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Comparison of three blood-sampling sites, for physiometabolic evaluation piglet

Comparación de tres sitios de muestreo sanguíneo para la evaluación fisiometabólica en el lechón

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ABSTRACT

Blood drawing of newborn piglets is necessary for physio-metabolic profiles assessment, and to determine gas change levels in it, what it is used in various phenomena diagnosis, such as, intrapartum asphyxia a non-infectious condition that causes a high rate of neonatal mortality in pigs. This study aimed to test three of the most common blood sampling sites used in piglets: umbilical cord (UC), retro-orbital sinus (ROS), and cava vein (VC), besides determining the differences among them, concerning to physio-metabolic variables and the acid-base balance. Thirty-two healthy non-asphyxiated piglets were randomly selected, coming from nine eutocic farrowing to carry out the three sampling sites. All of the physio-metabolic concentrations determined showed basal values within normal fetal ranges corresponding to the sampling site. However, UC values were more suitable for gas exchange diagnosis in asphyxia due to their similarity with physiological parameters of a normal newborn ($pCO2= 36.10 \pm 2.03$, $pO2= 32.68 \pm 3.03$), whereas for the metabolic profile (glucose and lactate) ROS values were more accurate for measuring a common lactate and glycaemia profile than the other sites. Therefore, when a physio-metabolic profile is required, the methodological approach of the blood-sampling site should be adequate to obtain the data needed for research following planned objectives and subsequent interpretation.

Keywords: newborn pig, blood gasses, umbilical cord, retro orbital sinus, vena cava.

RESUMEN

La extracción sanguínea en los lechones neonatos es necesaria para la evaluación fisiometabólica y para determinar alteraciones gaseosas útiles en el diagnóstico de varios fenómenos como la asfixia intraparto; siendo la causa principal no infecciosa que origina una alta tasa de mortalidad neonatal en cerdos. Nuestro objetivo fue evaluar tres de los sitios más comunes de muestreo sanguíneo usados en lechones: cordón umbilical (CU), seno retro orbital (ROS) y vena cava (VC); así como determinar las diferencias entre ellos con respecto a las variables fisiometabólicas y el equilibrio ácido-base. Treinta y dos lechones sanos provenientes de nueve partos eutócicos fueron aleatoriamente seleccionados para los tres sitios de muestreo. Todas las concentraciones fisiometabólicas mostraron valores basales de los parámetros fisiológicos fetales correspondientes al sitio de muestreo. Sin embargo, los valores de

UC resultaron más confiables para el diagnóstico del intercambio gaseoso durante la asfixia, debido a su similitud con los parámetros fisiológicos de un neonato sano (pCO_2= 36.10 ± 2.03, pO_2= 32.68 ± 3.03); mientras que para el perfil metabólico (glucosa y lactato) los valores de ROS fueron más precisos en determinar la glicemia y el lactato, comparados con los demás sitios. Por consiguiente, cuando el perfil fisiometabólico neonatal es requerido, el abordaje metodológico en el sitio de muestreo tiene que ser específico, de acuerdo a los objetivos planteados y a su subsecuente interpretación.
Palabras clave: lechones, gases sanguíneos, cordón umbilical, seno retroorbital y vena cava.

INTRODUCTION

In pig production, there is a high neonatal mortality rate, which has a direct impact on the economic sphere and on the welfare of the animals. In this regard, it is widely accepted that in a practical way there has not been a significant decrease in the number of stillbirths during the last thirty years, where it is estimated that the average mortality fluctuates between 16% and 20% (Baxter and Edwards, 2018). Of which 8% of deaths correspond to processes of fetal and neonatal asphyxia (Vanderhaeghe *et al*., 2013; van Dijk *et al*., 2005). Consequently, intrapartum asphyxia is considered as the main cause of non-infectious origin underlying the death of a considerable number of piglets within the first 72 hours postpartum (Baxter *et al*., 2011).

