro luteal cell culture (Thejaswini et al., 2024) and increase in the growth rate of the follicle, LH release and ovulation in buffalo at the dose rate of 10-20 µg/kg IV was reported (Hitesh and Kansal, 2022).

Non-hormonal strategies

Recombinant INFT: As previously mentioned, some cases of EEM in cattle may result from inadequate secretion of INFT by the conceptus, which fails to prevent CL regression. Studies have demonstrated that intrauterine administration of recombinant ovine IFNT (@100mcg, IU) or recombinant bovine IFNT (@200mcg, IU) twice daily from Days 14 to 24 of the estrous cycle prolongs CL lifespan, enhances CL lymphangiogenesis, extends interestrous intervals, and reduces OXTR-mediated uterine PGF2α release (Meyer et al., 1995; Spencer and Bazer, 1996; Nitta et al., 2011). Furthermore, unlike interferon-alpha (IFN-α), which induces hyperthermia (Plante et al., 1991), neither roIFNT nor rbIFNT triggered a hyperthermic response.

Endometrial receptor modulation: Successful implantation and early embryo survival are strongly influenced by modulation of endometrial receptors in response to maternal hormones and embryonic signals. Hormones and receptor changes are also central to initiating luteolysis. In the follicular phase, high levels of oxytocin receptors (OXTR), estrogen receptor alpha (ERα), and P4 receptors (PR) prime the endometrium for luteolytic signals. As the cycle progresses to the luteal phase, ERα and PR levels decline, weakening P4 production of the CL (Haaften et al., 1982), which enables prostaglandin F2α (PGF2α) to induce luteolysis through reduced suppression of luteolytic factors (Schramm et al., 1984).

Selective estrogen receptor modulators (SERMs) such as Tamoxifen, Raloxifene, Toremifene, and Bazedoxifene have not been shown to prevent luteolysis or sustain CL function based on current evidence.

Oxytocin receptor modulators (ORMs): Atosiban, a mixed oxytocin/vasopressin V1α receptor antagonist, improves pregnancy in women, (<$\mathbf{330}\ \boldsymbol{mg}$ administered IV) around embryo transfer (Huang, 2017). It may enhance implantation by reducing uterine contractions and improving endometrial receptivity (Huang 2017; Moraloglu 2010; Decleer 2012), and by inhibiting $PGF_2\alpha$ production, which may help prevent luteolysis.

Cytokines: Blocking immune responsive gene 1 (Irg1) reduces implantation by 80%, emphasizing its key role in establishing uterine receptivity (Cheon et al., 2003).
COX inhibitors: COX-2 is expressed in hatched bovine blastocysts, indicating its role in early embryonic development, while COX-1 is absent, highlighting COX-2's importance during implantation (Vasques et al., 2005). In

Table. 2. Various immunomodulators and their effective dosage for the treatment of SCE

S.N.	Immunomodulators	Effect and dosage	Reference
1	*E. coli* LPS	@100ug I/U in 20ml DW. Chemo attractants to PMN cells.	Singh et al., 2016
2	Autologous serum or plasma	@50ml I/U or 1/10th of total bacterial load. 75% recovery rate and 50% conception rate.	Methai,1999
3	PMN extracts and their components	Contains protein defensins (virucidal and bactericidal).	Hughes, 1988
4	Oyster glycogen	PMN migration, 75% recovery rate in cow affected with endometritis. @500mg single infusion I/U.	Krishna, 2011
5	LTB4	83.33% recovery from endometritis. Dose @50ml with conc. of 30nmol/L.	Krishnan, 2011
6	Carvacrol 0.1% and 0.1% Lugol's iodine	@30-50 ml I/U or according to uterine size half hr post AI. Antimicrobial property against most of the uterine pathogens except Pseudomonas species.	Lehimcioğlu et al., 2019
7	Neem oil extract	Non-specific herbal immunomodulator. @30-40 mL I/U showed recovery from endometritis, caused decline in bacterial load (96.02 ± 2.02 %) and higher conception rate. (71.4%).	Kumar et al., 2013

buffalo, meloxicam (COX-2 inhibitor, 0.5 mg/kg BW I/M on days 13–15 post-AI) was found to increase conception rates by 20% by reducing PGFM and PGF2α (Rajkumar et al., 2010). Similarly, the non-specific COX inhibitor flunixin meglumine improved conception rates in cattle.

