ASPRA Asociación Peruana de Reproducción Animal

SPERMOVA

Spermova 2015; 5(2): 168-188

Artículo de Revisión

PREGNANCY LOSS IN MARES

Perdida de preñez en yeguas

Ahmed Tibary[1], Lisa K Pearson[1]

http://dx.doi.org/10.18548/aspe/0002.37

[1] *DMV, MS, PhD, Dip. ACT Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, USA.*

E-mail: tibary@vetmed.wsu.edu

INTRODUCTION

La pérdida de preñez es un factor muy importante en la práctica de reproducción equina, debido a las pérdidas económicas y emocionales que ocasiona. La pérdida de preñez es frecuentemente dividido en dos categorías: Perdida de preñez temprana o muerte embrionaria (< 42 días de gestación) y tardía o perdida fetal (> 42 días de gestación). El diagnostico de las causas de perdida de preñez es frecuentemente muy difícil. Muchas de las causas de perdida de preñez temprana esta pobremente documentada, pero los estudios sobre el desarrollo embrionario y la interacción del embrión-útero han sido capaces de arrojar algo de luz sobre los factores predisponentes. Las pérdidas fetales o abortos están dominadas por causas infecciosas y particularmente placentitis bacteriana. Los detallados de la pérdida de preñez fueron publicados recientemente por los autores (Tibary *et al.*, 2012; Tibary y Pearson, 2012; Tibary *et al.*, 201). El objetivo de este artículo es proporcionar una visión general de la epidemiología, etiología, el diagnóstico y la prevención de la pérdida de preñez en la yegua.

INTRODUCTION

Pregnancy loss is an important aspect of equine practice due to the economic and emotional loss that it engenders. Pregnancy loss is often divided in two categories: early pregnancy loss (EPL) or embryonic death (ED) (first 42 days) and fetal losses (after 42 days). Diagnosis of the causes of pregnancy loss is often very challenging. Many of the causes of EPL remain poorly documented but studies on embryo development and embryo-uterine interaction have been able to shed some light on predisposing factors. Fetal losses or abortions are dominated by infectious causes and particularly bacterial placentitis. Detailed reviews of pregnancy loss were recently published by the authors (Tibary *et al.*, 2012; Tibary and Pearson, 2012; Tibary *et al.*, 2014). The objective of this paper is to provide an overview of the epidemiology, etiology, diagnosis and prevention of pregnancy loss in the mare.

EARLY PREGNANCY LOSS

Incidence and diagnosis of early pregnancy loss

The incidence of early pregnancy loss is usually determined based on ultrasonographic evaluation. Pregnancy diagnosis is scheduled 14 days, and rarely 12 days, after ovulation. The incidence of early pregnancy loss between day 12 and day 50 of pregnancy varies from 2.5% to 30% (Table 1). Embryo losses prior to day 12 are more difficult to estimate. However, studies based on embryo collection provided estimates of 10% for high fertility mares and up to 60 or 70% for aged mares (Woods *et al.*, 1986; Ball *et al.*, 1987; Vogelsang and Vogelsang, 1989; Davies-Morel *et al.*, 2005).

Clinically, early pregnancy loss is suspected based on serial ultrasonographic examinations. Prior to fixation, embryos with high risk of loss have smaller than normal size, irregular contour, or may be surrounded by endometrial edema or intra-uterine fluid. Post-fixation, the major signs are: abnormal location (uterine body or tip of the uterine horn), abnormal size of the amniotic vesicle, irregular contour, lack of fixation after day 17, lack of visualization of the embryo proper after day 22, abnormal orientation, fixation near a large uterine cysts, increased echogenicity, and abnormal development (Figures 1, 2, 3) (Adams et al., 1987; Ball and Wods, 1987; Bell and Bristol, 1987; Chevalier-Clement, 1989; Ginther, 1985; Ginther *et al.*, 1985). Diagnosis of the cause of EPL requires a complete breeding soundness examination including endometrial cytology, bacteriology and biopsy (Figure 4).

Table 1. Early pregnancy loss reported in various studies (adapted from Vanderwall 2008)

Interval post-conception (days)	Number of mares	Pregnancy loss (%)	Reference
13-52	1295	5,3	Chevalier-Clement and Palmer (1982)
14-63	326	4,0	Simpson *et al.* (1982)
11-50	154	18,5-24,0	Ginther (1985)
15-50	354	17,2	Villahoz *et al.* (1985)
14-48	404	10,4	Wods *et al.* (1985)
14-48	559	10,7	Wods *et al.* (1987)
18-42	437	3,0	Eilts *et al.* (1995)
15-56	2989	5,5	Chevalier-Clement (1989)
15-35	1085	7,8	Vogelsand *et al.* (19899
10-45	135	17,0	Villahoz *et al.* (1985)
17-42	179	9,5	Irvine et al. (1990)
12-50	54	13,0	Jobert *et al.* (2005)
11-40	85	23,5	Wods *et al.* (1990)
18-45	132	7,5	Lowis and Hyland (1991)
12-40	509	6,7	Meyers (1991)
24- term	1379	7,6	Bruck *et al.* (1993)
14-40	229	8,7	Tannus and Thun (1995)
14-30	168	10,1	Pycock and Newcombe (1996)
11-39	313	2,6	Newcombe (1997)
10-30	128	13,3	Papa *et al.* (1998)
14-50	349	8,9	Barbacini *et al.* (1999)
12-50	419	15,5	Carnevale *et al.* (2005)
15-35	1144	10,4	Morris and Allen (2002)
14 - term	391	12,5	Hemberg et al. (2004)
14-41	395	4,6	Blanchard *et al.* (2004)
16-50	201	20,9	Carnevale et al. (2005)
14-45	547	24,0	Newcombe and Wilson (2005)
15-42	3194	7,5	Allen et al. (2007)

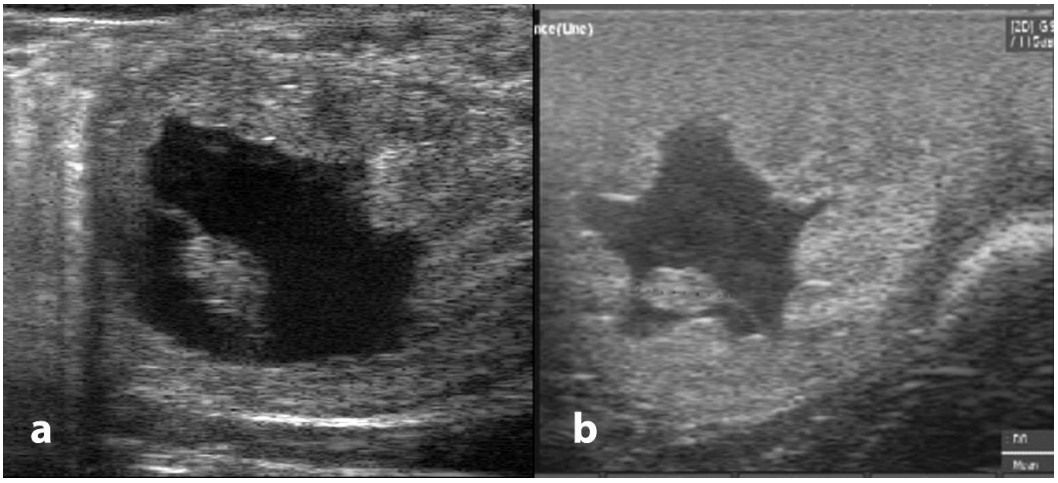

Figure 1: Pregnancy loss at 25 days. Note the increased uterine edema and abnormal orientation (b)

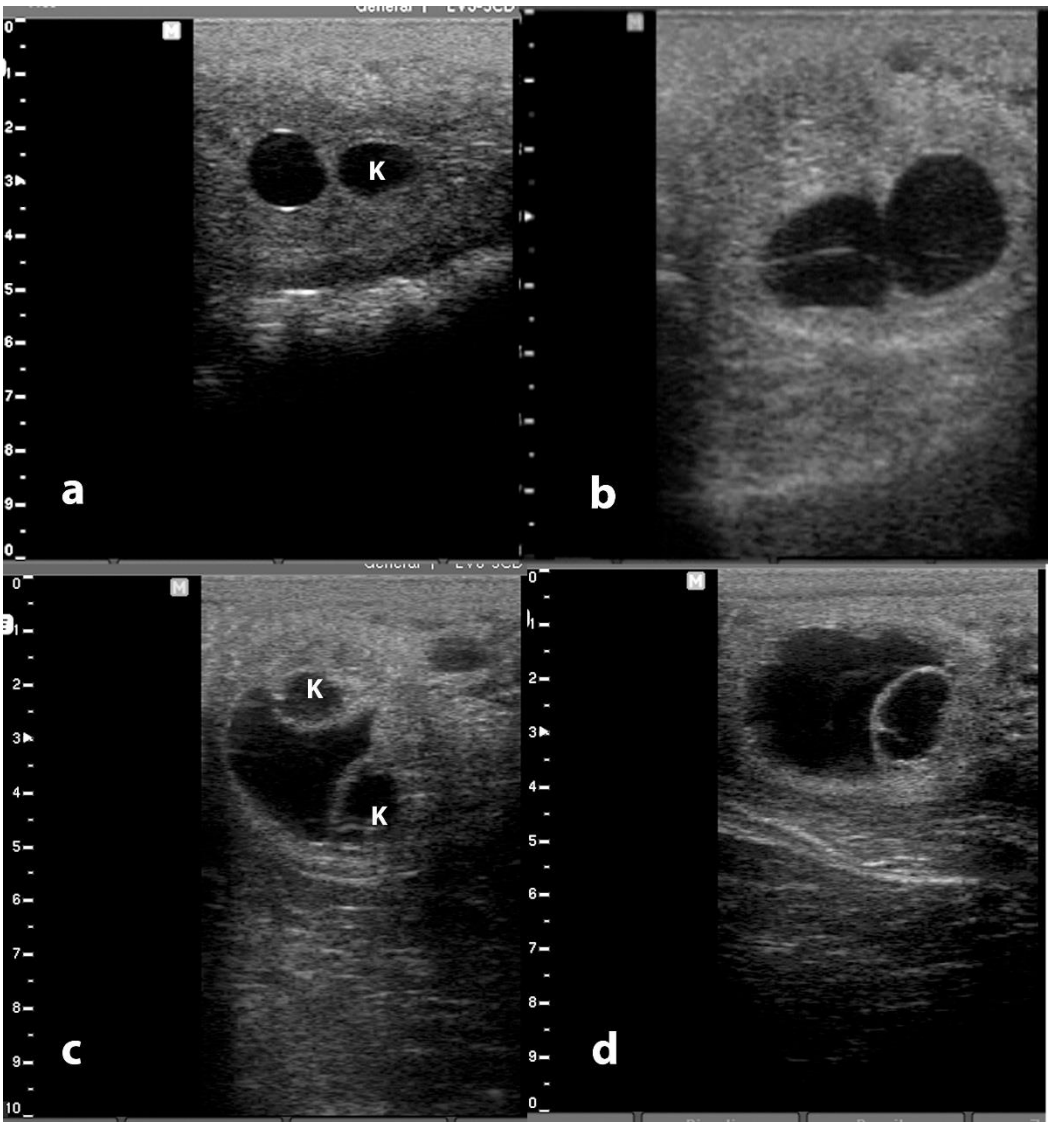

Figure 2: Ultrasonography of pregnancy and uterine cysts. a) 13-day embryonic vesicle near a cysts (k), b) twin embryonic vesicles at 14 days, c) 18-day embryonic vesicle fixed near two 2 uterine cysts, d) 18-day embryonic vesicle fixed near a cyst

