Ultrasonographic measurement of the adrenal gland in neonatal foals: reliability of the technique and assessment of variation in healthy foals during the first five days of life

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Adrenal glands ultrasonographic measurements in neonatal foals: reliability of the technique and assessment of variation in healthy foals during the first 5 days of life.

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Abstract

Background: Adrenal gland ultrasonographic measurements are useful in clinical evaluation of patients with adrenal dysfunction in several species. In human healthy neonates, ultrasonographic size of the adrenal glands decreases during the first days of life. Ultrasonography of adrenal glands was demonstrated to be feasible in neonatal foals.

The aims of this study were to describe a technique for ultrasonographic measurement of adrenal gland size , to test its reliability in neonatal foals, to assess any variation of ultrasonographic measurements during the first 5 days of life in healthy foals.

Methods: First, measurements of the adrenal glands were retrospectively obtained by three observers in 26 adrenal gland images of 13 healthy and sick neonatal foals. The inter- and intra-observer agreement were tested. Later, adrenal gland ultrasonographic images and measurements were acquired by one operator in 11 healthy neonatal foals at 1, 3 and 5 days of life and differences among the measurements obtained at the different time points were assessed.

Results: Inter-observer agreement ranged from fair to excellent (0.48–0.92), except for cortex width (<0.4); intra-observer agreement ranged from good to excellent (0.52-0.98). No significant differences were found among the measurements obtained at 1, 3 and 5 days of life.

Conclusion: Adrenal glands ultrasonographic measurements can be obtained consistently in equine neonates and, in contrast to humans, they do not vary during the first 5 days of life in healthy foals.

Introduction

Adrenal glands and hypothalamic-pituitary (HP) axis are involved in many vital processes in healthy neonate and adult horses. Hypothalamic-pituitary-adrenal (HPA) axis is involved in the homeostasis of metabolism, cardiovascular function, growth and immune response and it plays a central role in the organism response to stress and illness[1-3]. Furthermore, emerging evidence in equine medicine suggests that adrenocortical dysfunction, resulting in cortisol insufficiency, frequently develops during critical illness in neonatal foals contributing substantially to morbidity and mortality associated with the primary disease. These findings strongly encourage the evaluation of the adrenal gland function in neonatal equine patients[4].

Adrenal glands had been considered not detectable by ultrasound examination in neonatal foals for a long time[5,6]. Hoffman et alobtained and described adrenal gland ultrasonographic images in neonatal foals[7]for the first time. Recently, Beccati et al, described a simple and non-invasive ultrasonographic technique to image both adrenal glands in a transverse-oblique plan[8]. The structures and the shape of the adrenal glands were assessed and described, although only subjectively[8]. As far as we know, no data are reported on the objective or quantitative evaluation of the ultrasonographic size of the adrenal glands in neonatal foals.

Ultrasonographic examination has become the method of choice for evaluation of the appearance and size of the adrenal glands in men, dogs and cats; it is considered pivotal in clinical practice in the diagnosis of hyperadrenocorticism in dogs and in the diagnosis and treatment of congenital adrenal disorders in humans[9-13]. In human neonates, the normal variation in ultrasonographic measurements of the adrenal glands has been assessed, demonstrating a physiological decrease in ultrasonographic adrenal gland size in neonates throughout the first days of life[14].

The aims of this cohort study were: i) to describe an ultrasonographic measurement technique for adrenal glands in neonatal foals; ii) to test the reliability of the technique; iii) to assess any variation of the ultrasonographic size of the adrenal glands in the first 5 days of life in healthy neonatal foals.

Materials and Methods

Measurement technique and assessment of measurement reliability

A total of 26 two-dimensional (2D) ultrasonographic images of the left and right adrenal glands of 13 neonatal foals (5 healthy, 8 sick; 8 colts and 5 fillies, aging from 12 hours to 7 days) of various breeds were chosen from a pool of ultrasonographic images previously obtained during the foaling seasons 2014-2016 using the technique described by Beccati et al[8].