Treatment strategies for infectious causes of EM:

Rule out infectious causes and SCE: if any, then start with the following treatment:

Systemic antibiotic therapy: Penicillin, ceftiofur, ceftriaxone, etc.

a. Intrauterine antibiotic therapy: Oxytetracycline, levofloxacin + ornidazole/metronidazole suspension, etc.

b. Hormonal therapy: Cloprostenol (500 µg, I/M), Dinoprost (25 mg, I/M)

c. Combined hormonal + antibiotic therapy: Oxytetracycline (1500 mg, I/U) + Cloprostenol (500 µg, I/M)

d. Pre- and post-AI preventive measures: Penicillin G (10 lakh IU) in 20–30 ml DW, 5–6 hr pre-AI & 3–6 hr post-AI, Streptomycin-penicillin (1 g) in 20–30 ml DW, 5–6 hr pre-AI & 3–6 hr post-AI

e. Systemic antibiotics post-AI (timed for embryo protection): Administered on Day 4 (embryo entry into uterus) and Days 9–10 (zona hatching period).

Fig. 4. Amelioration of heat stress in buffaloes with cold water

f. Immunomodulators: Immunomodulators are substances that modify or regulate the immune system's activity. They can either enhance (immunostimulants) or suppress (immunosuppressants) immune responses, depending on the therapeutic need. Various immunomodulators, their effective dosage (Table 2).

Nutritional measures:

Proper nutritional management plays a critical role in supporting embryo development and survival. By adapting following strategies significantly reduce early embryonic mortality and enhance reproductive success in dairy can be achieved.

Fatty acid supplementation: Malnutrition significantly hinders fertility in bovines due to EM. Supplementation with omega-3 polyunsaturated fatty acids (n-3 PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish oil, has shown promise in reducing early pregnancy loss by lowering luteolytic $PGF_2\alpha$, enhancing P4, and improving uterine conditions. Fish oil supplementation in goat was found to improve estrus duration, follicular development, and conception rate (Mahla et al., 2017; Verma et al, 2018). It also reduced endometrial $PGF_2\alpha$ and altered prostaglandin gene expression (e.g., PTGS2, PGES), favouring implantation (Chaudhari et al., 2018). Furthermore, Teeli et al. (2019) showed that n-3 PUFAs increased P4 and ISG expression (ISG15, MX2) in peripheral blood leukocytes, indicating better embryo recognition, enhanced uterine receptivity and embryo signalling, thereby reducing EM in cow. These findings support n-3 PUFA supplementation as an effective strategy to improve reproductive success and reduce embryonic losses in bovines.

b. Glucogenic diets and insulin administration: Elevated insulin and glucose, promoting early postpartum ovarian activity and ovulation, especially in multiparous cows (Van Knegsel et al., 2007). Insulin administration (0.2 IU/kg BW on Days 8–10), followed by $PGF_2\alpha$ (0.75 mg on Day 12) and AI at 72–96 hrs, elevates P4 in pregnant cows, potentially through IGF-I-mediated enhancements in follicular and oocyte competence (Selvaraju et al., 2002). However, excess insulin may impair oocyte quality in over-conditioned cows. In contrast, lipogenic diets enhance estradiol synthesis and blastocyst development by supporting P4 precursor pathways (Leroy et al., 2017). A phased nutritional approach glucogenic feeding early postpartum followed by lipogenic pre-breeding optimizes ovarian function by increasing oocyte quality and so fertility (Ebdalabadi et al., 2014).

c. Others: Iodine supplementation, 8–12 days prior to estrus was found to enhance pituitary function and reduces incidence of RB (McDonald et al., 1962). Trace mineral deficiencies notably Cu, Se, Fe, Zn, and Mn impair fertility through oxidative stress, immunosuppression, and anaemia. β-Carotene supports CL function and P4 synthesis (Wang et al., 1988). Ragi feed supplementation was found to improve luteal activity, reduces blood urea nitrogen, and restores estrus expression (Gupta et al., 2008). Ascorbic acid and mineral mixtures support steroidogenesis and reproductive health, particularly via collagen biosynthesis and endocrine support.

