Causes of Pregnancy Loss: From the Laboratory to the Clinic

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Early pregnancy loss (EPL) conceptuses found to be aneuploid (loss or gain of whole chromosome) are highly likely to be nonviable. Given this condition is commonly associated with EPL, clinically treating compromised pregnancies with progesterone or progestins should be done with caution to avoid a delay in rebreeding. Authors' addresses: Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, NY 14850 (de Mestre) and Department of Pathobiology and Population Sciences, Royal Veterinary College, North Mymms, UK (Lawson); e-mail: amm43@cornell.edu. *Corresponding and presenting author.

1. Introduction

Pregnancy loss arises due to a pathology that can originate in the mare, the stallion, the embryo/feto-placental unit itself or due to an external insult (for example, a pathogen or teratogen) that impacts one of these three entities. Therefore, pregnancy loss is an outcome, not a diagnosis, and can be very frustrating for veterinarians to manage. One needs to either preemptively diagnose the pathology prior to the loss and then initiate preventative treatment(s) or retrospectively diagnose the causative pathology to accurately inform the management of future pregnancies. Establishment of the underlying cause of pregnancy loss is often not achieved, resulting in a reliance on more generic approaches to loss prevention such as supplemental progesterone/progestins. This review will discuss the causes of equine pregnancy loss, focusing on non-infectious causes and how the veterinarian can use aspects of the recent literature to inform clinical management of the mare and communicate and manage client expectations.

2. What Incidence of Pregnancy Loss Should Be Expected?

Equine pregnancies are most likely to fail in the first two months of gestation corresponding to the period of early embryogenesis, placental development, and organogenesis. Studies of research mares estimate that approximately 20% of pregnancies are lost prior to clinical detection,[1] observed by veterinarians as mares that fail to conceive. Epidemiological studies have quantified early pregnancy loss (EPL) after clinical detection in the US and abroad, consistently reporting that 5-15% of confirmed day 14-16 pregnancies are lost prior to 42 to 65 days.[2–5] Variability in loss rates can be attributed to geographical variation, breed, management, and inherent characteristics of each cohort. Specific examples of mare, pregnancy, stallion, and external risk factors are reviewed elsewhere.[6] Breeding method has been found to have a significant impact on EPL, with

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natural mating, artificial insemination, and artificial insemination combined with embryo flush and transfer all having a similar and lower incidence of early pregnancy loss (4.5–6%) when compared with pregnancies established using cryopreserved in vitro generated embryos (13%).[7]

The incidence of pregnancy loss beyond the first two months is also geographically variable and ranges between 5 and 15%.[2,3,8] Risk factors for pregnancy loss beyond day 70 are distinct from those associated with early pregnancy. The greatest risk factor for abortion is the mare having two or more previous abortions, but surprisingly, not mare age or status.[9] These results concur with other studies that show advancing maternal age is an important factor for embryo mortality in early gestation[10] but more likely impacts fetal morbidity (not mortality) in mid to late gestation.[11]

3. Causes of Pregnancy Loss

The underlying causes of pregnancy loss are heterogenous in nature. Ultimately most cases will be attributed to a primary pathology of the mare (such as endometrial pathology, endocrine function, immunopathology, and oocyte characteristics) or the embryo/feto-placental unit (inherited via the germline from the stallion or mare or acquired during development). In both cases, defects could be intrinsic to the tissue or alternatively the response of that tissue to environmental exposures such as pathogens and contaminants. While individual stallions can have a higher incidence of early pregnancy loss, several multivariable studies have shown, at the cohort level, the stallion contribution to variability in risk is not significant or low.[4,10,12]

4. Pregnancy Loss Prior to 70 Days

Genetic variants in equine pregnancies are more commonly associated with pregnancy loss in the first 70 days of gestation. Broadly, there are three main mechanisms by which genetic variants can impact the outcome of a pregnancy. The genetic characteristics of the mare can influence the environment she provides for the developing conceptus, the embryo can inherit lethal genetic variants via the germline (mare and stallion), or the embryo can acquire lethal variants in the early stages of development. Clinically, if a genetic cause of pregnancy loss is suspected, understanding these different types of genetic influences will be key to implementing the optimal clinical management as they do differ, with each discussed below.