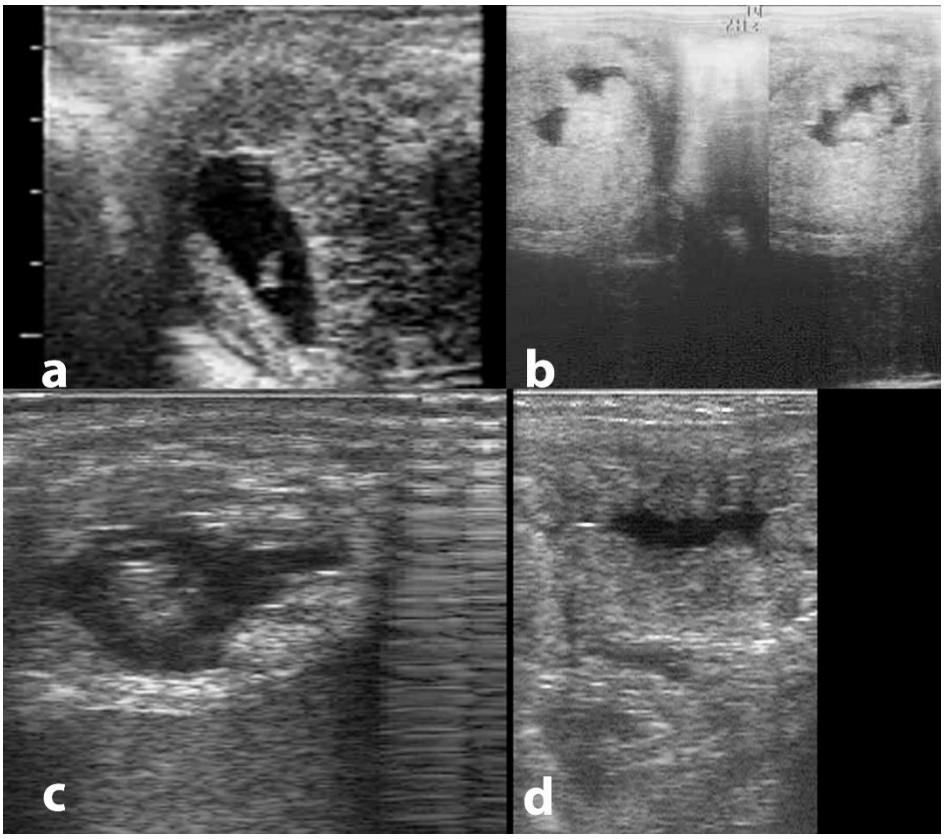

Figure 3: Ultrasonographic appearance of embryonic loss. A) 25 days embryonic vesicle with small irregular size, b) 30-day pregnancy, loss of fluid and lack of fetal heartbeat, c) 28-day pregnancy, irregularly shaped vesicle, d) same vesicle 2 days later

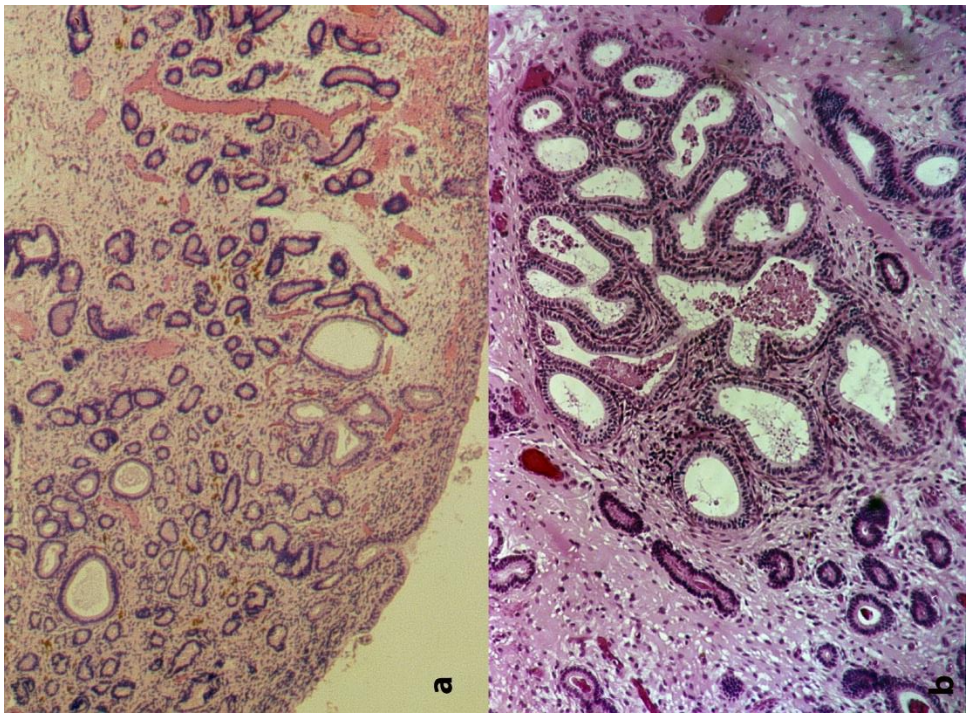

Figure 4: Severe endometrial degenerative changes (Endometrial biopsy grade III, periglandular fibrosis and glandular nesting) in a mare with recurrent early pregnancy loss.

CAUSES OF EARLY PREGNANCY LOSS

Factors involved in early pregnancy loss in mares are usually divided into: mare factors, embryo factors, semen or stallion factors and extrinsic factors (environment, nutrition, treatments etc.).

Mare factors

Factors intrinsic to the mare that are involved in early pregnancy loss include endocrine disorders, uterine diseases, history of infertility, age, chromosomal abnormalities, effect of postpartum and lactation, and twinning. The most common endocrine disorder incriminated in early pregnancy loss in practice is luteal insufficiency. However, evidence of the occurrence of primary luteal insufficiency is often difficult to obtain (Allen, 2001). The condition can be confirmed if pregnancy is maintained following progesterone supplement in mares with recurrent early pregnancy loss (Canisso *et al.*, 2013; Sieme *et al.*, 2015). Luteal insufficiency may be due to poor luteinization in mares with post-mating endometritis particularly after administration of cloprostenol. Diagnosis is primarily based on history and clinical impression (poor uterine tone or presence of uterine edema around the embryonic vesicle) (Ginther, 1985; Darenius *et al.*, 1989; Newcombe, 2000; Vanderwall *et al.*, 2000). Serum progesterone determination may be helpful in the decision to supplement mares with altrenogest. Ultrasonographic, particularly Doppler evaluation, may reveal altered blood flow to the corpus luteum. Mares with suspected luteal insufficiency can be supplemented with altrenogest (0.044 mg/kg per os once or twice daily) or progesterone (150 mg/day IM) starting on day 3 after ovulation and continuing until 100 to 120 days of pregnancy. Long acting injectable formulations of progesterone and altrenogest are available in some countries (Vanderwall *et al.*, 2007). Administration of the GnRH analogue, buserelin (40μg), 10 or 11 days after ovulation has been reported to improve luteal function and reduce EPL in some studies (Pycock and Newcombe, 1996).

Reproductive disorders, uterine and cervical pathologies in particular, play an important role in early pregnancy loss. Mares with a history of acute or chronic endometritis are at a higher risk for EPL (Allen *et al.*, 2007). In aged mares, the presence of endometrosis and development of uterine cysts compromise uterine-embryo interaction and result in embryonic death (Ousey *et al.*, 2012; Rebordao *et al.*, 2014; Tibary and Pearson, 2015). Large uterine cysts may hinder transuterine embryo migration resulting in a release of PGF2a from the endometrium and loss of the pregnancy (Eilts *et al.*, 1995; Miyakoshi *et al.*, 2012; Stanton *et al.*, 2004). Laser ablation is the technique of choice for treatment of endometrial cysts (Figures 5, 6) (Griffin, 2002).

The age of the mare influences embryo survival through deterioration of oocyte quality as well as an increase in the incidence of uterine and cervical disorders (Carnevale and Ginther, 1992). Several studies have shown that embryos produced in vivo and in vitro from aged mares have a reduced viability after transfer (Ball *et al.*, 1987; Carnevale *et al.*, 1993; Carnevale *et al.*, 2005). In one study, pregnancy rates at 12 days was 31% and 92% following transfer of oocytes from aged and young mares, respectively (Carnevale and Ginther, 1995). Oocytes from aged mares were shown to have several ultrastructural abnormalities such as presence of intracytoplasmic and nuclear vacuoles, reduced number of mitochondria, and abnormalities of mitochondria and chromatin that may explain the poor viability of embryos (Carnevale and Ginther, 1995; Rambags *et al.*, 2003; Rambags *et al.*, 2006).

The effect of the interval from foaling to breeding (foal heat breeding) on early pregnancy loss is a subject of debate (Bell and Bristol, 1987; Blanchard *et al.*, 2004; MacPherson and Blanchard, 2005; Meyers *et al.*, 1991; Morris and Allen, 2002). Mares with normal parturition and postpartum events do not sustain more EPL if ovulation occurs at least 10 days post-foaling (Blanchard *et al.*, 2004; Macpherson and Blanchard, 2005). The effect of lactation on early pregnancy loss remains controversial. While some studies have not shown any effect (Woods *et al.*, 1985; Woods *et al.*, 1987), others have shown a two fold increase in early pregnancy loss in lactating (31.4%) compared to non-lactating mares (15.4%) (Newcombe and Wilson, 2005).

Other factors that have been associated with increased EPL include systemic diseases, metabolic and endocrine disorders, twinning, location of the vesicle after fixation, and chromosomal abnormalities. Systemic diseases suspected to increase risks of EPL include primarily those resulting in endotoxemia or high fever (Daels *et al.*, 1991a; Daels *et al.*, 1991b). Mares that have undergone colic surgery in the first 40 days post-mating have a higher risk for loss of pregnancy (Drum *et al.*, 2013). Metabolic and endocrine disorders (insulin resistance, pituitary pars intermedia dysfunction) are suspected to affect embryo survival (Morresey, 2013). Twinning rate can be very high in some breeds (Thoroughbred and Warmblood). Twin embryos are at high risk for early and late pregnancy loss if they fix unilaterally (Fiala *et al.*, 2003; .Journée *et al.*, 2013; Wolfsdorf and Macpherson, 2010). Recurrent pregnancy loss in mares has been associated to a

number of chromosomal abnormalities including autosomal translocations (Lear *et al.*, 2008).

Embryo factors

Embryo quality and normal interaction with the uterus are important factors in the maintenance of pregnancy. Embryo quality is primarily determined by oocyte quality. Deterioration of oocyte quality is due to mare age, follicle age, and oocyte age after ovulation (Allen *et al.*, 2007; Carnevale and Ginther, 2005; Carnevale *et al.*, 2005). Delayed insemination after ovulation may result in acceptable early pregnancy rates but a large proportion of the embryos (34 to 38%) are lost between 15 and 40 days of pregnancy (Koskinen *et al.*, 1990; Newcombe and Cuervo-Arango, 2011). Embryo quality is also affected by in vitro manipulations such as biopsy, cryopreservation and advanced biotechnologies (ICSI, SCNT) (Hinrichs *et al.*, 2007; Vanderwall *et al.*, 2006).

Embryo fixation in the uterine body has been shown to affect embryo survival (Jobert et al., 2005). For mares bred in the postpartum period, fixation of the embryo in the non-fetal horn from the previous pregnancy results in a better survival rate (Sharma *et al.*, 2010).

Stallion or semen factors

The effect of the stallion or type of semen used (frozen-thawed, sexed) has long been debated. The effect of semen processing on embryo quality and survival has been investigated in several studies. There seems to be an interaction between stallion, semen processing, and embryo quality. Breeding to some stallions was shown to result in higher pregnancy loss. In one case, increased incidence of early pregnancy loss was attributed to slight dimorphism of heterochromatin in chromosome 1 (Blanchard *et al.*, 1994). In the authors' experience mares bred with donkey semen tend to experience more early pregnancy loss.

