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Salivary cortisol, heart rate and heart rate variability in healthy and diseased neonatal foals

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Summary: In this study, parameters indicative of adrenocortical and sympathoadrenal function were determined in foals (n = 43). Foals were assigned to the following groups: Healthy home (n = 10, born and kept at their home stud), Healthy clinic (n = 11, born to mares hospitalized for surveillance of foaling), Colostrum (n = 4, received colostrum by bottle or nasogastric tube, no signs of disease), Failure of passive transfer (n = 8, received hyperimmune serum, no signs of disease), Sepsis (n = 5) and Prematurity (n = 5). Saliva for cortisol analysis was collected four times daily and heart rate for analysis of heart rate variability (HRV) was recorded once daily during the first 5 days of life. On day 1, cortisol concentration was elevated ($p < 0.001$), reflecting the demands of neonatal adaptation, but did not differ among groups. On days 2–5, cortisol concentration in premature foals, was higher ($p < 0.05$) than in all other groups, indicating adrenal maturation. Cortisol concentration did not differ between foals that required frequent examination and treatment and healthy foals. This indicates that handling was not perceived as stressful. In none of the foal groups, salivary cortisol concentration was reduced compared to healthy foals. Heart rate in septic foals was elevated throughout the observation period ($p < 0.05$) but this was not associated with a poor outcome. Heart rate was not influenced by handling of the foals. The HRV did not differ among groups and sepsis was not associated with decreased HRV, at least if treatment is initiated early. In conclusion, adrenocortical and sympathoadrenal function of foals was neither consistently activated nor depressed by neonatal sepsis. Increased cortisol release in premature foals in the first days of life may reflect maturational processes that occur already shortly before birth in term foals.

Keywords: foal, salivary cortisol, heart rate variability, sepsis, premature

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Introduction

Birth and the demands of immediate adaption to the extrauterine environment thereafter have been postulated to be amongst the most stressful events of life. Stress responses include sympathoadrenal activity and activation of the hypothalamo-pituitary-adrenocortical axis with increased cortisol release (*Nagel* et al. 2015). The sympathoadrenal system stimulates cardiac function, pulmonary surfactant release, absorption of fluid from the lungs, energy mobilisation and thermogenesis (*Irestedt* et al. 1982, *Downing* and *Lee* 1983, *Padbury* et al. 1987). In foals, sympathetic and adrenocortical activity is not stimulated during the short expulsive phase of labour, but immediately after birth. This stress-like response once the foal is born has been suggested as a prerequisite for successful and rapid adaptation to the demands of extrauterine life (*Nagel* et al. 2015).

Despite major advances in equine perinatology in the 1980ies and 90ies, diseases in the neonatal period are still a considerable threat for survival and well-being of foals after birth with neonatal sepsis as the most frequent problem (e.g. *Koterba* and *Brewer* 1984, *LeBlanc* et al. 1992, *Sanchez* 2005). Only few studies have analysed physiological stress parameters in diseased neonatal foal. In septic foals between 4 hours and 7 days after birth, but not in foals younger than 4 hours, total and non-protein bound (free) cortisol were higher than in healthy controls (*Hart* et al. 2011). In another study, cortisol concentration in diseased foals was higher than in healthy foals and particularly elevated in premature neonates on days 2 and 3 of life (*Panzani* et al. 2009).

Hypothalamic-pituitary-adrenal (HPA) axis dysfunction is common in critically ill people and animals, particularly those with severe trauma or sepsis. This syndrome is often termed relative adrenal insufficiency (RAI) and can be defined as an inadequate cortisol response to an illness-related stress. The occurrence of RAI during sepsis is associated with an increased incidence of shock, multiple organ dysfunction and death in foals (reviewed by *Hart* et al. 2011). Adrenocortical and sympathoadrenal activity might therefore be correlated to survival of critically ill foals. Adrenocortical activity can be assessed by cortisol analysis in blood plasma but also in saliva (*Peeters* et al. 2011). Because only non-protein bound cortisol diffuses into saliva, salivary cortisol represents the free plasma cortisol fraction (*Kirschbaum* 2000).

