



## Transrectal ultrasonographic measurements of the combined thickness of the uterus and placenta in Spanish Purebred mares

Fernando D. Requena<sup>1</sup>, Estrella I. Agüera<sup>1</sup>, Francisco Requena<sup>1</sup>, Carlos C. Pérez-Marín<sup>2,3</sup>

<sup>1</sup>Cellular Biology, Physiology and Immunology Department, Veterinary Faculty, University of Cordoba, Cordoba, Spain.

<sup>2</sup>Animal Medicine and Surgery Department, Veterinary Faculty, University of Cordoba, Cordoba, Spain.

### Abstract

The present study was conducted to establish the normal values of the combined thickness of the uterus and placenta (CTUP) in Spanish Purebred mares during late pregnancy. A total of 107 mares were examined at 210, 240, 270 and 300 days of gestation, of which 13 had abnormal gestations (foetal death, abortion or stillbirth). In mares with healthy foals, CTUP measurements showed significant differences ( $P < 0.0001$ ) at the various gestational ages ( $4.83 \pm 0.59$  mm,  $6.12 \pm 0.60$  mm,  $7.41 \pm 0.61$  mm and  $10.45 \pm 0.92$  mm at 210, 240, 270 and 300 days, respectively). A high positive correlation was observed between CTUP and gestational age ( $r = 0.923$ ;  $P < 0.001$ ). In mares with abnormal gestation, CTUP measurements were significantly ( $P < 0.0001$ ) higher than in healthy gestations at day 270 (8.89 vs. 7.41 mm) and day 300 (14.17 vs. 10.45 mm). The age of the mare, fertilization time, type of semen and gestation length did not affect the CTUP. The results obtained suggest that the cut-off point for detecting abnormal pregnancies in Spanish Purebred mares is 7.75 mm on day 270 and 12.7 mm on day 300. The authors find that the incidence of placental thickness enlargement in the late gestation of Spanish Purebred is 14%, and a transrectal ultrasonographic examination is highly advisable at 270 days of gestation. Such timely placental assessment could reveal otherwise undetected disorders, thereby enabling early and proper treatments to be administered to prevent foetal loss.

**Keywords:** CTUP, equine, gestation, placentitis, ultrasonography.

### Introduction

In terms of population size, the Spanish Purebred (SPB) is the largest Spanish horse breed and the industry surrounding it in Spain exceeds other breeds according to the national census. There are approximately 215,000 SPB horses all over the world (Ministerio de Agricultura, Alimentación y Medio Ambiente - MAGRAMA, 2016), and they are bred in around sixty countries. The use of ultrasound to evaluate pregnancy and foetal wellbeing has been employed in horses for over 30 years (Palmer and Driancourt, 1980), and it helps to determine ascending placentitis, which is one major cause of abortion in mares (Giles *et al.*, 1993). This disorder occurs late in pregnancy, when bacteria reach the uterus from the lower reproductive tract (Cummins *et al.*, 2008),

causing infection in the cervical star and the chorioallantois region. This infection provokes ulceration and thickening of the chorioallantois (Platt, 1975), with high expression of pro-inflammatory cytokines and release of prostaglandins, inducing uterine contraction and early delivery (Dudley and Trautman, 1994; Pollard and Mitchell, 1996; LeBlanc *et al.*, 2002; LeBlanc, 2004). It may be associated with an early birth, the birth of a weak foal or abortion, and harmed mares often have difficulty in conceiving during the following breeding season (Troedsson *et al.*, 1997). To determine whether there is any risk of foetal loss, endocrine profiling (Rossdale *et al.*, 1991; Stawicki *et al.*, 2002; Morris *et al.*, 2007) and transrectal and transabdominal ultrasonography (Renaudin *et al.*, 1997; Bucca *et al.*, 2005; Carrick *et al.*, 2010; Löf *et al.*, 2010) are frequently used, and are reported during late gestation, at around 8-10 months. Bailey *et al.* (2012) also compared the ability of B-mode and Doppler ultrasonography to detect ascending placentitis in pony mares with experimentally induced disease.

