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Title

How to Perform Umbilical Cord Arterial and Venous Blood Sampling in Neonatal Foals

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Abstract:

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

Keywords:

Foal, Hypoxia, Perinatal Asphyxia Syndrome, Blood Gas Analysis, Umbilical Cord
1. Introduction

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

Our research group recently published a study in which reference intervals (RI) were determined for umbilical cord arterial and venous blood gas samples from healthy Thoroughbred foals [14]. The aim had been to evaluate the practicality of umbilical cord blood sampling from foals in a field-based setting and to determine RI values from normal, healthy foals. It was hypothesised that if RIs could be determined and the technique found to be feasible in a field-based setting, this assessment modality could then be used to evaluate for differences
between normal foals and foals at risk of PAS. During the study, the umbilical cord blood collection technique developed by the authors was found to be simple and minimally disruptive with consistent and accurate umbilical cord blood sampling. Furthermore, RIs were definitively identified for the group of healthy foals sampled in the study [14]. Because use of this technique in foals has not been reported since the publication by Rose et al. (1982) [15], the purpose of the present paper was to describe the protocol developed by the authors in greater detail. The authors believe that stall-side umbilical blood gas analysis may become a useful method for assessing *in utero* oxygenation and acid-base status in the equine neonate.

2. Materials and Methods

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

Thirty-five full-term gestation (>320 days old) foals were sampled in the study. All mares were from the same stud farm, and managed under similar circumstances. The mares foaled under constant supervision, which involved at least 2 trained staff members attending every parturition. One author (SSJ) attended all parturitions and took all umbilical cord blood samples. Whilst the mare was in second stage labour, preparations were made to ensure efficient and successful sampling and analysis (Figure 1). Two 1 ml pre-heparinised
Disposable blood gas syringes (RAPIDLyte, Cruinn medical Ltd, Dublin, Ireland) were prepared; one with a 23g x 1” (blue) needle and one with a 21g x 1” (green) needle. The colour coding system was to allow ease of identification of samples since the syringes were identical, with the green needle hub equating to the arterial sample and the blue needle hub equating to the venous sample. Foal identification details were entered into the blood gas analyser (Vetscan i-Stat®, Abaxis UK Ltd, York, United Kingdom) as stage 2 parturition commenced in order to expedite sample measurement. Sterile gloves (GAMMEX® moisturising latex, Ansell Healthcare Europe, Brussels, Belgium) were then put on in anticipation of umbilical cord blood sampling. Whilst not a sterile procedure, the authors deemed it appropriate to maximise cleanliness when handling the umbilical cord to help minimise risk of ascending umbilical infection.

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

All umbilical cord blood samples were taken as close to the foal’s body wall as possible, approximately one hand’s breath away from the body wall (Figure 3). Using the 21g needle, a 1 ml umbilical arterial blood sample was collected into the pre-heparinised blood gas syringe, followed by a 1 ml umbilical venous blood sample obtained into a second pre-heparinised blood gas syringe using the 23g needle. Due to the small gauge needles being used to sample both vessels, there was negligible bleeding from the puncture sites noted. Each foal then had an Apgar score assigned and a rectal temperature taken to temperature-correct the samples for blood gas analyses, which were performed without delay.

The portable Vetscan i-Stat® machine was used to analyse all samples using the CG4+ cartridge (Abaxis UK Ltd, York, United Kingdom). This analyser has previously shown to produce reliable, accurate and repeatable results as compared to laboratory-grade machines for the measurement of equine blood gas samples [16–20]. The parameters analysed included pH, PCO$_2$, PO$_2$, HCO$_3^-$, TCO$_2$, SO$_2$%, base excess/deficit and lactate. All samples were analysed in duplicate, with the arterial sample always measured first followed by the venous sample. Both the machine and cartridges were kept at room temperature to ensure the sensors would be calibrated at all times. As per the manufacturer’s guidelines, the well of the cartridge was filled to the line with the blood from the pre-heparinised syringe. The well portal was closed and inserted into the Vetscan i-Stat® gas analyser. Each sample took 120 seconds to run.
All foals had complete clinical examinations performed on days 1, 2, 3, 7, 14, 21 and 28 post-partum. This involved all vital parameters being assessed as well as a systematic clinical evaluation of all body systems. Clinical notes were documented on each animal after each examination. All foals also had a blood sample taken between 10–14 hours after parturition for measurement of serum IgG concentration.