Heart rate is regulated by complex interactions between the autonomous nervous system and cardiac pacemaker cells. This fine tuning of the cardiac beat-to-beat interval is known as heart rate variability (HRV) and increases during dominance of the parasympathetic branch of the autonomous nervous system. In response to stressful challenges, sympathetic influence increases and HRV decreases (*von Borell* et al. 2007). In human medicine, an increase in heart rate and a decrease in the HRV variable SDRR (standard deviation of

the beat-to-beat interval) is a useful parameter for early detection of neonatal sepsis (*Bohanon* et al. 2015). It has been suggested that systemic inflammation leads to decreasing HRV because of a partial uncoupling of cardiac pacemaker cells from autonomous nervous control (*Gholami* et al. 2012).

In conjunction with increased cortisol release, decreased HRV indicates stress responses in horses (*Schmidt* et al. 2010a, and b, *Erber* et al. 2012). In this study, we have determined physiological stress parameters indicative of adrenocortical and sympathoadrenal function in healthy foals and in neonates with different problems. We hypothesised that adrenocortical and sympathoadrenal function in foals is affected by endogenous and exogenous stressors such as disease, environment or handling.

Material and methods

Animals

A total of 43 foals (fillies n = 28, colts n = 15) were included into this study. Foals were from the following breeds: Warmblood (n = 25), Thoroughbred (n = 8), Quarter Horse (n = 3), Arab (n = 3), Icelandic Horse (n = 2), Paint horse (n = 1) and American Saddlebred (n = 1). Foals were assigned to different groups by history and clinical characteristics (place of birth, physical health, colostrum intake, plasma IgG values, hemogram, acute phase proteins, x-ray diagnostics; see Table 1). Foals of group Healthy home were born at their home stable at the Brandenburg State Stud at Neustadt (Dosse), Germany, and remained at the stud during the observation period. All foals of group Healthy clinic were born to mares with physiological gestations hospitalized for surveillance of foaling at the Animal Hospital of Vetmeduni Vienna. All other foals were either born at the clinic or admitted to the hospital within the first day of life (for details see Table 1). All but two premature foals euthanized on day 1 and 2 of life, respectively, were discharged healthy from the clinic.

Treatment

Foals at the Brandenburg State Stud were manipulated only for study purposes and thus had only little contact to humans in the first days of life. Foals at Vetmeduni Vienna were submitted to routine clinical examination twice daily. In all foals, plasma IgG concentration was determined either at arrival in the clinic or at 18 to 24 hours of life. At the Brandenburg State Stud, plasma IgG concentration was measured with a semiquantitative test (SnapFoal, IDEXX, Ludwigsburg, Germany). In foals born or admitted to Vetmeduni Vienna, plasma IgG concentration was determined by densimeter (Animal Reproductive Systems, Chino, California, USA). Foals of group Colostrum received good quality colostrum within the first 4 hours of life by bottle feeding or nasogastric tube but did not get any other treatment. Failure of passive transfer (FPT) foals were treated with 1 or 2 L of hyperimmune serum (Hypermune, Veterinary Immunogenics, Carleton Hill, Penrith, Cumbria, England) and got standard treatment (see below) if they were vulnerable to developing sepsis by their history (e.g. time of arrival in the clinic). All foals of groups Sepsis and Premature were treated with 1 or 2 L hyperimmune serum and standard treatment. Standard treatment included penicillin (20000–40000 IU/kg i.v. 4 times daily for 5–7 days; Penicillin-G-Natrium, Sandoz, Kundl, Austria) in combination with gentamicin (6.6 mg/kg i.v. once daily for 5–7 days; Gentavan, Vana, Vienna, Austria) and antiphlogistic treatment with flunixin-meglumine (1.1 mg/kg i.v. twice daily for 2–5 days, Finadyne, Essex, Munich, Germany). In addition, omeprazol was given for stomach protection (2–4 mg/kg once daily orally; Ratiopharm, Vienna, Austria). Depending on the severity of illness additional treatments (e.g. fluid therapy) were performed if needed.

Experimental design

For evaluation of stress parameters in foals, saliva for cortisol analysis was collected four times daily and heart rate for analysis of heart rate variability was recorded once daily during the first 5 days of life.