As the cervical star region is frequently altered in mares with ascending placentitis, transrectal ultrasonography is an excellent tool for monitoring foetal and placental changes. It has been suggested that measurements of the combined thickness of the uterus and placenta (CTUP) could be used to identify mares' risk of developing ascending placentitis or other placental disorders. Reanudin *et al.* (1997) described the CTUP evaluation technique, and reported normal values from day 180 to day 330 of pregnancy. Few animals have been involved in descriptive studies and usually different breeds were included in the same study. While the breed factor has been considered in many studies related to gestation, few horse breeds have been studied with a view to establishing their reference CTUP values.

From a clinical/practical perspective, it has been reported that utero-placental thickness should be no thicker than the gestational age of the foetus in mm plus 1, similar to that described in humans; however, this rule is excessively ambiguous and imprecise (Reef *et al.*, 1995).

In SPB pregnant mares, CTUP values are unknown and better understanding of placenta status could prevent late gestation disorders. Giles *et al.* (1993) reported that the incidence of foetus loss in different equine breeds due to ascending bacterial placentitis (foetal death, abortion and stillbirth) reached 20%, while others quantified these losses at 10% in a sample where the majority of cases were from Thoroughbred mares (Smith *et al.*, 2003). LeBlanc (2010) reported that placentitis affects approximately 3-

<sup>3</sup>Corresponding author: pv2pemac@uco.es

Received: November 16, 2017

Accepted: December 30, 2017



7% of pregnant mares, in line with Troedsson and Zent's (2004) findings in Thoroughbred mares. But there are no references describing the incidence of foetus loss due to placentitis in SPB mares.

The aim of this study was to monitor CTUP measurements during late gestation to establish a nomogram for SPB mares and determine the cut-off point for identifying ascending placentitis and initiating timely prophylactic therapies.

## Materials and Methods

### Animals

A total of 107 SPB mares, ranging from 3 to 17 years old, with body weights from 520 to 650 kg and body condition score between 3.5 and 4.5 (using a 5-point scale) were involved in this study. The animals were clinically and reproductively healthy, based on good vulvar conformation (with Caslick index lower than 100), absent of abnormal uterine fluid, negative uterine cytology, and normal ovarian activity before insemination; also, no abortions were reported in these mares during the last two years. They were located at the Miguel Angel Cardenas Equine Reproduction Centre, in Ecija (Seville, Spain; 15° 15' 51" N, 12° 54' 30" E). They were inseminated with both cooled and frozen semen, and they were treated for gastrointestinal parasites and for equine herpes virus (EHV) 1 and 4 (Duvaxyn® EHV 1,4 im, Fort Dodge Animal Health Europe) at five, seven and nine months of gestation. The animals were fed with 3 kg of feed/day, plus hay *ad libitum* and free access to water. A total of 94 mares exhibited normal gestation and healthy foals, while 13 mares had abnormal gestations (five aborted and eight gave birth to a weak foal or suffered stillbirth). Following the approach of Morris *et al.* (2007), no foeto-placental compromise was defined as delivery of a healthy foal and no gross signs of placentitis, while foeto-placental compromise was defined as birth of a non-viable, premature or dysmature foetus, or clear signs of placentitis.

### Transrectal ultrasonography

All the mares were examined at 210, 240, 270 and 300 days of gestation using ultrasonography equipment (Aquila, Esaote-Pie Medical, Maastricht, Netherlands). A 7.5-MHz linear transducer was located laterally at the cervical-placental junction. The CTUP was recorded from the ventral aspect of the uterine body (Troedsson *et al.*, 1997). The attached placenta was evaluated in the area adjoining the cervical star (Troedsson *et al.*, 1997). Three measurements were taken at each examination.

A total of 415 echographies were made and 1,245 CTUP measurements were taken.

### Statistical analysis

All statistical analyses were carried out using SPSS statistics v22.0.0 (Chicago, IL, USA). Data are shown as means  $\pm$ SD, and differences were considered

significant when  $P < 0.05$ .