Some characteristics of piglets, such as the integrity of the umbilical cord, the order of expulsion during parturition and birth weight, can predispose to the appearance of intrapartum asphyxia (Sánchez-Salcedo *et al*., 2019[b]). These alterations can cause changes in neonatal pulmonary ventilation, which in turn causes a decrease in oxygen flow to the brain (Herrera-Marschitz *et al.,* 2014), leading to possible neurological damage and a deficient acquisition of passive immunity , with the subsequent inability to gain weight during the postnatal period (Orozco-Gregorio *et al.,* 2012). In addition to the above, the costs associated with pre-weaning mortality, from gestation to delivery, are close to 45.72 dollars (Seddon *et al*., 2013). Therefore, there is an urgent need to develop therapeutic strategies aimed at the prevention or treatment of this condition.

For this reason, for the correct diagnosis of respiratory disorders in piglets and for adequate therapeutic intervention, it is necessary to assess the physiometabolic profile and blood gas concentrations, as a reliable indicator of the prognosis of postpartum viability of neonates. However, the extraction of blood from a newborn piglet is difficult, due to the inherent conditions of birth (presence of fluids in the body, small size and rapid movements); so the site and the sampling time must be precise. In this regard, blood gas analysis can be performed through arterial or venous blood, the venous option being the safest and easiest procedure in animals, such as pigs. However, being a species political, a wide range of physiometabolic variables can be expected at the time of its birth in different members of the same litter (Orozco-Gregorio *et al.,* 2012), which makes an individualized diagnosis necessary.

Therefore, the objective of the present investigation was to evaluate three different blood-sampling sites. For the determination of the physiometabolic profiles in newborn piglets without evidence of asphyxia and their differences between the various sampling sites: umbilical cord (UC), retro orbital sinus (ROS) and vena cava (VC) in order to determine its suitability at the time of decision making for the diagnosis of intrapartum asphyxia.

MATERIAL AND METHODS

Study conditions. Healthy piglets without evidence of suffocation (n = 32), born from 9 multiparous sows with eutocic farrowing (3.6 ± 0.32 farrowing; weight: 200-280 kg) of the Yorkshire-Landrace hybrid breed were used to carry out this work; in order to determine the baseline differences between the three sampling sites. All the sows were housed at the Center for Teaching, Research and Extension in Swine Production of the National Autonomous University of Mexico (CEIEPP-UNAM), under normal maternity conditions (standard diet and ad libitum water in individual maternity cages with temperature controlled temperature of 23 ± 2 ºC).
The Internal Committee approved all procedures carried out with animals for the Care and Use of Animals of the Faculty of Veterinary Medicine and Zootechnics (CICUA-FMVZ-UNAM), with the protocol approval number 062. In addition, during the performance National guidelines for the ethical use of experimental animals were for all tests followed.

Experimental procedures. The sows were randomly selected for the sampling of their piglets (UC: n = 12 piglets, ROS: n = 10 piglets and VC: n = 10 piglets). All deliveries were induced 24 hours prior to the probable delivery date (day 115 of gestation) with 1 mL intramuscular cloprostenol (Bioestrophan, Laboratorios Syva, Querétaro, Mexico), according to the routine practices of the production unit. At birth, no piglets received medical attention or therapeutic interventions, in order not to interfere with the natural process of parturition.
Immediately after birth and in a time no longer than 10 seconds, the UC and VC blood samples were taken by puncture with heparinized syringes (INHEPAR 5000 IU/mL, PiSA Farmaceutica, México; needles 23 g x 1 "); while the samples corresponding to ROS were taken through borosilicate capillary tubes also heparinized (Orozco-Gregorio *et al*., 2008; (Sánchez-Salcedo *et al*., 2019[b]). Immediately after blood extraction, glucose concentrations (mg/dL), lactate (mg / dL), pH and partial pressures of carbon dioxide [pCO_2 (mm Hg)] and oxygen [pO_2 (mm Hg)] were quantified, using an automated clinical gasometer (Epocal Inc., Ottawa, Canada).

