Mariella J, Iacono E, Lanci A, Merlo B, Palermo C, Morris L, Castagnetti C. Macroscopic characteristics of the umbilical cord in Standardbred, Thoroughbred and Warmblood horses. Theriogenology. 2018 Jun;113:166-170.

The final published version is available online at: 10.1016/j.theriogenology.2018.03.004

Revised highlighted

# MACROSCOPIC CHARACTERISTICS OF THE UMBILICAL CORD IN STANDARDBRED, THROUGHBRED AND WARMBLOOD HORSES.


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## Abstract

The umbilical cord (UC), the connection between mother and fetus through the umbilical vessels, carries nutrients and oxygenated blood to the fetus via the umbilical vein and removes deoxygenated blood and waste products via the umbilical arteries. It is designed to protect blood flow to the fetus during a term pregnancy. In equine medicine, only a few studies described the UC, mainly in the Thoroughbred. The present study aimed to describe and compare the macroscopic features of the equine umbilical cord in three different breeds and in relation to the foal's gender. Another aim is to investigate the correlation between UC features and maternal and perinatal factors. One hundred twenty four healthy mares with normal pregnancies were enrolled in the study and were divided into three groups according to the breed: 70 Standardbreds (STB), 38 Thoroughbreds (THB) and 16 Warmblood (WAB). The following data were recorded: mare's age and parity, gestation length, placental weight, presence of fetal membranes alterations, UC length and number of coils of amniotic and allantoic portions, and the Umbilical Coiling Index (UCI), which is the ratio between total coils and total UC length. The UCI has never been investigated in veterinary medicine. Furthermore, immediately after foaling, the APGAR score, foal's weight and sex were recorded. All the STB and WAB were housed in Italy and the THB were housed in New Zealand. The mean age of the mares was higher in WAB than in THB and STB; the latter had a significantly shorter gestation length. The foal's weight was positively correlated with placental weight in all breeds; and in STB, the foal's weight was positively related to parity and gestation length. Mean total UC lengths, were comparable to previous reports in THB, STB and WAB. The lengths of the two UC portions were statistically different between STB and THB, where the amniotic portion was longer than the allantoic one. In each breed, total UC length was correlated with total number of coils (THB and STB = 5±1; WAB = 6±1), the UC's amniotic length was positively correlated with the number of amniotic coils as the allantoic length with the number of allantoic coils. The UCI values were 0.09 in STB and THB and 0.1 in WAB. This study provides reference value for UCI that may be an important part of membrane evaluation and may have clinical value.

**Keywords:** umbilical cord, Umbilical Coiling Index, equine pregnancy, healthy foals

## 1. Introduction

The umbilical cord (UC) is an important connection between mother and fetus allowing the latter to receive oxygen and nutrients necessary for survival and development. The equine UC develops from the convergence of the two fetal sacs, the amnion and allantois around the remnants of the yolk sac and the vitelline duct to form a cord-like by amnion and attached to the fetus at the umbilicus; it contains two umbilical arteries and one umbilical vein, the urachus, and the vitelline vein remnant. The distal intra-allantoic portion is covered by the allantois and it is attached to the allantochorion. In this portion, the two major umbilical arteries become divergent and multibranched towards the chorion and the two umbilical veins remain close to these arteries. The two major veins unite at the proximal end of the allantoic portion or just inside the amniotic cavity. The normal attachment side to the allantochorion is between the two horns. The urachus connects the fetal urinary bladder to the allantoic cavity within loose stromal tissue in the amniotic portion [1-3].

Few studies have described the normal equine umbilical cord, mainly in the THB [1-3]. On the contrary, in human medicine the gross features of the UC has been intensively studied and one of its peculiar characteristics, the helical structure, focused the attention of bioengineers and physical scientists [4]. Strong et al., (1994) devised an Umbilical Coiling Index (UCI) calculated by dividing the total number of umbilical coils by the umbilical cord length (cm) for use during ultrasonographic assessment of the UC [5]. It was recently found [6,7] that the UCI is an effective indicator of perinatal outcome. A hypocoiled cord was strongly associated with preeclampsia in woman [8,9].

