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Cortisol and progestin release, heart rate and heart rate variability in the pregnant and postpartum mare, fetus and newborn foal

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Abstract

The mechanisms leading to parturition in the horse in many aspects differ from those in other species. Pregnancy is maintained not by progesterone but by 5α-pregnanes and the progestin precursor pregnenolone originates from the fetus. As parturition approaches, the fetal adrenal switches from pregnenolone to cortisol synthesis but it is not known whether cortisol crosses the placenta. We hypothesized that in parallel to fetal cortisol release, cortisol in the maternal circulation increases before foaling and this increase can be determined in both saliva and plasma. In addition, maternal, fetal and neonatal heart rate and heart rate variability were measured. In 25 pregnant mares, saliva for cortisol analysis was collected 4 times daily from 15 days before to 5 days after foaling. In 13 mares, in addition, fetomaternal electrocardiogram (ECG) recordings were made and blood samples for progestin and cortisol analysis were collected once daily. Heart rate (HR) was recorded until 5 days after foaling. The heart rate variability (HRV) variables standard deviation of the beat-to-beat (RR) interval (SDRR) and root mean square of successive RR differences (RMSSD) were calculated. From Days 15 to 4 before parturition, progestin concentration increased (peak 267 $\pm$ 42 ng/mL) and decreased thereafter ($P < 0.05$, day of foaling 113 $\pm$ 18 ng/mL). A prepartum increase in maternal cortisol concentrations was evident in blood ($P < 0.05$) and saliva ($P < 0.05$) and paralleled the decrease in progestin concentrations. In mares, HR remained constant during the last days of pregnancy but decreased within one day after parturition ($P < 0.05$) while maternal HRV did not change. In the fetus and neonate, HR increased from before to after birth ($P < 0.05$) indicating increasing demands on the cardiovascular system with adaptation to extrauterine life.

Keywords: Horse; Pregnancy; Progestins; Cortisol; Heart rate

1. Introduction

The mechanisms leading to parturition in the horse mare are in part still unknown and in many aspects differ from those in other species. Progesterone is undetectable in the blood of late-pregnant mares and pregnancy is apparently maintained by 5α-pregnanes. The concentration of these progestins increases during the last 20 to 30 days before foaling [1] and a decline of maternal progestin concentrations can only be observed during the last few days preceding parturition. Although progestin concentrations decline antepartum, mares foal with still high levels of circulating progestins [2,3] and treatment with the synthetic progestin altrenogest does not delay foaling [4].

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Unique to the horse, the precursor of maternal progestins, pregnenolone, originates from the fetal adrenal gland [1,5]. As parturition approaches, fetal adrenocortical sensitivity to adrenocorticotrophic hormone increases, and hormone production in the fetal adrenal switches from pregnenolone to cortisol [6]. The subsequent increase in fetal cortisol is essential for organ maturation [7] and in many other species is also part of the cascade which triggers parturition. In the horse, this marked rise in fetal cortisol synthesis occurs only within the last two to three days of gestation, coincident with the beginning decline in maternal progestins [6,8]. It is not known whether cortisol originating from the fetal adrenal crosses the placenta to the maternal side and may have direct effects on the mare.

Cortisol concentrations in plasma of pregnant mares are elevated compared to non-pregnant mares, but this increase occurs mainly at midpregnancy [9]. No antepartum increase in maternal cortisol concentrations has been reported so far [10–12] and under experimental conditions ^{14}C-labeled cortisol infused into late-gestation fetuses was found only in minimal amounts in maternal blood [10].

The induction of parturition by the fetus may be comparable to a chronic stress response which triggers the prepartum increase in cortisol. Acute stress can be questioned because fetal hypothalamo-pituitary-adrenocortical activation is not accompanied by sympathoadrenomedullary activity [13–15].

Cortisol in the horse can be measured either in blood or saliva [16,17]. Advantages of salivary cortisol determination are stress-free sample collection and the fact that only free, i.e., biologically active, cortisol is measured. In this study, we have analyzed changes in maternal salivary cortisol, blood cortisol and progestin concentrations before and after parturition. We hypothesized that in parallel to fetal cortisol concentrations, maternal cortisol increases before foaling and this increase can be determined in both saliva and plasma. An increase shortly before onset of foaling might allow assessment of fetal maturation and predict the onset of parturition. In addition, maternal, fetal and neonatal heart rate and heart rate variability were measured to determine stress-like changes in sympathoadrenal activity before and after foaling.

