#### **Animal Sciences**

# Ultrasonographic measurements of the combined thickness of the uterus and placenta in Colombian creole mares

Medidas ultrasonográficas del espesor conjunto de la unión útero-placentaria en yeguas criollas colombianas

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#### **Abstract**

This research aimed to determine the ultrasonographic values of the combined thickness of uterus and placenta (CTUP) from the fourth month to the end of gestation in 20 Colombian creole mares from 4 to 8 years with a mean weight 348 kg, where 640 measurements were taken for each examination. As such, transrectal and transabdominal examinations were conducted using a 6.0 MHz linear transducer and R40 3.5 MHz convex sector transducer respectively. Statistically significant differences ( $P \le 0.05$ ) were found throughout gestation between the parameters of CTUP, and differences when comparing the results with those reported in other equine breeds from the same gender. CTUP mean values increased with gestational age; finding for transrectal via a minimum of 3.1 ± 1.28 mm and a maximum of 7.3 ± 2.22 mm and for transabdominal via a minimum of 6.3 ± 1.28 mm and a maximum of 14.3 ± 2.22 mm.

**Key words**: Abortion, Colombian creole mares, equine, gestation, ultrasonography.

#### Resumen

En el trabajo se determinaron los valores ultrasonográficos del espesor conjunto de la unión úteroplacentaria (ECUP), desde el cuarto mes hasta el final de la gestación, en 20 yeguas criollas colombianas, con edades entre 4 y 8 años y un promedio de peso de 348 kg, para un total de 640 mediciones en cada examen. Para el efecto se hicieron exámenes transrectal y transabdominal con transductor lineal de 6.0 MHz y transductor convexo R40 de 3.5 MHz, respectivamente. Durante el periodo de gestación se encontraron diferencias (P  $\leq$  0.05) entre los parámetros ECUP y divergencias cuando se compararon con los resultados reportados en hembras equinas de otras razas. La media de los valores ECUP incrementó con la edad gestacional; para la transrectal se encontró un mínimo de 3.1  $\pm$  1.28 mm y un máximo de 7.3  $\pm$  2.22 mm, y para transabdominal estos valores fueron de 6.3  $\pm$  1.28 mm y 14.3  $\pm$  2.22 mm.

Palabras clave: Aborto, ecografía, equinos, gestación, yeguas criollas colombianas.

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#### Introduction

Although the technical advances in the reproductive diagnosis, in the Colombian equine gestation are still present miscarriages up to 15% due to alterations in placenta, embryo, fetus, the mother or the combination of these (Troedsson and Sage, 2001). Several of these causes happen without notice because the heath of the mare, ovaries and uterus is the only one controlled. To this problem are added the low conception rates since these equines are not selected by their fertility but, by their phenotype (Allen and Stewart, 2001).

There are several factors needed for the success of the equine production systems that can be translated in earnings or losses to the owner, among them are the advance pregnancy miscarriages that mean abortion after more than 4 month of pregnancy. Mare abortion has an incidence of 5% and 15%, higher than in other species (LeBlanc, 2003; Hafez and Hafez, 2002). On the other hand, those abortions after 5 months of pregnancy are a serious problem for the equine industry (Roberts, 1986; Troedsson and Sage, 2001) since the failure is not only to breed a foal by the reproductive age of the mare but, additionally the pregnancy rate is reduced (Córdova, 2006).

The normal development of the fetus relies on a functional placenta or fetus-placenta unit formed by the mother, placenta and fetus. Any anatomical or physiological alteration carries on a placental insufficiency and abortion, the most common result of an advanced pregnancy interruption (Troedsson and Sage, 2001; Bucca *et al.*, 2005).

The macroscopical evaluation of the placenta is a frequent practice after the delivery or when occurs an abortion or a disease in the new born; however this late exam is not useful to guarantee the fetus survival (Whitwell and Jeffcott, 1975b; Renaudin *et al.*, 1997; Troedsson and Sage, 2001). This exam could be performed by

ultrasonography or by endocrinological test. For the ultrasound of the uterine and placenta unit is used a 7.5 to 10 MHz transducer, preferably by sector or convex, that produces a triangular image with an increment in the visual field in the deep portion of the image (Reef et al., 1995). The normal values for the combined thickness of the uterus and placenta (CTUP), have been determined using 5 or 7.5 MHz transducers (Adams-Brendemuehl and Pipers, 1987; Reef et al., 1996.). Renaudin et al. (1997) found CTUP values between 3.5 mm and 4.69 mm in pregnancies between 4 and 9 months and, between 5.53 mm and 11.77 mm in pregnancies between 10 and 12 months, however, between 4 and 8 months there were not observable changes but significant increases each month from the last third of pregnancy. Reef et al. (1996) performed an echography exam of the placenta in the left and right cranial quadrants and the caudals of the same sides and found that using this technique the mares under normal pregnancy had minimum values for CTUP of 7.1 ± 1.6 mm and maximum of  $11.5 \pm 2.4$  mm. in further studies these researchers found that mare with high CTUP values frequently had abnormal foals.

