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The combined thickness of uterus and placenta (CTUP) and vulvar conformation of the mare

Andreza Morais de Souza, Gustavo Henrique Zimmermann Winter, Joana Weber Gregory, Maria Inês Mascarenhas Jobim, Ricardo Macedo Gregory and Rodrigo Costa Mattos

REPROLAB, Faculdade de Veterinária, UFRGS, Porto Alegre, RS, Brazil

Summary: Placentitis is a leading cause of abortion and neonatal death in horses. An increase in the combined thickness of uterus and placenta (CTUP) in the area of the cervical star is an indicator of ascending placentitis. The aim of this study was to characterize variations and relationship between the CTUP measurements and the vulvar conformation during mid- and late gestation in mares. Sixty-three healthy pregnant mares were studied. The mares were examined once every month, starting at 4th month of gestation and continuing until parturition. The Caslick Index (CI) was calculated. CTUP was obtained with ultrasonography at the cervical-placental junction, 2.5 to 5 cm cranial from the cervix, and the largest measurement was recorded. The CI was above 150 and within 100 and 150 in 1.6% and 2.8% of the measurements respectively. The age of mares influenced the CI. Mean CTUP did not change between months 4 and 7 of gestation but increased significantly from the 7th to 8th month of gestation, remained the same until the 9th month, then increased again until foaling. It was positively correlated with gestational age. There was no interaction between CTUP and mare's age or body condition. Thirty percent of the CTUP measurements exceeded the upper limit of the 95% confidence interval. No interactions were observed between CTUP and vulvar measurements and CI. We concluded that gestational age affects CTUP and its measurement in mares with correct vulvar conformation should not be the only parameter to estimate placental failure and impending abortion.

Keywords: Ultrasonography / placental failure / abortion / Caslick Index / mare / reproduction

Die kombinierte Dicke von Uterus und Plazenta (CTUP) und der Zustand der Vulva bei der Stute

Die Plazentitis ist die Hauptursache für Aborte und Neugeborenensterblichkeit bei Pferden. Eine Zunahme der Dicke von Uterus und Plazenta zusammen (CTUP) im Bereich des Zervixsterns ist ein Hinweis einer aufsteigenden Plazentitis. Ziel dieser Arbeit war es, Abweichungen und einen Zusammenhang zwischen CTUP-Maßen und dem Aufbau der Vulva bei Stuten während der mittleren und späten Trächtigkeit zu erfassen. Dreiundsechzig gesunde trächtige Stuten nahmen an der Studie teil. Ab dem 4. Monat der Trächtigkeit bis zur Geburt wurden sie einmal monatlich untersucht und der "Caslick-Index" bestimmt. CTUP wurde durch Ultraschalluntersuchungen von dem Zervix-Plazenta-Verbindungspunkt bestimmt (2.5 bis 5 cm cranial der Zervix), und die größte Messung wurde aufgezeichnet. Der Caslick-Index lag in 1.6% der Fälle über 150 und in 2.8% zwischen 100 und 150. Die Durchschnitte haben sich zwischen dem 4. und 7. Monat der Trächtigkeit nicht geändert, stiegen aber vom 7. zum 8. Monat deutlich, blieben bis zum 9. Monat gleich und nahmen bis zum Abfohlen wieder zu. Dies zeigte eine positive Korrelation mit der Tragezeit. Es gab keine Abhängigkeit zwischen CTUP und Alter der Stuten oder körperlicher Allgemeinzustand. Ein Drittel der CTUP-Maße hat die obere Grenze des 95%-Vertrauensintervalls überschritten. Zwischen CTUP und effektiver Länge der Vulva, Neigungswinkel der Vulva und Caslick-Index wurden keine Zusammenhänge beobachtet. Abschließend ist festzustellen, dass die Tragezeit der Stuten die CTUP-Messung beeinflusst, dass diese Messwerte, bei Stuten mit korrektem Aufbau der Vulva, jedoch nicht als einziger Kennwert für Plazentaversagen und Abort zu bewerten sind.