Statistical analysis. For the analysis of the results, the values were expressed as the mean ± SE for the blood concentrations of pCO_2, pO_2, glucose, lactate and pH in each of the three sampling sites. Data were analyzed using a one-way analysis of variance (ANOVA), followed by the Holm-Sidak test as post-hoc. For all variables the values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

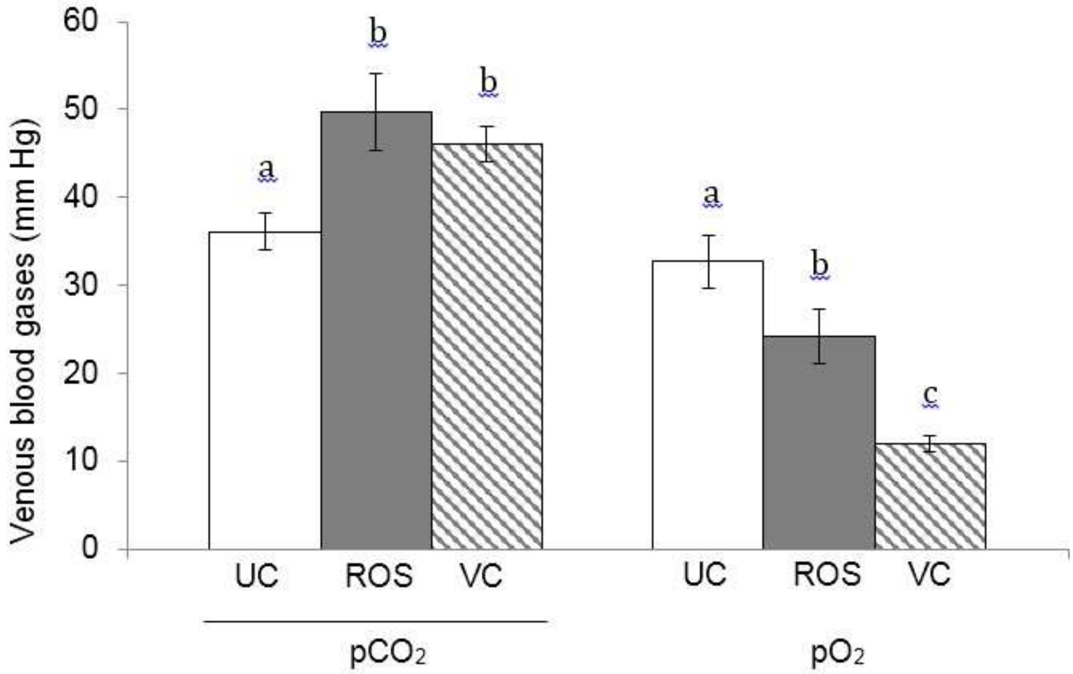

Figure 1. Basal values of gas exchange (mm Hg) at birth from three different sites in neonatal piglets without asphyxia. Different letters [a, b, c] within the same group indicate significant differences (P <0.05). Values are shown as means ± SE and Holm-Sidak test as *post-hoc*, (P <0.05)

All sows used for the physiometabolic evaluation of their piglets had eutocic farrowings, with approximate durations of 305.06 ± 50.5 minutes and an average of 11.80 ± 0.68 live piglets per litter (litters of 12.30 ± 0.59 piglets in total).
During the labor process, all fetuses experience periods of hypoxia of varying severity; which, if not interrupted by the onset of spontaneous respiration at the time of birth, can lead to neonatal apnea processes and lead to asphyxia (Sánchez-Salcedo *et al*., 2019[a]).

It is important to note that prior to delivery, the expected fetal pH is approximately 7.35, to later decrease in a physiological way to values close to 7.25; both values being considered normal. However, the pH values between 7.25 and 7.20 are taken into account as subnormal, so they require monitoring; while those under 7.20 indicate the imminent presence of fetal hypoxia (Sánchez-Salcedo *et al*., 2019[a]).

As could be evidenced in our results, only those pH values obtained from UC and VC were consistent with the profiles of non-asphyxiated piglets (table 1); while the samples taken from ROS showed average values suggestive of asphyxia (pH: 7.19 ± 0.06), without the piglets showing signs of the process. Therefore, this sampling site is not reliable for a diagnostic approach to this alteration, since we can infer that it could be even less, under conditions of asphyxia in newborns. Additionally, to diagnose a process such as asphyxia in which gas exchange is involved, the pH values will have to be directly related to oxygen and carbon dioxide.