Ventral CTUP was scanned and the nomogram for the last months of pregnancy was built. Linear regression (forward stepwise) was carried out for CTUP, including mare age, type of semen, gestation length and gestational age. Only gestational age was introduced into the model. Then, the best-fit regression equation was determined using data from term gestations (i.e. normal gestations), obtaining a linear regression model yielding the following equation:  $CTUP = (-8.21) + 0.06 \times \text{gestational age (days)}$  ( $R^2 = 0.853$ )

The receiver operating characteristic (ROC) curve provided information about the CTUP cut-off value, which offers the best predictive values for healthy gestation diagnosis at different stages of pregnancy. Sensitivity and specificity were calculated for different CTUP values. With this proposal, cut-off values were established for 95% confidence interval. Furthermore, predicted positive and negative values were calculated to determine the likelihood of the test accurately identifying the presence of abnormal gestation. Positive predictive value is defined as the proportion of animals that tested positive that were truly affected. Negative predictive value is defined as the proportion of animals that tested negative and were truly healthy.

The Mann-Whitney U test was used to determine if the age of mares and the intervals from insemination were significantly different comparing the mare population showing normal and abnormal gestations.

## Results

A high positive correlation was observed between CTUP and gestational age ( $r = 0.923$ ;  $P < 0.001$ ) in mares that showed normal term gestations, with a larger thickness increment as gestation advances. However, variables such as mare age, the interval between parturition and fertilization, type of semen or gestational length did not affect the placental thickness. Gestation length in healthy foaling mares ranged from 308 to 360 days, while mares with abnormal gestations lasted from 280 to 359 days.

As may be seen in Table 1, the monthly CTUP measurements in mares with healthy foals exhibited significant differences ( $P < 0.0001$ ). The CTUP measurements taken on a monthly basis showed a homogeneous mean increase of approximately 1.3 mm from 210 to 270 days. However, from 270 days onwards the mean increased almost threefold (3.0 mm/month) in comparison to the preceding period (Fig. 1). A nomogram for CTUP in SPB mares was undertaken, and data from abnormal pregnancies were subsequently used to determine the cut-off points for providing information about potential gestational defects. In the population studied, the incidence of abnormal gestation was 14%. Results showed that no significant differences appeared on days 210 and 240 (0.728 and 0.053, respectively), suggesting that the measurement of CTUP at these periods is unlikely to be able to discriminate

between healthy and non-healthy gestations. However, on days 270 and 300, i.e. nearer to the parturition, significant differences ( $P < 0.0001$ ) were detected, and it follows that CTUP values can play a role in predicting the success of gestation. The next step was to determine which cut-off value should be used in order to obtain the best diagnostic value. The authors decided to choose the cut-off value with the highest sensitivity and lowest rate of false positive results (calculated as  $1 - \text{specificity}$ ). On day 270, the best cut-off value was 7.750 mm, showing a sensitivity of 92.3% and a false positive percentage of 39.4% (Fig. 2). On day 300, results gave CTUP value of 12.7 mm and enabled a diagnosis of non-healthy pregnancy in all cases (i.e., sensitivity was 100%), with no false positives (Fig. 2).

Table 1 and Fig. 3 show that the increment of CTUP between 210 and 270 days in mares with abortions or weak foals at term was not homogeneous. The increment from day 240 to day 270 was higher

(2.4 mm) than that from day 210 to day 240 (1.6 mm), and in the following month (day 300) the thickness increased further (5.3 mm). However, this group of mares showed a similar CTUP measurement and ultrasound image at 210 and 240 days of gestation to those that gave birth to healthy foals (Fig. 3a, b, c and d). Highly significant differences ( $P < 0.001$ ) were observed when comparing the 270 and 300-day CTUP measurements between healthy and non-healthy gestations (Table 1, Fig. 3e, f, g and h). On comparing these increases, significant differences were found both in the results obtained in the period from 240 to 270 days and in that of 270 to 300 days, when comparing the two groups ( $P < 0.001$ ).

The mares that suffered abortion or placental failure were significantly older than the mares with normal gestation ( $11.7 \pm 4.2$  vs.  $9.1 \pm 3.9$ ;  $P = 0.025$ ), and exhibited longer intervals between parturition and insemination ( $220.0 \pm 183.0$  vs.  $110.1 \pm 160.7$  days;  $P = 0.025$ ).

