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1 **Title**

2 How to Perform Umbilical Cord Arterial and Venous Blood Sampling in

3 Neonatal Foals

4

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25

26 **Abstract:**

27 Umbilical cord arterial and venous blood gas analysis is a commonly performed  
28 procedure in human neonatal medicine to help ascertain a newborn infant's  
29 oxygenation and acid-base status prior to birth. Defined protocols for performing  
30 the procedure have been described in the medical literature. The aim of this  
31 report was to describe in detail the procedure for collecting paired blood samples  
32 from the umbilical artery and vein in newborn foals so that stall-side blood gas  
33 analysis could be carried out. Thirty-five Thoroughbred foals >320 days  
34 gestation from mares at one stud farm were sampled. Paired umbilical arterial  
35 and venous whole-blood samples were obtained in  $n=30$  foals, umbilical artery  
36 only samples obtained in  $n=3$  and umbilical vein only samples obtained in  $n=2$   
37 foals. There were no adverse events or clinical outcomes associated with the  
38 sampling protocol described. The authors found that umbilical cord blood  
39 collection for blood gas analysis was a practical clinical technique that  
40 potentially could be used as a stall-side method for assessing the *in utero*  
41 oxygenation and acid-base status of newborn foals.

42

43 **Keywords:**

44 Foal, Hypoxia, Perinatal Asphyxia Syndrome, Blood Gas Analysis, Umbilical  
45 Cord

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**51 1. Introduction**

52 The combination of Apgar scoring [1,2] and umbilical cord blood gas analysis is  
53 routinely used by human neonatologists to assess the likelihood that a hypoxic  
54 event effecting the human infant occurred *in utero*, either acutely during  
55 parturition or more chronically during the pregnancy [3,4,5]. Umbilical cord  
56 blood gas analysis is thought to be a particularly useful diagnostic technique, as  
57 it provides the most accurate insight into the neonate's acid-base status prior to  
58 birth [5]. These assessments help identify at-risk babies without delay, allowing  
59 for early medical intervention [6,7,8]. Mortality rates as well as adverse clinical  
60 sequelae both in the short and long-term, particularly in relation to  
61 neurodevelopmental disease, appear to be reduced with such early intervention  
62 [9,10,11]. Similar to the hypoxic syndromes seen in human neonates, perinatal  
63 asphyxia syndrome (PAS) in neonatal foals is likely caused by hypoxic-  
64 ischaemic damage that occurred during pregnancy or parturition [12,13].  
65 Although numerous risk factors have been identified for PAS, currently there are  
66 no confirmed biochemical parameters that can be used to inform on the risk of  
67 this disease being present [12].

68

69 Our research group recently published a study in which reference intervals (RI)  
70 were determined for umbilical cord arterial and venous blood gas samples from  
71 healthy Thoroughbred foals [14]. The aim had been to evaluate the practicality  
72 of umbilical cord blood sampling from foals in a field-based setting and to  
73 determine RI values from normal, healthy foals. It was hypothesised that if RIs  
74 could be determined and the technique found to be feasible in a field-based  
75 setting, this assessment modality could then be used to evaluate for differences

76 between normal foals and foals at risk of PAS. During the study, the umbilical  
77 cord blood collection technique developed by the authors was found to be simple  
78 and minimally disruptive with consistent and accurate umbilical cord blood  
79 sampling. Furthermore, RIs were definitively identified for the group of healthy  
80 foals sampled in the study [14]. Because use of this technique in foals has not  
81 been reported since the publication by Rose et al. (1982) [15], the purpose of the  
82 present paper was to describe the protocol developed by the authors in greater  
83 detail. The authors believe that stall-side umbilical blood gas analysis may  
84 become a useful method for assessing *in utero* oxygenation and acid-base status  
85 in the equine neonate.

86

## 87 **2. Materials and Methods**

88 The study was approved by the University College Dublin Animal Research  
89 Ethics committee, with informed written owner consent for all procedures. The  
90 methodology and results from the study for which the following technique is  
91 described can be found in further detail in the publication by Jeawon et al.  
92 (2018) [14].

