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Combined thickness of the uterus and placenta (CTUP) as indicator of placentitis in Thoroughbred mares

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Summary: Significant financial losses caused by ascending placentitis, frequently caused by bacterial infection in mares with poor perineal conformation, have stimulated interest in improved diagnostic and treatment tools. This study was designed to determine whether or not the presence of histologically evident placentitis was related to previously diagnosed thickened CTUP (combined thickness of the uterus and the placenta). During 30 days prior to their expected foaling date, 333 pregnant mares were examined on a single occasion. CTUP was obtained using transrectal ultrasonography. All parturitions were assisted and after foaling, the fetal membranes were weighed and macroscopically examined. Samples of allantochorion near the cervical star or from any area with macroscopical alterations were submitted to histological evaluation. A mare was considered with placentitis when the presence of neutrophils was detected outside the blood vessels after the complete examination of the slide. The data were analyzed by logistic regression with a statistical significance of $P < 0.05$. Only 3.9% of the mares suffered from ascending placentitis as demonstrated by the infiltration of neutrophils in the chorioallantoic tissues near the cervical star. No differences in mean values of CTUP were observed in mares with and without placentitis. All deliveries were eutocic and seven foals died within 24 h of birth. Five of these were born from mares with placentitis. In conclusion CTUP is not a good indicator of ascending placentitis during the final month of gestation in Thoroughbred mares.

Keywords: cervical star / infiltration of neutrophils / ultrasonography / uterus / placenta / placentitis / reproduction

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Introduction

The reduction of abortions through the effective management of twin pregnancies has reduced this cause of abortion, and placentitis has become one of the most common causes of abortion in late gestational mares (*Troedsson* 2003). Significant financial losses caused by placentitis have stimulated interest in improved diagnostic and treatment tools for affected mares (*Macpherson* and *Bailey* 2008a). The disease is most frequently caused by an ascending bacterial infection and by opportunist pathogens multiplying when the normal defenses of the pregnant uterus have broken down (*Platt* 1975). Affected mares often have poor perineal conformation or defects (*Macpherson* and *Bailey* 2008a).

Placental infection is manifested by thickening and ulceration of the chorioallantois (*Platt* 1975). The lesions are most severe and chronic in the chorion near the cervical star and in the posterior part of the uterine body (*Mays* et al. 2002). A suppurative, necrotizing inflammation of the cervical star portion of the chorioallantois, and presence of bacterial colonies are the predominant findings (*Hong* et al. 1993, *Mays* et al. 2002). Streptococcus zooepidemicus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa are isolated in the greatest number of cases (*Merkt* 1985, *Acland* 1993). A breed predilection has not been identified; however, the condition has been frequently diagnosed in Thoroughbreds (*Macpherson* and *Bailey* 2008b).

The measurement of the combined thickness of the uterus and the placenta (CTUP) has been proposed as an efficient way of diagnosing ascending placentitis prior to the development of overt clinical signs and placental failure (*Renaudin* et al. 1997, *Troedsson* et al. 1997, *Troedsson* 2001). A CTUP above 2 SD of the established normal range after 271 days of gestation suggests placental failure and pending abortion (8 mm between days 271 and 300; 10 mm between days 301 and 330; and 12 mm after day 330) (*Troedsson* et al. 1997). However, a controlled study of mares with experimentally induced placentitis treated with trimethoprim sulfamethoxazole, pentoxifylline and altrenogest did not demonstrate differences for development of vulvar discharge or CTUP when compared with non-treated mares (*Bailey* et al. 2010). These findings agree with Brazilian studies (*Souza* et al. 2010) which concluded that CTUP measurement in mares should not be the only parameter used to estimate placental failure and impending abortion.

This study was designed to determine whether or not the presence of histologically evident placentitis was related to previously diagnosed thickened CTUP.

Material and methods

Animals

The study was conducted on 11 commercial Thoroughbred stud farms in Southern Brazil in one breeding season. Healthy pregnant mares (n = 333), between 3 and 28 years old, were examined once during the last 30 days prior to their expected

Parturition

All parturitions were assisted. Immediately after birth all newborns were weighed, submitted to a clinical examination and their gestation length recorded. Mares that required assistance during the delivery of their foals were not included in the study. Placenta examination

After foaling, the fetal membranes were weighed and macroscopically examined. Samples of chorioallantois near the cervical star or from any area with macroscopically evident lesions suggestive of placentitis were submitted for histologi-

