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# Transrectal ultrasonographic evaluation of combined utero-placental thickness during the last half of pregnancy in Martina Franca donkeys

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## ABSTRACT

In the recent years, the donkey population decreased dramatically so that many breeds are presently considered as endangered. In comparison to the horse, the donkey placenta still remains not completely studied. In the horse, one of the diagnostic tools useful to identify pregnant mares at risk of abortion or premature delivery, include the transrectal ultrasound examination of the uterus and its contents; and especially of the combined thickness of the uterus and of the placenta (CUPT). Since the CUPT was never investigated in donkeys, the present study was aimed to define the transrectal CUPT values during the last half of pregnancy in 20 Martina Franca jennies. Foalings times, foals characteristics and placental gross appearance, and measurements were also evaluated and values resulted always within normality. Differently to the mare, a continuous significant CUPT increase between the sixth to the 12 months of pregnancy, and a substantial increase from the ninth to the 12th month of pregnancy, was found. Although statistically not evaluable, the CUPT values recorded from three jennies with pregnancy loss did not show evidence of CUPT increases. In conclusion, normal CUPT values from the sixth to the 12th month of pregnancy in Martina Franca donkeys are provided, but further investigations are needed to define possible breed or body-size CUPT specific differences, as well as the CUPT values during pregnancy disturbances or placental abnormalities.

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## 1. Introduction

In the horse mare, ascending bacterial placentitis is a recognized common cause of abortion, premature birth, or early neonatal death [1,2]. Under clinical conditions, the early diagnosis of every fetoplacental abnormality would be useful addressing for a more intensive pregnancy monitoring and possible treatments, leading to the birth of alive and viable foals [3].

Diagnostic tools for the detection of pregnant mares at risk of abortion or premature delivery include the transabdominal and transrectal ultrasound examination of the uterus and its contents [4–7]. The transrectal ultrasonography of the caudal uterine body, the region most frequently affected by ascending placentitis, was reported to be useful to identify mares with uteroplacental thickening or placental separation [6]. Under normal conditions, the uterus and the placenta are not distinguishable in the caudal uterine body, so that the combined thickness of the uterus and of the placenta (CUPT) is usually measured.

Normal values and timed changes of CUPT during normal gestation were reported for Quarterhorse mares [6],

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**Table 1**

Clinical data (mean  $\pm$  SD) about pregnancy length (PL), second stage of labor length (SS), placental expulsion time (PET), foal birth weight (BW), foal Apgar, foal time to stand (TSU), and time to first suck (TFS) obtained in the 17 jennies with normal pregnancy course.

PL (days)	SS (min)	PET (min)	BW (kg)	Apgar	TSU (min)	TFS (min)
368.5 $\pm$ 6.7	16.7 $\pm$ 10.8	38.5 $\pm$ 24.9	29.7 $\pm$ 4.0	9.2 $\pm$ 0.7	62.6 $\pm$ 20.5	101.8 $\pm$ 29.6

Standardbred mares [7] pony and Arabians mares [8], Thoroughbred mares [9], and for Dutch Warmblood mares [10]. An increase of CUPT in relation to the reference range for gestational age was reported to be associated to ascending placentitis, placental pathologies, or pending abortion [7,11,12].

In comparison to the horse, the donkey placenta still remains not completely studied [13–16]. In the Mediterranean countries, in the past, the donkey represented an important tool for the agricultural work and for transportation, but from the '50s of the last century, the donkey was replaced by the mechanical machines, so that the interest for this species decreased substantially in a short while, and the total donkey population dramatically decreased, reaching very low numbers in the last decades. At present, indeed, several donkey breed populations are considered by the FAO as endangered [14], and the Martina Franca donkey breed is in that list. However, recently, the donkey was reconsidered for several new purposes, such as the production of hypo-allergenic milk, the pet therapy, and the production of mules for the works in the national parks, where machines are banned. Therefore, because of this renewed effort to avoid some donkey breeds disappearance, and to increase some existing donkey breeds populations, the donkeys' reproductive efficiency must be optimized. Similarly to the horse, also in the donkey the birth of a viable foal at the end of a normal gestation is the prerequisite for a successful reproductive process [14].

To the authors' knowledge, the CUPT in donkey jennies was never investigated, and because of the importance of a prompt recognition of every potential pregnancy disturbance, the availability of the CUPT normal values in relation to gestational age, could be of interest for a correct management of pregnant jennies. The aim of the present study was therefore to define the transrectal ultrasonographic CUPT normal values during the last half of pregnancy in Martina Franca jennies.

## 2. Material and methods

### 2.1. Animals

The project was approved by the Committee on Animal Research and Ethics of the Universities of Chieti-Pescara and Teramo (<http://www.unich.it/unichieti/appmanager/federati/CEISA>), Protocol #45/2013/CEISA/COM, approval date July 16, 2013.

The study was conducted on 20 Martina Franca jennies, 5 to 11 (mean  $\pm$  standard deviation [SD]: 10.5  $\pm$  2.2) years old, 4 to 7 (mean  $\pm$  SD: 5.9  $\pm$  1.6) parity, 250 to 350 (mean  $\pm$  SD: 307  $\pm$  47.2) kg body weight, kept at the Chiareto estate, belonging to the Faculty of Veterinary Medicine, University of Teramo, Italy. All the jennies were healthy, dewormed before breeding, regularly vaccinated,

and fed with hay *ad libitum*. Follicular growth was monitored by ultrasonography, every 12 hours from the detection of a follicle greater than 30 mm in size [17], and artificially inseminated, every 48 hours, until ovulation, with semen collected by stallions of proven fertility [18]. Pregnancy was checked at 14 days after ovulation by transrectal ultrasonography, and confirmed at 45 days after ovulation [18]. The day of ovulation, considered as the first day of pregnancy, was used to define the gestational age. The normal pregnancy course, the normal development and well-being of the fetus were fully monitored until foaling [18]. Particular attention was paid to detect possible clinical signs of placentitis (i.e., premature udder development and lactation, or vulvar discharge) [19]. Pregnant jennies were kept in open paddock until the last month of gestation and, at udder enlargement, moved into individual foaling boxes, equipped with closed-circuit video camera systems for 24-hour round surveillance.

Starting from the sixth month of pregnancy, ranging between 151 and 180 days of gestation, all the jennies were monthly submitted to the ultrasonographic evaluation of CUPT, until foaling.

### 2.2. Combined thickness of the uterus and of the placenta measurement

Jennies were restraint in a stock, without sedation. The measurement of CUPT was performed by transrectal ultrasonography (Mindray DP-2200 Vet, Mindray DS USA Inc., USA), using a 7.5-MHz linear transducer. The probe was positioned just cranial to the cervix, and afterward moved laterally, to visualize the middle branch of the uterine artery, detectable at the ventral aspect of the uterine body. Measurements were obtained by the ventral aspect of the uterine body because, as reported by Renaudin et al. [6], in the horse mares, in the last month of pregnancy, the dorsal aspect of the uterine body can be edematous also under normal conditions. The CUPT was considered as the area included between the uterine artery and the allantoic fluid. On every occasion, for each jennies, three subsequent measurements were recorded, for a better calculation of the individual monthly average value. Attention was paid to the fetal fluids dynamics and to the fetus positioning that could affect the distension of the utero-placental unit at the cervical pole [7]. In fact, fetal limbs or skull can be easily observed to push against the cervical pole. Moreover, to ensure correct measurements, special attention was also paid to make sure that the amniotic membrane was not too close to the allantochorion.

### 2.3. Foaling and after foaling evaluation

At term, according to previous studies [20–22], jennies were allowed to foal spontaneously, under video

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