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Review

Embryonic and Fetal Mortality in Dairy Cows: Incidence, Relevance, and Diagnosis Approach in Field Conditions

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Abstract: Pregnancy loss (PL) in dairy cattle results in animal health and welfare disruption and has a great economic impact on farms, with decreases in fertility and increased culling. It can occur at any stage of embryonic or fetal development. Abortion occurring from the second half of pregnancy has a more negative impact on dairy farms. There are several infectious and non-infectious factors that can lead to PL and vary according embryonic or fetal stages. As this is a multifactorial or multi-etiological occurrence, it is important to identify the risk factors and the best diagnostic tools to approach these reproductive losses that can occur sporadically or by outbreaks. Reaching a final diagnosis can be challenging, especially when it occurs at a very early stage of pregnancy, where losses may not be detected and neonatal deaths may be related to alterations in the fetus in utero. Also, laboratorial results from animal samples should be interpreted according to the full clinical approach. This review aimed to highlight all these essential aspects, identifying the main infectious and non-infectious causes leading to PL, as well as the best veterinary practices for diagnosing it, mainly through transrectal palpation, ultrasound, and laboratory methods, in bovine dairy farms.

Keywords: abortion; dairy cattle; pregnancy loss; herd health management

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1. Introduction

Lactation is triggered by calving, which should occur every 360–400 days [1] to maximize milk yield during the productive lives of high-producing dairy cows [2,3]. Pregnancy loss (PL) is one of the major constraints in sustainability dairy farms, causing relevant impacts on animal health, welfare, and production. At an economic level, PL primarily represents a delay in calving interval, causing a suboptimization of milk yield production, cost of treatments, and new breeding and culling [4,5].

In field conditions, at animal and farm levels, the diagnosis and monitoring approach of PL is always a challenge for veterinarians due to the large number of causes, risk factors, and time occurrences of PL, as well as available technology and methodologies, including laboratorial support. PL can surge sporadically or by outbreaks, with or without a specific cause and even in an inapparent (silent) form. About half of the samples from abortion analyzed in laboratory remain inconclusive [6]. This highlights the role of the veterinarian on the farm to manage this reproductive issue.

This work aims to describe the importance of PL in bovine dairy farms and make a critical review mainly of its diagnosis approach in field conditions.

2. Embryonic and Fetal Mortality Definitions

In cattle, the pregnancy length is approximately 280 days with possible variations between 270 and 292 [7]. The "Committee on Bovine Reproductive Nomenclature" (1972) [8] defined the embryonic period as the first 42 days after fertilization, from conception to the end of embryo differentiation. Thus, any reproductive failure that occurs in this period is defined as embryonic mortality (EM) which, in turn, can also be subdivided into early (EEM) or late (LEM). This subdivision is established taking into account maternal recognition [9] and possible changes in the interval between estrus [10,11] (Figure 1).