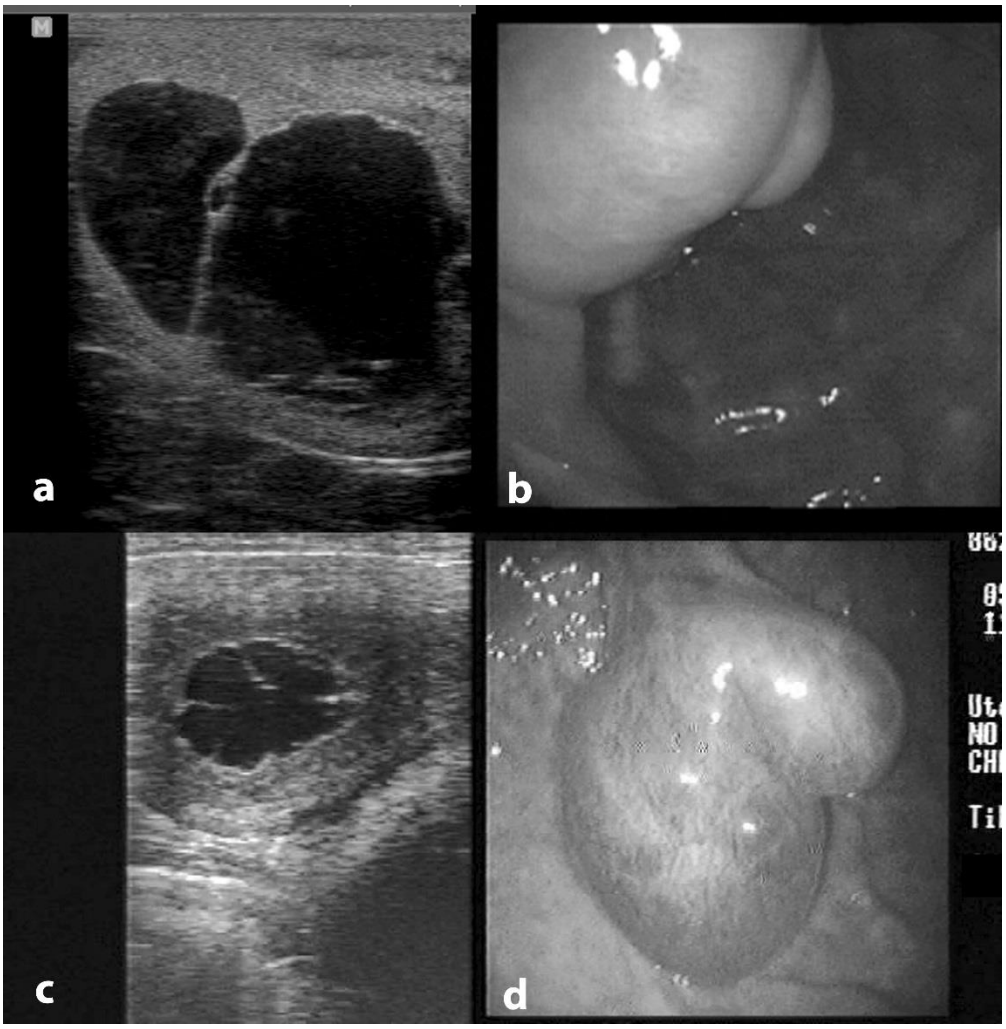

Figure 5: Large uterine cysts visualized by ultrasonography (a -b) and by hysteroscopy (c-d)

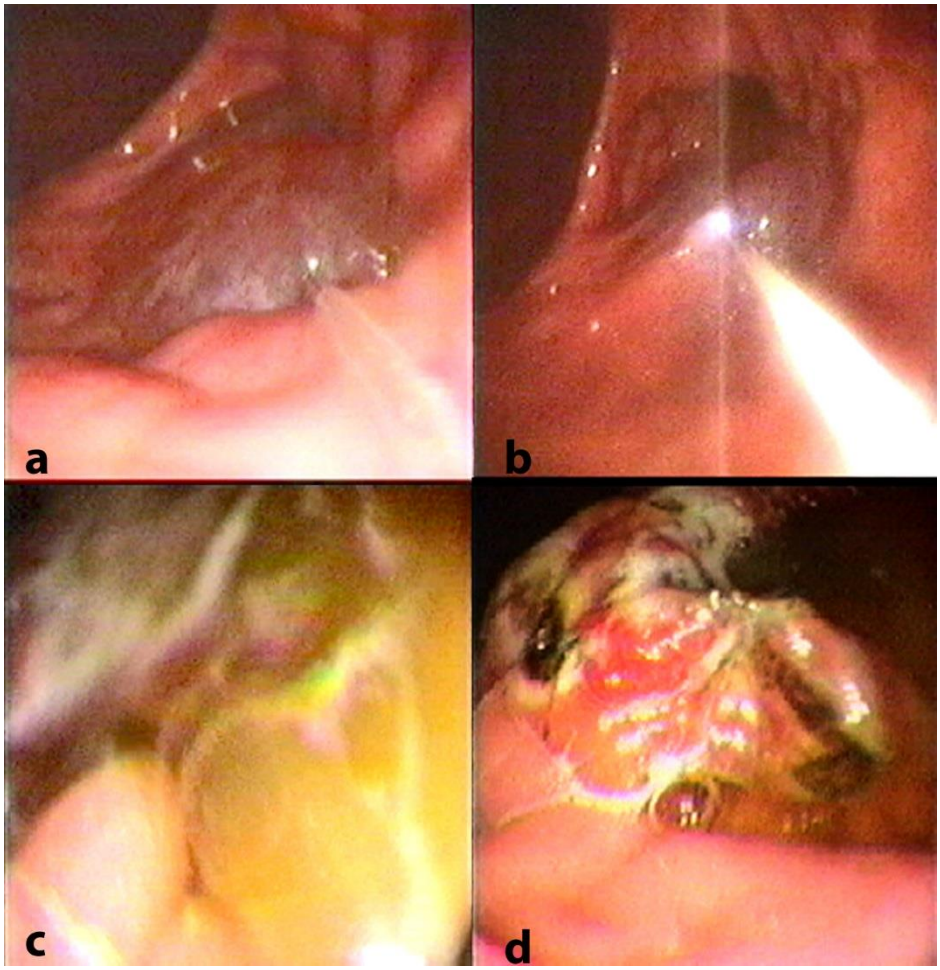

Figure 6: Laser ablation of large uterine cysts

Extrinsic factors

Nutrition and climatic conditions are the primary extrinsic factors involved in early pregnancy loss. Poor nutrition (protein restriction) (Niekerk van and Morgenthal, 1982; Niekerk van and Niekerk van, 1998) and significant weight loss (Henneke *et al.*, 1984; Newcombe and Wilson, 2005) in the first 30 days of pregnancy have been shown to result in an increased early pregnancy loss. The effects of climatic conditions on early pregnancy loss are not well documented. In the authors' clinical experience, heat stress (high ambient temperature and humidity) is detrimental to early pregnancy in the mare.

FETAL LOSS (ABORTION)

Mid to late term abortion rate varies from 2% to 5%; however, alarming abortion rates can be experienced in outbreaks of infectious diseases in naïve herds (Tibary *et al.*, 2014). Causes of equine abortion in several countries have been thoroughly reviewed recently by the authors (Tibary *et al.*, 2014). One-third to half of all equine abortions are estimated to be the result of infection (Table 2) (Giles *et al.*, 1993; Hong *et al.*, 1993; Laugier *et al.*, 2011). Numerous organisms have been associated with infectious abortion, including viruses, bacteria, and fungi (Tibary *et al.*, 2014). The prevalence of each organism differs geographically.

Bacterial Abortions and Placentitis

Placentitis is a significant cause of equine late-term abortion, premature delivery, and neonatal death (Tibary *et al.*, 2014). Bacterial placentitis is the most common. Fungal placentitis is reported in fewer than 10% of horses with placentitis. Placentitis is generally classified as one of three types: ascending, diffuse, and focal mucoid. With the exception of *Leptospira spp.* and nocardioform infection, most bacterial or mycotic placentitis in mares are the result of an ascending infection.

Table 2: Causes of abortion in 3 large studies

	Laugier *et al.* (2011) (France)	Smith *et al.* (2003) (UK)	Giles *et al.* (1993) (Kentucky USA
Number of observations	1822	1552	3514
Infectious causes (%)	47.7	19.7	35.5
Non-infectious causes (%)	27.2	72.5	50.5
Bacterial	*73.1*	*79.3*	*82.8*
Viral	*6.1*	*39.9*	*12.1*
Fungal	*6.9*	*1.5*	*5.2*
Undetermined (%)	25.1	7.8	16

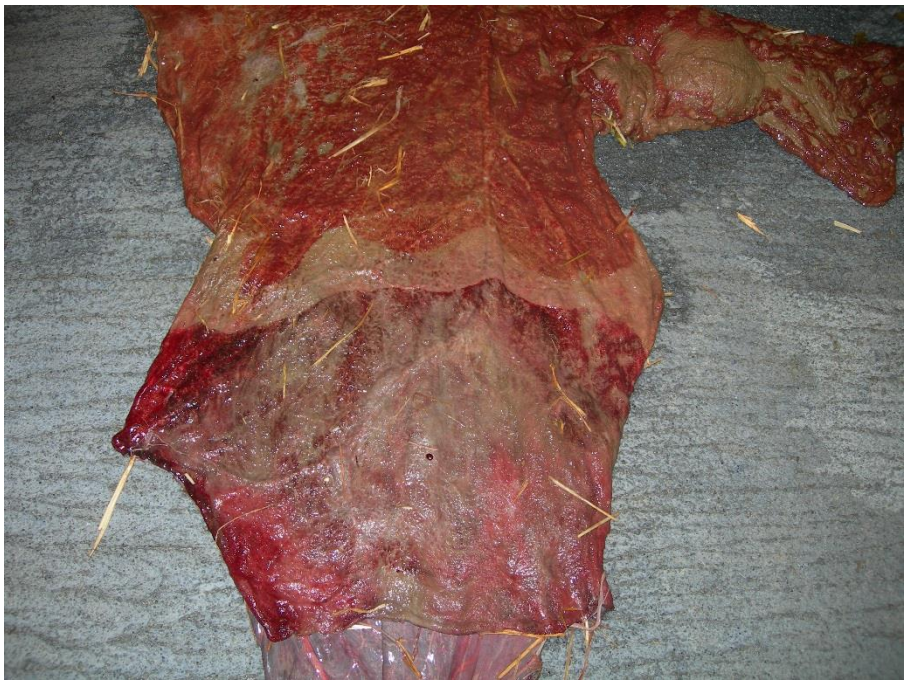

Figure 7: Ascendant placentitis. Note the thick congested appearance of the placenta at the cervical star

Ascendant Placentitis

Ascending placentitis is the most common type. *Streptococcus equi* subsp. *zooepidemicus*, *E. coli, Pseudomonas aeruginosa, Enterobacter* spp. and *Klebsiella pneumoniae* are the most frequent isolates (LeBlanc, 2010; Tibary *et al.*, 2014; Wolfsdorf and MacPherson, 2010). In a retrospective study of 954 cases of equine abortion in Kentucky, placentitis was recognized in 24.7% of all submissions (Hong *et al.*, 1993; Hörügel *et al.*, 2008). A bacterial or fungal organism was isolated in 68.6% of placentitis cases, and 57.4% of cases yielded bacteria both from the placenta and the fetal organs. Similarly, in a retrospective study on 1822 abortion submissions in France, fetoplacental infection represented 64% of the diagnoses with 80% due to bacterial infection and only 1.8% due to fungal infection (Laugier *et al.*, 2011). Isolation of more than one bacterium from cases of placentitis is not uncommon and the most common combinations are *S. equi* subsp. *zooepidemicus* with either *K. pneumoniae* or *E. coli.*

Bacterial placentitis most often induces abortion between 6 and 9 months of gestation. Placentitis resulting from *E. coli* tends to cause later abortion and more stillbirths. Placentitis from *S. equi* subsp. *zooepidemicus* tends to be acute and focal or diffuse. In acute bacterial placentitis the fetus is generally expelled before 8 months of pregnancy. Acute or diffuse placentitis may not be easy to recognize on gross examination of the placenta. Histologic evaluation of the allantochorion may reveal bacterial emboli with necrosis of chorionic villi or infiltration of neutrophils in the intervillous space. Chronic or focal placentitis typically results in birth of premature or weak foals or late-term abortions. Lesions tend to be located at the cervical star,

where discoloration and thickening are observed (Figure 7) (Laugier *et al.*, 2011; Tibary et al., 2014).