Table 1. Percentage of CTUP measurements in mares with healthy gestational periods and mares with abnormal pregnancy (with stillbirths, late abortions or weak foals) for each month of pregnancy.

Days of gestation	Type of gestation	n	Mean	SD	95% C.I.		P value
					Lower	Upper	
210	Healthy	94	4.83	0.59	4.71	4.95	0.727
	Abnormal	13	4.89	0.32	4.72	5.07	
240	Healthy	94	6.12	0.60	6.00	6.24	0.053
	Abnormal	13	6.47	0.41	6.25	6.69	
270	Healthy	94	7.41	0.61	7.28	7.53	0.0001
	Abnormal	13	8.89	1.39	8.14	9.65	
300	Healthy	94	10.45	0.92	10.26	10.63	0.0001
	Abnormal	11	14.17	1.27	13.42	14.92	

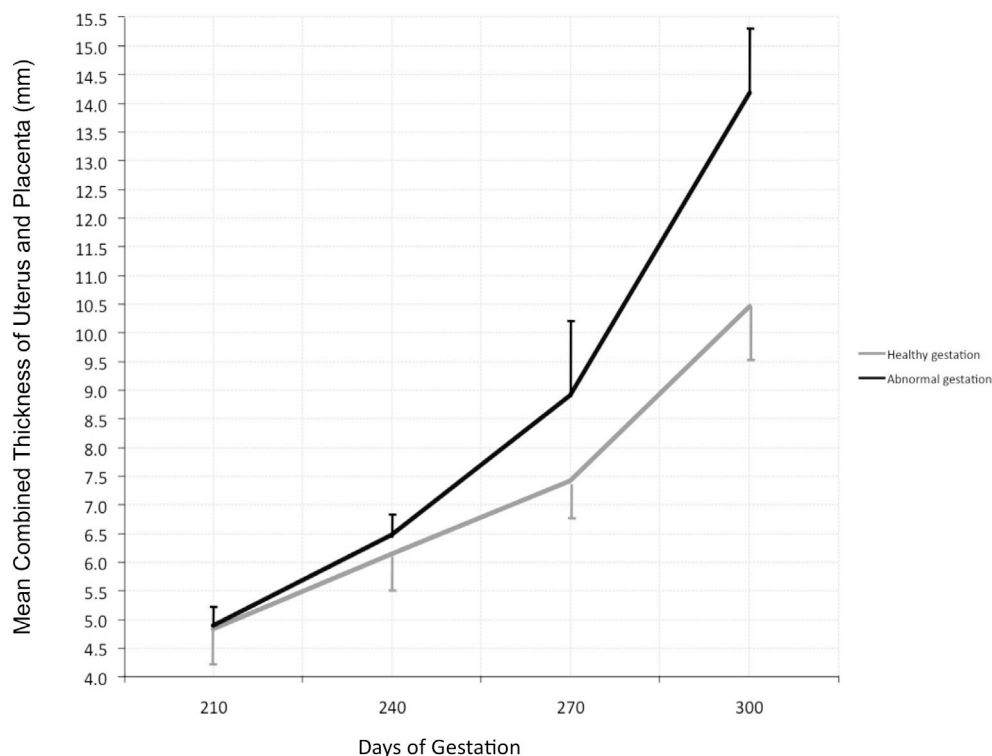


Figure 1. Monthly monitorization of CTUP records (mean  $\pm$  SD) by transrectal ultrasonography in Spanish Purebred mares with healthy (solid grey line) and non-viable (solid black line) gestation.

