

Alma Mater Studiorum Università di Bologna  
Archivio istituzionale della ricerca

Gross and histological examination of Wharton's Jelly in the equine umbilical cord

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

Lanci, A., Merlo, B., Grandis, A., Mariella, J., Castagnetti, C., Iacono, E. (2023). Gross and histological examination of Wharton's Jelly in the equine umbilical cord. THERIOGENOLOGY, 209, 184-192 [10.1016/j.theriogenology.2023.06.032].

*Availability:*

This version is available at: <https://hdl.handle.net/11585/935555> since: 2023-07-20

*Published:*

DOI: <http://doi.org/10.1016/j.theriogenology.2023.06.032>

*Terms of use:*

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).  
When citing, please refer to the published version.

(Article begins on next page)

1    **Gross and histological examination of Wharton's Jelly in the equine umbilical cord**

2    Lanci Aliai <sup>a\*</sup>, aliai.lanci2@unibo.it

3    Merlo Barbara <sup>ab</sup>, barbara.merlo@unibo.it

4    Grandis Annamaria <sup>a</sup>, annamaria.grandis@unibo.it

5    Mariella Jole <sup>a</sup>, jole.mariella2@unibo.it

6    Castagnetti Carolina <sup>ab</sup>, carolina.castagnetti@unibo.it

7    Iacono Eleonora <sup>ab</sup>, eleonora.iacono2@unibo.it

8

9    <sup>a</sup>Department of Veterinary Medical Sciences, University of Bologna, via Tolara di Sopra 50, 40064,  
10    Ozzano Emilia, Bologna, Italy

11    <sup>b</sup>Health Science and Technologies Interdepartmental Center for Industrial Research (CIRI-SDV),  
12    University of Bologna, Bologna, Italy

13

14    \*Correspondence to: Aliai Lanci, Department of Veterinary Medical Sciences, University of Bologna,  
15    via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy.

16    E-mail: aliai.lanci2@unibo.it

17

## 18    **Abstract**

19    Wharton's jelly (WJ) is fundamental for the well-being of the fetus, binding the umbilical vessels and  
20    protecting them from twisting and compression. Gross and microscopic studies have been undertaken  
21    on the umbilical cord (UC) of human placentae in both normal and high-risk pregnancies, while they  
22    are limited on the equine UC. The aim of this study was to describe microscopically and  
23    immunohistochemically the equine UC in normal pregnancies, with particular attention to WJ. Forty-  
24    seven healthy mares, with no complications during pregnancy, admitted to the hospital for attending  
25    delivery were enrolled. Clinical data was collected at foaling on foal health and placental  
26    characteristics. UC samples were collected from three sites (amniotic, allantoic and in the region of  
27    vein anastomosis) for histology. The thickness of different layers of arteries and veins and WJ in  
28    different UC portions were measured ( $\mu\text{m}$ ). Wharton's Jelly was weighted (g) and its sections were  
29    stained with Masson's trichrome stain, orcein technique and silver impregnation.  
30    Immunohistochemistry was undertaken using antibodies raised-against collagen type I, V, VI and  
31    fibrillin. Forty-seven UCs, from 19 colt and 28 filly foals, were analyzed for WJ weight and 8/47 UCs  
32    were examined histologically. Warton's jelly was only found in the amniotic portion of the UC closest  
33    to the foal's abdomen. The weight of WJ ( $4.0 \pm 3.3$  g) did not vary between colts and fillies and it was  
34    not correlated with any of the clinical or UC parameters measured. The tunica media of arteries and  
35    veins was thicker in the amniotic portion of the UC, as described in human UCs in late pregnancy.  
36    This finding could be an adaptation to aid in resisting compression because of fetal movements and  
37    UC twisting. The umbilical vein was thicker than the umbilical arteries in the tunica media and tunica  
38    adventitia in the sections examined throughout the length of the cord. This preliminary study  
39    describes gross and histological WJ's structure in the mare. However, further studies are required to  
40    better characterize UC's changes throughout pregnancy and in the presence of mare's or fetal disease.  
41    Keywords: equine; umbilical cord; histomorphology; Wharton's jelly; umbilical vessels layers;

42

43

## 1. Introduction

In equine medicine, neonatal risk identification should include a systematic evaluation of fetal membranes, using macroscopic and histopathologic evaluation to recognize placental alterations not observed during pregnancy [1]. For this reason, a complete examination of placenta and umbilical cord (UC), including their histopathological aspects, are important for identifying at-risk neonates and planning appropriate treatments.