Gross and histologic features of mycotic placentitis were described in detail by several authors (Hong *et al.*, 1993; Laugier *et al.*, 2011). Mycotic placentitis and abortion are most likely to occur in the late gestational period. Fungal organisms associated with equine abortion include *Aspergillus* spp., *mucoraceous fungi*, *Histoplasma caspulatum*, *Candida* spp., *Mucor* spp., *Coccidioides* spp., and *Cryptococcus neoformans*. Focally extensive placentitis is usually observed at the cervical star and adjacent area as a thick, leathery area. Histologically, except for histoplasmosis and candidiasis, the fungi induce a chronic, extensive placentitis characterized by extensive necrosis of the chorionic villi, neovascularization in the chorionic stroma, infiltration of neutrophils, mononuclear cells, or mixed inflammatory cells in the villi and chorionic stroma, and presence of fungal hyphae in the necrotic debris. Adenomatous hyperplasia with or without squamous metaplasia of the chorionic epithelium is frequently observed. *Histoplasma capsulatum* caused a multifocal granulomatous placentitis and abortion in one mare in the seventh month of gestation and in three mares in the tenth month. Four newborn foals died from severe granulomatous pneumonia within a few days of birth, and a weanling Thoroughbred developed granulomatous pneumonia and lymphadenitis at 5 months of age (Tibary *et al.*, 2014).

Placentitis caused by *Candida* spp. is generally diffuse, necrotizing, and proliferative with extracellular, yeast-like spores in the chorionic epithelium. Chronic, focally extensive placentitis is most common, with expulsion of the foal late in gestation.

Hematogenous Multifocal or Diffuse Placentitis

Multifocal or diffuse placentitis is less common than acute, focal placentitis and is usually a result of hematogenous spread of microorganisms to the uterus. This occurs with leptospirosis, salmonellosis, histoplasmosis, and candidiasis. A special focal mucoid form of placentitis, nocardioform placentitis, is emerging as common in several U.S. regions (Tibary *et al.*, 2014).

Leptospira spp. placentitis is characterized by diffuse lesions secondary to hematogenous spread. Leptospirosis as a cause of placentitis seems to be more frequently diagnosed in Kentucky and South America than in other regions of the world, probably because of specific regional characteristics and the difficulty in isolating or detecting the pathogen (Tibary *et al.*, 2014; Wolfsdorf and MacPherson, 2010). However the disease is increasingly reported in many areas of the world. In one retrospective study, *Leptospira* spp. were isolated in 16% of 364 cases of placentitis (Timoney *et al.*, 2011). Several species of Leptospira have been isolated from aborted equine fetuses (e.g., *L. pomona, L. grippotyphosa, L. interrogans* serovars *bratislava and pomona* type *kennewicki*, serovar *hardjo* type *hardjoprajitno*). *Leptospira interrogans* serovar *bratislava* has been reported as a possibile cause of abortion without systemic illness in Brazil (Marcolongo-Pereyra *et al.*, 2012). In North America the most common isolate is *L. interrogans* serogroup *pomona serovar kennewicki* which is carried by several wildlife species including the striped skunk, raccoon, whitetail deer and opossum and *L. kirschneri* serogroup gri*ppotyphosa* serovar *grippotyphosa.* Epidemiologic surveillance is emphasized in many areas because of the zoonotic nature of the infection as well as the impact on the equine industry (Tibary *et al.*, 2014).

Most leptospiral abortions occur between 6 and 9 months of gestation with no premonitory clinical signs in the mare. The affected placenta is thick, heavy, edematous, hemorrhagic, and occasionally covered with a brown mucoid material on the chorionic surface. Occasionally the affected placenta lacks detectable gross lesions. Green discoloration or cystic adenomatous hyperplasia of the allantois is observed in some cases. Funisitis, inflammation of the umbilical cord, has also been described in leptospiral abortion. The fetus often shows moderate autolysis and may present mild to moderate icterus, enlarged motted liver and renal edema. Fetal histopathological lesions may include various degrees of nephritis (microabcesses, mononuclear interstitial nephritis) and hepatitis (infiltration of portal triad). Fetal antibodies against *Leptospira* spp. may be detected in foals by the microagglutination test. Fetal leptospiral titers are considered significant findings. Spirochetes are present in large numbers in the placental sections and are detected using silver stains. Fluorescent antibody testing on placental impression smears or fetal tissue (kidney and liver) provide very good evidence of infection. Organisms may also be identified by immunohistochemistry or by PCR. High-titer agglutinating antibody (>1:6400) may be observed in mares, but interpretation of serologic tests remains difficult without confirmation of infection by culture and isolation. Antibiotic treatment (oxytetracycline 5 mg/kg IV SID or procaine penicillin G, 20,000 IU/kg IM BID) for 5 to 10 days has been reported to help prevent abortion during an outbreak. *Leptospira* spp. have been detected by PCR in ejaculates of a stallion. This observation merits further investigation on potential legal and health implications.

Nocardioform placentitis is a distinct type of equine placentitis first described in the United States in the late

1980s. Nocardioform actinomycetes induce a chronic placentitis that results in late-term abortion, stillbirth, or premature birth. Some mares may exhibit premature mammary gland development and lactation before abortion (Erol *et al.*, 2012; Tibary *et al.*, 2014). Various groups of gram-positive, filamentous, branching bacteria have been implicated as etiologic agents in mares with nocardioform placentitis, including *Nocardia* spp., *Rhodococcus rubropertinctus*, and *Amycolatopsis* spp. However, the most severe infections of this type are caused by the actinomycete *Crossiella equi*. Polyphasic taxonomic studies on actinomycete strains isolated from equine placentas from horses in Kentucky and the southern United States indicated that the isolates were members of the genus Amycolatopsis. It was proposed that these strains be classified as three novel species of the genus *Amycolatopsis* and named *Amycolatopsis kentuckyensis, Amycolatopsis lexingtonensis*, and *Amycolatopsis pretoriensis*. During the 2002 and 2003 foaling seasons, *Cellulosimicrobium (Cellumonas) cellulans* (formerly *Oerskovia xanthineolytica*) was isolated from fetal tissues or placentas from cases of equine abortion, premature birth, and term pregnancies in Kentucky (Donahue *et al.*, 2002; Labeda *et al.*, 2009). Significant pathologic findings included chronic placentitis and pyogranulomatous pneumonia. In addition, microscopic and macroscopic alterations in the allantochorion from four of seven cases of placentitis were similar to those caused by *Crossiella equi* and other nocardioform bacteria. A review of placentitis cases from Kentucky showed some bacterial isolates (*Pantonea agglomerans, Cellulosimicrobium cellulans, Pseudomonas* spp., *Enterobacter* spp., *Enterococcus* spp., and *Staphylococcus* spp.) produce placental gross lesions that are indistinguishable from nocardioform placentitis. During the 2011 foaling season in Kentucky 11.3% of the placental submissions were diagnosed as nocardioform placentitis. The most common gram positive branching actinomyces were *Amycolapsis* spp. (48.7%) and *C. equi* (28.9%). The association of both organisms was seen in 8% of the cases. The majority of abortion occurred in the last trimester of pregnancy (Erol *et al.*, 2012).

The lesion is an extensive and severe exudative, mucopurulent, and necrotizing placentitis centered on the junction of the placental body and horns rather than the cervical star (Figure 8). Infection of the placenta is generally thought to be a sequela of the hematogenous spread of the microorganisms from a primary port of entry. However the isolated nature of the lesions does not corroborate this theory. The bateria may originate from the caudal reproductive tract resulting in latent or slow developing infection. Attempts to induce this form of placentitis experimentally have been unrewarding, which limits studies on its pathophysiology (Canisso *et al.*, 2015). The fetus is often severely underdeveloped due to placental insufficiency and does not show any remarkable gross or histologic lesions. The placental lesion is focally extensive (15-30 cm) and frequently located at the base of the uterine horns or at the junction between the body and horns of the placenta. The affected area is thickened, and its chorionic surface is covered with brown, necrotic, mucopurulent exudate and dotted with white or yellow granular structures. Underneath this mucoid material, the chorionic surface is reddish white, mottled, and roughened. Villous necrosis and adenomatous hyperplasia of the allantoic epithelium and hyperplasia with or without squamous metaplasia of the chorionic epithelium are frequently observed.

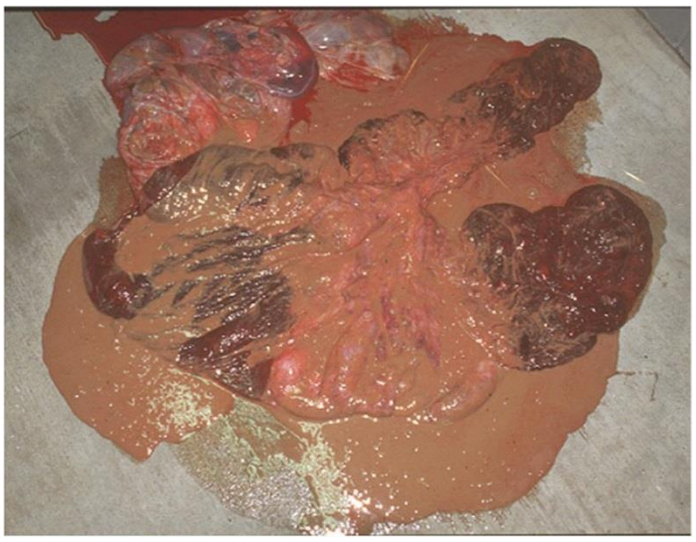

Figure 8: Nocardioform placentitis. (Photo courtesy Dr. Fairfield Bain, Colege of Veterinary Medicine, Washington State University).

Physiopathology and Diagnosis of Placentitis

Bacterial infection of the chorioallantois induces an increase in expression of pro-inflammatory cytokines (IL-6 and IL-8) in placental tissue. Subsequent release of PGE2 and PGF*2a* into the allantoic fluid leads to premature delivery. The premature delivery of the fetus is most likely caused by acceleration of the fetal maturation process induced by changes in placental function. The resulting endocrine changes lead to increased uterine contrations and increased intrauterine pressure, causing dilation of the cervix and induction of labor. A premature increase in maternal plasma progestins may be an indication of accelerated fetal maturation or fetal stress. Foals may survive if they are near term (>305 days) (Christiansen *et al.*, 2003; LeBlanc, 2010; Lyle, 2014).

Clinically, placentitis is suspected in mares with premature udder development or lactation and muccupurulent vaginal discharge. However, most mares with placentitis do not show any outward signs of infection (Tibary and Pearson, 2012; Woods *et al.*, 1985).

Placentitis may be diagnosed by transrectal and transabdominal ultrasound examination. Measurement of the combined thickness of the uterus and placenta (CTUP) by transrectal ultrasonography is particularly helpful in the diagnosis and monitoring of ascendant placentitis. The measurements are obtained 2.5 to 5 cm cranial to the cervical-placental junction using a 5- or 7.5-mHz linear transducer. The area measured should be on the ventral aspect of the uterine body just above the middle branch of the uterine artery. Normal CTUP for light horses is less than 8 mm between 271 and 300 days of gestation, less than 10 mm between 301 and 330 days of gestation, and less than 12 mm from 330 days of gestation to term. These measurements are slightly higher in Warmblood and Draft horses and lower in ponies (Barnes *et al.*, 2005). Placental malfunction has been associated with CTUP of greater than 15 mm in horses and greater than 12 mm in ponies after 310 days of gestation. During ultrasonographic evaluation, other features of infectious placentitis may be identified. These include placental separation, accumulation of purulent hyperechoic heterogenous fluid between the endometrium and the placenta, and increased echogenicity of fetal fluids. Increased echogenicity of the fetal fluids is caused by meconium, inflammatory debris, and hemorrhage (LeBlanc, 2010; Tibary *et al.*, 2006; Tibary *et al.*, 2014). In a recent study, mares with experimentally induced placentitis did not show a change in uterine artery blood flow but the CTUP was significantly increased. (Bailey *et al.*, 2012).

Transabdominal ultrasonographic evaluation allows assessement of fetal wellbeing as well as areas of the placenta near the body and the uterine horns. Features of placentitis include increased CTUP, increasing areas of placental edema or separation, fetal tachycardia (>130 bpm) or bradycardia (<50 bpm), fetal cardiac arrythmia, decreased fetal activity and tone, and increased fetal fluid echogenicity (cellular debris and meconium staining) (Tibary *et al.*, 2006).