All the images had been obtained by two different operators (F.B; E.L) with two different ultrasonographic machines (Logic C5, GE Healthcare, Milan, Italy; M-Turbo, FUJIFILM Sonosite, Bothell, USA), using a convex electronic transducer (4–5.5 MHz). The less expert operator (E.L) had obtained the images after a training period of 5 adrenal glands ultrasonographic examination under the guidance of the more expert one (F.B.). The depth setting had been fixed at 11 cm and maintained for all the foals; other parameters such as focus position and gains were adjusted individually for image optimization. The ultrasound examinations had been performed with the foals in the standing or sternal position and the haircoat over the region of the kidney was not clipped. Aorta and the left kidney and caudal vena cava and the right kidney had been used as landmarks for the correct identification of the left and right adrenal gland, respectively. Both images and videos had been obtained and stored in DICOM format. All the acquired images and frames from the video were reviewed by a single observer who selected one image for each adrenal gland of each foal. The

selection was based on the ability of the image or frame to allow the entire visualisation of the short and long axis of the adrenal gland

To assess the inter-observer agreement, the ultrasonographic images were than reviewed off-line, retrospectively, in a blinded fashion andindependently by three different observers To assess the intra-observer agreement, one observer repeated all the measurements twice at least one week apart.

Each observer measured (in cm) the following dimensions using the electronic calliper of a DICOM viewer:

- height and width of the left (Figure.1A) and the right (Figure.1B) whole adrenal gland;
- width of the cortex and medulla of the left (Figure. 2A) and the right (Figure. 2B) adrenal gland;
- height of the cortex and medulla of the left (Figure.3A) and the right (Figure. 3B) adrenal gland.

The height was defined as the maximum dorsoventral diameter and the measurement was made along the long axis of the gland; the width was defined as the maximum lateromedial diameter and the measurement was made perpendicular to the long axis of the gland. The width of the right adrenal gland was obtained in two sites, dorsal and ventral, because of its "peanut" shape (Figure 1B; Figure 2B; Figure 3B).

Each observer was allowed to record additional free comments to the images.

Assessment of variation in adrenal gland ultrasonographic size in the first 5 days of life

Definition criteria for foals

Eleven healthy neonatal foals (2 Warmbloods and 9 Standardbreds; 2 colts and 9 fillies) were included. The foals were privately owned, and were born from mares referred for attended delivery to the Equine Perinatology Unit of the University of Bologna, during the 2017–2018 foaling seasons.

All procedures on the animals were carried out with the approval of the Ethical Committee of the University of Bologna. The owners gave oral informed consent for their foals' inclusion in the study. The foals were included in the study when all the following criteria were met: at term birth (>320 days) and eutocic delivery, APGAR score ≥ 9 at birth[15], normal clinical examination, haematology (white blood cell count and leukogram, red blood cell and platelet counts, packed cell volume, haemoglobin concentration) and biochemistry (total proteins, albumin, total and direct bilirubin, glucose, urea, creatinine, alkaline phosphatase, lactate dehydrogenase, creatine kinase, gamma glutamyl transferase, aspartate aminotransferase, electrolyte concentrations and fibrinogen) within the normal range, and serum immunoglobulin G (IgG) concentrations >800 mg/dl at 18–24 hours of life.

Ultrasonographic protocol

Ultrasonographic examinations were performed after informed consent from horse owners. Two-D ultrasonographic images of the right and left adrenal glands were obtained at three different time-points: 1 (T1), 3 (T3) and 5 (T5) days of life. A single operator (F.F.) acquired all the images and videos using a convex transducer (1-8 MHz), (MyLabAlpha, ESAOTE, Genova, Italy). The technique described by Beccati et al[8] was employed after a training period of five adrenal gland ultrasound examinations under the guidance of an expert operator. The depth setting had been fixed at 11 cm and maintained for all the foals; other parameters such as focus position and gains were adjusted individually for image optimization.