Schlüsselwörter: Ultraschalluntersuchung / Plazentaverlust / Abort / Caslick Index / Reproduktion / Stute

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Correspondence: Andreza M. Souza, REPROLAB, Faculdade de Veterinária, UFRGS, Av. Bento Gonçalves 9090, 91570-000 Porto Alegre, RS, Brazil, E-mail: andreza@personalhorse.com.br

Introduction

Pregnancy losses during late gestation represent a great problem for the equine breeding industry (*Troedsson* 2001). Placentitis is a leading cause of fetal and neonatal death in horses (*Macpherson* 2006). Pathology results of more than 3,500 cases of abortion, stillborn and perinatal death in horses showed that bacterial placentitis caused nearly one third of all abortions or fetal mortalities (*Giles* et al. 1993). In the majority of placentitis cases, the route of infection is believed to be ascending through the cervix, and an area of the chorion adjacent to the cervical star shows characteristic pathology in aborting mares (*Platt* 1975, *Whitwell* 1988, *Troedsson* 2001). The lesions tended to be located at the cervical star area, where discoloration and thickening are frequently seen. Necrosis of the chorionic villi, infiltration of neutrophils in the chorion, and presence of bacterial colonies are the predominant findings (*Hong* et al. 1993). 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* 2008a).

Mares most commonly afflicted are pluriparous. Many have anatomical defects of the caudal reproductive tract such as pneumovagina, vestibule-vaginal reflux or cervical fibrosis, tears or adhesions (*LeBlanc* 2010). One method of quantifying the degree of variation from normalcy of the perineal conformation is by calculating the Caslick Index. This index is calculated by determining the degree of anterior slope of the vul-

va and the distance in centimeters that the dorsal commissure of the vulva is displaced dorsally in relationship to the pelvic brim (*Pascoe* 1979, *Bradecamp* 2011).

Combined thickness of the utero-placental unit at the cervical pole has been the focus of attention in recent years, (*Bucca* 2011). The combined thickness of the uterus and the placenta (CTUP) (*Renaudin* et al. 1997, 1999b) can be measured by transrectal ultrasonography and an increase in the CTUP or premature placental separations in the area of the cervical star are hallmarks of ascending placentitis (*Troedsson* 2001, *Renaudin* et al. 1999a, *Morris* et al. 2007). Long-term therapy in affected mares, from the onset of clinical signs to delivery of foal, with an antimicrobial, anti-inflammatories and progestin are needed to effectively combat placental infections (*Macpherson* and *Bailey* 2008a, 2008b).

The aim of this study was to characterize variations and the relationship between the CTUP measurements and the vulvar conformation during mid- and late gestation in mares.

Materials and methods

Animals

Sixty-three healthy pregnant mares with ages ranging between 4 and 22 years were studied. Data of mating, ovulation and foaling were known. The past breeding history from the mares was known and they had a previous history of at least one successful pregnancy. Mares were maintained under similar handling and feeding conditions during the trial. These mares were part of a commercial herd of a horse farm in Southern Brazil and were kept outside in a natural pasture, with ad libitum access to water and minerals throughout the pregnancy.

For the purpose of this project the gestational age was determined as: Month 4 = 91 to 120 d; Month 5 = 121 to 150 d; Month 6 = 151 to 180 d; Month 7 = 181 to 210 d; Month 8 = 211 to 240 d; Month 9 = 241 to 270 d; Month 10 = 271 to 300 d; Month 11 = 301 to birth. The pregnant mares were examined each month until parturition. Due to stud farm management, ultrasound scannings were performed with 20 to 40 days intervals and in this manner allowing the mares to be examined more than once per period. Not all mares entered the trial at 4th month as the routine examinations started after the end of reproductive season.

During the experiment no mare was treated with systemic antibiotics, anti-inflammatories, tocolytic drugs or submitted to a vulvar surgery.