Biochemical and endocrinological evaluation may also help in determining placental pathology and risk for abortion. Acute-phase proteins (serum amyloid A and haptoglobin) were shown to rapidely increase following experimental induction of ascendent placentitis (Canisso *et al.,* 2012). A change in serum progestin concentration (increase or decrease) by more than 50% or a value that is constantly out of the laboratory reference range signals placental pathology or fetal stress. Total progestins tend to decrease in aborting mares within 7 days whereas an increase is observed in chronic cases of placentitis. Levels of total estrogens below 1000 pg/mL between day 150 and 280 days of pregnancy were associated with a higher risk of abortion (Tibary and Pearson, 2012). After abortion or premature birth, most cases of chronic placentitis are easily recognized on gross examination, but microscopic histologic examination is important to determine the presence of acute placentitis. In acute placentitis the infection may be contained within the placenta, and the fetus is usually sterile. Some foals may be born alive with neonatal septicemia. Foals that are aborted, stillborn, or euthanized following experimental bacterial placentitis often display positive cultures from the airways and alveoli and some early pneumonia (Carrick *et al.*, 2010; LeBlanc, 2010).

Treatment of Placentitis

Treatment recommendations include tocolytic drugs to reduce uterine contractions, anti-inflammatory drugs to block the production of cytokines and prostaglandins, and antimicrobial therapy to control growth of bacteria. Antimicrobial therapy should be based on culture and sensitivity patterns of bacteria isolated from vaginal discharge or cervical swabs. Pharmacologic studies have shown that potentiated sulfonamides, gentamicin, and potassium penicillin can cross the placenta and reach MICs sufficient to control *S. equi* subsp. *zooepidemicus* (potassium penicillin 22,000 IU/kg every 6 hours [q6h] IV) and *E. coli* or *K. pneumoniae* (gentamicin, 6.6 mg/kg q24h IV). Trimethoprim-sulfamethoxazole (30 mg/kg q12h PO) presents an excellent choice for the treatment of placentitis caused by susceptible organisms because of its good uterine penetration (Bailey *et al.*, 2010; Graczyk *et al.*, 2006). However, ceftiofur crystalline free acid was not found in

sufficient therapeutic concentration in fetal fluids, placenta and fetal tissues following administration to pregnant mares and may not be a good choice for treatment of placentitis (MacPherson *et al.*, 2013). The most frequently used anti-inflammatory drugs are flunixin meglumine (0.5-1.1 mg/kg twice daily PO or IV) and phenylbutazone. However, dexamethasone and aspirin have also been used to control inflammation in mares with placentitis (Christian *et al.*, 2010). Pentoxifylline is often used to block the effects of endotoxin (8.5 mg/kg q12h PO) (Bailey *et al.*, 2010). Induction of uterine quiescence is obtained by administration of progestins to interfere with upregulation of prostaglandin and oxytocin and reduce myometrial activity. The oral synthetic progestin, altrenogest, is used at the label dose (0.044 mg/kg PO q24h), but twice as often (i.e. q12h), or twice the label dose (0.088 mg/kg PO q24h). Alternatively, progesterone in oil can be administered at 300 mg IM q24h. Long acting progesterone formulations (one injection lasting 12 or 30 days) are available through coumponding pharmacies but have not been thoroughly evaluated for use in mares with placentitis. In a report on 30 cases of placentitis, 29 delivered live foals following treatment. Of the foals born, 55% were healthy but small (between 311 to 347 days of gestation) while the remaining foals were compromised at birth (pregnancy length ranging from 299 to 355 days) (Barr, 2008). In one study, foals born to mares with placentitis were significanly more likely to have been born <320 days, and be diagnosed with perinatal asphyxia syndrome but had similar survival rates and discharge time as others. Treatment as outlined above reduced the risk for development of neonatal encephalitis, neonatal nephropathy and neonatal enteropathy (Carrick *et al.*, 2010; Palmer *et al.*, 2008).

Amnionitis and mare reproductive loss syndrome

Amnionitis and funistitis have been a feature of mares that aborted during the mare reproductive loss syndrome (MRLS) outbreak in Kentucky in 2000 and 2001 (Cawdell-Smith *et al.*, 2012; Todhunter *et al.*, 2013). This syndrome was associated with isolation of nonhemolytic *Actinobacillus* spp. and *a-Streptococcus* spp. in tissues of aborted fetuses. Mare reproductive loss syndrome was characterized by two types of pregnancy loss: early fetal loss between 40 and 80 days with a few losses up to 140 days; and late fetal losses between 10 months and term. Early fetal losses were characterized clinically by increased echogenicity of fetal fluids. Late pregnancy loss lesions included placentitis, funisitis, and perinatal fetal pneumonia. Some authors hypothesized that the pathogenesis of MRLS implicated hematogenous spread to the fetoplacental unit of bacteria carried from the oral cavity and intestinal tract by the setae, or stiff bristlelike barbed hairs, of the exoskeleton of the Eastern tent caterpillar. *Actinobacillus* spp. isolated from these cases were identical to the one found in the oral cavity and alimentary tracts of healthy horses which corroborates to some extent this hypothesis (Erol *et al.*, 2012; McDowell *et al.*, 2010). Abortion and similar clinical, pathological and bacteriological results were obtained recently in pregnant mares that received macerated whole processionary caterpillars (*Ochrogaster lunifer*) or their exoskeleton (Cawdell-Smith *et al.*, 2012; Todhunter *et al.*, 2013).

Equine Herpesvirus abortion

Despite the availability of inactivated and live herpesvirus vaccines, equine herpesvirus (EHV) remains a common cause of equine abortion. EHV-1 abortion storms continue to be reported in various areas of the world (Barbic *et al.*, 2012; Gryspeerdt *et al.*, 2011; Tibary *et al.*, 2014; Walter *et al.*, 2013). The principal mode of transmission from horse to horse is by direct contact with the virus through nasal secretions, reproductive tract discharge, placenta, or the aborted fetus. However, short-distance airborne spread of infection is possible. Farm personnel, equipment, feed, and water may act as fomites to facilitate transmission (Barbic *et al.*, 2012; Tibary *et al.*, 2014).

Some abortions are likely caused by reactivation of latent infection rather than primary exposure. The most common cause of equine herpesvirus abortion is EHV-1, although EHV-4 has also been isolated from some equine abortion cases. Both viruses cause similar lesions in the liver and lung; evaluation of the spleen is particularly useful for identification of red pulp necrosis caused by EHV-4 (Turan *et al.*, 2012). Traditionally, abortions have been attributed primarily to the non-neuropathogenic EHV-1; however, recently, neuropathogenic strains of EHV-1 have been detected in association with abortion (Forde *et al.*, 1987; Pronost *et al.*, 2010). Other equine herpesviruses (EHV-2 and EHV-5) have also been reported in a few cases of equine abortions. Regardless of which herpesvirus is involved, the pathogenesis of abortion is attributed to vascular necrosis. Viral nucleic acid can be demonstrated in endothelial cells of endometrial arterioles, within endometrial glands, and within placental microcotyledonary infarctions. Transplacental transfer of the virus may result in a virus-positive fetus, or severe endometrial vascular pathology (vasculitis and multifocal thrombosis) may result in abortion of a virus-negative fetus.

Abortion occurs most often during the last third of pregnancy. The risk of abortion depends on the strain and immune status of the dam (Gardiner *et al.*, 2012). The fetus is usually fresh and in good body condition. In

utero infection near term may result in the birth of a live infected foal that usually dies a few days later. The placenta is delivered along with the fetus or immediately after abortion and does not show any gross lesions. Gross fetal lesions may include accumulation of clear yellow fluid in the thoracic cavity, pericardial sac, mediastinum, peritoneal cavity and the perirenal areas. Many organs may have a hemorrhagic appearance. The lungs are often congested and edematous. The liver shows numerous pinpoint (1 to 2 mm) whitish foci of necrosis. Presence of fibrin within the airways is uncommon but considered pathognomonic. Microscopically, mutifocal areas of necrosis are observed in the lung, liver, adrenal glands, spleen, thymus and allantochorion. Herpetic intranuclear inclusion bodies may be present in infected cells (Tibary *et al.*, 2014).

For confirmation of herpesvirus abortion, antigen detection in combination with virus isolation, immunohistochemistry, or polymerase chain reaction (PCR) on fetal lung, liver, spleen, and thymus is recommended. Virologic and serologic investigation of the mare is also recommended (Easton et al., 2009; Pronost *et al.*, 2010).

Vaccines for the control of respiratory diseases caused by EHV-1 have been available for several decades, and currently more than a dozen commercial vaccines are available throughout the world (Bresgen, 2011). There are also several vaccines that claim protection against abortion caused by EHV-1. These vaccines should be administered according to the manufacturer's label instructions, usually at 5, 7, and 9 months of gestation. It is important to realize that EHV-1 abortion may occur despite regular vaccination. Causative factors include the mare's individual immunity, level of contamination, virulence of the viral strain, and the performance of available vaccine. Therefore, for maximum protection, vaccination strategies should be combined with appropriate biosecurity measures to minimize the likelihood of exposure of pregnant mares to EHV. Vaccination has been shown to supress EHV-1 nasal shedding and viremia (Goehring *et al.*, 2010). Serum neutralizing titers drop rapidly within the first 4 weeks of life in foals born to vaccinated mares (Bresgen, 2011).

Management practices that should be part of an EHV abortion prevention program include maintenance of small groups of horses segregated by age and by immune and physiologic status (pregnancy status and stage). Foaling mares should be segregated from the rest of the herd. Particular attention should be given to the risk of mixing equids from different species that may carry different susceptibility or strains of EHV. The most important epidemiologic risk is posed by introduction of new horses onto the farm. If introduction of new animals cannot be avoided, a 21-day quarantine is recommended. Analysis of nasopharyngeal swab samples with a PCR test may help detect infected horses.

An outbreak of EHV mandates early diagnosis of infected animals and interruption of viral transmission using strict sanitary measures for movement of personnel and animals between stables and paddocks, as well as use of disinfectants. Particular attention should be given to disposal of or shipping of fetuses and placenta to appropriate diagnostic laboratories. Mating activities should preferably be halted during an active outbreak.

Equine Viral Arteritis abortion

Equine viral arteritis (EVA) is a venereal disease of horses that can result in abortion usually late in gestation (Holyoak *et al.*, 2008; Hörügel *et al.*, 2008; Ruiz-Saénz, 2010). Abortion rates can approach 60% in a naive population as the result of direct impairment of placental function and severe fetal infection. Aborted fetuses may be fresh or autolyzed and show subcutaneous edema, petechial hemorrhages in the pleura and epicardium, and increases in pleural fluid volume. However, these changes are not necessarily present in all EVA abortions (Pronost *et al.*, 2010b). Gross pathologic changes have also been reported in a few affected placentas, including placentitis and full-thickness necrosis. Other nonpathognomonic histopathologic lesions in the fetus include vasculitis and perivasculitis in the heart, lung, and spleen; pneumonia and hemorrhage in alveoli; and inflammatory changes in the liver, spleen, and placenta. Diagnosis can be made by viral isolation, immunohistochemistry, PCR, or serology (seronutralization). Commercial ELISA tests have very poor sensitivity (26%) compared to the virus neutralization test which is considered to be the gold standard for serology. A new cELISA test seems to be more promising in terms of sensitivity and specifity (Chung *et al.*, 2013). Control of the disease is based on detection of the shedding stallion(s) and vaccination. The role of persistent infection in stallions in recent outbreaks in France, the United States, and other countries demonstrated the importance of testing all non-vaccinated stallions before breeding or shipping of semen for artificial insemination (Dennis *et al.*, 2014; Holyoak *et al.*, 2008; Hörügel *et al.*, 2008; Miszczak *et al.*, 2012; Pronost *et al.*, 2010b). Embryos from mares inseminated with semen from persistently infected stallions carry the virus (Broaddus *et al.*, 2011). It is important to note that transmission of the virus was possible despite embryo washing and trypsin treatment. Vaccination of pregnant mares in the face of an outbreak has been recently investigated. The vaccine is safe for healthy pregnant mares up to 3 months prior to

foaling. There is a significant risk for abortion if mares are vaccinated in the last 2 months of pregnancy (Broaddus *et al.*, 2011).