The ultrasound examinations were performed with the foals in the standing or sternal position and the haircoat over the region of the kidney was not clipped. Aorta and the left kidney and caudal vena cava and the right kidney were used as landmarks for the correct identification of the left and right adrenal gland, respectively. All the images and frames of the videos were stored in DICOM format.

From the acquired images only the image allowing the best visualisation of the adrenal gland was chosen the left and right adrenal gland of each foal at each time point.

Adrenal gland measurements were obtained off-line, retrospectively, in a blinded fashion by a single observer (E.L.) that, analysed the images in a random order at the end of the season.

The following measurements were obtained as previously described: height of the left and the right whole adrenal gland, width of the left and the right whole adrenal gland, and height and width of the left and the right adrenal medulla.

Statistical analysis

All measurements were tabulated in a Microsoft Excel worksheet and transferred to R Studio (version 3.4.3) and/or JASP (version 0.8.6.0) statistical software for analyses.

Inter- and intra-observer agreement for each measurement were assessed by calculating the intraclass correlation coefficient (ICC); a value of ICC less than 0.40 was considered poor, 0.40 to 0.59 fair, 0.60 to 0.74 good and 0.75 to 1.00 excellent[16].

The homoscedasticity of the variables was tested using the Shapiro–Wilk test (normal distribution) and Levene's test (homogenicity of the variance) and subsequent tests chosen as appropriate. Differences in ultrasonographic measurements of the adrenal glands at the different time points (T1, T3, T5) were tested using a one-way ANOVA for repeated measurements.

The mean value and the standard deviation were calculated for the measurements of the adrenal glands obtained at 1, 3 and 5 days of life.

Significance was set at $p<0.05$.

Results

Measurement technique and assessment of measurement reliability

All the observers judged the dorsoventral diameter of the adrenal cortex not easy to assess, especially in the left adrenal gland, because of a blurred demarcation between the cortex and the medulla. For this reason, the inter- and intra-observer reliability for the height of the cortex and medulla, were not calculated.

The overall inter-observer ICC ranged from poor (10%) to excellent (85%) depending on the measurements evaluated; specifically, the inter-observer ICC was poor for the left cortex width, right dorsal cortex width and right ventral cortex width, fair for the whole right adrenal gland height, good for the whole left adrenal gland height an left medulla width, excellent for all the other measurements considered. The overall intra-observer ICC varied from fair to excellent depending on the measurements evaluated; specifically, the ICC was fair for the right dorsal cortex width, good for the whole right ventral lobe and excellent for all the other measurements. Details of the ICC values are presented in Table 1.

Assessment of variation in adrenal gland ultrasonographic size in the first 5 days of life

The measurements of the adrenal glands in healthy foals did not change in the first 5 days of life ($p > 0.05$). Measurements obtained at each time-point are presented in Table 2.

Discussion

The results of this study demonstrate that objective ultrasonographic measurements of the adrenal glands in neonatal foals can be consistently obtained as previously described in dogs, cats and men[10,14,17]. However, in contrast to dogs, cats and men, where measurements are usually obtained in both the transverse and longitudinal plane, in this study the adrenal glands were visualized and measured using an ultrasonographic transverse-oblique plane that has been recently suggested as the

easiest approach for adrenal gland visualisation in foals since it offers clearer landmarks for gland identification and it avoids the acoustic shadowing due to the ribs[8,10,14,17]. In the transverse-oblique plane, it is not possible to assess the thickness and the length of the glands. For this reason, the measurement of the long axis corresponded to the height of the gland, and the measurement of the short axis had been defined as the width of the gland, in line with previous reports for kidney measurements in the transverse-oblique plane in the horse[7].