Machin et al. (2000) reported frequency of occurrence and clinical correlations of abnormally coiled cords in 1329 newborn humans. The principal clinical correlations found in hypercoiled and hypocoiled cords were fetal demise, fetal intolerance to labor, intrauterine growth retardation, and chorioamnionitis. Abnormal cord coiling was associated with thrombosis of chorionic plate vessels, umbilical venous thrombosis, and cord stenosis. The authors concluded that an abnormal cord coiling is a chronic state, established in early gestation, that may have chronic (growth retardation) and acute (fetal intolerance to labor and fetal demise) effects on fetal well-being [9]. The cause of abnormal cord coiling is currently unknown. In human medicine, the length of the UC and the number of coils are determined by ultrasonography during pregnancy and then the UCI can be calculated. The detection of an abnormal UCI could lead to elective delivery of fetuses at risk, thereby reducing the fetal death rate, so it is recommend that the UCI becomes part of the routine placental pathology examination.

In equine reproductive medicine, despite the macroscopic examination of the placenta after delivery being a fundamental feature of post-partum examination, the UCI has never been investigated.

In the equine, the twists affects all the structure of the UC and twisting is a generalized physiological phenomenon [10], sometimes with pathological implication. A large survey about equine abortion in UK on 1252 fetal and neonatal death, revealed that in 38.8% of the final diagnoses, the fetus died as a result of vascular compromise caused by twisted regions of the umbilical cord [11].

The present study aimed to describe the macroscopic features of equine umbilical cord, such as length and number of coils of each portion, to calculate the umbilical coiling index in normal pregnancy and healthy foals. These features were compared between three different breeds in relation to the foal’s gender. This study also aimed to verify if there is a correlation between the macroscopic UC characteristics and mare and perinatal features.

## 2. **Materials and Methods**

### *2.1 Animals and data collection*

One hundred twenty four healthy mares with normal pregnancies and normal parturition were enrolled in the study. Eighty of these were housed for attended delivery at the Equine Perinatology Unit (EPU) “Stefano Belluzzi” - Department of Veterinary Medical Sciences - University of Bologna, during six breeding seasons (2011-2016); 44 were housed in New Zealand, during the breeding season in 2013. Mares in New Zealand (all THB mares) were grazed at two stud farms, are grazed on ryegrass pasture and managed by one veterinarian. All the mares included in the study did not received any “light program” during hospitalization and were managed by a 24/7 foal watch team. All parturition occurred between February and August in the Northern hemisphere, while in the South hemisphere between November and December. No mare included in the study had placental retention and all the placenta were expelled within 2 hours from parturition.

The mares were divided into three groups according to the breed: 70 STB, 38 THB and 16 WAB.

Information about mare’s age and parity were recorded at admission. At delivery, the following data were registered: gestation length, placental weight, presence of fetal membranes alterations, UC length and number of coils of allantoic and amniotic portion. The coils were counted before the rupture of the UC, while the UC length was measured after his ruptured and the expulsion of the placenta, with a centimeter ruler (Fig.1). A coil is of 360-degree spiral course of umbilical vessels (Fig.2) [6]. The Umbilical Coiling Index (UCI), which is the ratio between total coils and total UC length, was then determined. Placental weight was measured, immediately after the expulsion, weighing the entire placenta along with the amnion and umbilical cord, as suggested by Elliott et al., (2009) [12]. Furthermore, within 5 minutes after birth, APGAR score [13] was calculated and foal’s weight and sex were recorded. Only healthy mares with normal deliveries and healthy foals were enrolled. The foals were classified as healthy when they had a normal clinical evaluation during the course of hospitalization, including a complete blood count and serum biochemistry at birth and an IgG serum concentration ≥800 mg/dL at 18 h of life.