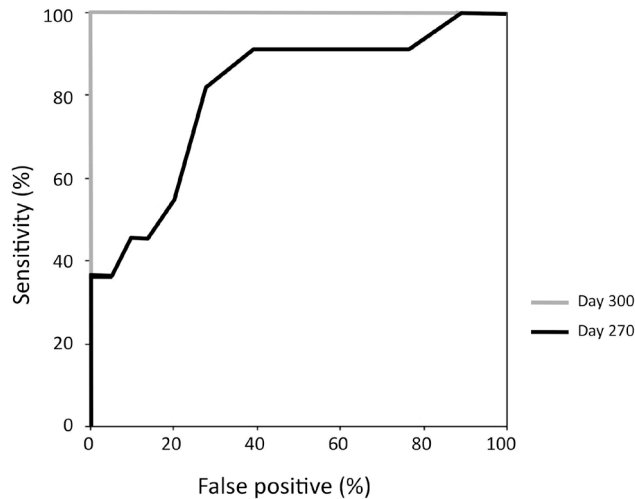
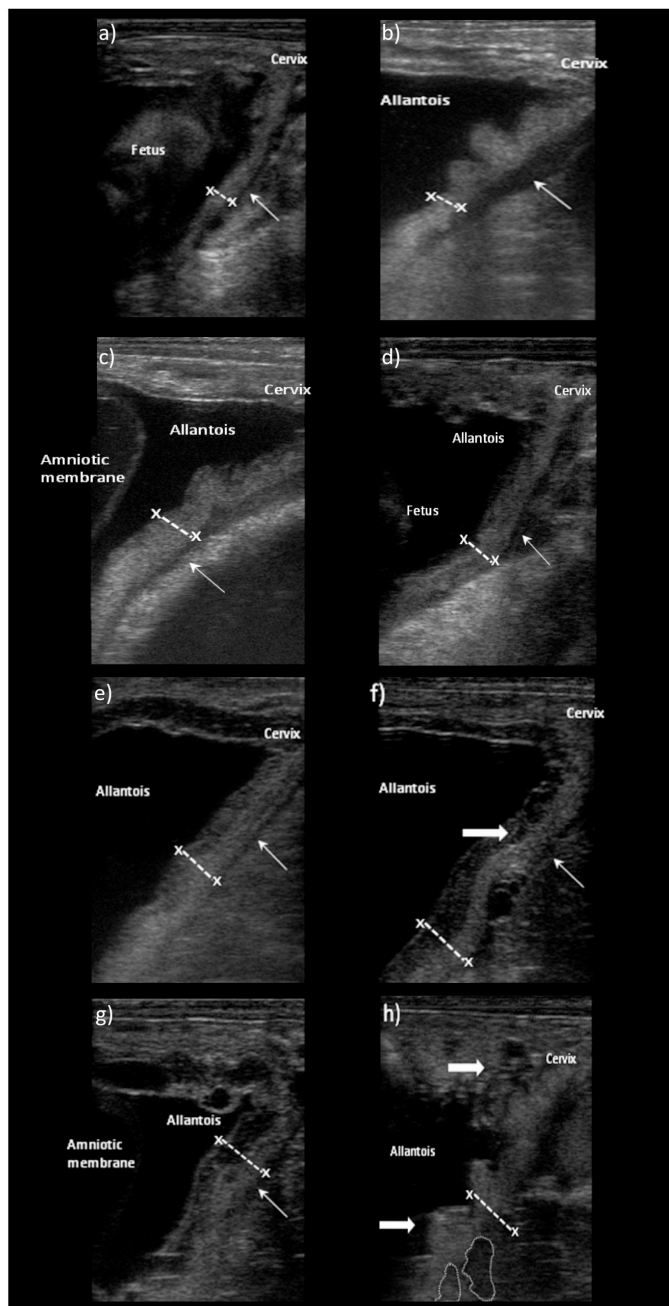


Figure 2. Receiver-operating characteristic (ROC) curves of combined thickness of uterus and placenta in SPB mares at day 270 (black line) and day 300 (grey line) of gestation.

Figure 3. Measurements of combined thickness of uterus and placenta (CTUP) at various phases of gestation. The dashed white line between X refers to CTUP. The thin white arrow indicates the position of the middle branch of the uterine artery in the ventral area of uterine body. The thick white arrow indicates areas of utero-placental separation. The ultrasound images on the left correspond to CTUP measurements in mares with healthy gestation at 210 (a), 240(c), 270(e) and 300(g) days of gestation. The ultrasound images on the right correspond to mares with non-viable gestation at 210(b), 240(d), 270(f) and 300(h) days of gestation. Figure 1h shows separation from the endometrium with exudative fluid (at the bottom, areas delimited by dashed white lines), indicating disorganization of the uterine-placenta junction, mainly in the dorsal area of the placenta.