93

94 Thirty-five full-term gestation (>320 days old) foals were sampled in the study.  
95 All mares were from the same stud farm, and managed under similar  
96 circumstances. The mares foaled under constant supervision, which involved at  
97 least 2 trained staff members attending every parturition. One author (SSJ)  
98 attended all parturitions and took all umbilical cord blood samples. Whilst the  
99 mare was in second stage labour, preparations were made to ensure efficient and  
100 successful sampling and analysis (Figure 1). Two 1 ml pre-heparinised

101 disposable blood gas syringes (RAPIDLyte, Cruinn medical Ltd, Dublin,  
102 Ireland) were prepared; one with a 23g x 1” (blue) needle and one with a 21g x  
103 1” (green) needle. The colour coding system was to allow ease of identification  
104 of samples since the syringes were identical, with the green needle hub equating  
105 to the arterial sample and the blue needle hub equating to the venous sample.  
106 Foal identification details were entered into the blood gas analyser (Vetscan i-  
107 Stat<sup>®</sup> 1, Abaxis UK Ltd, York, United Kingdom) as stage 2 parturition  
108 commenced in order to expedite sample measurement. Sterile gloves  
109 (GAMMEX<sup>®</sup> moisturising latex, Ansell Healthcare Europe, Brussels, Belgium)  
110 were then put on in anticipation of umbilical cord blood sampling. Whilst not a  
111 sterile procedure, the authors deemed it appropriate to maximise cleanliness  
112 when handling the umbilical cord to help minimise risk of ascending umbilical  
113 infection.

114

115 Once the foal was safely delivered, the time of foaling was noted. As soon as the  
116 foal was expelled, the umbilical structures were identified via visual and manual  
117 palpation (Figure 2). An incontinency pad (Laboratorios INDAS, Madrid, Spain)  
118 was placed underneath the umbilical cord to keep it clean and aid visualisation  
119 of the structures. The umbilical cord was not clamped for sampling, with the  
120 arterial sample always obtained first from the largest umbilical artery (Figure 3).  
121 The timing of each sample collection in relation to foetal expulsion was  
122 recorded. Sampling was best achieved by positioning oneself beside the foal’s  
123 lumbar spine region, then leaning over the foal to hold the umbilical cord in the  
124 non-dominant hand and using the dominant hand for sampling (Figure 3). Ease  
125 of umbilical structure identification was aided by the fact that a) the vein was

126 consistently the largest vessel and b) the largest artery usually had a palpable  
127 pulse in it (Figure 2).

128

129 All umbilical cord blood samples were taken as close to the foal's body wall as  
130 possible, approximately one hand's breath away from the body wall (Figure 3).

131 Using the 21g needle, a 1 ml umbilical arterial blood sample was collected into  
132 the pre-heparinised blood gas syringe, followed by a 1 ml umbilical venous  
133 blood sample obtained into a second pre-heparinised blood gas syringe using the  
134 23g needle. Due to the small gauge needles being used to sample both vessels,  
135 there was negligible bleeding from the puncture sites noted. Each foal then had  
136 an Apgar score assigned and a rectal temperature taken to temperature-correct  
137 the samples for blood gas analyses, which were performed without delay.

138

139 The portable Vetscan i-Stat<sup>®</sup> 1 machine was used to analyse all samples using  
140 the CG4+ cartridge (Abaxis UK Ltd, York, United Kingdom). This analyser has  
141 previously shown to produce reliable, accurate and repeatable results as  
142 compared to laboratory-grade machines for the measurement of equine blood  
143 gas samples [16–20]. The parameters analysed included pH, PCO<sub>2</sub>, PO<sub>2</sub>, HCO<sub>3</sub>,  
144 TCO<sub>2</sub>, SO<sub>2</sub>%, base excess/deficit and lactate. All samples were analysed in  
145 duplicate, with the arterial sample always measured first followed by the venous  
146 sample. Both the machine and cartridges were kept at room temperature to  
147 ensure the sensors would be calibrated at all times. As per the manufacturer's  
148 guidelines, the well of the cartridge was filled to the line with the blood from the  
149 pre-heparinised syringe. The well portal was closed and inserted into the  
150 Vetscan i-Stat<sup>®</sup> 1 gas analyser. Each sample took 120 seconds to run.

