# Evaluation of Premature Mammary Gland Development and Vaginal Discharge in a Mare in Late Gestation

**Raz, T.** [1,2] **and Haik, R.** [1]

[1] Koret School of Veterinary Medicine, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, P.O Box 12, Rehovot 76100, Israel

[2] Department of Biological Regulation, The Weizmann Institute of Science, P.O Box 26, Rehovot 76100, Israel

**Corresponding author:** Dr. Tal Raz, Tel: +972-3-9688588; Fax: +972-3-9604079; Email: talrazg@gmail.com

## CASE PRESENTATION

A nine year-old Quarter Horse pregnant mare (277 days in gestation) was presented to Department of Large Animals, the Koret School of Veterinary Medicine, the Hebrew University of Jerusalem, for evaluation of premature udder development and vaginal discharge. According to the owner, the mare was used as a pleasure riding horse, and was generally healthy. She delivered 4 healthy foals in previous years with no observed problems. The current pregnancy was achieved after the mare was artificially inseminated with fresh semen collected from a stallion of proven fertility. During this pregnancy, the owner did not observe any abnormalities; however, on the morning of the presenting day, he noticed udder development as well as vaginal discharge.

Upon presentation the mare was bright, alert and responsive. Physical examination revealed a body condition score of 5 on a 9-point assessment system, pink mucous membranes with a capillary refill time of <2 sec, and normal gastro-intestinal sound on auscultation. Heart rate was 44 beats per minutes, respiratory rate was 24 per minute, and body temperature was 37.6˚C. The mammary glands were both enlarged, and could be milked easily. Evaluation of the perineal area for conformation revealed that the vulvar lips were angled at about 35 degrees to the vertical, with suboptimal vulvar seal. Scant amount of dry white discharge was detected on the vulvar lips and on the tail hair nearby the perineal area. Transrectal palpation and ultrasonographic examinations of the reproductive tracts revealed a large uterus (appropriate for the gestation day) with a live fetus that moved during the examination. The cervix tone was moderately soft on palpation. Relevant ultrasonographic images that were taken during the transrectal examination are presented in Figure 1.

## QUESTIONS:

1. Based on the clinical presentation and ultrasonographic images, what is your diagnosis? What is the significance of this condition?
2. What is the pathophysiology for this condition?
3. What are the relevant diagnostic procedures?
4. What is the relevant treatment?

**CTUP = combined thickness or uterine and placenta**

**MUA = Middle uterine artery**

**PS = Placental separations**

**FOR DIAGNOSIS OF THIS CASE SEE THE FOLLOWING PAGES**

## DIAGNOSIS AND SIGNIFICANCE

### Ascending Equine Placentitis.

Equine placentitis is one of the most common causes of pregnancy loss in late gestation, worldwide (1,2). Placentitis, in addition to pregnancy loss, has further economic impact since it is a major cause of prematurity and neonatal sepsis (3). Furthermore, mares with placentitis are at increased risk for retained fetal membranes and may develop medical complications such as metritis, septicemia, shock, laminitis and death.

## THE PATHOPHYSIOLOGY OF ASCENDING EQUINE PLACENTITIS

Equine placentitis is commonly caused by bacteria ascending through the vagina, usually during the last trimester of pregnancy. Mares with poor perineal conformation or pneumovagina are predisposed to ascending placentitis due to the poor external barriers to fecal and skin contaminants. The most common bacterial pathogens of placentitis are *Streptococcus equi subspecies zooepidemicus*, *Escherichia coli*, *Klebsiella spp*., *Pseudomonas spp*., and *Staphylococcus aureus* (1,4). Infection generally begins via the caudal reproductive tract; however, hematogenous infection may occur as well. Infective bacteria invade and disrupt the intimate contact between the allantochorion and endometrium. If an infection is established, placental separation extends cranially from the cervical star along the body of the uterus. The affected areas of allantochorion may become thickened and necrotic. However, although bacterial infection initiates the disease, premature delivery is typically secondary to inflammation of the chorion rather than as a consequence of fetal infection. These inflammatory processes increase production of cytokines and prostaglandins (PGE2 and PGF2α), which stimulate myometrial contractility, thus resulting in abortion or preterm delivery.

Hematogenous bacterial placentitis is caused by the spread of microbial pathogens through the blood or lymphatic system to the uterus. Bacteremia in the mare is frequently associated with endotoxemia and prostaglandin release, which has negative effects on the pregnancy. The hematogenous route may cause systemic signs of illness in infected mares. Examples of hematogenous bacterial placentitis include *Leptospira* and *Salmonella* infections (2).