*2.3 Statistical analysis*

All parameters were tested for normal distribution by using the Kolmogorov–Smirnov test. Because the data showed non normal distribution, the variables were analyzed with nonparametric methods. Spearman's rank correlation coefficients were used to test the hypothesis that the anatomical features of the UC and the descriptive data related to mares and foals were correlated.

The Kruskal–Wallis test was performed to compare anatomical characteristics of the UC, placental weight and data relative to foals and mares between the 3 groups. When a statistically significant difference was found, the Bonferroni post-hoc procedure for all pair-wise comparisons was applied. To test the hypothesis that anatomical features of the UC, placental and foal's weight were related to foal's gender, the Wilcoxon's test was performed.

The Mann-Whitney test was used to compare the length of the allantoic and amniotic portion of the cord in each breed.

Descriptive statistics, including mean ± SD and range (min/max values) were calculated. A P value less than 0.05 was considered statistically significant. All analyses were carried out using the commercial software Analyse-it, version 2.03 (Analyse-it Software Ltd., Leeds, West Yorkshire, England).

## 3. Results

Data were collected in the three different breeds and significant differences were found for mare's age, gestation length, foal's weight, placenta's weight, and UC amniotic length. No differences between breeds were found for parity, total length and total coils of UC, allantoic length and coils of UC, amniotic coils of UC and UCI. Data are reported in Table 1.

Correlations were analyzed within each breed. In STB, there was a positive correlation between mare's age and parity ($P < 0.0001$; $rs = 0.80$), mare's age and foal's weight at birth ($P = 0.034$; $rs = 0.26$), foal's weight and parity ($P = 0.037$; $rs = 0.26$), foal's weight and gestation length ($P = 0.030$; $rs = 0.27$), foal's weight and placental weight ($P < 0.0001$; $rs = 0.59$), gestation length and placental weight ($P = 0.025$; $rs = 0.28$). A negative correlation was found between foals weight and UC allantoic length ($P = 0.013$; $rs = -0.32$). In THB, there was a positive correlation between foal's weight and placental weight ($P < 0.0001$, rs = 0.59) and a negative correlation between gestation length and placental weight (P = 0.012, rs = - 0.42). No other correlations were found.

In WAB, a positive correlation was found between mare's age and UC allantoic length (P = 0.03; rs = 0.56), foal's weight at birth and placental weight (P = 0.005; rs = 0.69), foal's weight at birth and number of total coils (P = 0.04; rs = 0.60), foal's weight at birth and allantoic coils (P = 0.002; rs = 0.74). A negative correlation was found between gestation length and placental weight (P = 0.025; rs = -0.54).Within each breed, UC total length was positively correlated with the number of total coils in STB ($p < 0.0001$, rs=0.53), in THB (p = 0.003, rs = 0.47), but not in WAB. The UC amniotic length was correlated with the number of amniotic coils only in THB (p = 0.047, rs = 0.33) and the UC allantoic length with the number of allantoic coils in STB (p = 0.002, rs = 0.5) and in THB (p = 0.0008, rs = 0.53). The UCI was similar in all breeds: in STB was 0.09 ± 0.03, in THB was 0.09 ± 0.02 and in WAB was 0.1 ± 0.03.

Analyzing gender-based features within each breed, significant differences were found in THB for foal's weight and placenta's weight and in STB for UC total length and UC allantoic length, higher in male than in female. The significant gender-based differences within each breed are summarized in Table 2.

## 4. Discussion

Many authors described the characteristics of the normal equine placenta but only few data were provided on the umbilical cord. Most of them are related to Thoroughbreds or to mixed group of breeds or to abnormal UC features [1-3, 14-16]. To our knowledge, only one recent study compare placental size, structure and function [17], but no one compare UC features, between equine breeds.