Table 1 Classification and treatment of foals in different groups | *Klassifizierung und Behandlungen von Fohlen der verschiedenen Gruppen*

Group (n)	Classification	Treatment
Healthy home (n=10)	born at home stable, healthy throughout study period, IgG at 24 h of life >800 mg/dl; suckled the mares udder within 2 h of life, mare with good quality colostrum (Brix >25%)	no antibiotic treatment
Healthy clinic (n=11)	born at the clinic, healthy throughout hospital stay, IgG at 24 h of life >800 mg/dl; suckled the mares udder within 2 h of life, mare with good quality colostrum (Brix >25%)	no antibiotic treatment
Colostrum (n=4)	born at the clinic, healthy throughout hospital stay; did not suckle the mares udder within 2 h of life or mare with poor colostrum quality (Brix < 20%)	colostrum (bottle/gastric tube) within 4 h of life, no antibiotic treatment
FPT (n=8)	born at the clinic (n=4) or referred 10.8±1.6 h after birth (n=4), healthy throughout hospital stay, no signs of leucopenia or leucocytosis, fibrinogen within reference values; did not suckle the mares udder within 6 h of life or IgG <400 at 12 h of life	Plasma transfusion, standard treatment if necessary
Sepsis (n=5)	born at the clinic (n=1) or referred 14.8±1.9 h after birth (n=4), clinical signs of infection (e.g. diarrhoea at the 1d of life), leucopenia or leucocytosis, fibrinogen ↑	Plasma transfusion (Hypermune), standard treatment
Premature (n=5)	born at the clinic (n=3) or referred 5.0±1.0 h after birth (n=2), clinical signs of prematurity, immature white blood count (neutrophil/lymphocyte ratio <2/1), immature ossification of carpal and tarsal joints (diagnosis by x-ray).	Plasma transfusion (Hypermune), standard treatment

Cortisol analysis

Saliva for cortisol analysis was collected with cotton rolls (Salivette cortisol, Sarstedt, Nümbrecht, Germany) as described (*Erber* et al. 2012). The Salivette was grasped with a surgical clamp and placed onto the tongue of the foal for one minute until it was well moistened. Sample collection was performed by one person and was well tolerated by all foals and no additional restraint was needed. Saliva samples were taken four times daily (06:00, 12:00, 18:00 and 24:00 h). In foals referred to Vetmeduni Vienna, no samples were available for time points before arrival of the foal at the clinic. After collection, samples were centrifuged at 1000 g for 10 min and at least 1 mL of saliva was obtained and frozen at -20 °C until analysis. For cortisol analysis an enzyme immunoassay established in our laboratory was used (*Schmidt* et al. 2010b). The antiserum cross-reacts with several cortisol metabolites and values have to be interpreted as cortisol immunoreactivity. The intra-assay and inter-assay coefficients of variation were 4.6 and 12.5%, respectively, and the minimal detectable concentration was 40 pg/mL.

Heart rate and heart rate variability

Heart rate recordings were made with the portable recording system Polar S 810i (Polar Electro, Vienna, Austria; *Nagel* et al. 2012) once daily in the morning for one hour between 06:00 and 09:00 h. The Polar system recorded beat-to-beat (RR) intervals in milliseconds (msec). Following established procedures a 5 min interval was chosen for analysis. Data was detrended and an artifact correction was made (*Tarvainen* et al. 2002, *Nagel* et al. 2010, *Schmidt* et al. 2010a, and b) using the Kubios HRV Software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland). From the recorded RR intervals, heart rate and the HRV variable SDRR (standard deviation of the RR interval) were calculated as described (*Nagel* et al. 2010, *Schmidt* et al. 2010a, and b).

Statistical analysis

The SPSS statistics programme (Version 24, IBM, Armonk, New York, USA) was used for statistical analysis. Because not all foals were available at all times (Table 2), numbers of animals vary at individual time points. All data were normally distributed (Kolmogorov-Smirnoff test). For comparison between groups on individual days, cortisol concentration, heart rate and SDRR were analysed by univariate ANOVA with Duncan's post-hoc test. In addition, values for all groups combined were compared among days with a general linear model repeated measures ANOVA and time as within subject factor. All values given are means ± standard error of mean.