Aneuploidy of the Conceptus Genome

The most common described genetic variant associated with equine early pregnancy loss is aneuploidy, the gain (trisomy) or loss (monosomy) of an entire chromosome. The horse has 31 pairs of autosomes, with aneuploidy associated of equine chromosomes 1, 4, 15, 20, combined 23/24, 26, 27, 30, and 31 all linked with lethality in the first two months of gestation.[13] It is worth noting that monosomies of some of the larger chromosomes are yet to be identified, perhaps suggesting these are lethal prior to clinical detection and may well be revealed once routine testing of equine blastocysts is established. Monosomies have never been reported in viable individuals in any species, including the horse. Trisomies found in failed pregnancies are also unique, with the exception of trisomy 30, which has also been described rarely in liveborn foals.[13,14] It is plausible that an extra copy of chromosome 30 can result in multiple outcomes (pregnancy loss or developmental disorders of the foal). Alternatively, the identification of Trisomy 30 in the lost pregnancy could be incidental. In a study that screened 55 primarily Thoroughbred clinical cases of EPL, aneuploidy of chromosomes 1-31 was identified in 11/55 (20%) feto-placental units, making it the most common cause of EPL described to date. To put the incidence in clinical context, just 13 liveborn foals have been described in the literature that are associated with aneuploidy of the small chromosomes, and wide scale screening of group sizes of 500 or more horses only identified three mosaic aneuploidies (i.e., the aneuploidy was only in some of the screened cells for that horse, not all of them).[14] Most aneuploid liveborn foals are euthanized at a young age due to developmental pathologies.[15–17]

Aneuploidy arises due to errors in gametogenesis (maternal or paternal gametes) or during early embryonic development, both reviewed elsewhere.[6] The balance of evidence currently available points towards maternal gametogenesis as the greatest contributor to equine aneuploid conceptuses, similar to what is observed in women.[6] Advanced maternal age is associated with increased incidence of aneuploidy of equine oocytes. Studies have found 56% of oocytes from older mares compared with 16% in younger mares are aneuploid.[18] It is important to note, aneuploid pregnancy losses can still be found in younger mares with 6/11 autosomal aneuploid EPLs described by Shilton et al.[13] identified in mares aged 10 years or younger.

Aneuploid pregnancies present clinically with a diverse range of pathologies, likely relating to which chromosome is affected. They have been linked with pregnancy losses ranging from gestation day 14 through 60. Only one of the twelve clinical aneuploid pregnancies described was found to be anembryonic. Other pathologies observed included marked edema and vascular defects and mismatch in developmental features of the fetus.[6,13] More recently, a case series of fetal cystic hygroma associated with equine pregnancy losses occurring between days 52 and 75 days were reported.[18,19] This condition is associated with aneuploidy in human conceptuses, but whether this is the case in the horse is still not known. Cystic hygroma can be detected via ultrasound and grossly after expulsion of the fetus (Fig. 1).[19] Cases present with a fetus with abnormal quantities of fluid initially in the neck region and later expanding to the abdomen (ascites) and pelvic limbs (Fig. 1D).