In regard to the range of UC length, data reported in the present study are comparable to that previously reported in THB [1,18], STB [19], and WAB [20]. What is surprising from the data is the wide range of length in the equine and that the human UC has the same length: in a recent study in woman with natural pregnancy UC length was 55.4 ±11.4 cm [21]. A preliminary study in infants with a short umbilical cord showed that they had lower tibia speed of sound (SOS) measurements compared with infants with a longer cord. The bone SOS is a parameter measured using quantitative ultrasound (QUS). The QUS bone measurement is related to the bone strength that reflects the bone density, architecture and mineralization. The authors postulated that this difference might be due to the restricted activity of the fetus with the short UC [22]. In human newborns, decreased fetal movements and activity have been associated with decreased infant bone mineralization [23]**.** This field of research was never been investigated in the equine neonates. From the first studies in the 1960's in human medicine, it is known that the cord's length is not correlated with maternal and fetal factors such as parity, age, weight, sex and presentation and that there is little growth of the cord during the last trimester [24]. Also Malpas (1964) found no correlation of cord length with placental or birth weight [25]. Miller et al. (1981) noted that cord length was related to the stretching placed on the cord by the developing embryo and fetus; the tension is determined by the availability of intrauterine space and the occurrence of fetal movement [23]. In a more recent study, the authors found that cord problems such as true cord knots and nuchal cords were significantly associated with male gender; they hypothesized that it is due to the longer cords and suggested that further studies should investigate umbilical cord length differences between male and female fetuses [26]. Also in veterinary medicine, this aspect has never been investigated. An excessively long UC was reported to be a frequent feature in aborted equine fetus [14]. Some authors suggested that the increased length of the UC in the equine fetus can predispose to other pathologic conditions such as excessive torsion, as illustrated by more than one report [27,28], and strangulation of the cord around the fetus [29]. In a study of Whitehead et al. (2005), short UC was not associated with fetal loss or other pathologic conditions. In the same study about placental characteristics in Standardbred mares [30], it was noted that there was an association between the opposite site attachment of the UC (the UC attachment was in the left horn but the gravid horn was the right one) and both Type II vascular pattern of the allantoic surface and an excessively long UC. In Type II vascular pattern, found in 20-23% of membranes examined, one artery supplies the gravid horn and the entire uterine body, while the other artery supplies the non-gravid horn. Anyway, it is not known the primary cause of relationship between longer UC and opposite site attachment. Whether the long UC allows the migration of the fetus to the opposite horn during gestation, or on the contrary, the long UCD develops due to fetal kinetics while the fetus is carried in the opposite uterine horn, is still not known. In the present study, the length of the two portions of the cord is statistically different in STB and THB, where the amniotic portion is longer than the allantoic one. The amniotic portion is longer in THB than in STB, probably because of the higher fetal weight. In the present study, only in STB the length of the UC and the allantoic portion are longer in colt than in filly. Since there is not weight difference between male and female in STB, the hypothesis is that the difference in the allantoic length could be due to other factors as described in human medicine. We can speculate that the amniotic portion is longer than the allantoic because is more affected by the stretching during the fetal movement.

In human medicine, the helical pattern, or coiling, of the umbilical cord is well studied and several studies have addressed the correlations between abnormal cord coiling and adverse pregnancy outcome [9,31]. The origin of the umbilical coiling is still poorly understood: the hypotheses include fetal activity, asymmetric blood flow, fetal and hemodynamic forces [4]. The umbilical coil is defined as one complete spiral of 360° of the umbilical vessels around each other. The coiling makes the umbilical cord a structure both flexible and strong, and provides resistance to external forces which could compromise blood flow. In the equine species, it seems that the number of the coils (total, allantoic and amniotic coils) is the same in each breed studied. Only in WAB, there is a positive correlation with the foals' weight at birth and it cannot be excluded that the fetal weight is one of the item that contribute to the origin of the umbilical coiling. Data obtained from the present study could be considered as a normal range of number of coils and UCI in the equine physiological pregnancies.