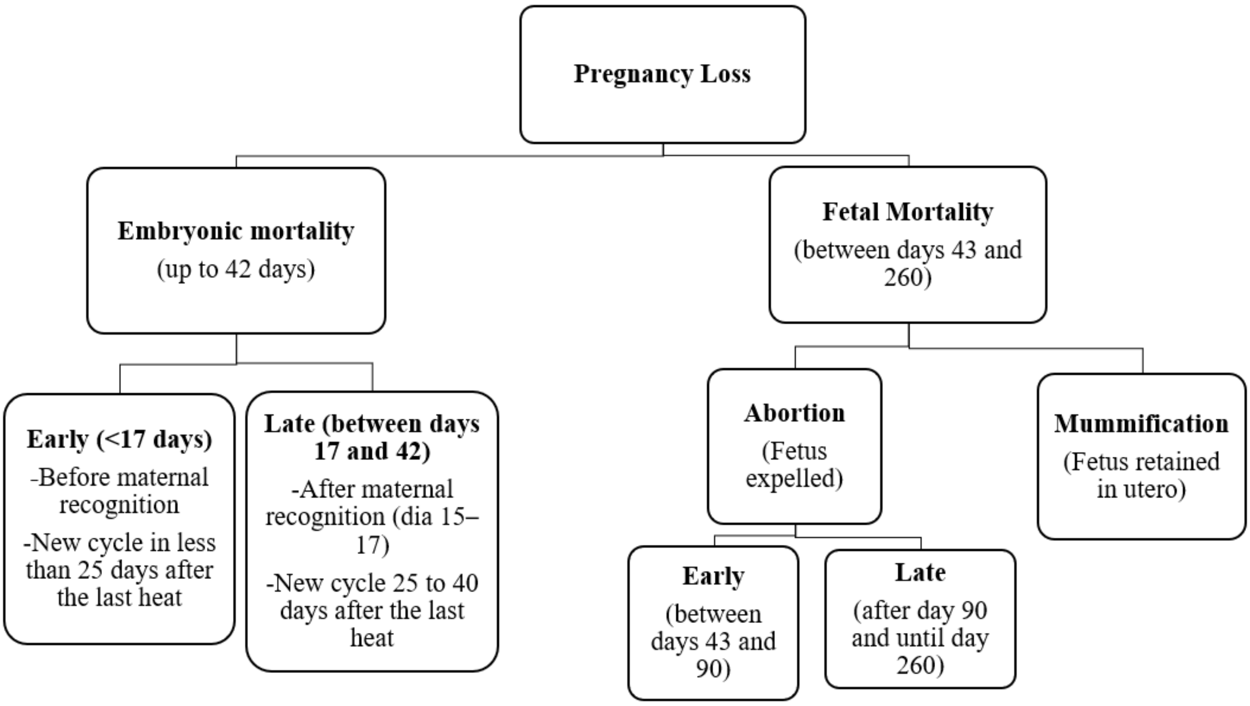

Figure 1. Representation of the different stages in the gestational interruption. Data adapted from [9–12].

EEM corresponds to the loss of an embryo that is not influenced by maternal recognition (between days 15 and 17 of pregnancy). In this case, the cow does not suffer any change in estrous cycle length, presenting a new estrus less than 25 days after its last one [9,13]. In LEM, the embryo is influenced by maternal recognition but does not progress beyond 42 days of gestation, leading to a later delay in luteolysis that consequently leads to a delayed return of estrus [9,14].

Fetal mortality (FM), commonly referred to as abortion, occurs between 43 days and 260 days of gestation, with the expulsion of a non-viable fetus [15]. The mummification process develops when, in this same time frame, there is a (aseptic) fetal death without expulsion and persistence of the corpus luteum (CL). It usually occurs after the second trimester of pregnancy, when the reabsorption of fetal bones and tissues is no longer possible, with a closed cervix remaining and no signs of systemic infection [16]. In the case of opening of the cervix, the entry of microorganisms can lead to fetal maceration and clinical symptoms in the mother, such as signs of metritis, fever, and decreased body condition [16]. One major difficulty with detecting EM is due to the fact that embryos are absorbed or expelled without being identified. In FM, the tissues are not easily absorbed and, when expelled, they are detected, excluding cases of mummification [16].

The end of pregnancy can occur at any stage. However, even with different results, several studies show that the phase with the greatest evidence of pregnancy loss (PL) is the early embryonic phase [17]. In cattle with multiple pregnancies, EEM may present as a normal process of elimination of inappropriate genotypes in each generation [10].

Progesterone (P4) is one of the main hormones that maintain pregnancy, and its production is carried out by CL [18]. Cows that produce higher amounts of milk tend to have higher embryonic mortality rates when compared to cows whose production is lower or in heifers. The high hepatic metabolism in these animals leads to an increased clearance of P4, causing less of this hormone in circulation [19].

3. Incidence of Embryonic and Fetal Mortality

The accurate estimation of EM and FM incidence in dairy farms is a challenge. This difficulty is mainly related to the under-detection or under-reporting of these events, especially in the early stages of pregnancy, as the detection of these losses is not always accessible. The lack of producers' knowledge, their perception to consider this process as "normal", or them not being aware that embryonic and/or fetal deaths on their farm can be high, leads to negligence and failure to register [6,20].

Cost–benefit is an obstacle to the search for causes for some producers, also leading to under-registration. Several studies have compared this same lack of notification, where only 38% of producers in Canada [21], 39% in France [22], and 5.5% of dairy herds in New Zealand [23] investigate the cause of the problem.

A zero incidence of PL is practically impossible, especially if we are dealing with a population of large animals. Thus, although there are different values when taking into account the country, region, and type of farm, "normal/acceptable" values of PL rates can be established [15]. The so-called "normal" abortion rates present, in most cases, lower values than the real ones. While in the "normal" rates only the observed abortions are considered, in the real rates the initial PLs are included, which are often not accounted for [15]. In practical terms, although there is disagreement among authors, detections of 3% EM and FM between 1 and 2% until the peri-partum period can be considered acceptable (thresholds) [24–26]. Recent studies have reported that, for further deep research, the PL can reach 6% in heifers and more than 3% in adult beef cows in Canada [27] and 10% in the United States of America (USA) [28]. In Europe (Ireland and UK), the values are lower with incidences between 1 and 4% on dairy and beef farms [29].