Managemental strategies and AI techniques:

A minimum voluntary waiting period of 45 to 60 days post-calving is recommended to allow for uterine involution, metabolic recovery, and ovarian rebound, so that animal passes transition phase disorder like RFM, LDA, milk fever, ketosis, laminitis, metritis, and mastitis, thus improving first-service conception rates (Stangaferro et al., 2018; Rasmussen et al., 2024). Insemination until at least 8–13 hrs before ovulation during mid to late estrus or timed AI can improves fertility (Stangaferro et al., 2018). A crystoscope to check the fern pattern in field conditions can be used to perform AI at an accurate time based on the observation of the fern pattern in cervical mucous. Adjunct interventions such as clitoral massage or cold-water stimulation post-AI may improve uterine contractions and aid sperm transport. Additionally, oxytocin administration (30–50 IU I/M post-AI) in cows with poor uterine tone can promote sperm movement toward the oviduct, potentially enhancing conception success.

Preventive measures for environmental factors:

Studies reinforce the critical role of heat mitigation in preventing EM (Singh et al., 2016). To protect buffaloes and cattle from thermal stress, housing design and microclimate control are essential. For heat stress, shade with reflective roofing and dark-coloured floors can reduce radiant heat load, while twice-daily cold-water spray, good ventilation, and fans enhance evaporative and convective cooling, (Chikkagoudara et al., 2022). Recent studies show that water immersion or pond access, combined with shade, significantly improves behaviour and physiological comfort in buffaloes (Ximenes et al., 2024). Buffaloes are especially vulnerable due to their dark skin and poor sweating ability but cool rapidly with access to water or fans (Marai and Haeeb, 2010). Long-term adaptations through selective breeding for heat-tolerant traits also show promise in enhancing reproductive resilience (Gupta et al., 2024). For cold stress, shelters with warm bedding and heat-insulated walls reduces heat loss, during winter. Although buffaloes are more cold-tolerant than often assumed, protection from wind and sudden temperature drops is critical for health and productivity (Yáñez-Pizaña et al., 2020).

Treating anti-sperm antibodies:

Antisperm antibodies were neutralized using chymotrypsin and galactose. Pregnancy rates were significantly higher in the chymotrypsin/galactose-treated group (25%) compared to an albumin control (3%) (Fedele et al., 1994). Galactose neutralizes antibodies by binding to sperm surface recognition sites, while the protease chymotrypsin cleaves and removes bound immunoglobulins (Katsoff and Check, 1997).

Assisted reproductive technique:

In the past 15-year ET with respect to AI, significantly increased conception rate in repeat breeders (Nowicki, 2021). When ET is performed on Day 7 post-estrus, either alone or following AI, it improves IFNT expression and MRP (Yaginuma et al., 2019), which helps sustain luteal function and reduce early luteolysis. Co-transfer strategies (AI + ET) result in higher pregnancy rates (e.g., 46.9%) and enhanced IFNT-mediated gene expression but are also associated with increased rates of twinning (6.25–18.4%) and associated complications such as abortion and dystocia, (Funeshima et al., 2019). While Et alone can avoid twin pregnancies if managed properly, thus AI + ET protocols must balance improved conception with the risk of reproductive complications (López-Gatius et al., 2022).

Future prospects

Future research on EEM in bovines should prioritize precision biomarkers like ISGs, PAGs, and P4 profiling for earlier pregnancy failure detection. Genomic and transcriptomic technologies can identify genetic factors and gene expression linked to reproductive inefficiency, enabling targeted breeding and therapy. Immunomodulation, via cytokine regulation or INFT analogues, could enhance maternal immune tolerance. Nutritional genomics may optimize feeding strategies to improve endocrine balance and uterine receptivity. Climate-resilient strategies, including heat-tolerant genetics and better housing, are needed to counter environmental stress. Integrating real-time monitoring tools like biosensors and AI can improve insemination timing. Refining assisted reproductive technologies, such as embryo transfer, is also critical.

Conclusions

In conclusion, early embryonic mortality (EEM) is a critical cause of decreased fertility, prolonged calving intervals, and major economic losses in dairy production. While managerial factors must first be addressed, specific treatments can be applied once a cause is hypothesized. However, due to EEM's multifactorial nature, identifying a single cause is often impossible. A case-by-case approach remains the most practical strategy. These interventions aim not only to resolve conception failure but also to improve the uterine environment for implantation. Given its substantial economic impact, continued research is essential to improve conception rates and overall herd reproductive efficiency.

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