Table 2 Number of foals from which data were available on different days of life | *Anzahl der an der Studie teilnehmenden Fohlen während der der ersten Lebenstage*

Group	day 1	day 2	day 3	day 4	day 5
Healthy home	10	10	10	10	10
Healthy clinic	11	11	7	5	3
Colostrum	4	4	4	3	1
FPT	8	8	8	4	4
Sepsis	5	5	5	5	5
Premature	5	4	3	3	3

Results

On day 1 of life, salivary cortisol concentration did not differ between healthy foals born at their home stable, healthy foals born at the clinic and diseased foals of all groups (Figure 1). On days 2 to 5, cortisol concentration in premature foals was significantly higher ($p < 0.05$) compared to foals of groups Healthy home, Healthy clinic, Colostrum, FPT and Sepsis. When all groups were combined, mean cortisol concentration decreased continuously from day 1 to 5 after birth ($p < 0.001$; e.g group Healthy home d1: 10.1 ± 1.2 ng/ml; d5: 4.1 ± 0.3 ng/ml).

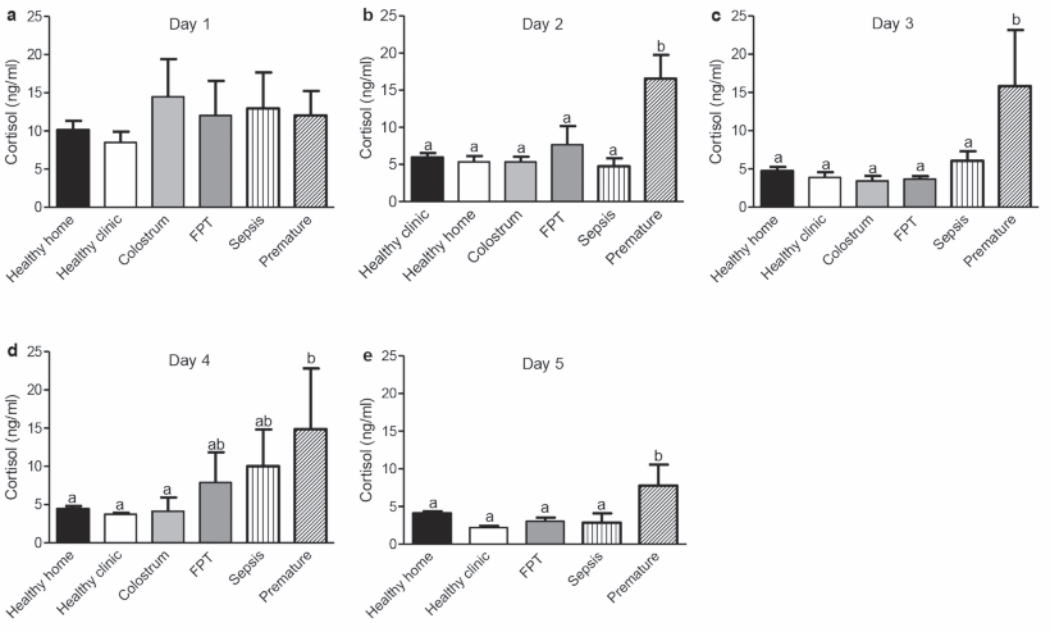

Fig. 1 Cortisol concentration (ng/ml) on days 1 to 5 of life (a-e) in foals of groups Healthy home, Healthy clinic, Colostrum, FPT, Sepsis and Premature. a,b Significant differences between individual groups on different days ($p < 0.05$). *Kortisolkonzentration (ng/ml) von Fohlen der Gruppen Healthy home, Healthy clinic, Colostrum, FPT, Sepsis und Premature vom 1. bis 5. Lebenstag (a-e). a,b Signifikante Gruppenunterschiede an den einzelnen Tagen ($p < 0.05$).*