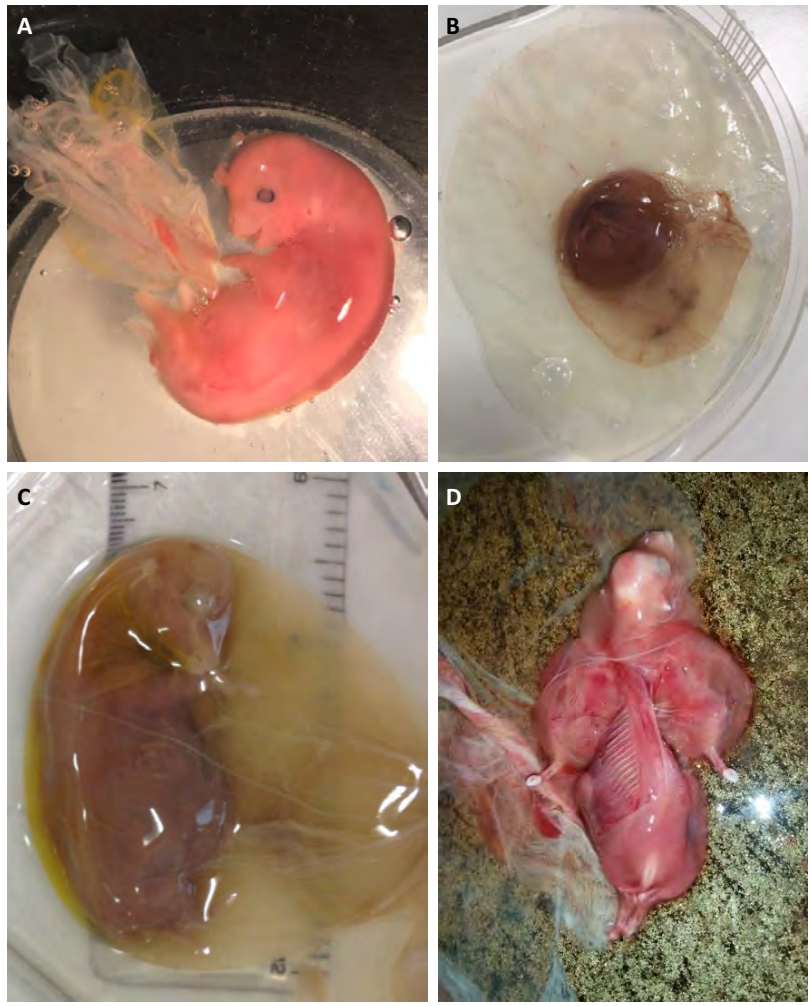

Fig. 1. Examples of gross presentation of clinical cases of EPL flushed from the uterus of Thoroughbred mares at gestation age 42 days (A), 41 days (B), 68 days (C) and a case of fetal cystic hygroma flushed from an embryo transfer recipient mare at gestation age 59 days (D). Photos from Equine Pregnancy Laboratory archive, (A–C) and generously provided by Dr. Santiago Martinez Zuviria (D).

There are currently limited options available to the clinician to avoid aneuploid pregnancies in naturally mated mares as it can only be diagnosed retrospectively using tissue from the conceptus. Recent studies have shown the validity of using DNA based screening over karyotyping for diagnosis of aneuploidy, providing advantages to the clinician as DNA is more readily isolated from autolytic tissues with viable dividing cells required for karyotyping. Due to the lethality of these variants, the best clinical outcome for the mare is to have the pregnancy lost as soon as possible so she can be rebred. Therefore, veterinarians are advised to consider carefully whether it is worth attempts to prolong a compromised pregnancy. In the case of embryo transfer, there are further options available to the clinician to prevent aneuploid embryos, although these are in their infancy in the horse. Embryos can be screened prior to transfer using a trophectoderm biopsy and only diploid blastocysts transferred to the recipient mare. Based on work in other species, it is important to note that ploidy status of one to two cells is not always reflective of chromosome number in all cells and can lead to false positives due to apoptosis of the aneuploid cell or detection of mosaic pregnancies with the aneuploidy restricted to certain tissue regions or compartments.

Translocations in the Mare Genome

Translocations are the breaking and fusing of chromosome segments between non-homologous chromosomes. Translocations involving the mare's genome have been found to increase the risk of that individual suffering recurrent EPL. Translocations are significantly less common variants when compared with aneuploidy, with just 13 mares identified in the literature in a recent review by two laboratories that regularly perform equine karyotyping screening.[14] In all cases the translocations were identified in the mare's genome, not that of the conceptus. The gametes that form in these mares can be unbalanced, leading to an imbalanced embryo genome, which is thought to lead in turn to a non-viable pregnancy. Less commonly, mares with a translocation can produce balanced gametes, which may then lead to a viable offspring. Veterinarians are advised to screen mares

for the presence of translocations when mares present with multiple EPLs and/or infertility. Diagnosis by qualified laboratories usually requires submission of 10 ml of peripheral blood in sodium or lithium heparin and EDTA. Owners of mares carrying these variants should be carefully counseled both of the likelihood of persistent untreatable subfertility/EPL but also the chance of an occasional live foal that will likely carry the variant. To date, no widespread screening of EPL conceptus tissue for translocations has been reported, so whether this condition can also arise in early embryonic development is not known.