Vulvar Conformation

Vulvar measurements were taken with an aluminum measuring device (*Pascoe* 1979). This consisted of a half protractor (0–90°), an arm calibrated in cm and pointer attached to and pivoted at the base of the protractor, which had a spirit level fixed to its upper edge. During the measurements, mares were standing on a horizontal floor. The parameters measured were: (1) total length (distance between the dorsal and ventral commissure of the vulva), (2) effective length (distance between the dorsal commissure of the vulva and the level of the ischial tuberosities) and (3) angle of declination of the vulva above the ischial tuberosities (*Pascoe* 1979).

The same cares described by *Pascoe* (1979) were taken during measurements, like not to upset the mare, as arching of the back, straining or placing the hind feet further forward than normal. Also, tails were raised to a uniform vertical position.

Perineal muscular tonus was obtained by the manual traction of the vulvar lips and classified using a score from 1 to 3, where 1 represents without or almost no resistance and 3 represents good muscular resistance promoting a good vulvar seal.

Caslick Index

The Caslick Index was calculated using the product of the effective length in cm multiplied by the angle of declination of the vulva (*Pascoe* 1979).

Ultrasonography

Each examination was performed by the same person and included a transrectal ultrasonography, using a Pie Medical Scanner 100 LC with an 8 MHz linear transducer. The transducer was positioned near the cervical-placental junction, 2.5 to 5 cm cranial from the cervix. When an optimal image was visualized it was frozen on the screen and measurements of the CTUP (*Renaudin* et al. 1997) were obtained from the ventral aspect of the uterine body above the blood vessel. Care was taken to ensure that only areas with no contact with the fetus or amniotic membrane were assessed. At each examination, CTUP were measured three times and the largest measurement was recorded as it is executed in routine examination on the stud farms. The images and measurements were printed (video printer UP-895MD-Sony) to allow for later detailed studies.

Body condition

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

Parturition

All parturitions occurred unaccompanied outdoors in the pasture, without human interference. Within the first 12 hours of birth all newborn foals were submitted to a clinical examination and their gestational length recorded.

Statistical analysis

Data were analyzed using ANOVA, with a statistical significance of $P < 0.05$ and results were expressed as means ± Standard Deviation. CTUP was used as main variable, and month of gestation, age of the mare and body condition, vulvar angle, vulvar total length and vulvar effective length, Caslick Index and their interactions as dependent variables. Means comparisons were performed using Tukey Test. Pearson's correlation coefficient was calculated for Caslick Index, CTUP, vulvar declination, vulvar effective length and mare's age. A 95% confidence interval with ± twice standard error of the mean (SE) was calculated (*Renaudin* et al. 1997, 1999b, *Troedsson* et al. 1997).

Results

All mares delivered viable foals at term with a mean gestation length of 330 ± 8.9 days. Mean age of mares was 10.8 ± 4.2 years and body condition score was 3.5 ± 0.4. Vulvar length, vulvar effective length, vulvar declination and Caslick Index during the pregnancy of the 63 mares are depicted in Table 1. The Caslick Index was above 150 and within 100 and 150 in 1.6% and 2.8% of the measurements respectively. Mare's age influenced Caslick Index ($P < 0.01$) with a weak correlation ($r = 0.21$).

Three hundred eighteen ultrasonographic measurements were obtained from the 63 mares. Mean CTUP did not change between months 4 and 7 of gestation but increased significantly from the 7th to 8th month, remained the same until the 9th month, then increased again until foaling (Table 2). It was positively correlated ($r = 0.69$, $P < 0.01$) with gestational age. There was no interaction between CTUP and mare's age ($P = 0.22$) or body condition ($P = 0.45$). A third of the CTUP measurements exceeded the upper limit of the 95% confidence interval (Table 3). No interactions were observed between CTUP and vulvar effective length ($P = 0.98$), vulvar declination ($P = 0.45$) and Caslick Index ($P = 0.68$).