The first third of pregnancy is a critical period, where there is the highest incidence of PL [30,31], with EM being the most common form, especially at an early stage [10]. As pregnancy progresses, the miscarriage rate tends to decrease [32]. Whitlock and Maxwell [9] indicated overall values between 35% and 40% of EEM; 20 to 50% of EEM can occur between days 5 and 8 of pregnancy (passage from morula to blastocyst) [33]. About 30% of EM between days 8 and 25 was also reported [34–36]. According to Pohler et al. [37], LEM can present an incidence between 5% and 10%. After 42 days of gestation, there is a decrease in pregnancy interruption with an incidence between 2.5% and 4.2% [9] that can reach 10% [38]. A meta-analysis study considering PL up to 90 days of pregnancy reported incidences of 27% EEM, 13% LEM, 7% early FM (EFM), and 2% late FM (LFM) in dairy cows [39]. In another meta-analysis involving beef cows, 28.4%, 3.9%, and 15.6% of EEM were observed by day 7, between days 7 and 16, and between days 16 and 32 after conception, respectively; only 5.8% of PL occurred between days 32 and 100 (LEM/EFM) [40]. These authors considered EEM and LEM up to days 28 and 45 of pregnancy, respectively.

4. Impact of Embryonic and Fetal Mortality on Dairy Farms

Productive and reproductive performance are forms of subsistence and profit on dairy farms [41]. The rate of profit on a farm should take into account milk production per cow/day, voluntary culling (e.g., taking into account herd size, milk production, or age), involuntary culling (e.g., due to illness, injury, or infertility), and also the costs of rearing and the rate of genetic progress [42].

A new pregnancy brings profit and a source of income to the farm, and this value can be defined as the difference between the money that comes in (through the production of milk and/or sale of the calf) and the expenses in the management and maintenance of a non-pregnant animal [43]. By the early/middle 2000s, Eicker and Fetrow [44] estimated that it takes an average of about USD 200 to start a new pregnancy, while Stevenson [45] and De Vries [43] presented values that can reach USD 275. Losses during pregnancy are one of the main causes of decreased fertility [46], and miscarriages in the last third of a pregnancy have a greater economic impact.

Predicting the economic impact presents several barriers, and it is not always easy to reach a conclusion. This is because EM and FM, especially when they occur at a very early stage of pregnancy, may not be detected, and neonatal fetal deaths may be related to in utero changes [25]. All cases must be analyzed, as not all animals that have presented abortion should go to waste [47], and there are several scenarios that can occur in the affected cow, namely (1) early slaughter, (2) permanence on the farm for having started a new lactation (even if it does not reach maximum production), or (3) its permanence by making new AI attempts until obtaining a new pregnancy [48].

Miscarriages in the last trimester of pregnancy have a higher incidence of endometritis (23.2%), prolonged uterine involution, a longer interval between PL and new insemination, and a greater predisposition to placental retention [43,49,50].

In addition to the changes previously mentioned, the occurrence of EM/FM leads to (1) changes in milk production [51,52]; (2) increases in the incidence of uterine infections [52]; (3) increases in the number of open days [53]; (4) decreases in the profit from the loss of the calf, and; (5) increases in the cost of acquiring new animals [54], as well as medical/veterinary costs [24] and the number of AI's to be performed until a new pregnancy is reached [52,55].

At the economic level, values may vary depending on the country, region, and race. It is estimated (2000s) that a midterm abortion can present losses between USD 600 and 1000 per case [24], and in the USA the values are around USD 555 [43]. According to studies carried out by Livingstone and Longbottom [56], in Europe, economic losses can reach EUR 40,000/year on a farm with about 60 adult cows and 20 heifers, and in the United Kingdom (UK) these values can reach EUR 250 million per year. More recent studies (around 2020) carried out by Cantón et al. [57] point to losses in the order of USD 1415/cow. Albuja et al. [48] confirmed what other previous studies had already shown: the later the abortion, the more costs are associated. In this study, between 45 and 90 days the losses are around USD 315/cow, between days 91 and 180 it is USD 580/cow, and after 180 days until the end of gestation it is around values that can reach USD 1320/cow.