Progesterone Concentration in Early Pregnancy

The importance of progesterone levels for pregnancy maintenance is incredibly well established, but the role of progesterone levels in pregnancy loss remains controversial. Luteal insufficiency in early pregnancy is categorically a cause of pregnancy loss, and these cases clearly benefit from progestin supplementation,[20] yet an old but well cited study suggested luteal insufficiency was uncommon.[21] A more recent study of closely-followed research mares found 6/11 singleton pregnancies were lost following luteolysis,[22] leading the field to ask again what role progesterone has in EPL. This interest in luteal insufficiency has been surpassed to some extent by studies assessing more nuanced effects of altered levels of progesterone on early pregnancy and focusing on the quality of the initial rise in progesterone following ovulation. What has been difficult to establish is a level of serum progesterone that is predictive of pregnancy loss. Progesterone levels of less than 4.3 ng/ml at day 5 postovulation are associated with a decreased pregnancy rate at day 14 when compared with progesterone levels 4.3 ng/ml and greater.[23] Further, there was no benefit for pregnancy rates at day 14 of higher levels of progesterone at day 5 post ovulation (5.8-7.6 or > 7.6 ng/ml compared with levels of 4.3 to 5.7 ng/ml).[23] Experimental models of subphysiological levels of progesterone from day 3 to 15 have demonstrated detrimental effects on endometrial function[24,25] as well as early embryonic organ development and placental growth.[25–27] Later stages of pregnancy now need to be assessed to determine if these detrimental changes lead to fetal morbidity or mortality. Further, empirical data has not been generated that shows progesterone supplementation (such as altrenogest) in the absence of absolute luteal insufficiency reduces the risk of EPL.

Endometrial Disease Associated with Early Pregnancy Loss

Endometrial bacterial infections[28] are associated with EPL prior to clinical detection and 5 to 16% of EPLs lost between days 15 and 65 of pregnancy.[3,29,30] This stresses the need to clinically monitor and manage endometritis to prevent EPL, particularly for those losses occurring prior to clinical detection. Endometrial degeneration can also lead to a compromised endometrial environment detrimental to embryonic development. Two independent risk factor studies have linked endometrial cysts to an increased risk of EPL.[10,31] The cysts may directly compromise the implantation and nutrition of the embryo or alternatively reflect other degenerative changes to the endometrium, reviewed elsewhere.[32] Interestingly, multiple risk factor studies have failed to link uterine treatments or presence of uterine fluid after cover with risk of EPL,[10,12] nor does the usage of treatments that target the endometrium align with the proportion of cases associated with endometrial disease.[3,30,33] Clearly, new diagnostics are urgently needed to better differentiate mares that warrant treatments that target and improve endometrial health and reduce a reliance on antimicrobials.

5. Pregnancy Loss Between Gestation Days 70 and Parturition

While in some cases clinical signs such as premature mammary gland development or discharge from the vulva precede abortion, in most instances the mare exhibits no indication of a failing pregnancy. The gold standard approach for clinical management of cases of abortion is the submission of the placenta and fetus (+/− mare serum) to a laboratory for diagnosis of the underlying cause. The limitation of field diagnosis was recently quantified in the context of nocardioform placentitis, where it was found 20% of mares recruited as controls were subsequently diagnosed as norcardioform placentitis by the laboratory and 18% of recruited placentitis mares were diagnosed by the pathologist to be free of nocardioform associated disease.[34] A recent UK study on the outcomes of Thoroughbred pregnancies after day 70 of gestation observed that only 62% of abortions were submitted for diagnostic investigation of any kind, and of those undergoing postmortem examination, 19% remained undiagnosed.[8] Hampering diagnostic submission was the absence of any abortus material in a third of these cases, ushering the question as to what causes abortion in these cases. Laboratory submission reports in the US paint an even more frustrating picture, with 70% of abortion and stillbirth submissions failing to have a cause attributed, despite placental tissue being submitted alongside the fetus in 77% of cases.[35] A lack of diagnosis, however, is not a failure of a post-mortem examination. The absence of identification of an infectious agent and inflammatory lesions provides valuable information to the attending veterinarian regarding the management of the mare and herd following an abortion.