The umbilical cord is an essential connection between mother and fetus and it is designed to protect blood flow to the fetus during pregnancy [2]. UC is composed of a vascular component and a gelatinous substance, called Wharton's jelly (WJ). In human medicine, WJ is a well-defined structure, composed of fibroblasts, myofibroblasts, mesenchymal stromal cells and collagen fibers, forming a network of interconnected cavities. The amorphous ground substance of the jelly is mainly made up of hyaluronic acid and glycosaminoglycans dissolved in an aqueous solution of salts, metabolites, and plasma proteins [3-8]. Wharton's jelly plays a trophic, depositing, and mechanical role in tensile and compressive strength of the UC. The thickness and rigidity of WJ influence the contraction or relaxation of the umbilical vessels, acting as a support and preventing excessive distention [4,9-10]. This tissue is fundamental for the well-being of the fetus, maintaining constant bidirectional blood flow between the mother and the fetus during fetal movements, avoiding tension, twisting, compression, and possible node formation [2,11].

During equine pregnancy, UC forms at the beginning of the fetal stage. Indeed, the membranes and associated vessels, that separate the yolk sac and the allantoic sac, combine from day 40 forming the UC, which lengthens until the fetus reaches the floor of the allantoic sac at day 48 of gestation [12]. To date, in equine species the most investigated and known aspects of the UC are the macroscopic appearance also in relation to its association with abortions [13-15] and the isolation of Mesenchymal stromal cells derived from WJ and UC blood [8,16-18]. Several studies described macroscopic characteristics of UC in different breeds [17, 19-23]. Furthermore, the main histological studies were

69 conducted on normal or abnormal placenta [24-26], while only two studies described some histolog-  
70 ical aspects of UC [27,28]. On the other hand, the microscopic aspects of human UC have been stud-  
71 ied more than in the horse, both in normal and high-risk pregnancies [6, 29,30]. In fact, any disease  
72 during pregnancy, which affects both the mother and the fetus, have a great impact on the UC mor-  
73 phological aspect [6, 29-31]. To the authors' knowledge, no studies are present about the quantitative  
74 and qualitative presence of WJ in equine species. This preliminary study aimed to describe the equine  
75 UC, with particular attention to WJ in normal pregnancy and healthy foals. For the first time in equine  
76 species, WJ was described macroscopically, histologically and immunohistochemically.

77

## 78 **2. Materials and Methods**

### 79 *2.1 Animals and data collection*

80 Healthy mares with normal pregnancies hospitalized at the Perinatology and Reproduction Unit of  
81 the Equine Clinical Service, Department of Veterinary Medical Sciences (DIMEVET), University of  
82 Bologna, during four foaling seasons (2016-2019), were included in the study. Mares were housed in  
83 wide straw bedding boxes and fed with hay ad libitum and concentrates twice a day. During the day,  
84 the mares were allowed to go to pasture. Information about mares' age, breed and parity have been  
85 recorded at admission. At foaling, the following data were registered: gestational length, fetal  
86 membranes' weight, fetal membranes alterations (allantochorion, allantoamnion and UC).  
87 Macroscopic evaluation of UCs was performed as previously described by our research group [17].  
88 The coils were counted before the rupture of the UC, while the UC length was measured after its  
89 rupture and the expulsion of the placenta, using a centimeter ruler. A coil is a 360-degree spiral course  
90 of umbilical vessels. The Umbilical Coiling Index (UCI), the ratio between total coils and total UC  
91 length, was then determined [17].

92 Furthermore, within 5 min after birth, the APGAR score [32] was calculated and the foal's weight and  
93 gender were recorded. Only healthy mares with normal pregnancy, eutocic delivery and healthy foal  
94 were enrolled. Foals were classified as healthy when they had an Apgar score  $\geq 9$  [32], a normal

clinical evaluation during hospitalization, including a complete blood count and serum chemistry at birth, and an IgG serum concentration > 800 mg/dL at 12-24 h of life.

## *2.2 UC's sampling*

All the UCs were collected entirely at foaling immediately after spontaneous breaking. The weight of WJ was obtained from 39/47 UCs at the Animal Reproduction and Biotechnology Laboratory of the Equine Clinical Service (DIMEVET, University of Bologna) and the microscopic evaluation was obtained from 8/47 UCs at the Laboratory of Normal Veterinary Anatomy (DIMEVET, University of Bologna).

## *2.3 Wharton's jelly weight*

Samples for WJ weight were stored in D-PBS (Dulbecco's phosphatase-buffered solution) containing penicillin (100 IU/mL) and streptomycin (100 mg/mL), at 4°C for at the latest 12h. Wharton's Jelly was isolated by cutting it with sterile surgical forceps and scissors, and weighed with an electronic balance.