2. Materials and methods

2.1. Animals

Twenty-five late-pregnant Warmblood brood mares with singleton pregnancies at the Brandenburg State Stud in Neustadt (Dosse), Germany, were available for this study. Mares were between 4 and 15 yrs of age (7.3 ± 0.7 yrs) and the average pregnancy length was 339 ± 2 days. Horses were housed in group stables on straw and were fed oats and hay twice daily. Mineral supplements and water were freely available at all times. Mares had daily access to an outdoor paddock. Approximately 15 days before the calculated day of parturition, mares were brought into the foaling unit, where they were housed in single boxes, also with daily access to the paddock together with their group mates. Mares in the foaling stable were observed 24 h per day. All mares were healthy throughout the study and during parturition no complications occurred and no assistance at foaling was needed. On clinical examination, all foals were mature and healthy and were able to stand at 47 ± 3 min and to suckle their dams' udder at 110 ± 12 min after birth.

2.2. Experimental design

In all mares and foals, saliva was collected at 6:00 AM, 12:00 AM, 6:00 PM and 12:00 PM. In mares, salivary samples were taken from 15 days before to 5 days after foaling. In nine foals, saliva was taken from the day of birth (Day 1) until Day 5 after birth. In addition to saliva collection, in a subgroup of mares (age 4–15 yrs, mean 7.1 ± 1.0 yrs, gestational length 338 ± 2 days) and their foals, cardiac beat-to-beat (RR) intervals were recorded for analysis of heart rate and heart rate variability (HRV) and from the mares, blood was collected once daily. Fetomaternal electrocardiogram (ECG) recordings were always performed at 6:00 PM for 1 h from Day 15 before delivery until the day of foaling. After parturition, heart rate in the mares and their neonatal foals was recorded from the day of birth (Day 1) until 4 days thereafter, for 1 h starting at 6:00 PM. Data were analyzed for Days -15, -10, -5, -4, -3, -2, -1, day of foaling (Day 1) and on Days 2–5. Because of individual deviation from the calculated day of foaling and because of stud management decisions, not all mares and foals were available at all times. The numbers of mares and foals and the parameters analyzed for individual days are summarized in Table 1.

2.3. Sample collection

Saliva for cortisol analysis was collected as described [18]. In brief, a cotton roll for saliva collection (Salivette for cortisol analysis, Sarstedt, Nümbrecht-Rommelsdorf, Germany) was grasped with a surgical arterial clamp, inserted gently into the horse's mouth at the angle of the lips and placed onto the horse's tongue

Table 1
Numbers of mares and foals included into the study at different time points.

Time relative to foaling	d -15	d -10	d -5	d -4	d -3	-48–-24 h	-24–0 h	0–24 h	24–48 h	d 3	d 4	d 5
Mares												
Saliva	10	16	22	23	24	25	25	16	16	16	16	16
Blood	5	9	13	13	13	13	13	9	9	9	9	9
Heart rate, HRV	5	9	13	13	13	13	13	9	9	9	9	9
Foals												
Saliva	—	—	—	—	—	—	—	9	9	9	9	9
Heart rate, HRV	9	9	9	9	9	9	9	9	9	9	9	9

for 1 min until the roll was well moistened. Collection of saliva was well tolerated by the animals without any restraint of the horse needed. 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 determination of plasma cortisol and progestins, blood was collected from one jugular vein into heparinized tubes (Vacuette, Greiner, Kremsmünster, Austria) at 6:00 PM once daily from the day the mares arrived at the foaling stable (Day -15) until 5 days after the foal was born. Immediately after collection, blood samples were centrifuged at 1200g for 10 min, the plasma was decanted and frozen at −20 °C until analysis.

2.4. Hormone analysis

Cortisol concentrations in saliva and in plasma were determined with an enzyme immunoassay established in our laboratory as described [18]. The antiserum cross-reacts with several cortisol metabolites and values have to be interpreted as cortisol immunoreactivity. The intraassay and interassay coefficients of variation were 5.0 and 6.7%, respectively, and the minimal detectable concentration was 0.3 pg/well.