Multiple studies describe the proportion of laboratory submissions ascribed to different causes of abortion and stillbirth;[29,35–38] however, a continued challenge of interpreting and comparing the data in these reports is that it is proportional. This means that the figures are relative to the other submitted causes. Therefore, for example, in areas with a high incidence of infectious causes of pregnancy loss, the relative percentage of other causes will be consequentially decreased. A more reliable way to compare the causes of pregnancy loss between equine populations is to study the causes at a cohort level, namely the percentage of all pregnancies that fail due to a

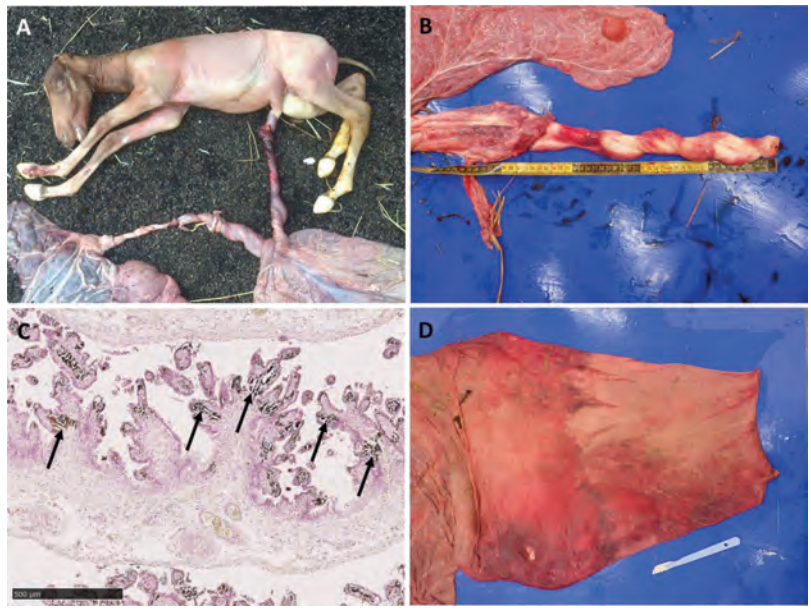

Fig. 2. A, Photograph of an aborted equine placenta and fetus showing excessive length of the umbilical cord, segmental reddening and twisting, B, the alternating pattern of reddened and blanched sections along the amniotic portion of the untwisted umbilical cord in a case of umbilical cord torsion abortion, and C, a histological section of equine chorioallantois stained with Von Kossa's stain depicting villous mineralization with calcium deposits; black arrows showing examples of mineralization within the section staining brown/black. Image D shows moderate ischemic necrosis of the chorioallantois at the cervical pole from an aborted equine pregnancy. Image A courtesy of Dr. F Barrelet and images B and D courtesy of Dr. A Foote.

particular cause as opposed to just those that fail and are submitted for postmortem examination. This allows for accurate comparison of figures between distinct populations and regions over time, enabling veterinarians and other stakeholders the ability to monitor the effects of interventions and other factors.

Noninfectious causes of pregnancy loss after day 70 of gestation include those inherent to the developing pregnancy (i.e., excessive torsion of the umbilical cord, twinning, genetic variants, dystocia arising from malformation/malpresentation, long cord with ischaemic necrosis) or those arising through the dam such as maternal disease or toxin ingestion (such as fescue toxicity leading to premature placental separation and expulsion of the pregnancy). Finally, there are those assigned as non-infectious but undiagnosed, which account for up to 65.4% of abortions and stillbirths.[35] One suggestion is that a more comprehensive investigation may be warranted in these cases,[35] or the possibility that a novel pathogen might be present which is not part of the routine screening of the laboratory; however, with an absence of lesions there is only a finite indication for continued investigation.

Umbilical Cord Torsion

Abortion can occur when there is excessive torsion of the umbilical cord, leading to vascular obstruction, fetal demise, and expulsion of the pregnancy by the mare (Fig. 2A). Until recently, umbilical cord torsion (UCT) abortion was seen to be a problem primarily affecting the UK equine population, accounting for 1.5% of all day 70 pregnancies and 44% of the pregnancy losses submitted for postmortem investigation.[8] However, the most recent analysis of laboratory submissions in California reported UCT to be the most common noninfectious cause of abortion and stillbirth in their population, although at levels almost 10-fold lower than the UK, at 4.4%.[35] The reason for this stark geographic variation in the proportion of UCTs remains unclear and will require direct comparisons of cohorts as opposed to laboratory submissions.