151

152 All foals had complete clinical examinations performed on days 1, 2, 3, 7, 14, 21  
153 and 28 post-partum. This involved all vital parameters being assessed as well as  
154 a systematic clinical evaluation of all body systems. Clinical notes were  
155 documented on each animal after each examination. All foals also had a blood  
156 sample taken between 10–14 hours after parturition for measurement of serum  
157 IgG concentration.

158

### 159 **3. Results and Discussion**

160 Paired umbilical arterial and venous whole-blood samples were obtained in  $n=30$   
161 foals, umbilical artery samples alone obtained in  $n=3$  foals and umbilical vein  
162 samples alone obtained in  $n=2$  foals. The average time from birth to the first  
163 umbilical cord sample acquisition was  $1.2\pm 0.8$  minutes, and the average time from  
164 sampling to analysis was  $5.0\pm 2.3$  minutes.

165

166 The location and timing of the umbilical cord blood samples were the same for all  
167 foals in the present study with the protocol based on human publications supporting  
168 the importance of considering these parameters [21,22]. A consistent increase in the  
169 arterial pH and  $PCO_2$  values and a decrease in the  $PO_2$  values have been  
170 demonstrated to occur in human neonates as blood moves from the area of placental  
171 attachment of the umbilical cord distally to the foetal attachment of the cord [21]. It  
172 is thus recommended that the umbilical cord blood sampling occur at a site as close  
173 to the foetus as possible, as was done in the present study. Researchers have  
174 identified that 60 minutes after birth umbilical cord blood gas measurements in  
175 human neonates will have altered from their original state with  $PCO_2$  and  $PO_2$



176 significantly decreasing and increasing, respectively, as compared to 5-minute post-  
177 birth samples [22]. A 30-minute sampling window has thus been proposed as optimal  
178 [23]. The sampling timings for both acquisition and analysis in this study were all  
179 within this optimal time-frame.

180

181 The results obtained allowed RIs to be determined for umbilical arterial and  
182 venous blood pH, PO<sub>2</sub>, PCO<sub>2</sub>, SO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, base-excess, TCO<sub>2</sub> and lactate [14].  
183 Umbilical arterial blood samples had lower pH ( $P<0.0001$ ), PO<sub>2</sub> ( $P=0.002$ ) and  
184 SO<sub>2</sub> ( $P<0.0001$ ) and higher PCO<sub>2</sub> ( $P<0.0001$ ) and lactate ( $P<0.0001$ ) than  
185 venous samples [14]. These consistently measured differences between the  
186 paired vessels along with the anatomical landmarks used to identify the different  
187 vessels supports the authors' conclusions that arterial and venous samples had  
188 been correctly and consistently obtained. The importance of obtaining paired  
189 arterial and venous blood samples for analysis is emphasised in the human  
190 medical literature, as the difference between the vessels' measured values can  
191 indicate whether there was an acute or chronic insult [5]. A large arterio-venous  
192 base-deficit difference has been demonstrated to indicate a more acute event,  
193 whilst a small arterio-venous base-deficit difference has been shown to indicate  
194 a more chronic problem; this is explained by the fact that it will take a  
195 significantly longer time for the venous sample to reflect changes due to the  
196 influence of the maternal circulation [5,24].

197

198 Umbilical arterial blood gas parameters for equine neonates were originally reported  
199 by Rossdale (1968) [25]. Rose et al. (1982) then published additional results from this  
200 work, reporting on 8 premature-induced and full-term-induced foals [15] with

201 umbilical arterial pH values identified to be similar to those reported by Jeawon et al.  
202 (2018) [14]. However, the umbilical arterial PO<sub>2</sub> values were higher and the PCO<sub>2</sub> and  
203 base-excess values lower for the foals from the study by Rose et al. (1982) as  
204 compared to the values reported by Jeawon et al. (2018) [14,15]. A likely reason for  
205 these differences is variations in experimental design between the two studies. The  
206 study reported by Rose et al. (1982) involved a much smaller sample population of  
207 foals, all of which were born after an induced parturition using fluprostenol [15].  
208 Furthermore, there were differences in the timing of sampling as well as differences in  
209 blood gas analysers used. Rose et al. (1982) also used a catheter to obtain umbilical  
210 arterial samples from an unknown umbilical cord location and did not acquire paired  
211 umbilical venous samples [15], all in contrast to the work of Jeawon et al. (2018) [14].