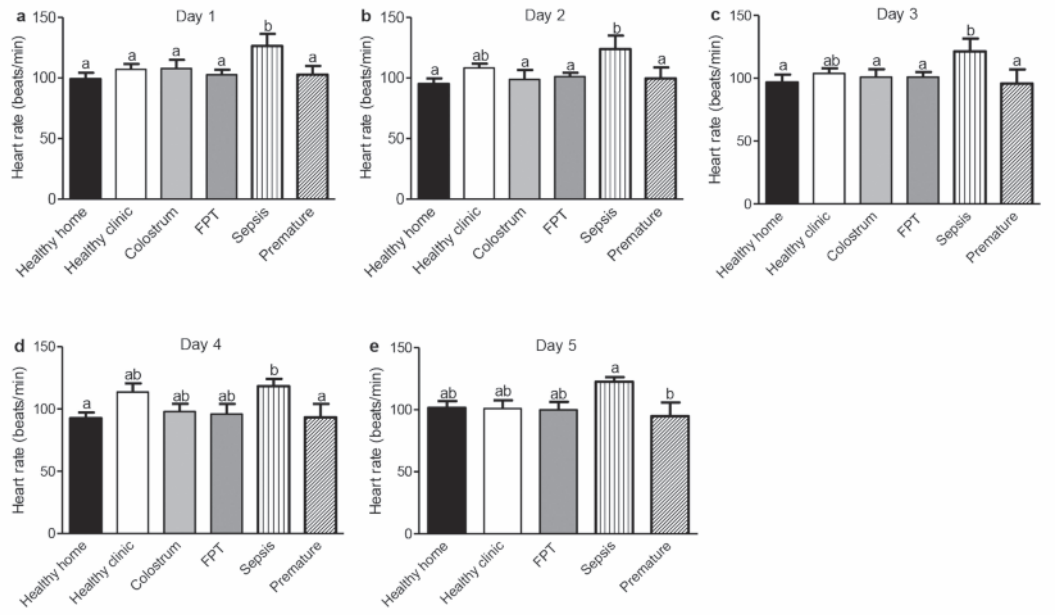

Fig. 2 Heart rate (beats/min) on days 1 to 5 of life (a-e) in foals of groups Healthy home, Healthy clinic, Colostrum, FPT, Sepsis and Premature. a,b. Significant differences between individual groups on different days ($p<0.05$). *Herzfrequenz (Schläge/min) von Fohlen der Gruppen Healthy home, Healthy clinic, Colostrum, FPT, Sepsis und Premature vom 1. bis 5. Lebenstag (a-e). a,b Signifikante Gruppenunterschiede an den einzelnen Tagen ($p<0.05$).*

Heart rate in foals of group Sepsis was at all times higher than in foals of groups Healthy home, Healthy clinic, Colostrum, FPT and Premature ($p < 0.05$; Figure 2; d1 Sepsis: 126 ± 10, Healthy home: 99 ± 5 beats/min). Mean heart rate in foals did not change during the first 5 days of life. The HRV variable SDRR differed neither among groups nor among days of life (Figure 3).

Discussion

In this study, salivary cortisol concentration was elevated in premature foals on days 2 to 5 after birth and septic foals had an elevated heart rate throughout the 5-day observation period. In none of the groups, a consistent stress response with increased adrenocortical and sympathoadrenal activity could be detected. Maturation of the fetal hypothalamo-pituitary-adrenocortical axis in horses occurs extremely late during gestation. Cortisol synthesis and adrenocortical responsiveness to ACTH increase markedly only during the last 3 to 4 days preceding birth (*Fowden* and *Silver* 1995). Foals born before term are often immature and have a poor prognosis in the immediate postnatum period (*Koterba* 1990, *Palm* et al. 2011). Elevated salivary cortisol concentration in premature foals without changes in heart rate and heart rate variability therefore most likely is not part of a stress response but may reflect ongoing adrenal maturation. In case these foals survive the first day, increasing cortisol release might enhance maturation of the neonate and improve further survival.

In all foal groups, cortisol concentration was elevated on the first day of life. Healthy newborn foals have highest cortisol concentration within 3 hours after birth and values decrease markedly thereafter (*Nagel* et al. 2015). Higher cortisol concentrations on the day of birth compared to subsequent days are in all foals a response to the demands of birth and neonatal adaptation. Cortisol concentration did not differ between foals that required frequent handling for examination and treatment but did not develop health problems (groups