5. Causes of Pregnancy Loss

5.1. Infectious Causes

Infectious causes can include the following: viral, bacterial, fungal, and parasitic. The transmission of microorganisms and clinical findings vary depending on the etiologic agent [10].

A study carried out in the USA in 1993 over 10 years in about 8962 cattle demonstrated the difficulty in reaching a diagnosis, since in 67.7% of cases it was not possible to determine a final diagnosis. In the remaining cases, 30.38% corresponded to infectious causes and 2.44% to non-infectious causes. Among the infectious causes, bacterial agents (14.49%) are more prominent, followed by viruses (10.57%) and fungi (5.31%). The most prevalent viral agents with values of 5.31% and 4.54% are, respectively, Bovine Herpesvirus type 1 (BHV-1) and Bovine Viral Diarrhea Virus (BVDv) [58]. The predominant bacteria were the following: *Trueperella pyogenes* (formerly known as Actinomyces pyogenes) (4.22%); *Bacillus* spp. (3.58%); *Listeria* spp. (1.35%); *Escherichia coli* (1.09%); and *Leptospira interrogans* (0.88%) [59].

More recent studies, carried out between 2010 and 2023 at an international level (15 countries), summarized in the article by Mee [32], present the most common infectious causes of PL. In this review, on average, approximately 42% of abortions are of infectious origin, with *Neospora caninum* being the most common cause, followed by *T. pyogenes*, BVDv, and some fungi. However, other agents such as BHV-1, *Coxiella burnetti*, *Chlamydia* spp., and *Leptospira* spp. continue to have a high prevalence, as does *Brucella abortus*, in countries where the disease is not eradicated [32].

The classification of these microorganisms is carried out taking into account their primary (primary bacteria) or secondary (secondary or opportunistic bacteria) effects on the reproductive system [60].

Opportunistic bacteria are mostly found either in the environment (*Bacillus* spp., *Pseudomonas* spp., *E. coli*, among others) or in the normal microflora present on the mucosal surfaces of the bovine species (*Arcanobacterium pyogenes*, *Pasteurella multocida*, *Mannheimia hemolytica*, *Haemophilus somnus*, *E. coli*, *Campylobacter* spp., *Staphylococcus* spp., and *Streptococcus* spp., among others) [59,61–63]. Taking into account several retrospective studies, up to 25% of fetal deaths in cattle can be caused by secondary bacteria [62,64,65]. Occasionally, through maternal blood, these ubiquitarian agents can culminate in problems such as placentitis and pregnancy interruption [60]. Opportunistic bacteria usually do not have contagious or transmissible potential [66]. The identification of more than one opportunistic bacterium in situations of abortive outbreaks or in continuous reproductive problems may indicate that there is a greater ease of entry of these microorganisms into the bloodstream and/or the existence of adjacent diseases. Factors such as subclinical or clinical acidosis, poor quality diet, or skin lesions potentiate the entry/dissemination of this type of bacteria [66].

Of the fungi known to have abortive capacity, *Aspergillus fumigatus* is the most common [66]. However, other fungi are often isolated, such as *Absidia* spp., *Rhizopus* spp., *Mucor* spp., *Candida* spp., and *Aspergillus* spp. [54]. Abortions of mycotic etiology are usually due to ubiquitous saprophytic fungi, often found in soil, hay, and silage of poor quality [67]. Placental and fetal infection occurs by the hematogenous route through the entry of the agent through the respiratory or gastrointestinal route. Most miscarriages occur between the sixth and eighth gestation [68,69]. However, a mycotic infection does not necessarily imply the death of the fetus, and live animal births may occur [54].

5.2. Non-Infectious Causes

Causes of non-infectious origin can be classified as genetic, toxic, nutritional, endocrine, and physical. Those that have a non-infectious origin external to the animal are described as stress agents [32].

In a review article by Yadav et al. [70], 10 to 60% of pregnancy interruptions were reported as non-infectious in nature. It should be mentioned that this review was based on studies carried out in economic and geographical realities and with different methodologies, hence the presence of such discrepant values. In older studies, carried out with a larger number of samples and large periods, values between 2% (8962 abortions and stillbirths examined) and 5% (2544 abortions and stillbirths examined) of non-infectious abortions were reported. In these studies, about 65% of the cases observed were attributed to an undetermined cause of abortion [58,71].