212

213 An understanding of normal foetal circulation is key to being able to understand  
214 the theory behind umbilical cord blood sampling. Furthermore, anatomical  
215 knowledge of the umbilical cord structures is vital for correctly performing the  
216 sampling technique as described in the present study. McGeady et al. (2017)  
217 [26] comprehensively outlined the intricacies of the foetal circulatory system in  
218 some of the common domestic species (Figure 4). During embryonic  
219 development the left and right umbilical veins initially convey blood from the  
220 allantoic cavity, through the septum transversum and into the sinus venosus. As  
221 the embryonic liver continues to grow, the veins become subdivided into cranial,  
222 middle and caudal segments. The cranial segments atrophy with the middle  
223 segments becoming incorporated into the hepatic vasculature to contribute to  
224 hepatic sinusoid formation. The left and right umbilical veins later fuse, whereby

225 the caudal segment of the right vein atrophies and the caudal segment of the left  
226 vein enlarges accordingly (Figure 5).  
227  
228 In most domestic species within the foetal circulation, oxygenated blood from the  
229 umbilical vein bypasses the liver via the *ductus venosus*; however, the full-term  
230 equine foetus does not have a *ductus venosus* with the umbilical venous blood passing  
231 through the foetal liver before emptying into the right atrium via the caudal *vena cava*  
232 [27,28]. Pressure and oxygenation gradients redirect most blood in the right atrium  
233 through the open *foramen ovale* into the left atrium, where it mixes with some blood  
234 coming from the non-functional foetal lungs via the pulmonary veins, before leaving  
235 the heart and entering the systemic circulation. Deoxygenated blood ultimately returns  
236 from the aorta to the placenta via the paired umbilical arteries (Figure 6). Since the  
237 umbilical vein carries oxygenated blood to the foetus and the two umbilical arteries  
238 carry deoxygenated blood away from the foetus, the umbilical arterial blood solely  
239 reflects the foetal acid-base and oxygenation status; in comparison, the umbilical  
240 venous blood reflects the foetal acid-base and oxygenation status that has been  
241 influenced by the maternal acid-base status [5,29].  
242  
243 The umbilical cord blood sampling technique detailed in this study became more  
244 refined as the sampling progressed, with the author taking the samples becoming  
245 increasingly more efficient at accurately identifying the individual vessels.  
246 Failure to obtain a paired arterial and venous sample in a foal was either due to  
247 the mare standing prematurely and rupturing the umbilical cord before the  
248 venous sample could be taken or due to the mare lying awkwardly against the  
249 wall as she foaled, preventing adequate access to the umbilical artery. Having

250 the mare's hindquarters well away from the wall allowed better access to the  
251 umbilicus once the foal had been delivered, making the sampling procedure  
252 easier to perform. The author found it easy to obtain the required amount of  
253 blood from each vessel for analysis.

254

255 The importance of calm, professional foaling practices was emphasised during  
256 the sampling process. Experienced staff helped to keep the mare calm and  
257 minimally stressed after the foal was born, ensuring the mare did not stand  
258 prematurely, inadvertently breaking the umbilical cord. Whilst it is normal  
259 practice in human obstetrics for the umbilical cord to be clamped after birth, the  
260 authors decided against this approach prior to sampling for two reasons. Firstly,  
261 it was noticed that the application of clamps to the cord was more time  
262 consuming than just taking the samples directly, given the often-awkward  
263 positioning of the umbilical cord between the mare's hind quarters/back legs and  
264 the foal itself. Secondly, as the amount of blood passed from the placenta to the  
265 foal through the umbilical cord in the minutes after birth may be as high as 30%  
266 of the total blood volume [26] the authors did not want to hinder this from  
267 occurring. Whilst the authors are not aware of any specific studies in the  
268 veterinary literature, the benefits of increased blood volume from placental  
269 transfer on neonatal health has been reported widely in for humans with the  
270 primary positive benefits related to increased total blood volume and reduced  
271 incidence of neonatal anaemia and iron deficiency [30,31]. Furthermore, delayed  
272 cord clamping in human neonates (performed approximately 2 minutes after  
273 birth) has not been shown to significantly change the blood gas findings when

274 compared with clamping at 10 seconds after birth [32]. This supports the  
275 sampling protocol described in the present study.