Discussion

The CTUP increased with gestational age but at a rate that differed from those reported by *Renaudin* et al. (1997) and *Bucca* et al. (2005). CTUP increased between 4 and 8 months, remained stable during the 9th month, and then increased again until foaling, but did not reach values reported by *Bucca* et al. (2005).

In the present study, over 33% of CTUP measures were higher than the upper limit of the 95% confidence interval, indicating that many mares could have been assumed to have ascending placentitis and to need treatment for the condition. The upper and lower limits that we used were similar to the 95% confidence interval described earlier by *Renaudin* et al.

Table 1 Mean ± SD of the vulvar length, vulvar effective length, vulvar declination and Caslick Index during the pregnancy of 63 mares.

	Mean ± SD	Lower	Upper
Vulvar length (cm)	8.2 ± 1.2	5	12
Effective vulvar length (cm)	3.8 ± 0.9	2	7
Vulvar declination (degrees)	10.4 ± 7.4	0	38.6
Caslick Index	40.6 ± 33.5	0	270
% of effective vulvar length in relation to total vulvar length	46.4 ± 7.4	25	67
Perineal muscular tonus	2.3 ± 0.6	1	3

Table 2 Mean combined thickness of uterus and placenta (CTUP) ± SD and Confidence Interval from the 4th month of gestation until term in 63

Month of gestation	CTUP (mm)				
	n	Mean ± SD	Lower measure	Upper measure	95% Confidence Interval
4	27	3.2 ± 0.6 [a]	1.8	4.5	3.0 – 3.5
5	31	3.4 ± 0.7 [a]	1.7	4.8	3.2 – 3.7
6	40	3.5 ± 1.1 [a]	1.5	5.8	3.1 – 3.8
7	32	3.9 ± 1.2 [ac]	2.2	6.1	3.5 – 4.4
8	53	5.1 ± 1.6 [b]	3.0	11.3	4.7 – 5.5
9	47	4.9 ± 1.2 [bc]	1.9	8.1	4.5 – 5.2
10	43	6.6 ± 1.6 [d]	3.2	10.6	6.1 – 7.0
11	45	7.2 ± 2.1 [d]	4.2	12.6	6.6 – 7.9

(a, b, c, d) different superscripts at column differ, $P < 0.01$.

Table 3 Percentage of CTUP measurements that exceeded the upper limit (suggesting placental failure or impending abortion) of the 95% confidence interval (assumed as twice SE) for each month of pregnancy.

Gestational age	Mares	Exceeded upper limit	
month	n	n	%
4	27	8	29.6
5	31	11	35.5
6	40	14	35.0
7	32	11	34.4
8	53	16	30.2
9	47	18	38.3
10	43	16	37.2
11	45	14	31.1
Total	318	108	33.9

(1997), but were higher than the upper risk values of *Troedsson* (2001) in 22.2% of cases. Although CTUP above the 95% confidence interval suggest impending abortion (*Troedsson* et al. 1997), no mare either aborted or presented with any signs of placentitis, such as premature udder development with or without the presence of a vulvar discharge (*Macpherson* and *Bailey* 2008a), like observed by *Souza* et al. (2010) and *Löf* et al. (2014). All foals were viable and born at term. These results are in agreement with those of *Bucca* et al. (2005), in that mares may exhibit thickening of CTUP without any negative effect on gestational development.

Diagnosis of placentitis at early stages and in mares that do not exhibit clinical signs of infection is difficult. Clinically, not all mares with ascending placentitis exhibit premonitory signs or gross placental pathology (*Mays* et al. 2002). Although thickening of CTUP has been advocated as a positive sign of placentitis, data from this study indicate that mares carrying a normal pregnancy may have a CTUP above the reference range and deliver a viable, 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. In a controlled study 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 no treated mares (*Bailey* et al. 2010). Probably many treatments based on the thickening of CTUP are unnecessary.