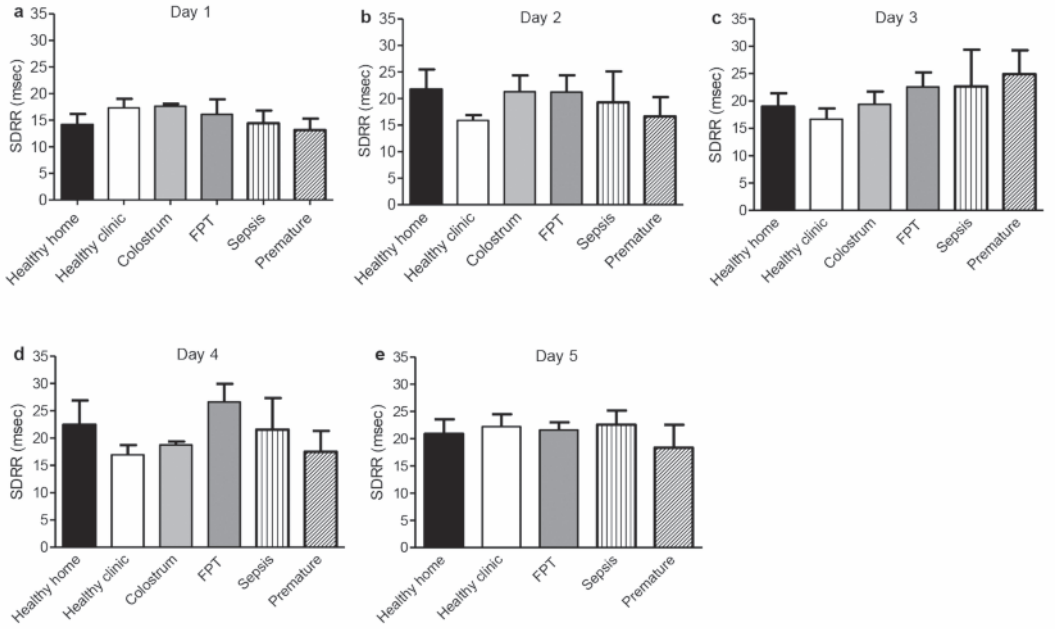

Fig. 3 SDRR (msec) on days 1 to 5 of life (a-e) in foals of groups Healthy home, Healthy clinic, Colostrum, FPT, Sepsis and Premature. No significant differences between groups. *SDRR (msec) von Fohlen der Gruppen Healthy home, Healthy clinic, Colostrum, FPT, Sepsis und Premature vom 1. bis 5. Lebenstag (a-e). Keine signifikanten Unterschiede zwischen den Gruppen.*

Colostrum and FPT) and healthy foals born either in a stud farm or a hospital environment. This indicates that handling and environment were not perceived as major stressors by the newborn foals of our study. Salivary cortisol concentration did also not differ between foals born in their stud or in the clinic and foals transported to the clinic on the first day of life. In adult horses, road transport is a stressor with a consistent increase in cortisol release (*Schmidt* et al. 2010a, and b). However, in newborn foals, any transport-related stress can be expected to be masked by a clearly more pronounced stress of neonatal adaptation (*Nagel* et al. 2015).

Interestingly, in none of the foal groups, basal salivary cortisol concentration was reduced compared to healthy foals. Even in premature foals, basal cortisol concentration was not lower than in healthy foals born at term. Major therapeutic effects of cortison or ACTH treatment in these foals are therefore unlikely. This does not exclude the occurrence of relative adrenal insufficiency (*Hart* et al. 2010) in foals. Low basal cortisol concentration and an inadequate cortisol release in response to treatment with synthetic ACTH has been demonstrated in septic foals with shock and multiple organ failure (*Hart* et al. 2009). Relative adrenal insufficiency, therefore, might occur shortly before death of critically ill foals but in our study was not present at the beginning or during the course of treatment.

Heart rate was elevated in septic foals compared to foals from all other groups. Although this is in partial contrast to previous findings from our group (*Palm* et al. 2011), sepsis is associated with pain, tissue hypoperfusion and often shock, i.e. factors that may all induce tachycardia in foals. *Palm* et al. (2011) evaluated only clinical data taken at admission of foals to the clinic while the present data obtained repeatedly over a 5-day period give a more detailed picture. All foals of the present study were discharged healthy from the clinic. Thus, a higher heart rate in septic foals cannot be associated with poor outcome. As for cortisol, average heart rate was neither influenced by the frequency and intensity of handling of the foals nor by a stud farm or veterinary hospital environment.