Other infectious causes of abortion

Abortion in mares has been associated with a variety of other organisms (see review, Tibary *et al.*, 2014). Protozoa, including *Trypanosoma evansi*, *Trypanosoma equiperdum, Babesia* spp. (*Babesia caballi and Babesia equi* now *Theleiria equi*) and *Neospora* spp. have been reported as causes of abortion. However, studies on the pathogenesis of abortion caused by these parasites are scarce. Vertical, transplacental transmission of Theleiria equi has been demonstrated. Genital chlamydiosis of horses is reported to result in occasional abortion. Equine abortions have been associated with *Salmonella abortus equi*, *Shigella* spp., *Brucella abortus*, *Brucella melitensis*, and *Brucella suis*. However most of these reports have been based on serology and lack strong scientific evidence. Neorickettsia risticii can be transmitted transplacentally and has also been identified as a cause of fetal resorption, abortion, and birth of weak foals in horses. Abortion from *Aeromonas hydrophila*, a bacterium found in stagnant water, has been reported in a few horses. An unusual case of abortion in a 6-year-old mare has been associated with a spirochete (*Borrelia parkei–B. turicatae*) transmitted by ticks in California. The spirochete was isolated from the fetus, suggesting transplacental transmission.

Non-infectious causes of abortion

Non-infectious equine abortions are usually sporadic in nature. Causes include twinning (Figure 9), umbilical cord anomalies (torsions) (Figure 10), abnormal placentation (avillous areas) and severe fetal congenital abnormalities (Bencharif *et al.*, 2010; Mizushima, 2005; Smith *et al.*, 2003; Snider, 2007).

Diagnostic Approach for Equine Abortion

Diagnosis should be pursued in any case of abortion, premature birth, or birth of a compromised or septicemic foal (Schlafer, 2004; Tibary *et al.*, 2014). Diagnosis of the cause of abortion or in utero infection can be made in most cases with proper history, clinical observations, and collection and submission of all required samples (Table 3). The importance of placental examination in the diagnosis of abortion, stillbirths, or premature births cannot be overemphasized. Normal and abnormal characteristics of the placenta and descriptions of proper examination of the equine placenta and its pathology have been described elsewhere. Weight of the placenta should be determined; normal placental weight is approximately 11% of the foal weight. The placenta should be gently cleaned of any bedding material, grass, or dirt with cold water, then laid out flat and all surfaces examined. The umbilical cord should be of normal length. The amniotic sac and chorionic surface is examined from the cervical star to the tips of the horns on all sides. Samples should be obtained from areas of grossly normal and abnormal placenta and submitted for histopathology, immunohistochemistry, and culture. Some morphologic characteristics of the placenta may allow immediate exclusion of infectious causes of abortion (e.g., umbilical cord torsion, body pregnancy, twin pregnancies). Chronic infections are generally easy to detect because of the thick, leathery nature of the placenta. Lesions of placentitis appear tan or brown and thick and may have overlying tenacious, fibrinonecrotic exudate. Cytologic evaluation of a contact smear may reveal inflammatory cells and responsible organisms. Lesions of ascending placentitis are usually located on the cervical star, whereas hematogenous placentitis, as in leptospirosis, may cause diffuse lesions. Nocardioform placentitis has characteristic lesions on the cranial ventral uterine body.

Although it is possible to perform fetal or neonatal necropsy in the field when necessary, it is preferred that the entire carcass be sent to a diagnostic laboratory if possible. If fetal necropsy is performed in the field, fetal weight and size (crown-rump length) should be determined. Proper precautions should be taken to document lesions, prevent contamination, and obtain appropriate samples. Fetal blood (heart blood) samples as well as pericardial, pleural and peritoneal fluids should be obtained. Stomach contents provide an excellent sample for bacteriology. Tissue samples from any abnormal-appearing tissues and from all major organs (e.g. liver, lung, kidney, adrenal gland, placenta, heart, thymus, brain, spleen, small intestine) should be submitted for histopathologic evaluation.

CONCLUSION

Pregnancy loss causes significant economic loss to the equine industry. The diagnosis of the cause of pregnancy loss is often complicated and frustrating for both the veterinarian and client. Several factors may contribute to early pregnancy loss however age and uterine health are the most important. Mares with recurrent early pregnancy loss should undergo a complete breeding soundness examination including endometrial biopsy and cytogenetic evaluation. Mares at risk for pregnancy loss should be identified and other methods of reproduction (i.e. embryo transfer) should be contemplated if possible. The most common infectious cause of abortion is bacterial placentitis which can be detected and managed clinically. Client education regarding collection of data and all samples for

laboratory submission is extremely important. The veterinary practitioner should always be aware of the epidemiological situation of infectious causes of abortion in their region or practice. Veterinarians need to develop a protocol with their clients and diagnostic labs for handling of samples and biosecurity procedures.
.

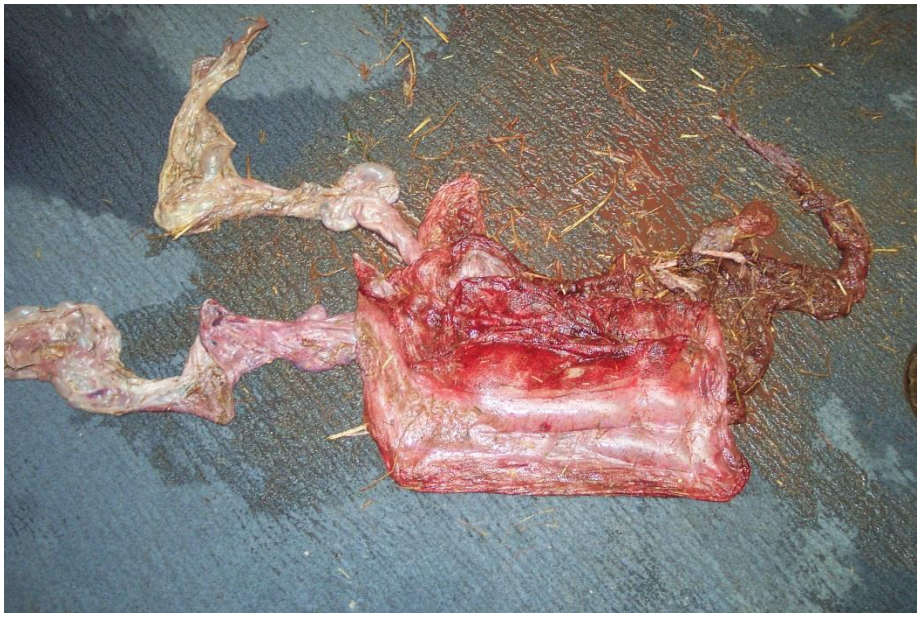

Figure 9: Twin placenta showing a large avillous area where the two placentae were adjacent to each other.

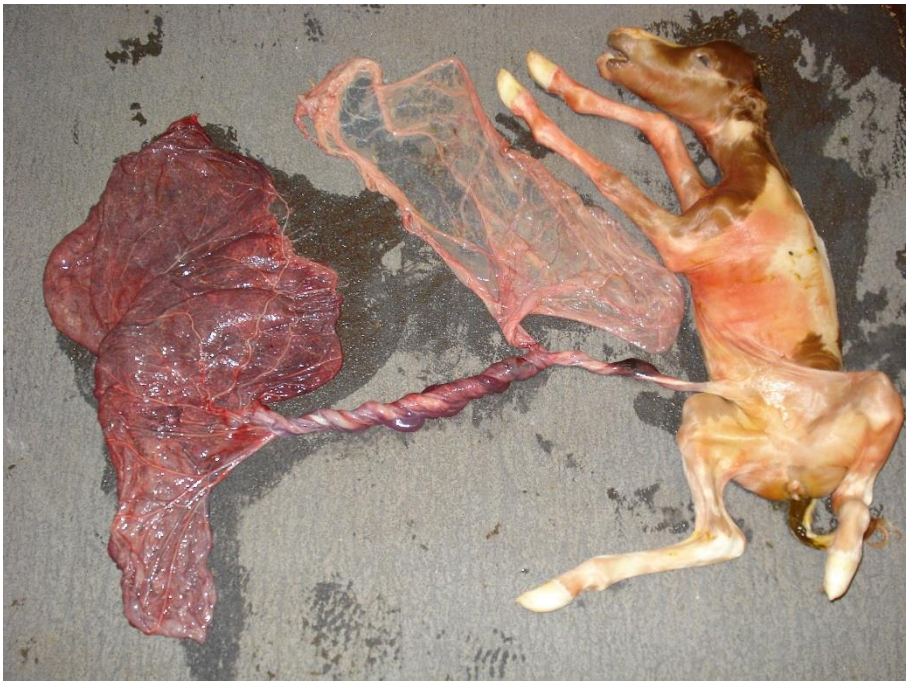

Figure 10: Abortion due to umbilical cord torsion in a miniature mare. Note the excessive number of twists, edema and hemorrhage within the cord.

Table 3: Gross fetal and placental lesions associated with some causes of abortion

Gross lesions	Differential diagnoses
Fresh fetus and placenta	EHV
Underdeveloped fetus	Twinning, IUGR
Mummification	Twinning
Pinpoint white foci on liver	EHV
Fetal pneumonia	EHV, placentitis, MRLS
Thickened placenta at the cervical star	Ascendant placentitis (bacterial or fungal)
Thickened placenta with area of diffuse placentitis	Leptospirosis, salmonellosis
Placentitis in the uterine body and base of the uterine horns	Nocardioform placentitis
Large avillous areas	Twinning, uterine fibrosis
Umbilical cord edema and/or funisitis	Umbilical cord torsion, MRLS
Placental edema, allantoic cysts	Uterine abnormalities

REFERENCES

- Adams G, Kastelic J, Bergfelt D, Ginther O. Effect of uterine inflammation and ultrasonically detected uterine pathology on fertility in the mare. J Reprod Fertil 1987; 35(Suppl):445-54.
- Allen WR. Luteal deficiency and embryo mortality in the mare. Reprod Domest Anim. 2001; 36:121-31.
- Allen WR, Brown L, Wright M, Wilsher S. Reproductive efficiency of flatrace and national hunt Thoroughbred mares and stallions in England. Equine Veterinary Journal 2007; 39:438-45.
- Bailey CS, Macpherson ML, Pozor MA, Troedsson MHT, Benson S, Giguere S, *et al.* Treatment efficacy of trimethoprim sulfamethoxazole, pentoxifylline and altrenogest in experimentally induced equine placentitis. Theriogenology; 2010, 74:402-12.
- Bailey CS, Heitzman JM, Buchanan CN, Bare CA, Sper RB, Borst LB, *et al.* B-mode and Doppler ultrasonography in pony mares with experimentally induced ascending placentitis. Equine Veterinary Journal; 2012. 44:88-94.
- Ball B, Hillman R, Woods G. Survival of equine embryos transferred to normal and subfertile mares. Theriogenology; 1987. 28: 167-74.
- Ball B, Little T, Webb B, Woods G. 1989. Survival of day-4 embryos from young, normal mares and aged, subfertile mares after transfer to normal recipient mares. J Reprod Fertil, 1989; 85:187-94.
- Ball BA, Woods GL. Embryonic Loss and Early-Pregnancy Loss in the Mare. Comp Cont Educ Pract. 1987; 9:459-471.
- Barbacini S, Gulden P, Marchi V, Zavaglia G. Incidence of embryo loss in mares inseminated before or after ovulation. Equine Veterinary Education; 1999. 11:251-254.
- Barbic L, Lojkic I, Stevanovic V, Bedekovic T, Staresina V, Lemo N, *et al.* Two outbreaks of neuropathogenic equine herpesvirus type 1 with breed-dependent clinical signs. Vet Rec; 2012. 170:227.
- Barnes M, Fite C, Tibary A. Trans-rectal ultrasonographic evaluation of the placenta in Arabian and pony mares in mid-to-late gestation. Theriogenology; 2005. 64:787.
- Barr BS. The outcome of foals born to mares treated for placentitis. 1Havemeyer Foundation Monograph Series 2008. 19; 49-50.
- Bell R, Bristol F. Fertility and pregnancy loss after delay of foal oestrus with progesterone and oestradiol-17b. J Reprod Fertil;Suppl 1987. 35:667-669.
- Bencharif D, Tainturier D, Amirat L, Betti E, Guintard C. Case study of abortion in a mare: coelosomy with urachal dilatation following umbilical cord torsion. J Equine Vet Sci 2010; 30:208-212.
- Blanchard T, Thompson J, Brinsko S, Stich K, Wendt K, Varner DD *et al.* Mating mares on foal heat: a five-year retrospective study. Lexington: American Association of Equine Practitioners (AAEP); 2004. 525-530.
- Blanchard TL, Varner DD, Fernandez M, Buoen L, Zhang TQ, Womack JE, *et al.* Stallion karyotype variability and lack of association with abortion: a case report. Theriogenology; 1994. 41:777-784.
- Bresgen C. Efficacy of different vaccines against infections with equine herpesvirustype 1 (EHV-1). Thesis, Freie Universität Berlin. 2011.
- Broaddus CC, Balasuriya UBR, Timoney PJ, White JLR, Makloski C, Torrisi K, *et al.* Infection of embryos following insemination of donor mares with equine arteritis virus infective semen. Theriogenology; 2011. 76:47-60.
- Broaddus CC, Balasuriya UBR, White JLR, Timoney PJ, Funk RA, Holyoak GR. Evaluation of the safety of vaccinating mares against equine viral arteritis during mid or late gestation or during the immediate