3. Results and Discussion

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

The location and timing of the umbilical cord blood samples were the same for all foals in the present study with the protocol based on human publications supporting the importance of considering these parameters [21, 22]. A consistent increase in the arterial pH and PCO\(_2\) values and a decrease in the PO\(_2\) values have been demonstrated to occur in human neonates as blood moves from the area of placental attachment of the umbilical cord distally to the foetal attachment of the cord [21]. It is thus recommended that the umbilical cord blood sampling occur at a site as close to the foetus as possible, as was done in the present study. Researchers have identified that 60 minutes after birth umbilical cord blood gas measurements in human neonates will have altered from their original state with PCO\(_2\) and PO\(_2\).
significantly decreasing and increasing, respectively, as compared to 5-minute post-
birth samples [22]. A 30-minute sampling window has thus been proposed as optimal
[23]. The sampling timings for both acquisition and analysis in this study were all
within this optimal time-frame.

The results obtained allowed RIs to be determined for umbilical arterial and
venous blood pH, $PO_2$, $PCO_2$, $SO_2$, $HCO_3^-$, base-excess, $TCO_2$ and lactate [14].
Umbilical arterial blood samples had lower pH ($P<0.0001$), $PO_2$ ($P=0.002$) and
$SO_2$ ($P<0.0001$) and higher $PCO_2$ ($P<0.0001$) and lactate ($P<0.0001$) than
venous samples [14]. These consistently measured differences between the
paired vessels along with the anatomical landmarks used to identify the different
vessels supports the authors’ conclusions that arterial and venous samples had
been correctly and consistently obtained. The importance of obtaining paired
arterial and venous blood samples for analysis is emphasised in the human
medical literature, as the difference between the vessels’ measured values can
indicate whether there was an acute or chronic insult [5]. A large arterio-venous
base-deficit difference has been demonstrated to indicate a more acute event,
whilst a small arterio-venous base-deficit difference has been shown to indicate
a more chronic problem; this is explained by the fact that it will take a
significantly longer time for the venous sample to reflect changes due to the
influence of the maternal circulation [5,24].

Umbilical arterial blood gas parameters for equine neonates were originally reported
by Rossdale (1968) [25]. Rose et al. (1982) then published additional results from this
work, reporting on 8 premature-induced and full-term-induced foals [15] with
umbilical arterial pH values identified to be similar to those reported by Jeawon et al. (2018) [14]. However, the umbilical arterial PO$_2$ values were higher and the PCO$_2$ and base-excess values lower for the foals from the study by Rose et al. (1982) as compared to the values reported by Jeawon et al. (2018) [14,15]. A likely reason for these differences is variations in experimental design between the two studies. The study reported by Rose et al. (1982) involved a much smaller sample population of foals, all of which were born after an induced parturition using fluprostenol [15]. Furthermore, there were differences in the timing of sampling as well as differences in blood gas analysers used. Rose et al. (1982) also used a catheter to obtain umbilical arterial samples from an unknown umbilical cord location and did not acquire paired umbilical venous samples [15], all in contrast to the work of Jeawon et al. (2018) [14].

An understanding of normal foetal circulation is key to being able to understand the theory behind umbilical cord blood sampling. Furthermore, anatomical knowledge of the umbilical cord structures is vital for correctly performing the sampling technique as described in the present study. McGeady et al. (2017) [26] comprehensively outlined the intricacies of the foetal circulatory system in some of the common domestic species (Figure 4). During embryonic development the left and right umbilical veins initially convey blood from the allantoic cavity, through the septum transversum and into the sinus venosus. As the embryonic liver continues to grow, the veins become subdivided into cranial, middle and caudal segments. The cranial segments atrophy with the middle segments becoming incorporated into the hepatic vasculature to contribute to hepatic sinusoid formation. The left and right umbilical veins later fuse, whereby
the caudal segment of the right vein atrophies and the caudal segment of the left vein enlarges accordingly (Figure 5).