Troedsson et al. (1997) found in pure english breed mares that increases in the CTUP at the middle or end part of pregnancy indicated placental insufficiency and imminent abortion; on the opposite, a normal thickness of the placenta had a normal pregnancy. In normal management conditions it is suggested that a CTUP higher than 8 mm between days 271 and 300, 10 mm between days 301 and 330 and, 12 mm after day 330, indicate placental insufficiency and imminent abortion. Schott (1991) found than a CTUP > 17.5 mm represents placentitis. Renaudin et al. (1997) when studying transrectally the monthly variations in the CTUP in mares with normal pregnancy notice significant differences in the CTUP measurement between pregnancy months. Using the transabdominal way, the CTUP is not increase constantly thus the confidence of this measurement is under questioning.

This study had the objective of establishing the minimum and maximum ultrasonographic measurements for CTUP, from the fourth month till the end of pregnancy in Colombian creole mares with normal pregnancy and, to compare these results with the ones observed in equine breeds.

#### Materials and methods

The study was done in the town of Ibague (4°15´ N and 75° 00' W), department of Tolima, Colombia, at 1285 MASL and temperature of 21°C. In this work were pregnant monitored 20 mares from commercial breeding places, which during their clinic history did not report any reproductive alteration, they were between 4 and 8 years old proved by registry or dental chronometry with an average weight of 348 kg and with a minimum time of pregnancy of about 4 months confirmed by ultrasound measurement of the fetal orbital diameter as proposed by Renaudin et al. (2000). These mares were monitored each month till the end of the pregnancy and, for selection, age and pregnancy were not taken into account since these parameters affect significantly the development and foal weight at the time of birth (Hendriks et al., 2009).

Monthly ultrasonographic follow up was done between the fourth and eleventh month of pregnancy using the ultrasound Auila Vet®, with R40 convex probe, 3.5 MHz frequency for transabdominal examination and a lineal transducer with 6.0 MHz frequency in the transrectal examination for a total of 1280 CTUP measurements, 640 measurements per examination type.

For transrectal examination the uterus was divided in left and right cranial quadrants and caudals in the same sides according to recommendations of Reef et al. (1996). For the transabdominal examination, the maternal abdomen was divided in four compartments as well from the mammary gland to the xiphoid. The CTUP average for both ways was obtained with three measurements in different places of the corresponding quadrant. In the statistical analysis (SAS version 9.1) descriptive measurements (mean, standard deviation, minimum, maximum and interval of confidence at 95%) were calculated for the combined thickness of the uterus and placenta (CTUP, mm); additionally, an analysis of variance was performed to determine the differences between the means in each pregnancy time and the Tukey's multiple comparison test and, the Pearson's correlation coefficient was calculated to establish the association degree between the CTUP and the pregnancy time.

#### Results and discussion

## Transrectal examination

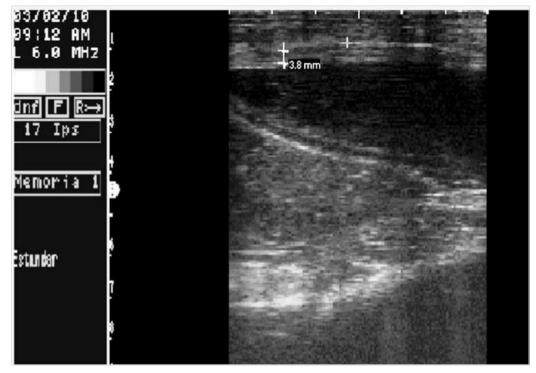
In the Table 1 are included the results of the CTUP for this examination and in Picture 1 is observed an ultrasound of a 4 month pregnancy mare. In the Table 1 is observed that as the pregnancy age increases the CTUP average also increases on a proportional manner, as well as the variability and dispersion of the data through the time. This finding is associated with the fetus growth which is accompanied of an increase in the amount of blood flux to meet the metabolic needs; consequently the placenta increases its thickness and the contact area with the uterus.