Gas exchange is a fundamental aspect for the physiometabolic evaluation of neonatal piglets, since these variables allow us to assess the degree of asphyxia during parturition, at the same time as to predict survival in the postpartum period (Orozco-Gregorio *et al*., 2008). Regarding the gas exchange variables in the present work (Figure 1), the pCO_2 values were significantly higher in the samples obtained from ROS and VC (49.76 ± 4.35 and 46.06 ± 2.03, respectively), compared to those from UC (36.10 ± 2.03, P <0.05). This is possibly due to the fact that the neonatal acid-base state is better reflected in the umbilical arterial circulation; while the gaseous content from umbilical venous blood (as taken in this protocol), depends mainly on the maternal acid-base state and on placental functions; but not directly from the newborn (Yli y Kjellmer, 2016). Consequently, the values obtained for pCO_2 from the UC samples are not of diagnostic utility for the accepted criteria for an asphyctic neonate (hypercapnia and acidosis), since they do not agree with the pH of the same sampling site, which corresponds to a healthy profile (7.34 ± 0.04), and even with values above the mean. On the contrary, the values reported from ROS and VC for pCO2 can be associated with those described by Orozco-Gregorio *et al*., (2012) for healthy piglets not asphyxiated during parturition and 24 hours later (60.4 ± 18.7 and 42.1 ± 2.2 mm Hg, respectively).

In contrast, the pO_2 values of UC were approximately three times higher than those obtained from VC (32.68 ± 3.03 mm Hg *VS* 12.01 ± 0.89 mm Hg, *P* <0.001, respectively). In our study, the values of healthy piglets from CV were lower than those reported for neonates with asphyxia (21.7 ± 10.5 mm Hg) by Orozco-Gregorio *et al*., (2012), without an asphyctic profile in its entirety (values pH and pCO_2 within normal ranges); which confirms that these animals were not asphyxiated neonates; however, its pO_2 was the lowest. On the contrary, ROS sampling showed values 50% higher than VC for the same variable (pO_2: 24.12 ± 3.09 mm Hg VS 12.01 ± 0.89 mm Hg, P <0.05), but lower than UC (32.68 ± 3.03 mm Hg, P <0.05). The oxygen in ROS, in the same way as in VC, exhibited concentrations suggestive of intrapartum asphyxia (<26.4 ± 17.7 mm Hg), which can generate confusion, and therefore lead to an erroneous diagnosis, since our data in set did not match the diagnostic inclusion criteria of a piglet with suffocation. However, it is widely accepted that childbirth increases the risk of compromised oxygenation by the fetus, which can lead to interruptions in gas exchange, where certain degrees of hypoxemia and acidemia are normal; even in healthy fetuses during a eutocic delivery (Yli y Kjellmer, 2016).

In our findings for pO_2, the differences between the three sampling sites correspond more to a physiological condition than a pathological one. In UC blood, the highest concentration of oxygen delivered to the fetus is at a pressure of only 30-35 mm Hg. Subsequently, 50% of the venous blood from UC passes through the hepatic circulation and mixes with poorly oxygenated blood, to pass from the anterior vena cava to the pulmonary circulation; therefore, the sample obtained from VC (12-24 mm Hg) will normally be less oxygenated (Hall, 2016). Conversely, the upper part of the body in fetuses (ROS) is exclusively with blood from the left ventricle supplied, which has a slightly higher pO_2 than the blood perfused towards the lower part of the fetus (Hall, 2016).

When suboptimal oxygen concentrations are present in any tissue, there is a loss of oxidative phosphorylation capacity and a resulting transition from an aerobic to an anaerobic metabolic state. Under anaerobic conditions, pyruvate is reduced to lactate, leading to inefficient energy transfer in the neonate (Yli and Kjellmer, 2016).