276

277 Complete clinical examinations performed by one of two authors (SSJ or NPG) over  
278 the first month for each of the foals in the present study revealed no adverse events  
279 or clinical consequences of the umbilical cord blood sampling for any of the foals.

280 Clinical exams were within normal limits at all time-points, with all foals exhibiting  
281 normal immediate post-foaling behaviour including the ability to stand, nurse and  
282 pass meconium and urine. All foals had a serum IgG of >800mg/dl, with none of the  
283 foals developing umbilical haemorrhage in the immediate post-parturient period nor  
284 omphalophlebitis over the follow-up time-frame.

285

#### 286 **4: Conclusion**

287 The described protocol for obtaining umbilical cord blood samples from foals in  
288 a field-setting was shown to be an effective and simple technique, with minimal  
289 disruption to the foaling environment. Umbilical cord blood sampling of  
290 neonatal foals is a practical technique that can be employed in the field.

291

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298

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379

380 Figure Legends

381

382 Figure 1: Image of the sampling equipment used (clockwise from top left): Vetscan  
383 iStat® 1 machine, CG4+ sampling cartridge, incontinency pad, colour-coded blood  
384 gas syringes, sterile gloves.

385

386 Figure 2: Image of the umbilical cord structures prior to sampling. a = umbilical vein;  
387 b = smaller umbilical artery; c = larger umbilical artery.

388

389 Figure 3: Image of the umbilical cord blood sampling procedure being performed. a =  
390 umbilical vein; b = smaller umbilical artery; c = larger umbilical artery.

391

392 Figure 4: Diagram of equine foetal circulation. Arrows indicate direction of blood  
393 flow.

394

395 Figure 5: Close-up of the gross image of the fusion of the left and right umbilical  
396 veins (\*). a = left umbilical vein; b= right umbilical vein.

397

398 Figure 6: Schematic of the umbilical cord vessels running between the placenta and  
399 foal. Arrows indicate the direction of blood flow.