## Discussion

To determine whether a given placental thickness is normal or not, normal placental thickness must be defined for a gestational age. Since this parameter may vary among different populations (Table 2), breed-specific nomograms are needed. Renaudin *et al.* (1997) defined abnormal CTUP as measurements that deviated from 95% confidence interval of previously defined normal values. However, those normal values

were taken from nine mares of different breeds (Quarterhorse, Thoroughbred and American Paint Horse). Data obtained here demonstrate that CTUP measurements in pregnant mares is really effective to discriminate healthy and abnormal gestations since day 270, in accordance with the findings of Colon (2008), although placental abnormalities (perhaps associated to ascending placentitis) might occur and be evident sooner. In Criollo mares however, differences have been detected at eight months (240 days; Souza *et al.*, 2010).

Table 2. CTUP measurements (mm) in normal gestations.

Breed	n	Days of pregnancy					
		180	210	240	270	300	330
Quarterhorse, Thoroughbred and American Paint Horse (Renaudin <i>et al.</i> , 1997)	9	3.84	3.91	4.33	4.38	5.84	7.35
Arabian and ponies (Barnes <i>et al.</i> , 2005)	20	6.3	5.0	7.7	7.9	8.4	-
Unknown breed (Bucca <i>et al.</i> , 2005)	150	4.7	5.0	5.2	7.7	8.3	9.56
Thoroughbred (Colon 2008)	106	-	-	-	5.5	7.1	8.5
Criollo breed (Souza <i>et al.</i> , 2010)	63	3.47	4.02	5.17	4.87	6.53	7.3
Warmblood (Coutinho <i>et al.</i> , 2013)	15	-	-	-	-	8.8	9.7
Data obtained in the present study	107	-	4.83	6.12	7.41	10.45	-

The percentage of mares with measurements over the upper limit (based on the 95% confidence interval) was 33.9% in Criollo mares (Souza *et al.*, 2010), but 3.1% (Troedsson and Zent, 2004) and 15% (Colón, 2008) in others. In the present study, non-healthy pregnancies in SPB mares represented 14% of the total, and the CTUP average was significantly higher than in healthy pregnancies from day 270. In this context, Souza *et al.* (2010) argue that many mares may be assumed to have placentitis and thus to require treatment, even though in many cases no abortion or symptoms of placentitis are observed. Bucca *et al.* (2005) observed that mares may have increased CTUP without any negative effect on gestational development.

The age of mares did not affect the CTUP in SPB mares, a finding in line with that previously described (Souza *et al.*, 2010). However, it was noted that the group of mares that suffered abortion or placental failure was significantly older than mares with normal gestation and took longer to become pregnant. In the present study, the later gestations of mares exhibiting abnormal CTUP were monitored. One mare presenting abnormal CTUP measurements repeated this condition in her subsequent pregnancy, but this was not noted in others.

Gestation losses are a major problem for the equine industry, and for decades attempts have been made to address the difficulty by treating the diseases causing them. One of those cited is placentitis, with a general incidence of 3-7% (LeBlanc, 2010). In recent years ultrasonography has been recognized as providing a useful technique for estimating the state of the placenta and foetus (Troedsson and Zent, 2004). In the present study, the changes in the CTUP measurement in the last months of gestation were recorded, since this is considered to be an indicator of placentitis, as well as alterations in the gestation and/or abortions (Giles *et al.*, 1993), with the aim of establishing normal values of this measurement for Spanish Purebred (SPB) mares. The

ultrasonography was performed in the area of the cervical star, which is the region most affected by placentitis (Renaudin *et al.*, 1997; Calderwood *et al.*, 2002; Macpherson, 2005). In the present study, CTUP measurements were monitored at day 210, 240, 270 and 300 of gestation, mindful of the fact that several authors have reported that ascending placentitis causing alterations in gestation occurs over the last months of pregnancy (Troedsson and Zent, 2004; Cummins *et al.*, 2008). No differences between healthy and abnormal gestations were observed until day 270, and it is therefore recommended that placental ultrasonographic inspection be initiated no later than this date.