Progestins in plasma were determined with a commercial enzyme immunoassay for progesterone (ADI-900-011, Assay Designs, Ann Arbor, MI, USA) following the manufacturer's recommendations. The antiserum cross-reacts 100% with 5α-pregnane-3,20-dione, thus besides progesterone measuring accurately the most important pregnancy-specific equine progestin. According to the manufacturer's information, cross-reactivity is 3.5% with 17-OH-progesterone and <1% for all other steroids tested. Serial dilutions of equine plasma showed good parallelity to the progesterone standard curve and recovery of standard added to equine samples before assaying was 96%. Depending on the concentration to be expected from preliminary assays, plasma was diluted from 1:20 to 1:500 before analysis. The intraassay coefficient of variation was 7.5 and 9.0%, respectively, for low- and medium-range reference plasma, and the interassay coefficient of variation was 10.1 and 8.5%, respectively for the low- and medium-reference plasma. The minimal detectable concentration of the assay was 8.6 pg/mL.

2.5. Heart rate and heart rate variability

Fetomaternal ECG recordings were made with the Televet 100 system (version 4.1.3, Kruuse, Marslev, Denmark) as described [14]. This ECG device uses a filter allowing to display and analyze maternal and fetal cardiac action both combined and separately and to amplify the fetal signal for evaluation. Data were transferred to a computer via Bluetooth. Recordings in mares after parturition were made with the same position of ECG electrodes. During recordings, mares remained in their normal surroundings. Heart rate recordings in newborn foals were made with a portable recording system (S810i, Polar) as described [18,19] and during recordings foals remained together with their dams in the individual foaling boxes.

For HRV analysis, the Kubios HRV Software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was used. For determination of heart rate (HR) and HRV variables, from all records of the mare, the fetus and the neonatal foal, three 5-min intervals from the start, middle and end of each 1 h recording were selected taking in account also signal quality. For all further calculations mean values from these three intervals were used. To remove trend components, data were detrended and, in addition, an artifact correction was made as in previous studies on horses [18,20] following established procedures [21]. From the recorded beat-to-beat (RR) intervals, heart rate and the HRV variables standard deviation of the RR interval (SDRR) and root mean square of successive RR differences (RMSSD) were calculated for mares, fetuses and foals.

2.6. Statistical analysis

Statistical analysis was performed with the PASW 17.0 statistics package (SPSS, Chicago, IL, USA) using non-parametric tests throughout. In mares, for parameters determined once daily (plasma cortisol, progestins, heart rate, HRV) throughout the observation period (Days -15 to +5) on all days and for salivary cortisol concentrations from 48 h before to 48 h after foaling, the last value obtained before foaling was compared to all other time points by Wilcoxon test. Heart rate and HRV in fetuses and foals were analyzed in the same way. In foals, salivary cortisol concentrations for the first 48 h after birth were compared to the first time point after birth by Wilcoxon test. For analysis of salivary cortisol concentrations over the whole observation period, for each day the mean of the 4 values (6:00 AM, 12:00 AM, 6:00 PM and 12:00 PM) was calculated and means were compared to the mean of the last 4 values before foaling (mares) or mean of the first 4 values after birth (foals). Data given are means ± SEM. A P-value below 0.05 was considered significant.

In the figures, values obtained between 48 h before and 48 h after foaling are aligned to foaling as Time 0. With the 6 h sampling interval, salivary cortisol data for this period are thus grouped into 6 h time windows. All data collected before or after that period are given for the respective days with the day of foaling defined as Day 1.

3. Results

3.1. Progestins

Progestin concentrations in blood plasma of mares increased until Day 4 before foaling and decreased continuously thereafter (Fig. 1). On Day 15 before parturition, blood progestin concentration averaged 112.3 ± 31.8 ng/mL and until Day 4 before parturition had reached a peak mean value of 266.7 ± 42.2 ng/mL. Subsequently a continuous decrease occurred to the last sample taken before foaling (113 ± 18.1 ng/mL; $P < 0.05$ vs. days -10 to -3 and 24–48 h before foaling). After foaling, progestin concentrations decreased further and on Day 5 after foaling were 2.5 ± 0.3 ng/mL (last sample before foaling vs. all values after foaling $P < 0.05$).