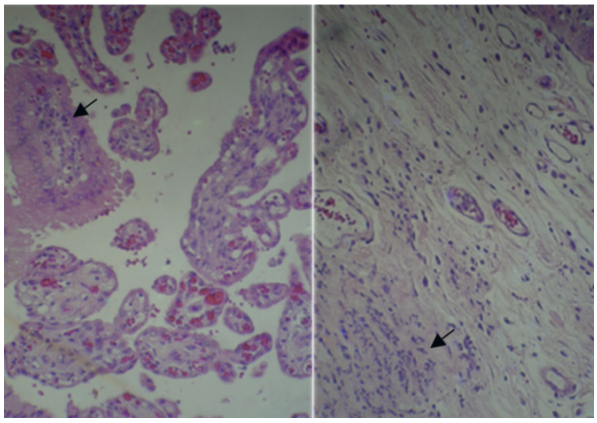

Figure 1 (A) – Chorionic villi with cellular infiltration (arrow). (B) Chorioallantois with mild neutrophilic infiltration (arrow). / *Chorionzotten mit zellulärer Infiltration (Pfeil). (B) Chorionallantois mit geringgradiger Infiltration neutrophiler Granulozyten (Pfeil)*

foaling date. Of the total mares 275 had a previous history of at least one successful pregnancy (range 1 to 13) and the remainder carried their first gestation. Mares were maintained under similar handling and feeding conditions during the trial. These mares were kept outside on a pasture, fed with oats, alfalfa hay and a commercial supplement twice a day and had ad libitum access to water and minerals throughout the pregnancy.

Body condition

Body condition (BC) of each mare was evaluated before examination using a score varying from 1 to 5 (*Malschitzky* et al. 2001).

Ultrasonography

Each examination included a transrectal ultrasonography, using an Aloka 500 with a 5 MHz linear transducer. The transducer was positioned at the cervical-placental junction, 2.5 to 5 cm cranial to the cervix with care to be certain that the amniotic membrane was not adjacent to the allantochorion. When an optimal image was visualized, the image was frozen on the screen and measurements of the CTUP (*Renaudin* et al. 1997) were obtained from the ventral aspect of the uterine body. During each examination, CTUP were measured three times and the mean was recorded. Mares previously treated for placentitis or with systemic antibiotics, anti-inflammatories, or tocolytic drugs were not included in the study.

Table 1 – Means (± SEM) and P values from CTUP, fetal membrane and foal weights in 333 Thoroughbred mares with and without placentitis. / *Arithmetisches Mittel (± Standardabweichung) und P-Werte von CTUP, Placenta fetalis und Fohlengewicht bei 333 Vollblutstuten mit und ohne Plazentitis*

	Mares without placentitis (n=320)	Mares with placentitis (n=13)	P
CTUP (mm)	7.8 ± 0.1	7.3 ± 0.6	0.29
Fetal membrane weight (kg)	6.52 ± 0.1	7.0 ± 0.5	0.37
Foal birth weight (kg)	55.4 ± 0.4	57.1 ± 2.2	0.55

Table 2 – Frequency of histological evidence of ascending placentitis in mares with CTUP that exceeded the upper limit 10 mm of the 95% confidence interval (assumed as twice SE). / *Häufigkeit einer histologisch nachweisbaren Plazentitis bei Stuten mit CTUP, die die Grenze von 10 mm des 95% Konfidenzintervalls (angenommen als doppelte Standardabweichung) übersteigt*

CTUP (mm)	Mares (n)	Mares with placentitis	
		n	%
< 10	279	12 [a]	4.3
> 10	54	1 [a]	1.8

(a, a) values not different, P = 0.7.

Table 3 – Correlation coefficient (r) between CTUP, mare age, body condition (BC), placenta weight and foal weight using a multiple regression analysis. / *Korrelationskoeffizient (r) zwischen CTUP, Stutenalter, body condition (BC), Plazentagewicht und Fohlengewicht unter Verwendung einer multiplen Regressionsanalyse*

	Mare Age	BC	Placenta weight	Foal weight
CTUP	16.0%[a]	NS	NS	15.2%[a]
Mare Age		NS	21.7%[a]	30.2%[a]
BC			12.1%[a]	11.8%[a]
Placenta weight				41.6%[a]

a (P<0.05) represent significant difference, NS = not significant.

cal evaluation. The samples were fixed in 10% buffered formalin, embedded in paraplast, sectioned (5 μm thickness) and stained with hematoxylin-eosin (HE). Placenta samples were evaluated in a blind manner depending on the presence of inflammatory cells. A mare was considered with placentitis when the presence of neutrophils was detected outside the blood vessels after the complete examination of the slide.

Statistical analysis

The data were analyzed by single logistic regression with a statistical significance of $P < 0.05$ using the SPSS 16.0 software. The histopathological result was used as main variable and body condition, CTUP, and the foal and membrane weight were used as response variables. A correlation coefficient was determined using a multiple regression analysis. CTUP, mare age, mare body condition, placenta weight and foal weight were used as dependent variables and as independent variables.