postpartum period. Javma-J Am Vet Med A; 2011. 238:741-750.
- Bruck I, Anderson G, Hyland J. Reproductive performance of thoroughbred mares on six commercial stud farms. Aust Vet J; 1993. 70:299-303.
- Canisso IF, Troedsson MH, Cray C, Ball BA. Serum amyloid A and haptoglobin concentrations in pregnant mares with experimentally induced ascending placentitis. Proceeding of the 58th Annual Convention of AAEP. 2012. 524-525.
- Canisso IF, Beltaire KA, Bedford-Guaus SJ. Premature luteal regression in a pregnant mare and subsequent pregnancy maintenance with the use of oral altrenogest. Equine Veterinary Journal; 2013. 45:97-100.
- Canisso IF, Ball BA, Erol E, Claes A, Scoggin KE, McDowell KJ, *et al*. Attempts to induce nocardioform placentitis (Crossiela equi) experimentally in mares. Equine Veterinary Journal; 2015. 47:91-95.
- Carnevale E, Ginther O. Relationships of age to uterine function and reproductive efficiency in mares. Theriogenology; 1992. 37:1110-5.
- Carnevale E, Griffin P, Ginther O. Age-associated subfertility before entry of embryos into the uterus of mares. Equine Veterinary Journal;Suppl 1993. 15:31-35.
- Carnevale E, Ginther O. Defective oocytes as a cause of subfertility in old mares. Biol Reprod;Mono 1995. 1:209-214.
- Carnevale E, Coutinho da Silva M, Panzani D, Stokes J, Squires E. Factors affecting the success of oocyte transfer in a clinical program for subfertile mares. Theriogenology; 2005. 64:519-527.
- Carrick JB, Axon JE, Collins N. The association of placentitis with clinical signs at admission, diagnosis and outcome of foals admitted to an equine neonatal intensive care unit. Australian Equine Veterinarian; 2010. 29:69.
- Cawdell-Smith AJ, Todhunter KH, Anderson ST, Perkins NR, Bryden WL. Equine amnionitis and fetal loss: Mare abortion following experimental exposure to Processionary caterpillars (Ochrogaster lunifer). Equine Veterinary Journal; 2012. 44:282-288.
- Chevalier-Clement F, Palmer E. Ultrasonic echography in the mare. J Reprod Fertil;Suppl 1982. 32:423-430.
- Chevalier-Clement F. Pregnancy Loss in the Mare. Anim Reprod Sci; 198920:231-244.
- Christiansen DL, Moulton K, Walters FK, Cooley AJ, LeBlanc MM, Ryan P. Evidenced-based medicine approach to develop efficacious therapies for late-gestation mares presenting with uterine infections using an experimentally-induced placentitis model. Animal Reproduction Science; 2010. 121:345-346.
- Chung CW, Wilson C, Timoney P, Adams E, Adams DS, Chung JS, *et al*. Comparison of an improved competitive enzyme-linked immunosorbent assay with the World Organization for Animal Health-prescribed serum neutralization assay for detection of antibodey to Equine arteritis virus. J Vet Diagn Invest; 2013. 25:182-188.
- Daels P, Stabenfeldt G, Hughes J, Odensvik K, Kindahl H. Evaluation of progesterone deficiency as a cause of fetal death in mares with experimentally induced endotoxemia. Am J Vet Res; 1991a 52:282-288.
- Daels P, Stabenfeldt G, Hughes J, Odensvik K, Kindahl H. Effects of flunixin meglumine on endotoxin-induced prostaglandin F2 alpha secretion during early pregnancy in mares. Am J Vet Res; 1991b 52:276-281.
- Darenius K, Fredriksson G, Kindahl H. Allyl trenbolone and flunixin meglumine treatment of mares with repeated embryonic loss. Equine Veterinary Journal;Suppl 1989. 8:35-39.
- Davies Morel M, Newcombe J, Swindlehurst J. The effect of age on multiple ovulation rates, multiple pregnancy rates and embryonic vesicle diameter in the mare. Theriogenology; 2005. 63:2482-2493.
- Dennis S, Pearson LK, Campbell AJ, Tibary A. Interstate Equine Semen and Embryo Shipment Regulations in the United States and Their Implications on Control of Disease Transmission. J Equine Vet Sci; 2014. 34:897-902.
- Donahue JM, Williams NM, Sells SF, Labeda DP. Crossiella equi sp. nov. isolated from equine placentas. Int J Syst Evol Microbiol; 2002. 52:2169-2173.
- Donahue JM, Sells SF, Bolin DC. Classification of Actinobacillus spp. isolates from horses involved in mare reproductive loss syndrome. American Journal of Veterinary Research; 2006. 67:1426-1432.
- Drumm NJ, Embertson RM, Woodie JB, Ruggles AJ, Hopper SA, Fimmers R, *et al*. Factors influencing foaling rate following colic surgery in pregnant Thoroughbred mares in Central Kentucky. Equine Veterinary Journal; 2013. 45:346-349.
- Easton C, Fuentealba NA, Paullier C, Alonzo P, Carluccio J, Galosi CM. Immunohistochemical and molecular detection of equine herpesvirus 1 in Uruguay. Rev Sci Tech Oie; 2009. 28:1085-1090.
- Eilts B, Scholl D, Paccamonti D, Causey R, Klimczak J, Corley J. Prevalence of endometrial cysts and their effect on fertility. Biol Reprod;Mono 1995. 1:527-532.
- Erol E, Sells SF, Williams NM, Kennedy L, Locke SJ, Labeda DP, *et al*. An investigation of a recent outbreak of nocardioform placentitis caused abortions in horses. Vet Microbiol; 2012. 158:425-430.

- Fiala SM, Trein CR, Meirelles L, Malschitzky E, Winter G, Garbade P, et al. Reduction of twin pregnancy in thoroughbred mares either before or after fixation. Acta Scientiae Veterinariae; 2003. 31:145-148.
- Forde D, Keenan L, Wade J, O'Connor M, Roche J. Reproductive wastage in the mare and its relationship to progesterone in early pregnancy. J Reprod Fertil;Suppl 1987. 35:493-495.
- Fritsche AK, Borchers K. Detection of neuropathogenic strains of Equid Herpesvirus 1 (EHV-1) associated with abortions in Germany. Vet Microbiol; 2011. 147:176-180.
- Gardiner DW, Lunn DP, Goehring LS, Chiang YW, Cook C, Osterrieder N, *et al*. Strain impact on equine herpesvirus type 1 (EHV-1) abortion models: Viral loads in fetal and placental tissues and foals. Vaccine; 2012. 30:6564-6572.
- Giles RC, Donahue JM, Hong CB, Tuttle PA, Petrites-Murphy MB, Poonacha KB, *et al*.. Causes of abortion, stillbirth and perinatal death in horses: 3527 cases (1986-1991). Journal of the American Veterinary Medical Association; 1993203:1170-1175.
- Ginther O. Embryonic loss in mares: incidence, time of occurrence, and hormonal involvement. Theriogenology; 1985. 23:77-89.
- Ginther O. Embryonic loss in mares: nature of loss after experimental induction by ovariectomy or prostaglandin F2a. Theriogenology; 1985. 24:87-98.
- Ginther O, Bergfelt D, Leith G, Scraba S. Embryonic loss inmares: incidence and ultrasonic morphology. Theriogenology; 1985. 24:73-86.
- Goehring LS, Wagner B, Bigbie R, Hussey SB, Rao S, Morley PS, *et al*. Control of EHV-1 viremia and nasal shedding by commercial vaccines. Vaccine; 2010. 28:5203-5211.
- Graczyk J, Macpherson ML, Pozor MA, Troedsson MHT, Eichelberger AC, LeBlanc MM, *et al*. Treatment efficacyof trimethoprim sulfamethoxazole and pentoxifylline in equine placentitis. Animal Reproduction Science; 2006. 94:434-435.
- Griffin RL BS. Nd:YAG laser photoablation of endometrial cysts: a review of 55 cases (2000-2001). Proceedings 48th Annual Convention of AAEP; 2002. 48: 58-60.
- Gryspeerdt A, Vandekerckhove A, Van Doorsselaere J, Van de Walle GR, Nauwynck HJ. Description of an unusually large outbreak of nervous system disorders caused by equine herpesvirus 1 (EHV1) in 2009 in Belgium. Vlaams Diergen Tijds; 2011. 80:147-153.
- Hemberg E, Lundeheim N, Einarsson S. Reproductive performance of thoroughbred mares in Sweden. Reprod Domest Anim; 2004. 39:81-5.
- Henneke D, Potter G, Kreider J. Body condition during pregnancy and lactation and reproductive efficiency of mares. Theriogenology; 1984. 21:897-909.
- Hinrichs K, Choi Y, Walckenaer B, Varner D, Hartman D. In vitro produced equine embryos: Production of foals after transfer, assessment by differential staining and effect of medium calcium concentrations during culture. Theriogenology; 2007. 68:521-529.
- Holyoak GR, Balasuriya UBR, Broaddus CC, Timoney PJ. Equine viral arteritis: current status and prevention. Theriogenology; 2008. 70:403-414.
- Hong CB, Donahue JM, Giles RC, Petrites-Murphy MB, Poonacha KB, Roberts AW, *et al*. Etiology and pathology of equine placentitis. J Vet Diagn Invest; 1993. 5:56-63.
- Hong CB, Donahue JM, Giles RC, Petritesmurphy MB, Poonacha KB, Roberts AW, *et al*. Equine Abortion and Stillbirth in Central Kentucky During 1988 and 1989 Foaling Seasons. J Vet Diagn Invest; 1993. 5:560-6.
- Hörügel U, Dietz U, Muluneh A, Hardt M, Wensch HJ, May M. Outbreak of equine virus arteritis (EVA) on two stud farms in Saxonia - a case report. Praktische Tierarzt; 2008. 89:484-491.
- Irvine C, Sutton P, Turner J, Mennick P. Changes in plasma progesterone concentrations from Days 17 to 42 of gestation in mares maintaining or losing pregnancy. Equine Veterinary Journal; 1990. 22:104-106.
- Jobert ML, LeBlanc MM, Pierce SW. Pregnancy loss rate in equine uterine body pregnancies. Equine Veterinary Education; 2005. 17:163-165.
- Journée SL, Ruijter-Villani Md, Hendriks WK, Stout TAE. Efficacy of transvaginal ultrasound-guided twin reduction in the mare by embryonic or fetal stabbing compared with yolk sac or allantoic fluid aspiration. Theriogenology; 2013. 80:346-349.
- Koskinen E, Lindeberg H, Kuntsi H, Ruotsalainen L, Katila T. Fertility of mares after postovulatory insemination. Zentralbl Veterinarmed A; 1990. 37:77-80.
- Labeda DP, Price NP, Kroppenstedt RM. Streptomyces atriruber sp. nov. and Streptomyces silaceus sp. nov., two novel species of equine origin. Int J Syst Evol Microbiol; 2009. 59:2899-903.
- Laugier C, Foucher N, Sevin C, Leon A, Tapprest J. A 24-Year Retrospective Study of Equine Abortion in Normandy (France). J Equine Vet Sci; 2011. 31:116-123.
- Lear TL, Lundquist J, Zent WW, Fishback WD, Jr., Clark A. Three autosomal chromosome translocations associated with repeated early embryonic loss (REEL) in the domestic horse (Equus

