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Determination of reference intervals for umbilical cord arterial and venous blood gas analysis of healthy Thoroughbred foals

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ABSTRACT

Although umbilical cord blood gas analysis is considered the best way to assess *in utero* oxygenation in human neonates, there is limited evaluation of this method in equine neonatology. Our objectives were to assess the practicality of obtaining umbilical cord blood gas samples in the field and to determine umbilical cord arterial and venous blood gas reference intervals (RI) for healthy, newborn foals. Thoroughbred foals >320 days gestation from healthy mares with uneventful pregnancies at one stud farm were evaluated. All parturitions were observed, with paired umbilical arterial and venous whole-blood samples obtained immediately following parturition for blood gas and lactate concentrations measured in duplicate. Apgar scores were assigned immediately and 10 min after birth, with all foals subsequently examined on days 1–28 to monitor for development of perinatal asphyxia syndrome. Foals were excluded from analysis based on abnormalities of stage 2 labour, Apgar scores and gross and histological placental assessment. Data was analysed using a Student's *t*-test, Pearson's correlation and the Robust method with $P \leq 0.05$ significant. Umbilical cord samples were simple to obtain with minimal disruption to the foaling environment. Of the $n = 34$ foals assessed, $n = 7$ were excluded based on premature placental separation deliveries. The mean time for stage 2 labour and blood gas analysis after parturition was 17.3 ± 5.1 min and 5.0 ± 2.3 min, respectively. RI were identified for umbilical arterial and venous pH (7.19–7.42 *vs.* 7.34–7.44), PO_2 (15.5–48.39 mmHg *vs.* 16.6–52.7 mmHg), PCO_2 (49.5–82.29 mmHg *vs.* 45.4–63.1 mmHg), SO_2 (9.19–76.89% *vs.* 39.9–84.88%), bicarbonate (27.3–38.7 mmol/l *vs.* 27.7–37.8 mmol/l), base excess (0.36–12.9 mmol/l *vs.* 1.97–13.1 mmol/l), TCO_2 (28.99–40.3 mmHg *vs.* 29.0–39.5 mmHg) and lactate (1.4–7.3 mmol/l *vs.* 1.3–4.9 mmol/l). Umbilical arterial 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. The initial Apgar score was positively correlated to umbilical arterial SO_2 ($r = 0.4$, $P = 0.05$) and negatively with umbilical arterial TCO_2 ($r = -0.6$, $P = 0.004$). Overall, umbilical cord sampling was simple and minimally disruptive, with RI obtained for blood gas measurements. RI for umbilical blood gas measurements from a larger population of healthy and unhealthy foals is required to evaluate the accuracy of this method for assessing *in utero* oxygenation.

1. Introduction

Arterial and venous blood gas analysis can be used as a diagnostic tool in veterinary medicine to help evaluate an animal's oxygenation, ventilation and acid-base status [1]. In human neonatology, umbilical cord blood gas analysis immediately at the time of birth is thought to provide the most sensitive representation of neonatal acid-base status prior to birth [2]. Hypoxic syndromes have been described in human neonates [3,4] with the combination of Apgar scoring [5,6] and umbilical cord blood gas analysis used by neonatologists to assess the likelihood that a hypoxic event occurred *in utero*, either during parturition or more chronically during the pregnancy [2,7,8]. These assessments help

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identify at-risk neonates, allowing for early medical intervention [9–11], reduction in morbidity and mortality rates as well as adverse clinical sequelae, in particular related to neurodevelopmental disease [12–14].

Similar to the hypoxic syndromes seen in human neonates, perinatal asphyxia syndrome (PAS) in neonatal foals is likely caused by hypoxic-ischemic damage that occurred during pregnancy or parturition [15]. Whilst originally thought to primarily affect just the central nervous system, it is now known to affect multiple other body systems [16]. The overall occurrence of PAS is cited to be between 1 and 2% of all foals born [17]. Although numerous risk factors have been identified for PAS, currently there are no confirmed pathognomonic biological or biochemical parameters that can be used to indicate the presence of this disease [18]. Researchers have described associations between decreased thyroid hormones [19], increased systemic creatinine and decreased systemic glucose concentrations [17,20] with risk of neonatal illness such as PAS. Ringger et al. (2011) also reported on increased concentrations of the biomarker UCHL1 in foals affected by PAS [21]. However, affected foals are more commonly not identified until after clinical signs develop, at which point significant systemic damage by inflammatory mediators may have already occurred [15].