The heart rate variability parameter SDRR at no time differed among groups. In healthy foals during the first 120 minutes after birth, SDRR was lower than at the end of the first week of life (*Nagel* et al. 2015) and a postnatal increase in HRV occurs already 4 to 6 hours after birth (*Wulf* et al. 2017). Analysis of HRV in foals of the present study was performed at a time when a transient HRV decrease during immediate postnatal adaptation can no longer be detected. In contrast to findings in newborn human babies, sepsis in foals does not seem to be associated with a decrease in HRV. This is at least true if veterinary intervention starts at an early stage and the foals respond positively to treatment. Nevertheless, this does not exclude a decrease in HRV in association with severe endotoxemia and poor outcome in foals. As for cortisol, average heart rate and HRV were neither influenced by the frequency and intensity of handling of the foals nor by a stud farm or veterinary hospital environment.

In conclusion, adrenocortical and sympathoadrenal function of foals was neither consistently activated nor depressed by neonatal sepsis. Only heart rate was increased in septic foals. Increased cortisol release in premature foals in the first days of life may mimic maturational processes that occur already shortly before birth in term foals. Neither frequent handling nor a hospital environment induced a stress response in newborn foals.

Conflict of interest statement

None of the authors of this paper have financial or personal relationships with other people or organizations that could inappropriately influence or bias the content of the paper.

Animal welfare statement

The study was approved by the competent authority for animal experimentation in Brandenburg State, Germany (Landesamt für Umwelt, Gesundheit und Verbraucherschutz, license number V3-2347-14-2011).

References

Bohanon F. J., Mrazek A. A., Shabana M. T., Mims S., Radhakrishnan G. L., Kramer G. C., Radhakrishnan R. S. (2015) Heart rate variability analysis is more sensitive at identifying neonatal sepsis than conventional vital signs. Am. J. Surg. 210, 661-667; DOI 10.1016/j.amjsurg.2015.06.002

Downing S. E., Lee J. C. (1983) Analysis of cardiac adrenergic mechanisms in hypoxic lambs. Am. J. Physiol. 244, 222-227

Erber R., Wulf M., Becker-Birck M., Kaps S., Aurich J. E., Möstl E., Aurich C. (2012) Physiological and behavioural responses of young horses to hot iron branding and microchip implantation. Vet. J. 191, 171-175; DOI 10.1016/j.tvjl.2011.08.008

Fowden A. L., Silver M. (1995) Comparative development of the pituitary-adrenal axis in the fetal foal and lamb. Reprod. Dom. Anim. 30, 170-177: DOI10.1111/j.1439-0531.1995.tb00141.x

Gholami M., Mazaheri P., Mohamadi A., Dehpour T., Safari F., Hajizadeh S., Moore K. P., Mani A. R. (2012) Endotoxemia is associated with partial uncoupling of cardiac pacemaker from cholinergic neural control in rats. Shock 37, 219-227; DOI 10.1097/SHK.0b013e318240b4be

Hart K. A., Slovis N. M., Barton M. H. (2009) Hypothalamic-pituitary-adrenal axis dysfunction in hospitalized neonatal foals. J. Vet. Intern. Med. 23,901–912; DOI 10.1111/j.1939-1676.2009.0323.x

Hart K. A., Barton M. H., Ferguson D. C., Berghaus R., Slovis N. M., Heusner G. L., Hurley D. J. (2011) Serum free cortisol fraction in healthy and septic neonatal foals. J. Vet. Intern. Med. 25, 345-355; DOI 10.1111/j.1939-1676.2010.0667.x

Irestedt L., Lagercrantz H., Hjemdahl P., Hägnevik K., Belfrage P. (1982) Fetal and maternal plasma catecholamine levels at elective cesarean section under general or epidural anesthesia versus vaginal delivery. Am. J. Obstet. Gynecol. 142, 1004-1010

Kirschbaum C. (2000) Salivary cortisol. In: Fink, G. (Ed.), Encyclopedia of Stress. Academic Press, San Diego, California, USA, pp. 379–383