It is worth remembering that observing twists in the umbilical cord is not diagnostic of UCT with 5 and 6 twists normal in Thoroughbred/Standardbred and Warmblood pregnancies respectively.[39] Failed pregnancies tend to show mild to moderate autolytic changes and a congested fetus with hemorrhagic thoracic and or peritoneal effusions.[29,38,40] Once untwisted, pathologists look for an alternating pattern of reddened and blanched/constricted portions along the umbilical cord,[40] as shown in Fig 2B. Cord pathology tends to be focused on the amniotic portion of the umbilical cord with edema, surface intimal tears, or "stretch marks," urachal dilations and excessive length all associated with UCT.[29,37,40,41] It should be noted that excessive amniotic length is not a required diagnostic feature with cord lengths within the normal range observed in cases of UCT. Histological analysis often reveals mineralization of the stroma and chorioallantoic blood vessels (Fig 2C), as well as villous core degeneration.[36,40] To date there have only been limited, descriptive studies of UCT abortions; therefore, further research is warranted to ascertain diagnostic criteria that can be universally accepted. Only then can the risk factors and interventions to prevent the occurrence of UCT abortions be explored.

A second diagnosis of mid- to late-term pregnancy loss involving the umbilical cord that warrants attention is that of cervical pole necrosis, associated with excessive umbilical cord length. Fifty-seven cases of cervical pole necrosis were diagnosed by the University of Kentucky Veterinary Diagnostic Laboratory between 2013 and 2022, accounting for under 2% of their submissions.[42] Although cervical pole necrosis is currently an idiopathic cause of abortion and perinatal morbidity, excessive umbilical cord length does seem to be associated with the disease.[42] Pathogenesis is hypothesized to arise from necrosis of the chorioallantois (Fig 2D) following detachment, or as a result of an ischemic insult likely from vascular compromise due to excessive umbilical cord length.[40] Fetal hypoxia and/or catastrophic rupture of the necrosed fetal membranes are most likely the terminal cause of the pregnancy failing. Like UCT, further research into cervical pole necrosis is warranted to better understand the risk factors and pathology of this condition.

Non-Infectious Placental Disease

Pregnancy failure due to non-infectious placental disease was the second highest diagnosed cause of pregnancy loss in a cohort of day 70 pregnancies in the UK, accounting for 11.2% of investigated losses and an incidence risk of 0.4% of all pregnancies.[8] The lesions or pathologies noted in this category of loss included placental edema, villous atrophy, mineralization of the chorioallantois, and ischaemic necrosis not associated with umbilical pathology or infectious agents. Comparatively, non-infectious placental disease only accounted for 0.7% of the lab submissions out of the California Animal Health and Food Safety Laboratory System over the last 32 years.[35] Further work is needed to understand the etiology of these lesions and to reach a consensus on what constitutes sufficient pathology to be ascribed as the cause of loss.

Twinning

In the most recent studies on the causes of abortion and stillbirth in the US and the UK, twinning was a rare[35] or absent[8] diagnosis of loss, supporting the notion that early ultrasound examination and reduction have largely abolished this cause of pregnancy failure. It is worth noting, however, that in less intensively managed equine populations twinning is still a significant cause of abortion.[43] Further, an increasing number of monozygotic multiple pregnancies are being reported in the equine literature, representing 1.6% of in vitro produced embryos in one study.[44] The exact reason monozygotic twins develop is not known. In human embryos, it has been reported to be associated with intracytoplasmic sperm injection (ICSI).[45] Other plausible explanations may be suboptimal culture conditions or embryo cryopreservation.[45]

Genetics Variants Causing Lethality in Late Pregnancy

There are two single nucleotide polymorphisms (SNPs) reported in the equine literature associated with lethality in utero and/or immediately following parturition.[6,46,47] While these are not common causes of abortion and stillbirth, they can present as a problem for farms if carriers are bred to one another. Clinical management of breeding mares who are known carriers of these SNPs can be rewarding, as pregnancy loss attributed to these SNPs can be avoided with mating of carrier mares to non-carrier stallions. Should mating of carrier mares and carrier stallions occur, this would result in 25% of fetuses/foals being homozygous for the mutation, 25% free of the mutation, and 50% carriers of the mutation. For non-Thoroughbred breeds, pregnancy loss can be avoided when mating two carriers using embryo recovery combined with pre-implantation genetic testing (trophectoderm biopsy) that allows selection and subsequent transfer of just the 25% of embryos that fail to carry the mutation.