5.2.1. Endocrine Causes

Involuntary termination of pregnancy in cattle can result from the administration or abnormal endogenous concentrations of certain hormones [32].

Theoretically, any factor that causes placental dysfunction or decreased concentration of circulating P4 can cause PL [72]. Despite having a significant impact on "commercial" herds, diagnosis of hormonal imbalances is not feasible; therefore, their prevalence is unknown [32]. P4 is an essential hormone for the maintenance of pregnancy depending on the continuity of its serum levels [18]. This hormone is mainly produced by CL but also, in more advanced stages, by the placenta [73].

Elevated metabolism in intensive production dairy cows causes lower levels of circulating P4 (increased clearance) [19]. In addition, increases in cortisol levels consequently lead to the release of estrogens and PGF2α (prostaglandin F2α) by the placenta, resulting in luteolysis and decreased P4 [74,75]. Therefore, in bovine species, a circulating P4 deficit contributes to the occurrence of EMs. In order to avoid these situations, P4 (progestogens) can be administered exogenously as long as the cervix is closed, and there is the presence of a live fetus to avoid situations of mummification, maceration, or late delivery of dead fetuses [76].

Accidental administration of hormones, such as PGF2α, glucocorticoids, estrogens, and oxytocin, has the ability to induce an iatrogenic termination of pregnancy [70,77–81].

Up to 150 days of gestation, the isolated administration of PGF2α induces EM/FM because P4 is secreted only by CL, causing luteolysis. The same does not occur after this period, as P4 is also produced by the placenta and can be necessary to combine it with dexamethasone for miscarriage to occur [70,76].

In livestock, stress factors induce high levels of blood glucocorticoids and consequently a higher probability of embryonic and fetal death [82].

In some countries, oxytocin is administered with the intention of letting the milk down. However, according to Murtaza et al. [83], its application negatively affects reproductive performance in dairy herds, and Abdullah et al. [84] reported PL at the beginning of pregnancy in about 50% of the animals injected with oxytocin.

5.2.2. Physical Causes

Usually, farmers attribute physical causes of PL in situations of falls or conflict between animals [32]. However, any physical factor (e.g., a cow's trauma or hyperthermia), which affects the integrity of the amniotic membrane and damages the fetal heart or fetal blood vessels, can cause miscarriage [85,86].

Re-inseminations or intrauterine lavages between 6 and 70 days of pregnancy and gross transrectal palpation can also result in losses, although their prevalence is unknown [32,82].

In general, stress has an effect on the activation of the hypothalamic–pituitary–adrenal axis, with the release of ACTH (corticotropin-releasing hormone) and corticosteroids. Heat stress leading to a state of hyperthermia in the mother affects reproductive performance, through hormonal imbalance, decreased oocyte quality, poor semen quality, and decreased embryo development and survival [70,87,88]. Hyperthermia situations trigger fetal stress due to hypotension, hypoxia, and fetal acidosis, with the development of the steroid response necessary for delivery [25,89]. This negative effect is evident mainly in the first two weeks of gestation, as there is an alteration in protein synthesis, oxidative cell damage, and reduced maternal recognition [90,91]. Heat stress in addition to EM can also lead to abortions and stillbirths, particularly in Holstein-Friesland cows [52].

Multiple pregnancies have a higher rate of miscarriage, especially if both embryos are in the same uterine horn [88,92]. Even if miscarriages do not occur, there is a greater possibility of dystocic cases that can lead to stillbirths [79]. Cases of multiple ovulations are associated with high milk production rates [32].

Excessive twisting or winding of the umbilical cord around the ends of the fetus can disrupt blood flow at the umbilical level. These situations are rare; however, they can cause abortion [70].

5.2.3. Genetic Causes

Genetic mutations, chromosomal abnormalities, and genetic and polygenic defects, among others, are associated with PL [70], and genetic predisposition to abortion is due to the amplification of AI, situations of inbreeding, and high selection rates [32,93]. The concentration of lethal genes due to inbreeding is probably one of the reasons behind EM, FM, and stillbirths [85].

Birth defects are often dismissed as "accidents" occurring randomly. Delay in detecting hereditary defects or failure to identify allows for a greater distribution of deleterious alleles [94].