## *2.4 Microscopic description and measurement of different layers*

Samples for UC microscopic evaluation were stored in PBS (Phosphate Buffer Solution), containing 0.1% (v/v) sodium-azide and 30% (v/v) sucrose solution, at 4°C until analysis. Ten sections of 15 µm in three different portions for each UCs (amniotic portion, allantoic portion and at the site of vein anastomosis and in particular at the division of the two umbilical veins) (Figure 1) were obtained by cryostat and mounted in gelatin-coated glass slides. Umbilical cord's sections were stained with Masson's trichrome stain. The thickness of different layers of umbilical arteries and veins and WJ were measured (µm) in each UC's portion using image analysis software (ImageJ, processing and analysis in Java, Version 1.6). In particular, the values of different layers of the largest and smallest vessel were recorded for both arteries and veins (see Table S1 in the Supplementary Material) and averages were calculated.

121 For an accurate WJ analysis, a section was also stained with orcein technique to identify elastic fibers  
122 and with silver impregnation technique to identify reticular fibers.

### 123 *2.5 Immunohistochemical analysis*

124 The immunohistochemical analysis was conducted, giving particular attention to WJ. After three  
125 washes for 10 mins in PBS, sections were incubated with 1% H<sub>2</sub>O<sub>2</sub> in PBS for 30 mins at room  
126 temperature (RT) for eliminating endogenous peroxidase activity. Sections were then rinsed in PBS  
127 three times for 10 mins and incubated in PBS plus 10% (v/v) normal goat serum (Colorado Serum,  
128 Denver, CO, #CS 0922) and 0.5% Triton X-100 (Merck, Darmstadt) for 2 hours at RT.

129 Thereafter, sections were incubated for 48 h at 4°C with rabbit anti-bovine collagen type I polyclonal  
130 antibody (dilution 1:80; Chemicon, Temecula, CA-USA, batch NG1804950), rabbit anti-human  
131 collagen type V polyclonal antibody (dilution 1:40; Chemicon, Temecula, CA-USA, batch  
132 0604027824), rabbit anti-collagen type VI polyclonal antibody (dilution 1:10-1:40; Chemicon,  
133 Temecula, CA-USA, batch NG 1833210) and anti-fibrillin clone 689 purified mouse monoclonal  
134 antibody (dilution 1:200; Chemicon, Temecula, CA-USA, batch NRG1758239). The chosen  
135 antibodies were previously used and validated in the equine species [33]. The primary antibody was  
136 diluted in a solution (1.8% NaCl in 0.01 M PBS containing 0.1% sodium azide) containing 1% normal  
137 goat serum and 0.5% Triton X-100.

138 After three washes in PBS, the sections were incubated in goat biotinylated anti- rabbit 10µg/ml  
139 (Vector Laboratories, Burlingame, CA, USA, BA-1000) or goat biotinylated anti- mouse 10µg/ml  
140 (Vector Laboratories, Burlingame, CA, USA, BA-9200) for 2 hours at RT. The secondary antibody  
141 was diluted in PBS plus 1% v/v normal goat serum and 0.5% v/v Triton X-100.

142 The sections were transferred to avidin–biotin complex (ABC kit Vectastain, PK-6100, Vector  
143 Laboratories, Burlingame, CA) for 30 mins and the immunoperoxidase reaction was developed by  
144 3,30-diaminobenzidine (DAB kit, SK-4100, Vector Laboratories, Burlingame, CA). Slides were dried  
145 overnight, dehydrated in ethanol, cleared in xylene, and coverslipped with Entellan (Merck,  
146 Darmstadt, Germany). All the incubations were performed in a humid chamber. Masson trichrome

147 staining was also performed to better highlight the fibroblasts.  
148 Negative controls were produced by replacing the primary antibodies with PBS.  
149 All sections were observed under a Zeiss Axioplan microscope (Carl Zeiss, Oberkochen, Germany).  
150 Images were recorded by a Polaroid DMC digital photcamera (Polaroid Corporation, Cambridge,  
151 MA, USA) and DMC 2 software.  
152 In WJ, the density of fibroblast cells was calculated as the number of cells/mm<sup>2</sup> in each section sep-  
153 arately. Fibroblast cells were counted at 20× magnification in 10 fields of view in each specimen.  
154 Only cells with an evident nucleus were counted.

155

## 156 *2.6 Statistical analysis*

157 Data were analyzed for normality using a Shapiro-Wilk test. Since distribution was normal, data were  
158 expressed as mean ± standard deviation.

159 Student T-test was used for evaluating the difference between WJ's weight and foal's gender.

160 Pearson's test was performed to compare WJ weight and clinical data (mare's age and parity, gesta-  
161 tional length, foal's weight, fetal membranes' weight) and UC characteristics (total length, total coils,  
162 UCI, amniotic and allantoic length and coils).