3.2. Cortisol

During the last 15 days before parturition, concentration of cortisol increased both in blood plasma and saliva (Figs. 1 and 2). On Day 15 before parturition, concentration of cortisol in plasma was 11.4 ± 1.8 ng/mL and in saliva was 0.9 ± 0.18 ng/mL (samples taken at 6:00 PM, i.e., same time as blood samples). On Day 4 before delivery, cortisol values were 17.8 ± 1.6 and 1.5 ± 0.2 ng/mL in blood and saliva, respectively. Thereafter, a marked increase in cortisol concentrations occurred. Highest values in plasma were measured in the last sample collected before foaling (24.3 ± 2.1 ng/mL) while highest values in saliva were measured in the first postpartum sample (6.1 ± 1.6 ng/mL). Plasma cortisol concentration in the last sample taken before foaling was significantly higher than on days -15 to -4 and in all postpartum values ($P < 0.05$; Fig. 1).

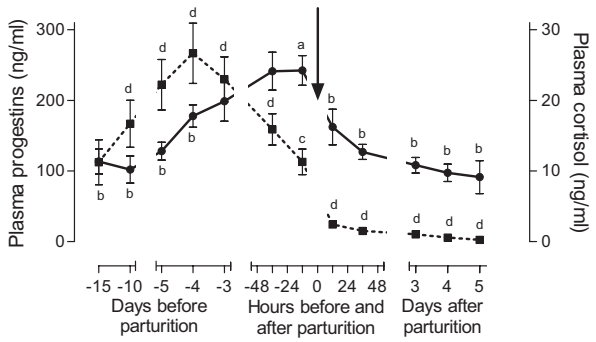

Fig. 1. Concentration of progestins (■) and cortisol (●) in plasma of mares from 15 days before to 5 days after parturition (n = 5–13), arrow = time of parturition, values are mean ± SEM a, b: For cortisol values marked with b differ significantly from the last antepartum value (marked a; $P < 0.05$); c, d: for progestins, values marked with d differ significantly from the last antepartum value (marked c; $P < 0.05$).

While blood was collected once daily, cortisol in saliva was determined 4 times per day. For the last 8 samples (corresponding to the last 48 h), before foaling data were analyzed in relation to the time of foaling. On average, the last sample was taken 162 ± 20 min before foaling. Salivary cortisol values in the last antepartum sample (4.5 ± 1.0 ng/mL) were significantly ($P < 0.05$) higher than during the time window from 48 to 24 h before foaling (between 1.6 ± 0.1 and 2.6 ± 0.7 ng/mL). During foaling, salivary cortisol concentrations increased further (first sample after foaling 6.1 ± 1.6 ng/mL at 170 ± 29 min postpartum). After foaling, the first 8 samples, aligned with the time of parturition, did not differ significantly from the last antepartum value (Fig.2b). When average 24 h salivary cortisol concentrations were compared, the mean of the last 24 h before foaling differed significantly ($P < 0.05$) from all values except the first 24 h period after foaling (Fig. 2a).