Intrauterine colonization of bacteria may be a source of placentitis. Mares that suffer from chronic uterine infection and conceived may develop placentitis in mid- to late gestation, with the same organism that was cultured at the time of breeding. It is hypothesized, therefore, that some mares maintain pregnancy with an inactive infection that may flare up and cause placentitis in late gestation. Bacteria such as *Nocardia actinomycete* are hypothesized to operate through this mechanism. Nocardiform placentitis, which is prevalent in central Kentucky, is caused by several genera of gram-positive, branching, filamentous actinomycetes (i.e. *Crossiella equi*, *Amycolatopsis spp., Streptomycin spp.*) that reside in the soil (1,4). The typical placental lesion in equine Nocardiform placentitis is located at the bifurcation of the uterine horns and body, the most dependent portion of the uterus, and not at the cervical star.

## DIAGNOSTIC EXAMINATION OF A MARE SUSPECTED OF PLACENTITIS

Mares suffering from ascending placentitis are typically multiparous and middle-aged to aged, although young nulliparous mares may be affected as well. No breed predilection has been reported. Affected mares often have poor to suboptimal perineal conformation. The most common clinical presentation for the mare with placentitis is premature udder development, with or without vulvar discharge. As the condition progresses, mares may show signs of labour.

Procedures for evaluating the health of a late-term pregnancy in suspected mares include [1] physical examination, [2] transrectal and [3] transabdominal ultrasonographic evaluation of the reproductive tract, the placenta, and the fetus, [4] vaginal speculum examination if there is vaginal discharge, and [5] hormonal analysis (e.g. estrogen and progestins) if applicable (4).

### Physical examination

Physical examination of a mare suspected of having placentitis includes evaluation of vital signs (TPR), body condition and the musculoskeletal system, palpation of the udder, and assessment of the perineal area for conformation and discharge. Mares with ascending placentitis typically have no systemic clinical signs, nor do they have any alterations in blood counts or in clinical chemistry parameters (1). However, occasionally, mares may be febrile and/or dehydrated. Furthermore, mares having uterine contractions preceding abortion may have other applicable clinical signs such as restlessness, sweating, and an elevated heart rate.

The most common clinical presentation for a mare with placentitis is premature udder development. In the normal