163 Student T-test was used for evaluating the difference between arteries and veins in different vessels'  
164 layers (amniotic epithelium, tunica intima, media and adventitia) in three different UC portions (am-  
165 niotic, allantoic portion and at anastomosis site).

166 One-way ANOVA was used for evaluating the three different UC portions (amniotic, allantoic por-  
167 tion and at anastomosis site) for each vessels layer (amniotic epithelium, tunica intima, media and  
168 adventitia, total thickness) of arteries and veins.

169 All analyses were carried out using the software IBM SPSS Statistics 23 (IBM Corporation, Milan,  
170 Italy). Significance was assessed for P<0.05.

171

## 172 **3. Results**



Forty-seven mares (37 Standardbred, 7 Italian Saddlehorse, 2 Arabian and 1 Quarter Horse) were included in the study. Recorded data are reported in Table 1. Fetal membranes alterations were not present. Thirty-nine UCs were analyzed for WJ's weight, while 8/47 UCs were analyzed for microscopic description, measurement of different layers, immunohistochemical analysis and fibroblast cells count.

In all the collected UCs, WJ was found only in the intramniotic portion, close to the foal, while it was absent in the allantoic portion (Figure 1). Foals were 19 males and 28 females and WJ's weight was  $4.0 \pm 3.1$  g and  $4.1 \pm 3.4$  g respectively. No differences were found between WJ's weight and foal's sex. The amount of WJ (g) was not correlated with any of the clinical and umbilical registered data.

### *3.1 Microscopic description and measurement of different layers*

Eight/47 UCs have been microscopically evaluated. All the vessels showed the three typical layers: tunica intima, tunica media and tunica adventitia. The endothelium of tunica intima was regular and well developed, having a single layer of cells (Figure 2a) resting on a basement membrane. The sub-endothelial layer was composed of connective tissue with some smooth muscle fibers between the collagen fibers (Figure 2a, 2b). The tunica media was thicker than tunica intima and it had multiple concentric smooth muscle and few collagen fibers (Figure 2b, 2c). The tunica adventitia was the thickest layer and the collagen fibers progressively increased going towards the periphery, while smooth muscle fibers decreased (Figure 2c, 2d). A perivascular tissue, composed of dense collagen fibers arranged concentrically, surrounded the tunica adventitia (Figure 2f, 3a). This tight tissue was composed of dense and well-organized connective tissue. On the contrary, WJ was loose connective tissue: collagen fibers were arranged to create a loose reticular texture (Figure 2d, 4, 5). Other cells observed in WJ were fibroblasts (Figure 4e) and white blood cells (Figure 5). Finally, the UC was externally surrounded by amniotic membrane (Figure 2d, 3a), composed by two thin layers: amniotic epithelium and subamnion, an amniotic connective tissue with collagen fibers and fibroblasts (Figure 3a insert).

198 The orcein staining showed the absence of elastic fibers in the WJ. They were less concentrated with  
199 an uneven pattern around the vessels (Figure 6a). Finally, the silver impregnation staining revealed a  
200 dense network of reticular fibers in the entire section of the cord (Figure 6b).

201 The mean measurements of the tunica intima, media and adventitia of the umbilical arteries and veins  
202 are reported in Table 2 and 3, respectively. The measurement of the tunica intima, media and adven-  
203 titia of the largest and smallest umbilical vessels are reported in Table S1 in Supplementary materials.

204 The differences between arteries and veins in each umbilical portion are reported in Figure 7.

205 Mean number of fibroblasts counted in the WJ were  $57 \pm 9$  cells/mm<sup>2</sup> and the mean thickness of WJ  
206 was  $649 \pm 474$   $\mu$ m.

207

### 208 *3.2 Immunohistochemical analysis of WJ*

209 The immunohistochemical analysis revealed the presence of fibroblast cells positive for antibodies  
210 anti-type I, V and VI collagen and anti-fibrillin (Figure 8).

211

## 212 **4. Discussion**

213 In the present study, the microscopic features of UCs collected at delivery of healthy foals born after  
214 normal pregnancies were investigated. The histological description focused on the complete analysis  
215 of WJ, a still poorly known tissue in the equine species. The present study describes, equine WJ for  
216 the first time, focusing on its amount in healthy newborn foals and its histological aspects, such as  
217 thickness and fibroblasts concentration.