For both adrenal glands, the width of the whole gland, medulla and cortex, and the height of the whole gland were successfully obtained. The height of the cortex and the medulla was not assessed because of the absence of a clear-cut corticomedullary demarcation in the long axis of the left gland due to a blurred image appearance. Image blurring is often unavoidable in ultrasound images and, as reported in previous studies, it could be a consequence of the poor lateral resolution secondary to an excessive divergence of the ultrasonographic beam. In this study the image blurring was noticed especially in the left adrenal gland, possibly because of the greater distance between the left adrenal gland and the transducer in comparison to the right gland[18-20].

The values of the ICC demonstrate that the technique can be considered reliable for all measurements considered in the study except for the cortex measurements, and that the technique should be able to be easily applied by other clinicians with a short training. The poor agreement obtained for the cortex is likely to be due to its small size, which increases the error in positioning the electronic calliper used for the measurements. With this consideration, we excluded the cortex from subsequent measurements. However, in the authors' opinion, the lack of cortex measurement does not affect the utility of the assessment of the adrenal gland size since the whole adrenal gland measurements have been demonstrated to be useful markers of the gland and cortex function in other species[9,17]. The fair agreement obtained for the height of the whole right adrenal gland, in contrast with the good to

excellent agreement obtained for the other measurements, can be explained by the fact that movements of the lung could make it difficult to obtain high-quality images of the right gland[8].

In contrast to reports in newborn children, in which a rapid decrease in the size of the adrenal glands was demonstrated between day 1 and day 5 of life, in this study no changes in adrenal size were found in neonatal foals[14]. These differences can reflect the different histological development of the adrenal glands in horses and men. In fact, the human fetal adrenal gland is characterized by the presence of a fetal zone, which is responsible for DHEA synthesis and androgen production in general, throughout the gestation period[21,22]. The fetal zone occupies about 80% of the gland in newborn children and it is demonstrated to involute rapidly in the first few weeks of life[14]. The fetal zone characterizes the fetal adrenal glands of men and other primates, it has been described in sheep and cats, but is not present in the horse. In horses, steroidogenic enzymes are all expressed in the cortical cells and their expression increases significantly just before or after birth, corresponding to the increase in plasma cortisol that occurs very close to birth[22,23].

In this study, the cross-section of the aorta and the caudal vena cava represented two important markers in the identification of the left and right adrenal gland, respectively. Accordingly with the previous study by Beccati et al[8], left adrenal gland was always identified ventrally to the aorta and medially to kidney, while the right adrenal gland was identified located between the right kidney and the caudal vena cava, confirming that the aorta and caudal vena cava represent two important ultrasonographic markers in the identification of the left and right adrenal gland, respectively, in the transversal-oblique plane.

Two main limitations are present: first of all, the repeatability of the image acquisition has not been tested; the operators, the variability intrinsic to image collection and the employ of two different ultrasonographic machines could have potentially affected the results. However, the operators involved in the study underwent the same training and, furthermore, videos, in addition to images,

were obtained; videos were subsequently reviewed by one single observer with the purpose of selecting images allowing the entire visualisation of the short and long axis of the gland. In light of this, in authors' opinion, the variability linked to images collection and its effect on final results are limited. The second limitation is represented by the fact that no comparison was made between ultrasonographic size of the adrenal glands and morphometric measurements on gross specimen. The technique and the peculiar anatomical localisation of the adrenal glands in the body could affect the correspondence between ultrasonographic and gross anatomical size of the adrenal glands. Small changes in dimensions could have been missed and, although not detected by the ultrasonographic examination, changes in gross dimensions of the adrenal glands in the first 5 days of life in healthy foals cannot be excluded. The assessment of variation in gross anatomical size of the adrenal glands, was, however, beyond the purpose of this study.

Conclusions

This study demonstrates that 2Dultrasonographic measurements of the adrenal glands in neonatal foals can be reliably obtained and that the ultrasonographic size of both the adrenal glands does not vary significantly during the first 5 days of life in healthy neonatal foals. This finding suggests that the same measurement ranges could be used as reference for healthy neonatal foals indistinctly from birth to the 5th day of life. In line to what previously reported in other species, further studies are recommended to investigate the correlation between measurements and sex, age and weight of the foals and to further investigate the applicability of adrenal glands ultrasonographic measurements in the clinical evaluation of foals.