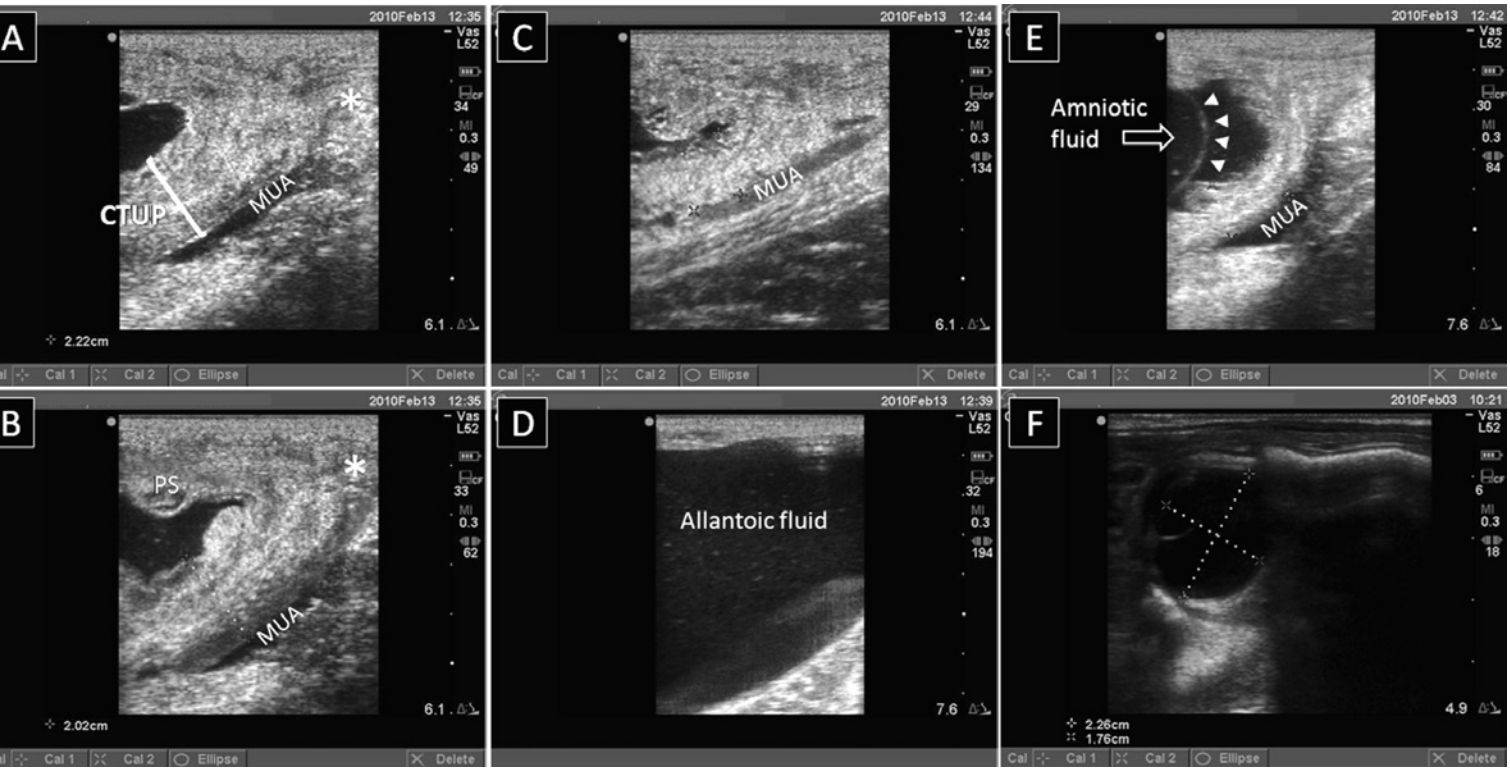


**Figure 1: Ultrasonographic images taken during transrectal examination of a pregnant mare (277 days in gestation) presented with premature udder development and vaginal discharge.** In all images left is cranial, right is caudal. Images A,B,C and E were taken at the cervical star area, whereas images D and F were taken more cranially. The middle uterine artery (MUA) was identified, and the combined thickness of the uterus and placenta (CTUP) was measured to be 20-22mm (Normal CTUP for the gestation day should be <8mm). The echodensity of the CTUP showed increased heteroginesity. A compartment with increased echodensity (*) was significant at the area of the internal cervical os and caudally. Local placental separations (PS) were observed. Amniotic and allantoic fluids had a persistent increased echodensity. The thickness of the amnion (marked by arrow heads in image E) was within the normal range. Image E presents measurement of the fetal orbit size, which was appropriate for the gestation day.

pregnant mare, the mammary gland usually develops 2-5 weeks prior to parturition. Premature mammary gland development, however, is most frequently attributed to twin pregnancies or placental abnormality. Vaginal discharge is never normal or desirable in a pregnant mare. Although vaginal discharge is often present in mares with ascending placentitis, it is an inconsistent clinical finding; this is because discharge may be produced in scant quantity that is difficult to detect without careful inspection, or it is easily being removed from the vulva by the tail. The severity of the clinical signs and the amount of vaginal discharge, however, do not necessarily determine the outcome of the pregnancy.

## Transrectal palpation and ultrasongraphy

Transrectal palpation and ultrasonographic examination of the caudal reproductive tract is a routine diagnostic tool for equine ascending placentitis, as it enables direct examination of the cervical star region which is typically affected in these mares (5). It is also practical for the evaluation of fetal activity, fetal fluid characteristics, the amnion, as well as for the measurement of fetal orbit size as a mean for estimation of gestational age (6,7).

During the transrectal examination, the cervical star area is evaluated, and the combined thickness of the uterus and placenta (CTUP) is measured in the ventral portion of the uterus, just cranial to the cervix. For 271 to 300, 301 to 330, and >330 days of gestation, normal CTUP measurement should be <8, <10, and <12 mm, respectively (1,4,6). However, mares with ascending placentitis have thicker CTUP measures (e.g. ≥12 mm at 9 month; ≥15 mm at 11 months). Moreover, purulent pockets may appear as hyperechoic compartment between the chorioallantois and the

endometrium, in areas where separation of the membranes had occurred. The amnion may also be thicker than normal, and fetal fluid might show altered echogenisity. The allantoic fluid is normally hypoechoic (black), whereas amniotic fluid is slightly more echodense (light gray); however, even in the normal pregnancy, characteristics of either fluid compartment might be effected by fetal *in utero* movements that agitate cellular material. In the evaluation of mares suspected of having placentitis, amniotic and/or allantoic fluid that persistently have increased echodensity strongly suggest increased cellularity due to inflammation or infection.