218 In women, physiological variations in WJ amount can occur in normal pregnancies: WJ decreases  
219 significantly with advancing gestation and its water content is significantly lower in term than in  
220 preterm neonates, with a progressive reduction from 30 weeks' gestation to term [11, 34-38]. Further-  
221 more, a significant positive correlation between WJ weight and both birth weight and placental weight  
222 has been reported [39], with a significant negative correlation observed between the WJ density and  
223 both birth weight and placental weight [39]. Moreover, it has also been reported that male fetuses

224 have a higher amount of WJ than females, but the density remains the same [40]. Biochemical and  
225 morphological alterations of WJ may cause some prenatal diseases [29, 35, 41-43] and a quantitative  
226 variation of WJ seems to be associated with pathological conditions such as hypertensive disorders  
227 [29], gestational diabetes [44], foetal distress [40], and foetal growth restriction [35,37,45]. In women  
228 with preeclampsia, WJ has more glycosaminoglycans and type III collagen, whereas hyaluronic acid  
229 is reduced. Because hyaluronic acid is highly hydrophilic, the amount in the WJ is particularly im-  
230 portant for the UC's mechanical properties and macroscopic appearance [29,42,46]. Furthermore, the  
231 total absence of WJ has been associated with foetal death [47]. The quantitative decrease of WJ could  
232 determine UC torsion, compression or stretching that would adversely affect foetal blood flow [2,48].  
233 Contrary to what expected, in the present study the amount of WJ at term was not correlated with the  
234 mare's age and other investigated clinical and UC's parameters. The average age of the mares in-  
235 cluded in the present study is 11 years, age at which they are in full reproductive activity. It would be  
236 interesting to make a comparison between young and old mares, with particular attention to old maid-  
237 ens. Regarding gestational age, the mean included in the present study is within the normal range and,  
238 since this is very wide, it could be difficult to find a significant correlation. Based on these results, it  
239 would be interesting to compare them with umbilical cords of healthy animals born at different ges-  
240 tational ages. Umbilical cord parameters have not been found to vary with the amount of WJ during  
241 normal pregnancy, but it is likely that other parameters come into play. On the other hand, in human  
242 medicine, research on this topic has been in progress for more than 40 years, while in equine medicine  
243 it is just starting.

244 Accordingly to the only one study that had briefly described the WJ characteristics in 6 Thoroughbred  
245 foals [28], in the present study, reactive fibroblasts containing I, IV and V collagen, and fibrillin  
246 surrounded by a dense network of reticular fibers were described, as in the human umbilical cord  
247 [49]. Fibroblasts and myofibroblasts are the main cellular component of human WJ, contributing to  
248 its elasticity through the synthesis of collagen fibers, which affects the regulation of blood flow  
249 through the UC [4]. The same would also appear to be true for the horse. Differently from human [2],

250 in the equine UC, elastic fibers are less concentrated with an uneven pattern around the vessels. Fi-  
251 nally, in the equine species, as well as in human [2,49], a dense network of reticular fibers pervades  
252 the entire tissue.

253 For the first time, the vessel layers in each portion of the UC were compared for both arteries and  
254 veins and the different thicknesses between artery and vein were also observed. These original results  
255 cannot be compared with any other study, since Kumar et al. [28] did not report a statistical analysis.

256 As for human vessels, also the equine tunica media of arteries and veins resulted to be thicker in the  
257 amniotic portion s as pregnancy advances [50,51]. This because the amniotic portion, closely related  
258 to the fetus, is affected by its movements and usceptible to excessive twisting of the UC, apart from  
259 a partial occlusion of blood flow. For this reason, the tunica media may be thicker to better resist any  
260 compression caused by the fetus. The first detectable equine fetal limb movements occur from day  
261 46, when the fetus changes presentation, position and location with great vigor in the first trimester  
262 of pregnancy, even about 5 times/hour [52]. It is likely that the extensive fetal activity and mobility  
263 in this species during early pregnancy play a role in fetal development of muscle and nerve coordi-  
264 nation. The amniotic and the allantoic portion of the UC could become coiled as early as day 68 of  
265 gestation [53,54]. The UC coils are attributable to changes in fetal presentation, and it seems that a  
266 coil in the allantoic portion of the UC represents a presentation change of the fetal-amniotic unit, and  
267 a coil in the amniotic portion represents a presentation change of the fetus within the amnion [54].

268 The umbilical coiling makes the UC a structure both flexible and strong and provides resistance to  
269 external forces which could compromise blood flow [17]. The umbilical coiling could be an evolu-  
270 tionary purpose whereby cord shortening would decrease the distance between the fetus and the uter-  
271 ine attachment in a caudal horn when fetal mobility and changes in presentation have reduced [54].

272 Probably, the shortening of this distance, the presence of the WJ, and maybe even the increased thick-  
273 ness of the tunica media in the UC vessels of the amniotic portion could reduce entanglements be-  
274 tween the UC and the active and long fetal hindlimbs.