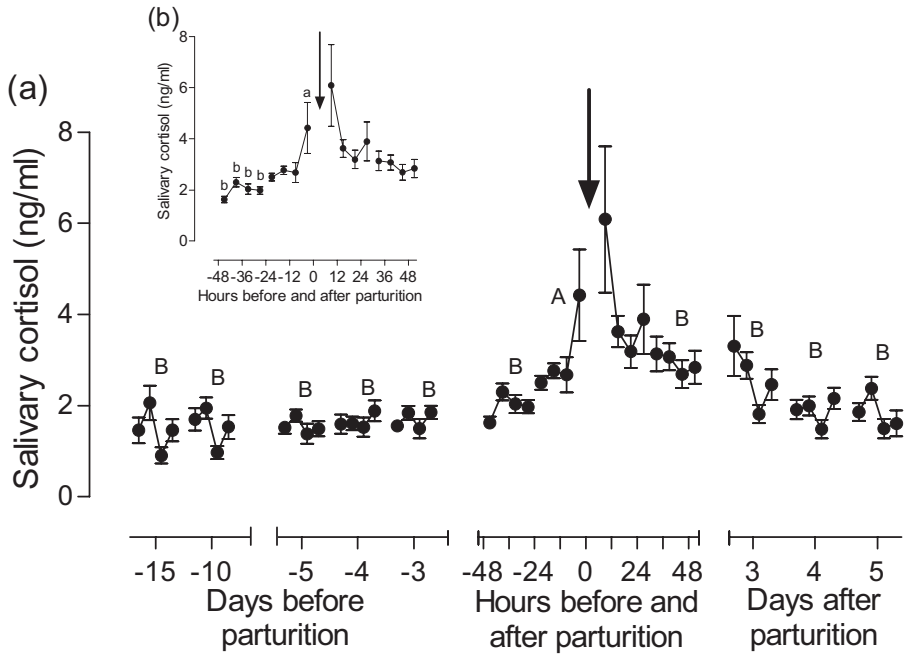

Fig. 2. (a) Salivary cortisol concentration in mares (n = 10–25) from 15 days before to 5 days after foaling. Samples were taken at 6:00 AM, 12:00 AM, 6:00 PM and 12:00 PM. For the last 48 h before and after foaling, values are aligned to the time of parturition and for all other times are given for the respective days, arrow = time of parturition, values are mean ± SEM A, B: mean values for days marked B differ significantly from the mean of the last 4 antepartum values (marked A; $P < 0.05$). (b: insert). Within the time window from 48 hours before to 48 h after foaling, values marked with b differ significantly from the last value before foaling (marked a; $P < 0.05$).

Salivary cortisol concentrations in newborn foals were determined for the first time at 190 ± 40 min after birth and were 39.9 ± 12.5 ng/mL. Thereafter, a significant decrease in salivary cortisol concentration occurred with the first postnatal value being significantly higher than all subsequent values during the first 48 h of life ($P < 0.05$) and the mean of the first 24 h of life being significantly higher than mean values for Hours 24–48 and Days 3, 4, and 5 (e.g., Day 5: 4.0 + 0.3 ng/mL; $P < 0.05$; Fig. 3).

3.3. Heart rate and heart rate variability

In the pregnant mares, heart rate was relatively constant during the last 15 days of pregnancy. Maternal heart rate decreased significantly from the last recording before to the first recording after foaling ($P < 0.05$; Fig. 4a). Mean heart rate during the prepartum period averaged 53 ± 1 beats/min, whereas mean heart rate during the postpartum period was 42 ± 1 beats/min. Maternal HRV did not change over the last 15 days before parturition. There was also no significant change in SDRR and RMSSD postpartum (Fig. 4b, 4c).

Heart rate in the fetus and neonate increased from Day 5 before parturition to Day 5 after birth. Heart rate recorded for one h during the second 24 h interval after birth (96 ± 3 beats/min) and on Day 5 after birth (102 ± 6 beats/min) was significantly higher than within 24 h before birth (79 ± 3 beats/min; $P < 0.05$; Fig. 5a). The HRV variable SDRR recorded during the last 24 h before birth was higher than on day -10 ($P < 0.05$) and RMSSD during the last 24 h before birth was higher than on day -10 and +5 ($P < 0.05$; Fig. 5b, 5c).

4. Discussion

In this study, a clear prepartum increase of maternal cortisol concentrations in horse mares was found. This increase was evident in both blood plasma and saliva and occurred very late in gestation. An increase in cortisol release during the last 4 to 5 days of pregnancy has been described in the equine fetus [6], but has not been demonstrated in the antepartum mare so far [11,22]. Total cortisol in blood can be divided into bound and unbound cortisol. Only unbound cortisol is biologically active and accounts for approximately 10 to 15% of total plasma cortisol [23]. Measurements of salivary cortisol reflect the unbound part of total cortisol which readily diffuses into saliva. In our study,