Results

The mean age of mares was 10.1 ± 4.1 years. Gestation length was from 329.1 ± 18.5 days and the mean interval between examination/parturition was from 23 ± 12.9 days. Their BC was 4.1 ± 0.6. Of 333 mares only 3.9% (13) suffered from ascending placentitis as demonstrated by the infiltration of neutrophils in the allantochorionic tissues near the cervical star (Figure 1). However, 16 (5%) mares without placentitis presented gross lesions, such as brown mucoid material on the chorionic surface with thickening of the placenta (4), edema thickening of the placenta (2), thickening at the cervical star (2), hemorrhagic placenta (2), ischemic areas (1), friable placentas (1) and others (4).

Table 1 shows mean values of CTUP and weights of fetal membranes and foals. The mean CTUP was 7.9 ± 0.1 mm, ranging from 3.3 to 16.7 mm. The incidence of ascending placentitis in mares whose CTUP was above or below 10 mm (*Troedsson* et al. 1997) is depicted in Table 2. The correlation coefficients between CTUP, mare age, body condition (BC), placenta weight and foal weight are represented in Table 3. No correlation was observed between CTUP and placenta weight.

All deliveries were eutocic. Seven foals died within 24 h of birth. Five of these were born from mares with histological placentitis ($P < 0.01$). All the foals born from mares with placentitis died within 24 h of birth.

Discussion

Multifocal leukocyte infiltrates, predominantly consisting of neutrophils, were observed in all placentitis cases. The lesions were most severe in the chorion adjacent to the internal os of the cervix and the posterior part of the uterine body, suggesting an ascending infection through the cervix and into the uterus. These results are similar to those observed by *Platt* (1975) and *Hong* et al. (1993). Of the five dead foals, two were premature, delivered at 313 and 309 days respectively, prior to the normal 320 days of gestation (*Rossdale* 1993) and the other three died within 9 hours of parturition.

It has become routine in veterinary practice to examine pregnant mares for early evidence of ascending placentitis, before the onset of clinical signs (*Macpherson* and *Bailey* 2008b). Such examinations usually involve the measurement of CTUP near the cervix by means of transrectal ultrasonography. If the CTUP is increased, placental insufficiency is likely present and abortion may ensue (*Cummins* et al 2008). The main considerations in the medical management of mares with placentitis include treating the infectious agent, suppressing inflammation and preventing myometrial contractions. However, treatment of mares with suspected placentitis is often empirical (*Bain* and *Zent* 2009). We were unable to establish a significant correlation between the thickening of the CTUP and the histopathological diagnosis of placentitis. Also CTUP did not correlate with placenta weight. These results, obtained during the last month of pregnancy, indicate that many mares with placental thickening probably do not require such therapy, which significantly increases costs of breeding. That agrees with observations in Criollo Horses (*Souza* et al. 2010) where mares carrying a normal pregnancy may have a CTUP above the reference range and deliver a healthy foal at term. The application of mathematical values to biological models and the clinical importance of single CTUP measurements throughout gestation should be reconsidered (*Souza* et al. 2010). In this study 320 placentas were diagnosed without placentitis (absence of inflammatory cells outside blood vessels) and 16 presented gross lesions, from which eight showed thickening or edema. These findings can, in part, explain why the thickening of the uterus and the placenta unity is not only caused by an inflammatory process. We did not evaluate the potential value of CTUP measurements during earlier months of gestation. It may be possible that most of the mares at risk of developing ascending placentitis had already aborted by the time mares were selected for this study.

In conclusion, the CTUP alone is not a good indicator of ascending placentitis during the last month of gestation in Thoroughbred mares.

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Conflict of interest statement

None of the authors have any conflict of interest to declare

Author contributions

H. K. Löf designed the study, collected samples, carried out the analyses and prepared the manuscript. J. W. Gregory car-

ried out histological analyses. A. P. Neves collected samples. M. I. M. Jobim, R. M. Gregory and R. C. Mattos participated in the design of the study and in the preparation of the manuscript.

References

Acland H. M. (1993) Abortion. In: McKinnon AO, Voss JL (eds), Equine reproduction. Lea & Febiger Philadelphia 554-561

Bailey C. S., Macpherson M. L., Pozor M. A., Troedsson M. H. T. , Benson S., Giguere S., Sanchez L. C., LeBlanc M. M., Vickroy T. W. (2010) Treatment efficacy of trimethoprim sulfamethoxazole, pentoxifylline and altrenogest in experimentally induced equine placentitis. Theriogenology 74, 402-412

Bain F. T., Zent W. W. (2009) Placentitis. In: Robinson NE, Sprayberry KA (eds), Current therapy in equine medicine. Sauders, Saint Louis, 834-835

Cummins C., Carrington S., Fitzpatrik E., Duggan V. (2008) Ascending placentitis in the mare: A review. Irish Vet. J. 61, 307-313

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., Swerczekl T. W. (1993) Etiology and pathology of equine placentitis. J. Vet. Diagn. Invest. 5, 56-63