Our lactate concentrations obtained in ROS were significantly higher (60.66 ± 8.51 mg/dL), than those of UC and VC (41.36 ± 5.08 mg/dL and 35.21 ± 7.51 mg/dL, P <0.01, respectively). However, our ROS values are consistent with those reported by Orozco-Gregorio *et al*., (2008) for healthy neonates (65.3 ± 15.5 mg/dL), which were also collected from ROS. In this regard, it is known that lactate can also function as a brain energy substrate in a healthy fetus under transient hypoxic conditions inherent to a typical labor, consuming even more substrate than oxidative metabolism (Boardman and Hawdon, 2015).

Table 1. Blood variables at delivery taken from three different sampling sites in neonatal piglets without evidence of asphyxia

Variable	UC	ROS	VC
pH	7.34 ± 0.04 a	7.19 ± 0.06 a	7.27 ± 0.01 a
Glucose (mg/dL)	38.91 ± 2.63 a	43.60 ± 5.37 a	38.20 ± 3.89 a
Lactate (mg/dL)	41.36 ± 5.08 a	60.66 ± 8.51 b	35.21 ± 7.51 a

Mean ± SE. UC = umbilical cord ROS = retro orbital sinus VC = vena cava. [a, b] Different letters in the same row indicate significant differences between the sampling site (P <0.001)

Glucose is considered the main source of energy for newborn piglets, because these animals are born with limited energy storage; in such a way that, if glucose supplementation and glucose requirements are balanced, fetuses will obtain adequate oxygenation to metabolize glucose aerobically and transfer the energy required for organ functions (Yli and Kjellmer, 2016). However, the blood glucose concentrations reported by different studies are not comparable with our findings, since they are considerably higher [UC: 52.25 ± 0.12 mg/dL (Rootwelt *et al*., 2014); ROS: 89.3 ± 11.4 mg/dL (Orozco-Gregorio *et al*., 2008); CV: 62.3 ± 8.9 mg/dL (Trujillo-Ortega *et al*., 2007)], in comparison with our results (table 1). In this regard, it is necessary to take into account that our blood samples were obtained immediately after birth, with the piglets still apnea (<10 seconds postpartum).

It is widely accepted that a significant drop in glucose during labor demonstrates how quickly the stores of this energy substrate are depleted. At this point, the fetus (and later the neonate) needs to maintain normoglycemia through anaerobic glycolysis, due to its hypoxic state (Martz *et al.,* 2017); therefore, the immature neonatal brain, compared to a mature adult brain, is relatively more resistant to injuries caused by hypoglycemia (glucose <40 mg/dL). All this coping with the alteration by decreasing brain energy requirements, increasing the blood flow at the brain level and the mobilization of glucose; consequently improving the ability to use lactate as an alternative energy source (Basu *et al.,* 2009).

The results of this report indicate that among the three blood sampling sites for the physiometabolic evaluation of neonatal piglets, the main difference lies in the dynamics of blood gases, due to a normal physiological process. Another considerable aspect is the methodological approach used, where taking blood samples from UC turns out to be the only non-invasive procedure in newborn pigs, with the limitation of being able to obtain mixed blood (arterial and venous), compared to ROS and VC. Both can confirm a venous sample, but not the welfare of the animals, if untrained personnel perform the sampling. In the same way, both ROS and VC can provide significantly elevated and decreased values respectively in case of requiring a metabolic diagnosis of the neonate, based on glucose and lactate, so their use should be determined according to the needs of the research, since they would not be fully functional in assessing the respiratory capacities of newborns. However, and regardless of the sampling site, the correct diagnosis of intrapartum asphyxia necessarily requires compliance with the criteria of hypercapnia, acidosis and hypoxia, to be considered as a useful tool in production.

CONCLUSION

In order to determine the ideal blood-sampling site for the diagnosis of intrapartum asphyxia, it can be concluded that the umbilical cord values were more reliable for the diagnosis of gas exchange during asphyxia. All this, it is due to their similarity with the physiological parameters of a healthy neonate (pCO_2 = 36.10 ± 2.03, pO2 = 32.68 ± 3.03); while for the metabolic profile, the retro-orbital sinus values were more accurate in determining glycemia and lactate.

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