caballus). Cytogenetic and Genome Research; 2008. 120:117-122.
- LeBlanc MM. Ascending Placentitis in the Mare: An Update. Reprod Domest Anim; 2010. 45:28-34.
- Lowis T, Hyland J. Analysis of post-partum fertility in mares on a thoroughbred stud in southern Victoria. Aust Vet J; 1991. 68:304-306.
- Lyle SK. Immunology of infective preterm delivery in the mare. Equine Veterinary Journal; 2014. 46:661-668.
- MacPherson ML, Blanchard TL. Breeding mares on foal-heat. Equine Veterinary Education; 2005. 17:44-52.
- Macpherson ML, Giguère S, Hatzel JN, Pozor M, Benson S, Diaw M, *et al*. Disposition of desfuroylceftiofur acetamide in serum, placental tissue, fetal fluids, and fetal tissues after administration of ceftiofur crystalline free acid (CCFA) to pony mares with placentitis. Journal of Veterinary Pharmacology and Therapeutics; 2013. 36:59-67.
- Marcolongo-Pereira C, Adrien MD, Ladeira SRL, Soares MP, Assis-Brasil ND, Schild AL. Equine abortion in Southern Brazil: study of 72 cases. Pesquisa Vet Brasil; 2012. 32:22-26.
- McDowell KJ, Webb BA, Williams NM, Donahue JM, Newman KE, Lindemann MD, *et al*. The role of caterpillars in mare reproductive loss syndrome: a model for environmental causes of abortion. Journal of Animal Science; 2010. 88:1379-1387.
- Meyers P, Bonnett B, Mckee S. Quantifying the Occurrence of Early Embryonic Mortality on 3 Equine Breeding Farms. Can Vet J; 1991. 32:665-672.
- Miszczak F, Legrand L, Balasuriya UBR, Ferry-Abitbol B, Zhang J, Hans A, *et al*. Emergence of novel equine arteritis virus (EAV) variants during persistent infection in the stallion: origin of the 2007 French EAV outbreak was linked to an EAV strain present in the semen of a persistently infected carrier stallion. Virology; 2012. 423:165-174.
- Miyakoshi D, Shikichi M, Ito K, Iwata K, Okai K, Sato F, *et al*. Factors Influencing the Frequency of Pregnancy Loss among Thoroughbred Mares in Hidaka, Japan. J Equine Vet Sci; 2012. 32:552-557.
- Mizushima C. Late-term abortion associated with umbilical cord torsion in the mare: case report. J Equine Vet Sci; 2005. 25:162-163.
- Morresey P. Metabolic diseases – do we really know if they affect reproduction? Clinical Theriogenology; 2013. 5:334-338.
- Morris LHA, Allen WR. Reproductive efficiency of intensively managed Thoroughbred mares in Newmarket. Equine Veterinary Journal; 2002. 34:51-60.
- Newcombe J. Observations on early pregnancy diagnosis and early embryonic loss in the mare. Irish Vet J; 1997. 50:534-536.
- Newcombe J. Spontaneous oestrous behaviour during pregnancy associated with luteal regression, ovulation and birth of a live foal in a part Thoroughbred mare. Equine Veterinary Education; 2000. 2:111-114.
- Newcombe J, Wilson M. Age, body weight, and pregnancy loss. J Equine Vet Sci; 2005. 25:188-194.
- Newcombe JR, Cuervo-Arango J. The effect of time of insemination with fresh cooled transported semen and natural mating relative to ovulation on pregnancy and embryo loss rates in the mare. Reprod Domest Anim; 2011. 46:678-681.
- Niekerk van CH, Morgenthal JC. Fetal loss and the effect of stress on plasma progestagen levels in pregnant Thoroughbred mares. Journal of Reproduction and Fertility; Suppl. 1982. 32:453-457.
- Niekerk van F, Niekerk van C. The effect of dietary protein on reproduction in the mare. VII. Embryonic development, early embryonic death, foetal losses and their relationship with serum progestagen. J S Afr Vet Assoc; 1998. 69:150-5.
- Ousey JC, Kolling M, Newton R, Wright M, Allen WR. 2012. Uterine haemodynamics in young and aged pregnant mares measured using Doppler ultrasonography. Equine Veterinary Journal; 44:15-21.
- Palmer JE, Sertich PL, Cornman BI, Wilkins PA, Boston RC. Placentitis associated neonatal problems and the effect of therapeutic interventions. Havemeyer Foundation Monograph Series, 2008; 19; 45.
- Papa F, Lopes M, Alvarenga M, Meira C, Luvizotto M, Langoni H. Early embryonic death in mares: clinical and hormonal aspects. Braz J Vet Res Anim Sci; 1998. 35:170-173.
- Pronost S, Léon A, Legrand L, Fortier C, Miszczak F, Freymuth F, *et al*. Neuropathogenic and non-neuropathogenic variants of equine herpesvirus 1 in France. Vet Microbiol; 2010. 145:329-333.
- Pronost S, Pitel PH, Miszczak F, Legrand L, Marcillaud-Pitel C, Hamon M, *et al*. Description of the first recorded major occurrence of equine viral arteritis in France. Equine Veterinary Journal; 2010b. 42:713-720.
- Pycock J, Newcombe J. The effect of the gonadotrophin-releasing hormone analog, buserelin, administered in diestrus on pregnancy rates and pregnancy failure in mares. Theriogenology; 1996. 46:1097-1101.
- Rambags B, Colenbrander B, Stout T. Early pregnancy loss in aged mares: probable causes and

possible cures. Pferdeheilkunde; 2003. 19:653-656.
- Rambags B, van Boxtel D, Tharasanit T, Lenstra J, Colenbrander B, Stout T. Oocyte mitochondrial degeneration during reproductive ageing in the mare. Havemeyer Foundation Monograph Series; 2006. 18:25-27.
- Rebordao MR, Galvao A, Szostek A, Amaral A, Mateus L, Skarzynski DJ, *et al*. Physiopathologic Mechanisms Involved in Mare Endometrosis. Reprod Domest Anim; 2014. 49:82-87.
- Ruiz-Sáenz J. Equine viral arteritis: epidemiological and intervention perspectives. Revista Colombiana De Ciencias Pecuarias; 2010. 23:501-512.
- Schlafer DW. Postmortem examination of the equine placenta, fetus, and neonate: methods and interpretation of findings, Proceedings of the 50th Annual Convention of AAEP; 2004. 144-161.
- Sharma S, Morel MCGD, Dhaliwal GS, Dadarwal D. The pattern of embryonic fixation and its relationship to pregnancy loss in thoroughbred mares. Reprod Domest Anim; 2010. 45:e61-e7.
- Sieme H, Lüttgenau J, Sielhorst J, Martinsson G, Bollwein H, Thomas S, *et al*. Improving the formation and function of the corpus luteum in the mare. Revista Brasileira de Reprodução Animal; 2015. 39:117-120.
- Simpson D, Greenwood R, Ricketts S, Rossdale P, Sanderson M, Allen W. Use of ultrasound echography for early diagnosis of single and twin pregnancy in the mare. J Reprod Fertil;Suppl 1982. 31:431-439.
- Smith KC, Blunden AS, Whitwell KE, Dunn KA, Wales AD. A survey of equine abortion, stillbirth and neonatal death in the UK between 1988 and 1997. Equine Veterinary Journal; 2003. 35:496-501.
- Snider TA. Umbilical cord torsion and coiling as a cause of dystocia and intrauterine foal loss. Equine Veterinary Education; 2007. 19:532-534.
- Stanton M, Steiner J, Pugh D. Endometrial cysts in the mare. J Equine Vet Sci; 2004. 24:14-19.
- Tannus RJ, Thun R. Influence of endometrial cysts on conception rate of mares. Journal of Veterinary Medicine Series A; 1995. 42:275-283.
- Tibary A, Fite C, Sghiri A, Bakkoury M. Pregnancy complication and evaluation for fetal well-being. Proceeding 9th WEVA, January 22-26, 2006, Marrakech, Morocco, 196-206.
- Tibary A, Pearson L, Sghiri A, Rodriguez J. Petes embryonnaires précose chez la jument. In. Tibary A and Sghiri A (ed). Jument et Poulains: Suivi de la gestatin, du poulinage et du nouveau-né, Actes Editions, Rabat-Instituts. 2012. 81-102
- Tibary A, Pearson LK. Mare problems in the last month of pregnancy. Proceedings of the 58th Annual Convention of AAEP; 2012; 350-358.
- Tibary A, Pearson LK. Les endométrites dégénératives (endométroses) (Degenerative endometritis – endometrosis in the mare. Le Nouveau Praticien Vétérinaire Equine. In press. 2015.
- Tibary A, Pearson LK, Fite CL. Chapter 8 - Reproductive Tract Infections. In: Sellon DC, Long MT, editors. Equine Infectious Diseases (Second Edition). St. Louis: W.B. Saunders; pages. 2014. 84-106.
- Timoney JF, Kalimuthusamy N, Velineni S, Donahue JM, Artiushin SC, Fettinger M. A unique genotype of Leptospira interrogans serovar Pomona type kennewicki is associated with equine abortion. Vet Microbiol; 2011. 150:349-353.
- Todhunter KH, Perkins NR, Wylie RM, Chicken C, Blishen AJ, Racklyeft DJ, *et al*. Equine amnionitis and fetal loss: The case definition for an unrecognised cause of abortion in mares. Aust Vet; 2008. 87:35-38.
- Todhunter KH, Muscatello G, Blishen AJ, Chicken C, Perkins NR, Gilkerson JR, *et al*. Bacteria isolated from field cases of equine amnionitis and fetal loss. Aust Vet J; 2013. 91:138-142.
- Turan N, Yildirim F, Altan E, Sennazli G, Gurel A, Diallo I, *et al*. Molecular and pathological investigations of EHV-1 and EHV-4 infections in horses in Turkey. Res Vet Sci; 2012. 93:1504-1507.
- Vanderwall D, Squires E, Brinsko S, McCue P. Diagnosis and management of abnormal embryonic development characterized by formation of an embryonic vesicle without an embryo in mares. J Am Vet Med Assoc; 2000. 217:58-63.
- Vanderwall D, Woods G, Roser J, Schlafer D, Sellon D, Tester D. Equine cloning: applications and outcomes. Reprod Fertil Dev; 2006. 18:91-98.
- Vanderwall D, Marquardt J, Woods G. Use of a compounded long-acting progesterone formulation for equine pregnancy maintenance. J Equine Vet Sci; 2007. 27:62-66.
- Villahoz M, Squires E, Voss J, Shideler R. Some observations on early embryonic death in mares. Theriogenology; 1985. 23:915-924.
- Vogelsang M, Vogelsang S, Lindsey B, Massey J. Reproductive performance in mares subjected to examination by diagnostic ultrasound. Theriogenology; 1989. 32:95-103.
- Vogelsang S, Vogelsang M. Influence of donor parity and age on the success of commercial equine embryo transfer. Equine Vet J;Suppl 1989. 1:71-72.
- Walter J, Seeh C, Fey K, Bleul U, Osterrieder N. Clinical observations and management of a severe equine herpesvirus type 1 outbreak with abortion and encephalomyelitis. Acta Vet Scand; 2013. 55:19.
- Wolfsdorf KE, Macpherson ML. Management of twins. Clinical Theriogenology; 2010. 2:326-331.

- Woods G, Baker C, Bilinski J, Hillman R, Mank E, Sluijter F. A field study on early pregnancy loss in Standardbred and Thoroughbred mares. J Equine Vet Sci; 1985. 5:264-267.
- Woods G, Hillman R, Schlafer D. Recovery and evaluation of embryos from normal and infertile mares. Cornell Vet; 1986, 76:386-394.
- Woods G, Baker C, Baldwin J, Ball B, Bilinski J, Cooper W. Early pregnancy loss in brood mares. J Reprod Fertil;Suppl 1987. 35:455-459.
- Woods J, Bergfelt D, Ginther O. Effects of time of insemination relative to ovulation on pregnancy rate and embryonic loss rate in mares. Equine Vet J; 1990.22:410-415.