In Figure 1 is displayed the relation between CTUP by transrectal examination and the pregnancy age which has a correlation coefficient (r = 0.74) corresponding to a direct lineal relation between both variables. Among the variables pregnancy time and CTUP the lineal regression model showed a positive relationship ( $r^2 = 0.55$ ) were only the 55% of the total variation in the CTUP is

Table 1.	Combin	ed	thickness of	the ut	erus and	l placenta	. (CT	UP, mm	ı) in 1	the quadra	ants of t	the
	uterus	of	Colombian	creole	mares,	between	the	fourth	and	eleventh	month	of
	pregnar	ncy	. Transrectal	ultras	ound wit	h 6 MHz p	robe	· .				

Pregnancy month	Maximum (mm)	Minimum (mm)	Average± S. D. (mm)	IC
4	4.2	1.8	3.1 ± 0.66 h*	2.83 -3.41
5	5.1	2.5	$3.6 \pm 0.76 \text{ g}$	3.33 - 4.00
6	5.5	2.8	$4.1 \pm 0.80 \text{ f}$	3.78 - 4.49
7	6.2	3.0	$4.4 \pm 0.88$ e	4.10 - 4.86
8	7.1	3.1	4.9 ± 1.14 d	4.42 - 5.41
9	8.1	3.7	5.6 ± 1.35 c	5.01 - 6.20
10	8.8	4.3	$6.4 \pm 1.47 \text{ b}$	5.77 - 7.05
11	9.9	5.4	7.3 ± 1.53 a	6.72 - 8.05

<sup>\*</sup> Average values followed by equal letters do not significantly differ (P < 0.05) according to Duncan's test. iC = interval of confidence.



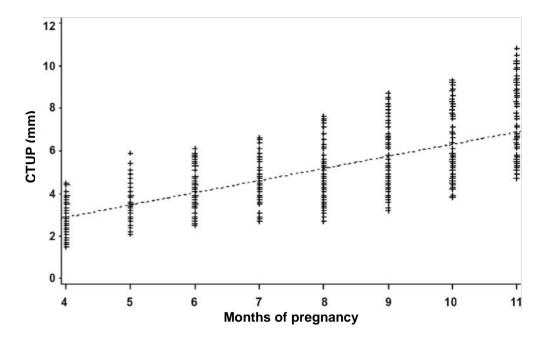
**Picture 1.** Transrectal ultrasound with 6MHz probe of the combined thickness of the uterus and placenta of a 4 months pregnancy Colombian creole mare with normal pregnancy. The combined uterus and placenta with a thickness of 3.8 mm is indicated with the (+) symbol.

specified by the pregnancy time. The values of the CTUP growth can be determined by the equation:

CTUP rectal = 
$$0.622 + 0.569x$$
 (1)

where, x = months of pregnancy

For each month of pregnancy the CTUP increases, in average, 0.569 mm. The ultrasonographic exam by the endorectal way with 6.0 MHz transducer in Colombian creole mares with normal pregnancy showed a CTUP with minimum average



**Figure 1.** Regression of the combined thickness of uterus and placenta (CTUP, mm) in the uterus quadrants of Colombian creole mares between the fourth and eleventh month of pregnancy by transrectal examination with 6.0 Mhz probe.

value of 3.1 ± 1.28 mm and maximum of 7.3 ± 2.22 mm (Table 1). Souza *et al.* (2010) at the South of Brazil worked with creole mares and found minimum values of 3.21 ± 0.62 mm and maximum values of  $7.3 \pm 2.1$ mm, without significant changes between the fourth and seventh month of pregnancy; however, from the seventh month till the eighth they observed increases until reaching stability at the ninth month and increase again till the end of pregnancy. These results agree with the ones found in this study, possibly because both studies were referring to equine creole breeds with similar height and weight, conversely, they differ from the results found by Hendriks et al. (2009) in Dutch Warmblood mares, Bucca et al. (2005) in Standardbred mares, Renaudin et al. (1997) Cuarto de Milla, Thoroughbred and American Paint Horse mares.

#### Transabdominal examination

In this examination, similar to the trasnrectal study, it was observed a proportional increment of the pregnancy age and the CTUP values (P < 0.05) (Table 2 and Picture 2).