Although the use of arterial and venous blood gas analysis in equine neonatology has been described in the literature [22–24], to-date, the use of umbilical cord blood gas analysis in equine neonates has only been reported by one group in which umbilical arterial blood gas parameters were evaluated in premature-induced and term-induced foals [25]. Therefore, the purpose of this study was a) to investigate the practicality of obtaining umbilical cord blood samples from newborn foals on a stud farm and b) determine umbilical cord arterial and venous blood gas reference intervals (RI) in a group of healthy, newborn Thoroughbred (Tb) foals.

2. Materials and methods

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

2.1. Sample population

During the 2017 breeding season, Thoroughbred broodmares with no history of systemic illness or placentitis during pregnancy and a gestational length >320 days were included in the study; exact gestational length was recorded for each mare once they foaled. All mares were from the same stud farm and managed under similar circumstances. The mares were closely monitored by on-site staff 24-hours a day, with at least 2 trained staff members attending each foaling. After foaling, data from foals of mares in the study group were retrospectively excluded from analysis if there was any abnormality of the second stage of parturition (e.g., premature placental separation [PPS]), if the Apgar score of the foal was <6 at each assessment, if the clinical appearance of the foal after parturition and/or the gross and histopathological appearance of the placenta were abnormal. Data from excluded foals are only reported for comparison.

2.2. Experimental protocol

All mares were observed from outside the stable as they went through stage 1 labour. The time of chorioallantoic membrane rupture was noted, with the mares then allowed to progress through the second stage of labour with minimal interference, although all mares had one staff member guiding the foal's head and one applying gentle traction on its legs until the foal was safely delivered. The duration of stage 2 of parturition was recorded for every animal, calculated from the time of chorioallantoic membrane rupture to the birth of the foal.

One author (SSJ) attended all parturitions and obtained all immediate post-partum clinical and umbilical cord blood samples. As soon as the foal was expelled, the umbilical arteries and vein were identified via visual inspection and manual palpation. For each blood gas sample measurement, 1 ml of blood was collected anaerobically into a heparinised disposable blood gas syringe (RAPIDLyte, Cruinn Medical Ltd, Dublin, Ireland). The umbilical cord was not clamped for sampling purposes, with the arterial sample always obtained first from the largest umbilical artery (as this was the easiest one from which to obtain a blood sample) using a 21 g needle, followed by venous blood collection using a 23 g needle. The timing of each sample collection in relation to foetal expulsion was recorded. Rectal temperature was taken from each foal to temperature-correct blood gas analysis.

Immediately following umbilical cord blood collection, a 4-parameter Apgar score was assigned to the foal using a modified system previously described by Vaala (1994) [26] (Table 1), with a second Apgar score assigned 10 min after the initial one; scores of ≥6 were considered to be within normal limits. Immediately after completion of the initial Apgar score, umbilical blood samples were evaluated in duplicate using a portable blood gas analyser (Vetscan i-Stat® 1, Abaxis UK Ltd, York, United Kingdom). Parameters measured included pH, PCO_2, PO_2, HCO_3, TCO_2, SO_2, base excess/deficit and lactate. The arterial sample was always evaluated first followed by the venous sample. The time from blood sampling to analysis was recorded.

For all mares, the time to passage of the foetal membranes was recorded, with the membranes immediately collected for analysis. The membranes were initially laid out on a designated table to visually inspect that the entire placenta had been passed, with the presence of any tears noted before being weighed on-site with commercial weighing scales. The foetal membranes were then submitted for full gross and histopathological evaluation. If parturition occurred during the weekend, the foetal membranes were stored in a cold room until the subsequent Monday. All gross and histopathological evaluations were performed by one author (UMF) blinded to any information about the parturition other than the date of foaling and weight of the foetal membranes. Approximately 2 cm^2 of pregnant horn/non-pregnant horn body pouch, cervical area, allantoamnion and amniotic cord were taken for histopathological examination. Any pathological findings were recorded as absent, mild, moderate or severe (Supplementary Tables 1–3). The foetal membranes were then classified into group 1 (absent or mild pathological findings) or group 2 (at least one section characterised by moderate-to-severe pathological findings). Data from foals with foetal membranes placed into group 2 were excluded from analysis.

Complete physical examinations were carried out on all foals at days 1, 2, 3, 7, 14, 21 and 28 by 1 of 2 authors (SSJ or NPG) to monitor for development of PAS. A blood sample was taken from all foals between 10 and 14 h after birth for measurement of serum IgG concentrations.

2.3. Data analysis

Calculations and analyses were performed using MedCalc statistical software (www.medcalc.org, MedCalc Software, v. 13.1.00, Mariakerke, Belgium) and SPSS (IBM SPSS Statistics for Windows, v. 24.0, Armonk, NY: IBM Corp). Measurements were evaluated for normality using a D'Agostino-Pearson test for normal distribution. Boxplots and histograms were assessed and any extreme outliers identified. The measurements were further assessed using Reed