The actual incidence of abortion in cattle due to genetic factors is unknown. While in some cases birth defects are visible, in others they may not have phenotypically recognizable lesions [95]. According to Ghanem et al. [96], mummified fetuses are often carriers of mutant alleles. Additionally, gamete-level mismatch plays a role in reproductive failures in cattle [32]. Few studies are available on the topic of genetic/chromosomal defects that lead to bovine abortion, and there is a need to develop more advanced tests to identify and verify the incidence of these problems [70]. Phenotypic anomalies of 3 to 7% were found in 2544 abortions and stillbirths [71], 9.2% in 931 aborted fetuses [97], and 1.7% in 595 abortions [98].

5.2.4. Nutritional Causes

Nutritional deficits in selenium, copper, zinc, iodine, and vitamin A are related to pregnancy interruption and decreased fertility [70]. Because complex processes are involved in the exchange of nutrients in the fetus–maternal axis, it is not always easy to determine what nutritional cause, if any, causes miscarriage [82].

Insufficient levels of iodine are usually due to its absence in the mother's diet but may be secondary to a high intake of calcium and/or plants belonging to the genus *Brassica*. In these cases, in stillbirths or weak calves, an increase in thyroid size is visible [86].

Vitamin A or its precursor (beta-carotene) plays a role in maintaining the integrity of the epithelia and mucous membranes, and its deficiency causes degenerative changes in these tissues. For this reason, an increase in the prevalence of abortions has also been observed in farms whose diet is precarious in this compound [86,99], and calves born to cows nutritionally deficient in vitamin A are commonly weak and blind [24]. Amino acids also play an important role in maintaining pregnancy. For example, methionine supplementation (rumen-protected) reduces PL between days 28 and 61, according to Toledo et al. [100]. Recently, it has been proposed that the risk of abortion associated with nutrition could be used to create a metabolic profile to monitor cows with a history of abortion [101].

5.2.5. Toxic Causes

Any substance that interferes with normal biological processes and causes adverse effects upon contact by ingestion, inhalation, and skin is considered a toxin [102]. Toxins with harmful reproductive potential are capable of causing infertility, subfertility, EM, teratogenic effects, and stillbirths [103].

The main poisonings in cattle are due to the ingestion of phytotoxins present in plants (such as *Conium maculatum*, *Oxytropis* and *Astragalus*, *Pinus ponderosa*, *P. contorta* and *Juniper communis*, *Gutierrezia microcephala* and *G. sarothrae*), the consumption of nitrates/nitrites in plants that accumulate them and in fertilizers, and also the ingestion of feed contaminated with mycotoxins [86,104].

Ruminants are resistant to the effects of mycotoxins (secondary metabolites of fungi) due to rumen microorganisms that allow effective degradation and deactivation and binding to toxic molecules [105,106]. However, high concentrations of mycotoxins in the diet may play an abortive role [70]. The incidence is unknown because testing is not routinely carried out [32].

The bovine species is particularly sensitive to nitrate poisoning [70]. Poisoning mainly involves ingesting water contaminated with nitrate/nitrite and using fertilizers with the same compound directly on forage or hay grown in areas close to the animals. Consumption of plants that accumulate nitrate in low amounts leads to birth defects [107]. Plants of the genera *Oxytropis* and *Astragalus* have a major negative economic impact on the bovine reproductive sector. Continued intake between 4 and 6 weeks is responsible for losses at any stage of gestation and teratogenesis [104].

When consumed in the last trimester of pregnancy, species such as *P. ponderosa*, *P. contorta*, and *J. communis*, also known as "pine needles", can lead to the occurrence of miscarriages [108]. The acid present in these plants (isocupressic acid) is responsible for the severe constriction of blood circulation to the pregnant uterine horn, causing hypoxia and fetal stress [109].

5.2.6. Risk Factors

There are numerous risk factors that can influence the normal continuation of pregnancy (Table 1). Someone's trauma, genetic factors, and fetus–maternal incompatibility, among other factors [16], can also be direct causes of PL.

Table 1. Risk factors of pregnancy losses in dairy cows.