### Transabdominal ultrasonography

Transabdominal ultrasonography examination in late gestation is performed by systematically evaluating the quadrants of the abdomen. This is done in order to rule-out twin pregnancy, to evaluate the placenta and the fetal membranes, as well as to assess fetal viability parameters such as fetal heart rate, tone, activity, and size (1,4,6). Locating the fetus(es) is achieved by identifying the fetal thorax ("fetal ribs shadows"), which narrows into the cervical spine at its cranial aspect. Confirmation of twins is generally made by identifying two fetal thoraces, and/or two beating hearts. The active fetal heart can be visualized at the cranial-most aspect of the fetal thorax, or alternatively be determined using feto-maternal electrocardiography (for more details see (8)). In singleton pregnancy, typical fetal heart rates in late gestation are around 75 - 85 bpm. Sustained heart rate elevations over 110 bpm or rates below 50-60 bpm may suggest fetal compromise. Fetal heart rate should normally increase when the fetus is moving (exercising) and should decrease during uterine contractions. A non-reactive heart rate may indicate fetal hypoxemia.

The placenta and fetal fluids are also evaluated using transabdominal ultrasonograpy. The chorioallantois should be intimately associated with the endometrium, and the CTUP measurement should normally be less than 12 mm in late gestation. Evaluation of the caudal allantochorion, near the cervix, is not accurate using the transabdominal approach in cases of ascending placentitis. However, transabdominal evaluation of fetal membranes is very useful for identifying placental abnormalities in mares with hematogenously-induced or nocardiform placentitis, in which placental separation and purulent material are located at the base of the gravid horn and the junction of the uterine body.

### Vaginal Examination

The pregnant mare should have good perineal conformation, a pale dry vagina with tacky light coloured mucus, and a tight cervix with no discharge. Appropriate vaginal examination using sterile speculum should be preformed if there is vaginal discharge. A double-guarded swab can be used through the speculum to sample the discharge near the cervix. Cytology (stained with Giemsa or Diff-Quick) should be evaluated for the presence of neutrophils and bacteria. Samples should be submitted to identify the organism and determine antimicrobial sensitivity patterns.

### Hormonal analysis

**Progesterone/ total progestins**: Some commercial progesterone ELISA's and radioimmunoassays have cross-reactivity with fetoplacental progestins, and therefore, can be used to monitor alterations in total progestins produced by the feto-placental unit in mares (1,5). Hormonal concentrations may indicate a compromised pregnancy or potential premature delivery, and can be used to evaluate the efficacy of treatment in mares with placentitis. Serum progestagen concentrations may increase or decrease on a daily basis, hence the suggestion is to monitor the levels over time. Significantly elevated progestagen concentrations indicate fetal stress such as placentitis; a sudden drop in serum concentration is often associated with impending abortion within just few days.

**Total Estrogens:** In the pregnant mare estrogen production requires both the fetus and placenta, as the fetal gonads provide the precursors for estrogen formation by the placenta. A total estrogen concentration greater than 1000 ng/ml between 150 and 280 days of gestation is considered to be normal, while concentrations lower than 500 ng/ml have been associated with a severely-compromised or dead fetus; levels between 500–800 ng/ml indicate a compromised fetus (1). Still, the practical value of this measurement in assessing mare with placentitis is yet to be studied.

## TREATMENT OF A MARE WITH ASCENDING PLACENTITIS

Bacterial infection commonly initiates equine placentitis; however, premature delivery of the fetus may occur secondary to inflammation of the chorioallantois rather than as a direct consequence of fetal infection. Pro-inflammatory cytokines in fetal membranes and fluids, as well as the production

of prostaglandins (PGE2 and PGF2α), may increase uterine contractions and lead to pre-term delivery. Therefore, treatment objectives are to [1] stop spread of microbial invasion; [2] block or reduce inflammation; and [3] maintain uterine quiescence by using tocolytic drugs (1,4).

A common treatment regimen for the mare with ascending placentitis includes antibiotics, non-steroidal anti-inflammatory drugs (NSAID), pentoxifylline (which has anti-cytokine activity), and altrenogest (synthetic progestin; tocolytic) (1,9) (Table 1). Treatment must be initiated early, soon as clinical signs appear, as this might be the difference between a successful case, in which a viable foal is delivered, to an unsuccessful case. Treated mares should be evaluated every few days or more frequently, depending on case severity, to monitor progression of the disease and treatment efficacy, and to ensure that the fetus has not died *in utero*.