Caslick Index increases with age and can vary with nutritional status (*Pascoe* 2007) especially if loss of body condition occurs. In the present study, age influenced the Calick Index in a weak correlation. This can be explained because the mares used were young (mean age of 10.8 years) with good body condition during the gestation (mean of 3.5 ± 0.4). Caslick Index above 100 is associated with a normal anatomic arrangement and better fertility, than a score approaching 150 or higher (*Pascoe* 1979, 2007) when they must to be sutured. The mares of our study had not been submitted to a vulvar surgery. Only 4.4% (7 mares) presented Caslick Index higher than 100 and 1.6% of the measurements obtained a Caslick Index as higher as 150 (2 mares). Despite vulvar measurements occurred during pregnancy the moment when many mares presented a shorter effective and total vulvar length and a decrease in angle as well (*Lieux* 1972), the absence of abortions during the experiment can partially explain these results. The studied mares present a high percentage of vulvar length over the ischial tuberosities, but the Caslick Index was lower due to the vulvar declination. The vulvar angle can be influenced by the perineal muscular tonus and body condition, allowing a greatest body fat accumulation in the perineum.

We conclude that gestational age affects CTUP and its measurement in mares with correct vulvar conformation should not be the only parameter used to estimate placental failure and impending abortion.

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Conflict of interest statement

None of the authors have any conflict of interest to declare.

Animal welfare statement

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Erweiterte Zusammenfassung

Die Plazentitis ist die Hauptursache für Aborte und Neugeborenensterblichkeit bei Pferden. Eine Zunahme der Dicke von Uterus und Plazenta zusammen (CTUP) im Bereich des Zervixsterns gilt als Hinweis auf eine aufsteigende Plazentitis. Ziel dieser Arbeit war es, Abweichungen und einen Zusammenhang zwischen CTUP-Maßen und dem Aufbau der Vulva bei Stuten während der mittleren und späten Trächtigkeit zu charakterisieren.