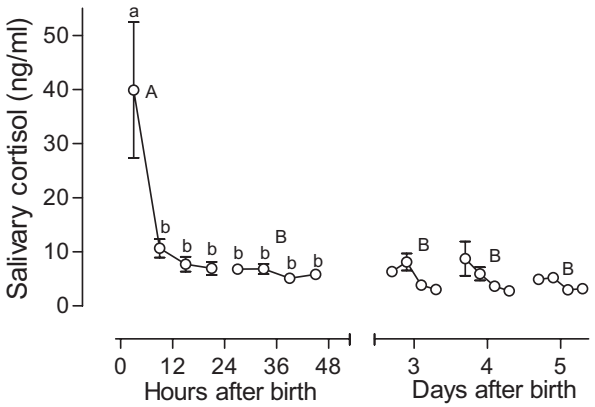

Fig. 3. Salivary cortisol concentration in foals (n = 9) until Day 5 after birth at 6:00 AM, 12:00 AM, 6:00 PM and 12:00 PM. For the first 48 h, values are aligned to the time of birth and for all other times are given for the respective days, values are mean ± SEM a, b: Within the first 48 h after birth, values marked with b differ significantly from the first postnatal value (marked a; $P < 0.05$); A, B: mean values for days marked B differ significantly from the mean of the first postnatal values (marked A; $P < 0.05$).

blood plasma cortisol concentration increased from Day 4 before parturition onwards and a pronounced increase in salivary cortisol could be detected during the last 2 days before foaling.

The concentration of corticosteroid-binding globulin in horses changes during stress situations, leading to an increase in free cortisol while total cortisol remains unchanged [24]. In late-pregnant mares, plasma cortisol increased approximately 1 day earlier than salivary cortisol concentrations. This non-parallel increase may indicate that in the antepartum mare, the cortisol binding capacity changes causing a shift towards higher amounts of free cortisol. Similar changes in cortisol binding capacity have been described in the prepartum equine fetus [6].

Cortisol release into plasma in mares is not only elevated during the immediate postpartum period but to a lesser degree already throughout pregnancy with even a decrease towards the end of gestation [9]. However, in that study, no samples were taken during the last 7 to 14 days before foaling. Higher cortisol concentrations in late-pregnant vs. non-pregnant mares can be also found in saliva. Salivary cortisol concentrations in pregnant mares 15 days before foaling were markedly higher than in samples from non-pregnant mares analyzed with the same assay (our own unpublished data). In pregnant women, both total cortisol and cortisol binding protein increases with each trimester of pregnancy while the concentration of free cortisol in plasma increases only in the second and third trimester [25].

In this study, a clear increase in maternal cortisol concentrations was found during the last days before foaling and thus at the same time as an increase in cortisol release in the fetus [6]. The increase in mater-