In the Figure 2 a trend for CTUP monthly increase with the increase in pregnancy time is observed when measured by transabdominal examination. The coefficient of correlation between the CTUP and pregnancy time variables (r = 0.80) indicates a direct and high association between the increment in CTUP and the increment in pregnancy time. The values of the increment on CTUP can be predicted using the equation:

CTUP transabdominal = 
$$1.26 + 1.05x$$
 (2)

where, x = months of pregnancy

In each month of pregnancy the CTUP increased, in average, 1.05 mm. Among the variables pregnancy time and CTUP the lineal model showed a correlation of  $r^2$  = 0.64, where the 64% of the total variation of CTUP is determined by the pregnancy time, this suggests the enrolment of other variables or individual traits. When comparing this result with the one obtained by the

Table 2.	Combined thickness of	the uterus and placenta	(CTUP, mm) in the quadrants of the			
	uterus of Colombian	creole mares, between	the fourth and eleventh month of			
pregnancy. Transabdominal ultrasound with 3.5 MHz R40 convex probe.						

	Minimum (mm)	Maximum (mm)	Average ± S. D. (mm)	IC
4	4.4	8.7	6.3 ± 1.28 a*	5.83 - 6.95
5	4.8	9.1	$6.8 \pm 1.28 \text{ b}$	6.31 -7.43
6	5.2	9.5	$7.4 \pm 1.29 \text{ c}$	6.83 - 7.97
7	5.7	9.8	$7.9 \pm 1.31 d$	7.37 - 8.53
8	6.1	10.2	8.5 ± 1.36 e	7.93 -9.13
9	7.1	12	9.5 ± 1.49 f	8.88 - 10.19
10	9	14.7	12.0 ± 1.71 g	11.32 - 12.82
11	10.5	17.7	14.3 ± 2.22 h	13.37 - 15.31

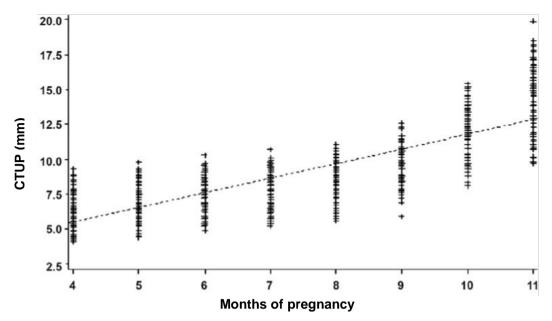
<sup>\*</sup> Average values followed by equal letters do not significantly differ (P < 0.05) according to Duncan's test. iC = interval of confidence.



**Picture 2.** Transrectal ultrasound with 6MHz probe of the combined thickness of the uterus and placenta of a 4 months pregnancy Colombian creole mare with normal pregnancy. The combined uterus and placenta indicated between (+) measures 7.7 mm and it is in the same quadrant of the 4 months pregnancy mare in the ultrasound of Picture 1. R40 convex probe 3.5 MHz was used.

model in the equation 1 ( $r^2 = 0.55$ ) that includes the CTUP variable by transrectal ultrasound, is observed that the model by the equation 2 has a better fit because of a higher coefficient  $r^2 = 0.64$ , indicating that the CTUP variable obtained by transabdominal examination is safer when identifying pregnancy problems in mares. In the transabdominal ultrasound examination of the placenta with the R40 convex probe at 3.5 MHz in the previously defined quadrants was found a CTUP minimum

average value of  $6.3 \pm 1.28$  mm and maximum of  $14.3 \pm 2.22$  mm (Table 2). These values differ from the ones found in other breeds by Reef *et al.* (1996), Renaudin *et al.* (1997) and Bucca *et al.* (2005). The data in this study show that CTUP both, in the transrectal and transabdominal ultrasound, increases on a lineal way; opposite to the reports of Renaudin *et al.* (1997), Bucca *et al.* (2005), Hendriks *et al.* (2009) and Souza *et al.* (2010).



**Figure 2.** Regression of the combined thickness of uterus and placenta (CTUP, mm) in the uterus quadrants of Colombian creole mares between the fourth and eleventh month of pregnancy by transabdominal examination with a R40 sectorial probe at 6.0 MHz.

When comparing the results in the Table 1 is observed that as the pregnancy time increased the CTUP values by the transabdominal examination, the values obtained by transrectal examination increased approximately two times. The CTUP values established in this study by transrectal and transabdominal ultrasonograph from the fourth month till the end of the pregnancy in Colombian creole mares with normal pregnancies are highly useful in the research of placental pathologies and, allow the on time diagnosis and treatment of possible losses in advance pregnancies. They also facilitate indirect monitoring of the fetal growth and predict the increase in CTUP or determine the pregnancy time using the equations developed in this study.

# Conclusion

 The combined thickness of the uterus and placenta (CTUP) in Colombian creole mares increased on a lineal and continuous manner through the time of pregnancy, being higher the increment in the last third of pregnancy.

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