275 The umbilical vein was found to have greater thickness than the umbilical artery in almost all layers  
276 in the three sections of the UC, in agreement with what reported in human medicine [31]. The reason  
277 for this difference can only be assumed and some hypotheses can be made, such as the larger caliber  
278 of the umbilical vein compared with the umbilical artery, the anastomosis of the two umbilical veins,  
279 or the greater resistance. Therefore, even in the equine species, umbilical veins could have a greater  
280 thickness because, by carrying oxygenated blood, they actually act as arteries.

#### 281 4.1 Conclusion

282 Histological characteristics of WJ, its physiological amount in healthy animals and, particularly, for  
283 the first time in the equine species, the measurements of umbilical vessel layers at different locations  
284 were described. Contrary to authors' expectations, no correlations were found with the examined pa-  
285 rameters in the studied population. Further studies enrolling animals with more variable parameters  
286 (such as age, gestational age and UC's parameters) could help to better characterize qualitative and  
287 quantitative differences throughout the entire pregnancy and, subsequently, to compare physiological  
288 and high-risk pregnancies.

289

#### 290 5. Acknowledgments

291 The authors would like to thank all vets and students of the Equine Perinatology and Reproduction  
292 Unit, University of Bologna, for their help in attending parturitions.

293

#### 294 6. References

- 295 1. Schlafer DH. Postmortem examination of the equine placenta, fetus, and neonate: methods and  
296 interpretation of findings. *Proc Am Assoc Equine Pract* 2004;50:144-161.
- 297 2. Ferguson VL, Dodson RB. Bioengineering aspects of the umbilical cord. *Eur J Obstet Gynecol*  
298 *Reprod Biol* 2009;144S:S108-S113.

- 299 3. Klein J, Meyer FA. Tissue structure and macromolecular diffusion in umbilical cord  
300 immobilization of endogenous hyaluronic acid. *Biochim Biophys Acta Gen Subj* 1983;755:400-  
301 11.
- 302 4. Takechi K, Kuwabara Y, Mizuno M. Ultrastructural and immunohistochemical studies of  
303 Wharton's jelly umbilical cord cells. *Placenta* 1993;14:235–45.
- 304 5. Vizza E, Correr S, Goranova V, Heyn R, Angelucci PA, Forleo R, Motta PM. The collagen  
305 skeleton of the human umbilical cord at term. A scanning electron microscopy study after 2N-  
306 NaOH maceration. *Reprod Fertil Dev* 1996;8:885–94.
- 307 6. Franc S, Rousseau JC, Garrone R., van der Rest M, Moradi-Améli M. Microfibrillar composition  
308 of umbilical cord matrix: characterization of fibrillin collagen VI and intact collagen V. *Placenta*  
309 1998;19:95-104.
- 310 7. Ghezzi F, Raio L, Di Naro E, Franchi M, Balestreri D, D'Addario V. Nomogram of Wharton's  
311 jelly as depicted in the sonographic cross section of the umbilical cord. *Ultrasound Obstet*  
312 *Gynecol* 2001;18:121-5.
- 313 8. Merlo B, Teti G, Mazzotti E, Ingrà L, Salvatore V, Buzzi M, et al. Wharton's jelly derived  
314 mesenchymal stem cells: comparing human and horse. *Stem Cell Rev Rep* 2018;14:574-84.
- 315 9. Gogiel T, Bankowski E, Jaworski S. Proteoglycans of Wharton's jelly. *Int J Biochem Cell Biol*  
316 2003;35:1461–9.
- 317 10. Can A, Karahuseyinoglu S. Concise Review: Human Umbilical Cord Stroma with Regard to the  
318 Source of Fetus-Derived Stem Cells. *Stem Cells* 2007;25:2886-95.
- 319 11. Weissman A, Jakobi P, Bronshtein M, Goldstein I. Sonographic measurements of the umbilical  
320 cord and vessels during normal pregnancies. *J Ultrasound Med* 1994;13:11-4.
- 321 12. Ginther OJ. Equine pregnancy: physical interactions between the uterus and conceptus. In *Proc*  
322 *Am Assoc Equine Pract* 1998;44:73-104.
- 323 13. Frazer GS. Umbilical cord compromise as a cause of abortion. *Equine Vet Educ* 2007;19:535-37.