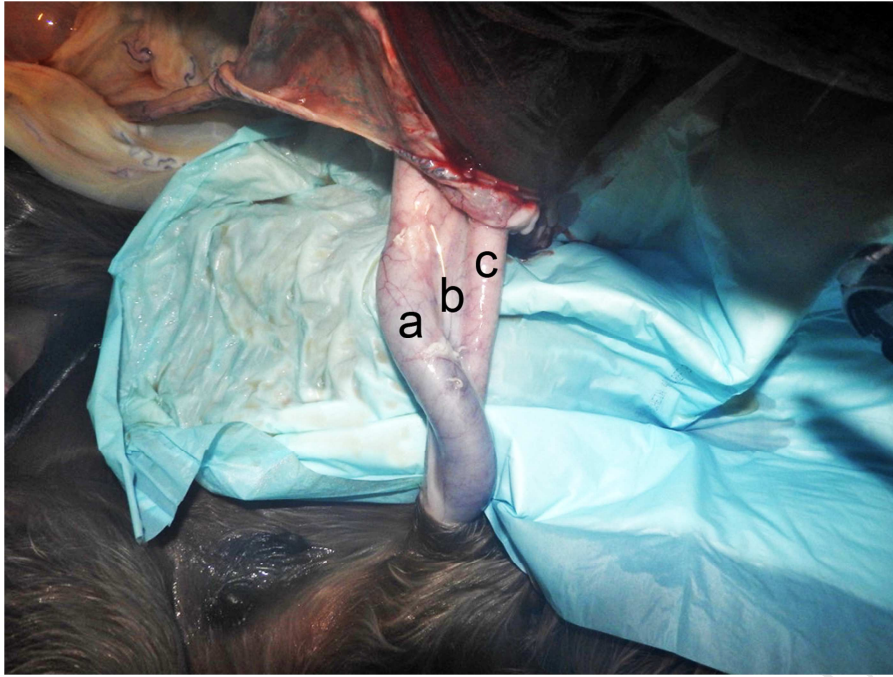
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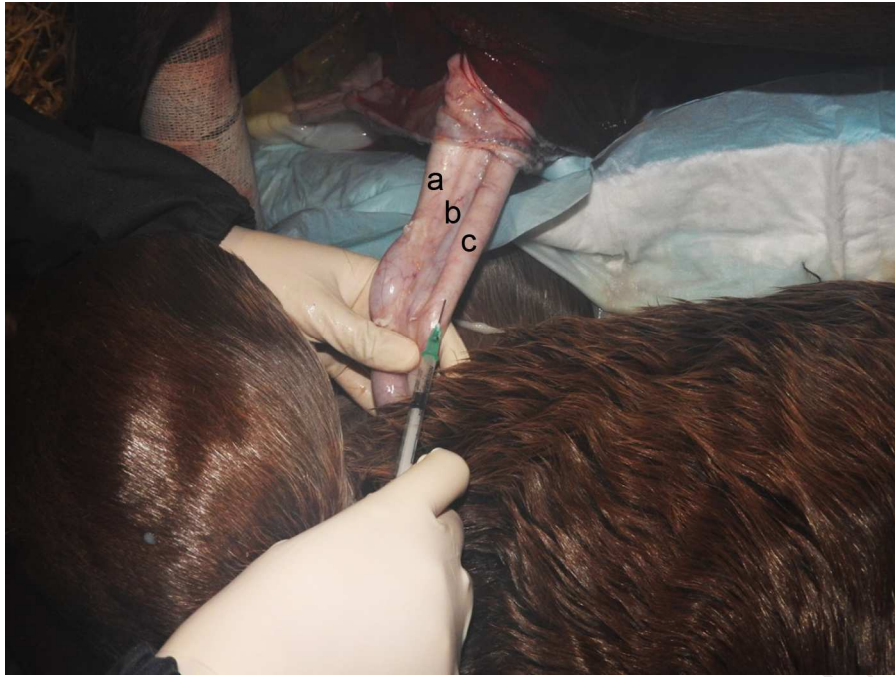