Dreiundsechzig gesunde trächtige Stuten wurden beobachtet. Ab dem 4. Monat der Trächtigkeit bis zur Geburt wurden die Stuten einmal monatlich untersucht. Die Maße der Vulva wurden mittels eines Aluminium-Winkelmessers bestimmt. Während der Messung standen die Stuten auf horizontalem Fußboden. Der "Caslick-Index" errechnet sich aus dem Ergebnis der effektiven Länge (Entfernung zwischen dem dorsalen Schamwinkel und der Höhe des Tuber ischiadicum) in cm multipliziert mit dem Neigungswinkel der Vulva über dem Tuber Ischiadicum. CTUP wurde durch Ultraschalluntersuchungen von dem Zervix-Plazenta-Verbindungspunkt bestimmt (2.5 bis 5 cm cranial der Zervix), und die größte Messung wurde aufgezeichnet. Der körperliche Allgemeinzustand aller Stuten wurde vor jeder Untersuchung bewertet (Grad zwischen 1 und 5). Daten wurden mit ANOVA analysiert (statistischen Bedeutung von $P < 0.05$), und die Ergebnisse wurden als Mittel ± Standardabweichung dargestellt. CTUP wurde als Hauptvariable eingesetzt, und Monat der Trächtigkeit, Alter der Stute, körperlicher Allgemeinzustand, Neigungswinkel der Vulva, Gesamtlänge der Vulva, effektive Länge der Vulva, "Caslick Index" und ihre Interaktionen als abhängige Variablen verwendet. Mittelvergleiche wurden mit dem Tukey-Test durchgeführt. Der Korrelationskoeffizient von Pearson wurde für den Caslick-Index, CTUP, Neigungswinkel, effektiven Länge der Vulva und Alter der Stuten berechnet. Ein 95% Vertrauensintervall mit ± zweimal Standardfehler des Durchnitts (SE) wurde berechnet. Alle Stuten fohlten ohne Begleitung auf den Weiden, ohne menschliche Interferenz. Innerhalb der ersten 12 Stunden der Geburt wurden alle neugeborenen Fohlen klinisch untersucht und die Dauer der Trächtigkeit wurde registriert. Alle Stuten haben lebensfähige Fohlen auf die Welt gebracht mit einer durchschnittlichen Tragezeit von 330 ± 8.9 Tagen. Das Durschnitssalter der Stuten betrug von 10.8 ± 4.2 Jahre und der Körperzustand lag bei 3.5 ± 0.4. Der Caslick-Index war über 150 in 1.6% der Fälle und innerhalb 100 und 150 in 2.8% der Messungen. Das Alter der Stuten beeinflusste den Caslick Index ($P < 0.01$) mit einer schwachen Korrelation ($r = 0.21$). Dreihundertachtzehn Ultraschalluntersuchungen wurden von den 63 Stuten gewonnen. Die CTUP-Durchschnitte haben sich zwischen dem 4. und 7. Monat der Trächtigkeit nicht geändert, stiegen aber deutlich vom 7. zum 8. Monat, blieben gleich bis zum 9. Monat und nahmen dann wieder bis zum Abfohlen zu. Dies zeigte eine positive Korrelation ($r = 0.69$, $P < 0.01$) mit der Tragezeit. Es gab keine Einwirkung zwischen CTUP und Alter der Stuten ($P = 0.22$) oder körperliche Allgemeinzustand ($P = 0.45$). Ein Drittel der CTUP-Maße überschritt die obere Grenze des 95%-Vertrauensintervalls. Es wurden keine Einwirkung zwischen CTUP und effektiver Länge der Vulva ($P = 0.98$), Neigungswinkel der Vulva ($P = 0.45$) und Caslick-Index ($P = 0.68$) beobachtet. Die Diagnose Plazentitis im Anfangsstadium bei Stuten ohne klinische Hinweise der Infektion ist schwierig. Nicht alle Stuten mit aufsteigender Plazentitis zeigten klinische Hinweise oder Plazenta Pathologie. Obwohl die Verdickung der CTUP als positives Zeichen für Plazentitis befürwortet wurde, zeigten die Ergebnisse dieser Studie, dass Stuten mit normaler Trächtigkeit einen CTUP- Wert über dem Referenzbereich haben können und trotzdem ein lebensfähiges und gesundes Fohlen gebären. Die Anwendung mathematischer Werte, um biologische Modelle zu erzielen, und die klinische Bedeutung einer einzigen CTUP- Messung während der gesamten Trächtigkeit sollte kritisch überdacht werden. Wahrscheinlich sind viele Behandlungen auf Basis der Verdickung der CTUP nicht erforderlich. Der Caslick-Index steigt mit dem Alter und kann mit dem Ernährungszustand variieren (*Pascoe* 2007) vor allem dann, wenn ein Verlust der Körperkondition auftritt. In der vorliegenden Studie, beeinflusste das Alter den Calick-Index in schwacher Korrelation. Dies kann sich daraus erklären, dass die verwendeten Stuten jung waren (Durchschnittsalter von 10.8 Jahren) und während der Trächtigkeit einen guten Körperzustand aufwiesen (Mittelwert von 3.5 ± 0.4). Der Caslick-Index über 100 ist mit einem normalen anatomischen Aufbau und besserer Fertilität verbunden und Werte um 150 oder höher, benötigen eine Vulvoplastik. Keine der Stuten unserer Studie wurden einer Operation unterzogen. Nur 4.4% der Stuten (7 Tiere) zeigten einen Caslick-Index höher als 100 und 1.6% (2 Stuten) der Messungen ergaben einen Caslick-Index höher als 150.

Die Stuten zeigten einen hohen Prozentsatz der vulvaren Länge über den Tuber Ischiadicum, jedoch war der Caslick-Index wegen der Neigung reduziert. Der Neigungswinkel kann vom Tonus der Muskulatur des Perineum und der Körperbedingung der Stuten beeinflusst werden. Abschließend stellten wir fest, dass die Tragezeit der Stuten die CTUP-Messung beeinflusst, dass diese Messwerte, bei Stuten mit korrektem Aufbau der Vulva, jedoch nicht als alleinigen Kennwert für Plazentaversagen und Abort heranzuziehen sind.