Koterba A. M. (1990) Prematurity In: Koterba A. M., Drummond W. H., Kosch P, (Eds), Equine Clinical Neonatology. Lea and Febiger, Philadelphia, Pennsylvania, USA, pp. 769-778

Koterba A. M., Brewer B. D., Tarplee F. A. (1984) Clinical and clinicopathological characteristics of the septicaemic neonatal foal: review of 38 cases. Equine Vet. J. 16, 376-382

LeBlanc M. M., Tran T., Baldwin J. L., Pritchard E. L. (1992) Factors that influence passive transfer of immunoglobulins in foals. J. Am. Vet. Med. Assoc. 200, 179-183

Nagel C., Aurich J., Aurich C. (2010) Determination of heart rate and heart rate variability in the equine fetus by fetomaternal electrocardiography. Theriogenology 73, 973-983; DOI10.1016/j.theriogenology.2009.11.026.

Nagel C., Erber R., Bergmaier C., Wulf M., Aurich J., Möstl E., *Aurich C.* (2012) Cortisol and progestin release, heart rate and heart rate variability in the pregnant and postpartum mare, fetus and newborn foal. Theriogenology 78, 759-767; DOI10.1016/j.theriogenology.2012.03.023.

Nagel C., Erber R., Ille N., Wulf M., Aurich J., Möstl E., Aurich C. (2015) Heart rate and salivary cortisol concentrations in foals at birth. Vet. J. 203, 250-252; DOI 10.1016/j.tvjl.2014.11.013

Padbury J., Agata Y., Ludlow J., Ikegami M., Baylen B., Humme J. (1987) Effect of fetal adrenalectomy on catecholamine release and physiologic adaptation at birth in sheep. J. Clin. Invest. 80, 1096-1103

Palm F., Aurich C., Nachengast I., Aurich J. (2011) Primary diagnosis, survival and long-term outcome in young horses with problems in the neonatal period. Pferdeheilkunde 27, 695-700; DOI 10.21836/PEM20110618

Panzani S., Villani M., McGladdery A., Magri M., Kindahl H., Galeati G., Martino P. A., Veronesi M. C. (2009) Concentrations of 15-ketodihydro-PGF2alpha, cortisol, and progesterone in the plasma of healthy and pathologic newborn foals. Theriogenology. 72, 1032-1040; DOI 10.1016/j.theriogenology.2009.06.015

Peeters M., Sulon J., Beckers J.F., Ledoux D., Vandenheede M. (2011) Comparison between blood serum and salivary cortisol concentrations in horses using an adrenocorticotropic hormone challenge. Equine Vet. J 43, 487-493; DOI 10.1111/j.2042-3306.2010.00294.x

Sanchez L. C. (2005) Equine neonatal sepsis. Vet. Clin. North. Am. Equine Pract. 21, 273-293

Schmidt A., Biau S., Möstl E., Becker-Birck M., Morillon B., Aurich J., Faure J. M., Aurich C. (2010a) Changes in cortisol release and heart rate variability in sport horses during long distance road transport. Domest. Anim. Endocrinol. 38, 179-189; DOI 10.1016/j.domaniend.2009.10.002.

Schmidt A., Möstl E., Wehnert C., Aurich J., Müller J., Aurich C. (2010b) Cortisol release and heart rate variability in horses during road transport. Horm. Behav. 57, 209-215; DOI10.1016/j.yhbeh.2009.11.003.

Tarvainen M. P., Ranta-Aho P. O., Karjalainen P. A. (2002) An advanced detrending method with application to HRV analysis. IEEE Trans. Biomed. Eng. 49, 172-175

von Borell E., Langbein J., Despres G., Hansen S., Leterrier C., Marchand-Forde J., Marchand- Fonde R., Minero M., Mohr E., Prunier A., Valance D., Veissier I. (2007) Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals – a review. Physiol. Behav. 92, 293-316; DOI 10.1016/j.physbeh.2007.01.007

Wulf M., Erber R., Ille N., Beythien E., Aurich J., Aurich C. (2017) Effects of foal sex on some perinatal characteristics in the immediate neonatal period in the horse. J. Vet. Behav. 18, 37-42; DOI 10.1016/j.jveb.2016.12.010