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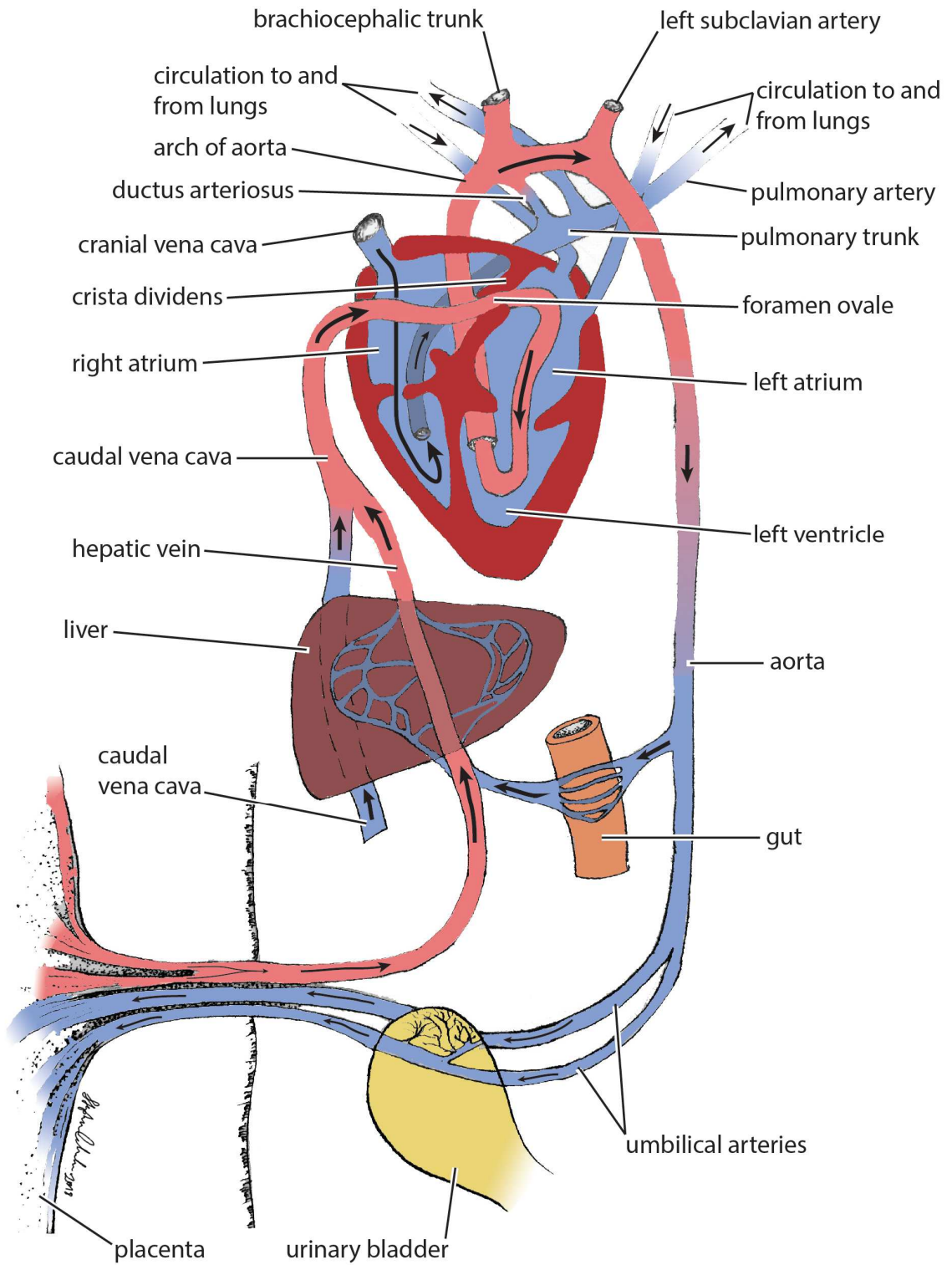