- 324 14. Roach JM, Verheyen KLP, Smith KC, Molyneux M, Bryan J, Foote A, de Mestre AM. Incidence  
325 and Pathology of Abortions Associated with Umbilical Cord Torsions. *Journal of Equine*  
326 *Veterinary Science* 2018;66:230.
- 327 15. Roach JM, Foote AK, Smith KC, Verheyen KL, de Mestre AM. Incidence and causes of  
328 pregnancy loss after Day 70 of gestation in Thoroughbreds. *Equine Vet J* 2021;53:99661003.
- 329 16. Iacono E, Pascucci L, Rossi B, Bazzucchi C, Lanci A, Ceccoli M, et al. Ultrastructural  
330 characteristics and immune profile of equine MSCs from fetal adnexa. *Reproduction*  
331 2017;154:509-19.
- 332 17. Mariella J, Iacono E, Lanci A, Merlo B, Palermo C, Morris L, et al. Macroscopic characteristics  
333 of the umbilical cord in Standardbred, Thoroughbred and Warmblood horses. *Theriogenology*  
334 2018;113:166e70.
- 335 18. Merlo B, Teti G, Lanci A, Burk J, Mazzotti E, Falconi M, Iacono E. Comparison between adult  
336 and foetal adnexa derived equine post-natal mesenchymal stem cells. *BMC Veterinary Research*,  
337 2019;15:1-15.
- 338 19. Whitwell KE, Jeffcott LB. Morphological studies on the fetal membranes of the normal singleton  
339 foal at term. *Res Vet Sci* 1975;19:44e55.
- 340 20. Whitehead AE, Foster R, Chenier T. Placental characteristics of Standardbred mares. In: Powell  
341 DG, Furry D, Hale G, editors. *Proceedings of the workshop on the equine placenta*. Lexington,  
342 KY: University of Kentucky; 2003. p. 71-6.
- 343 21. Govaere J, Hoogewijs C, Schawer De, Roels K, Vanhaesebrouck E, De Lange V, et al. Placenta  
344 evaluation in Warmblood horses. *J Equine Vet Sci* 2014;34:237.
- 345 22. Robles M, Peugnet PM, Valentino SA, Dubois C, Dahirel M, Aubri\_ere MC, et al. Placental  
346 structure and function in different breeds in horses. *Theriogenology*, 2017;108:136e45.
- 347 23. Wilsher S, Bowker A, Silva J, Allen WET. Morphological characteristics of the placenta and  
348 umbilical cord of arabian mares foaling in the United Arab Emirates. *J Equine Vet Sci*  
349 2020;91:103124.

- 350 24. Bianco C, Pirrone A, Boldini S, Sarli G, Castagnetti C. Histomorphometric parameters and fractal  
351 complexity of the equine placenta from healthy and sick foals. *Theriogenology* 2014;82:1106-12.
- 352 25. Pazinato FM, Curcio BR, Fernandes CG, Feijó LS, Schmith RA, Nogueira CEW. Histological  
353 features of the placenta and their relation to the gross and data from Thoroughbred mares. *Pesq*  
354 *Vet Bras* 2016;36:665-70.
- 355 26. Pazinato FM, Curcio BR, Fernandes CG, Santos CA, Feijo LS, Varela AS, et al. CEW  
356 histomorphometry of the placental vasculature and microcotyledons in thoroughbred mares with  
357 chronic laminitis. *Theriogenology* 2017;91:77-81.
- 358 27. Corradetti B, Lange Consiglio A, Barucca M, Cremonesi F, Bizzaro D. Isolation and  
359 characterization of size-sieved mesenchymal stem cells from perivascular and intervascular  
360 Wharton's Jelly of horse umbilical cord. *Reprod Fertil Dev* 2010;22:347–8.
- 361 28. Kumar P, Gulati BR, Deep A, Kumar R, Mohanty N, Anand T, et al. Gross anatomy,  
362 histomorphology and histochemistry of equine umbilical cord. *Indian J Vet Anat* 2013;25.
- 363 29. Bańkowski E, Sobolewski K, Romanowicz L, Chyczewski L, Jaworski S. Collagen and  
364 glycosaminoglycans of Wharton's jelly and their alterations in EPH-gestosis. *Eur J Obstet*  
365 *Gynecol Reprod Biol* 1996;66:109-17.
- 366 30. Kilman HJ. Umbilical cord. In: Knobil E, Neill J, editors. *Encyclopedia of Reproduction*. New  
367 York: Academic Press; 2006. pp. 915–23.
- 368 31. Chillakuru S, Velichety SD, Rajagopalan V. Human umbilical cord and its vessels: a  
369 histomorphometric study in difference severity of hypertensive disorders of pregnancy. *Anat Cell*  
370 *Biol* 2020;53:68.
- 371 32. Vaala WE. Perinatology. In: Higgins AJ, Snyder JR, editors. *The equine manual* second ed. W.B.  
372 Saunders; 2006. pp. 803-4.
- 373 33. Brooks DE, Komaromy AM, Garcia-Fernandez MC, Cutler TJ, Samuelson DA, Kallberg ME.  
374 Immunohistochemistry of the extracellular matrix of the normal equine lamina cribrosa. *Vet*  
375 *Ophthalmol* 2000;3:127-32.