Macpherson M. L., Bailey C. S. A. (2008a) Clinical approach to managing the mare with placentitis. Theriogenology 70, 435-440

Macpherson M. L., Bailey C. S. (2008b) Treating the mare with placentitis: a clinical approach. Equine Vet. Scie. 28, 703-708

Malschitzky E., Schilela A., Meirelles L. S;, Mattos A. L. G., Gregory R. M., Mattos R. C. (2001) Artificial photoperiod in pregnant mares and its effect on pregnancy length and postpartum reproductive performance. Pferdeheilkunde 17, 565-569

Mays M. B. C., LeBlanc M. M., Paccamonti D. (2002) Route of fetal infection in a model of ascending placentitis. Theriogenology 58, 791-792

Merkt H. (1985) Trächtigkeitverluste beim Pferd und die Möglichkeiten ihrer Reduzierung (Pregnancy interruption in the mare and its reduction). Tierärztl. Umsch. 40, 428-435

Platt H. (1975) Infection of the horse fetus. J. Reprod. Fertil. (Suppl) 23, 605-610

Renaudin C. D., Troedsson M. H. T., Gillis C. L. (1999) Transrectal ultrasonographic evaluation of the normal equine placenta. Equine Vet. Educ. 11, 75-76

Renaudin C. D., Troedsson M. H. T., Gillis C. L., King V. L., Bodena A. (1997) Ultrasonographic evaluation of the equine placenta by transrectal and transabdominal approach in the normal pregnant mare. Theriogenology 47, 559-573

Rossdale P. D. (1993) Clinical view of disturbances in foetal maturation. Equine Vet. J. (Suppl) 14, 3-7

Souza A. M., Winter G. H. Z., Garbade P., Wolf C. A., Jobim M. I. M., Gregory R. M., Mattos R. C. (2010) Ultrasonographic evaluation of the Criollo mare placenta. Animal Reprod. Sci. 121, 320-321

Troedsson M. H. T. (2001) Ultrasonographic evaluation of the equine placenta. Pferdeheilkunde 17, 583-588

Troedsson M. H. T. (2003) Placentitis. In: Robinson N. E (ed), Current therapy in equine medicine. Sauders Philadelphia, pp.297-300

Troedsson M. H. T., Renaudin C. D., Zent W. W., Steiner J. V. (1997) Transrectal ultrasonography of the placenta in normal mares and mares with pending abortion: a field study. Proc. Am. Assoc. Equine Pract. 43, 256-258

Zusammenfassung

Die bedeutenden finanziellen Verluste durch aufsteigende Plazentitiden, häufig bedingt durch Bakterien bei Stuten mit schlechter perinealer Ausbildung, haben das Interesse an verbesserten diagnostischen und therapeutischen Möglichkeiten geweckt. Ziel dieser Studie war es festzustellen, ob eine Beziehung besteht zwischen einer histologisch erkennbaren Plazentitis und einer zuvor diagnostizierten CTUP (combined thickness of the uterus and the placenta = kombinierte Verdickung von Uterus und Plazenta). An einem Tag im Laufe der letzten Tage vor dem erwarteten Abfohltermin wurden 333 trächtige Stuten untersucht. Eine CTUP wurde durch transrektale Sonographie ermittelt. Alle Geburten wurden unterstützt, die Plazenta fetalis postpartal gewogen und makroskopisch begutachtet. Proben aus dem Allantochorion in der Nähe des Zervixsterns oder aus jedem Bereich mit makroskopischen Veränderungen wurden einer histologischen Beurteilung unterzogen. Die Diagnose Plazentitis wurde gestellt, wenn nach der vollständigen Prüfung des gesamten Schnittes neutrophile Granulozyten außerhalb der Blutgefäße nachgewiesen werden konnten. Die Daten wurden mit Hilfe der logistischen Regression mit einer statistischen Signifikanz von $p < 0{,}05$ analysiert. Nur 3,9 % der Stuten litt an einer aufsteigenden Plazentitis, die sich durch eine Infiltration von neutrophilen Granulozyten im Chorioallantoisgewebe in der Nähe des Zervixsterns auszeichnete. Keine Unterschiede der Mittelwerte der CTUP wurden zwischen Stuten mit und ohne Plazentitis beobachtet. Alle Geburten verliefen ungestört, sieben Fohlen starben innerhalb von 24 h nach der Geburt. Fünf dieser Fohlen wurden von Stuten mit einer Plazentitis geboren. Zusammenfassend ist festzustellen, dass die CTUP kein guter Indikator für eine aufsteigende Plazentitis während des letzten Trächtigkeitsmonats bei Vollblutstuten ist.

Schlüsselwörter: Zervikalstern / Infiltration von neutrophilen Granulozyten / Sonographie / Reproduktion / Plazentitis