Conflict of Interest Statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Conflicts of interest statement:

No competing interests have been declared.

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Tables

Table 1. Intra- and inter-observer reliability for ultrasonographic measurements obtained, retrospectively, in 13 healthy and sick neonatal foals.

Measurement	Intraobserver ICC (95% CIs)	Interobserver ICC (95% CIs)
Whole left adrenal gland height	0.91 (0.72–0.97)	0.60 (0.27–0.84)
Whole left adrenal gland width	0.91 (0.72–0.97)	0.81 (0.59–0.93)
Left cortex width	0.89 (0.66–0.97)	0.27 (-0.067–0.65)
Left medulla width	0.98 (0.93–0.99)	0.63 (0.31–0.86)
Whole right adrenal gland height	0.93 (0.79–0.98)	0.48 (0.14–0.77)
Whole right dorsal lobe width	0.97 (0.90–0.99)	0.76 (0.52–0.91)
Right dorsal cortex width	0.52 (-0.02–0.82)	0.17 (-0.13–0.56)
Right dorsal medulla width	0.86 (0.60–0.96)	0.82 (0.62–0.93)
Whole right ventral lobe width	0.73 (0.32–0.91)	0.85 (0.68–0.94)
Right ventral cortex width	0.86 (0.60–0.96)	0.22 (-0.09–0.60)
Right ventral medulla width	0.84 (0.56–0.95)	0.84 (0.65–0.94)

ICC, intraclass correlation coefficient; CI, confidence intervals.

Table 2. Serial ultrasound measurements obtained in healthy neonatal foals (n = 11) at T1, T3 and T5. Data are presented as mean value and standard deviation.

Measurement (cm)	T1 (mean ± SD)	T3 (mean ± SD)	T5 (mean ± SD)	P-value
Whole left adrenal gland height	2.5 ± 0.48	2.6 ± 0.58	2.86 ± 0.52	0.2
Whole left adrenal gland width	0.69 ± 0.21	0.63 ± 0.25	0.73 ± 0.19	0.2
Left medulla width	0.52 ± 0.17	0.40 ± 0.18	0.49 ± 0.13	0.1
Whole right adrenal gland height	2.78 ± 0.35	3.08 ± 0.4	3.28 ± 0.58	0.6
Whole right dorsal lobe width	0.77 ± 0.26	0.75 ± 0.09	0.74 ± 0.15	0.2
Right dorsal medulla width	0.55 ± 0.19	0.53 ± 0.15	0.49 ± 0.13	0.1
Whole right ventral lobe width	0.79 ± 0.24	0.79 ± 0.14	0.83 ± 0.23	0.3
Right ventral medulla width	0.67 ± 0.26	0.54 ± 0.14	0.56 ± 0.21	0.1

* significative difference in row.

SD = standard deviation

Figure legends

Figure 1. Ultrasound image obtained in a transverse-oblique plane in a standing neonatal foal. D = dorsal; M = medial. The figure illustrates the measurement of the width (white solid line) and the height (white dashed line) of the left (Figure 1A) and the right (Figure 1B) whole adrenal gland.

Figure 2. Ultrasound image of the adrenal gland obtained in a transverse-oblique plane in a standing neonatal foal. D = dorsal; M = medial. The figure illustrates the measurement of the width of the cortex (white asterisks) and the medulla (white solid line) of the left (Figure 2A) and the right (Figure 2B) adrenal gland.

Figure 3. Ultrasound image obtained in a transverse-oblique plane in a standing neonatal foal. D = dorsal; M = medial. The figure illustrates the measurement of the height of the cortex (white asterisks) and the medulla (white dashed line) of the left (Figure 3A) and the right (Figure 3B) adrenal gland.