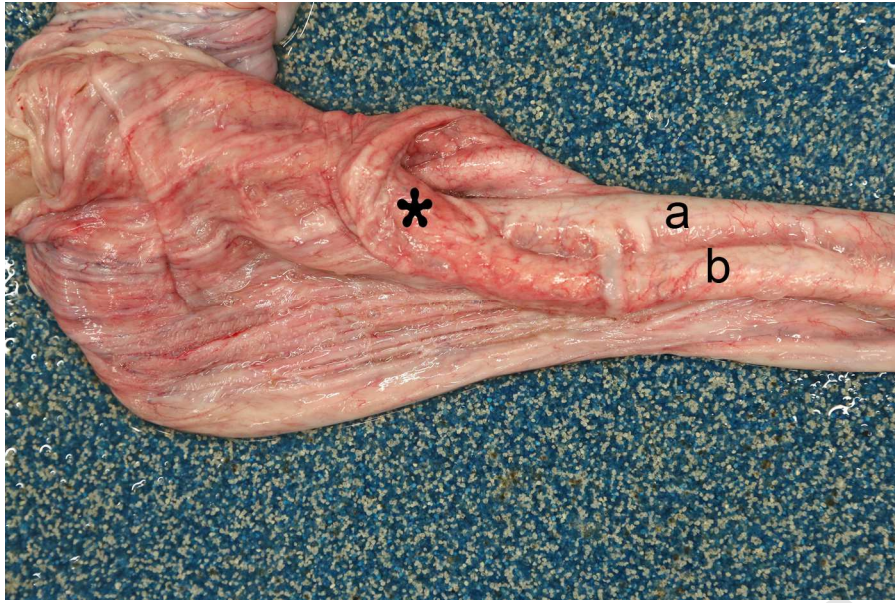
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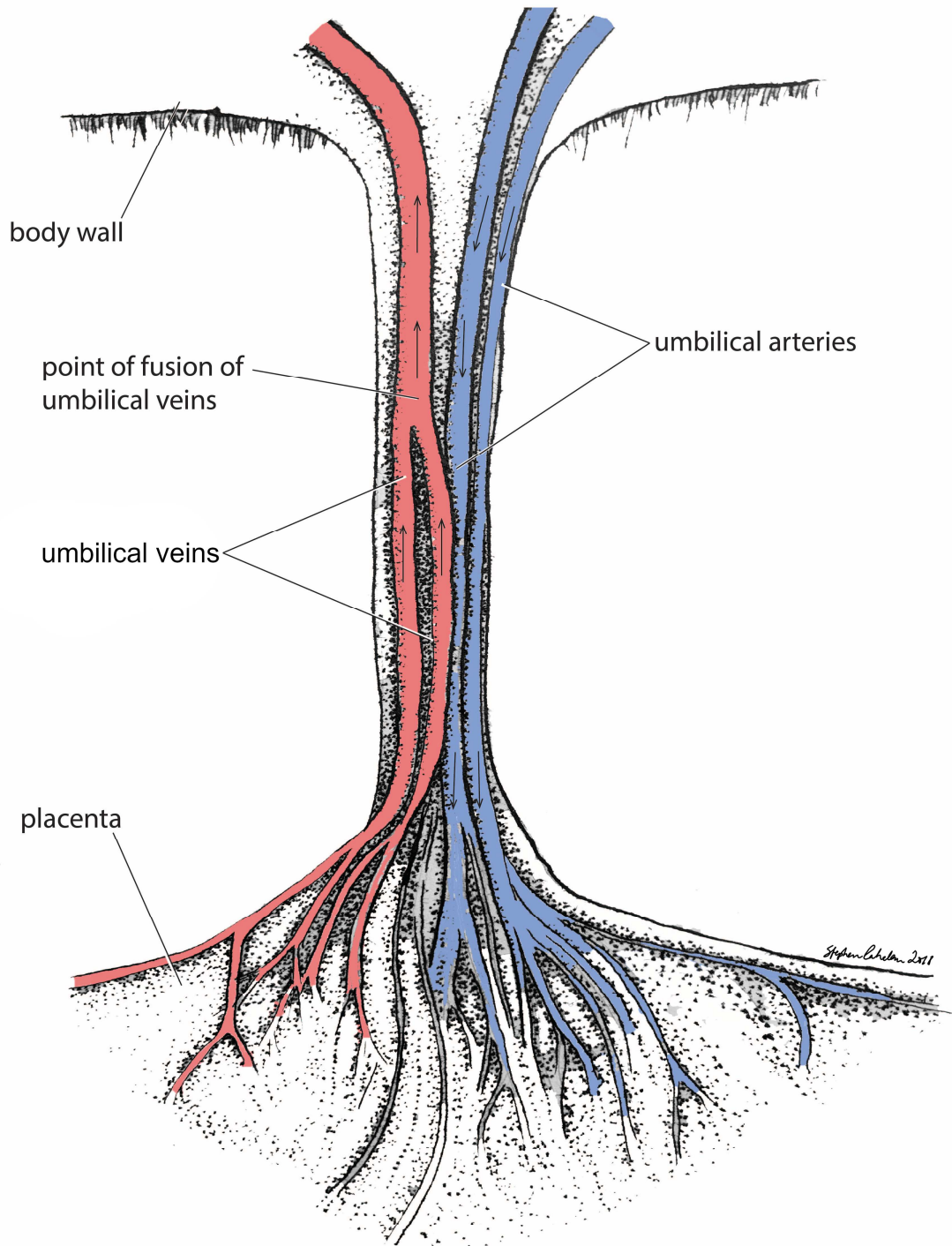
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**Ethical Statement**

University College Dublin's Animal Research and Ethics Committee approved this study. Owner consent was also granted.

ACCEPTED MANUSCRIPT

**Conflict of Interest Statement**

The authors declare no conflict of interest associated with this paper.

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