- 376 34. Sloper KS, Brown RS, Baum JD. The water content of the human umbilical cord. *Early Hum Dev*  
377 1979;3:205-10.
- 378 35. Bruch JF, Sibony O, Benali K, Challer C, Blot P, Nessmann C. Computerized microscope  
379 morphometry of umbilical vessels from pregnancies with intrauterine growth retardation and  
380 abnormal umbilical artery Doppler. *Hum Pathol* 1997;28:1139–45.
- 381 36. Raio L, Ghezzi F, Di Naro E, Gomez R, Mueller MD, Maymon E, et al. Sonographic  
382 measurements of the umbilical cord and fetal anthropometric parameters. *Eur J Obstet Gynecol*  
383 *Reprod Biol* 1999;83:131–5.
- 384 37. Ghezzi F, Raio L, Di Naro E, Franchi M, Balestreri D, D'addario V. Nomogram of Wharton's jelly  
385 as depicted in the sonographic cross section of the umbilical cord. *Ultrasound Obstet Gynecol*  
386 2001;18:121-5.
- 387 38. Togni FA, Araujo Ju'nior E, Vasques FA, Moron AF, Torloni MR, Nardoza L.M. The cross-  
388 sectional area of umbilical cord components in normal pregnancy. *Int J Gynaecol Obstet*  
389 2007;96:156-61.
- 390 39. Filiz AA, Rahime B, Keskin HL, Esra AK. Positive correlation between the quantity of Wharton's  
391 jelly in the umbilical cord and birth weight. *Taiwan J Obstet Gynecol* 2011;50:33-6.
- 392 40. Goodlin RC. Fetal dysmaturity, "lean cord," and fetal distress. *Am J Obstet Gynecol*  
393 1987;156:1357.
- 394 41. Clausen I. Umbilical cord anomalies and antenatal fetal deaths. *Obstet Gynecol* 1989;44:841-  
395 55.
- 396 42. Inan S, Sancı M, Can D, Vatansever S, Oztekin O, Tinar S. Comparative morphological  
397 differences between umbilical cords from chronic hypertensive and preeclamptic pregnancies.  
398 *Acta Med Okayama* 2002;56:177–86.
- 399 43. Debebe SK, Cahill LS, Kingdom JC, Whitehead CL, Chandran AR, Parks WT, et al. Wharton's  
400 jelly area and its association with placental morphometry and pathology. *Placenta* 2020;94:34-8.

- 401 44. Weissman A, Jakobi P. Sonographic measurements of the umbilical cord in pregnancies  
402 complicated by gestational diabetes. *J Ultrasound Med* 1997;16:691-4.
- 403 45. Ghezzi F, Raio L, Duwe DG, Cromi A, Karousou E, Dürig P. Sonographic umbilical vessel  
404 morphometry and perinatal outcome of fetuses with a lean umbilical cord. *J Clin Ultrasound*  
405 2004;33:18-23.
- 406 46. Sharony R, Keltz E, Biron-Shental T, Kidron D. Morpho-metric characteristics of the umbilical  
407 cord and vessels in fetal growth restriction and pre-eclampsia. *Early Hum Dev* 2016;92:57-62.
- 408 47. Kulkarni ML, Matadh PS, Ashok C, Pradeep N, Avinash T, Kulkarni AM. Absence of Wharton's  
409 jelly around the umbilical arteries. *Indian J Pediatr* 2007;74:787-9.
- 410 48. Kurita M, Hasegawa J, Mikoshiba T, Purwosunu Y, Matsuoka R, Ichizuka K, Sekizawa A, Okai  
411 T. Ultrasound evaluation of the amount of Wharton's jelly and the umbilical coiling index. *Fetal*  
412 *Diagn Ther* 2009;26:85-9.
- 413 49. Van Der Rest M, Garrone R. Collagen family of proteins. *FASEB J* 1991;5:2814-23.
- 414 50. Sexton AJ, Turmaine M, Cai WQ, Burnstock G. A study of the ultrastructure of developing human  
415 umbilical vessels. *J Anat* 1996;188:75-85.
- 416 51. Malas MA, Sulak O, Gökçimen A, Sari A. Morphology of umbilical vessels in human fetuses: a  
417 quantitative light microscope study. *Eur J Morphol* 2003;41:167-74.
- 418 52. Griffin PG, Ginther OJ. Uterine and fetal dynamics during early pregnancy in mares. *Am J Vet*  
419 *Res* 1991;52:298–306.
- 420 53. Vandeplasse M, Lauwers H. The twisted umbilical cord: an expression of kinesis of the equine  
421 fetus? *Anim Reprod Sci* 1986;10:163-75.
- 422 54. Ginther OJ. Physical activities and morphologic aspects of the equine fetus during Days 40 to  
423 150. *J Equine Vet Sci* 2022;112:103891.