Currently, there are no data about the correlation between the number of coils and the adverse pregnancy outcome in the equine, whereas in human medicine it is well known: undercoiling may give way to kinking and compression, whereas overcoiling may give way to occlusion. This may help to explain the association with the low Apgar Score in undercoiled cords and with low arterial pH and asphyxia with overcoiled cords [9,31].

From the present study emerged that the mean age of mares included in the study was higher in WAB than in THB and STB. This probably reflects the different approach to breeding management in different breeds: STB and THB begin their reproductive career earlier than WAB. Only in STB, the mare's age was correlated with parity. This probably reflects the management of three different breeds: STB and THB start their racing career at just two years old and some of them retire by the age of four. The female of these two breeds were then used for reproduction purpose with less use of reproductive biotechnologies as embryo-transfer (ET) and ovum-pick-up (OPU). Warmblood dominate dressage and jumper competition and can continue to work and have value well into their teens. This is probably the reasons because in an Italian study about OPU clinical program, the most representative breed is Warmblood [32].

As recently reported [33], normal gestation length in the THB mares included in this study ranged between 330 and 396 days, with a mean of 352 days. All THB included in the present study were housed in the Waikato region, New Zealand, where the hours of daylight ranged from 11.20 to 14.30, while in Bologna, Italy, daylight ranged from 11 to 15.25 (http://www.timeanddate.com/sun/new-zealand/auckland). In the mare, gestation length is influenced by daylight [34,35]. The exposure to more hours of light is probably the reason because STB, all housed in Italy except for eight, had a shorter gestation lengths than THB, with a mean of 343 days. It is worth noting that the STB gestation length reported in the present study is comparable to THB gestation length reported in studies from the United Kingdom [36]. In the Southern Hemisphere in New Zealand two studies in STB foaling at pasture, reported a mean gestation length of 349 days. The reasons why in this study mares in New Zealand appear to have longer gestations than the Northern Hemisphere counterparts could be due to differences in genetics, management, nutrition, climatic conditions and latitude as suggested also by other authors [37,38].

Foal's weight was correlated with placental weight in all breeds. Normal placental weight at term is approximately 11% of the foal weight [2,39], as further confirmed in the present study. Only in THB, foal's weight and subsequently the placental weight is higher in colt than in filly and probably this is due to a more pronounced sexual dimorphism as reported by Hintz et al. (1979) [40]. It is worth noting that in STB, which had a higher number of foaling than the other breeds in this study, foal's weight is related to parity and gestation length. The hypothesis suggested first by Wilsher and Allen (2002, 2003) [41,42] and then by Elliott et al. (2009) [12] is that the equine uterus needs to be in some way primed by a first pregnancy before it can achieve its full potential in terms of facilitating fetal growth. Wilsher and Allen (2003) found that placentas from primiparous mares have reduced microcotyledon surface densities coupled with reduced chorionic volumes resulting in less total available area for haemotrophic exchange of nutrients and gases, than secundiparous and multiparous mares [42]. This has been confirmed by two recent studies of the same research group [17,43].

A weak correlation between gestation length and foal's weight resulted only in STB and is not in accordance with the current knowledge about the equine pregnancy length. Foals born after a prolonged pregnancy are defined postmature and they usually show weak suckle reflex, poor thermoregulation and glucose regulation, poor postural reflexes, fully erupted incisors and are thin with poor muscle development [44]. Since the gestation length ranges reported in the present study in healthy mares with healthy foals are particularly wide, in the authors' opinion, it is difficult to define what a prolonged pregnancy is in the equine species. Also Palmer (1998) reported that normal gestation length may vary from 315 days to more than 390 days [44].

## Conclusions

This study have established the reference values of the UC length in three different breeds of horses and have introduced a new parameter, the UCI, that may have clinical relevance. The UCI as well as the entire UC, should be considered during the evaluation of equine placenta immediately after foaling. Further studies are needed to understand if an abnormal UCI should be related to high-risk pregnancy and adverse fetal outcome in the equine.

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