International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
NAAS Rating (2025): 4.61
VET 2025; 10(8): 106-108

www.veterinarypaper.com
Received: 27-05-2025
Accepted: 29-06-2025

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An insight study on ultrasonography-assisted diagnosis of embryonic loss and its management in crossbred cow

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DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i8b.2457

Abstract
Embryonic losses significantly impact reproductive efficiency in dairy animals. This study presents clinical observations of embryonic mortality in a *Vrindavani* crossbred cow diagnosed through sequential ultrasonography imaging. Findings revealed an amniotic vesicle devoid of an embryo, disintegrating embryonic membranes, and luteal regression followed by uterine fluid clearance and subsequent follicular development. The cow was successfully conceived after therapeutic intervention with intrauterine antibiotics and given a breeding of one cycle. Findings highlight the importance of ultrasonography in detecting embryonic losses, facilitating timely interventions to reduce pregnancy wastage, thereby reducing economic losses.

Keywords: Embryonic mortality, ultrasonography, cattle

Introduction
Embryonic losses contribute significantly to pregnancy wastage in dairy animals. These losses are categorized into early (18-24 days) and late embryonic loss (25-42 days post-artificial insemination), with maternal embryo signaling and implantation as the demarcating factor. The origin of pregnancy loss may be attributed to the embryo itself or the withdrawal of luteal support (Domingues *et al*., 2022) [1]. The causes of embryonic loss are diverse, encompassing infectious and non-infectious ones. Besides multiple risk factors, both maternal and non-maternal factors such as environment, parity, body condition score, and retained foetal membranes are associated with embryonic loss (Diskin *et al*., 2012) [2].
The majority of embryonic losses occur during the early embryonic period; detection of these losses needs monitoring of pregnancy-signalling molecules such as interferon-tau (INF-T) and pregnancy-associated glycoproteins (Domingues *et al*., 2022) [1]. In contrast, late embryonic and early fetal losses are often diagnosable and may be incidentally discovered through reproductive ultrasonography (Lokavya *et al*., 2020) [3].
Embryonic death after maternal recognition of pregnancy results in delayed luteal regression with clinical outcomes varying based on timing, underlying causes, and diagnostic methods. These losses typically manifest as delayed return to estrus, extended open days, decreased conception rates, and potential uterine infections (Madoz *et al*., 2013) [4]. All of these factors collectively contribute to reduced reproductive efficiency and farm economics.
The present study aimed to diagnose late embryonic mortality in a *Vrindavani* crossbred cow through sequential ultrasonographic imaging and its management using intrauterine antibiotic treatment and breeding rest.

Materials and Methods
A Vrindavani crossbred cow in its third parity, which had been inseminated 40 days earlier, was presented for pregnancy assessment at C&B Farm, Indian Veterinary Research Institute. The reproductive tract was examined using real-time B-mode ultrasonography (EXAGO™, USA) with a transrectal probe set to 7.5 MHz frequency and 8 cm depth.

After initial diagnosis of embryonic mortality, sequential examination was done at 48 hrs and 72 hrs later. Therapeutic management was initiated with an intrauterine antibiotic (Lenovo APTM -60 ml), and the animal was given a breeding cycle. The same has been inseminated at the subsequent estrus, and pregnancy was confirmed by ultrasonography on 35 days post AI.

Results and Discussion

At first examination, ultrasound imaging revealed an empty amniotic vesicle devoid of embryo (Fig. 1a), disintegrating embryonic membranes (Fig. 1b), and a regressing corpus luteum on the right ovary was evident (Fig. 1c). A follow-up assessment within 48 hours aimed to evaluate uterine and ovarian changes. The examination showed no remnants of embryonic membranes but displayed multiple anechoic fluid pockets across various uterine cross-sections (Fig. 2a & 2b) with minimal blood perfusion in corpus luteum (Fig. 2c). Third examination at 72 hours, revealed a uterus free of fluid residues (Fig. 3a). Ovarian examination revealed a development of dominant follicle seen on the left ovary (Fig. 3b). A positive effect of an intrauterine antibiotic and breeding rest for a cycle was recorded. The same has been demonstrated through the successful attainment of pregnancy as confirmed by ultrasonography on 35 days (Fig. 4).

Clinical observations and history of the cow presented in the current study are suggestive of having undergone late embryonic mortality. Incidence of late embryonic losses is reported between 4.7% to 29% depending on the method and duration window of detection (Rodriguez *et al*., 2019) [5]. Since reproductive efficiency is a function of both conception rate and pregnancy rate, pregnancy loss greatly affects later outcomes. Ultrasonography is the most practical method for the early detection of pregnant and non-pregnant animals. The present investigation demonstrated the absence of definitive signs of pregnancy, such as a developing embryo with a heartbeat in the amniotic vesicle. However, signs of embryonic losses such as detaching chorio-allantoic membranes, reduced placental fluids, and regressing luteal tissue are evident (Lopez-Gatius and Garcia-Ispierto, 2010) [6]. Multiple factors can trigger pregnancy loss, including sub-uterine function during the late embryonic period. Detecting these losses is challenging clinically and requires sequential ultrasonography, P4, or PAG estimation (Domingues *et al*., 2022) [1]. Doppler ultrasonography can provide valuable information on luteal activity during pregnancy diagnosis by detecting luteal blood flow (Lopez-Gatius and Garcia-Ispierto, 2010) [6]. The exact timeline of events surrounding the pregnancy loss remains largely unknown. Luteal regression has been observed to occur within 3 days of embryonic loss (Kastelic *et al*., 1991) [7], presenting a potential area for further research. In the present case, embryonic mortality had already occurred by the time of diagnosis, with luteal regression continuing for 72 hours, followed by the emergence of a dominant follicle on the contralateral ovary. Although impending embryonic losses cannot be treated, they can be effectively managed by employing early pregnancy diagnosis in bred animals, where such cases can be identified. Also, the most important intervention in such cases includes breeding rest that reduces the open day period, thereby by the losses associated with production and repeated inseminations.

Fig 1: Ultrasonographic findings depict uterine and ovarian changes in the cow undergoing embryonic mortality. a: Empty embryonic sac, 1b: Disintegrating embryonic membranes, and 1c: Regressing corpus luteum.

Fig 2: Follow-up assessment at 48 hr. later revealed reduced fluid pockets (Fig. 2a, 2b) & decreased blood perfusion to the corpus luteum is still evident (2c).

Fig 3: Depicts the uterus cleared from the remnants and fluids (Fig. 3a), and dominant follicle development on the right ovary (Fig. 3b) is evident 72 hours later

Fig 4: A developing embryo in the amniotic vesicle is at 35 days during Pregnancy. Diagnosis is evident at the subsequent AI after breeding rest of one cycle.

Conclusion

The above short report documents detailed insights into late embryonic loss in a *Vrindavani* crossbred cow by assessment of events post-embryonic loss using real-time ultrasonography and follow-up examinations. This study emphasizes the utility of ultrasonography for diagnosing and managing embryonic loss, thereby minimizing economic impacts associated with reduced conception rates, prolonged open days, and repeated inseminations.

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How to Cite This Article
Gangula ALR, Baghel MS, Sahu UK, Nivetha, Mahala P, Vadera V, *et al*. An insight study on ultrasonography-assisted diagnosis of embryonic loss and its management in crossbred cow. International Journal of Veterinary Sciences and Animal Husbandry. 2025; 10(8): 106-108.