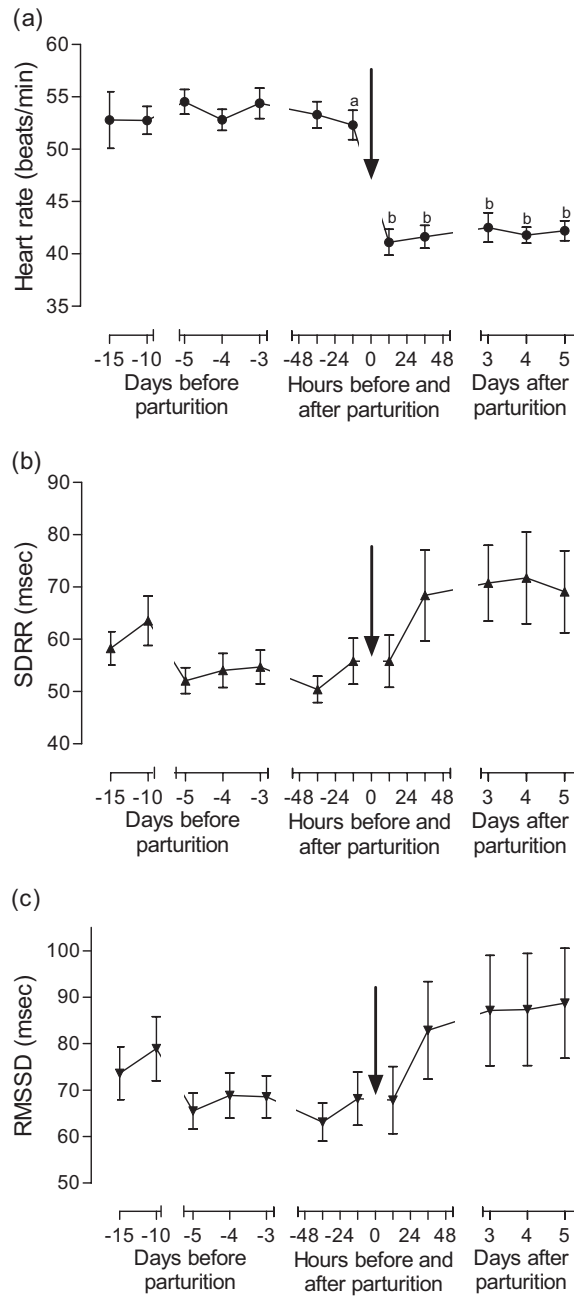

Fig. 4. (a) Maternal heart rate and HRV variables (b) SDRR and (c) RMSSD from 15 days before to 5 days after parturition (mares prepartum n = 5–13, mares postpartum n = 9), arrow = time of parturition, values are mean ± SEM a, b: Values marked with b differ significantly from the last antepartum value (marked a; $P < 0.05$).

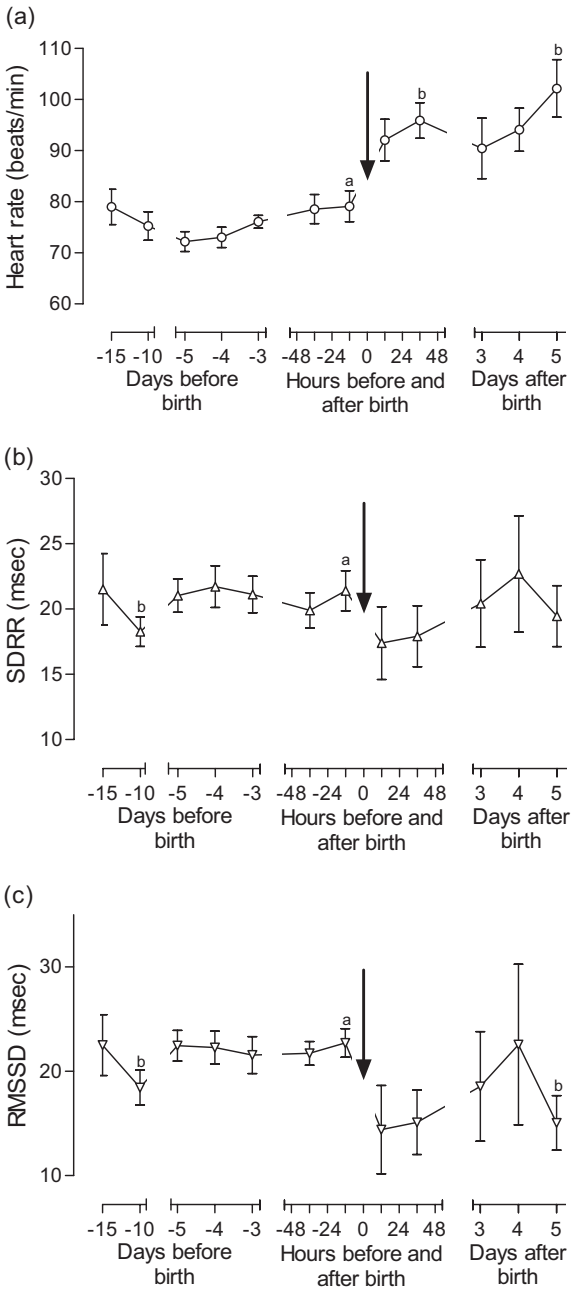

Fig. 5. (a) Fetal and neonatal heart rate and HRV variables (b) SDRR and (c) RMSSD from 15 days before to 5 days after birth (fetuses prenatal n = 5–13, foals postnatal n = 9), arrow = time of birth, values are mean ± SEM a, b: Values marked with b differ significantly from the last value before birth (marked a; $P < 0.05$).