Fragile Foal Syndrome (FFS), initially described as Warmblood Fragile Foal Syndrome as it was first identified in a Warmblood,[46] is a monogenic disorder with an autosomal inheritance of a single missense mutation (c.2032G>A) in the gene procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (*PLOD1*). To date, clinical cases of pregnancy loss associated with this mutation have only been reported in the literature in Warmbloods[48] and the Thoroughbred.[49] This breed distribution is likely to expand in the future with carriers of the mutation identified in other breeds including American Sports Pony (1/12 tested), Haflinger (2/48), and Knabstrupper (3/46).[50] Phenotypically, fetuses/foals homozygous for the mutation die in utero or are euthanized shortly after delivery. Stillbirth is the most common presentation, with the 11/14 cases in one study presenting after 320 days[48] but abortion observed as early as 9 months of gestation. Fetal pathology results from a disruption of collagen biosynthesis and includes significant open skin defects at the level of the deep dermis/subcutaneous fascia that are present at parturition, distal limb laxity or contracture and spinal scoliosis. In some cases, incomplete closure of the abdominal wall was noted.[48,49]

Congenital hydrocephalus is a clinical condition reported across multiple breeds associated with abortion, stillbirth, or perinatal death. Phenotypically it is well characterized in Friesian foals as of the internal or communicative type involving dilation of all four ventricles, a decreased ratio of white to grey matter, and likely arising due to stenosis of the jugular foramen.[51] While the condition has been reported widely across breeds for many decades, more recently in Friesians and Belgium drafts, it has been directly linked to a mutation in β-1,3-N-acetylgalactosaminyltransferase 2 (*B3GALNT2*) gene.[47] In these two breeds, affected foals are either aborted from 7 months gestation, present as stillborn, or are euthanized immediately following birth.[52,53] Studies of Friesian populations in Mexico and Netherlands indicate a 10–17% carrier frequency for the mutation, possibly explaining the higher clinical presentation of the condition in this breed. It is plausible, like FFS, the mutation is present in other breeds at a lower frequency, so clinicians

should keep an open mind when considering whether congenital hydrocephalus in other breeds is associated with this mutation.

Infectious Causes of Mid- and Late-Pregnancy Loss

Infectious causes of abortion and stillbirth can be grouped into bacterial, viral, fungal, or mixed. At the forefront of most clinicians' minds when discussing infectious causes of pregnancy loss will be placentitis and equine herpesvirus 1 (EHV-1). Placentitis, inflammation of the placenta, is a symptom rather than a diagnosis of the cause of pregnancy loss. Attribution of the inciting cause is important due to the different treatment and management strategies dependent on the infectious agent. In a recent scoping review, EHV-1 was the most frequently published on cause of mid- and late-term pregnancy loss for every decade since 1960.[54] However, although EHV-1 is a highly researched cause of abortion and stillbirth, the incidence is relatively low, accounting for 4.6% of a Californian laboratory' submissions[35] and 0.3% of day 70 pregnancies in the UK.[8] The elevated research interest likely reflects the high stakes consequences to the wider population on the farm, with the aborted fetus and fetal membranes being a source of infection to other pregnant mares, as opposed to the relative risk of individual occurrence. Nevertheless, vaccination and appropriate herd management are warranted to mitigate risk of loss and to continue to prevent EHV-1 contributing more significantly in the future.[55,56] Current AAEP guidelines are for pregnant mares to receive an inactivated EHV-1 vaccine at five, seven and nine months of gestation.[a]

The most recent report of the diagnoses of laboratory submissions reported single agent bacterial infections (primarily *Streptococcus* spp. at 7.2% and *Leptospira* spp. at 1.0%) to be the most common diagnosis of pregnancy loss in a US population, accounting for 11.8% of submissions.[35] Of these infections, placentitis was the most commonly characterized gross lesion.[35] Similarly, in the UK, 9% of laboratory submissions of abortus/stillbirth material received between 2013 and 2017 were associated with infectious placentitis.[8] However, these figures may not be a true reflection of what is happening on farms, with a cohort study of Thoroughbred pregnancies in the UK observing only 0.3% of day 70 pregnancies failed due to infectious placentitis.[8] A further study of UK Thoroughbred mares found 4.9% of pregnancies were clinically diagnosed with placentitis by a veterinarian,[33] and none of these cases aborted, suggesting in that population at least, it is more a disease of pregnancy rather than necessarily a major contributor to abortion. The fact that ascending bacterial placentitis is one of the few causes of abortion and stillbirth that can be diagnosed premortem makes it an ideal candidate for research due to the possibility of understanding the etiology and providing opportunities for preventative treatments.