Renaudin *et al.* (1997) obtained a non-significant increase in the monthly CTUP measurement from four to eight months of gestation, but the increase was very significant from 10 to 11 months (1.51 mm) and from 11 to 12 months of gestation (2.17 mm). Similar results in the last months were observed in the present study, with an increase in CTUP from eight to nine months of 1.23 mm, and from nine to 10 months of 3.06 mm in healthy gestations. The same trend with higher measurements was observed in abnormal gestations (2.86 and 4.705 mm from eight to nine months and from nine to ten months, respectively). This is in accordance with the findings reported by Hendriks *et al.* (2009), who found no important differences in the CTUP measurement until the fifth month of gestation, with the fastest enlargement of the placenta occurring in the eighth month. This may be attributable to foetus growth in the third phase of gestation being exponential, vascularization increasing as a consequence in order to meet the needs of the foetus. This also occurs in other species, such as jennies (Crisci *et al.*, 2014). Macpherson and Bailey (2008) reported that clinical signs do not occur until the disease is in an advanced stage. The mares that suffered abortion in the present study did not present any detectable external signs. However, they did undergo an increase in their CTUP or



a separation of the endometrium membranes due to their purulent matter (hyperechoic; see Fig. 3h), which accords with other findings (Macpherson and Bailey, 2008). Likewise, in the same image an exudative fluid can be observed in the uteroplacental space (delimited by white dashes). From 210 to 240 days of gestation, both the CTUP measurements and the echography images were similar in both groups. Figure 3f shows, in addition to an increase in the CTUP measurement, an edema in the uteroplacental union in which the placental structure is distinguished as being more hypoechogenic than the uterus. However, in Fig. 3g, in spite of there being an edema both on the dorsal and ventral edge of the uterus corpus, it is apparent that this is a physiological situation in the last month of gestation. It should be noted that in this study it was estimated that 8.5% of the evaluated mares aborted, or gave birth to a weak foal, a percentage which, although based on a small sample of the population, could be representative of SPB mares in general, since it does not differ from what has been reported by other authors (Giles *et al.*, 1993; Smith *et al.*, 2003; Troedsson and Zent, 2004; LeBlanc, 2010).

In equine reproduction, the use of transrectal ultrasonography is a minimally invasive routine procedure. In the present study it did not cause any adverse effects on scanned pregnant mares and this is in accordance with the findings of Renaudin *et al.* (1997). Since most placentitis occurs by upward routes, the infection very often first affects the cervical star (LeBlanc, 2004; Troedsson and Zent, 2004), and for this reason the use of transabdominal ultrasonography is not advisable, since it is unable to provide scans of the area in question. The present authors found that transrectal echography may be readily evaluated, in accordance with other findings (Cummins *et al.*, 2008).

These results are a preliminary attempt to determine CTUP cut-off values in Spanish mares, and indicate the importance of carrying out the transrectal evaluation of placental thickness from the ninth month of gestation. As has been highlighted by others authors (Bucca *et al.*, 2005; Colón, 2008), it is important to monitor foetal status throughout pregnancy if signs of problems are to be detected in a timely manner. However, this was not the aim of the present study.

In conclusion, in SPB mares, a monthly transrectal ultrasonographic examination is recommended from 210 days of gestation, with the aim of assessing the thickness of the placenta by means of CTUP measurement; furthermore, this study demonstrated that differences between healthy and abnormal gestations are more evident since day 270 of gestation. A placental thickness nomogram has been defined for SPB mares during late-term gestation and around 14% of pregnant mares has been diagnosed as placental thickness enlargement.

#### Acknowledgements

The authors are grateful to Miguel Angel Cárdenas (R.I.P.) and his staff for allowing them to do this research in his stud.

#### Conflict of interest

None of the authors have any conflict of interest to declare. There has been no financial support for this work that could have influenced its outcome.

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