nal cortisol concentrations also occurred together with a decrease in progestin concentrations. Assuming that the precursors of maternal progestins originate from the fetus [11], it is thus possible that the increase in cortisol and decrease in progestins in the mare is caused by changes in fetal adrenal function, leading to high amounts of cortisol which then pass the placenta. Because at least part of ^{14}C-labeled cortisol infused into the fetus diffused into the maternal circulation during the last weeks of gestation [10] placental transfer of endogenous cortisol from the fetus to the mother is possible. It may increase with changes in placental structure and steroid metabolism in the immediate antepartum period. However, we cannot exclude that maternal cortisol originates at least in part from the mare itself. Because of a cross-reactivity of cortisol and cortisone in our assay, we can also not exclude that part of maternal cortisol immunoreactivity is in fact cortisone. Activity of the enzyme 11β-hydroxysteroid dehydrogenase which converts biologically active cortisol into inactive cortisone has been demonstrated in the equine placenta [26] and may limit placental cortisol transfer.

In non-pregnant, adult horses [27,28] as well as in prepubertal young horses [19], cortisol concentrations show a clear diurnal rhythm with highest values in the morning and a decrease throughout the day. In horse mares, in agreement with cortisol release in pregnant women [25], this diurnal rhythm is apparently absent in the peripartum period. The loss of a diurnal rhythm in mares might indicate that the maternal rhythm is overridden and masked by increasing fetal cortisol.

Although cortisol concentration in the mare increased, changes in prepartum maternal HR and HRV were not found, confirming previous studies on feto-maternal HR and HRV from Day 170 of gestation until foaling [15]. The lack of changes in maternal HRV does not support the interpretation of a stress-induced cortisol release, because acute as well as chronic stress results in an HRV decrease [29]. Although cortisol release in preparturient mares is elevated compared to non-pregnant mares, absolute levels are still considerable lower than in horses exposed to known stressors, such as transport [18,20,30] or in critically ill horses (e.g., undergoing abdominal surgery, our own unpublished data). Preparation for foaling is thus neither associated with a sympathoadrenal stress response in the mare nor with an extremely high cortisol release. Changes in cortisol concentrations are most likely part of a physiological cascade triggering maturation and preparation for parturition.

A slow but continuous increase in heart rate during pregnancy [14] together with the rapid decrease after foaling in the present study indicates that pregnancy has a considerable impact on the cardiovascular system of the mare. In contrast to heart rate, HRV in the mare showed only minor, non-significant changes during

pregnancy [14] and after foaling. In the fetus, HR increased at foaling while HRV remained unchanged. Although this does not indicate a major stress response because of missing HRV changes, it nevertheless indicates adaptive processes of the fetal cardiovascular system associated with preparation for birth.

For the first time, heart rate and HRV were determined in the antepartum fetus and in the same animals after birth. Heart rate increased after birth, presumably caused by increasing demands on the cardiovascular system with adaptation to extrauterine life. After birth, the HRV variable RMSSD decreased transiently. The RMSSD reflects activity of the parasympathetic (vagal) branch of the autonomous nervous system [29]. A decreased RMSSD indicates a low vagotonus. Immediately after delivery, sympathoadrenal activity is markedly elevated in both humans [31] and calves [32] but this immediate postnatal period was not investigated in the current study.

The progesterone assay in this study shows 100% cross-reactivity with 5α-pregnane-3,20-dione and thus not only progesterone but also one of the major pregnancy-specific progestins in horses was determined. As described [1,12] and in contrast to most other species delivery in the mare takes place under high concentrations of progestins. Although onset of foaling is associated with a decline in total progestin concentration, initiation of parturition in the horse does not require a decrease in progestins to near-zero values. This is further underlined by the fact that treatment with the synthetic progestin altrenogest does not prevent parturition in mares [4]. In other species, e.g., the dog, exogenous progestin treatment in pregnant bitches has to be terminated well before the expected day of whelping to prevent delayed parturition [33]. In horse mares, removal of the progesterone block and thus an increased sensitivity to uterotonic hormones, may be regulated by other mechanisms than a decrease in progestin release.

5. Conclusions

In conclusion, cortisol concentrations increase in the antepartum mare. Although progestin concentrations decrease shortly before foaling, mares give birth when progestin concentrations are still elevated. We suggest that cortisol in the maternal circulation could in part be of fetal origin. Changes in maternal, fetal and neonatal heart rate and HRV indicate that pregnancy has considerable impacts on the maternal cardiovascular system and adaptation to extrauterine life in the foal is associated with a marked sympathoadrenal response.

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