Risk Factor	References
Loss of body condition score in the post-partum period	[110]
High milk production, especially in Holstein-Friesian cows	[31,111]
Reduced period between calving and re-insemination	[87,112]
Cohabitation with other species such as dogs (risk of infection by *Neospora caninum*)	[113]
Negative energy balance	[16]
Heat stress	[87,88]
Corrective hoof trimming in the last month of gestation	[92]
Mastitis and high somatic cell count	[30,110]
History of dystocia, retained placenta, and recurrent uterine infections	[110,114]
History of miscarriages	[87,115]
Multiple pregnancies	[88,92]
Lack of access to running water	[6]
Absence of vaccination	[6,115]
Inadequate progesterone levels	[16]
Aged oocytes from persistent follicles	[16]

6. Approaching a Diagnosis of Pregnancy Losses in Bovine Dairy Farms

For a long time, the main method of determining a pregnancy was transrectal palpation, which is still used today. The position and flexibility of the cow's reproductive organs allow a trained professional, through digital rectal examination, to detect changes that confirm or rule out a pregnancy [116]. The presence of embryonic vesicle(s), palpation of placentomas, positive slippage of membranes (slipping of chorio-allantoic membranes) from 35 to 40 days of gestation, and, in more advanced stages, palpation of the fetus are fundamental indicators for the diagnosis of pregnancy through transrectal palpation [117]. As a rule, the pregnancy diagnosis should be performed after 30 days after AI [117]. There are several studies that show that physical manipulation at the level of the embryonic vesicle especially before 30 days can induce iatrogenic lesions, resulting in EM [118,119]. Nonetheless, it was observed by Romano et al. [120] that transrectal palpation of the amniotic sac performed between days 34 and 45 after breeding does not induce PL or fetal abnormalities.

The use of ultrasound methods emerged later in the 1980s [121]. Its use, also transrectally, allows the evaluation of the morphological characteristics of the embryo/fetus (shape, size, sex, echogenicity, and heartbeat) and uterine alterations in a fast, practical, and accurate way, providing indicators of embryonic/fetal viability [122]. A positive diagnosis by this diagnostic means must be based on the presence of an embryonic vesicle with a heartbeat (present from 21 days onwards [123]), the existence of intact embryonic membranes, and the normal amount of chorio-allantoic fluid present in the uterine horn ipsilateral to the ovary in which the CL is located [124–126]. The presence of an embryonic vesicle allows a positive pregnancy diagnosis to be made but not multiple (twin). In this way, performing a scan of both uterine horns is essential to detect the presence of twin pregnancies. This type of pregnancy can have some consequences, such as freemartin, a greater probability of embryo/fetal loss (of one or both), and dystocic deliveries [127,128]. It is also important to distinguish unilateral (twins in the same uterine horn) or bilateral (one fetus in each uterine horn) twinning. Unilateral twinning seems to be a relevant non-infectious risk factor of PL during mid-to-late pregnancy of dairy cows [129,130].

There is a high probability of early termination of pregnancy in the absence of embryonic heartbeat or presence of weak heartbeat, alterations in the embryonic membranes, a reduced amount of vesicular fluid, or the presence of echogenic floating debris [131].

A second pregnancy diagnosis can be performed between 50 and 60 days of gestation as a way of evaluating fetal anomalies and heartbeat and fetal sexing [12].

The ultrasound Doppler association, starting 20 days after AI, allows for the visualization of the blood flow present in the LC. There is a direct relationship between decreased CL blood flow and circulating progesterone levels [132–134]. Compared to transrectal palpation, ultrasound has advantages in terms of being an earlier, less invasive method with a lower number of false positives [135].

The collection of information from both the affected cow and the herd to determine the cause of an abortion is extremely important, even if it may be an isolated case. It should be noted that cases of sporadic miscarriage can lead to outbreaks [16]. Systematic research can avoid high economic losses on the farm, allowing the producer/veterinarian to be determined and directed to the most likely causes (infectious or non-infectious origin).

Most of the tissues analyzed come from fetuses in more advanced pregnancy stages, because at an early stage it is not possible to access samples [16]. In herds whose management is weak, agents, even with low virulence, can be the problem, with the possibility of the presence of more than one microorganism/cause involved [16,57]. Vaginal/cervical secretions should be collected from the mother for microbiological evaluation. The blood samples should be collected from the affected animal, those that have recently presented PL, and from unaffected cows (samples from 10% of the herd with a minimum of 10 animals), thus making the serological sample more meaningful [16,24]. Also, the introduction of APM (abortions and perinatal mortalities) monitoring programs, based on free on-farm sample collection and an extensive pathogen screening panel [136], can improve the success of reproductive herd health management in dairy farms.

The visualization of macroscopic lesions and the collection of samples for laboratory analysis during the necropsy procedure allow additional information to be obtained or a presumptive/final diagnosis to be established. From the fetus, samples of brain, skeletal muscle, thyroid, skin, ileum, adrenal gland, lung, spleen, liver, kidney, ocular fluid, abomasal contents, heart, and fetal membranes can be collected, directing laboratory tests to suspicious etiologies [16].