Equine viral arteritis (EVA) can cause abortion in mares, and in some instances produces abortion storms with multiple animals rapidly compromised.[57,58] While not a significant cause of loss in the equine population, accounting for 0.2% of laboratory submissions over the last 32 years,[35] EVA does have a high morbidity rate, leading to 71% of mares aborting following exposure in one study.[59] Knowledge of the EVA status of both the mare and covering stallion is important, allowing for implementation of the correct protocols following AAEP Vaccination Guidelines.[a,60,61]

In addition to the aforementioned infectious causes, leptospirosis, chlamydial, and Nocardioform placentitis have garnered the attention of the scientific community over the last three decades.[54] Interestingly, to date, there have been no published reports of chlamydial abortion occurring in the US, likely reflecting the native avian population. However, given the zoonotic nature of the disease, continued monitoring is warranted, and there could be merit in assessing archived abortion material to confirm this belief.[62] Leptospirosis, on the other hand, is a more common cause of abortion and stillbirth, showing both geographical and temporal variation. Infection is via exposure to urine, contaminated water sources, or infected aborted material. As with EHV, diagnosis of leptospiral abortion allows for correct management of the mare following loss, minimizing the risk of infection to other pregnant mares. An approved vaccination for the prevention of leptospirosis due to *Leptospira interrogans* serovar *pomona* is available with the schedule and details available via the AAEP vaccination guidelines.[a]

Nocardioform placentitis is another disease exhibiting both temporal and geographic variation.[34,63,64] It is thought to arise through infection by gram-positive branching actinomycetes, primarily *Amycolatopsis* spp., *Crossiella equi*, or *Streptomyces* spp.; however, the exact pathogenesis remains elusive.[63,65,66] Although the chorionic surface of the placenta tends to exhibit a classic brown/tan mucoid material at the ventral portion of the base of the horns and body, gross field analysis is not always reliable. In a recent study, a considerable proportion of cases were missed or misdiagnosed,[34] supporting the necessity for additional histopathology at a diagnostic laboratory on abortus/stillbirth tissues. While the pathogenesis of the disease is currently unknown, prevention is not possible. Empirical implementation of antimicrobial therapy is recommended; however, there is limited evidence that any of the current treatments are effective in preventing abortion.

6. Conclusion

The most common cause of pregnancy loss in the first two months of gestation is autosomal aneuploidy, with current evidence suggesting it primarily originates in maternal meiosis. Autosomal aneuploid pregnancies are non-viable, and most are lost within the first two months of gestation. Active management to reduce the chance of an aneuploid embryo is possible in embryo transfer programs but not in naturally mated mares. Management of mares to promote optimal endometrial health remains an important priority

for veterinarians, but it should be noted that endometrial disease is likely to contribute to approximately 15-30% of cases of EPL in well managed mares, and other causes should therefore be carefully considered. There is growing evidence for more nuanced effects of sub-physiological levels of progesterone, although equally an absence of evidence that shows progesterone supplementation is beneficial in preventing early pregnancy loss.

In mid- to late-pregnancy, it is important to remember that placentitis is a symptom of inflammation of the placenta, and attribution of the inciting cause is key to both clinical management and monitoring of infectious disease outbreaks. While abortion associated with EHV-1 infection rightly remains an intense focus of the research community due to the devastating effects it can have after an abortion storm, EHV-1 infection is only responsible for a very small proportion of cases of abortions seen by veterinarians in many jurisdictions. Umbilical cord torsion is a significant contributor to non-infectious causes of abortion not only in the UK but also in the US. Submission of abortus material remains the gold standard of diagnosing the underlying cause of pregnancy loss. Genetic causes are also relevant to mid- to late-pregnancy loss, although their relative contribution is significantly lower when compared with the first two months of gestation.

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Declaration of Ethics

The authors have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

Conflict of Interest

The authors have no conflicts of interest.

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