In most domestic species within the foetal circulation, oxygenated blood from the umbilical vein bypasses the liver via the *ductus venosus*; however, the full-term equine foetus does not have a *ductus venosus* with the umbilical venous blood passing through the foetal liver before emptying into the right atrium via the caudal *vena cava* [27,28]. Pressure and oxygenation gradients redirect most blood in the right atrium through the open *foramen ovale* into the left atrium, where it mixes with some blood coming from the non-functional foetal lungs via the pulmonary veins, before leaving the heart and entering the systemic circulation. Deoxygenated blood ultimately returns from the aorta to the placenta via the paired umbilical arteries (Figure 6). Since the umbilical vein carries oxygenated blood to the foetus and the two umbilical arteries carry deoxygenated blood away from the foetus, the umbilical arterial blood solely reflects the foetal acid-base and oxygenation status; in comparison, the umbilical venous blood reflects the foetal acid-base and oxygenation status that has been influenced by the maternal acid-base status [5,29].

The umbilical cord blood sampling technique detailed in this study became more refined as the sampling progressed, with the author taking the samples becoming increasingly more efficient at accurately identifying the individual vessels. Failure to obtain a paired arterial and venous sample in a foal was either due to the mare standing prematurely and rupturing the umbilical cord before the venous sample could be taken or due to the mare lying awkwardly against the wall as she foaled, preventing adequate access to the umbilical artery. Having
the mare’s hindquarters well away from the wall allowed better access to the 
umbilicus once the foal had been delivered, making the sampling procedure 
easier to perform. The author found it easy to obtain the required amount of 
blood from each vessel for analysis.

The importance of calm, professional foaling practices was emphasised during 
the sampling process. Experienced staff helped to keep the mare calm and 
minimally stressed after the foal was born, ensuring the mare did not stand 
prematurely, inadvertently breaking the umbilical cord. Whilst it is normal 
practice in human obstetrics for the umbilical cord to be clamped after birth, the 
authors decided against this approach prior to sampling for two reasons. Firstly, 
it was noticed that the application of clamps to the cord was more time 
consuming than just taking the samples directly, given the often-awkward 
positioning of the umbilical cord between the mare’s hind quarters/back legs and 
the foal itself. Secondly, as the amount of blood passed from the placenta to the 
foal through the umbilical cord in the minutes after birth may be as high as 30% 
of the total blood volume [26] the authors did not want to hinder this from 
occurring. Whilst the authors are not aware of any specific studies in the 
veterinary literature, the benefits of increased blood volume from placental 
transfer on neonatal health has been reported widely in for humans with the 
primary positive benefits related to increased total blood volume and reduced 
incidence of neonatal anaemia and iron deficiency [30,31]. Furthermore, delayed 
cord clamping in human neonates (performed approximately 2 minutes after 
birth) has not been shown to significantly change the blood gas findings when
compared with clamping at 10 seconds after birth [32]. This supports the sampling protocol described in the present study.

Complete clinical examinations performed by one of two authors (SSJ or NPG) over the first month for each of the foals in the present study revealed no adverse events or clinical consequences of the umbilical cord blood sampling for any of the foals. Clinical exams were within normal limits at all time-points, with all foals exhibiting normal immediate post-foaling behaviour including the ability to stand, nurse and pass meconium and urine. All foals had a serum IgG of >800mg/dl, with none of the foals developing umbilical haemorrhage in the immediate post-parturient period nor omphalophlebitis over the follow-up time-frame.

4: Conclusion

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

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sampling site along the umbilical artery significant? Gynecol Obstet Invest 2002;54:172–175.


Figure Legends

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

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

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

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

Figure 5: Close-up of the gross image of the fusion of the left and right umbilical veins (*). a = left umbilical vein; b = right umbilical vein.
Figure 6: Schematic of the umbilical cord vessels running between the placenta and foal. Arrows indicate the direction of blood flow.
Ethical Statement

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

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