The set of macroscopic lesions, microscopy, and isolation with moderate to intense pure bacterial growth and other laboratorial analytic methodologies are essential for establishing a diagnosis. The tissues that are usually required for analysis are the placenta, fetal abomasal content, lung, and liver. Even though there is a strong possibility that placental culture media are contaminated by the environment and vaginal fluids, it is imperative to analyze them, as the bacterial agent may be solely confined to this tissue [66]. At the macroscopic level, a calf that has died in utero may present hepatic (pale and soft liver) and renal (friable kidneys in various states of autolysis) lesions, fluids in the body cavities, and edematous tissues of reddish color. At the cutaneous level, raised plaques adhering to the skin, especially in the periocular region, may indicate a possible fungal cause [16]. As an example, findings such as placentitis and fibrin involving the viscera are frequently detected, and, in cases caused by *Bacillus* spp., it affects the heart with epicarditis [66]. Signs of suppurative and/or fetal fibrinous bronchopneumonia, placentitis with signs of overactive necrosis, and multifocal hepatic necrosis are microscopic lesions most frequently detected in these infections [137].

Even through the correct performance of all procedures, it is not always possible, at the laboratory level, to reach a diagnosis. The collection of samples from fetuses that are in a high state of autolysis and contamination makes the process of identifying the cause difficult [16]. At laboratory, serological and histological methods, smears, and PCR (polymerase chain reaction) are some of the routine methods used to identify microorganisms, toxins, antibodies, and other biomarkers. The analysis of the results must be carried out carefully. For example, a positive serological result only indicates that the animal has already been in contact with a certain agent and may not be the cause of the abortion [16]. Laboratory results need be interpreted regarding herd health management programs, epidemiology of the farm and region, and the clinical approach to individuals and animal groups.

7. Conclusions

The main causes of pregnancy loss have an infectious component (viral, bacterial, parasitic, and mycotic). However, it is still a multifactorial problem where, in addition to infectious causes which may be present in isolation, there are also non-infectious factors (genetic, hormonal, metabolic, or developmental).

It is the veterinarian's responsibility, when carrying out reproductive examinations, to detect any changes that may indicate early abortion by rectal palpation and/or ultrasound. Nonetheless, it is not always easy to make a definitive diagnosis or to determine the cause of embryonic/fetal death. Often, animal samples subjected to laboratory tests are negative or positive for several agents, making it difficult to determine which is the primary cause of abortion. The presence of several microorganisms can pose a greater risk to the survival of the embryo and fetus due to synergistic effects.

By principle, the larger the herd, the greater the risk of miscarriage. There is a concept known as reproductive compliance, i.e., implementing the right reproductive program in order to accurately detect heat and inseminate the right animal at the right time. The same concept can be applied in different ways to the management of embryonic and fetal deaths, i.e., detecting a cow that has aborted as early as possible, effectively identifying the cause and preventing the occurrence, in order to decrease losses.

It is extremely important to properly manage animals that have aborted and to understand whether it is feasible to re-inseminate them until they become pregnant again and how many of these are acceptable and economically viable or whether it is preferable to scrap these animals.

It would be beneficial to carry out laboratory analyses on aborted animals more routinely and in larger numbers. However, the financial factors involved in carrying out such tests and researching the causes can be a barrier. Often, the high cost of laboratory analysis to carry out a diagnostic plan makes it unfeasible.

In short, reproductive medicine must always look at the whole picture, as an isolated case can very easily trigger a miscarriage outbreak. It is therefore equally important to adopt a multifactorial approach, using a series of measures that can solve or minimize the problem. Therefore, the benefits of regular reproductive monitoring of the farm should be made known to the owner, as it will allow a better assessment of the impact of this type of problem, thus achieving greater profitability for the farm.

For proper regular reproductive monitoring, it is advisable to: (1) conduct at least three reproductive assessments during gestation (via transrectal palpation and/or ultrasound); (2) Even in isolated cases of abortions, fluids and/or samples of aborted tissues should always be sent for laboratory analysis, and the aborted cows should be kept apart from the other cows in the herd to prevent potential dissemination of pathogens; (3) conduct routine laboratory testing on a randomized selection of animals in order to early confirm and identify suspected causes of PL, and; (4) consistently evaluate the reproductive performance (reproductive indices) of the dairy the herd.

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