Length of antibiotic, non-steroidal and altrenogest administration is controversial. Some practitioners administer antibiotics and non-steroidal anti-inflammatory therapy for two to three weeks, while others maintain the treatment until delivery. Prolonged antibiotic administration may possibly lead to antibiotic resistance, while prolonged non-steroidal anti-inflammatory treatment may result in gastro-intestinal disturbances in the mare. Administration of altrenogest is discontinued approximately at day 325 of gestation to prevent prolonged second-stage labour and more neonatal complications.

**Table 1:** Drugs commonly used to treat mares with ascending placentitis

| |
|---|
| **Anti-microbial therapy*** |
| Trimethoprim sulpha (30 mg/kg, PO, q12h) |
| Potassium penicillin G (22 000 IU/kg, IV, q6h) |
| Gentamicin (6.6 mg/kg, IV, q24h) |
| Ceftiofur Sodium (2.2 mg/kg, IV or IM, q12h) |
| **Anti-inflammatory therapy**** |
| Flunixin meglumine (1.1 mg/kg, PO/IV,q12 or 24h) |
| Phenylbutazone (2.2 mg/kg, PO, q12h or 24h) |
| Pentoxifylline (8.5 mg/kg, PO, q12h) |
| **Tocolytics** |
| Altrenogest (0.088 mg/kg, PO, q24h) |
| Isoxsuprine (0.4–0.6 mg/kg, PO, q24h) |
| Clenbuterol (0.8 µg/kg, PO/IV, q12h) |

* Antimicrobial therapy should be adjusted according to culture and sensitivity results.

** Pentoxifylline is commonly combined with either Flunixin meglumine or Phenylbutazone.

Foals born to mares with placentitis are at significant risk of suffer from neonatal diseases such as septicemia. Therefore, antimicrobial therapy is indicated in foals delivered from mares having placentitis (4). Also, it is important to ensure proper nursing of colostrum to establish adequate passive transfer of immunity. Assessment of IgG level in the neonate is commonly recommended in such foals at 12-24 hr post foaling.

## REFERENCES


1. LeBlanc, MM.: Ascending placentitis in the mare: an update. Reprod. Domest. Anim. 45 Suppl 2:28-34, 2010.
2. Hong, C.B., Donahue, J.M., Giles, R.C/, Jr., Petrites-Murphy, M.B., Poonacha, K.B., Roberts, A.W., Smith, B.J., Tramontin, R.R., Tuttle, P.A. and Swerczek, T.W.: Equine abortion and stillbirth in central Kentucky during 1988 and 1989 foaling seasons. J. Vet. Diagn. Invest. 5:560-566, 1993.
3. Koterba, A.M., Brewer, B.D. and Tarplee, F.A.: Clinical and clinicopathological characteristics of the septicaemic neonatal foal: review of 38 cases. Equine Vet. J. 16:376-382, 1984.
4. Macpherson, M.L. and Bailey, C.S.: A clinical approach to managing the mare with placentitis. Theriogenology 70:435-440, 2008.
5. Morris, S., Kelleman, A.A., Stawicki, R.J., Hansen, P.J., Sheerin, P.C., Sheerin, B.R., Paccamonti, D.L. and LeBlanc, M.M.: Transrectal ultrasonography and plasma progestin profiles identifies fetoplacental compromise in mares with experimentally induced placentitis. Theriogenology 67:681-691, 2007.
6. Renaudin, C.D., Troedsson, M.H., Gillis, C.L., King, V.L. and Bodena, A.: Ultrasonographic evaluation of the equine placenta by transrectal and transabdominal approach in the normal pregnant mare. Theriogenology 47:559-573, 1997.
7. Renaudin, C.D., Gillis, C.L., Tarantal, A.F. and Coleman, D.A.: Evaluation of equine fetal growth from day 100 of gestation to parturition by ultrasonography. J Reprod Fertil Suppl 56:651-660, 2000.
8. Nagel, C., Aurich, J. and Aurich, C.: Determination of heart rate and heart rate variability in the equine fetus by fetomaternal electrocardiography. Theriogenology 73:973-983, 2010.
9. Bailey, C.S., Macpherson, M.L., Pozor, M.A., Troedsson, M.H., Benson, S., Giguere, S., Sanchez, L.C., Leblanc, M.M. and Vickroy, T.W.: Treatment efficacy of trimethoprim sulfamethoxazole, pentoxifylline and altrenogest in experimentally induced equine placentitis. Theriogenology 74:402-412, 2010.